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Plasma Tryptophan-Kynurenine Metabolites Are Altered in Human Immunodeficiency Virus Infection and Associated With Progression of Carotid Artery Atherosclerosis

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Background. It is unknown whether disrupted tryptophan catabolism is associated with cardiovascular disease (CVD) in human immunodeficiency virus (HIV)–infected individuals.

Methods. Plasma tryptophan and kynurenic acid were measured in 737 women and men (520 HIV+, 217 HIV–) from the Women's Interagency HIV Study and the Multicenter AIDS Cohort Study. Repeated B-mode carotid artery ultrasound imaging was obtained from 2004 through 2013. We examined associations of baseline tryptophan, kynurenic acid, and kynurenic acid-to-tryptophan (KYNA/TRP) ratio, with risk of carotid plaque.

Results. After a 7-year follow-up, 112 participants developed carotid plaque. Compared to those without HIV infection, HIV-infected participants had lower tryptophan ($P < .001$), higher KYNA/TRP ($P = .01$), and similar kynurenic acid levels ($P = .51$). Tryptophan, kynurenic acid, and KYNA/TRP were correlated with T-cell activation (CD38+HLA-DR+) and immune activation markers (serum sCD14, galectin-3) but had few correlations with interleukin-6, C-reactive protein, or CVD risk factors (blood pressure, lipids). Adjusted for demographic and behavioral factors, each standard deviation (SD) increment in tryptophan was associated with a 29% (95% confidence interval [CI], 17%–38%) decreased risk of carotid plaque ($P < .001$), while each SD increment in kynurenic acid ($P = .02$) and KYNA/TRP ($P < .001$) was associated with a 34% (6%–69%) and a 47% (26%–73%) increased risk of carotid plaque, respectively. After further adjustment for CVD risk factors and immune activation markers, these associations were attenuated but remained significant.

Conclusions. Plasma tryptophan-kynurenine metabolites are altered in HIV infection and associated with progression of carotid artery atherosclerosis.

Keywords. association study; atherosclerosis; HIV infection; metabolite.

Cardiovascular disease (CVD) has become a major concern for people who live with human immunodeficiency virus (HIV) infection [1]. Although prior studies have provided insights into the virologic, inflammatory, immunologic, treatment-related, and traditional risk factors that may affect HIV-related CVD risk, an understanding of its pathophysiology remains incomplete [2]. Recently, there has been an interest in examining the role of the tryptophan-kynurenine pathway, which is closely related to inflammation and immune activation, in the development of CVD [3]. Disrupted tryptophan catabolism has been noted in HIV infection and associated with HIV disease progression [4, 5]; however, its relationship with CVD risk in HIV infection has been explored only to a limited extent [6].

Tryptophan is an essential amino acid that is used for protein synthesis and the biosynthesis of serotonin and melatonin; it also serves as the sole source of kynurenine pathway metabolites [3]. In the kynurenine pathway, tryptophan is catabolized into kynurenine and several downstream metabolites (eg, kynurenic acid, anthranilic acid), mainly regulated by indoleamine 2,3-dioxygenase 1 (IDO-1), which is induced by Th1-type cytokines (eg, interferon-γ) during inflammation and immune activation [7]. In HIV-uninfected populations, several studies have linked plasma kynurenine-to-tryptophan (KYN/TRP) ratio, a measure that reflects IDO-1 activity, with carotid artery subclinical atherosclerosis [8–10] and CVD risk [11–14]. Little is known about this relationship in HIV-infected individuals. One small study of 105 participants showed an association between the declining plasma KYN/TRP ratio over 6 months of treatment and lower single time point measurement of carotid artery intima media thickness (cIMT) [6].

