Serum oxidized low-density lipoprotein decreases in response to statin therapy and relates independently to reductions in coronary plaque in patients with HIV

Eric Nou[a], Michael T. Lu[b], Sara E. Looby[a], Kathleen V. Fitch[a], Elli A. Kim[a], Hang Lee[c], Udo Hoffmann[b], Steven K. Grinspoon[a] and Janet Lo[a]

Objective: Circulating oxidized low-density lipoprotein (oxLDL) levels are elevated in HIV-infected patients and have been associated with atherosclerosis. Statins have been shown to reduce plaque on coronary computed tomography angiography (cCTA) in HIV-infected individuals. Thus, we investigated the effect of statins on serum oxLDL levels and the relationship between changes in oxLDL and coronary atherosclerosis on cCTA in patients with HIV.

Design: We previously conducted a 12-month randomized, placebo-controlled trial with atorvastatin in 40 HIV-infected patients on stable antiretroviral therapy with subclinical coronary atherosclerosis and low-density lipoprotein (LDL)-cholesterol less than 130 mg/dl.

Methods: In the current analysis, patients underwent cCTA and measurements of serum oxLDL, sCD14, sCD163, lipoprotein phospholipase-A_2, and fasting lipids at baseline and end of the study.

Results: Nineteen patients were randomized to atorvastatin and 21 patients to placebo. Serum oxLDL decreased −22.7% (95% CI −28.7 to −16.7) in the atorvastatin group and increased 7.5% (95% CI −3.3 to 18.4) in the placebo group ($P < 0.0001$). Change in oxLDL significantly correlated with changes in noncalcified plaque volume, total plaque volume, positively remodeled plaque, and low attenuation plaque. The association between changes in oxLDL and noncalcified plaque volume was independent of the baseline 10-year Framingham risk, LDL, $CD4^+$ cell count, and viral load.

Conclusion: Statins lower oxLDL levels in HIV-infected patients, and reductions in oxLDL are related to improvements in coronary atherosclerosis, independent of traditional cardiovascular risk factors. Reductions in oxLDL may be one mechanism through which statins exert beneficial effects on reducing atherosclerosis in HIV-infected individuals.

AIDS 2016, **30**:583–590

Keywords: atherosclerosis, cardiovascular, HIV, oxidized LDL, statin

Introduction

Cardiovascular disease (CVD) is a major cause of mortality in the HIV population, especially coronary heart disease (CHD) [1,2]. Even after controlling for traditional CVD risk factors, the risk of myocardial infarction (MI) is about 50% greater in HIV-infected patients than uninfected controls [3,4]. Moreover, the

[a]Program in Nutritional Metabolism, [b]Cardiovascular Imaging Section, Department of Radiology, and [c]Biostatistics Center, Massachusetts General Hospital, Boston, Massachusetts, USA.

Correspondence to Janet Lo, MD, MMSc, Biostatistics Center, Massachusetts General Hospital, LON-207, 55 Fruit Street, Boston, MA 02114, USA.

Tel: +1 617 724 3425; fax: +1 617 724 8998; e-mail: jlo@mgh.harvard.edu
Received: 19 August 2015; revised: 14 October 2015; accepted: 15 October 2015.

DOI:10.1097/QAD.0000000000000946

prevalence of subclinical atherosclerosis measured by coronary computed tomography angiography (cCTA) is greater in HIV-infected patients despite adjusting for traditional risk factors [5,6]. These data show that HIV-related mechanisms result in accelerated development of atheromatous lesions, including unstable plaque. Oxidized low-density lipoprotein (oxLDL) is a pro-inflammatory form of low-density lipoprotein (LDL) and thus represents a unifying link between lipids, inflammation, and atherosclerosis. A potential role of oxLDL in relationship to this increased risk of atherosclerosis in HIV-infected individuals is suggested by two studies that have reported higher levels in HIV-infected patients compared with uninfected controls [7,8]. Given the potentially unique mechanisms of advanced atherosclerosis in HIV, including immune activation and inflammation [9], further investigation of this pathway among HIV-infected patients is warranted.

OxLDL is thought to play a central role in atherosclerotic development at the vascular intima as it is involved in foam cell generation as well as endothelial and smooth muscle cell dysfunction [10]. Circulating oxLDL levels are about 70 times lower than concentrations within atheromatous lesions [11] but have been associated with atherosclerosis in HIV and non-HIV cohorts. In the general population, circulating oxLDL has been associated with unstable, high-risk atherosclerosis including vulnerable, macrophage-rich carotid lesions [11], noncalcified coronary plaque on cCTA [12], acute coronary syndrome [13,14], and risk of incident MI or sudden cardiac death [15]. These data from the general population suggest that circulating oxLDL levels in the HIV-infected population may be a useful marker of atherosclerosis, especially unstable plaque that results in MI, but little is known regarding the relationship of oxLDL to plaque in the HIV population. In one HIV-infected cohort, a cross-sectional study reported a significant correlation between oxLDL and the prevalence of subclinical atherosclerosis measured by carotid intima–media thickness [16]. Whether reductions in oxLDL are associated longitudinally with improvements in CHD in patients with HIV, however, remains unknown.

