

# Soluble levels of receptor for advanced glycation endproducts and dysfunctional high-density lipoprotein in persons infected with human immunodeficiency virus

## ACTG NWCS332

Theodoros Kelesidis, MD, PhD<sup>a,\*</sup>, Michelle A. Kendall, MS<sup>b</sup>, Ann Danoff, MD<sup>c,d</sup>, Judith A. Aberg, MD<sup>e</sup>, Judith S. Currier, MD, Msc<sup>a</sup>, Ann Marie Schmidt, MD<sup>f</sup>

### Abstract

The role of high-density lipoprotein (HDL) function and advanced glycation end products (AGEs) in HIV-related atherosclerotic cardiovascular disease (CVD) is unclear. Both glycation and oxidation (HDL<sub>ox</sub>) are major modifications of HDL that can alter its composition and function. Therefore, we explored the longitudinal association of HDL<sub>ox</sub> with progression of glycation, as evaluated by measurement of circulating forms of receptor for AGE that predict morbidity (soluble Receptors for Advanced Glycation Endproducts [sRAGE], endogenous secretory Receptors for Advanced Glycation Endproducts [esRAGE]), in people with HIV-1 (PWH; HIV-1<sup>+</sup>) and uninfected (HIV-1<sup>-</sup>) individuals.

We retrospectively assessed if levels of plasma sRAGE and esRAGE and HDL function (reduced antioxidant function is associated with increased HDL lipid hydroperoxide content; HDL<sub>ox</sub>) in a subset of participants (n=80) from a prospective 3-year study (AIDS Clinical Trials Group A5078). Primary outcomes were baseline and yearly rates of change over 96 of 144 weeks ( $\Delta$ ) in HDL<sub>ox</sub> in HIV-1<sup>+</sup> versus uninfected HIV-1<sup>-</sup> controls (noted as HIV-1<sup>-</sup>).

Higher baseline levels of sRAGE in PWH on effective anti-retroviral therapy and with low CVD risk, but not in HIV-1<sup>-</sup> persons, were independently associated with higher HDL<sub>ox</sub>. EsRAGE, but not sRAGE, had consistent inverse relationships with  $\Delta$ HDL<sub>ox</sub> in both HIV-1<sup>+</sup> and HIV-1<sup>-</sup> persons at baseline. In HIV-1<sup>-</sup> but not in HIV-1<sup>+</sup> persons,  $\Delta$ HDL<sub>ox</sub> had positive and inverse relationships with  $\Delta$ RAGE and  $\Delta$ esRAGE, respectively.

Glycation and oxidation of HDL may contribute to impaired HDL function present in PWH.

**Abbreviations:**  $\Delta$ esRAGE = yearly rates of change over 96/144 weeks ( $\Delta$ ) in endogenous secretory receptors for advanced glycation endproducts,  $\Delta$ HDL<sub>ox</sub> = yearly rates of change over 96/144 weeks ( $\Delta$ ) in oxidized high-density lipoprotein,  $\Delta$ RAGE = yearly rates of change over 96/144 weeks ( $\Delta$ ) in receptors for advanced glycation endproducts, ACTG = AIDS Clinical Trials Group, AGER = the gene encoding Receptors for Advanced Glycation Endproducts (RAGE), AGEs = advanced glycation end products, AIDS = acquired immune deficiency syndrome, ART = antiretroviral therapy, BMI = body mass index, CD4 = cluster of differentiation 4, CIMT = carotid intima-media thickness, CVD = atherosclerotic cardiovascular disease, DHR = dihydrorhodamine 123, ELISA = enzyme-linked immunosorbent assay, esRAGE = endogenous secretory receptors for advanced glycation endproducts, FU = fluorescence units, HBS = N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid buffered saline, HDL = high-density lipoprotein, HDLox = oxidized high-density lipoprotein, HEPES = N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid, HIV-1<sup>-</sup> = seronegative people not infected with human immunodeficiency virus 1, HIV-1<sup>+</sup> = seropositive people infected with human immunodeficiency virus 1, HIV-HDL = high-density lipoprotein in chronic, treated HIV infection, hs-CRP = high-sensitivity C-reactive protein, LDL = low-density lipoprotein, MDFCM = monocyte-derived foam cell formation, Min = Minute, MMPs = matrix metalloproteinases, PI = Protease

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<sup>a</sup> Department of Medicine, Division of Infectious Diseases, UCLA David Geffen School of Medicine, Los Angeles, CA, <sup>b</sup> Center for Biostatistics in AIDS Research, Harvard TH Chan School of Public Health, Boston, MA, <sup>c</sup> Department of Medicine, CPL Michael J Crescenzo VA Medical Center, <sup>d</sup> Department of Medicine, Division of Endocrinology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, <sup>e</sup> Department of Medicine, Division of Infectious Diseases, Icahn School of Medicine at Mount Sinai, <sup>f</sup> Department of Medicine, Division of Endocrinology, Diabetes and Metabolism, New York University School of Medicine, New York, NY.

\* Correspondence: Theodoros Kelesidis, Department of Medicine, Division of Infectious Diseases, David Geffen School of Medicine at UCLA, 10833 Le Conte Ave. CHS 37-121 Los Angeles, CA 90095 (e-mail: tkelesidis@mednet.ucla.edu).