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Our prior work in the Women's Interagency HIV Study (WIHS) and the Multicenter AIDS Cohort Study (MACS) has demonstrated greater progression of carotid artery atherosclerosis in HIV-infected individuals, even in those with viral suppression during antiretroviral therapy (ART), compared to HIV-uninfected individuals [15]. In the present study, we profiled plasma levels of tryptophan and kynurenic acid in 737 WIHS women and MACS men, approximately 65% of whom were HIV infected, and examined their associations with risk of carotid artery plaque, assessed by repeated B-mode carotid artery ultrasound imaging over a 7-year period. Since generation of kynurenic acid rather than kynurenine has been suggested to reflect tryptophan consumption [16], we calculated the kynurenic acid-to-tryptophan (KYNA/TRP) ratio as an indicator of tryptophan catabolism [17] in the current study.

METHODS

Study Participants

Participants were from 2 prospective multicenter cohort studies of women and men with or at risk for HIV infection, the WIHS and MACS. Details on study design and methods have been described previously [18, 19]. Every 6 months, WIHS and MACS participants complete a core visit with a comprehensive physical examination; participants provide biological specimens and complete interviewer-administered questionnaires. Since 2004, the WIHS and MACS have collaborated on a uniform carotid artery imaging protocol [20], scanning the cohorts at 2- to 4-year intervals to ascertain progression of subclinical carotid artery atherosclerosis (the WIHS/MACS CVD study) [15]. In 2015, a plasma metabolomics study, which aimed to examine plasma metabolites in relation to cardiometabolic risk in the context of HIV infection, was initiated among participants aged ≥35 years who underwent carotid artery imaging for plaque assessment at a baseline visit (2004–2006) and at a follow-up visit (2011–2013) in the WIHS/MACS CVD study. As our primary outcomes included incident carotid artery plaque and incident diabetes, participants with prevalent carotid artery plaques and prevalent diabetes at baseline were excluded from plasma metabolomics profiling. In the current analysis, we included 737 women and men with data on plasma tryptophan and kynurenic acid and incident carotid artery plaque. Characteristics of included participants and those not included in the current study were generally similar and comparable (Supplementary Table 1), although participants who were included had lower prevalence of antihypertensive and lipid-lowering medication use compared to those not included. All individuals provided informed consent, and each site's institutional review board approved the study.

Carotid Artery Plaque Ascertainment

Participants underwent high-resolution B-mode carotid artery ultrasound in order to image 6 locations in the right carotid artery: the near and far walls of the common carotid artery (CCA), carotid bifurcation, and internal carotid artery. A standardized protocol was used at all sites [15]. Focal plaque measures were obtained at a centralized reading center (University of Southern California). Coefficients of variation of repeated measurements of IMT at the CCA have been published [21], and replicate image acquisition and interpretation studies were repeated to ensure consistency over time. We defined a focal plaque as an area with localized IMT >1.5 mm in any of the 6 imaged carotid artery locations [22].

Plasma Tryptophan and Kynurenic Acid Measurement

Plasma tryptophan and kynurenic acid were profiled from stored frozen plasma specimens that had been collected at the core study visit closest to the baseline carotid artery imaging study visit using liquid chromatography-tandem mass spectrometry at the Broad Institute Metabolomics Platform (Cambridge, Massachusetts). Briefly, metabolites were profiled using a Nexera X2 Ultra-High Performance Liquid Chromatography (UHPLC)(Shimadzu Corp., Marlborough, Massachusetts) coupled to an Exactive Plus mass spectrometer (Thermo Fisher Scientific, Waltham, Massachusetts). Metabolite peaks were identified and confirmed using authentic reference standards. Metabolites were quantified using area-under-the-curve of the peaks. Raw data were processed using TraceFinder software (Thermo Fisher Scientific, Waltham, Massachusetts) and Progenesis QI (Nonlinear Dynamics, Newcastle upon Tyne, United Kingdom). Detailed methods of plasma metabolite measurement are described in the Supplementary Material.

