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Association of Plasma Eicosanoid Levels With Immune, Viral, and Cognitive Outcomes in People With HIV

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Abstract

Background and Objectives

To determine whether plasma eicosanoid levels are associated with immune, viral, and cognitive outcomes in people with HIV (PWH).

Methods

We measured 42 eicosanoids in a longitudinal study of 95 PWH and 25 demographically comparable uninfected participants. Routine clinical chemistry, virologic, immune markers, and a neuropsychological test battery assessing 7 cognitive domains were administered to all participants at 2 study visits over an average of 6.5 months.

Results

Plasma eicosanoid concentrations were elevated in PWH (n = 95) compared with seronegative controls (n = 25) (100% prediction power at 5% false discovery rate [FDR], $\alpha = 0.0531$) and were negatively associated with lower current and nadir CD4 lymphocyte counts. Higher levels of eicosanoids were associated with impairments in working memory, verbal fluency, and executive function. Higher plasma viral load was associated with elevated proinflammatory eicosanoids (24% prediction power at 5% FDR and 42.4% prediction power at 10% FDR, $\alpha = 0.10$). Longitudinal analyses showed that eicosanoid levels were correlated with viral load and with plasma creatinine. Despite associations of eicosanoids with viral loads, elevated plasma eicosanoids were similar in virally suppressed and not fully suppressed PWH.

Discussion

These data show that HIV infection is associated with a robust production of eicosanoids that are not substantially reduced by antiretroviral therapy (ART). The sustained elevation of these oxylipins in PWH despite ART may contribute to an accelerated aging phenotype that includes earlier than expected brain and peripheral organ damage.

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Glossary

AA = arachidonic acid; **ART** = antiretroviral therapy; **CI** = cognitive impairment; **DHA** = docosahexaenoic acid; **EPA** = eicosapentaenoic acid; **GDS** = Global Deficit Score; **HIV_{CI}** = HIV cognitively impaired; **HIV_{CN}** = HIV cognitively normal; **HIV_{SN}** = HIV seronegative; **iNOS** = inducible nitric oxide synthase; **LA** = linoleic acid; **NO** = nitric oxide; **PWH** = people with HIV; **SIP** = speed of information processing; **SPE** = solid-phase extraction; **TLR** = toll-like receptor.

Antiretroviral therapy (ART) has proven to be effective in controlling replication of the human immunodeficiency virus (HIV).[1] Despite the efficacy of ART to suppress viral replication, immune dysfunction, chronic inflammation, organ damage, and cognitive impairment (CI) are still apparent in many people with HIV (PWH).[2] Although the precise mechanisms for these residual pathologies have been elusive, considerable clinical and experimental evidence has associated chronic inflammation with immune dysfunction and damage to multiple organ systems including brain.[3]

It is well known that the inflammatory cytokines elevated in PWH are largely, but not completely, reduced with suppressive ART.[4] However, very little is known about the impact of ART on eicosanoid metabolism. Eicosanoids are bioactive lipids derived from the metabolism of polyunsaturated fatty acids (PUFAs) that are processed in 1 of 3 major pathways: cyclooxygenase, lipoxygenase, or cytochrome p450 epoxide pathways that each produce multiple biologically active metabolites. Eicosanoids play important roles in the regulation of numerous physiologic responses including those associated with vascular function, inflammation, and immune modulation[5] (among others). Two papers in the early 1990s reported elevated CSF levels of the eicosanoids PGE2, PGF2α, PGD2, LTB4, and TXB2 in ART-naive PWH compared with HIV-negative control participants.[6,7] Since that time, a number of studies have identified elevated levels of COX-1 and COX-2 metabolic products including prostaglandins, prostacyclins, and thromboxanes in HIV model systems and in human samples from PWH,[8,9] but no studies to date have addressed the potential dysregulation of lipoxygenase and cytochrome p50 epoxide metabolic pathways in HIV. Similarly, the effects of ART on eicosanoid metabolism in PWH are unknown, as are the associations of eicosanoids with cognition in PWH. Here, we performed an extensive analysis of plasma eicosanoids with a focus on lipoxins and epoxides in ART-naive PWH, ART suppressed PWH, and uninfected healthy controls and report associations with clinical chemistry and cognitive functions.

Methods

Human Plasma Sample Collection

Plasma samples from PWH were obtained from the CNS HIV Antiretroviral Therapy Effects Research (CHARTER) study. Participant selection was based on a case review of ~3,500 clinical visits from 430 study participants as previously described.[10,11] In brief, neurocognitive change was defined using a multivariable change score approach. A Z-score was generated for each of 15 neuropsychological variables that reflect how well or poorly the participant performed at follow-up, relative to the expectation for someone with the same baseline neuropsychological and other relevant characteristics. Z-scores were summed to provide a summary regression change score. The central 80% of the summary regression change score distribution was defined as cognitively "stable." The top 10% were defined as cognitively "improved," and the bottom 10% defined as cognitively "declined."[10] All participants were carefully assessed for neuromedical and neuropsychiatric comorbidities and excluded if confounds not related to HIV were likely to be primary contributors to cognitive impairment as previously described.[12]

Standard Protocol Approvals, Registrations, and Patient Consents

The collection of human plasma samples was approved by the Institutional Review Board at each performance site. All participants provided informed consent for all study procedures, including future use of their stored specimens and data for research.

Neuropsychological Testing

Neuropsychological testing was conducted by trained neuropsychometricians and consisted of tests covering 7 cognitive domains: executive function, learning and delayed recall, working memory, verbal fluency, speed of information processing, and motor skills. The best available normative standards were used to convert the scores to demographically corrected standard scores (T-scores), which correct for effects of age, education, sex, and ethnicity.[13] The presence and severity of CI were determined using the Global Deficit Score (GDS) approach, where a GDS ≥ 0.5 was impaired.[14] All follow-up visits were corrected for practice effects.[15]

Sample Preparation

Each plasma sample (200 μL) was spiked with deuterium labeled internal standards representing several major classes of eicosanoids: AA-d8, 13-HODE-d4, 15(S) HETE-d8, LXA4-d5, PGB2-d4, and LTC4-d5 (1 μg/mL each). Solid-phase extraction (SPE) of eicosanoids was performed using Trace N, 15 mg, 10 μm columns (SPEware Corporation) connected to a positive pressure SPE CEREX SYSTEM 48 processor (SPEware Corporation). Columns were preconditioned using 2 mL of methanol followed by 2 mL of water. Samples were loaded onto columns, washed with 2 mL of water and methanol mixture (95:5, vol/vol), and then eluted with 1 mL of pure methanol. Methanol eluent was dried under a stream of nitrogen using a

Multivap nitrogen evaporator (Model 118, Organomation Associates Inc) and stored at −80°C. Dried extracts were resuspended in pure methanol just before analysis.

Eicosanoids Analysis by LC-MS/MS

Plasma eicosanoids levels were measured by using LC-ESI-MS/MS. Chromatographic separation was performed by reverse-phase liquid-chromatography using a Luna 3 μm C18 250 × 2-mm column (Phenomenex), coupled to a Shimadzu liquid chromatography (LC) (Shimadzu Scientific Instruments, Inc). Eluted samples were introduced into a triple stage quadrupole linear ion trap (4000QTRAP) mass spectrometer (Applied Biosystems) using multiple reaction monitoring in negative electrospray ionization mode (ESI). Instrument control and data acquisition were performed using Analyst 1.5.1 software. Data processing was performed using Multiquant 1.2 software (Applied Biosystems).