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inhibitor therapy, PWH = people with HIV-1, RNA = ribonucleic acid, sRAGE = soluble receptors for advanced glycation endproducts.

**Keywords:** cardiovascular disease, glycation, HDL, HDL function, HIV, inflammation, oxidation

## 1. Introduction

Despite potent antiretroviral therapy, people with chronic HIV-1 infection (PWH) have increased risk of atherosclerotic cardiovascular disease (CVD), but the mechanisms are unclear.<sup>[1]</sup> High-density lipoprotein cholesterol (HDL-C) function, rather than absolute level, may more accurately predict atherosclerosis.<sup>[1]</sup> In HIV-seronegative (HIV-1<sup>-</sup>) persons, abnormal HDL function strongly correlates with clinical CVD.<sup>[2]</sup> Inflammatory states such as chronic HIV-1 infection can induce protein and lipid compositional changes that impair HDL function, reduce anti-inflammatory and antioxidant proteins, and increase HDL lipid hydroperoxide content and redox activity (HDL<sub>ox</sub>).<sup>[3]</sup>

Glycation is a major modification of HDL that can alter its composition and function.<sup>[4–6]</sup> Under aerobic conditions, glycation and oxidation interact to form glycooxidation, or advanced glycation end products (AGEs), on proteins and lipids. Numerous studies suggest that circulating levels of soluble Receptors for Advanced Glycation Endproducts (RAGE) that bind to and transduce the signal stimulated by AGEs are implicated in the pathogenesis of chronic diseases such as diabetes and atherosclerosis.<sup>[7,8]</sup> In humans, 2 soluble forms of RAGE are detectable, including soluble (sRAGE, a form of the extracellular receptor cleaved from the cell surface via the actions of various proteases such as matrix metalloproteinases [MMPs] that are also regulated by oxidized lipoproteins<sup>[9]</sup>) and endogenous secretory (esRAGE, the translation product of a splice variant of *AGER*, the gene encoding RAGE).<sup>[7]</sup> We have previously shown that RAGE suppresses macrophage cholesterol efflux in diabetes,<sup>[10]</sup> and dysfunctional HDL from PWH with low CVD risk promotes monocyte-derived foam cell formation (MDFCM) *in vitro*.<sup>[11]</sup> However, little is known about the role of RAGE, HDL function, and the cross-talk between glycation and HDL function in chronic treated HIV-1 infection.

NWCS332 was a sub-study of AIDS Clinical Trials Group (ACTG) A5078, which was designed to study the role of protease inhibitor (PI) therapy and HIV infection on the risk of development of subclinical atherosclerosis (as evaluated by carotid intima-media thickness, CIMT) and its progression.<sup>[12]</sup> In NWCS332, we previously reported that serum HDL<sub>ox</sub> (assessed by a biochemical assay that quantifies the antioxidant function of HDL; higher HDL<sub>ox</sub> indicates HDL dysfunction<sup>[13]</sup>) declined over 3 years but was not associated with progression of CIMT;<sup>[14]</sup> baseline (e)sRAGE levels were not significantly different between the HIV-1<sup>+</sup> and HIV-1<sup>-</sup> groups;<sup>[15]</sup> there were positive yearly rates of change in sRAGE within the HIV-1<sup>+</sup> group and in esRAGE within both groups;<sup>[15]</sup> and increased odds of CIMT progression were associated with lower baseline esRAGE in HIV-1<sup>+</sup> group.<sup>[15]</sup>

Although both oxidation and glycation are modifications of HDL that may underlie some of the atherogenic properties of impaired HDL, it is unknown whether complex interactions between glycation and HDL may underlie some of the atherogenic properties of HIV-HDL. The overall goal of this sub-study was to investigate a role for (e)sRAGE as biomarkers and/or mediators of HDL function in PWH and as

a possible driver of atherosclerotic risk in chronic, treated HIV infection.

Our objectives were to be the first to explore the longitudinal association of HDL<sub>ox</sub> with changes in glycation, as evaluated by measurement of circulating forms of receptors for glycation products that predict morbidity (sRAGE, esRAGE), in HIV-1<sup>+</sup> and HIV-1<sup>-</sup> individuals using data from NWCS332. We hypothesized that with chronic inflammation resulting from HIV-1 infection, sRAGE and esRAGE would increase over time<sup>[16]</sup> in association with higher HDL<sub>ox</sub>.

## 2. Methods

### 2.1. Study design and participants

The present study is a subset analysis of samples obtained from a prospective, matched cohort study<sup>[14,15]</sup> in which participants were enrolled as risk factor-matched triads of HIV-1<sup>+</sup> individuals with HIV-1 viremia <500 RNA copies/mL with (n=29) or without (n=26) use of PI therapy, and HIV-1-uninfected individuals (n=36) from 41 triads. Individuals were excluded if they had diabetes mellitus, family history of early myocardial infarction in first-degree relatives, a history of coronary heart disease or stroke, uncontrolled hypertension, untreated hypothyroidism, or obesity. Individuals requiring systemic chemotherapy, radiation therapy, or systemic steroids were also excluded, as were individuals with a serum creatinine >1.5 mg/dL or alanine or aspartate aminotransferases >2.5× upper limit of normal. Individuals were excluded from NWCS332 if they had HIV RNA ≥500 copies/mL or missing stored samples (weeks 0, 72, and 144 (or week 96 if week 144 was missing; thus, “week 96/144”). The 80 participants who had the required HIV-1 RNA level and sufficient residual sample to be included in this analysis were from 40 triads (Group 1: n=25, Group 2: n=21, and Group 3: n=34), of which 9 triads were complete. Given that we did not find notable differences in (e) sRAGE among the 2 HIV groups (with or without PIs),<sup>[15]</sup> the HIV groups were combined. The A5078 informed consent document included the provision for future testing of stored samples for ACTG-approved AIDS-related research and the study was approved by the local institutional review boards.