Statins have been shown to lower oxLDL levels in the general population [17]. However, data in HIV are limited to a single abstract that suggested an effect of rosuvastatin on oxLDL, but change in coronary plaque was not measured [18]. In a 12-month randomized, placebo-controlled trial in antiretroviral therapy (ART) treated HIV-infected individuals, we previously reported significant reductions with atorvastatin in noncalcified plaque volume, total plaque volume, positively remodeled plaque, and low attenuation plaque on cCTA [19], findings which have all been associated with culprit lesions in acute coronary syndrome [20]. These reductions were not significantly associated with decreases in LDL. Thus, the primary goal of this study was to examine the effects of atorvastatin on circulating oxLDL levels in our HIV-infected study population and investigate whether observed changes in coronary atherosclerosis on cCTA related to changes in serum oxLDL. We hypothesized that atorvastatin would lower circulating oxLDL levels and that reductions in oxLDL would be related to decreases in coronary artery lesions on cCTA, especially in plaques with high-risk morphologic characteristics that have been associated with acute MI. To our knowledge, this is first investigation relating change in oxLDL to coronary plaque in the context of statin therapy. Our data suggest that change in oxLDL relates strongly to changes in coronary plaque, independent of LDL, in HIV-infected patients receiving statin therapy, thus extending our knowledge of the potential mechanisms of CVD and of the effects of statins in the HIV population.

Methods

Study design

We previously conducted a randomized, double-blind, placebo-controlled clinical trial between 13 November 2009 and 13 January 2014, as previously reported [19]. We recruited 40 men and women with HIV on stable ART, no prior history of CVD or cardiac symptoms, LDL cholesterol between 70 and 130 mg/dl, and evidence of subclinical coronary atherosclerosis on cCTA as previously defined [19]. Patients were randomized in 1 : 1 ratio to either atorvastatin (starting at a dose of 20 mg per day and escalating to 40 mg per day at 3 months if study drug was well tolerated) or placebo for 12 months. All participants provided written informed consent, and the study was approved by the institutional review board. The trial is registered on ClinicalTrials.gov (NCT00965185).

Coronary computed tomography angiography protocol and analysis

ECG-gated cCTA was performed on a Somatom Definition Flash 128-slice dual source CT (Siemens Medical Solutions, Forchheim, Germany) according to a standardized protocol [19,21] at enrollment and at 1-year follow-up. Assessment of coronary plaque was performed by an experienced cardiac radiologist blinded to clinical data and randomization. Coronary plaque volume (calcified, noncalcified and total) as well as high-risk plaque features including low attenuation (<40 Hounsfield units) plaque and positive remodeling of the vessel wall (ratio of plaque to reference segment outer diameter > 1.05) were assessed for each coronary segment as previously described [19].

Lipid, inflammatory, metabolic, and biochemical assessments

All blood samples were drawn after a 12-h fast. Serum oxLDL was measured by enzyme-linked immunosorbent assay (ELISA) (Mercodia, Uppsala, Sweden). The intra

and inter-assay variability were 6.3 and 4.7%, respectively. Soluble CD163 (Trillium Diagnostics, intra-assay variability 3–6% and inter-assay variability 5–8%) and soluble CD14 (R&D Systems, intra-assay variability 4.8–6.4% and inter-assay variability 4.8–7.4%) were measured by ELISA. Direct LDL, total cholesterol, high-density lipoprotein (HDL), triglycerides, glucose, hemoglobin A1c, and lipoprotein-associated phospholipase-A_2 (Lp-PLA_2) were measured as previously described [19].

Immune function

Current $CD4^+$ T-cell count and viral load data were obtained, but documentation of nadir $CD4^+$ cell count was not available for this study.

Statistical analysis

Mean and 95% confidence intervals (CI) are reported to describe changes in continuous variables with normal distribution, and otherwise, median (IQR) are used. Comparisons between groups (atorvastatin vs. placebo) were performed using the Student's *t*-test for normally distributed continuous variables and the Wilcoxon rank-sum test for non-normally distributed continuous variables. Intention to treat analysis was carried out for between group comparisons using all obtainable data including the last available value for those who did not complete the study. For investigation of bivariate linear relationships between two continuous variables, a Pearson's correlation coefficient was assessed when both variables were normally distributed. Otherwise, a Spearman's rank correlation coefficient was determined. Multivariate regression modeling was performed to examine the effects of change in oxLDL on change in noncalcified plaque volume while controlling for baseline $CD4^+$ cell count, log viral load, and 10-year estimated Framingham risk – a composite score of traditional CVD risk factors known to affect plaque, as well as secondary models additionally including change in direct LDL with and without change in HDL. As LDL and oxLDL are collinear, partial correlation coefficients were also assessed to determine respective partial relationships of oxLDL and LDL to noncalcified plaque volume. As change in Lp-PLA_2 is likely an intermediary variable along the causal biological pathway of oxLDL's effects on plaque, regression modeling including these two variables simultaneously was not performed. Statistical tests with two-tailed $P < 0.05$ were considered significant. All statistical analyses were performed using SAS JMP and SAS (SAS Institute Inc., Cary, North Carolina, USA).

Results

Study population

Eighty-one HIV-infected patients were screened and 40 were enrolled and assigned to receive atorvastatin ($n = 19$) or placebo ($n = 21$), as previously reported [19]. The groups were similar at baseline (Table 1). In the overall cohort, the mean age was 51 years with a 10-year Framingham risk estimate of 6.1%. The majority were male, white, and nonsmokers. All patients were on ART

Table 1. Baseline demographics and characteristics.

