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# Changes in Plasma Levels of Oxidized Lipoproteins and Lipoprotein Subfractions with Atazanavir-, Raltegravir-, Darunavir-Based Initial Antiviral Therapy and Associations with Common Carotid Artery Intima-Media Thickness: ACTG 5260s

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## Abstract

**BACKGROUND—**The role of oxidized lipoproteins (high-density [HDLox] and low-density [LDLox]) and total lipoprotein particle (Lp) number and size in HIV-related cardiovascular disease (CVD) is unclear. The goal of this study was to evaluate changes of these biomarkers and their associations with rate of carotid intima media thickness progression over 3 years (ΔCIMT) in chronic HIV infection.

**METHODS—**Prospective study of 234 HIV-infected antiretroviral treatment naïve participants without CVD who were randomized to receive tenofovir-emtricitabine plus atazanavir/ritonavir, darunavir/ ritonavir, or raltegravir (RAL) and achieved plasma HIV-1 RNA <50 copies/ml by week 24 and thereafter. Biomarker changes over 24, 48 or 96 weeks from baseline and pairwise treatment group comparisons were examined. Associations of these biomarkers with ΔCIMT were analyzed with mixed effects linear regression.

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**RESULTS—**HDLp number increased with both protease inhibitors (PIs) over 48 weeks, while LDLp number declined with RAL; Lp size did not change. Over 96 weeks, normalized HDLox declined with both PIs; LDLox increased in all groups. Few treatment group differences were observed across all biomarkers. Associations between ΔCIMT and oxidized lipoproteins at all timepoints were not apparent ($p \geq 0.10$). There was some evidence of slower ΔCIMT for higher HDLp number ($p=0.06$) and for lower LDLp number ($p=0.08$) measured at baseline.

**CONCLUSIONS—**Unexpectedly, LDLox increased modestly in all treatment groups after ART initiation. Associations of plasma HDLox and LDLox with ΔCIMT were not apparent. While plasma levels of abnormal lipoproteins have been shown to be associated with CVD outcomes, clear associations with sub-clinical atherosclerosis progression were not apparent in our study.

### Keywords

Oxidized Lipoproteins; Lipoprotein Subfractions; cardiovascular disease; Human Immunodeficiency Virus; Inflammation; Protease inhibitors; Integrase inhibitors; lipoprotein function; HIV-1 infection

## INTRODUCTION

Since the advent of effective antiretroviral therapy (ART), HIV-1 infected individuals have improved survival but continue to be at increased risk of non-AIDS complications, such as cardiovascular disease (CVD), when compared to their uninfected peers [1, 2]. In HIV-1 Infection, there is incomplete reversal of immune activation during ART [3] and measures of immune activation despite ART have been associated with CVD progression [4]; the exact mechanisms remain unclear. Oxidative stress is involved in pathogenesis of inflammatory diseases [5] including chronic HIV-1 infection [6]. During oxidative stress, oxidized phospholipids (OxPLs) acquire different biological activities (such as the ability to regulate immunity), and these properties may contribute to the pathogenesis of many diseases including CVD [7]. Oxidized low density lipoprotein (LDLox) is a proinflammatory lipoprotein [8] that carries oxPLs and has pleomorphic atherogenic effects [9]. While High Density Lipoprotein (HDL) counteracts OxPLs and protects against CVD, HDL can become dysfunctional in the setting of inflammation and contribute to increased CVD risk [10]. This modified HDL has decreased levels and activity of anti-inflammatory antioxidant factors, increased pro-inflammatory proteins, lipid hydroperoxide content and redox activity (HDLox), reduced potential to efflux cholesterol and diminished ability to prevent LDL oxidation [8, 10].

HIV-1 infected ART-treated individuals have a higher prevalence of dyslipidemia [11] and an atherogenic lipid profile that includes low high-density lipoprotein cholesterol (HDL-C) [12] and is a predictor of CVD [10]. We have found that HDL in individuals with ART-treated infection have impaired antioxidant function (HDLox) and increased lipid hydroperoxide content [13, 14]. HIV-1 infected individuals have also been shown to have impaired lipoprotein processing [15, 16] and increased levels of plasma LDLox [17, 18] and HDLox that are associated with CVD[14, 19]. The size and number of lipoprotein particles (HDLp, LDLp), measured by Nuclear magnetic resonance (NMR), have been shown to predict CVD risk and HDL function [20] in both the general population [20] and in HIV-1

infected subjects [21, 22],. However there is limited data in the setting of ART-initiation during a prospective randomized study whether plasma levels of oxidized lipoproteins and plasma measures of lipoprotein function (such as NMR lipoprotein biomarkers) may predict surrogate measures of CVD. Moreover, elucidating in a prospective study how different antiretrovirals affect lipoproteins and immune responses in HIV-1 infection will contribute to increased understanding of the pathogenesis of HIV-1 related CVD.

The objectives of the present analysis were to characterize and evaluate changes in levels of oxidized lipoproteins and of lipoprotein composition longitudinally among treatment-naïve individuals following suppressive ART initiation with an integrase-based regimen containing raltegravir (RAL) or a protease inhibitor (PI) -based regimen containing either atazanavir/ritonavir (ATV/RTV) or darunavir/ritonavir (DRV/RTV) in the AIDS Clinical Trial Group (ACTG) A5260s study. We sought to compare these changes by ART regimen and hypothesized that raltegravir (RAL) would have favorable effects on oxidized lipoproteins and NMR lipoprotein biomarkers compared to both PIs. This is in light of an earlier analysis of the ACTG A5257 study (parent study of A5260s) where modest increases in triglycerides, low-density lipoprotein (LDL-C) and non-HDL-C levels were observed in both PI groups compared to decreases with RAL [23]; HDL-C modestly increased in all treatment arms [24]. We also sought to examine how ART-associated changes in these lipoprotein-related biomarkers were associated with progression of carotid intima media thickness (CIMT). We hypothesized that higher baseline levels of oxidized lipoproteins, lower number of HDL particles, higher number of LDL particles and small lipoprotein particles would be associated with faster progression of atherosclerosis in HIV-1 infected individuals.