### 2.2. Data collection

Clinical data, CVD-related measurements, (e)sRAGE, and HDL<sub>ox</sub> have previously been described.<sup>[14,15]</sup> Fasting glucose, insulin, lipids, cardiovascular/metabolic disease-related measurements (including homocysteine and high-sensitivity C-reactive protein [hs-CRP]), CD4+ cell counts, and HIV-1 RNA levels were collected at A5078 study entry. In this secondary analysis utilizing stored A5078 samples, levels of plasma sRAGE and esRAGE were measured at weeks 0, 72, and 96/144 in individuals who also had HIV RNA <500 copies/mL and CIMT results at all time points.

### 2.3. Determination of sRAGE and esRAGE

In this study, plasma sRAGE and esRAGE levels were assayed on stored samples using enzyme-linked immunosorbent assay

(ELISA) kits in accordance with the manufacturer's protocol (R&D Systems Quantikine Immunoassay Minneapolis, MN; and B-Bridge ELISA, B-Bridge International, Cupertino, CA). Stored samples from baseline, week 72, and week 144 (or 96 if week 144 was missing) were assayed.

#### 2.4. Reagents

Dihydrorhodamine 123 (DHR) was obtained from Molecular Probes (Eugene, OR). DHR was prepared as a stock of 50 mmol/L in dimethyl sulfoxide as previously described.<sup>[14]</sup> Iron-free N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES)-buffered saline (HBS, HEPES 20 mmol/L, NaCl 150 mM, pH 7.4) was prepared as previously described.<sup>[14]</sup> The DHR stock was diluted 1:1000 in HEPES saline solution to prepare a working solution of 50  $\mu$ mol/L.

#### 2.5. HDL purification

HDL was isolated using precipitation with polyethylene glycol and ultracentrifugation, aliquoted, and stored as previously described. HDL cholesterol was quantified using a standard colorimetric assay (Thermo DMA Co., San Jose, CA) as previously described.<sup>[14]</sup>

#### 2.6. Biochemical cell-free assay of HDL function

Quadruplicates of HDL (2.5  $\mu$ g of cholesterol unless otherwise specified) were added to 96-well plates (polypropylene, flat bottom, black, Fisher Scientific). HBS was added to each well to a final volume of 150  $\mu$ L, followed by addition of 25  $\mu$ L of the 50  $\mu$ mol/L DHR working solution, for a total volume of 175  $\mu$ L (final DHR concentration of 7  $\mu$ mol/L). Immediately following DHR addition, the plate was protected from light and placed in the fluorescence plate reader (at 37°C). The fluorescence of each well was assessed at 2-minute intervals during an hour with a Synergy 2 Multi-Mode Microplate Reader (Biotek, Winooski, VT), using a 485/538 nm excitation/emission filter pair with the photomultiplier sensitivity set at medium. Determination of oxidation rate of DHR and the slope of oxidation of HDL (HDL<sub>ox</sub>) was performed by measuring the slope of fluorescence increase during 50 minutes after addition of a specific amount of lipid (HDL) as previously described.<sup>[14]</sup> HDL oxidative function was calculated as the mean of quadruplicates for the wells containing the HDL sample.

#### 2.7. Statistical methods

Using SAS version 9.4 with SAS/STAT version 14.1, by-group comparisons of baseline characteristics were assessed using Fisher exact and Wilcoxon tests as appropriate. Spearman rank correlation evaluated correlations. Mixed models regression analyses evaluated whether baseline measurements of HDL<sub>ox</sub> were associated with baseline (e)sRAGE. Repeated measures regression analyses evaluated associations between yearly rates of change over 96/144 weeks ( $\Delta$ ) in HDL<sub>ox</sub> and (e)sRAGE. Regression models were fit within the HIV-1<sup>+</sup> and HIV-1<sup>-</sup> groups separately. Mixed models regression analyses with triad as a random effect evaluated whether baseline (week 0) measurements of sRAGE and esRAGE were associated with baseline HDL<sub>ox</sub>. Repeated measures regression analyses with triad as a random effect evaluated associations with yearly rates of change over 144 weeks (or 96 weeks if the week 144 sample