Assessments of HIV Infection and Other Variables

Demographic, clinical, and laboratory variables were collected using standardized protocols at semiannual core study visits [15]. HIV infection was ascertained using enzyme-linked immunosorbent assay and confirmed using Western blot. HIV-specific parameters included CD4+ T-cell counts, HIV-1 viral load, and detailed information on ART. Hepatitis C virus (HCV) infection status was based on antibody and viral RNA testing. Inflammation and immune activation markers included serum-soluble [s]CD14, sCD163, galectin-3 [Gal-3], Gal-3 binding protein [Gal-3BP], C-reactive protein (CRP), and interleukin (IL)-6 [23]. CD4+ T-cell and CD8+ T-cell activation (CD38+HLA-DR+) and senescence (CD28-CD57+) markers were measured in a subsample of women using methods that have been previously described [24]. In the current analysis, these variables and biomarkers were assessed at baseline and were not updated at the time of the follow-up visit of carotid imaging.

Among HIV-infected participants, we defined persistent virologic suppression as consistent plasma HIV RNA levels <80 copies/mL simultaneous with continuous ART use over the study period (up to 16 measurements per participant). We allowed for 1 virologic "blip" during the period as long as it was <500 copies/mL, based on evidence that blips ≥500 copies/mL

are associated with virologic rebound [25]. Participants were allowed to miss no more than 1 core visit to be eligible for being categorized as "persistently virologically suppressed." In addition, HIV infection tests were also performed routinely for all HIV-uninfected participants during semiannual visits, and those included in the current analysis remained HIV uninfected over the study period.

Statistical Analyses

Characteristics of participants were compared by HIV serostatus in women and men separately. Raw values of tryptophan, kynurenic acid, and KYNA/TRP ratio were natural-log transformed to approximate a normal distribution before analysis. Linear regression was used to compare metabolite levels among HIV-uninfected, HIV-infected aviremic (undetectable viral load ≤80 copies/mL), and HIV-infected viremic (viral load >80 copies/mL) individuals, adjusting for age and sex. We calculated age- and sex-adjusted partial Spearman correlations of metabolite levels with CD4+ T-cell counts, viral load, inflammation and immune activation markers, markers of CD4+ T-cell and CD8+ T-cell activation and senescence, and conventional CVD risk factors. Poisson regression models with robust variance estimates were used to calculate risk ratios (RRs) and 95% confidence intervals (CIs) of carotid artery plaque per standard deviation (SD) increment in log-transformed metabolites. We tested interactions by sex and by HIV serostatus with generalized score statistics. We adjusted for age, sex, race/ethnicity, education, study site, current smoking, history of HIV infection, HIV serostatus, HIV treatment status and baseline viral load, body mass index (BMI), systolic blood pressure, total cholesterol, high-density lipoprotein (HDL) cholesterol, antihypertensive medication, lipid-lowering medication, sCD14, sCD163, Gal-3, Gal3-BP, and IL-6 in models. The associations between metabolites and incident carotid artery plaque were further examined in the following 3 strata: HIV-uninfected participants, HIV-infected participants with persistent virologic suppression, and HIV-infected participants without persistent virologic suppression, with generalized score statistics for the interaction tests. Analyses were performed using SAS software, version 9.3 (SAS Institute, Cary, North Carolina), and a 2-sided $P < .05$ was considered statistically significant.

RESULTS

Participant Characteristics

At baseline, HIV-infected (291 women and 229 men) and HIV-uninfected (107 women and 110 men) groups were generally similar in terms of demographic and behavioral variables, although HIV-infected participants were more likely to have previously injected drugs and have a history of HCV infection (Table 1). HIV-infected participants were more likely to use antihypertensive medication (women only) and lipid-lowering medication and had lower BMI (women only) and lower total cholesterol and HDL cholesterol levels. The majority of HIV-infected individuals reported using highly active ART at baseline (74% in women and 83% in men), and 46% of women and 66% of men had undetectable HIV-1 viral load.