## METHODS

### Study Design and Participants

ACTG A5260s, a cardiovascular substudy of ACTG A5257, is a prospective, 144-week longitudinal evaluation of 328 ART-naïve, HIV-infected adults without known CVD or diabetes mellitus, uncontrolled thyroid disease or use of lipid-lowering medications. Participants were randomized equally to one of three regimens of tenofovir disoproxil fumarate-emtricitabine (TDF/FTC) plus either ATV/RTV, DRV/RTV, or RAL [25]; randomization was stratified by screening HIV RNA level (>100,000 or ≤100,000 copies/mL) and Framingham 10-year Coronary Heart Disease Risk Score (<6% or ≥6% risk). The virologic, tolerability and metabolic outcomes of these regimens on the parent study were previously reported [23, 25]. The primary CIMT outcomes for A5260s have also been reported elsewhere [26]. The parent study and substudy (clinicalTrials.gov identifiers: NCT00811954 and NCT00851799) were approved by the Institutional Review Boards at all participating institutions, and all participants provided written informed consent. For the present analysis, the A5260s population was restricted to a cohort of virologically suppressed individuals in order to minimize potential confounding due to uncontrolled viremia. This cohort included participants who remained on randomized treatment throughout substudy follow-up (no ART interruptions >7 days) and who achieved and maintained HIV-1 RNA suppression of <50 copies/ml by study week 24 and thereafter.

### Carotid Artery Ultrasonography

B-mode images of the distal right common carotid artery (CCA) and the right carotid artery bifurcation were acquired with a high-resolution linear array ultrasound transducer with simultaneous electrographic tracings before ART initiation and after 48, 96, and 144 weeks. Primary results and detailed methodology are reported elsewhere [26].

### Biomarker and Laboratory Assessment

Blood samples were drawn in a fasting state at all study visits. HDL-C and LDL-C were measured in batch and have been previously described [27]. Lipoproteins were quantified at entry and week 48 by nuclear magnetic resonance spectroscopy at LipoScience (Raleigh, NC). Oxidized LDL (entry and weeks 24 and 96) was quantified using ELISA (Mercodia) according to manufacturer instructions. Oxidized HDL (entry and weeks 24 and 96) was quantified using a novel and previously validated fluorometric cell-free biochemical assay that measures HDL lipid peroxidation based on the oxidation of the fluorochrome Amplex Red [14]. To reduce experimental variability [14] and adjust for HDL amount, we also normalized HDLox by the HDLox of a pooled plasma control and by concurrent HDL cholesterol concentration level using the following calculation: “normalized” oxidized HDL (nHDLox) = HDLox x 40 mg/dl] / [HDL-C (mg/dL)], where 40 mg/dL represents HDL-C measure of the pooled plasma control. Throughout, oxidized HDL is presented as both unadjusted [HDLox] and normalized [nHDLox] measures to reflect the lack of or adjustment for HDL cholesterol concentration, respectively.

### Statistical Analyses

Biomarkers were analyzed using all available measures at study entry (baseline) and further examined at weeks 24 and 96 (oxidized lipoproteins) and week 48 (NMR lipoproteins). Biomarker changes over time were calculated as the mean difference of the on-treatment level compared to baseline on the log10 scale and back-transformed to represent mean fold change from baseline. Evidence for change over time was assessed according to the bounds of the 95% confidence interval (CI) with one indicating no change. Shifts in the distribution of changes from baseline for all pairwise treatment group comparisons were evaluated using Wilcoxon rank sum tests and described as relative fold-change. To provide error rate control for the three pairwise comparisons, effect sizes are presented with 97.5% CIs and inference assessed against a type I error of 2.5%. Associations between biomarkers and CIMT progression were examined using mixed effects linear regression models with random intercept and slope and unstructured covariance matrix on the random effect consistent with the study primary analysis [26]. The CIMT outcome vector included measures at weeks 48, 96 and 144. Biomarkers were examined as a three-level categorical covariate based on the upper quartile (≥Q3), combined middle quartiles (Q1–<Q3), and lower quartile (<Q1) of the respective biomarker distribution at baseline. The biomarker effect of primary interest was the difference in the annual rate of change in CIMT progression (i.e., the slope) between higher biomarker levels (≥Q3) relative to lower levels (<Q1) and modeled as an interaction with time. The overall type 3 statistic for the biomarker effect and the directionality of the biomarker effect for the combined middle quartiles (compared to the upper quartiles) were used to guide primary inferences. Inference was assessed with a 5% type I error and 95%

confidence intervals. All analyses adjusted for baseline CIMT and randomization stratification factors; time was modeled continuously in years. In ART-associated analyses with baseline and early on-treatment biomarkers, all models also adjusted for randomized treatment. Evidence that a given biomarker may mediate observed treatment group differences was evaluated by attenuation of the estimated treatment group difference upon addition of the biomarker to the model. Of note, we did not formally adjust for multiple comparisons since the study was not powered to detect effect sizes with adjustment for multiple comparisons. Rather, inference was guided by nominal p-values as well as consideration of the consistency, direction and magnitude of the effect sizes and confidence intervals. All analyses were performed with SAS, version 9.4 (SAS Institute, Cary, North Carolina, USA).

## RESULTS

### Baseline characteristics

Baseline characteristics and biomarker distributions of the 234 participants in the virologically suppressed cohort are shown in Table 1. Briefly, the cohort was 29% non-Hispanic black and 19% Hispanic with a median age of 36 years. The group overall had a low cardiovascular disease risk; only 12% of participants had a 10 year risk of hard coronary heart disease ≥6%, 33% were current smokers; 13% had metabolic syndrome; and prevalence of carotid lesions was low (9%). Participants were similar across treatment groups and representative of the full substudy population [26].

