Changes in lipidomic profile by anti-retroviral treatment regimen
An ACTG 5257 ancillary study

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Abstract
High cardiovascular disease risk in people living with HIV is partly attributed to antiretroviral therapy (ART). Lipid response to ART has been extensively studied, yet, little is known how small molecule lipids respond to Integrase inhibitor-based (INSTI-based) compared to Protease inhibitor-based (PI-based) ART regimens.

Ancillary study to a phase 3, randomized, open-label trial [AIDS Clinical Trial Group A5257 Study] in treatment-naive HIV-infected patients randomized in a 1:1:1 ratio to receive ritonavir-boosted atazanavir (ATV/r), ritonavir-boosted darunavir (DRV/r) (both PI-based), or raltegravir with Tenofovir Disoproxil Fumarate-TDF plus emtricitabine (RAL, INSTI-based).

We examined small molecule lipid response in a subcohort of 75 participants. Lipidomic assays of plasma samples collected pre- and post-ART treatment (48 weeks) were conducted using ultra-performance liquid chromatography coupled to time-of-flight mass spectrometry. The effect of ART regimens was regressed on lipid species response adjusting for the baseline covariates (lipids, age, sex, race, CD4 level, BMI, and smoking). Results were validated in the Centers for AIDS Research Network of Integrated Clinical Systems study (N=16).

Out of 417 annotated lipids, glycerophospholipids (P= .007) and sphingolipids (P= .028) had a higher response to ATV/r and DRV/r compared to RAL. The lysophosphatidylcholine (LPCs16:1), (17:1), (20:3) and phosphatidylcholine species (PCs40:7), (38:4) had an opposite response to RAL versus ATV/r in the discovery and validation cohort. The INSTI-based regimen had an opposite response of ceramide species ((d38:1), (d42:2)), PCs(35:2), (38:4)), phosphatidylethanolamines (PEs(38:4), (38:6)), and sphingomyelin(SMd38:1) species compared with the PI-based regimens. There were no differences observed between 2 PI-based regimens.

We observed differences in response of small molecule lipid species by ART regimens in treatment-naive people living with HIV.

Abbreviations: A5257 = AIDS Clinical Trial Group A5257, ART = antiretroviral treatment, ATV/r = ritonavir-boosted atazanavir, BMI = body mass index, CNICS = Centers for AIDS Research Network of Integrated Clinical Systems, CVD = cardiovascular disease, DAD = Data Collection on Adverse events of Anti-HIV Drugs, DRV/r = ritonavir-boosted darunavir, EMR = electronic medical

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MRI and ETO conceptualized the project and provided direction to the proposal development and conduct. MRI and TK established the lipidomic methodology and acquired the lipidomic data. HENW and NSC participated in the statistical analysis and the interpretation of data. NSC and MRI jointly wrote the initial draft of the manuscript. ALW, MSS, SS, NF, and ETO participated in critical revisions for intellectual content.

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1. Introduction

Antiretroviral therapy (ART) for human immunodeficiency virus (HIV)-1 infection has been transformative in improving the length and quality of life of people living with HIV (PLWH).¹ Since the first ART agent was approved more than 30 years ago, there have been improvements in the potency, tolerability, simplicity, and availability of ART.² With the reduction in AIDS-related deaths and increases in the lifespan of PLWH, aging-related comorbidities, including cardiovascular disease (CVD), have emerged as a significant public health burden in this population.³⁻⁴ CVD is currently one of the most common causes of death (after cancer) among PLWH.⁵⁻⁶ PLWH have a significantly higher rate of hospitalization for coronary artery disease and higher risk of myocardial infarction (MI) than HIV-negative (HIV-) individuals of the same age and gender.⁷⁻⁸ Thus, the prevention and treatment of CVD and its risk factors are priorities for HIV clinical care and management.

Increased CVD risk is related, at least in part, to plasma lipid alterations linked to both HIV infection and long-term exposure to ART.⁹ Clinical trial data have demonstrated that various ART classes influence clinical lipids differently.¹⁰⁻¹¹ Changes in lipids usually occur rapidly, within 8 to 12 weeks, after beginning ART, with ongoing minor changes thereafter in the first year of ART exposure.¹² Protease inhibitor (PI-based) regimens have been associated with notable clinical lipid changes and a higher risk of CVD.¹³⁻¹⁷ Among PI-based therapies, atazanavir alone or in combination with ritonavir has a proven virological efficacy but also has been associated with dyslipidemia.¹⁸ Currently recommended first-line therapies for HIV treatment (Integrase strand transferase inhibitor (INSTI-based) regimens) have a more favorable effect on lipid profiles.¹⁹⁻²¹ For example, raltegravir is a well-tolerated INSTI-based treatment which some studies have shown may even have beneficial effects on specific lipid classes in some patient groups.¹⁰,²²,²³