Tryptophan, Kynurenic Acid, and HIV Infection

Plasma levels of tryptophan, kynurenic acid, and KYNA/TRP ratio were positively correlated with age (all $P < .001$), higher in men than in women (all $P < .001$), higher in HCV-infected participants than in those without HCV infection (all $P < .001$), and higher in HIV-infected participants than in those without HIV infection (all $P < .001$). After adjusting for age, sex, and HCV infection, HIV-infected participants had lower tryptophan ($P < .001$), higher KYNA/TRP ratio ($P = .01$), and similar kynurenic acid levels ($P = .51$) compared to those without HIV infection. We further examined these metabolites in HIV-infected aviremic (undetectable viral load ≤80 copies/mL) and HIV-infected viremic (viral load >80 copies/mL) individuals compared to those without HIV infection (Figure 1). There was a significantly decreased trend in levels of tryptophan and kynurenic acid across the 3 groups (both $P < .001$), with the lowest levels in HIV-infected viremic participants and the highest levels in HIV-uninfected participants.

Tryptophan, Kynurenic Acid, HIV Infection Parameters, Inflammation and Immune Activation Markers, and CVD Risk Factors

Among HIV-infected participants, CD4+ T-cell counts were positively correlated with tryptophan (r = 0.22; $P < .001$) and inversely correlated with KYNA/TRP ratio (r = −0.10; $P = .006$; Supplementary Figure 1). Tryptophan, kynurenic acid, and KYNA/TRP ratio were more closely correlated with specific monocyte activation and macrophage inflammation markers (serum sCD14, Gal-3), surface markers of CD4+ T-cell activation, and CD8+ T-cell activation and senescence, rather than global inflammation markers (IL-6, CRP) or conventional CVD risk factors (systolic blood pressure, total cholesterol, HDL cholesterol, BMI; Supplementary Figure 1).

Tryptophan, Kynurenic Acid, and Risk of Carotid Artery Plaque

After a median follow-up of 7 years (ranging from 6 to 9 years; 85% of participants had a follow-up of 6.5 to 7.5 years), 112 participants developed carotid artery plaques (IMT >1.5 mm) in any of the 6 imaged carotid artery locations. After adjustment for demographic and behavioral variables (model 1), higher plasma tryptophan was significantly associated with a lower risk of carotid artery plaque (RR, 0.71 [95% CI, 0.62, 0.84] per SD; $P < .001$), while higher plasma kynurenic acid (RR, 1.34 [95% CI, 1.06, 1.69] per SD; $P = .02$) and KYNA/TRP ratio (RR, 1.47 [95% CI, 1.26, 1.73] per SD; $P < .001$) were significantly associated with an increased risk of carotid artery plaque (Table 2). Results were consistent between women and men (all P for interaction > .05), and between HIV-infected and HIV-uninfected participants (all P for interaction > .05).

Table 1. Baseline Characteristics of Participants by Human Immunodeficiency Virus Serostatus