Characteristics	Placebo ($n = 21$)	Atorvastatin ($n = 19$)
Age (years)	50.0 (5.6)	52.2 (3.8)
Male	17 (81%)	15 (79%)
Race or ethnic group		
White	13 (68%)	13 (68%)
Black	3 (16%)	3 (16%)
Hispanic	1 (5%)	1 (5%)
Asian	1 (5%)	0
More than one	1 (5%)	2 (11%)
Framingham 10-year risk estimate (%)	5.4 (4.4)	6.9 (4.1)
Hypertension	2 (10%)	4 (21%)
Systolic blood pressure (mmHg)	119 (16)	117 (13)
Diastolic blood pressure (mmHg)	76 (10)	73 (8)
Diabetes mellitus	2 (10%)	2 (11%)
Fasting plasma glucose (mg/dl)	88.6 (6.9)	85.8 (11.8)
Hemoglobin A1c (%)	5.5 (0.3)	5.6 (0.4)
Current smoker	6 (29%)	5 (26%)
Positive family history of CHD	10 (48%)	13 (68%)
$CD4^+$ T-lymphocyte count (cells/μl)	590 (289)	522 (263)
HIV RNA viral load (copies/ml)	<48 (<48–48)	<48 (<48–48)
Undetectable HIV RNA (<48 copies/ml)	17 (81%)	16 (84%)
Duration since HIV diagnosis (years)	15.0 (6.9)	16.8 (5.1)
Duration of antiretroviral therapy (years)	11.4 (5.8)	12.4 (3.7)
Current protease inhibitor treatment	8 (38%)	11 (58%)
Current NNRTI treatment	12 (57%)	7 (37%)
Current NRTI treatment	21 (100%)	17 (89%)

Data presented as mean (SD), median (IQR), or *n* (%). CHD, coronary heart disease; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside or nucleotide reverse transcriptase inhibitor. *P* value more than 0.05 for all between group comparisons.

for an average duration of 11–12 years, and most had undetectable viremia. At baseline, there were no discernible differences in oxLDL, Lp-PLA_2, lipids, monocyte/macrophage activation markers, or plaque characteristics between the two groups (Table 2). Discontinuation rates were similar with one patient that self-discontinued in the placebo group and two patients that were lost to follow-up in the atorvastatin group as previously described [19]. Adherence to treatment was determined by pill count and was similar between groups with an overall compliance of more than 90%. Patients were increased from 20 to 40 mg as per protocol at 3 months, after which one patient in the atorvastatin group required a dose reduction to 10 mg a day and one patient in the placebo group had a dose reduction to 20 mg a day. The remaining patients continued on 40 mg a day until study completion.

Change in lipids, inflammatory markers, and plaque characteristics by treatment group

Atorvastatin significantly lowered oxLDL with a change in oxLDL of −14.9 U/l (95% CI −20.0 to −9.9) with atorvastatin vs. 4.2 U/l (95% −3.0 to 11.3) with placebo ($P < 0.0001$ for comparison of change between groups) (Table 2). This corresponds to a mean percentage change in oxLDL of −22.7% (95% CI −28.7 to −16.7) with treatment and 7.5% (95% CI −3.3 to 18.4) with placebo ($P < 0.0001$) (Fig. 1). Atorvastatin also significantly decreased direct LDL ($P < 0.0001$), total cholesterol ($P < 0.0001$), and Lp-PLA_2 ($P = 0.005$) (Table 2). The atorvastatin group had significant reductions in non-calcified plaque volume ($P = 0.03$), total plaque volume ($P = 0.02$), positively remodeled plaque ($P = 0.04$), and low attenuation plaque ($P = 0.03$) compared with increases in the placebo group as previously reported [19] (Table 2).

Relationships between plaque characteristics, lipids, and inflammatory markers

The change in oxLDL significantly correlated with changes in direct LDL ($r = 0.70$, $P < 0.0001$), total cholesterol ($r = 0.64$, $P < 0.0001$), and Lp-PLA_2 ($r = 0.34$, $P = 0.04$) but not sCD14, sCD163, HDL, nor triglycerides. Changes in oxLDL were also significantly related to changes in noncalcified plaque volume ($\rho = 0.50$, $P = 0.002$), total plaque volume ($\rho = 0.34$, $P = 0.04$), positively remodeled plaque ($\rho = 0.34$, $P = 0.047$), and low attenuation plaque ($\rho = 0.41$, $P = 0.02$) (Table 3). There were no significant associations between change in plaque characteristics and change in direct LDL, total cholesterol, triglycerides, sCD14, or sCD163. Changes in HDL had a significant correlation with noncalcified plaque volume ($\rho = -0.32$, $P = 0.05$). Change in Lp-PLA_2 had significant associations with noncalcified plaque volume ($\rho = 0.44$, $P = 0.007$), total plaque volume ($\rho = 0.34$, $P = 0.04$), positively remodeled plaque ($\rho = 0.34$, $P = 0.04$), and low attenuation plaque ($\rho = 0.36$, $P = 0.03$).

Table 2. Baseline and between group changes in lipids, inflammatory markers, and plaque characteristics on cCTA.