Statistical Analyses

Because imputation of values below the limit of detection with a constant value can adversely affect statistical analyses by increasing the likelihood of biased parameter estimations that distort sample distribution and impair statistical power, in this study, undetectable values for each eicosanoid were replaced with a left-censored stochastic minimal value approach which imputes data by random draws from a Gaussian distribution centered in the minimal value which was estimated as being the qth quantile of the observed values (imputeLCMD package in R). Two-group comparisons were performed using the Wilcoxon test, and multiple group comparisons were accomplished using the Kruskal-Wallis test with the Dunn test for post hoc comparisons. Correlation analyses were performed using the Spearman rank correlation method, and the p values were corrected using the Benjamin-Hochberg (B-H) method for multiple comparison tests. The value of the effect size is considered low if the value of Spearman r correlation value was around 0.1, medium if around 0.3, and large if it is greater than 0.5. p values less than 0.05 were considered statistically significant. The power analysis was performed using MetaboAnalyst 5.0 to estimate sample size and the effect size of this study data to achieve prediction power greater than 80% at 5% false discovery rate false discovery rate [FDR].

Results

Participant Characteristics

This study included 120 participants grouped from baseline cognitive function as (i) HIV seronegative (n = 25), (ii) HIV cognitively normal (n = 41), and (iii) HIV cognitively impaired (n = 54). Age-matched and sex-matched plasma from healthy control participants (HIV seronegative, n = 25) were obtained from the Johns Hopkins University performance site. Demographic and clinical characteristics of the participants for cross-sectional analyses are summarized in Table 1, ART naive vs ART stable in eTable 1 (links.lww.com/WNL/C210), and longitudinal analyses in eTable 2. In cross sectional analyses, nadir CD4 was lower in HIV cognitively impaired compared with HIV cognitively normal ($p < 0.024$). Average age, current CD4, plasma viral load, and AIDS status were not statistically different between the groups (Table 1). There were overall group differences in current CD4 levels ($p < 0.028$), with HIV stably impaired cognition > HIV stably normal cognition > HIV worsening cognition > HIV impaired cognition and plasma viral load ($p < 0.032$) with HIV impaired cognition > HIV worsening cognition > HIV stably impaired cognition > HIV stably normal cognition, when participants were arranged according to longitudinal change in cognition (eTable 2).

Table 1 Baseline Clinical and Demographic Characteristics

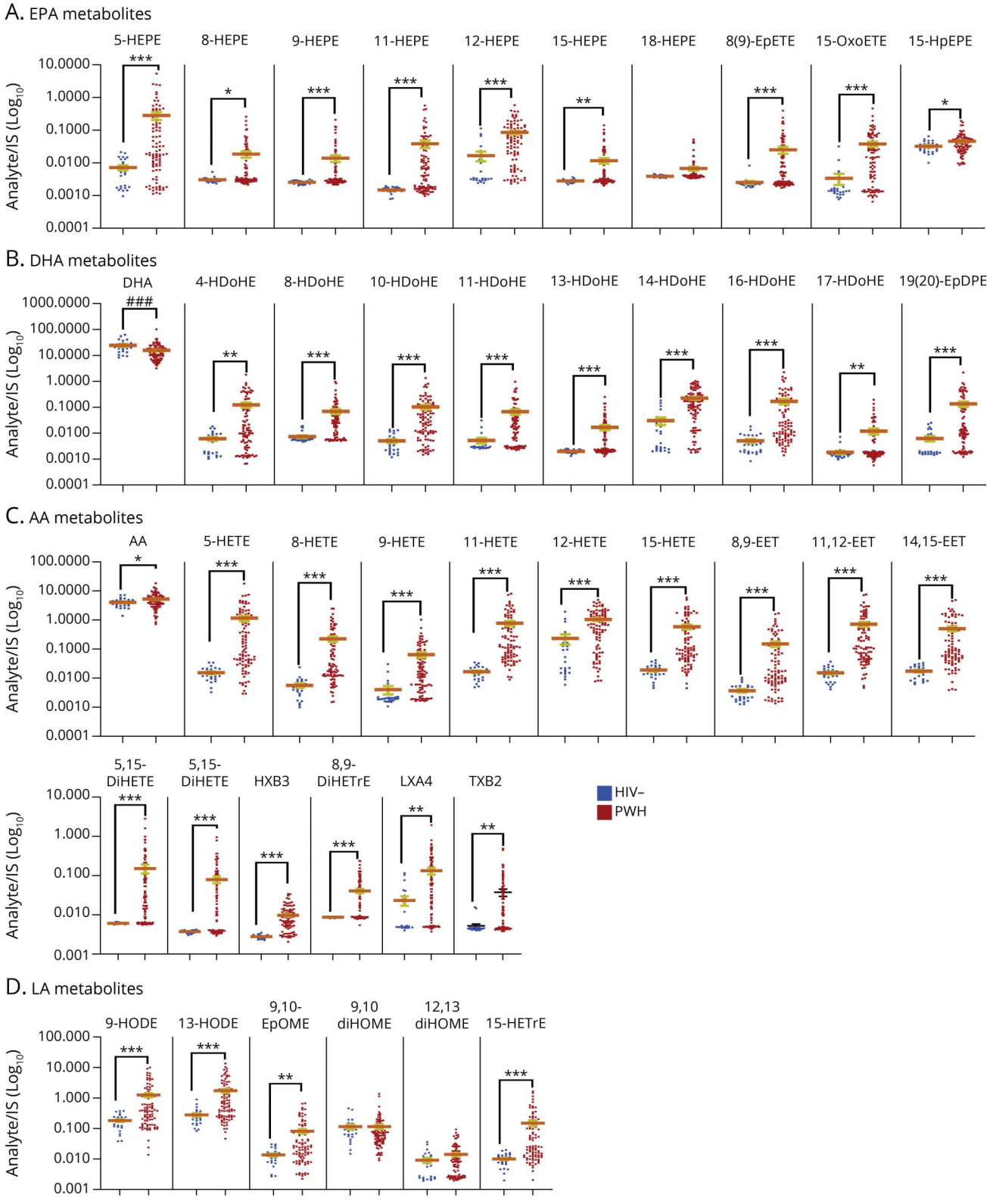
Baseline characteristics	HIV_{SN} (n = 25)	HIV_{CN} (n = 41)	HIV_{CI} (n = 54)
Age (y)	42.26 (12.55)	44.12 (9.54)	45.17 (7.78)
Education (y)	nd	13.05 (1.96)	13.11 (2.73)
Sex (male)	56	92.7	77.8
Ethnicity (Caucasian)	60	78	79.6
AIDS	nd	63.4	74.1
Current CD4 (cells/mm^3)	nd	389.73 (226.38)	450.09 (280.67)
Nadir CD4 (cells/mm^3)	nd	192.85 (194.08)	162.24 (160.85)
Current ART	nd	68.3	74.07
Plasma viral load (≤50 copies/mL)	nd	69.2	72.2
CSF viral load (≤50 copies/mL)	nd	40.5	40.7

Abbreviations: ART = antiretroviral therapy; HIV_{CI} = HIV cognitively impaired; HIV_{CN} = HIV cognitively normal; HIV_{SN} = HIV seronegative; nd = no data. Data are expressed as % mean (±SD) as appropriate.