Most existing knowledge concerning HIV-related lipid abnormalities has been quantified using clinical assays for total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and triglyceride (TG) concentration. Importantly, these clinically measured lipids may not as accurately predict risk for CVD in PLWH as compared with the general population.²⁴⁻²⁵ For example, data from the Data Collection on Adverse Events of Anti-HIV Drugs (DAD) Study demonstrated each 2-fold increase in TG concentration was associated with only an 11% increase in the risk of MI after control for clinical and HIV-specific covariates.²⁶ Since measures of CVD risk in the general population can underestimate risk in PLWH receiving ART, novel CVD risk markers remain needed for this group.²⁷⁻²⁸

Emerging data profiling small molecule lipids, “lipidomics,” has the potential to significantly improve risk stratification beyond conventional lipid measures used in clinics.²⁹⁻³¹ For this reason lipidomic studies of CVD endpoints are increasing and results have highlighted novel lipids (many including phosphatidylcholines (PCs), lysophosphatidylcholines (LPCs), and sphingomyelin) associated with incident CVD, CVD mortality, MI, and atherosclerosis in the general population.³² Fewer studies have been conducted in PLWH³³⁻³⁴ and we sought to investigate lipidomic changes after initiation of PI- or INSTI-based ART.

The current study was facilitated by a collaboration with AIDS Clinical Trial Group A5257 (A5257) (ClinicalTrials.gov: NCT00811954), a randomized trial that links clinical data to stored specimens from PLWH receiving treatment with PI or INSTI-based ART.³⁵ Using liquid chromatography-mass spectrometry (LC-MS)-based assays on existing specimens from ART-naive HIV-infected patients we measured response to ritonavir-boosted atazanavir (ATV/r, PI-based), ronitavir-boosted darunavir (DRV/r, PI-based), and raltegravir (RAL, INSTI-based) over 48 weeks. Changes in lipid species were evaluated to identify lipids that responded differently to the individual therapies. We replicated the findings in the Centers for AIDS Research Network of Integrated Clinical Systems (CNICS) study.

2. Methods

2.1. Study population

2.1.1. Discovery cohort. The A5257 study included PLWH receiving care in the United States and Puerto Rico with plasma HIV-1 RNA levels greater than 1000 copies/mL who had received 10 or fewer days of ART. Participants were randomly assigned in a 1:1:1 ratio to receive 1 of 3 regimens: 300 mg of atazanavir with 100 mg of ritonavir both once daily (ATV/r, PI-based); 800 mg of darunavir with 100 mg of ritonavir, both once daily (DRV/r, PI-based); or 400 mg of raltegravir twice daily, each with a fixed-dose combination of 300 mg of TDF plus 200 mg of emtricitabine (RAL, INSTI-based). The study population and randomization protocol have been fully described elsewhere.³¹,³⁵ The current research leveraged data and specimens (baseline and 48 week time points) from the A5257 dataset and repository collected on 75 total participants (25 from each arm) who were fasting at each visit and randomly selected from the original cohort.³³ Fasting was defined as nothing to eat or drink for at least 8 hours except for water or decaffeinated black coffee and required prescription medications. We excluded participants who failed to achieve viral load suppression by 24 weeks and maintain suppression through week 48; who initiated lipid-lowering therapy in the first 48 weeks; participants with the diagnosis of diabetes or coronary artery disease; and participants with samples unavailable at the 2-time points.

2.1.2. Validation cohort. PLWH participants from the CNICS study electronic medical record (EMR) linked biorepository were included for validation of findings from the A5257 study. The EMR-linked biorepository has been previously described.³⁶ In
brief, comprehensive clinical data regarding a patient’s prior ART history from all outpatient and inpatient encounters, including standardized HIV-related information, was collected at enrollment (initial clinic visit). Medication data were entered into the EMR by clinicians and/or prescription fill/refill data were uploaded directly from pharmacy systems and verified through medical record review. For the current evaluation, pre and post first ART (i.e., treatment-naive) plasma samples for 3 RAL (INSTI-based), 5 ATV/r (PI-based), and 8 DRV/r (PI-based) CNICS study participants were identified at the University of Alabama at Birmingham Study site. Stored nonfasting plasma samples both before first ART and 12 weeks to 1 year posttreatment (i.e., 2 samples per participant) were used for lipidomics assays. Participants were excluded if viral load suppression was not achieved by week 24 of ART initiation, participants have either fasting glucose >126 mg/dL or non-fasting >200 mg/dL or were taking oral antidiabetic medication, were currently taking lipid-lowering medication, or had a body mass index (BMI) >30 kg/m².