was missing) in sRAGE, esRAGE, and HDL<sub>ox</sub>. The baseline covariates considered in the univariable analyses were age, sex, race, fasting glucose, fasting lipid measurements (total cholesterol, low-density lipoprotein [LDL] cholesterol, and triglycerides), use of lipid-lowering drugs, use of statins, body mass index [BMI], waist circumference, waist/hip ratio, insulin, hs-CRP, and homocysteine. For the HIV-1<sup>+</sup>, additional covariates included CD4<sup>+</sup> cell count and HIV RNA. The unadjusted associations of the above covariates with HDL<sub>ox</sub>, sRAGE, and esRAGE were previously described.<sup>[15]</sup> Here, the univariable associations between sRAGE and esRAGE (predictors) and baseline HDL<sub>ox</sub> and yearly rate of change in HDL<sub>ox</sub> over 96/144 weeks (outcomes) were adjusted for baseline HDL cholesterol and, in the yearly rate of change analysis, also week 96/144 HDL. The multivariable analysis was adjusted for baseline age, sex, HDL cholesterol, glucose, and, for the HIV-1<sup>+</sup>, CD4<sup>+</sup> cell count and HIV RNA; the yearly rate in change analyses was also adjusted for HDL cholesterol, CD4<sup>+</sup>, and HIV RNA at week 96/144. Covariates with  $P < .20$  in the univariable analysis were examined together in multivariable analysis and reduced using the backward elimination method, with the final multivariable model containing covariates with  $P < .05$ . Results with 2-sided  $P < .05$  were deemed significant; no adjustments were made for multiple comparisons.

### 3. Results

#### 3.1. Baseline characteristics

Participant characteristics are summarized in Table 1. The participants were 91% male, 75% white/non-Hispanic, with a median age of 41 years. The 46 HIV-1<sup>+</sup> had significantly ( $P < .05$ ) higher waist-to-hip ratio, more use of lipid lowering drugs, and lower homocysteine levels compared to the HIV-1<sup>-</sup> participants. Owing to matching, the groups were similar with respect to age, sex, and race. As previously published, baseline HDL<sub>ox</sub> and (e) sRAGE levels were not significantly different between the groups ( $P > .2$ ).<sup>[14,15]</sup> Median baseline levels of HDL<sub>ox</sub>, sRAGE, and esRAGE were 243,753 FU/min, 1039 pg/mL, and 233 pg/mL, respectively.

#### 3.2. Baseline relationships between HDL function and (e) sRAGE

Baseline HDL<sub>ox</sub> was correlated with baseline esRAGE overall and in HIV-1<sup>+</sup> participants (Fig. 1). However, in the more robust univariable regression analysis in PWH, baseline HDL<sub>ox</sub> was marginally associated with esRAGE ( $P = .051$ ), but significantly associated with sRAGE ( $P = .039$ ; Table 2). In an adjusted multivariable regression analysis in HIV-1<sup>+</sup> participants, baseline HDL<sub>ox</sub> was marginally associated with baseline sRAGE ( $P = .056$ ) (Table 3). Assuming fixed levels of the covariates used to adjust the model (details provided in Table 3), an increase in baseline sRAGE by 100 pg/mL (~10% of median baseline level) in HIV-1<sup>+</sup> participants would result in, on average, an increase in baseline HDL<sub>ox</sub> by 0.55 per 10,000 FU/min (~2% of median baseline level).

#### 3.3. Relationships between changes in HDL function and (e) sRAGE

As previously published, longitudinal levels of HDL<sub>ox</sub> and (e) sRAGE were not significantly different between the groups