Baseline Associations Between Plasma Eicosanoids, HIV Status, and ART

We detected and quantified 42 individual eicosanoids in plasma that were categorized based on the precursor molecule from which they were derived. These included metabolites of (i) eicosapentaenoic acid (EPA), (ii) docosahexaenoic acid (DHA), (ii) arachidonic acid (AA), and (iv) linoleic acid (LA). Most eicosanoids detected (38 of 42) were elevated in PWH compared with HIV seronegatives (Figure 1A). These included the EPA metabolites 5-HEPE, 8-HEPE, 9-HEPE, 11-HEPE, 12-HEPE, 15-HEPE, 18-HEPE, and 8, (9)-EpETE (Figure 1B); DHA metabolites 4-HDoHE, 8-HDoHE, 10-HDoHE, 11-HDoHE, 13-HDoHE, 14-HDoHE, 16-HDoHE, 17-HDoHE, and 19(20)-EpDPF (Figure 1B); AA metabolites 5-HETE, 8-HETE, 9-HETE, 11-HETE, 12-HETE, 15-HETE, 15-OxoETE, 8,9-DilHETrE, 5,12-DilHETE, 5,15-DilHETE, HXB3, LXA4, 8, (9)-EET, 11, (12)-EET, 14, (15)-EET, TXB2 (Figure 1C); and the LA metabolites 9-HODE, 13-HODE, 9,10-EpOME, and 15-HETrE (Figure 1D). The data shown prediction power of 100% at 5% FDR ($\alpha = 0.0531$). Several of these EPA metabolites including 8-HEPE, 9-HEPE, and 11-HEPE were below detectable limits in most HIV

Figure 1 Baseline Analysis of Plasma Eicosanoids in PWH and Healthy Seronegative Control Participants



Box plots show (A) EPA metabolites, (B) DHA metabolites, (C) AA metabolites, and (D) LA metabolites. Data show median and range with individual data points. HIV seronegative (n = 25) and PWH (n = 95). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. The nonparametric Wilcoxon signed-rank test with Benjamini-Hochberg FDR correction performed for 2-group comparisons. AA = arachidonic acid; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; LA = linoleic acid; PWH = people with HIV.

seronegatives and were well above detectable limits in plasma from PWH. These data demonstrate that HIV infection is associated with a robust increase in plasma eicosanoids. When we separated groups based on ART use, there were no group differences in plasma eicosanoids between ART naive and PWH stably treated with ART (Figure 2, A–D). These data suggest that HIV infection is associated with a robust increase in plasma eicosanoids that is not fully resolved by ART.

Baseline Associations Between Plasma Eicosanoids and Cognition

We next compared the baseline plasma levels of eicosanoids with cognitive impairment status in PWH. We found that plasma levels of most eicosanoids were elevated in both HIV cognitively normal and HIV cognitively impaired compared with HIV seronegative (Figure 3, A–D). Eight EPA metabolites 5-HEPE, 8-HEPE, 9-HEPE, 11-HEPE, 12-HEPE, 15-HEPE, 18-HEPE, and 8, (9)-EpETE were elevated in HIV cognitively normal and HIV cognitively impaired compared with HIV seronegative (Figure 3A). DHA was decreased, and 10 DHA metabolites including 4-HDoHE, 8-HDoHE, 10-HDoHE, 11-HDoHE, 13-HDoHE, 14-HDoHE, 16-HDoHE, 17-HDoHE, and 19, (20)-EpDPF were elevated in HIV cognitively normal and HIV cognitively impaired compared with HIV seronegative (Figure 3B). We did not observe group differences in AA, but all 16 AA metabolites 5-HETE, 8-HETE, 9-HETE, 11-HETE, 12-HETE, 15-HETE, 15-OxoETE, 8, 9-DilHETE, 5, 12-DilHETE, 5, 15-DilHETE, HXB3, LXA4, 8, (9)-EET, 11, (12)-EET, 14, (15)-EET, and TXB2 were elevated in HIV cognitively normal and HIV cognitively impaired compared with HIV seronegative (Figure 3C). Four of 6 LA metabolites including 9-HODE, 13-HODE, 9, 10-EpOME, and 15-HETrE were also elevated in HIV cognitively normal and HIV cognitively impaired compared with HIV seronegative (Figure 3D). The data showed prediction power of 35% at 5% FDR ($\alpha = 0.05$) and 100% prediction power at 10% FDR ($\alpha = 0.10$). These data demonstrate that most plasma eicosanoids are elevated in PWH regardless of cognitive status.

Baseline Associations of Plasma Eicosanoids With Virologic and Immunologic Markers

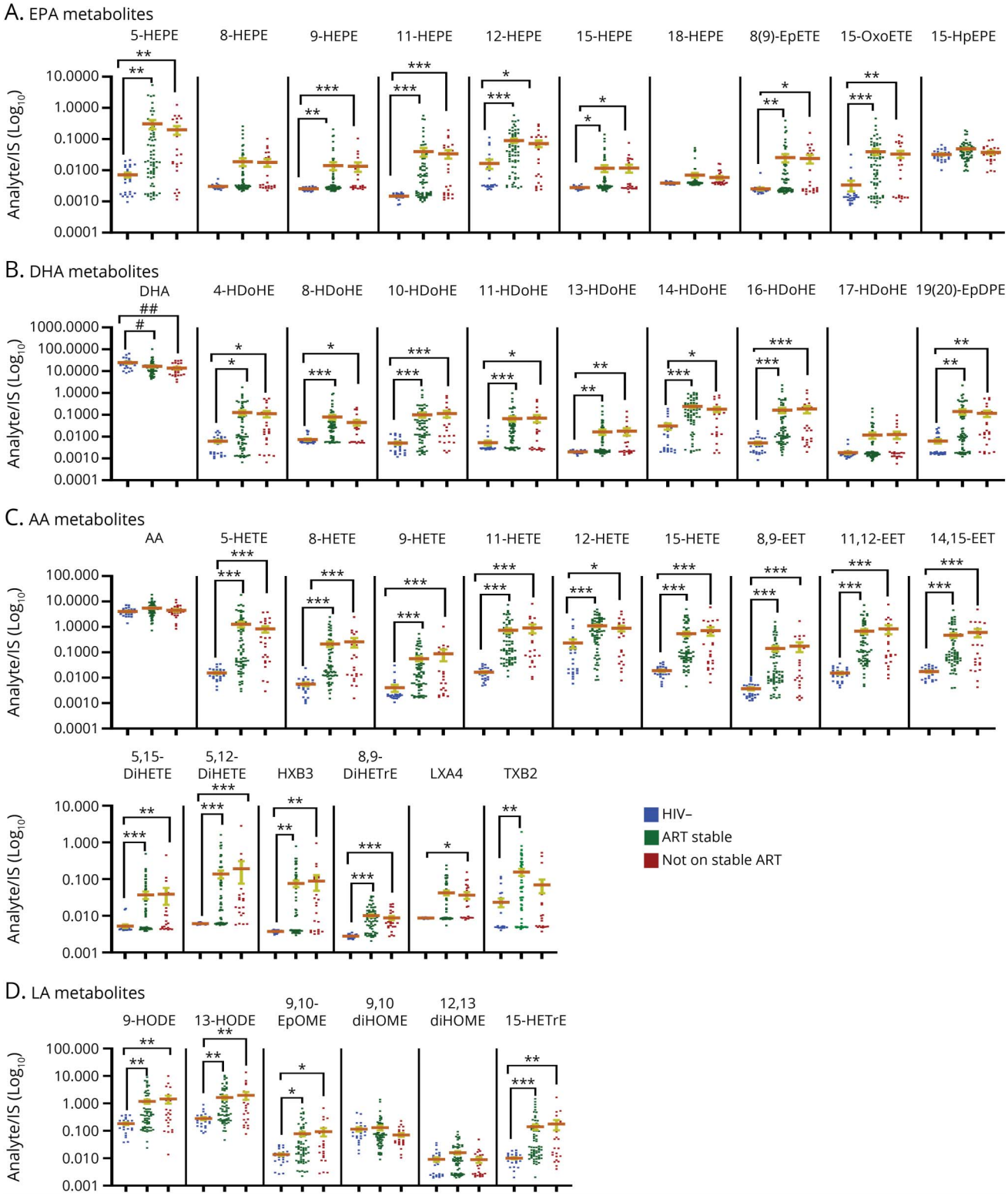
In exploratory analyses, we found negative associations between baseline plasma levels of eicosanoids and current CD4 (8 EPA, 7 DHA, 15 AA, 4 LA), nadir CD4 (9 DHA, 6 EPA, 15 AA, 4 LA), 1 AA (TXB2) metabolite was associated with creatinine, 1 LA (12, 13 diHOME) metabolite was associated with bilirubin, 1 EPA and 1 AA metabolite were associated with aspartate aminotransferase, and 1 DHA, and 2 AA metabolites were associated with alanine transminase. One DHA (4-HDoHE) metabolite was associated with cholesterol, high density lipoprotein (1 DHA, 7 AA, 1 LA); a single DHA metabolite was associated with white blood cell; 1 EPA and 1 DHA metabolites were associated with RBC; hemoglobin (2 EPA, 3 DHA, 1LA), hematocrit (1 EPA, 3DHA, 1LA), and 3 DHA metabolites were associated with MCHC; 2 DHA metabolites were associated with platelets, total lymphocyte count (4 DHA, 9 EPA, 13 AA, 4 LA); and a single LA metabolite was associated with basophil (Figure 4). There were also positive associations between eicosanoids and age (1EPA, 2DHA, 1AA), education (3 DHA, 3 EPA), glucose (1 AA), calcium (1 EPA, 4 DHA, 2 AA, 4 LA), 1 AA and 1 LA metabolite associated with total protein, a single AA metabolite was associated with triglyceride levels, a single AA metabolite was associated with platelet count, neutrophil counts (1EPA, and 3AA), and eosinophil count associated with (3 DHA, 1 AA, and 1 LA) (Figure 4). The most robust associations show that low current CD4, nadir CD4, high density lipoprotein, and total lymphocyte counts are associated with higher levels of proinflammatory eicosanoids. They also show that higher calcium levels and neutrophil counts are associated with higher levels of plasma eicosanoids (Figure 4). Many of these associations did not remain after FDR correction with the exceptions of current and nadir CD4 association with multiple eicosanoids (eFigure 1, links.lww.com/WNL/C210). The data shown prediction power of 100% at 5% FDR ($\alpha = 0.02$).