2.1.3. Study approval. The study is approved by Institutional Review Board at the University of Alabama at Birmingham.

2.2. Lipidomic assays
2.2.1. Materials and extractions. Lipidomic assays were conducted at the West Coast Metabolomics Center (WCMC). The methods of lipid extraction have been described elsewhere.[17,38] In brief, a minimum of 400 μL aliquots of plasma were sent to WCMC. Samples were thawed on ice and added to 225 μL of cold methanol, mixed, and shaken for 10 s. Subsequently, 750 μL of methyl tert-butyl ether (MTBE) was added with 10 minutes of continuous shaking. Finally, 188 μL of LC-MS grade water was added and mixed by shaking. The suspension was centrifuged for 2 minutes at 14,000 ref. Three hundred fifty μL from the supernatant was dried and suspended with 110 μL of methanol/toluene (9:1, v/v) prior to LC-MS analysis. This extraction protocol extracts all main lipid classes in plasma with high recoveries, specifically glycerolipids, glycerophospholipids, sphingolipids, sterol lipids, fatty acids, and acylcarnitines. Lipid standards were purchased from Avanti Polar Lipids (Alabaster, USA).

2.2.2. Instrumentation. Measurements were carried out in positive and negative electrospray (ESI) mode on an Agilent 1290 Infinity UHPLC/6530 QTOF MS and Agilent 1290 Infinity UHPLC/6550 QTOF MS.[18] Samples were separated on a Waters Acquity charged surface hybrid technology (CSH) C18 2.1 × 100 mm, 1.7 μm column, protected with a Waters Acquity VanGuard CSH C18 2.1 × 5 mm, 1.7 μm pre-column. For positive ionization mode the mobile phase A consisted of (60:40 ACN:water +10 mM ammonium formate +0.1% formic acid) and mobile phase B consisted of (90:10 IPA:ACN +10 mM ammonium formate +0.1% formic acid). For negative ionization mode 10 mM ammonium acetate modifier was used to increase peak responses for low abundant compounds. The instrument was tuned using the Agilent tune mix and internal reference compounds were used for quality control. For the positive ESI mode the gas temperature was 325°C, gas flow 81/min, nebulizer 35 psig, sheath gas temperature 350°C, sheath gas flow 11, capillary voltage 3500 V, nozzle voltage 1000 V, fragmentor voltage 120 V, and skimmer 65 V. In negative ionization mode the gas temperature was 200°C, gas flow 14 L/min, fragmentor voltage 175 V, while all other parameters remained identical to the positive ionization mode. Data were collected in centroided mode at 2 scans per second. The injection volume was 1.7 μL for the positive mode and 5 μL for the negative mode. The liquid chromatography gradient used a 0.6 mL/min linear velocity flow rate. The gradient started at 15% B, ramped to 30% at 2 minutes, 48% at 2.5 minutes, 82% at 11 minutes, 99% at 11.5 minutes, and kept at 99% B until 12 minutes before ramping down to 15% B at 12.1 minutes which was kept isocratic until 15 minutes to equilibrate the column. The total run time was 15 minutes. For quality control, Bioreclamation and NIST SRM 1950 pooled plasma as well as isotope labeled internal standards were used.

2.2.3. Data processing and identification. The LC-MS/MS data were analyzed by the Agilent MassHunter Qualitative Analysis (7.0) and MS-Dial data processing software to obtain accurate masses and retention times and peak heights.[39] The analytical output included the identification of 417 known lipid species used in subsequent analysis. Lipid molecules were annotated by matching MS/MS spectra and retention times against an in-house library of authentic lipid standards and the NIST14 and LipidBlast libraries. For lipid isomers with the same carbon number and degree of unsaturation which were separated by chromatography the isomers were designated as compound (A) and (B), for example, PC (38:4) A and PC (38:4) B.

2.3. Demographic, lifestyle, and Clinical Data
In the A5257 study, race, age, and gender were recorded during the initial study visit. Current smoking status (Yes/No) was self-reported. BMI was estimated as weight (kg) divided by Height (m²). Fasting plasma lipid measurements evaluated included TC, HDL-C, TG, and LDL-C (calculated as TC – [HDL-C + (TG/5)]), if TG <400 mg/dL). Per protocol, these parameters were assessed at weeks 0 and 48. Fasting lipid profile levels were performed at any Clinical Laboratory Improvement Amendments–compliant laboratory. Additional covariates collected from baseline were blood pressure measurements, estimated glomerular filtration rate, family history of diabetes, CD4 count (cells/μL), CD8 count (cells/μL), and HIV-1 RNA levels (measured as log10 copies/mL). Comparable data were collected from the UAB CNICS site including age, sex, race, BMI, CD4 count (cells/μL), and fasting lipid levels extracted from the EMR.[36]