### Changes over time in plasma levels of oxidized lipoproteins

HDL-C and LDL-C levels increased over time in all treatment groups (Figure 1). Levels of normalized HDLox declined to 88% of baseline after week 96 of ART in both PI/r groups whereas 96-week levels with RAL were similar to baseline measures (mean fold-change of 0.97). Post-baseline levels of unadjusted HDLox increased with RAL but remained relatively unchanged in the ATV/RTV and DRV/RTV treatment groups. Post-baseline levels of LDLox increased after 24 weeks and remained elevated compared to baseline levels after 96 weeks across all treatment groups (Figure 1). In general, there was minimal variability among participants in the magnitude of change for both oxidized lipoproteins.

Pairwise treatment group differences for unadjusted and normalized oxidized HDL were evident for the ATV/RTV versus RAL and DRV/RTV versus RAL comparisons at week 24 ($p \leq 0.012$). The magnitude of these differences (that suggested greater declines for the PIs compared to RAL) remained evident at week 96 for both comparisons, however these did not all reach formal statistical significance at a 2.5% level. Treatment group differences for oxidized LDL were not apparent (Table 2).

### Changes over time in NMR lipoproteins

Of the NMR lipoprotein particles, changes after ART initiation were only apparent for total HDL particle number and total LDL particle number (Figure 1). Specifically, levels of total HDL particle number after 48 weeks increased 22% and 26% from baseline in the ATV/RTV and DRV/RTV groups, respectively; no change was apparent with RAL. In

contrast, total LDL particle number declined with RAL. There were no changes in lipoprotein size over 48 weeks of ART. The pairwise treatment group difference evident after 48 weeks suggested a smaller decline in total LDL particle number for DRV/RTV versus RAL (p=0.004) and ATV/RTV versus RAL (P=0.026). No other statistically significant differences were observed (p≥0.046) (Table 2).

### Associations between oxidized lipoproteins and CCA IMT progression

No associations between CIMT progression and unadjusted HDLox at baseline and after 24 weeks of ART were apparent when comparing the highest versus lowest quartiles of the data (p≥0.10). These conclusions were unchanged when also considering outcomes for the middle two quartiles (based on type 3 statistics). These observations remained consistent upon further adjustment for the effects of lipoprotein cholesterol concentration and particle number (p≥0.10) (Figure 2). Associations with CIMT progression were also not evident for normalized HDLox (p≥0.67) or LDLox (p≥0.28) (Figure 2), regardless of adjustment for the effect of treatment (Figure 3a, 3c). The effect of oxidized lipoprotein also did not impact pairwise treatment group differences of CIMT progression (Figure 3b, 3d).

### Associations between NMR lipoproteins and CCA IMT progression

No statistically significant associations between CIMT progression and the NMR lipoproteins at baseline or on-treatment at week 48 were apparent (p>0.05) (Figure 2). However, relative to the lowest quartile, there was marginal evidence of slower CIMT progression for higher baseline levels of total HDL-P (−5.01 [−10.25, 0.23] μm/year; p=0.06) and faster CIMT progression for higher baseline levels of total LDL-P (4.62 [−0.55, 9.79] μm/year, p=0.08). The evidence for these findings was strengthened after adjustment for the previously described treatment group differences; total HDL-P (−5.20 [−10.36, −0.04] μm/year; p=0.048) and total LDL-P (5.43 [0.34, 10.52] μm/year; p=0.037) (Figure 3a). Adjustment for these baseline biomarkers did not change the magnitude of the previously reported treatment group comparisons (Figure 3b, 3d).

## DISCUSSION

In this prospective study of ART-naïve participants who initiated ART with TDF/FTC and RAL, ATV/RTV or DRV/RTV and successfully maintained virologic suppression on these regimens, we found modest increases in levels of oxidized LDL that remained elevated after 96 weeks across all treatment groups. Small decreases in normalized oxidized HDL levels were observed with PI treatment that were not apparent in the RAL treated group. While there was minimal change in total number of LDL particles in both PI groups, a modest decrease in LDL particle number was observed with RAL. In contrast, a modest increase in HDL particle number was seen with PI treatment but not with RAL. There were no consistent pairwise treatment group differences for all oxidized and NMR lipoproteins across timepoints. While our prior findings demonstrated a slower rate of CIMT progression with ATV/RTV compared to the RAL and DRV/RTV groups [26], these findings do not appear to be explained by the changes in these NMR or oxidized lipoprotein biomarkers. However, our data provide some evidence of associations between higher pre-treatment total

HDL particle number and lower LDL particle number with slower CIMT progression. We found no associations between levels of oxidized LDL and HDL and CIMT progression.

To our knowledge, this is the most comprehensive prospective study describing changes in oxidized and NMR lipoproteins after successful ART initiation with regards to measures of subclinical atherosclerosis (CIMT). Contrary to our hypothesis, we found that successful initiation of ART increased rather than decreased levels of LDLox. Notably, in placebo-controlled trial that evaluated the effect of rosuvastatin in HIV-infected adults on stable ART with LDL less than 130 mg/dl and increased inflammation, LDLox levels increased after initially declining and were not different from placebo at week 48, suggesting that LDLox was influenced by some unclear mechanism in treated and suppressed patients with HIV[28]. A prospective cohort of virologically suppressed HIV-infected patients on stable ATV/r versus efavirenz (EFV)-based first-line therapies also found an increase (compared to baseline before ART) in LDLox in both ATV/r- and EFV- based regimens[29]. In addition, it has been shown that LDLox levels are increased in HIV Infection and may drive monocyte activation[17]. It is known that initiation of ART among HIV-infected patients incompletely reduces markers of systemic inflammation and immune activation [3] and therefor it is surprising to observe a rise in LDLox in this setting. Possible mechanisms for the rise in LDLox in this setting could include production of free radical species, mitochondrial dysfunction and alterations in antioxidant systems [30–33]. Finally, successfully treated HIV-1 infected individuals are known to have persistently elevated proinflammatory monocytes (CD14+CD16+) that may contribute to oxidation of lipoproteins [34]. Given data from randomized, placebo-controlled trials that increased levels of LDLox may be one mechanism that contribute to atherosclerosis in HIV-infected individuals[35], further prospective studies are needed to definitely address the important question whether ART may increase plasma levels of LDLox.