We further compared the plasma eicosanoids level in HIV seronegative participants, virally suppressed (<50 copies/mL) PWH, and not fully suppressed (≥50 copies/mL) individuals (Figure 5). Elevated plasma eicosanoids were similar in virally suppressed and not fully suppressed individuals, and none of the eicosanoids measured were different between these 2 groups. These data suggest that most plasma eicosanoids are elevated in PWH regardless of viral suppression.

Plasma Eicosanoids Are Negatively Associated With Performance on Working Memory Tasks in Exploratory Analyses

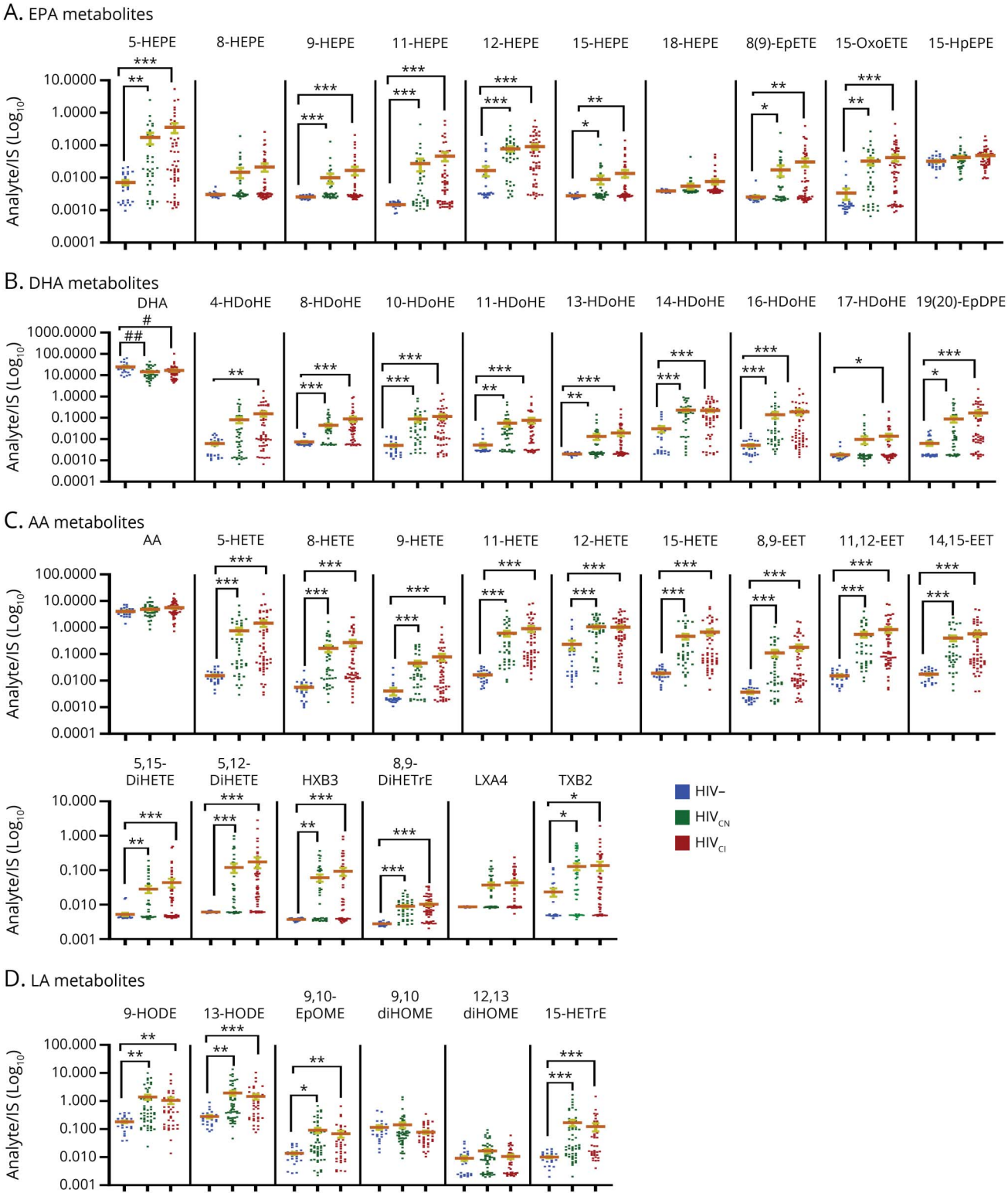
We next determined in exploratory analyses whether baseline plasma levels of eicosanoids were cross-sectionally associated with cognition in PWH. There were no associations between baseline plasma eicosanoid levels and global deficit score, speed of information processing (SIP), learning, recall, or motor function. Several eicosanoids were negatively associated with verbal fluency, executive function, and working memory. Seven eicosanoid metabolites (3 DHA, 3 AA, 1 LA) were associated with verbal fluency, 4 eicosanoid metabolites (2 EPA, 2 DHA) were associated with executive function, and striking 30 eicosanoid metabolites (5 EPA, 7 DHA, 13 AA, 5 LA) were associated with working memory performance (Figure 6A). When we only included HIV cognitively normal in the analysis, we observed only a small number of associations between plasma eicosanoids and cognitive domain performance. A single AA metabolite was negatively associated with global deficit score, and 1 EPA and 1 AA metabolite were negatively associated with executive function (Figure 6B). There were also positive associations between eicosanoids and global deficit score (1 DHA, and 1 LA), SIP (1 LA), and motor function (1 EPA, 1 AA, 1 LA) (Figure 6B). However, when we only included HIV cognitively impaired in the analysis, a negative association of 4 eicosanoids (3 DHA, 1AA) with verbal fluency and 13 eicosanoids (3 EPA, 9 AA, and 1LA) with working memory performance remained (Figure 6C). There were also positive associations between eicosanoids and learning (2 DHA) and recall (3 DHA, 3 AA, 1 LA) (Figure 6). These findings suggest that elevated plasma levels of eicosanoids in PWH may be associated with worse