Variable	Baseline placebo ($n = 21$)	Baseline atorvastatin ($n = 19$)	Between group *P* value	Change in placebo ($n = 20$)	Change in atorvastatin ($n = 17$)	Between group *P* value
OxLDL (U/l)	63.5 (13.1)	62.2 (15.9)	0.78	4.2 [−3.0 to 11.3]	−14.9 [−20.0 to −9.9]	**<0.0001**
Lp-PLA_2 (ng/ml)	272.5 (73.7)	285.6 (75.0)	0.58	−13.3 [−32.8 to 6.2]	−52.2 [−70.4 to −34.0]	**0.005**
Direct LDL (mg/dl)	124.5 (32.1)	123.6 (36.8)	0.93	11.4 [1.5 to 21.3]	−38.5 [−53.3 to −23.7]	**<0.0001**
Total cholesterol (mg/dl)	191.7 (27.1)	198.6 (37.7)	0.52	5.9 [−3.4 to 15.1]	−46.2 [−57.4 to −35.0]	**<0.0001**
HDL (mg/dl)	50.8 (14.9)	51.9 (19.4)	0.84	−1.0 [−5.9 to 4.0]	0.9 [−3.9 to 5.7]	0.58
Triglycerides (mg/dl)	113 (92 to 136)	120 (97 to 204)	0.39	8.0 (−36.0 to 32.0)	−10.0 (−39.5 to 38.3)	0.55
sCD14 (ng/ml)	1953 (350 to 2400)	2100 (1088 to 2552)	0.48	125 (−238 to 495)	−162 (−780 to 201)	0.15
sCD163 (ng/ml)	2440 (1080 to 3476)	2503 (980 to 3778)	0.94	417 (−834 to 1342)	−51 (−829 to 165)	0.29
Noncalcified plaque volume (mm^3)	66.1 (14.8 to 94.8)	33.7 (19.6 to 83.5)	0.72	6.7 (−6.5 to 29.8)	−8.2 (−18.3 to 3.5)	**0.03**
Total plaque volume (mm^3)	81.1 (30.6 to 134.9)	55.2 (23.0 to 153.6)	0.63	12.0 (0.8 to 100.4)	−0.8 (−16.8 to 14.2)	**0.02**
Positively remodeled plaque (# segments)[a]	2.1 (1.8)	2.0 (2.4)	0.59	0.4 (−0.1 to 0.8)	−0.2 (−0.4 to 0.1)	**0.04**
Low attenuation plaque (# segments)[a]	0.4 (0.6)	0.8 (1.0)	0.14	0.4 (0.0 to 0.7)	−0.2 (−0.6 to 0.2)	**0.03**

Data are mean (SD) and median (IQR) for baseline values and mean (95% CI) for change columns. Significant *P* values are shown in bold. cCTA, coronary computed tomography angiography; HDL, high-density lipoprotein; LDL, low-density lipoprotein; Lp-PLA_2, lipoprotein phospholipase-A_2; oxLDL, oxidized LDL; sCD14, soluble CD14; sCD163, soluble CD163. With intention to treat analysis and using all available data, sample sizes for change columns in oxLDL, Lp-PLA_2, total cholesterol, HDL, and triglycerides were 21 for placebo and 18 for atorvastatin.
[a]For interpretability, data presented as mean (SD) and mean (95% CI) despite being non-normally distributed but *P* value by nonparametric comparisons.

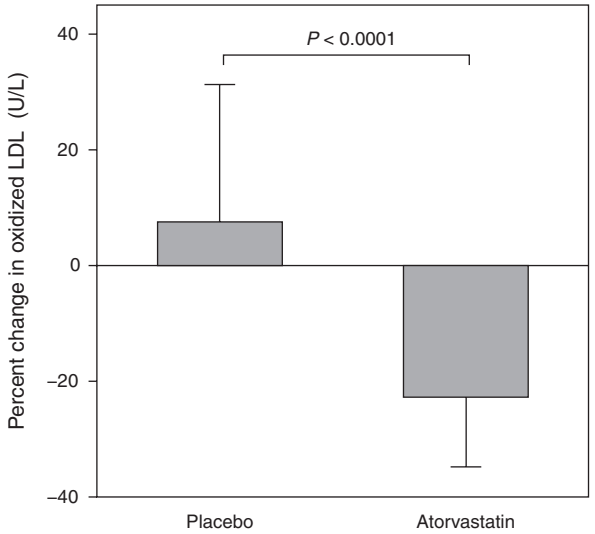

Fig. 1. Mean percentage change in oxidized LDL over 12 months between study groups. *P* value calculated using Student's *t* test. Mean (bar) and SD (error bar).

Effect of oxidized low-density lipoprotein on coronary atherosclerosis

We performed multivariate modeling to see if the relationship between changes in oxLDL and noncalcified plaque volume occurred independent of traditional CVD risk factors and HIV-specific factors. Change in oxLDL significantly associated with change in noncalcified plaque volume ($P = 0.02$) after controlling for the baseline 10-year Framingham risk estimate, $CD4^+$ cell count, and log viral load (Table 4A). In a second model that also included direct LDL, change in oxLDL significantly associated with change in noncalcified plaque volume ($P = 0.02$) after controlling for baseline 10-year Framingham risk estimate, $CD4^+$ cell count, log viral load, and change in direct LDL (Table 4B).

Respective partial relationships of oxidized low-density lipoprotein and low-density lipoprotein with noncalcified plaque volume

As biologically expected, collinearity existed between change in oxLDL and change in direct LDL. The standard error for change in oxLDL with change in noncalcified plaque volume was modified by 34% when change in

Table 3. Spearman correlations between change in plaque characteristics and change in lipids and inflammatory markers.