There are limited data regarding the effect of different ART regimens on oxidized lipoproteins, lipoprotein particle number and size and HDL function [36]. Contrary to our original hypothesis, raltegravir did not appear to have more favorable effects on the oxidized lipid biomarkers than PI treatment. We found that LDLox increased in all three-treatment regimens (even in the ATV/r group) after 96 weeks of ART. As previously noted, patients on ATV/r have been shown to have lower increase in LDLox relative to those on EFV[29]. These data and our findings that ATV/r did not reduce plasma LDLox (despite that elevated bilirubin may have a beneficial role in counteracting oxidative stress[29]), suggest that the differential effects of ART on LDLox may be more complex than initially thought.

Normalized HDLox increased with RAL at week 24, but decreased in both PI regimens. After 96 weeks, normalized HDLox continued to decrease in both PI regimens relative to baseline levels whereas RAL returned back to baseline levels. The exact enzymatic or non-enzymatic oxidative mechanisms that initiate or regulate lipoprotein oxidation *in vivo* are unclear [34, 37]. The possibility of a differential effect of RAL on monocytes and macrophages (M/M) [38], and GI tissue, which contribute to oxidation of lipoproteins and HDL function [34, 39, 40], may contribute to the differential effects of RAL compared to PIs on HDLox. Differences in the structures of lipoproteins (HDL lipids are oxidized in preference to those in LDL when human plasma is exposed to aqueous ROS [41] and HDL

also has antioxidant groups [8]) and in regulation of their *in vivo* oxidation may explain our discrepant results regarding the effect of ART on HDLox compared to LDLox levels.

The size and number of lipoprotein particles (HDLp, LDLp), measured by NMR, has been shown to predict CVD risk and HDL function [20]. Studies in the general population suggest that smaller LDL-p size, greater number of small LDL-p and total LDLp, or lower number of total HDLp are associated with an increased risk of coronary disease [20]. Baseline HDLp, but not LDLp, predicted CVD risk in HIV-1 infected subjects in SMART [21]. In a subgroup of participants not taking ART at study entry who were randomized in the SMART trial to immediately initiate ART or to defer ART, HDL lipoprotein particle concentrations increased following ART initiation to a degree that depends on the degree of inflammation present at entry, suggesting that activation of inflammatory pathways contribute to HIV-associated changes in HDL [22]. Consistent with these data, we also found evidence suggesting slower progression of CIMT with higher total HDL particle number and lower total LDL particle number at baseline. Interestingly, we did not find associations of on-treatment levels of these markers and CIMT progressions.

We did not find evidence of associations of oxidized lipoproteins with progression of CIMT; in particular these biomarkers do not appear to help explain the slower rate of CIMT progression previously reported among participants that were randomized to ATV/RTV in this study. This contrasts with data from randomized trials of statin interventions based on which it has been suggested that reductions in LDLox may be one mechanism through which statins exert beneficial effects on reducing atherosclerosis in HIV-infected individuals[28,35]. It is noted that the statin effect on LDLox in these studies was much greater than the effect of ART seen in the present study which may explain the differences in our results.

We modeled HDLox using two different approaches that represented a) a normalized fraction of HDL that is oxidized, and b) a single level of oxidized HDL with adjustment for level of HDL-C concentration [14, 42] or total HDL particle number in our statistical models. Our observations were consistent regardless of approach. These results parallel our results from a prior small matched cohort study of HIV-1-infected participants with low cardiovascular risk profiles. In this study, HDLox changed over time and was independently associated with anthropometric parameters of obesity but not with progression of CIMT [43]. In retrospective studies, both HDLox and LDLox have been associated with progression of CVD in HIV infection [14, 19, 44]. We previously showed that HDLox in HIV-1 infected subjects on long term ART and without clinical CVD are i) associated with *in vivo* progression of CVD [14] ii) may stimulate endothelial cells to induce M/M chemotaxis, a measure of HDL function [13, 45, 46] iii) correlated positively with non-calcified coronary atherosclerotic plaque [19] iv) and correlated with sCD163 [19], a marker of M/M activation that has been linked to CVD in HIV disease [47]. Thus, larger studies involving other surrogate markers of CVD such as coronary artery calcium scoring and coronary CT angiography or other clinical endpoints are needed to further study the role of oxidized lipoproteins in HIV-associated CVD.

Our study had several limitations. This analysis of oxidized and NMR lipoproteins was exploratory and not the primary outcomes of the A5260s study. The study was therefore not specifically powered to detect clinically meaningful treatment differences in these biomarkers or associations of these biomarkers with CIMT progression. Moreover, it is not clear what clinically meaningful effect sizes are for both the treatment differences and CIMT associations. With that in mind, we focused our association analyses on the differences in progression between the highest and lowest quartiles of response to assist in interpretation and to reflect where we anticipated observing the largest effect. While this approach is not optimal for the power of the study (as it effectively halved our sample size), the overall type 3 statistics that consider the full biomarker distribution were examined as part of these analyses and did not impact inferences. The power of the analysis to detect clinically meaningful associations of CIMT progression may have also been impacted by the very low cardiovascular disease risk profile of the analysis population (median age of 36 years; 9% with carotid lesions). While we also did not control for concomitant medication use (e.g., statins) during study follow-up that may contribute to changes in oxidized lipoproteins, these medications precluded eligibility at study entry and incident use across study follow-up was limited to a handful of participants. It is also noted that our study population was predominantly men, and all study participants received TDF/FTC. Further research is needed to investigate whether the results observed differ in female populations or with alternative nucleoside reverse transcriptase inhibitor backbones. Changes in levels of oxidized lipoproteins were measured in the setting of initiation of ART, and thus during a period where there may be major changes in systemic inflammation and oxidative stress. This may have further increased between subject variability and compromised our ability to detect differences in measures of oxidized lipoproteins in this study. HDL-C has been used in numerous studies to adjust the HDLox measurement for HDL amount. Increased levels of this adjusted measure of HDL function have been associated with worse outcomes [14, 19, 42, 48, 49]. However, we did not examine other established measures of HDL function, such as HDL cholesterol efflux, due to limited sample availability. Further limitations are also recognized when using cryopreserved rather than fresh samples for biochemical assays of HDL function and measurement of oxidized lipoproteins [13, 14, 50].