Figure 2 Baseline Analysis of Plasma Eicosanoids in Healthy Seronegative Control Participants and People with HIV Who Are ART Naive Or on a Stable ART Regimen



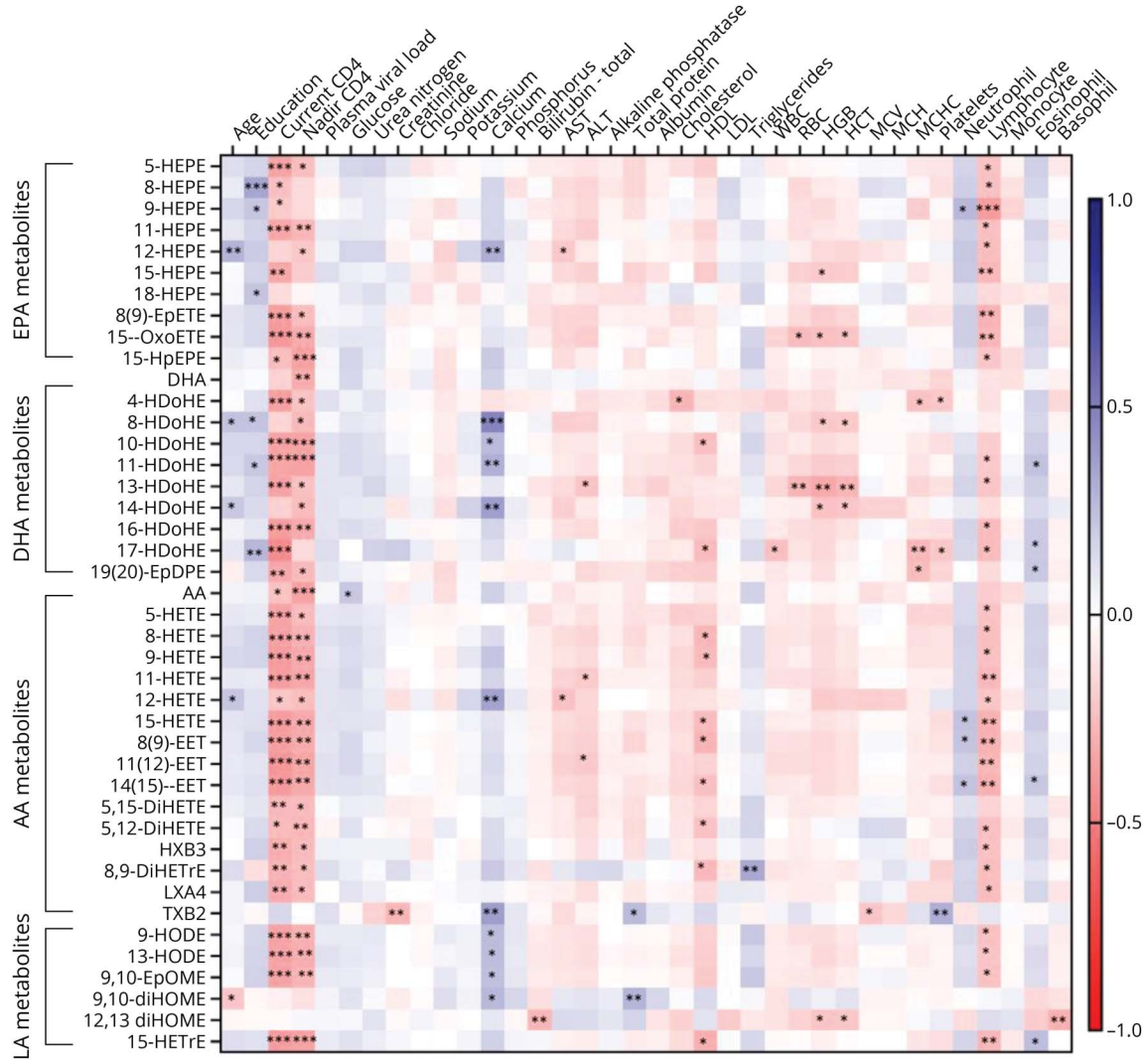
Box plots show (A) EPA metabolites, (B) DHA metabolites, (C) AA metabolites, and (D) LA metabolites Data show median and range with individual data points. HIV seronegative (n = 25), ART stable (n = 66), and ART naive (n = 29). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ increased compared with HIV seronegative. $^{\#\#\#}p < 0.001$ decreased compared with HIV seronegative. The Kruskal-Wallis test with the Dunn post hoc comparison test performed for 3-group comparisons. AA = arachidonic acid; ART = antiretroviral therapy; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; LA = linoleic acid.

Figure 3 Baseline Analysis of Plasma Eicosanoids in Healthy Seronegative Control Participants, Cognitively Normal, and Cognitively Impaired



People with HIVBox plots show (A) EPA metabolites, (B) DHA metabolites, (C) AA metabolites, and (D) LA metabolites. Data show median and range with individual data points. HIV seronegative (n = 25), HIV_{CN} = cognitively normal (n = 41), and HIV_{CI} = cognitively impaired (n = 54). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ increased compared with HIV seronegative. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ decreased compared with HIV seronegative. The Kruskal-Wallis test with the Dunn post hoc comparison test performed for 3-group comparisons. AA = arachidonic acid; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; LA = linoleic acid.

Figure 4 Correlation Plot Showing the Relationships of Plasma Eicosanoid Levels to Demographic, Virologic, and Clinical Markers



The value of effect size of Spearman correlation and the direction of the relationship is depicted by color from 1.0 in dark blue to −1.0 in dark red. The value of the effect size is considered low if the value of r is around 0.1, medium if around 0.3, and large if r is greater than 0.5. Most effect sizes in this analysis were in the medium to high value range. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Spearman rank correlation.

performance on neuropsychological tests assessing working memory, and to a lesser extent verbal fluency, and executive function. None of these associations remained after FDR correction (eFigure 2, links.lww.com/WNL/C210). The data shown prediction power of 100% at 5% FDR ($\alpha = 0.0419$).