**Table 1****Participant' characteristics. Except otherwise indicated, data represent N (%) of participants.**

|                                    |                               | Group                      |                            |                           | P                 |
|------------------------------------|-------------------------------|----------------------------|----------------------------|---------------------------|-------------------|
|                                    |                               | Total (N=80)               | HIV-uninfected (N=34)      | HIV-infected (N=46)       |                   |
| Sex                                | Male                          | 73 (91%)                   | 30 (88%)                   | 43 (93%)                  | .451*             |
|                                    | Female                        | 7 (9%)                     | 4 (12%)                    | 3 (7%)                    |                   |
| Race/ethnicity                     | White Non-Hispanic            | 60 (75%)                   | 25 (74%)                   | 35 (76%)                  | .659*             |
|                                    | Hispanic (regardless of race) | 15 (19%)                   | 8 (24%)                    | 7 (15%)                   |                   |
|                                    | Asian, Pacific Islander       | 3 (4%)                     | 1 (3%)                     | 2 (4%)                    |                   |
|                                    | Other/unknown                 | 2 (3%)                     | 0 (0%)                     | 2 (4%)                    |                   |
|                                    |                               |                            |                            |                           |                   |
| Age, y                             | Median (Q1, Q3)               | 41 (36, 45)                | 40 (36, 45)                | 41 (37, 45)               | .949 <sup>†</sup> |
| Glucose, mg/dL                     | Median (Q1, Q3)               | 86 (79, 93)                | 85.5 (75, 92)              | 86 (81, 94)               | .335 <sup>†</sup> |
| Total Cholesterol, mg/dL           | Median (Q1, Q3)               | 195 (162, 231)             | 184 (163, 206)             | 202 (159, 240)            | .261 <sup>†</sup> |
| LDL cholesterol, mg/dL             | # Missing                     | 2                          | 0                          | 2                         | .691 <sup>†</sup> |
|                                    | Median (Q1, Q3)               | 115.5 (90, 149)            | 114 (88, 149)              | 116 (95.5, 150.5)         |                   |
| HDL cholesterol, mg/dL             | Median (Q1, Q3)               | 42 (35, 48.5)              | 43 (38, 50)                | 41 (35, 48)               | .566 <sup>†</sup> |
| Triglycerides, mg/dL               | Median (Q1, Q3)               | 128 (91.5, 199)            | 112 (89, 153)              | 142.5 (96, 221)           | .068 <sup>†</sup> |
| Use of lipid-lowering drugs        | None                          | 70 (88%)                   | 34 (100%)                  | 36 (78%)                  | .004*             |
|                                    | Any                           | 10 (13%)                   | 0 (0%)                     | 10 (22%)                  |                   |
| Use of statins                     | None                          | 73 (91%)                   | 34 (100%)                  | 39 (85%)                  | .019*             |
|                                    | Any                           | 7 (9%)                     | 0 (0%)                     | 7 (15%)                   |                   |
| Body mass index, kg/m <sup>2</sup> | Median (Q1, Q3)               | 24.7 (23.4, 27.2)          | 24.8 (23.7, 27.7)          | 24.0 (23.4, 26.8)         | .690 <sup>†</sup> |
| Waist circumference                | ≥90 cm                        | 43 (54%)                   | 19 (56%)                   | 24 (52%)                  | >.999*            |
|                                    | <90 cm                        | 36 (45%)                   | 15 (44%)                   | 21 (46%)                  |                   |
|                                    | Missing                       | 1 (1%)                     | 0 (0%)                     | 1 (2%)                    |                   |
|                                    |                               |                            |                            |                           |                   |
| Waist/hip ratio                    | # Missing                     | 1                          | 0                          | 1                         | .008 <sup>†</sup> |
|                                    | Median (Q1, Q3)               | 0.90 (0.85, 0.93)          | 0.89 (0.83, 0.92)          | 0.92 (0.88, 0.94)         |                   |
| Insulin, mU/L                      | # Missing                     | 6                          | 3                          | 3                         | .300 <sup>†</sup> |
|                                    | Median (Q1, Q3)               | 6.35 (5.1, 8.0)            | 6.1 (5.0, 7.7)             | 6.50 (5.5, 10.4)          |                   |
| Homocysteine, μmol/L               | # Missing                     | 6                          | 3                          | 3                         | .018 <sup>†</sup> |
|                                    | Median (Q1, Q3)               | 8.95 (7.4, 10.1)           | 9.7 (8.7, 11.0)            | 8.1 (6.8, 10.0)           |                   |
| hs-CRP, mg/L                       | # Missing                     | 6                          | 3                          | 3                         | .184 <sup>†</sup> |
|                                    | Median (Q1, Q3)               | 1.0 (0.5, 2.1)             | 0.9 (0.5, 1.7)             | 1.1 (0.5, 3.3)            |                   |
| HIV-1 RNA                          | <50 copies/mL                 | —                          | —                          | 38 (83%)                  | —                 |
|                                    | ≥50 copies/mL                 | —                          | —                          | 8 (17%)                   |                   |
| CD4, cells/mm <sup>3</sup>         | Median (Q1, Q3)               | —                          | —                          | 514 (344, 679)            | —                 |
| Duration of ART, wk                | Median (Q1, Q3)               | —                          | —                          | 175 (124, 269)            | —                 |
| Ritonavir use                      | None                          | —                          | —                          | 34 (74%)                  | —                 |
|                                    | Any                           | —                          | —                          | 11 (24%)                  |                   |
|                                    | Missing                       | —                          | —                          | 1 (2%)                    |                   |
|                                    |                               |                            |                            |                           |                   |
| NRTI use                           | None                          | —                          | —                          | 1 (2%)                    | —                 |
|                                    | Any                           | —                          | —                          | 44 (96%)                  |                   |
|                                    | Missing                       | —                          | —                          | 1 (2%)                    |                   |
| NNRTI use                          | None                          | —                          | —                          | 20 (43%)                  | —                 |
|                                    | Any                           | —                          | —                          | 25 (54%)                  |                   |
|                                    | Missing                       | —                          | —                          | 1 (2%)                    |                   |
|                                    |                               |                            |                            |                           |                   |
| HDL <sub>ox</sub> , FU/min         | Median (Q1, Q3)               | 243,753 (202,048, 280,312) | 236,089 (192,379, 270,475) | 248,455 (212,56, 280,859) | .274 <sup>†</sup> |
| sRAGE, pg/mL                       | Median (Q1, Q3)               | 1039 (797, 1298)           | 1030 (797, 1265)           | 1064 (792, 1433)          | .517 <sup>†</sup> |
| esRAGE, pg/mL                      | Median (Q1, Q3)               | 233 (139, 337)             | 236 (128, 343)             | 229 (143, 331)            | .778 <sup>†</sup> |

ART = antiretroviral therapy, esRAGE = endogenous secretory receptors for advanced glycation endproducts, HDL = high-density lipoprotein, HDL<sub>ox</sub> = oxidized high-density lipoprotein, hs-CRP = high-sensitivity C-reactive protein, LDL = low-density lipoprotein, NNRTIs = Non Nucleoside Reverse Transcriptase Inhibitors, sRAGE = soluble receptors for advanced glycation endproducts.