2.4. Statistical analyses
The A5257 treatment groups were compared using Chi-square test and one-way analysis of variance (ANOVA) tests. For descriptive purposes, we grouped the individual lipid species into their major lipid class by summing their signal intensities and defined 6 lipid classes as glycerolipids, glycerophospholipids, sphingolipids, sterol lipids, fatty acids, and acylcarnitines. We compared the lipids classes across the treatment groups using one-way ANOVA. For the association analysis, we calculated the outcome as the response for each lipid species as a proportional difference ((post-treatment – pretreatment value)/pretreatment value). To achieve an approximate normal distribution of the outcome for regression analysis we added 1 to each value and took the natural log transform of that value. We then modeled the effect of the treatment group on lipid species response (outcome) adjusting for the fasting lipid measure, age, sex, race, baseline CD4 level, BMI, and smoking category. A P value <.05 for the
treatment coefficient was considered significant. We replicated the results in the CNICs study using a parallel approach. Results for lipids that were significant ($P < .05$) in A5257 and marginally significant (at least $P < .1$) with the same direction of effect in the validation cohort (CNICs) are presented.

Among the lipids that met this significance criterion, we conducted secondary analyses in the A5257 data. First, we compared the mean change in intensity (48 weeks baseline) by treatment group using one-way ANOVA tests. Second, since a low baseline CD4 count is associated with increased CVD risk, we assessed the lipid correlation with baseline CD4 level. Finally, we measured the correlation between small molecule lipid response and clinical lipid response by treatment group. All the analyses were conducted using SAS v9.4 statistical software (SAS Institute, Cary, NC). The plots were generated using the R “ggplot2” package.

### 3. Results

Descriptive characteristics of the 75 discovery participants from A5257 are presented in Table 1. The treatment groups were balanced by age (mean age 35.7 [SD = 10.0] years), race, and biological sex in the randomization. The majority of participants were male (93%), smokers (61%), and had a mean BMI of 23.7 (SD = 3.2). The median CD4 count was 346.0 (p25-p75 = 253.0–423.0). On average, blood pressure and clinical lipid levels were within the normal range. Participants (N = 16) in the validation cohort were mostly African-Americans (n = 9), males (n = 11), overweight (mean BMI 27.2 [SD = 3.6] kg/m²), with mean age of 35.6 (SD = 11.7) years and median CD4 count of 451.0 cells/µL (p25<p75 = 270.0–811.0) (Supplemental Table 1, http://links.lww.com/MD2/A276).

The annotated lipids include 5 lipid classes and 417 lipid identifiers (Supplemental Table 2, http://links.lww.com/MD2/A277). The lipid classes and the number of lipid identifiers per class are presented in Figure 1 and include glycerophospholipid class (208), sphingolipids (92), glycerolipids (82), fatty acids (19), sterols (9), and acylcarnitines (7). Figure 1 shows the mean response to the randomized treatments (ATV/r, RAL, and DRV/r) by lipid class. As a class, glycerophospholipids ($P = .007$) and sphingolipids ($P = .028$) responded differently by ART regimen where the response was higher for PI-based ART (ATV/r and DTV/r) by lipid class. A class, glycerophospholipids ($P = .007$) and sphingolipids ($P = .028$) responded differently by ART regimen where the response was higher for PI-based ART (ATV/r and DTV/r) compared to INSTI-based (RAL). The association of ART regimen with individual lipid species response in the discovery set and the validation set is presented in Table 2 (for lipids with $P < .05$ in discovery and $P < .1$ in validation for at least 1 treatment comparison). Results for all 417 lipids are presented in Supplemental Table 2, http://links.lww.com/MD2/A277. The mean change in intensity (48 week-baseline) by treatment for each lipid species from Table 2 is presented in Supplemental Table 2, http://links.lww.com/MD2/A277. In total, differences in response for 2 ceramides, 3 LPCs, 3 PCs, 2 phosphatidylethanolamines (PEs), 1 SM, and 5 TG species were observed as shown in Table 2. LPC (16:1), LPC (17:1), LPC (20:3), PC (40:7), and PC (38:4) were each associated with an opposite response to RAL (negative) versus ATV/r (positive) in each population (Table 2).

### Table 1

Descriptive characteristics of the AS257 discovery sample.