	Change in noncalcified plaque volume (mm^3)	Change in total plaque volume (mm^3)	Change in positively remodeled plaque (# segments)	Change in low attenuation plaque (# segments)
Change in oxLDL (U/l)	**$\rho = 0.50$; $P = 0.002$**	**$\rho = 0.34$; $P = 0.04$**	**$\rho = 0.34$; $P = 0.047$**	**$\rho = 0.41$; $P = 0.02$**
Change in Lp-PLA_2 (ng/ml)	**$\rho = 0.44$; $P = 0.007$**	**$\rho = 0.34$; $P = 0.04$**	**$\rho = 0.34$; $P = 0.04$**	**$\rho = 0.36$; $P = 0.03$**
Change in direct LDL (mg/dl)	$\rho = 0.26$; $P = 0.12$	$\rho = 0.27$; $P = 0.11$	$\rho = 0.17$; $P = 0.31$	$\rho = 0.14$; $P = 0.41$
Change in HDL cholesterol (mg/dl)	**$\rho = -0.32$; $P = 0.05$**	$\rho = -0.20$; $P = 0.23$	$\rho = -0.20$; $P = 0.24$	$\rho = -0.30$; $P = 0.07$

Data are Spearman's rank correlation coefficients. Significant *P* values are shown in bold. HDL, high-density lipoprotein; LDL, low-density lipoprotein; Lp-PLA_2, lipoprotein phospholipase-A_2; oxLDL, oxidized LDL. Data relating change in oxLDL to plaque parameters exclude two outliers in the placebo group. Sensitivity analyses including these patients show similar results in terms of directionality and significance of relationship for noncalcified plaque volume.

Table 4. Effects of oxLDL on noncalcified plaque volume.

A. Primary model assessing the effect of oxidized LDL on noncalcified plaque volume while controlling for HIV-related and traditional CVD risk factors

	Change in noncalcified plaque volume (mm^3)	
	β estimate	*P* value
Change in oxLDL (U/l)	1.6	**0.02**
Baseline estimated 10-year Framingham risk	−0.7	0.72
Baseline $CD4^+$ cell count (cells/μl)	0.0	0.87
Baseline log viral load (copies/ml)	−18.7	0.67
B. Secondary model that additionally controls for change in direct LDL		
Change in oxLDL (U/l)	2.2	**0.02**
Baseline estimated 10-year Framingham risk	−0.6	0.78
Baseline $CD4^+$ cell count (cells/μl)	0.0	0.91
Baseline log viral load (copies/ml)	−25.4	0.57
Change in direct LDL (mg/dl)	−0.3	0.38

Significant *P* values are shown in bold. CVD, cardiovascular disease; LDL, low-density lipoprotein; oxLDL, oxidized LDL. When change in high-density lipoprotein is added to model B, change in oxLDL remains significantly associated with noncalcified plaque volume ($P = 0.02$).

direct LDL was introduced into the multivariate modeling. Thus, partial correlation coefficients were determined for both change in oxLDL and change in direct LDL in relation to change in noncalcified plaque volume. The partial correlation coefficient was considerably stronger for oxLDL than for direct LDL ($r = 0.40$, $P = 0.02$ vs. $r = -0.16$, $P = 0.38$, respectively).

Discussion

This study is the first prospective trial in any study population, HIV or non-HIV, to investigate the effects of statins on oxLDL in relationship to coronary plaque on cCTA among patients with subclinical coronary atherosclerotic disease. In our randomized, placebo-controlled trial in ART-treated HIV-infected patients, atorvastatin over 1 year significantly lowered circulating oxLDL concentrations. Furthermore, we show for the first time a significant relationship between reductions in oxLDL and improvement in high-risk coronary plaque features including noncalcified plaque volume, total plaque volume, low attenuation plaque, and positively remodeled plaque. Even after adjusting for the 10-year estimated Framingham risk, $CD4^+$ cell count, viral load, and direct LDL, the relationship between changes in oxLDL and noncalcified plaque volume remained significant. In addition, changes in serum oxLDL had a stronger relationship with noncalcified plaque volume than LDL, suggesting that oxLDL may be a more relevant biomarker to assess for efficacy or mechanistic insights into the effects of statins.

This study demonstrates the efficacy of statin therapy in lowering oxLDL levels in HIV-infected patients. The percentage change in oxLDL with atorvastatin 40 mg was −22.7% (95% CI −28.7 to −16.7) over 12 months. The magnitude of reduction appears to be generally similar to the reductions seen in the general population. In a randomized, placebo-controlled study of non-HIV patients with metabolic syndrome that used a similar oxLDL assay, atorvastatin 10 and 80 mg led to reductions of 24 and 39% in oxLDL, respectively, over 12 weeks from baseline oxLDL levels of 56 ± 19 U/l and 55 ± 22 U/l, respectively [17]. Baseline oxLDL concentrations in our study may be higher, suggesting the possible effect of HIV infection on raising oxLDL as consistent with prior reports [7,8]. Further studies are needed to understand the mechanisms involved in elevated oxLDL levels in HIV. Moreover, even more potent statin treatments may lead to even greater reductions in oxLDL in HIV-infected patients in the future.