Characteristic	Women		Men	
	HIV+ (N = 291)	HIV− (N = 107)	HIV+ (N = 229)	HIV− (N = 110)
Age, years	42 (38–46)	42 (38–47)	46 (43–50)	47 (45–54)
Race/ethnicity				
White/Other	9	5	56	64
Hispanic	31	27	11	10
African American	60	68	33	26
Education				
Less than high school	40	33	9	5
High school	30	31	14	15
College and above	30	36	77	81
Annual income <$30 000 per year	84	83	52	36
Current smoking	47	60	36	23
Current alcohol use				
Abstainer	57	42	22	10
Light (<3 drinks/week, WIHS; 1–3, MACS)	33	30	54	53
Moderate (3–13, WIHS; 4–13, MACS)	7	19	19	30
Heavier (14+ drinks/week)	3	9	5	7
Current crack/cocaine use	8	18	15	8
History of injection drug use	31	26	11	3
History of hepatitis C infection	34	22	15	6
Body mass index, kg/m^2	27.2 (24.1–31.5)	29.1 (24.8–34.4)	25.1 (22.6–28.1)	25.8 (23.8–28.2)
Systolic blood pressure, mm Hg	115 (106–124)	116 (105–126)	122 (115–129)	126 (117–132)
Diastolic blood pressure, mm Hg	74 (67–81)	72 (66–80)	73 (68–78)	74 (69–80)
Total cholesterol, mg/dL	175 (152–207)	182 (160–203)	187 (156–213)	198 (172–222)
High-density lipoprotein cholesterol, mg/dL	47 (38–57)	56 (46–66)	43 (36–52)	49 (42–58)
Antihypertensive medication use	17	10	16	17
Lipid-lowering medication use	4	0	23	14
HIV-specific characteristics				
CD4+ T-cell count, cells/mm^3	438 (289–619)	n/a	519 (359–689)	n/a
HIV-1 viral load, copies/mL	160 (80–4700)	n/a	40 (40–1280)	n/a
Undetectable viral load (≤80 copies/mL)	46	n/a	66	n/a
Highly active ART use in past 6 months	74	n/a	83	n/a
Cumulative exposure of potent ART, years	3.5 (2.0–6.5)	n/a	4.9 (2.4–7.3)	n/a
of protease inhibitors, years	2.5 (0–4.5)	n/a	2.5 (0–6.1)	n/a
of nonnucleoside reverse transcriptase inhibitors, years	1.0 (0–3)	n/a	1.5 (0–4.3)	n/a
of nucleoside reverse transcriptase inhibitors, years	5.5 (2.5–8.5)	n/a	6.4 (3.1–8.8)	n/a

Data are median (interquartile range) or %, assessed at baseline unless otherwise noted.

Abbreviations: ART, antiretroviral therapy; HIV, human immunodeficiency virus; MACS, Multicenter AIDS Cohort Study; n/a, not applicable; WIHS, Women's Interagency HIV Study.

Further adjustment for HIV infection and related factors (model 2) did not change the significance of the results. After further adjustment for conventional CVD risk factors (model 3), the associations with risk of carotid artery plaque were slightly attenuated but remained significant (all $P < .05$). Even after additional adjustment for 5 inflammation and immune activation markers (model 4), the association results remained significant (all $P < .05$).

We further examined these associations in HIV-infected participants stratified by status of persistent virologic suppression over the study period, along with HIV-uninfected participants (Figure 2). Overall, the associations with risk of carotid artery plaque were generally consistent, in the same directions, across the 3 groups (all P for interaction > .05).

DISCUSSION

While most previous studies have used KYN/TRP ratio [8–13], we evaluated KYNA/TRP ratio as an indicator of tryptophan catabolism [16, 17]. Despite this difference, our data led to the same conclusion of previous non-HIV population studies [8–13], namely, that the ratio of tryptophan and its metabolic products, which indicate tryptophan catabolism, is associated with CVD risk. Moreover, we found consistent association results for KYNA/TRP ratio and progression of carotid artery atherosclerosis between persistently virologically suppressed and unsuppressed individuals. This is in line with our prior work that suggested increased CVD risk in both virologically suppressed and unsuppressed HIV-infected individuals compared to those without HIV infection [15].

