# Lack of Associations of Oxidized Lipoproteins With Atherosclerosis in HIV: ACTG A5260s

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## BACKGROUND

- The role of oxidized lipoproteins (ox-lipids) in the pathogenesis of cardiovascular disease (CVD) in chronic HIV infection remains poorly defined.
- In retrospective studies, both oxidized low-density lipoprotein (LDLox) and oxidized high-density lipoprotein (HDLox) have been associated with progression of CVD in HIV infection (1-3).
- We previously showed that HDLox in HIV-1 infected subjects on long term antiretroviral (ART) and without clinical CVD are i) associated with in vivo progression of CVD (1) ii) may stimulate endothelial cells to induce M/M chemotaxis, a measure of HDL function (4) iii) correlated positively with non-calcified coronary atherosclerotic plaque (2) iv) and correlated with sCD163, a marker of M/M activation that has been linked to CVD in HIV disease (2).

**ACTG A5260s Study Design and Participants**

- Prospective, 144-week (3-year) longitudinal evaluation of 328 ART-naïve HIV-infected adults without known CVD or diabetes mellitus, or use of lipid-lowering medications who enrolled in a randomized antiretroviral therapy treatment trial (ACTG A5257).
- Randomization into ACTG A5257 was stratified by screening HIV-1 RNA level (> or ≤100,000 copies/ml), Framingham 10-year coronary heart disease risk score (<6% or ≥6% risk) and ACTG A5260s participation.
- Randomly assigned to tenofovir/emtricitabine plus atazanavir/ritonavir (**ATV/RTV**, a pharmacologically boosted protease inhibitor [PI/r]), darunavir/ritonavir (**DRV/RTV**, a PI/r), or raltegravir (**RAL**, an integrase inhibitor).

## OBJECTIVES

**To characterize and evaluate changes longitudinally in levels of plasma oxidized lipoproteins.**

**To examine the associations of plasma oxidized lipoprotein biomarkers with progression of carotid intima media thickness (CIMT).**

## Acknowledgements

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**References:** 1. Kelesidis et al. PLoS One 2014,9:e111716 2. Zanni et al. Antivir Ther 2014,19:805-811, 3. Para et al. HIV Med 2010,11:225-231 4. JKelesidis et al. Lipid Res 2011,52:2341-2351

## METHODS

**Study Population**

- For the present analysis, the A5260s population was restricted to 234 participants (71%) who remained on randomized treatment (no ART interruptions >7 days) and who achieved HIV-1 RNA suppression of <50 copies/ml by study week 24 and maintained for three years.
- **ATV/RTV** (n=68), **RAL** (n=82), **DRV/RTV** (n=84).

**CIMT Testing**

- CIMT images were obtained by ultrasonography at 26 U.S sites twice prior to study drug initiation (baseline), then at weeks 48, 96, and 144
- The right common carotid artery (CCA) and bifurcation segments were imaged using the jugular vein/carotid artery stacking method

**Biomarker Assessment**

- All blood samples were drawn fasting.
- Biomarkers were assayed at baseline and weeks 24 and 96.
- Plasma oxidized LDL was determined by ELISA (Mercodia).
- Oxidized HDL was determined by a previously validated fluorometric biochemical assay that measures HDL lipid peroxidation based on the oxidation of the fluorochrome Amplex Red.
  - To reduce experimental variability and adjust for HDL amount, HDLox was normalized by the HDLox level of pooled plasma control and by concurrent HDL level.

**Data Analysis**

- **Biomarker Changes over Time** were estimated 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 was assessed by the bounds of the 95% confidence interval (CI); one indicates no change.
- **Pairwise Treatment Group Comparisons** used Wilcoxon rank sum tests
  - Inference was assessed against a type I error of 2.5%.
- **Association Analyses** with CIMT rate of progression over 3 years used mixed effects linear regression with random intercept and slope and unstructured covariance matrix on the random effect.
  - Biomarkers were examined as a three-level categorical covariate: 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 between higher biomarker levels (≥Q3) relative to lower levels (<Q1); modeled as an interaction with time.
  - All analyses adjusted for baseline CIMT and randomization stratification factors; time was modeled continuously in years.
  - Inference was assessed with a 5% type I error, 95% CIs.
- Formal adjustments for multiple comparisons were not performed. Inference was guided by nominal p-values and consideration of the consistency, direction and magnitude of the effect sizes and confidence intervals.