In conclusion, contrary to prior data that oxidized lipoproteins are associated with subclinical atherosclerosis, we did not find evidence of an association between early on-treatment levels of HDLox or LDLox and CIMT progression over three years among treatment naïve individuals who achieved and sustained virologic suppression after initiating and successfully maintaining ART regimens of TDF/FTC with RAL, ATV/RTV or DRV/RTV. Similar to the SMART study, we saw some evidence that higher pre-treatment total HDL particle number and lower total LDL particle number were associated with slower progression of CIMT in treated HIV-1 infection. HDLox declined with ATV/RTV and DRV/RTV over 96 weeks; LDLox increased in all groups. While levels of abnormal lipoproteins have been shown to be associated with CVD outcomes, clear associations with sub-clinical atherosclerosis progression were not apparent in our study. Larger studies with longer-term treatments that also measure oxidized lipids at the tissue level and examine other CVD endpoints are needed to further understand the role of oxidized lipoproteins and lipoprotein particles in HIV-associated cardiovascular disease.

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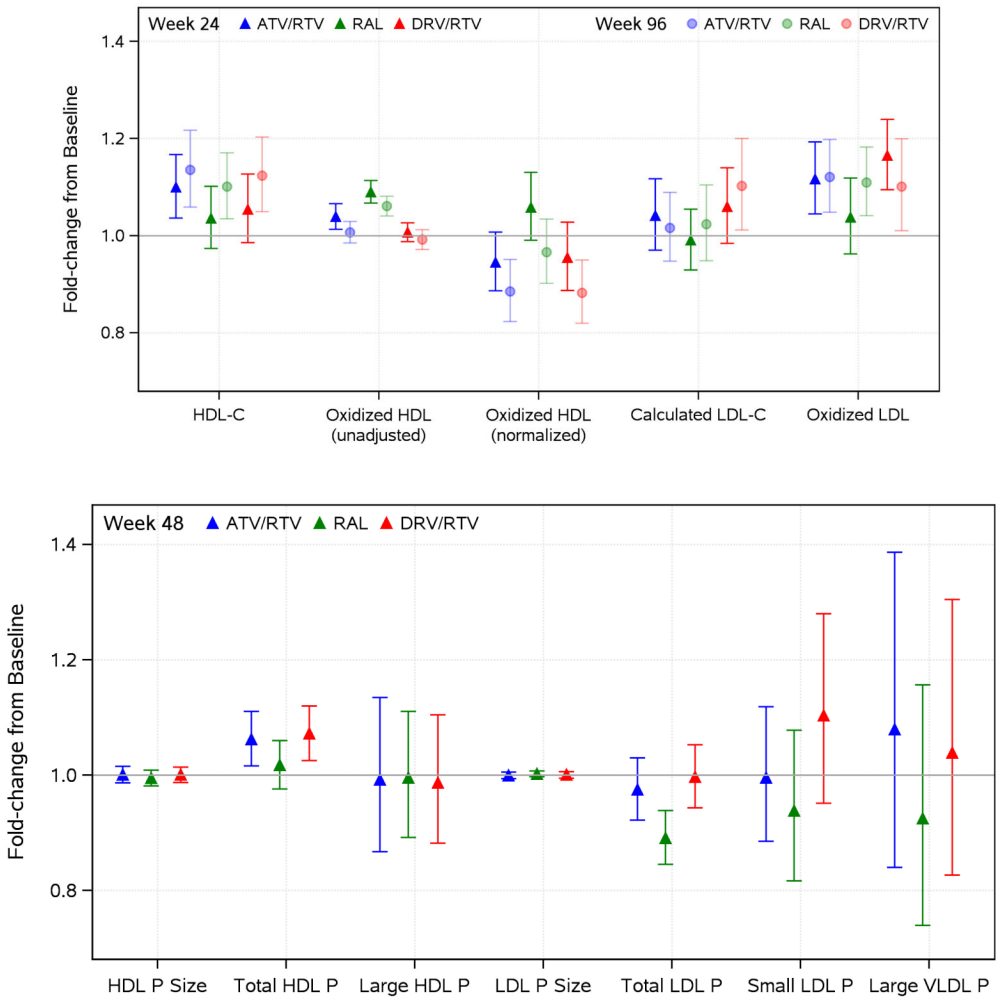


**Figure 1. Mean fold change (95% CI) from baseline over time by treatment group. A. Changes in levels of plasma oxidized lipoproteins over time. B. Changes in levels of plasma NMR lipoproteins over time**

HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; VLDL, large very low-density; P, particle.