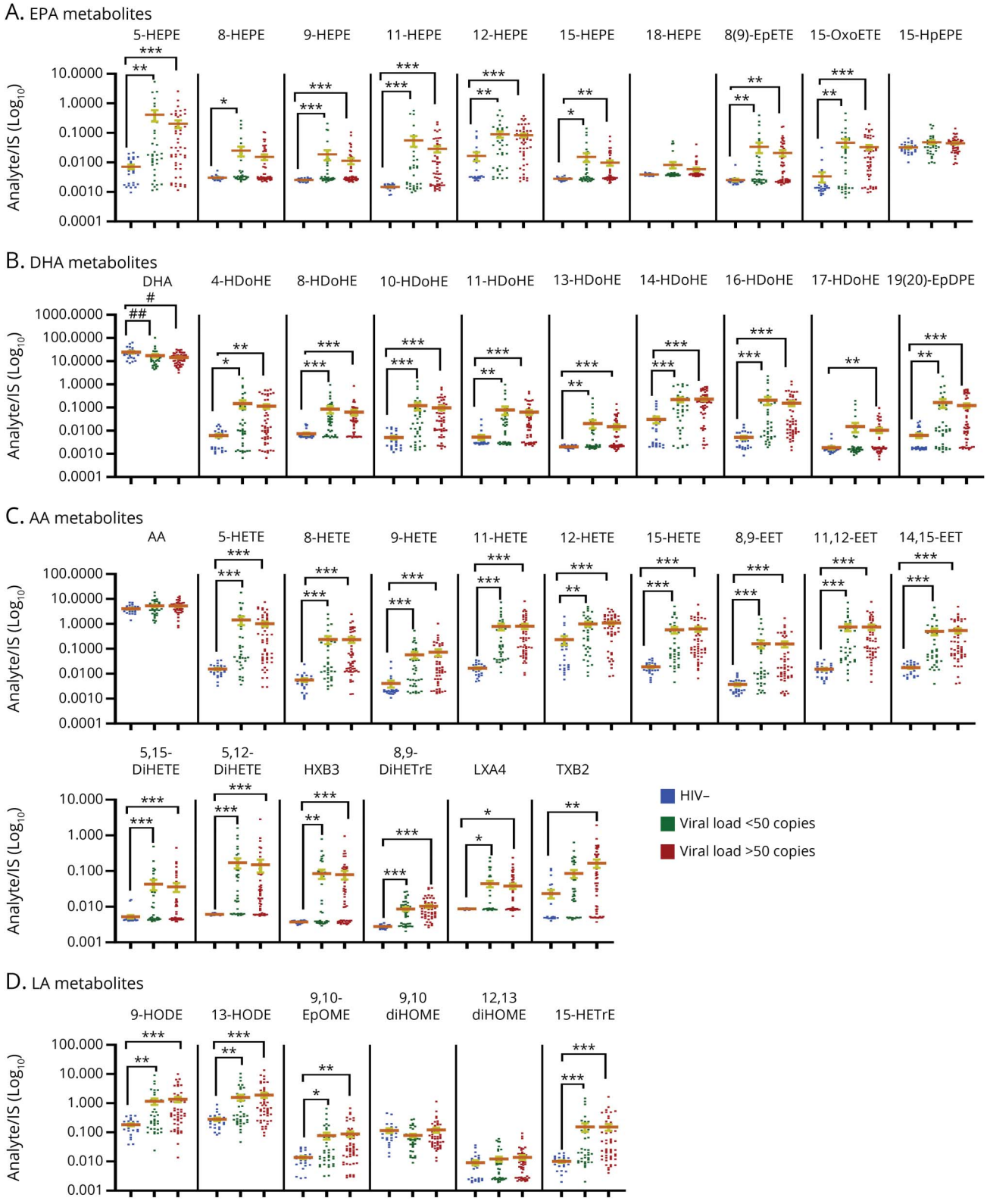
Longitudinal Associations Between Plasma Eicosanoids, Cognition, and Plasma Markers

In initial longitudinal analyses, we sought to determine whether plasma eicosanoid levels at baseline were prognostic indicators of cognitive function at the next study visit. All but 1 plasma eicosanoids were similar between all groups (eFigure 3, A-D, links.lww.com/WNL/C210). The DHA metabolite 17-HDoHE was elevated at baseline in plasma of HIV improving cognition compared with HIV stably normal cognition (eFigure 3B). These data are consistent with the protective and anti-inflammatory properties of these omega 3 DHA metabolites and suggest that plasma levels of most eicosanoids are not prognostic indicators for change in cognition.

We next determined whether plasma levels of eicosanoids accompanied changes in cognition using plasma samples from visit 2. None of the eicosanoids measured at visit 2 were different across the 4 HIV change groups (eFigure 4, A–D, links.lww.com/WNL/C210). The data shown prediction power of 2% at 5% FDR ($\alpha = 0.05$) and a maximum prediction power of 37% at $\alpha = 0.297$. These findings suggest that plasma eicosanoid levels are not associated with changes in cognition after the change in cognitive function has occurred.

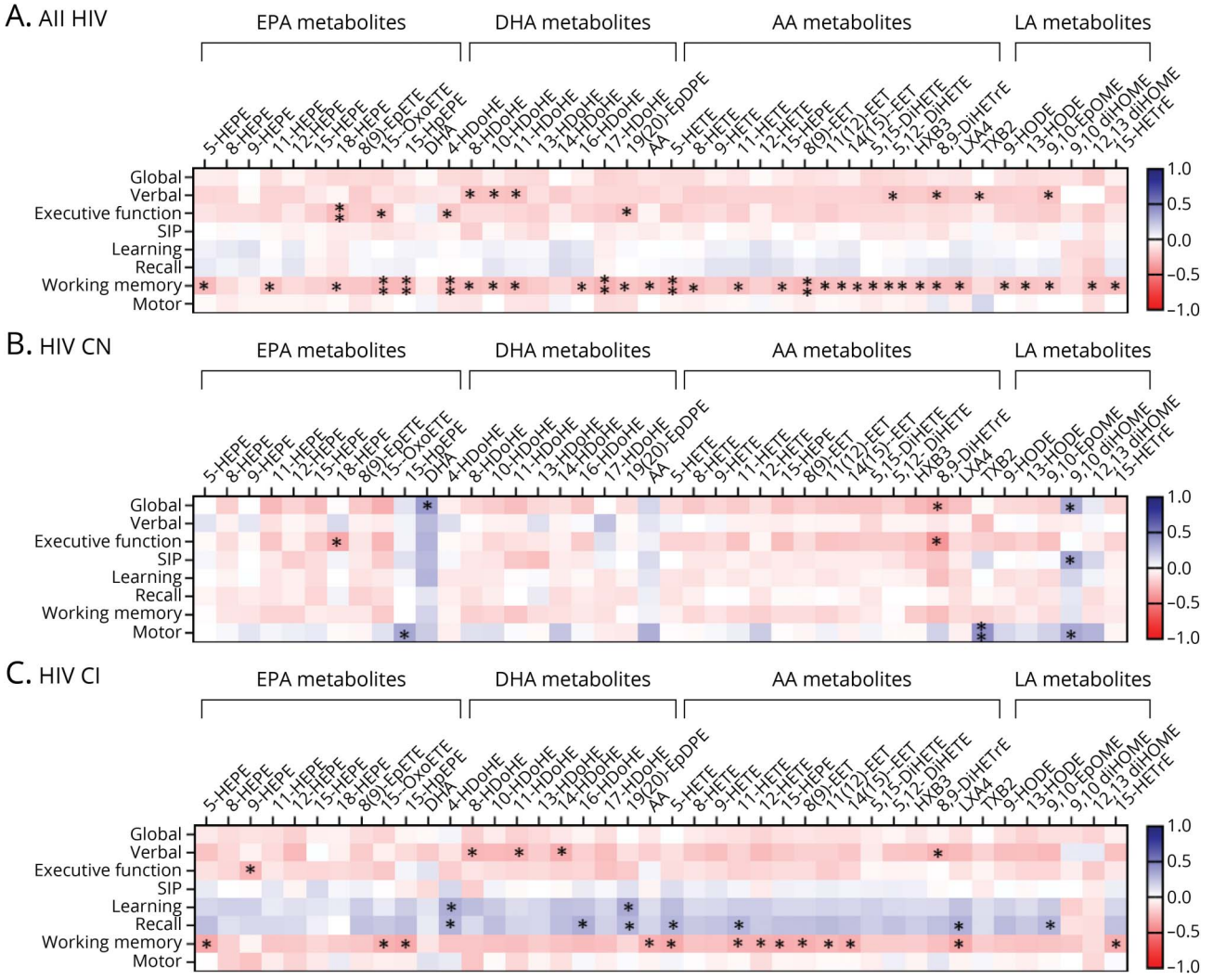
When we looked at associations between change in virologic/blood markers and change in plasma eicosanoid concentrations,

Figure 5 Baseline Plasma Eicosanoids in Healthy Seronegative Control Participants and People with HIV With Virally Suppressed (<50 Copies/mL) and Not Fully Suppressed (>50 Copies/mL) Individuals



Box plots show (A) EPA metabolites, (B) DHA metabolites, (C) AA metabolites, and (D) LA. Data show median and range with individual data points. HIV seronegative (n = 25), viral load <50 copies (n = 43), and viral load >50 copies (n = 52). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ increased compared with HIV seronegative. The Kruskal-Wallis test with the Dunn post hoc comparison test performed for 3-group comparisons. AA = arachidonic acid; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; LA = linoleic acid.