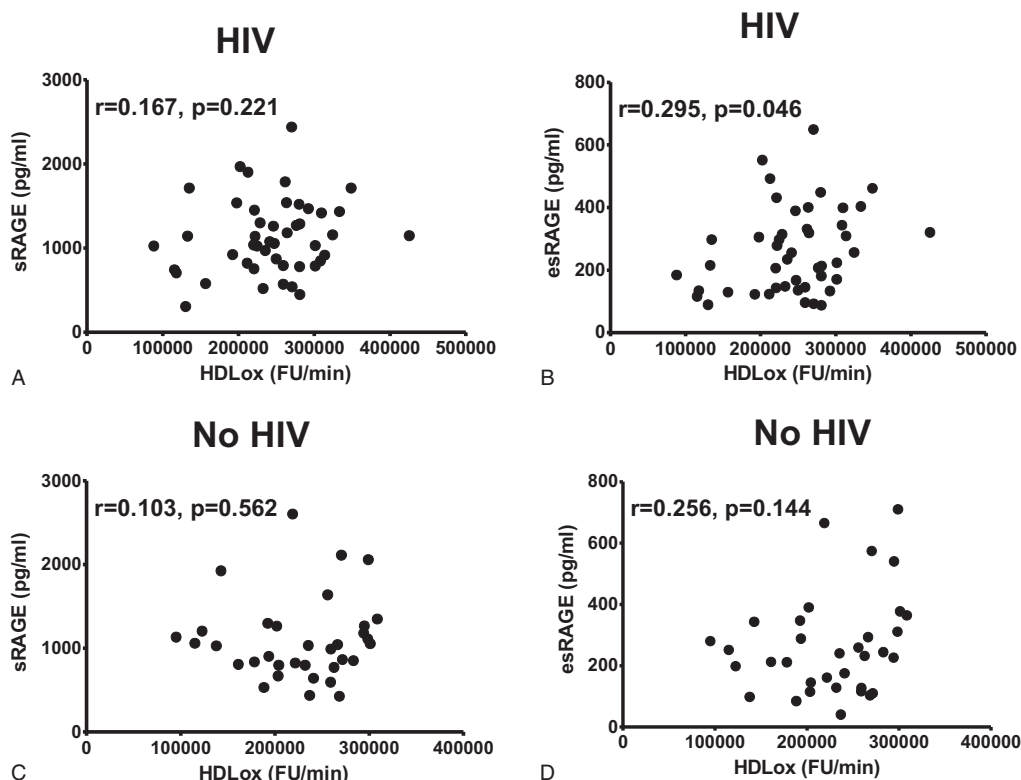
\* Fisher exact test.

<sup>†</sup> Wilcoxon test.

( $P > .2$ ).<sup>[14,15]</sup> In univariable regression analysis,  $\Delta$ HDL<sub>ox</sub> was significantly associated with baseline esRAGE ( $P < .05$ ), but not baseline sRAGE ( $P > .1$ ; Table 2) in both groups. In the adjusted multivariable regression analysis in HIV-1<sup>+</sup> participants, positive  $\Delta$ HDL<sub>ox</sub> was associated with lower baseline esRAGE ( $P = .012$ ), white race ( $P = .031$ ) and lower baseline BMI ( $P < .001$ ) (Table 3). Assuming fixed levels of the other covariates in the model, a decrease in baseline esRAGE by 100 pg/mL (~43% of median baseline level) in HIV-1<sup>+</sup> participants would result in, on average, an increase in HDL<sub>ox</sub> by 0.37 per 10,000 FU/min/year (~1.5% of median baseline level). In the adjusted multivariable regression

analysis HIV-1<sup>-</sup> persons, positive  $\Delta$ HDL<sub>ox</sub> was significantly associated with positive  $\Delta$ sRAGE ( $P < .001$ ), negative  $\Delta$ esRAGE ( $P < .001$ ), and waist circumference  $\geq 90$  cm ( $P = .003$ ). Assuming fixed levels of the other covariates in the model, including  $\Delta$ esRAGE and  $\Delta$ sRAGE as applicable, for every 100 pg/mL increase in sRAGE over time (~10% of median baseline level), HDL<sub>ox</sub> would, on average, increase by 0.26 per 10,000 FU/min/year (~1% of median baseline level) or for every 100 pg/mL decrease in esRAGE over time (~43% of median baseline level), HDL<sub>ox</sub> would, on average, increase by 0.10 per 10,000 FU/min/year (~0.5% of median baseline level), respectively.





**Figure 1.** Spearman rank correlations between baseline levels of HDL<sub>ox</sub>, sRAGE (A, C) and esRAGE (B, D) between HIV-1<sup>+</sup> (A, B) and HIV-1<sup>-</sup> (C, D) participants. Baseline HDL<sub>ox</sub> was correlated with baseline esRAGE (Spearman rank  $r=0.274$ ,  $P=.014$ ) and remained correlated in the HIV-1<sup>+</sup> group ( $P=.046$ ). esRAGE= endogenous secretory receptors for advanced glycation endproducts, HDL<sub>ox</sub>=oxidized high-density lipoprotein, sRAGE=soluble receptors for advanced glycation endproducts.