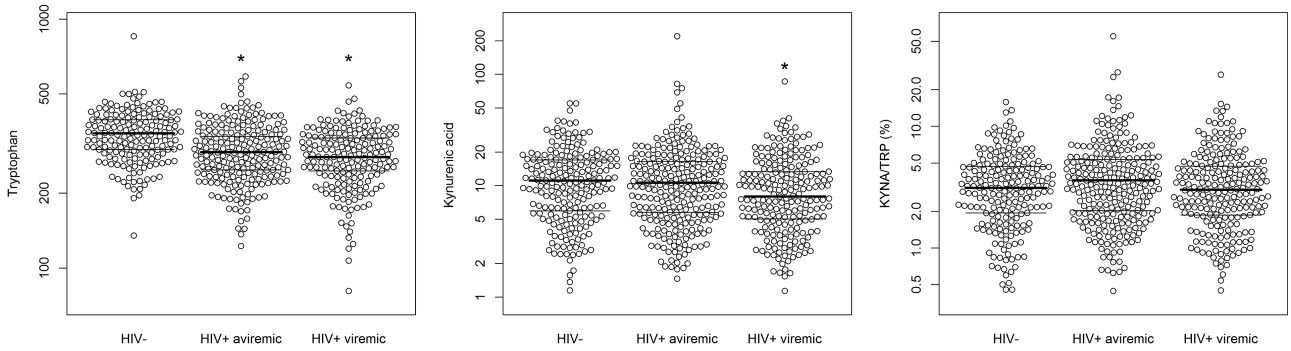

Figure 1. Plasma tryptophan, kynurenic acid and kynurenic acid-to-tryptophan (KYNA/TRP) ratio between human immunodeficiency virus (HIV)–infected and HIV-uninfected participants. Data are raw values (area under the curve of metabolite peaks) of plasma tryptophan, kynurenic acid, and KYNA/TRP ratio in a logarithmic scale among 217 HIV-uninfected, 290 HIV-infected aviremic (undetectable viral load ≤80 copies/mL), and 230 HIV-infected viremic (viral load >80 copies/mL) individuals. *P* for trend <0.001, <0.001, and = 0.86 for tryptophan, kynurenic acid, and KYNA/TRP ratio, respectively, across 3 groups. **P* < .05 for pairwise comparison between HIV+ aviremic group or HIV+ viremic group vs HIV-uninfected group, controlling for multiple testing. Abbreviations: HIV, human immunodeficiency virus; KYNA/TRP, kynurenic acid-to-tryptophan.

Table 2. Associations of Plasma Tryptophan, Kynurenic Acid, and Kynurenic Acid-to-Tryptophan Ratio With Risk of Carotid Artery Plaque

	All		Women	Men		HIV+	HIV−	
	RR (95% CI)	*P* Value	RR (95% CI)	RR (95% CI)	*P* for Interaction	RR (95% CI)	RR (95% CI)	*P* for Interaction
Number of participants	112/737		45/398	67/339		90/520	22/217	
Tryptophan								
Model 1[a]	0.71 (0.62, 0.84)	<.001	0.66 (0.54, 0.80)	0.78 (0.63, 0.96)	.27	0.76 (0.63, 0.91)	0.77 (0.51, 1.14)	.99
Model 2[b]	0.76 (0.65, 0.89)	<.001	0.69 (0.56, 0.84)	0.84 (0.67, 1.04)	.20	0.76 (0.64, 0.90)	0.77 (0.51, 1.14)	.96
Model 3[c]	0.82 (0.69, 0.97)	.02	0.77 (0.62, 0.96)	0.87 (0.70, 1.08)	.45	0.84 (0.70, 1.01)	0.67 (0.44, 1.02)	.38
Model 4[d]	0.81 (0.67, 0.99)	.04	0.78 (0.60, 1.00)	0.85 (0.67, 1.09)	.56	0.82 (0.67, 1.02)	0.75 (0.47, 1.19)	.72
Kynurenic acid								
Model 1[a]	1.34 (1.06, 1.69)	.02	1.49 (1.12, 1.98)	1.13 (0.81, 1.59)	.25	1.32 (1.03, 1.68)	1.42 (0.93, 2.17)	.76
Model 2[b]	1.33 (1.08, 1.64)	.007	1.40 (1.08, 1.81)	1.23 (0.88, 1.70)	.54	1.31 (1.05, 1.65)	1.42 (0.93, 2.17)	.70
Model 3[c]	1.27 (1.03, 1.55)	.02	1.32 (1.03, 1.68)	1.18 (0.85, 1.64)	.60	1.27 (1.03, 1.58)	1.23 (0.79, 1.90)	.86
Model 4[d]	1.29 (1.02, 1.63)	.03	1.37 (1.02, 1.83)	1.16 (0.82, 1.66)	.48	1.32 (1.03, 1.70)	1.13 (0.71, 1.79)	.54
Kynurenic acid-to-tryptophan ratio								
Model 1[a]	1.47 (1.26, 1.73)	<.001	1.54 (1.30, 1.83)	1.33 (0.97, 1.82)	.42	1.40 (1.18, 1.67)	1.57 (1.01, 2.44)	.64
Model 2[b]	1.41 (1.22, 1.63)	<.001	1.44 (1.23, 1.69)	1.34 (0.98, 1.83)	.68	1.39 (1.19, 1.62)	1.57 (1.01, 2.44)	.57
Model 3[c]	1.30 (1.11, 1.53)	.002	1.32 (1.10, 1.59)	1.26 (0.93, 1.72)	.81	1.29 (1.09, 1.54)	1.38 (0.88, 2.16)	.84
Model 4[d]	1.34 (1.10, 1.63)	.004	1.39 (1.10, 1.74)	1.25 (0.90, 1.74)	.60	1.36 (1.11, 1.68)	1.20 (0.74, 1.93)	.62