Figure 6 Correlation Plots Showing the Relationships of Plasma Eicosanoid Levels to Global Cognitive Function and Performance in Individual Cognitive Domains



(A) Relationship of baseline plasma eicosanoid levels in all HIV-infected patients to overall cognitive function (global) and performance in verbal, executive function, SIP, learning, recall, working memory, and motor function. (B) Relationship of baseline plasma eicosanoid levels in cognitively normal HIV-infected patients (HIV_{CN}) to overall cognitive function (global) and performance in the indicated cognitive domains. (C) Relationship of baseline plasma eicosanoid levels in cognitively impaired HIV-infected patients (HIV_{CI}) to overall cognitive function (global) and performance in the indicated cognitive domains. The value of effect size of Spearman correlation and the direction of the relationship is depicted by color from 1.0 in dark blue to −1.0 in dark red. The value of the effect size is considered low if the value of r is around 0.1, medium if around 0.3, and large if r is greater than 0.5. Most effect sizes in this analysis were in the medium value range. *p < 0.05 and **p < 0.01. Spearman rank correlation. AA = arachidonic acid; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; LA = linoleic acid; SIP = speed of information processing.

we found associations between change in viral load and change in 6 of the 42 eicosanoids that included the 1 EPA metabolite 15-HpEPE; 3 DHA metabolites 13-, 17-HDoHE, and DHA; 1 AA metabolite AA; and 1 LA metabolites 9, 10-diHOME (eFigure 5, links.lww.com/WNL/C210). There were similarly strong associations between change in plasma creatinine and change in 35 of the 42 eicosanoids that included the 9 EPA metabolites 5-HEPE, 8-, 9-, 11-, 12-, 15-, 8, (9)-EpETE, 15-OxoETE, and 15-HpEPE; 9 DHA metabolites 4-, 10-, 11-, 13-, 14-, 16-, 17-HDoHE, 19, 20-EpDPE, and DHA; 14 AA metabolites 5-, 8-, 9-, 11-, 12-, 15-HETE, 8, (9)-, 11(12)-, 14, (15)-EET, 5, (15)-, 5, (12) DiHETE, HXB3, 8, (9)-DiHETrE, and AA; and 3 LA metabolites 13-HODE, 9, 10-EpOME, and 15-HETrE (eFigure 5). A single association was found between change in the LA metabolite 9, 10-diHOME and bilirubin, and 3 LA metabolites (9-, 13-HODE, and 12, 13-diHOME) were associated with change in triglyceride levels (eFigure 5). There were 2 associations found between change in low density lipoprotein levels and 2 LA metabolites that included 9, 10- and 12, 13-diHOME. Two associations between white blood cell count and 2 eicosanoids that included DHA and AA. There were 6 associations between hemoglobin and 1 EPA metabolite (5-HEPE), 4 DHA (11-, 13-, 17-HDoHE, and DHA), and 1 AA metabolite included AA. Two associations between neutrophil and 1 EPA (12-HEPE) and 1 DHA (8-HDoHE) metabolite were also observed (eFigure 5). Looking at change in eicosanoid levels and change in virologic/blood markers, we found that 6 of 42 eicosanoids were correlated with plasma viral load and 35 of 42 eicosanoids were

correlated with creatinine (eFigure 5). The most robust associations suggest that increases in plasma viral load were strongly associated with increases in plasma eicosanoids, reductions in kidney function (as suggested by creatinine reductions). None of these associations remained after FDR correction (eFigure 6). The data shown prediction power of 24% at 5% FDR ($\alpha = 0.05$) and a maximum prediction power of 42.4 at 10% FDR ($\alpha = 0.10$).

Discussion

HIV infection results in a robust inflammatory response with increased plasma levels of cytokines, chemokines, and eicosanoids (see reference 4 for a review). A prominent role for these inflammatory mediators is to regulate the immune response to HIV infection. ART suppresses HIV replication, largely stabilizes immune function and decreases levels of most (but not all) cytokines and chemokines. However, the possible effects of ART on eicosanoid metabolism have not been studied. Here, we provide evidence that ART may not substantially reduce elevated levels of eicosanoids in PWH, suggesting that a sustained immune dysregulation with ART may involve chronically elevated levels of proinflammatory eicosanoids. Plasma eicosanoid levels were negatively associated with current CD4, nadir CD4, and lymphocyte counts. Eicosanoids with proinflammatory effects (LA and AA metabolites) were positively associated with plasma viral load. Longitudinal analyses showed that eicosanoid levels rise in accordance with viral load and with plasma creatinine, implicating elevations of eicosanoids with impairments in kidney function. Despite the associations of eicosanoid levels with viral loads, elevated plasma eicosanoids were similar in virally suppressed (<50 copies/mL) and not fully suppressed (>50 copies/mL) individuals. These data suggest that HIV infection is associated with a robust production of eicosanoids that is not substantially reduced by ART and is associated with impairments in cognition, immune dysregulation, and damage to peripheral organs.

Eicosanoids are a family of bioactive lipids produced primarily through the metabolism of AA, LA, EPA, and DHA. These bioactive lipids serve a wide variety of signaling functions including regulation of the inflammatory response, immune function, and vasodilation/constriction, among others (see reference 5 for a recent review). Eicosanoid metabolism is complex, often involving multiple metabolic steps within a single cell. Each of the metabolites produced in a metabolic pathway have independent functions, with some metabolites produced later in series opposing the functions of earlier metabolites, thus serving to limit the duration of some physiologic responses such as an inflammatory reaction. However, prolonged activation of these metabolic pathways under conditions such as those produced by HIV infection can result in a deregulated inflammatory and immune response. Although we cannot precisely determine a mechanism for this sustained eicosanoid response in ART-treated PWH from our results, there is a considerable amount of evidence that the gut microbiome may play an important role. $CD4^+$ cells lining the gut are largely lost with HIV infection and do not fully re-establish with ART. This loss of $CD4^+$ cells in PWH increases gut permeability and results in bacterial-derived lipopolysaccharide leaking into circulation.[16] Lipopolysaccharides in addition to viral infection are potent activators of toll-like receptors (TLRs) that are linked with eicosanoid metabolism.