**4. Discussion**

In this exploratory sub-study of PWH with low CVD risk, we found that higher baseline levels of the sRAGE biomarker of glycation were associated with reduced baseline HDL antioxidant

function (higher HDL<sub>ox</sub>). This association was not found in the matched HIV-1<sup>-</sup> controls. EsRAGE, but not sRAGE, had consistent inverse relationships with  $\Delta$ HDL<sub>ox</sub> in both PWH and HIV-1<sup>-</sup> persons at baseline. These data complement our previous

**Table 2**  
**Adjusted univariable associations of HDL<sub>ox</sub> with sRAGE and esRAGE.**

| Covariate  | HIV-1-infected participants (n=46) |       | HIV-1-uninfected participants (n=34) |        |
|--|------------------------------------|-------|--------------------------------------|--------|
|  | Parameter estimate (95% CI)        | P     | Parameter estimate (95% CI)          | P      |
| Baseline HDL <sub>ox</sub> (per 10,000 FU/min; per ~5% of median baseline level)   |                                    |       |                                      |        |
| Baseline sRAGE (per 100 pg/mL; per ~10% change in median baseline level)   | 0.477 (0.115, 0.838)               | 0.039 | -0.008 (-0.375, 0.358)               | 0.970  |
| Baseline esRAGE (per 100 pg/mL; per ~43% change in median level)   | 1.421 (0.265, 2.578)               | 0.051 | 0.724 (-0.360, 1.810)                | 0.266  |
| Yearly rate of change in HDL <sub>ox</sub> (per 10,000 FU/min/year; per ~5% change above median baseline level per year) |                                    |       |                                      |        |
| Baseline sRAGE (per 100 pg/mL; per ~10% change above median baseline level per year)                                     | -0.080 (-0.161, 0.002)             | 0.110 | -0.039 (-0.135, 0.056)               | 0.492  |
| Yearly rate of change in sRAGE (per 100 pg/mL/year; per ~10% change above median baseline level per year)                | -0.060 (-0.141, 0.021)             | 0.221 | 0.319 (0.270, 0.367)                 | ≤0.001 |
| Baseline esRAGE (per 100 pg/ml; per ~43% change in median level)   | -0.323 (-0.563, -0.084)            | 0.028 | -0.332 (-0.598, -0.066)              | 0.042  |
| Yearly rate of change in esRAGE (per 100 pg/mL/year; per ~43% change above median baseline level per year)               | -0.051 (-0.204, 0.101)             | 0.577 | -0.179 (-0.245, -0.113)              | ≤0.001 |
| White Race   | 1.626 (0.824, 2.429)               | 0.001 | -                                    | -      |
| BMI, kg/m <sup>2</sup>   | -0.148 (-0.266, -0.030)            | 0.040 | -                                    | -      |
| Waist circumference <90 cm   | -                                  | -     | -1.475 (-2.337, -0.614)              | 0.007  |

BMI=body mass index, CI=confidence interval, esRAGE=endogenous secretory receptors for advanced glycation endproducts, HDL<sub>ox</sub>=oxidized high-density lipoprotein, sRAGE=soluble receptors for advanced glycation endproducts.

**Table 3****Adjusted multivariable associations of HDL<sub>ox</sub> with sRAGE and esRAGE.**

| Covariate  | HIV-1-infected participants (n=46) |        | HIV-1-uninfected participants (n=34) |        |
|--|------------------------------------|--------|--------------------------------------|--------|
|  | Parameter estimate (95% CI)        | P      | Parameter estimate (95% CI)          | P      |
| Baseline HDL <sub>ox</sub> (per 10,000 FU/min; per ~5% of median baseline level)   |                                    |        |                                      |        |
| Baseline sRAGE (per 100 pg/mL; per ~10% change in median baseline level)   | 0.552 (−0.020, 1.124)              | 0.056  | –                                    | –      |
| Baseline esRAGE (per 100 pg/mL; per ~43% change in median level)   | –                                  | –      | –                                    | –      |
| Yearly rate of change in HDL <sub>ox</sub> (per 10,000 FU/min/year; per ~5% change above median baseline level per year) |                                    |        |                                      |        |
| Baseline sRAGE (per 100 pg/mL; per ~10% change above median baseline level per year)                                     | –                                  | –      | –                                    | –      |
| Yearly rate of change in sRAGE (per 100 pg/mL/year; per ~10% change above median baseline level per year)                | –                                  | –      | 0.259 (0.204, 0.614)                 | ≤0.001 |
| Baseline esRAGE (per 100 pg/mL; per ~43% change in median level)   | −0.374 (−0.662, −0.086)            | 0.012  | –                                    | –      |
| Yearly rate of change in esRAGE (per 100 pg/mL/year; per ~43% change above median baseline level per year)               | –                                  | –      | −0.095 (−2.587, −0.053)              | ≤0.001 |
| White race   | 1.720 (0.168, 3.273)               | 0.031  | –                                    | –      |
| BMI, kg/m <sup>2</sup>   | −0.272 (−0.415, −0.129)            | ≤0.001 | –                                    | –      |
| Waist circumference <90 cm   | –                                  | –      | −1.593 (−2.587, −0.599)              | 0.003  |

BMI = body mass index, CI = confidence interval, esRAGE = endogenous secretory receptors for advanced glycation endproducts, HDL<sub>ox</sub> = oxidized high-density lipoprotein, sRAGE = soluble receptors for advanced glycation endproducts.

data that lower baseline levels of esRAGE were associated with  $\Delta$ CIMT in chronic treated HIV-1 infection;<sup>[14,15]</sup> RAGE directly contributes to atherogenic properties of macrophages;<sup>[10]</sup> HDL in chronic, treated HIV infection (HIV-HDL) is dysfunctional and oxidized even in PWH with low CVD risk;<sup>[14]</sup> and HIV-HDL from PWH with low CVD risk promotes MDFCM in vitro.<sup>[11]</sup> Collectively, these data suggest that complex interactions between glycation and HDL may underlie some of the atherogenic properties of HIV-HDL. This is the first longitudinal study investigating a role for (e)sRAGE as biomarkers and/or mediators of HDL function in PWH and as a possible driver of atherosclerotic risk in chronic, treated HIV infection. Elucidating the mechanisms driving modifications of HDL and glycation may identify dysfunctional HDL and glycation as potential therapeutic targets to reduce HIV-related CVD risk.