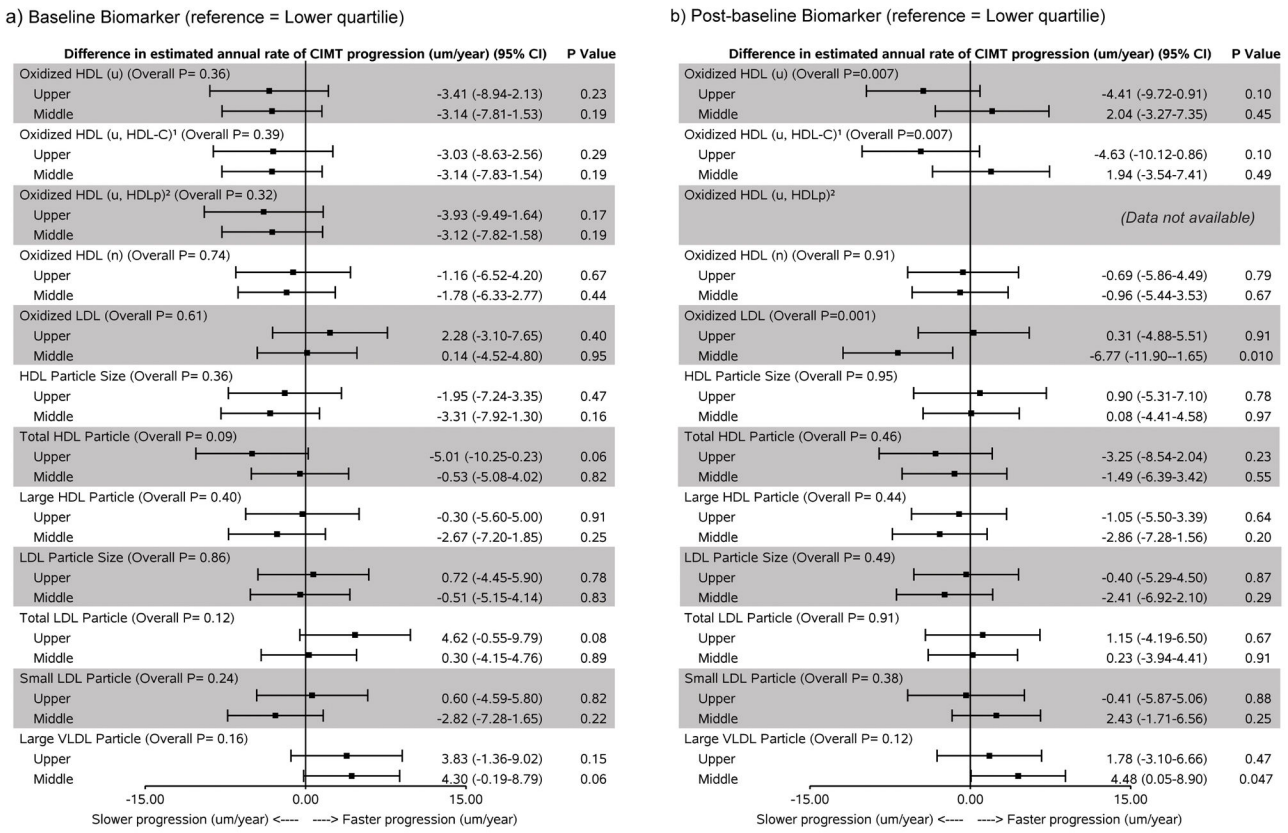


**Figure 2. Oxidized lipoproteins and NMR particles associations with CCA IMT progression (no adjustment for treatment)**

All analyses adjusted for baseline CCA IMT and screening HIV-1 RNA and Framingham risk scores; time modeled continuously in years.

[1]Analysis also adjusted for HDL cholesterol concentration (u, HDL-C).

[2]Analysis also adjusted for total HDL particle number (baseline biomarker only) (u, HDLp).

Biomarker effects reflect the estimated difference in the annual rate of CIMT change for the upper quartile (≥Q3) and the combined middle quartiles (Q1–Q3) versus the lower quartile (<Q1) with 95% CIs. Overall p value reflects the overall type 3 statistic for each biomarker. CCA IMT, common carotid artery intima media thickness; Oxidized HDL (u), unadjusted oxidized HDL; Oxidized HDL (n), normalized oxidized HDL.