| Demographics | All (n = 75) | ATV/r (n = 25) | RAL (n = 25) | DRV/r (n = 25) |
|--------------|-------------|---------------|-------------|---------------|
| Age          | 35.7 (9.9)  | 35.1 (10.0)   | 35.8 (9.1)  | 36.0 (10.0)   |
| Sex          |             |               |             |               |
| Males        | 70 (93.3)   | 25 (100.0)    | 24 (96.0)   | 21 (84.0)     |
| Females      | 5 (6.7)     | 0 (0.0)       | 1 (4.0)     | 4 (16.0)      |
| Ethnicity    |             |               |             |               |
| NH White     | 38 (50.7)   | 15 (60.0)     | 10 (40.0)   | 13 (52.0)     |
| NH Black     | 20 (26.7)   | 5 (20.0)      | 6 (24.0)    | 9 (36.0)      |
| Hispanic     | 15 (20.0)   | 5 (20.0)      | 7 (28.0)    | 3 (12.0)      |
| Asian        | 2 (2.6)     | 0 (0.0)       | 2 (8.0)     | 0 (0.0)       |
| BMI          | 23.7 (3.2)  | 24.4 (2.9)    | 23.4 (3.2)  | 23.3 (3.4)    |
| Smoking status | 46 (61.3) | 18 (72.0)     | 16 (64.0)   | 12 (48.0)     |
| Baseline comorbidities | |               |             |               |
| Systolic blood pressure | 115.6 (13.1) | 114.3 (13.9) | 117.5 (40.1) | 114.8 (12.4) |
| Diastolic blood pressure | 73.3 (9.5) | 72.6 (8.3) | 73.2 (10.6) | 74.3 (8.6) |
| Total cholesterol | 155.4 (32.7) | 148.2 (30.2) | 163.4 (40.1) | 154.6 (25.7) |
| HDL cholesterol | 39.4 (11.2) | 38.8 (13.0) | 38.2 (8.4) | 41.3 (11.1) |
| Triglycerides | 117.2 (63.4) | 124.7 (70.9) | 119.1 (71.8) | 107.7 (45.2) |
| LDL cholesterol | 92.6 (26.7) | 84.5 (23.3) | 101.4 (32.1) | 91.8 (21.9) |
| Metabolic syndrome | 9 (12.0) | 4 (16.0) | 3 (12.0) | 2 (8.0) |
| Family history of diabetes | 9 (12.0) | 4 (16.0) | 4 (16.0) | 1 (4.0) |
| Baseline measures | |               |             |               |
| eGFR (mL/min/1.73m²) | 108.7 (22.4) | 109.7 (23.7) | 107.1 (23.7) | 109.3 (20.5) |
| CD4 count (cells/µL) | 346.0 (199.0–423.0) | 292.0 (211.0–495.0) | 354.0 (241.0–416.0) | 338.0 (182.0–384.0) |
| CD8 count (cells/µL) | 739.0 (572.0–994.0) | 791.0 (578.0–994.0) | 739.0 (607.0–1101.5) | 689.0 (557.0–819.0) |
| hiv-1 RNA (log10 copies /mL) | 4.6 (0.7) | 4.6 (0.7) | 4.6 (0.7) | 4.5 (0.8) |

$P$ value

$\text{ATV/r} = \text{ritonavir-boosted atazanavir, BMI} = \text{body mass index, DRV/r} = \text{ritonavir-boosted darunavir, eGFR} = \text{estimated glomerular filtration rate, HDL} = \text{high-density lipoproteins, LDL} = \text{low-density lipoproteins, NH} = \text{non-Hispanic, RAL} = \text{ribonucleic acid.}$

$^a$Data is presented as mean (standard deviation) or median (p25-p75) for continuous variables as appropriate, and N (percent) for categorical variables.
Two TGs (50:5, 56:8) had a negative response to RAL versus a positive response to ATV/r, while TG (52:6) had a larger decrease for RAL versus ATV/r (Table 2, Supplemental Table 3, http://links.lww.com/MD2/A278). Similarly, we detected a decrease versus increase for 2 ceramides (d38:1, d42:2), PC (38:4), 2 PEs (38:4, 38:6), SM (d38:1), and 5 TG species (50:5, 52:6, 54:6, 56:4 and 56:8) when comparing RAL to DRV/r (Supplemental Table 3, http://links.lww.com/MD2/A278). When comparing PIs (ATV/r to DRV/r), no significant differences were observed.

We further determined whether the baseline CD4 level was associated with the baseline level of lipid species presented in Table 2. We identified 5 lipid species [LPC (17:1), LPC (20:3), PE (38:6), SM (d38:1), and TG (52:6)] that were significantly correlated with baseline CD4 level (Fig. 2). LPC (17:1), LPC (20:3), and SM (d38:1) were positively correlated while TG (52:6) and PE (38:6) were negatively correlated with baseline CD4 count (Supplemental Table 4, http://links.lww.com/MD2/A279). When we compared baseline CD4 count with change in lipid species (for lipids in Table 2), only 1 lipid species SM (d38:1) was significantly correlated (Supplemental Table 4, http://links.lww.com/MD2/A279). Supplemental Figure 1, http://links.lww.com/MD2/A280 displays the correlations between clinical lipid (HDL-C, LDL-C, TG, TC) and lipid species response to treatment (again among those lipids in Table 2). For some species, the response was consistent with the clinical lipid response across treatment regimens (e.g., 5 TG species were positively correlated with clinical TG and negatively correlated with HDL). There were also differences between the lipid species response and clinical lipid response across treatment regimens (Supplemental Figure 1, http://links.lww.com/MD2/A280). For example, the LPC (16:1), and LPC (17:1) responses were positively correlated with clinical TG response for ATV/r but negatively correlated to clinical TG response to RAL and DRV/r.