In vitro studies indicate that oxidative modification of HDL may impair cholesterol efflux activity. We showed that dysfunctional HDL (higher HDL<sub>ox</sub>) in PWH without clinical CVD was associated with in vivo progression of CVD (CIMT);<sup>[17]</sup> may stimulate endothelial cells to induce monocyte/macrophage chemotaxis;<sup>[13,17]</sup> was positively correlated with non-calcified coronary atherosclerotic plaque;<sup>[18]</sup> and was independently correlated with several markers of inflammation, immune activation,<sup>[19]</sup> and with impaired HDL remodeling.<sup>[20]</sup> Here we show that biomarkers of glycation are associated with impaired HDL function, consistent with previous data about a role of glycation in HDL dysfunction in diseases.<sup>[4,5]</sup> The ligands of RAGE may interact with distinct non-RAGE receptors, such as toll receptors that are also receptors for (oxidized) lipoproteins. sRAGE is cleaved from the cell surface via the actions of MMPs that are also regulated by oxidized lipoproteins.<sup>[9]</sup> It is possible that higher HDL glycation may lead to sequestration of esRAGE; alternatively, HDL glycation may suppress the alternative splicing of RAGE and reduce levels of esRAGE. The directionality of these complex interactions depends on the inflammatory milieu and is probably specific to diseases.<sup>[7,8,21–23]</sup>

In contrast to our study, the majority of previous studies regarding RAGE were cross-sectional<sup>[24,25]</sup> and do not report both sRAGE and esRAGE. Single time point analysis may not take into account the effects of exacerbations of disease in superimposed chronic diseases such as HIV. Consistent with this hypothesis, sRAGE levels may change over time with disease activity<sup>[16]</sup> and we found that higher baseline levels of esRAGE were associated with higher baseline HDL<sub>ox</sub>, but lower baseline esRAGE levels were associated with positive  $\Delta$ HDL<sub>ox</sub> and lower baseline esRAGE levels were associated with increased odds of CIMT progression in the HIV-infected participants suggesting that lower levels of soluble forms of esRAGE may be a biomarker of mechanisms that perpetuate vascular inflammation.<sup>[15]</sup>

The strengths of our study are the careful covariate phenotyping of our study population, including novel measures of HDL function and glycation. However, this study has several limitations. The study population overall had a low risk for CVD, which may have diminished our ability to study changes in levels of (e)sRAGE and HDL<sub>ox</sub>. All assays of HDL function have limitations<sup>[26]</sup> and cholesterol efflux assays were not performed. Other limitations are known with any biomarker<sup>[27]</sup> and also include the small sample size and use of cryopreserved samples. The clinical impact of measures of glycation and HDL function is unknown.

In conclusion, our data provide important insight into the role of glycation and dysfunctional HDLs as possible drivers of increased atherosclerotic risk in this population. Because of the role of RAGE and dysfunctional HDL in atherosclerosis, diabetes, inflammation, and in view of the prevalence of CVD in chronic treated HIV infection, further investigation of the cross-talk between RAGE and dysfunctional HDL in the setting of HIV is indicated.

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## Author contributions

**Conceptualization:** Theodoros Kelesidis, Ann Danoff, Judith A Aberg, Judith Currier, Ann Marie Schmidt.

**Data curation:** Theodoros Kelesidis, Michelle A Kendall, Ann Danoff, Judith A Aberg, Judith Currier, Ann Marie Schmidt.

**Formal analysis:** Theodoros Kelesidis, Michelle A Kendall.

**Funding acquisition:** Theodoros Kelesidis, Judith A Aberg, Ann Marie Schmidt.

**Investigation:** Theodoros Kelesidis, Ann Danoff, Judith A Aberg, Judith Currier.

**Methodology:** Theodoros Kelesidis, Michelle A Kendall, Ann Danoff, Judith A Aberg, Judith Currier, Ann Marie Schmidt.

**Project administration:** Judith A Aberg.

**Resources:** Theodoros Kelesidis.

**Software:** Michelle A Kendall.

**Supervision:** Theodoros Kelesidis, Michelle A Kendall, Ann Danoff, Judith A Aberg, Judith Currier, Ann Marie Schmidt.

**Validation:** Theodoros Kelesidis, Michelle A Kendall, Ann Danoff, Judith A Aberg, Judith Currier, Ann Marie Schmidt.

**Visualization:** Theodoros Kelesidis, Michelle A Kendall, Ann Danoff, Judith A Aberg, Judith Currier, Ann Marie Schmidt.

**Writing – original draft:** Theodoros Kelesidis, Michelle A Kendall, Ann Marie Schmidt.

**Writing – review & editing:** Theodoros Kelesidis, Michelle A Kendall, Ann Danoff, Judith A Aberg, Judith Currier, Ann Marie Schmidt.

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