We report for the first time a significant relationship between changes in circulating oxLDL levels and coronary plaque on cCTA in ART-treated HIV-infected individuals receiving a statin. We previously showed that 12 months of atorvastatin treatment led to significant improvement in coronary atherosclerosis on cCTA in comparison to plaque progression in the placebo group [19]. Now, our current study shows that the effect of statins on reducing coronary plaque is strongly related to its effects on lowering oxLDL. In our study, the change in oxLDL significantly related to changes in noncalcified plaque volume, low attenuation plaque, and positively remodeled plaque. This finding is also supported by a cross-sectional study in the general population that showed patients with exclusively noncalcified plaque on cCTA had higher levels of serum oxLDL than those with calcified plaque despite lower cardiovascular risk factors [12]. These plaque characteristics measured by cCTA are clinically relevant in the general population as they have all been associated with unstable lesions [20]. In addition, HIV infection has been associated with accelerated development of noncalcified plaque, low attenuation plaque, and positively remodeled plaque [9]. Thus, our data suggest the importance of investigating oxLDL in the HIV-infected population, in whom unique mechanisms and progression to advanced atherogenesis have been identified.

Interestingly, the relationship between changes in oxLDL and coronary atherosclerosis was independent of LDL. Cross-sectional data from non-HIV cohorts also support this finding. For example, one study reported higher circulating oxLDL levels in patients with acute MI compared with those with stable angina or healthy controls whereas serum levels of HDL, triglycerides, and LDL did not differ between these groups [13]. Thus, our findings suggest that in HIV-infected patients, serum oxLDL may be a better marker than LDL of treatment response to statins in reducing vulnerable coronary lesions, which may help to prevent future incident MIs.

The data from this study suggest potential mechanistic insight into the effects of statins on CVD in patients with HIV. Indeed, the stronger relationship by partial correlations analysis between noncalcified plaque and oxLDL than LDL suggests that reductions in oxLDL may be another pathway through which statins exert anti-inflammatory actions in HIV-infected individuals as oxLDL and noncalcified plaque have both been associated with statin-responsive markers of immune activation in patients with HIV. Rosuvastatin, for example, decreases sCD14 levels in HIV-infected patients [22]. Soluble CD14 is a marker of monocyte/macrophage activation that is elevated in patients with HIV and associated with circulating oxLDL levels, progression of subclinical atherosclerosis, and clinical CVD events [8,23–26]. Furthermore, statins have been shown to decrease tissue factor (TF) expression on monocytes in HIV-infected individuals [22]. In cross-sectional studies, oxLDL has been correlated with intermediate ($CD14^+ CD16^+$) monocytes expressing TF, and this expression profile is increased in HIV-infected individuals and non-HIV

patients with acute coronary syndrome compared with healthy, uninfected controls [8,27]. In addition, in an in-vitro experiment, oxLDL but not LDL stimulated an increase in intermediate monocytes including TF expression on this subset [8]. These data suggest that statins may mitigate oxLDL-mediated monocyte/macrophage activation, which in turn could improve CHD via this inflammatory pathway. Although we did not see a relationship between oxLDL and other markers of monocyte activation, including sCD14, further mechanistic studies relating change in oxLDL to specific monocyte subsets and markers of immune activation in HIV-infected patients are needed.

In this study, atorvastatin significantly lowered Lp-PLA_2 in addition to oxLDL. Moreover, the change in Lp-PLA_2 was also significantly associated with changes in noncalcified plaque volume, as well as total plaque volume and high-risk plaque features. One potential explanation for these results is that Lp-PLA_2 and oxLDL share a common biologic pathway. Indeed, the formation of oxidized phospholipids in LDL stimulates Lp-PLA_2 activity [34], which in turn hydrolyzes these oxidized phospholipids, resulting in metabolites such as lysophospholipids that are pro-inflammatory and pro-atherogenic [35]. These data further support the role of this pathway as a target through which statins could mediate cardioprotective effects, potentially modifying the inflammatory milieu within the arterial wall through reductions in oxLDL and Lp-PLA_2.

Relatively few data are available on the clinical utility of reducing Lp-PLA_2 on cardiovascular events. Two clinical trials, STABILITY and SOLID-TIIMI 52, showed no reductions in primary CVD endpoints associated with darapladib, an Lp-PLA_2 inhibitor, in addition to standard therapy for non-HIV patients with stable CHD and recent acute coronary syndrome [28,29]. The STABILITY trial though did show significant reductions in the rate of major coronary events and total coronary events, and in both trials, the vast majority of patients were already on statin therapy, which may have minimized the effects of darapladib. In fact, the LIPID study showed that in non-HIV patients with stable CHD randomized to pravastatin, changes in Lp-PLA_2 accounted for over half of the reduction in CHD death and nonfatal MIs [30]. Thus, the effect of reducing oxLDL and Lp-PLA_2 with statin therapy on primary prevention of CHD in patients with HIV remains unknown and an area for future investigation.

There are limitations to our study. First, due to limitations in sample size, we were not able to adequately determine relationships between changes in monocyte/macrophage activation and changes in oxLDL. Future studies will be needed to determine whether reductions in oxLDL directly affect monocyte/macrophage activation and the potential mechanisms thereof. In addition, future studies are needed to determine the clinical relevance of our findings for both oxidized LDL and changes in coronary plaque measurements with respect to clinical cardiovascular events in HIV-infected patients.