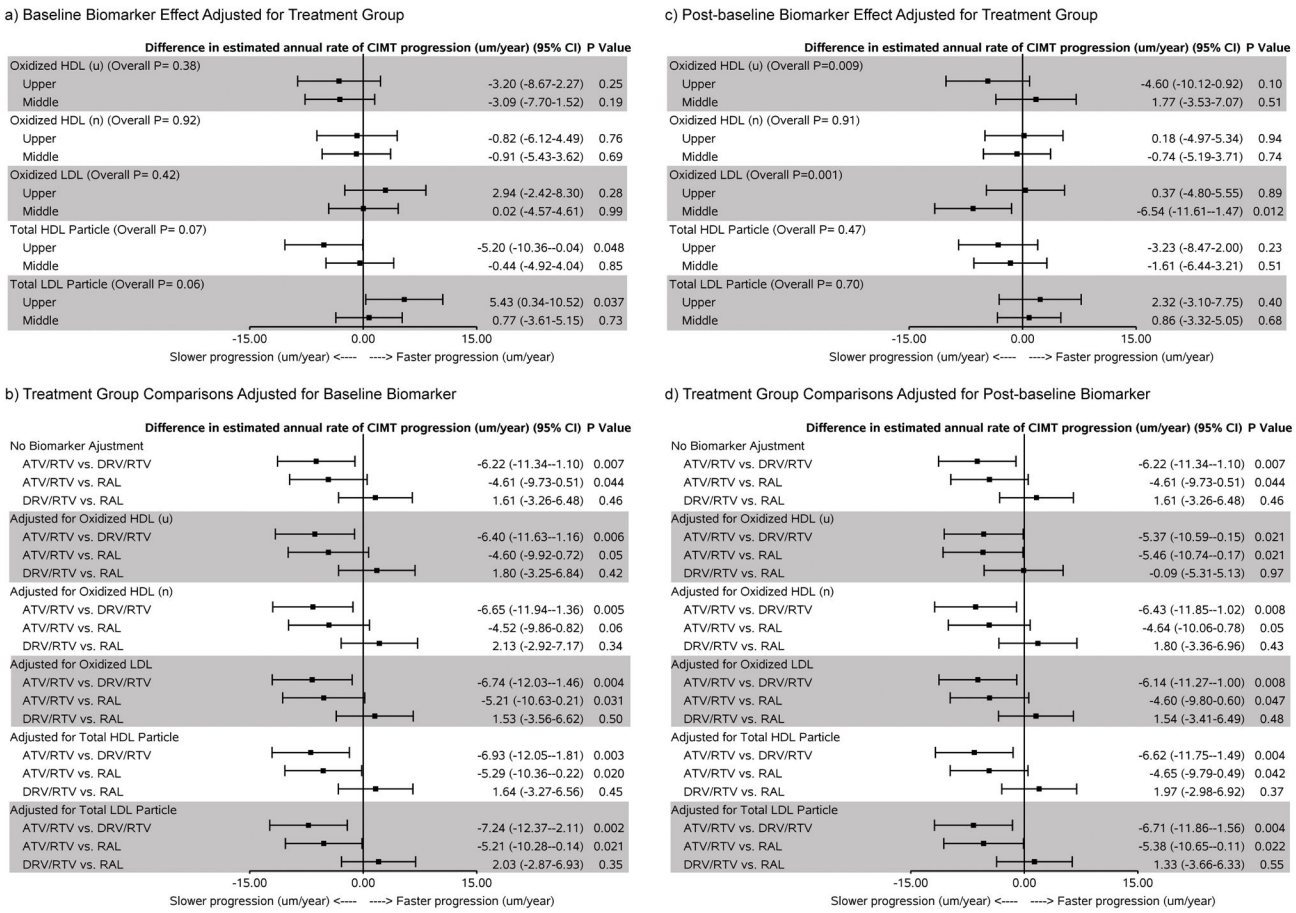


**Figure 3. Oxidized lipoproteins and NMR particles associations with CCA IMT progression (with adjustment for treatment)**

All analyses adjusted for baseline CCA IMT, screening HIV-1 RNA and Framingham risk scores and treatment group; time modeled continuously in years. Biomarker effects reflect the estimated difference in the annual rate of CIMT change for the upper quartile (≥Q3) and the combined middle quartiles (Q1–Q3) versus the lower quartile (<Q1) with 95% CIs.

Overall p value reflects the overall type 3 statistic for each biomarker.

Pairwise treatment group comparisons reflect the estimated difference in the annual rate of CIMT change for Treatment A -Treatment B with 97.5% CIs.

CCA IMT, common carotid artery intima media thickness; (u), unadjusted HDLox; (n), normalized HDLox; ATV/RTV, atazanavir/ritonavir, DRV/RTV, darunavir/ritonavir; RAL, raltegravir.

## Table 1

Baseline characteristics by randomized treatment group

| | Total (N=234) | ATV/RTV (n=68) | RAL (n=82) | DRV/RTV (n=84) |
|---|---|---|---|---|
| **Demographics** | | | | |
| **Sex** | | | | |
| Male | 210 (90%) | 63 (93%) | 73 (89%) | 74 (88%) |
| Female | 24 (10%) | 5 (7%) | 9 (11%) | 10 (12%) |
| **Age** | 36 (28–45) | 38 (31–44) | 36 (27–45) | 36 (28–47) |
| **Race/Ethnicity** | | | | |
| Non-Hispanic White | 112 (48%) | 35 (51%) | 36 (44%) | 41 (49%) |
| Non-Hispanic Black | 68 (29%) | 21 (31%) | 23 (28%) | 24 (29%) |
| Hispanic | 45 (19%) | 11 (16%) | 16 (20%) | 18 (21%) |
| Asian/Other/More than one race | 8 (3%) | 1 (1%) | 6 (7%) | 1 (1%) |
| **CD4+ cell count [/mm$^3$]** | 338 (191–448) | 294 (180–461) | 347 (246–450) | 337 (172–424) |
| **HIV-1 RNA ($log_{10}$ copies/ml)** | 4.6 (4.0–5.0) | 4.8 (4.0–5.2) | 4.5 (4.0–4.9) | 4.6 (4.0–5.0) |
| **10-year risk of hard CHD** | | | | |
| Low (<6%) | 205 (88%) | 61 (90%) | 75 (91%) | 69 (82%) |
| Medium/High (≥6%) | 29 (12%) | 7 (10%) | 7 (9%) | 15 (18%) |
| **Current smoker** | 77 (33%) | 22 (32%) | 28 (34%) | 27 (32%) |
| **Metabolic syndrome** | 31 (13%) | 8 (12%) | 11 (13%) | 12 (14%) |
| **Baseline CCA IMT [μm]** | 0.65 (0.59– 0.71) | 0.67 (0.60–0.72) | 0.64 (0.59–0.69) | 0.64 (0.59–0.71) |
| **Evidence of carotid lesions** | 22 (9%) | 4 (6%) | 7 (9%) | 11 (13%) |
| **Biomarkers** | | | | |
| **Oxidized Lipoproteins** | | | | |
| Oxidized HDL [unadjusted] | 0.95 (0.90– 1.00) | 0.95 (0.90–1.00) | 0.95 (0.90–1.02) | 0.94 (0.91–0.99) |
| Oxidized HDL [normalized] | 0.99 (0.84– 1.22) | 1.04 (0.86–1.21) | 0.94 (0.83–1.15) | 0.99 (0.82–1.25) |
| Oxidized LDL [U/L] | 49.0 (39.5– 59.6) | 53.0 (40.2–64.6) | 45.5 (37.9–58.0) | 49.2 (40.0–59.7) |
| **NMR Lipoprotein Particles** | | | | |
| HDL Particle Size [nm] | 8.9 (8.6–9.2) | 8.7 (8.6–9.1) | 8.9 (8.6–9.1) | 8.9 (8.6–9.3) |
| Total HDL Particles [umol/L] | 29.5 (25.7– 33.2) | 29.4 (25.0–32.5) | 30.5 (26.2–33.2) | 29.4 (24.9–33.4) |
| Large HDL Particles [umol/L] | 3.2 (2.2–4.9) | 2.8 (2.1–3.9) | 3.6 (2.2–5.0) | 3.2 (2.2–4.9) |
| LDL Particle Size [nm] | 20.5 (20.1– 20.9) | 20.4 (20.2–20.7) | 20.6 (20.2–21.0) | 20.5 (19.9–20.9) |
| Total LDL Particles [umol/L] | 1094 (911– 1345) | 1193 (938–1364) | 1050 (899– 1363) | 1103 (899–1301) |
| Small LDL Particles [umol/L] | 680 (454–917) | 704 (522–844) | 631 (451–826) | 702 (428–979) |
| Large VLDL Particles [umol/L] | 2.0 (1.1–4.4) | 2.3 (1.1–6.0) | 1.8 (1.1–3.8) | 2.1 (1.3–4.6) |
| **Lipid Panel** | | | | |
| Total cholesterol [mg/dL] | 156 (136–178) | 154 (135–179) | 159 (137–178) | 158 (135–175) |
| Triglycerides [mg/dL] | 105 (74–142) | 112 (79–151) | 94 (73–137) | 98 (74–144) |
| HDL cholesterol [mg/dL] | 38 (32–45) | 36 (32–43) | 40 (34–45) | 37 (32–46) |
| Calculated LDL cholesterol [mg/dL] | 92 (75–110) | 93 (75–113) | 92 (78–110) | 89 (75–109) |
| Non-HDL cholesterol [mg/dL] | 115 (97–138) | 116 (98–146) | 113 (97–135) | 115 (94–136) |