4. Discussion

HIV and its treatment are associated with metabolic changes that increase cardiovascular risk. Clinical lipid response to ART has been extensively studied but few studies have examined the molecular lipids that compose lipoproteins (lipidomic species) in PLWH or their response to ART. In the current study we examined 417 annotated small molecule lipids and their response to 3 ART regimens (ATV/r, DRV/r, and RAL) in a subset of 75 participants (25 in each treatment group) from the A5257 trial. In total, 16 lipids responded differently for at least 1 treatment comparison (8 for RAL vs ATV/r and 12 for RAL vs DRV/r, in common) in the discovery and validation population. Of those 16 lipids, 5 lipid species [LPC (17:1), LPC (20:3), PE (38:6), SM (d38:1), and TG (52:6)] were correlated with pretreatment CD4 count. If validated in additional studies these treatment responsive lipids should be further examined for association with CVD risk as a potential modifiable risk factor.

Our findings corroborate the evidence that plasma lipidomic profile changes differentially in response to specific ART agents.
We observed no statistically significant differences in lipidomic response when comparing the 2 PI-based regimens. We did, however, observe several lipids with a differing response when comparing PI-based to INSTI-based treatment (RAL) especially for RAL versus DRV/r. In the literature PI-based ART has been consistently linked to a less favorable lipid profile which may be due to decreased lipoprotein lipase (LPL)-mediated hydrolysis of VLDL bound triglycerides\textsuperscript{13} impaired free fatty acid uptake into adipose and skeletal muscle tissue\textsuperscript{13,14} and reduced fatty acid oxidation in the mitochondria\textsuperscript{15}. In our study, overall, RAL had a lower lipid species response compared with PI-based ART for the lipids highlighted in Table 2. This observation is consistent with the trends for clinical lipids (e.g., LDL, TG) by treatment class in the parent trial.\textsuperscript{11} However, when we examined the

| Lipids          | ATV/r v DRV/r (ref) |           | RAL v DRV/r (ref) |           | RAL v ATV/r (ref) |           |
|-----------------|---------------------|-----------|-------------------|-----------|-------------------|-----------|
|                 | \( \beta \)         | \( P \)   | \( \beta \)       | \( P \)   | \( \beta \)       | \( P \)   |
| Ceramide (d38:1)|                     |           |                   |           |                   |           |
| D               | \(-0.08\)           | .42       | \(-0.21\)         | .03       | \(-0.13\)         | .16       |
| V               | \(-0.38\)           | .17       | \(-0.67\)         | .047      | \(-0.29\)         | .27       |
| Ceramide (d42:2)|                     |           |                   |           |                   |           |
| D               | \(-0.06\)           | .49       | \(-0.22\)         | .01       | \(-0.16\)         | .06       |
| V               | \(-0.41\)           | .53       | \(-1.23\)         | .08       | \(-0.82\)         | .26       |
| LPC(16:1)       |                     |           |                   |           |                   |           |
| D               | 0.19                | .12       | \(-0.05\)         | .69       | \(-0.24\)         | .047      |
| V               | \(-0.06\)           | .80       | \(-0.87\)         | .01       | \(-0.81\)         | .01       |
| LPC(17:1)       |                     |           |                   |           |                   |           |
| D               | 0.28                | .27       | \(-0.49\)         | .06       | \(-0.77\)         | .002      |
| V               | 0.25                | .29       | \(-0.42\)         | .14       | \(-0.67\)         | .02       |
| LPC(20:3)       |                     |           |                   |           |                   |           |
| D               | 0.12                | .36       | \(-0.14\)         | .27       | \(-0.26\)         | .04       |
| V               | 0.12                | .76       | \(-1.01\)         | .06       | \(-1.13\)         | .04       |
| PC(35:2)        |                     |           |                   |           |                   |           |
| D               | 0.11                | .43       | \(-0.38\)         | .008      | \(-0.49\)         | <.001     |
| V               | \(-0.81\)           | .14       | \(-1.01\)         | .05       | \(-0.20\)         | .67       |
| PC(38:4)        |                     |           |                   |           |                   |           |
| D               | \(-0.04\)           | .53       | \(-0.18\)         | .005      | \(-0.14\)         | .02       |
| V               | \(-0.07\)           | .84       | \(-0.79\)         | .07       | \(-0.72\)         | .08       |
| PC(40:7)        |                     |           |                   |           |                   |           |
| D               | 0.14                | .23       | \(-0.28\)         | 0.02      | \(-0.42\)         | .001      |
| V               | 0.32                | .19       | \(-0.27\)         | 0.28      | \(-0.59\)         | .03       |
| PE (38:4)       |                     |           |                   |           |                   |           |
| D               | 0.02                | .90       | \(-0.39\)         | 0.02      | \(-0.41\)         | .01       |
| V               | \(-0.22\)           | .38       | \(-0.73\)         | 0.02      | \(-0.51\)         | .14       |
| PE(38:6)        |                     |           |                   |           |                   |           |
| D               | \(-0.05\)           | .71       | \(-0.48\)         | 0.001     | \(-0.43\)         | 0.002     |
| V               | \(-0.64\)           | .07       | \(-0.59\)         | 0.015     | 0.05              | .84       |
| SM(d38:1)       |                     |           |                   |           |                   |           |
| D               | \(-0.13\)           | .22       | \(-0.23\)         | 0.020     | \(-0.11\)         | .29       |
| V               | \(-0.20\)           | .25       | \(-0.62\)         | 0.009     | \(-0.42\)         | .04       |
| TG(50:5)        |                     |           |                   |           |                   |           |
| D               | 0.09                | .72       | \(-0.61\)         | 0.020     | \(-0.70\)         | .007      |
| V               | \(-0.58\)           | .11       | \(-1.23\)         | 0.010     | \(-0.65\)         | .09       |
| TG(52:6)        |                     |           |                   |           |                   |           |
| D               | 0.13                | .55       | \(-0.63\)         | 0.005     | \(-0.76\)         | <.001     |
| V               | \(-1.62\)           | .14       | \(-2.02\)         | 0.068     | \(-0.40\)         | .07       |
| TG(54:6)        |                     |           |                   |           |                   |           |
| D               | 0.13                | .65       | \(-0.64\)         | 0.028     | \(-0.77\)         | .007      |
| V               | \(-1.26\)           | .16       | \(-1.85\)         | 0.070     | \(-0.59\)         | .47       |
| TG(56:4)        |                     |           |                   |           |                   |           |
| D               | 0.22                | .24       | \(-0.40\)         | 0.044     | \(-0.62\)         | .001      |
| V               | \(-0.37\)           | .04       | \(-0.38\)         | 0.04      | \(-0.01\)         | 0.96      |
| TG (56:8)       |                     |           |                   |           |                   |           |
| D               | \(-0.09\)           | .59       | \(-0.40\)         | 0.015     | \(-0.31\)         | .05       |
| V               | 0.03                | .85       | \(-0.31\)         | 0.09      | \(-0.34\)         | .06       |