These data from a randomized, placebo-controlled study with well phenotyped patients assessed for sensitive indices of coronary plaque and high-risk plaque morphology add new information to the field. Our findings show that statin therapy can decrease oxLDL levels in patients with HIV and changes in oxLDL in response to statins relate strongly to changes in coronary plaque in HIV-infected patients, even more strongly than changes in LDL. The relationship of oxLDL to changes in specific vascular inflammatory markers such as Lp-PLA_2 and to high-risk plaque features suggest that statins may exert cardioprotective benefits partly as a result of lowering oxLDL and its effects on downstream inflammatory pathways in HIV. Future therapeutic strategies to reduce oxLDL may provide important benefits in treating and preventing coronary artery disease in the HIV-infected patient population.

Acknowledgements

We thank the patients who generously donated their time to participate in this study and the nurses and bionutritionists at the MGH Clinical Research Center of the Harvard Clinical and Translational Science Center.

Authors' contributions: study conception S.K.G., J.L.; study design S.K.G., J.L.; patient recruitment and implementation of the protocol S.E.L., J.L.; data acquisition and analysis and database management E.N., M.T.L., S.E.L., K.V.F., E.A.K., U.H., S.K.G., J.L.; statistical analysis and interpretation E.N., H.L., S.K.G., J.L.; drafting of the manuscript E.N., J.L.; critical revision of the manuscript E.N., M.T.L., S.E.L., K.V.F., E.A.K., H.L., U.F., S.K.G., J.L.; and supervision of the study S.K.G, J.L. All other authors have read and approved the text submitted.

Source of funding: This work was supported by the National Institutes of Health (5T32DK007028-40 to E.N., K23HL092792 to J.L., 5T32HL076136 to M.T.L., 5K24HL113128 to U.H., and R01HL095123 and P30 DK040561 to S.K.G.); Massachusetts General Hospital Executive Committee on Research Fund for Medical Discovery; and Harvard Clinical and Translational Science Center from the National Center for Research Resources (1 UL1 RR025758-04). Funding sources had no role in the design of the study, data analysis, or writing of the manuscript.

Conflicts of interest

U.H. received grant support from Siemens Healthcare, American College of Radiology Imaging Network, and

HeartFlow Inc, unrelated to this manuscript. S.K.G has consulted with Navidea, AstraZeneca, NovoNordisk, Theratechnologies, Bristol Myers Squibb, Merck, and Gilead, and received grant support from Gilead, Amgen, KOWA Pharmaceuticals, and Theratechnologies, unrelated to this manuscript. J.L. has consulted with Gilead, unrelated to this manuscript. All other authors declare no competing interests.