TLRs belong to a family of pattern recognition receptors that play key roles in initiating an innate immune response that involves a rapid activation of arachidonic acid metabolism in leukocytes, platelets, and dendritic cells[17,18] (among others). The HIV coat protein gp120 interacts with TLR2 and TLR4 in the presence of heparan sulfate[19] and inhibits the activation of TLR9 in plasma dendritic cells (this latter effect presumably limits the ability of the host to produce some antiviral and inflammatory mediators).[20] HIV ssRNA binds and activates TLR7 and TLR8 in mononuclear cells during viral internalization.[21,22] After HIV infection, the expression of both TLR2 and TLR4 is increased in monocyte-derived macrophages, PBMCs, and dendritic cells.[23,24] A number of other viral components interact with TLRs including p17 and gp41 that activate TLR1 and TLR2, p24 activates TLR2 and TLR6,[25] and Tat directly binds TLR4 mRNA to more than double its half-life with a resultant decrease in TLR4 expression.[26] Although it is not entirely clear which viral components contribute to the maintenance of the eicosanoid response in virally suppressed PWH, there is a great deal of evidence suggesting that nonstructural proteins such as Tat continue to be produced despite viral suppression.[27]

In addition to initiating an innate immune response, eicosanoids play important roles in regulating the interactions between innate and adaptive immune responses including activation, proliferation, migration, differentiation, antibody and cytokine production in lymphocytes, and other leukocytes.[28] In this study, we found a negative association between current CD4 count and plasma eicosanoids levels and a positive association between viral load and eicosanoid levels. The association between high viral loads and low CD4 counts is a common finding in PWH because the virus replicates in and kills $CD4^+$ T cells. Presumably, a higher viral load would also increase the production of eicosanoids through activation of TLRs as described above, but it is also possible that eicosanoids directly regulate the activity of $CD4^+$ cells. Both $CD4^+$ and $CD8^+$ cells express enzymes for arachidonic acid metabolism including COX1, COX2, 5-LOX, and PGG2.[29] PGE2 can differentially regulate apoptosis and inhibit proliferation depending on the subpopulation and activation status of CD4 and CD8 cells.[30] LTB4 signaling through BLT1 promotes activation, cytokine production, chemotaxis, endothelial adhesion, and migration of macrophages and $CD4^+$ cells into tissues including brain.[31] Indeed, the elevated numbers of macrophages and T cells in brain parenchyma of PWH is a common finding.[32] We also observed that ART did not substantially reduce circulating levels of eicosanoids in addition to a negative association with

low nadir CD4. Together, these data suggest that the initial damage to the immune system may have long-term consequences that delimit the eicosanoid response to viral infection. Indeed, low nadir CD4 has been consistently associated with faster disease progression and worse cognitive outcomes in multiple cohorts of PWH.[33,34]

Although the mechanisms responsible for a deregulated eicosanoid response are likely multifactorial, in exploratory analyses, we found evidence for reduced antioxidant capacity as evidenced by a negative association between plasma bilirubin and eicosanoid levels. Bilirubin is a powerful antioxidant that suppresses inflammation by inhibiting the activities of secretory phospholipase A_2 ($sPLA_2$).[35,36] Secretory PLA_2 is responsible for the production of AA from membrane phospholipids that are subsequently metabolized into numerous proinflammatory eicosanoids through COX, LOX, or cytochrome P450 pathways. It is also possible that reduced bilirubin levels contribute to increased eicosanoids indirectly through a disinhibition of inducible nitric oxide synthase (iNOS). Bilirubin has been shown to inhibit hepatic iNOS expression and nitric oxide (NO) production in response to endotoxin in rats,[37] and previous studies have shown that NO increases the production of eicosanoids.[38-40] NO together with cGMP promote COX-2 expression and the production of PGE2 in human granulosa cells through cAMP response element-binding protein signaling,[40] and PGE2 has been shown to further promote NO production though IFNγ in cultured rat microglia.[41] In PWH, a negative association of bilirubin and cardiovascular disease, insulin resistance, and DNA damage has been previously reported.[42,43] These data combined with the current findings suggest that reduced levels of circulating bilirubin may contribute to the overproduction of eicosanoids.

There is a strong association between peripheral immune activation, inflammation, eicosanoid metabolism, and neurologic dysfunction that is apparent in multiple neurodegenerative conditions that include a rich literature on roles for eicosanoids in regulating cognitive performance.[44-46] Peripheral immune activation and inflammation are known to activate brain resident glia with consequent increases in the expression of PLA2, the rate-limiting enzyme in AA production. In this study, we found a negative correlation between most of the plasma eicosanoids we measured and working memory, with a smaller number of eicosanoids showing a negative correlation with executive function. In exploratory analyses, the observation that worse performance on tasks assessing working memory and executive functions was associated with higher levels of almost all eicosanoids suggests a deregulated inflammatory response, despite the production of eicosanoids whose function is to limit the duration and extent of the response. In addition to prominent roles for eicosanoids in inflammation, they also play important roles in vasal tone and platelet activation. For example, HETEs exhibit prohypertensive effects through vasoconstriction and antihypertensive effects through natriuresis.[47,48] Platelet activation and apoptotic pathways are increased in PWH, despite virologic suppression,[49] and thrombocytopenia is a common finding in PWH. In addition to roles in vascular and tissue repair, they play central roles in innate immune activation by directly interacting with leukocytes and secreting cytokines and chemokines. These data suggest that elevated eicosanoids in PWH may not only directly contribute to cognitive impairments but also restrict neurovascular flow and promote platelet activation.

Although we believe the findings from this study provide a potential mechanistic explanation for sustained inflammation in virally suppressed PWH, there are some shortcomings. This study was conducted with a relatively small number of samples from PWH that were obtained from a single cohort. The PWH of this study were almost exclusively male. Based on known sex differences in the prevalence and etiology of autoimmune diseases, inflammatory diseases, and neurodegenerative conditions, the associations of eicosanoids with clinical outcomes and cognition that we report here may not be directly applicable to HIV-infected women. Future studies should re-evaluate potential roles of eicosanoids as drivers of sustained inflammation in a larger sample size obtained from multiple cohorts with diverse demographics and risk factors.

Our findings suggest that HIV infection is associated with a robust increase in plasma eicosanoids that is not resolved by ART. Although there was an association between low nadir CD4, low current CD4, high viral load, and elevated eicosanoids, those individuals with viral suppression below detectable limits did not completely resolve plasma eicosanoid levels. Elevated eicosanoids were associated with poorer cognitive performance. Our data suggest impairment in the self-limiting aspects of an inflammatory eicosanoid response.

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Disclosure

The authors report no relevant disclosures. Go to Neurology.org/N for full disclosures.

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Appendix Authors

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Pragney Deme, PhD	Department of Neurology, Johns Hopkins University School of Medicine, Baltimore, MD	Drafting/revision of the manuscript for content, including medical writing for content; Study concept or design; Analysis or interpretation of data; Additional contributions: prepared figures, performed statistical analyses, reviewed and edited the manuscript. Interpreted experimental findings and wrote the initial draft of the manuscript.
Mohammed Moniruzzaman, PhD	Department of Neurology, Johns Hopkins University School of Medicine, Baltimore, MD	Provided samples from the CHARTER cohort, contributed to the analysis of cognitive testing data, interpretation of data and editing of the manuscript.
David Moore, PhD	HIV Neurobehavioral Research Program and Departments of Neurosciences and Psychiatry, School of Medicine, University of California, San Diego, La Jolla, CA	Provided samples from the CHARTER cohort and directs the HNRC at UCSD. Edited the manuscript
Robert Heaton, PhD	HIV Neurobehavioral Research Program and Departments of Neurosciences and Psychiatry, School of Medicine, University of California, San Diego, La Jolla, CA	Provided samples from the CHARTER cohort, oversaw sample collection and contributed to interpretation of data and editing of the manuscript.
Ronald Ellis, MD, PhD	HIV Neurobehavioral Research Program and Departments of Neurosciences and Psychiatry, School of Medicine, University of California, San Diego, La Jolla, CA	Provided samples from the CHARTER cohort, contributed to interpretation of data and editing of the manuscript
Scott Letendre, MD	HIV Neurobehavioral Research Program and Departments of Neurosciences and Psychiatry, School of Medicine, University of California, San Diego, La Jolla, CA	Oversaw all aspects of experimental design, analyses, data interpretation, and manuscript preparation.
Norman Haughey, PhD	Department of Neurology, Johns Hopkins University School of Medicine; Department of Psychiatry, Johns Hopkins University School of Medicine, Baltimore, MD	Drafting/revision of the manuscript for content, including medical writing for content; Analysis or interpretation of data; Additional contributions: prepared figures, performed statistical analyses, reviewed and edited the manuscript.

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