\( \beta \) = \text{beta coefficient}, ATV/r = ritonavir-boosted atazanavir, D = discovery cohort, DRV/r = ritonavir-boosted darunavir, RAL = raltegravir, V = validation cohort; positive \( \beta \) coefficient indicate larger increase or smaller decrease in the exposure treatment group compared to the reference treatment group; negative coefficient indicates smaller increase or a larger decrease; please see Supplemental Table 3, http://links.lww.com/MD2/A278 for absolute intensity values corresponding to parameter estimates; \( P \) values from linear regression model adjusted for the fasting lipid measure, age, sex, race, baseline CD4 level, BMI, and smoking. Bold italics values met statistical significant threshold of \( P < 0.05 \).
correlation between lipidomic species response and clinical lipid response (HDL, LDL, TG, TC) we observed several differences by treatment group (Supplemental Figure 1, http://links.lww.com/MD2/A280). These findings provide evidence that the change in the lipidomic profile upon ART initiation varies by treatment regimen and could provide useful information beyond clinical lipids about CVD risk.

Other studies have investigated lipidomic (and/or metabolomic) response to ART. In a secondary analysis of 35 treatment naive PLWH participants and 13 participants without HIV matched by age and sex, the saturated LPC species, particularly LPC (17:0), LPC (18:0), LPC (20:0), were higher in PLWH than individuals without HIV at baseline and after 48 weeks of treatment with raltegravir. There were fewer differences with regard to LPCs with monounsaturated and polyunsaturated fatty acids, but the study did report higher LPC (20:1) at baseline in PLWH compared with participants without HIV. In comparison, we observed a significantly lower response to raltegravir for LPC (16:1), LPC (17:1), and LPC (20:3), compared with atazanavir. Among these findings, LPC (17:1) and LPC (20:3) are of particular importance due to their previous association with CVD in a case-control study set among PLWH (odds ratio (OR) = 2.96, 95% CI (1.27–6.88) for LPC (20:3) and OR = 3.52, 95% CI (1.4–8.8) for LPC (17:1)). These 2 lipids were also associated with baseline CD4 count in our study. In another study, plasma lipid profiling was performed on 115 ART-naive PLWH participants randomized to tenofovir/emtricitabine with efavirenz, ATV/r, or zidovudine/abacavir. After 48 weeks 72 lipid species and 7 classes were observed to be different by ART group. Findings that overlapped with the current study included differences in response to treatment for the PC class overall [overlapping with glycerophospholipids from our study and individual species PC (38:4) (efavirenz vs ATV/r and efavirenz vs zidovudine/abacavir), PC (40:7) (efavirenz vs zidovudine/abacavir) and LPC (20:3) (efavirenz vs ATV/r, ATV/r vs zidovudine/abacavir, and efavirenz vs zidovudine/abacavir)]. Other lipids highlighted in Table 2 have been previously associated with lower odds for HIV infection (SM (d38:1)) in 1 study and baseline IL6 levels (LPC (16:1)) in another study. These lipid findings require further validation for their role in treatment response, HIV infection, and CVD risk in the setting of HIV.