References

1. Miller CJ, Baker JV, Bormann AM, Erlandson KM, Huppler Hullsiek K, Justice AC, *et al*. **Adjudicated morbidity and mortality outcomes by age among individuals with HIV infection on suppressive antiretroviral therapy.** *PLoS One* 2014; **9**:e95061.
2. Morlat P, Roussillon C, Henard S, Salmon D, Bonnet F, Cacoub P, *et al*. **Causes of death among HIV-infected patients in France in 2010 (national survey): trends since 2000.** *AIDS* 2014; **28**:1181–1191.
3. Triant VA, Lee H, Hadigan C, Grinspoon SK. **Increased acute myocardial infarction rates and cardiovascular risk factors among patients with human immunodeficiency virus disease.** *J Clin Endocrinol Metab* 2007; **92**:2506–2512.
4. Freiberg MS, Chang CC, Kuller LH, Skanderson M, Lowy E, Kraemer KL, *et al*. **HIV infection and the risk of acute myocardial infarction.** *JAMA Intern Med* 2013; **173**:614–622.
5. Lo J, Abbara S, Shturman L, Soni A, Wei J, Rocha-Filho JA, *et al*. **Increased prevalence of subclinical coronary atherosclerosis detected by coronary computed tomography angiography in HIV-infected men.** *AIDS* 2010; **24**:243–253.
6. Post WS, Kingsley BM, Palella L, Witt FJ, Li Xiuhong MD, George RT, *et al*. **Associations between HIV infection and subclinical coronary atherosclerosis: The Multicenter AIDS Cohort Study (MACS).** *Ann Intern Med* 2014; **160**:458–467.
7. Duong M, Petit JM, Martha B, Galland F, Piroth L, Walldner A, *et al*. **Concentration of circulating oxidized LDL in HIV-infected patients treated with antiretroviral agents: relation to HIV-related lipodystrophy.** *HIV Clin Trials* 2006; **7**:41–47.
8. Zidar DA, Juchnowski S, Ferrari B, Clagett B, Pilch-Cooper HA, Rose S, *et al*. **Oxidized LDL levels are increased in HIV infection and may drive monocyte activation.** *J Acquir Immune Defic Syndr* 2015; **69**:154–160.
9. Zanni MV, Schouten J, Grinspoon SK, Reiss P. **Risk of coronary heart disease in patients with HIV infection.** *Nat Rev Cardiol* 2014; **11**:728–741.
10. Itabe H, Ueda M. **Measurement of plasma oxidized low-density lipoprotein and its clinical implications.** *J Atheroscler Thromb* 2007; **14**:1–11.
11. Nishi K, Itabe H, Uno M, Kitazato KT, Horiguchi H, Shinno K, *et al*. **Oxidized LDL in carotid plaques and plasma associates with plaque instability.** *Arterioscler Thromb Vasc Biol* 2002; **22**:1649–1654.
12. Bamberg F, Truong QA, Koenig W, Schlett CL, Nasir K, Butler J, *et al*. **Differential associations between blood biomarkers of inflammation, oxidation, and lipid metabolism with varying forms of coronary atherosclerotic plaque as quantified by coronary CT angiography.** *Int J Cardiovasc Imaging* 2012; **28**:183–192.
13. Ehara S, Ueda M, Naruko T, Haze K, Itoh A, Otsuka M, *et al*. **Elevated levels of oxidized low density lipoprotein show a positive relationship with the severity of acute coronary syndromes.** *Circulation* 2001; **103**:1955–1960.
14. Anselmi M, Garbin U, Agostoni P, Fusaro M, Pasini AF, Nava C, *et al*. **Plasma levels of oxidized-low-density lipoproteins are higher in patients with unstable angina and correlated with angiographic coronary complex plaques.** *Atherosclerosis* 2006; **185**:114–120.
15. Koenig W, Karakas M, Zierer A, Herder C, Baumert J, Meisinger C, *et al*. **Oxidized LDL and the risk of coronary heart disease: results from the MONICA/KORA Augsburg Study.** *Clin Chem* 2011; **57**:1196–1200.
16. Parra S, Coll B, Aragones G, Marsillach J, Beltran R, Rull A, *et al*. **Nonconcordance between subclinical atherosclerosis and the calculated Framingham risk score in HIV-infected patients: relationships with serum markers of oxidation and inflammation.** *HIV Med* 2010; **11**:225–231.
17. Singh U, Devaraj S, Jialal I, Siegel D. **Comparison effect of atorvastatin (10 versus 80 mg) on biomarkers of inflammation and oxidative stress in subjects with metabolic syndrome.** *Am J Cardiol* 2008; **102**:321–325.
18. Hileman CO, Semba RD, Turner R, Storer NJ, Labbato DE, McComsey GA. **Rosuvastatin lowers oxidative LDL in HIV-infected persons on antiretroviral therapy: SATURN-HIV [Abstract 750].** *21st Conference on Retroviruses and Opportunistic Infections (CROI)*. Boston, Massachusetts; 2014.
19. Lo J, Lu MT, Ihenachor EJ, Wei J, Looby SE, Fitch KV, *et al*. **Effects of statin therapy on coronary artery plaque volume and high-risk plaque morphology in HIV-infected patients with subclinical atherosclerosis: a randomised, double-blind, placebo-controlled trial.** *Lancet HIV* 2015; **2**:e52–e63.
20. Maurovich-Horvat P, Ferencik M, Voros S, Merkely B, Hoffmann U. **Comprehensive plaque assessment by coronary CT angiography.** *Nat Rev Cardiol* 2014; **11**:390–402.
21. Raff GL, Abidov A, Achenbach S, Berman DS, Boxt LM, Budoff MJ, *et al*. **SCCT guidelines for the interpretation and reporting of coronary computed tomographic angiography.** *J Cardiovasc Comput Tomogr* 2009; **3**:122–136.
22. Funderburg NT, Jiang Y, Debanne SM, Labbato D, Juchnowski S, Ferrari B, *et al*. **Rosuvastatin reduces vascular inflammation and T-cell and monocyte activation in HIV-infected subjects on antiretroviral therapy.** *J Acquir Immune Defic Syndr* 2015; **68**:396–404.
23. Li JZ, Arnold KB, Lo J, Dugast AS, Plants J, Ribaudo HJ, *et al*. **Differential levels of soluble inflammatory markers by human immunodeficiency virus controller status and demographics.** *Open Forum Infect Dis* 2015; **2**:ofu117.
24. Sandler NG, Wand H, Roque A, Law M, Nason MC, Nixon DE, *et al*. **Plasma levels of soluble CD14 independently predict mortality in HIV infection.** *J Infect Dis* 2011; **203**:780–790.
25. Kelesidis T, Kendall MA, Yang OO, Hodis HN, Currier JS. **Biomarkers of microbial translocation and macrophage activation: association with progression of subclinical atherosclerosis in HIV-1 infection.** *J Infect Dis* 2012; **206**:1558–1567.
26. McKibben RA, Margolick JB, Grinspoon S, Li X, Palella FJ Jr, Kingsley LA, *et al*. **Elevated levels of monocyte activation markers are associated with subclinical atherosclerosis in men with and those without HIV infection.** *J Infect Dis* 2015; **211**:1219–1228.
27. Funderburg NT, Zidar DA, Shive C, Lioi A, Mudd J, Musselwhite LW, *et al*. **Shared monocyte subset phenotypes in HIV-1 infection and in uninfected subjects with acute coronary syndrome.** *Blood* 2012; **120**:4599–4608.
28. O'Donoghue ML, Braunwald E, White HD, Lukas MA, Tarka E, Steg PG, *et al*. **Effect of darapladib on major coronary events after an acute coronary syndrome: the SOLID-TIMI 52 randomized clinical trial.** *JAMA* 2014; **312**:1006–1015.
29. White HD, Held C, Stewart R, Tarka E, Brown R, Davies RY, *et al*. **Darapladib for preventing ischemic events in stable coronary heart disease.** *N Engl J Med* 2014; **370**:1702–1711.
30. White HD, Simes J, Stewart RA, Blankenberg S, Barnes EH, Marschner IC, *et al*. **Changes in lipoprotein-associated phospholipase A_2 activity predict coronary events and partly account for the treatment effect of pravastatin: results from the Long-Term Intervention with Pravastatin in Ischemic Disease study.** *J Am Heart Assoc* 2013; **2**:e000360.