Data are adjusted RR and 95% CI on risk of carotid artery plaque per standard deviation increment in log-transformed metabolite variables.

Abbreviations: CI, confidence interval; HIV, human immunodeficiency virus; RR, risk ratio.

[a]Adjusted for age, sex, race/ethnicity, education, study site, current smoking, and history of hepatitis C virus.

[b]Further adjusted for human immunodeficiency virus (HIV) serostatus, HIV treatment status, and baseline viral load level.

[c]Further adjusted for systolic blood pressure, total cholesterol, high-density lipoprotein cholesterol, antihypertensive medication use, lipid-lowering medication use, and body mass index (total sample size was reduced to 705 due to missing data on cardiovascular disease risk factors).

[d]Further adjusted for serum levels of soluble(s) CD14, sCD163, galectin-3, Gal-3 binding protein, and interleukin-6 (total sample size was reduced to 673 due to missing data on inflammation and immune activation markers).

It is not surprising that the observed association between disrupted tryptophan catabolism and progression of carotid artery atherosclerosis only slightly changed after adjusting for traditional CVD risk factors, since plasma tryptophan, kynurenic acid, and KYNA/TRP ratio showed very weak correlations with these CVD risk factors. However, it should be noted that diabetes status, which is a risk factor for carotid artery atherosclerosis in this HIV-infected population [15], was not examined as a covariate in the current study, because participants with prevalent diabetes were excluded at baseline. Of note, diabetes status has been found to be associated with increased plasma levels of tryptophan and kynurenines in HIV-uninfected populations, and diabetes status may amplify the association between kynurenines and risk of CVD [26, 27].

We also found the independent association between tryptophan catabolism and carotid artery atherosclerosis after controlling for serum markers of monocyte activation and macrophage inflammation, which have themselves been associated