Numerous studies indicate lipidomic profiling can improve upon conventional lipids as a biomarker of future CVD in general population-based studies and studies of populations with other comorbidities such as T2D or HIV. The large prospective population-based Bruneck study demonstrated that TGs and CEs with a low carbon number and double-bond content (including TG (54:2) and CE (16:1)) are strongly associated with future CVD events. The study demonstrated that the addition of these significant lipid species to a prediction model of traditional risk factors resulted in improved risk discrimination compared with traditional risk factors alone. Also in a subset (N = 3779) of the Action in Diabetes and Vascular Disease: Preterax and Diamicron-MR Controlled Evaluation (ADVANCE) study the addition of 7 lipid species [PC (O-36:1), CE (18:0), PE (O-36:4), PC (28:0), PC (35:4), LPC (20:0), LPC (18:2)] to a base model (including 14 traditional risk factors and medications) to predict CVD events improved the model with the C statistic increasing from 0.680 (95% confidence interval [CI], 0.678–0.682) to 0.700 (95% CI, 0.698–0.702; P < .0001). In another case-control study in the Malmo Diet and Cancer study (N = 427) LPC (16:0) and LPC (20:4) were protective while SM (38:2) increased risk for CVD. Finally, a prospective study of 2 multicenter HIV
Additional populations treated for HIV over longer periods with INSTI classes. Future analysis should validate these lipids in differences in response when comparing the 2 classes of PIs. The comparing the INSTI-based regimen to the PI-based, the most with the 2 PI-based regimens (ATV/r and DRV/r). When more in-depth research to understand the stability of these lipidomic species may be less stable over time than clinical lipids, restrictions of sample size, we did not adjust for multiple testing extremes of weight. Limitations included the small sample size helped us identify lipidomic markers not in

Results for lipids validated using data and samples from an EMR-agnostic discovery of small molecule lipids. We only present Lipids were measured using an untargeted approach providing an response in well-characterized ART-naive PLWH randomized to of future CVD risk including among PLWH.

Our study has several strengths. We examined treatment response in well-characterized ART-naive PLWH randomized to 3 individual treatments that were carefully followed for 48 weeks. Lipids were measured using an untargeted approach providing an agnostic discovery of small molecule lipids. We only present results for lipids validated using data and samples from an EMR-based population of comparable study participants. Our study population had normal BMI on average which should have helped us identify lipidomic markers not influenced by the extremes of weight. Limitations included the small sample size available for validation and inability to identify fasting specimens from treatment naive participants in CNICS. Also, due to the restrictions of sample size, we did not adjust for multiple testing lending our results to future validation efforts. Finally, since lipidomic species may be less stable over time than clinical lipids, more in-depth research to understand the stability of these metabolites is warranted.

In conclusion, this study identified 16 lipids that responded differently to ATV/r, RAL, and/or DRV/r. In particular, the lipids changed less on the INSTI-based regimen (RAL) in comparison with the 2 PI-based regimens (ATV/r and DRV/r). When comparing the INSTI-based regimen to the PI-based, the most notable differences were observed for DRV/r. There were no differences in response when comparing the 2 classes of PIs. The responsive lipids belonged to the ceramide, LPC, PC, PE, SM, and TG classes. Future analysis should validate these lipids in additional populations treated for HIV over longer periods with data on CVD events.

Author contributions

Conceptualization: Edgar T Overton, Marguerite R. Irvin. Data curation: Howard W. Wiener. Formal analysis: Ninad S Chaudhary, Howard W. Wiener. Funding acquisition: Edgar T Overton, Marguerite R. Irvin. Methodology: Tobias Kind, Nicholas Funderburg, Edgar T Overton, Marguerite R. Irvin. Resources: Amanda L. Willig, Michael S. Saag, Sadeep Shrestha, Edgar T Overton. Supervision: Tobias Kind, Edgar T Overton, Marguerite R. Irvin. Visualization: Ninad S Chaudhary, Marguerite R. Irvin. Writing – original draft: Ninad S Chaudhary, Marguerite R. Irvin. Writing – review & editing: Ninad S Chaudhary, Tobias Kind, Amanda L. Willig, Michael S. Saag, Sadeep Shrestha, Nicholas Funderburg, Edgar T Overton, Marguerite R. Irvin.

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