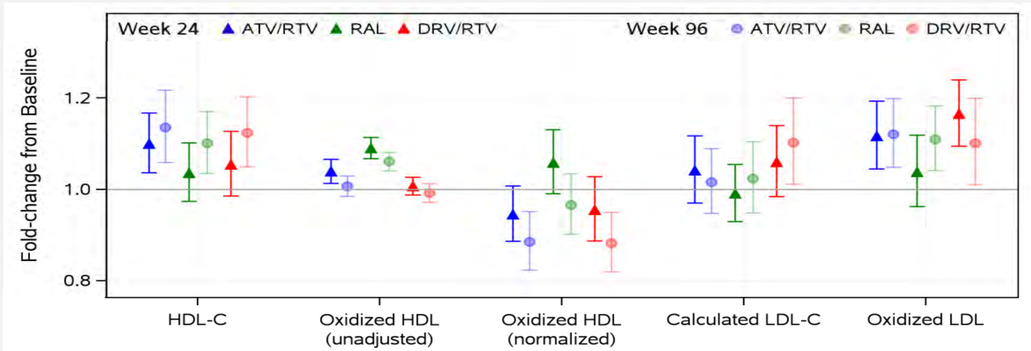
## RESULTS

**Table 1. Baseline characteristics (n=234)**

| | |
|---|---|
| **Sex** | |
| **Male** | 210 (90%) |
| **Female** | 24 (10%) |
| **Age** | 36 (28–45) |
| **Race/Ethnicity** | |
| **Non-Hispanic White** | 112 (48%) |
| **Non-Hispanic Black** | 68 (29%) |
| **Hispanic** | 45 (19%) |
| **Asian/Other/More than one race** | 8 (3%) |
| **CD4+ cell count [/mm$^3$]** | 338 (191–448) |
| **HIV-1 RNA ($log_{10}$ copies/ml)** | 4.6 (4.0–5.0) |
| **Low (<6%) 10-year risk of coronary heart disease** | 205 (88%) |
| **Current smoker** | 77 (33%) |
| **Metabolic syndrome** | 31 (13%) |
| **Baseline CIMT [µm]** | 0.65 (0.59–0.71) |
| **Evidence of carotid lesions** | 22 (9%) |
| **Oxidized Lipoproteins** | |
| **Oxidized HDL [unadjusted]** | 0.95 (0.90–1.00) |
| **Oxidized HDL [normalized]** | 0.99 (0.84–1.22) |
| **Oxidized LDL [U/L]** | 49.0 (39.5–59.6) |
| **Lipid Panel** | |
| **Total cholesterol [mg/dL]** | 156 (136–178) |
| **Triglycerides [mg/dL]** | 105 (74–142) |
| **HDL cholesterol [mg/dL]** | 38 (32–45) |
| **Calculated LDL cholesterol [mg/dL]** | 92 (75–110) |
| **Non-HDL cholesterol [mg/dL]** | 115 (97–138) |

Median (1st -3rd quartiles) or number (%).

**Figure 1. Mean fold change (95% CI) from baseline over time by treatment group**




**Biomarker Changes over Time (Figure 1)**

- HDL-C and LDL-C levels increased over time in all treatment groups.
- Post-baseline levels of unadjusted HDLox increased with RAL but remained unchanged with the ATV/RTV and DRV/RTV groups.
- Normalized HDLox declined to 88% of baseline levels after week 96 in both PI/r groups; week 96 levels with RAL were similar to baseline measures (mean fold-change of 0.97).
- Post-baseline levels of LDLox increased after 24 weeks across all treatment groups and remained elevated after 96 weeks (10% greater than baseline).

**Pairwise Treatment Group Comparisons**

- Evidence of greater declines for both PI/r groups compared to RAL for unadjusted and normalized HDLox were apparent at week 24 (p≤0.025).
  - The magnitude of these differences remained consistent at week 96, but were not statistically significant (p≥0.06).
- Treatment group differences for LDLox were not observed (p≥0.05).

**Association Analyses (Table 2)**

- Associations between CIMT rate of progression and all oxidized lipoproteins at baseline and after 24 weeks of ART were not evident when comparing the highest versus lowest quartiles of the data.
  - Normalized HDLox (p≥0.40); LDLox (p≥0.79).
  - These conclusions were unchanged when also considering outcomes for the middle quartiles.
- These observations remained consistent upon further adjustment for the effect of lipoprotein cholesterol concentration (p≥0.28).

**Table 2. Oxidized lipoprotein associations with CIMT rate of progression**

| | Estimated Rate of CCA IMT Change (µm/year) | |
|---|---|---|
| **Oxidized HDL** | **Estimate [95% CI]** | **p** |
| **Baseline** | | |
| **Upper quartile** | -1.16 [-6.52, 4.20] | 0.67 |
| **Middle quartiles** | -1.78 [-6.33, 2.77] | 0.44 |
| **Week 24** | | |
| **Upper quartile** | 2.28 [-3.10, 7.65] | 0.40 |
| **Middle quartiles** | 0.14 [-4.52, 4.80] | 0.95 |
| **Oxidized LDL** | **Estimate [95% CI]** | **p** |
| **Baseline** | | |
| **Upper quartile** | -0.69 [-5.86, 4.49] | 0.79 |
| **Middle quartiles** | -0.96 [-5.44, 3.53] | 0.67 |
| **Week 24** | | |
| **Upper quartile** | 0.31 [-4.88, 5.51] | 0.91 |
| **Middle quartiles** | -6.77 (-11.90, -1.65) | 0.010 |

Reference: group: Lower quartile

## CONCLUSIONS

- To our knowledge, this is the most comprehensive prospective study describing changes in oxidized lipoproteins after successful ART initiation with regards to measures of subclinical atherosclerosis (CIMT).
- 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 IMT progression over three years among treatment naïve individuals who achieved and sustained virologic suppression after initiating and successfully maintaining ART regimens of tenofovir/emtricitabine with RAL, ATV/RTV or DRV/RTV.
- Larger studies with longer-term treatments that also measure oxidized lipids at the tissue level and also examine other surrogate markers of CVD such as coronary CT angiography or other clinical endpoints are needed to further study and understand the role of oxidized lipids in HIV-associated cardiovascular disease.

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