Median (first – third quartiles) or number (%). CCA IMT, common carotid artery intima-media thickness; CHD, coronary heart disease; HDL, high-density lipoprotein; LDL, low-density lipoprotein; VLDL, large very low-density.

## Table 2

Pairwise treatment group comparisons by biomarker and study week

| | Week 24 or 48 | | Week 96 | |
|---|---|---|---|---|
| | **Mean (97.5% CI)** | ***P*** | **Mean (97.5% CI)** | ***P*** |
| **ATV/RTV vs. DRV/RTV** | | | | |
| Oxidized HDL (unadjusted) | 1.03 (1.00, 1.07) | 0.05 | 1.02 (0.98, 1.05) | 0.37 |
| Oxidized HDL (normalized) | 0.99 (0.88, 1.11) | 0.96 | 1.00 (0.89, 1.13) | 0.77 |
| Oxidized LDL | 0.96 (0.86, 1.06) | 0.40 | 1.02 (0.90, 1.16) | 0.84 |
| HDL Particle Size | 1.00 (0.98, 1.02) | 0.87 | -- | -- |
| Total HDL Particles | 0.97 (0.77, 1.23) | 0.99 | -- | -- |
| Large HDL Particles | 1.01 (0.83, 1.23) | 0.79 | -- | -- |
| LDL Particle Size | 1.00 (0.99, 1.01) | 0.90 | -- | -- |
| Total LDL Particles | 0.98 (0.90, 1.07) | 0.52 | -- | -- |
| Small LDL Particles | 0.90 (0.72, 1.12) | 0.50 | -- | -- |
| Large VLDL Particles | 1.04 (0.71, 1.53) | 0.95 | -- | -- |
| **ATV/RTV vs. RAL** | | | | |
| Oxidized HDL (unadjusted) | 0.95 (0.92, 0.99) | <0.001 | 0.95 (0.92, 0.98) | <0.001 |
| Oxidized HDL (normalized) | 0.89 (0.80, 0.99) | 0.012 | 0.92 (0.82, 1.03) | 0.11 |
| Oxidized LDL | 1.08 (0.96, 1.21) | 0.28 | 1.01 (0.91, 1.12) | 0.64 |
| HDL Particle Size | 1.01 (0.98, 1.03) | 0.87 | -- | -- |
| Total HDL Particles | 1.16 (0.92, 1.45) | 0.06 | -- | -- |
| Large HDL Particles | 1.00 (0.82, 1.21) | 0.68 | -- | -- |
| LDL Particle Size | 1.00 (0.99, 1.01) | 0.61 | -- | -- |
| Total LDL Particles | 1.09 (1.00, 1.19) | 0.026 | -- | -- |
| Small LDL Particles | 1.06 (0.86, 1.31) | 0.43 | -- | -- |
| Large VLDL Particles | 1.17 (0.80, 1.71) | 0.38 | -- | -- |
| **DRV/RTV vs. RAL** | | | | |
| Oxidized HDL (unadjusted) | 0.92 (0.89, 0.95) | <0.001 | 0.93 (0.91, 0.97) | <0.001 |
| Oxidized HDL (normalized) | 0.90 (0.81, 1.01) | 0.012 | 0.91 (0.81, 1.02) | 0.06 |
| Oxidized LDL | 1.12 (1.01, 1.25) | 0.05 | 0.99 (0.88, 1.12) | 0.35 |
| HDL Particle Size | 1.01 (0.98, 1.03) | 0.77 | -- | -- |
| Total HDL Particles | 1.19 (0.95, 1.49) | 0.046 | -- | -- |
| Large HDL Particles | 0.99 (0.83, 1.19) | 0.93 | -- | -- |
| LDL Particle Size | 1.00 (0.99, 1.01) | 0.57 | -- | -- |
| Total LDL Particles | 1.12 (1.03, 1.22) | 0.004 | -- | -- |
| Small LDL Particles | 1.18 (0.93, 1.48) | 0.19 | -- | -- |
| Large VLDL Particles | 1.12 (0.78, 1.62) | 0.41 | -- | -- |

Pairwise treatment group comparisons reflect estimated relative mean fold change from baseline (97.5% confidence interval); p-values using Wilcoxon rank sum test.