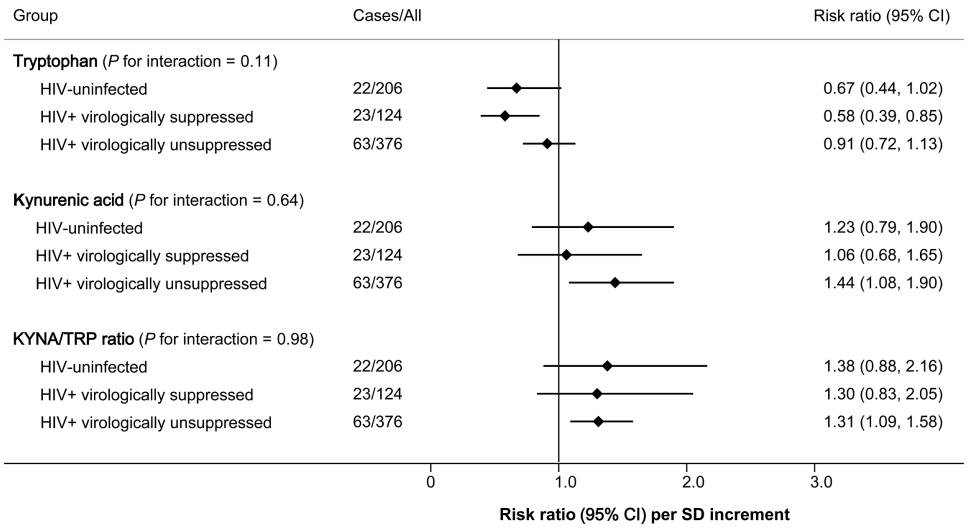

Figure 2. Associations of plasma tryptophan, kynurenic acid, and kynurenic acid-to-tryptophan ratio with risk of carotid artery plaque in human immunodeficiency virus (HIV)–uninfected, HIV+ persistently virologically suppressed, and HIV+ virologically unsuppressed participants. Data are risk ratio (95% confidence interval) on risk of carotid artery plaque per standard deviation increment in log-transformed metabolite variables, adjusted for age, sex, race/ethnicity, education, study site, current smoking, history of hepatitis C virus, systolic blood pressure, total cholesterol, high-density lipoprotein cholesterol, antihypertensive medication use, lipid lowering medication use, and body mass index. HIV+ persistently virologically suppressed participants were defined as participants who had consistent plasma HIV RNA levels <80 copies/mL simultaneous with continuous antiretroviral therapy use over the study period. Abbreviations: CI, confidence interval; HIV, human immunodeficiency virus; KYNA/TRP, kynurenic acid-to-tryptophan; SD, standard deviation.

with CVD risk in HIV infection [23, 28–30]. In this study, serum sCD14, a marker that potentially reflects monocyte and macrophage activation by host inflammatory cytokines and microbial components (eg, lipopolysaccharide) [31, 32], was positively correlated with KYNA/TRP ratio. This is in line with the fact that tryptophan catabolism can be regulated by both host inflammation and microbial exposure–induced IDO-1 activity [4]. Emerging evidence also suggests that gut microbiota altered during HIV infection might contribute to disrupted tryptophan catabolism through encoding tryptophan catabolism–related enzymes rather than through inducing host IDO-1 activity [33].

The role of tryptophan-kynurenine metabolites in the development of CVD remains unclear. As the precursor to serotonin and melatonin, tryptophan may contribute to CVD risk through depression and sleep disturbance [34, 35]. Kynurenic acid and other downstream kynurenines have been examined in few studies. In support of our results, Pedersen et al [26] found that higher plasma levels of kynurenic acid, along with the other 3 kynurenines, were associated with increased risk of acute myocardial infarction in patients with stable angina pectoris. However, in another study, it was reported that plasma kynurenine and 3-hydroxykynurenine, but not kynurenic acid or other kynurenines, were associated with risk of acute coronary events in community-dwelling elderly individuals [27]. Data from experimental studies are controversial [16, 36, 37]. A recent study demonstrated that kynurenic acid, but not kynurenine, may promote atherosclerotic plaque by inhibiting IL-10 production in mice, which may explain the relationship between high IDO-1 activity and atherosclerosis [16].

To the best of our knowledge, this is the first study in HIV-infected individuals to report significant associations between plasma levels of tryptophan catabolism–related metabolites and subclinical carotid atherosclerosis in a prospective analysis. Given the moderate magnitude of our effect estimates (eg, a 29% decreased risk of carotid plaque per SD increment in tryptophan; a 47% increased risk of carotid plaque per SD increment in KYNA/TRP ratio) and lack of data on incident CVD in this HIV-infected population, our findings should be interpreted with caution. A few previous studies in non-HIV populations have suggested that tryptophan catabolism–related metabolites serve as predictors for incident CVD [13, 14, 26, 27]. For example, among 2380 individuals with stable coronary artery disease, each SD increment of KYN/TRP ratio was associated with a 28% increased risk of major coronary events [14]. However, the stability of these metabolite levels in the prediction of CVD remains unclear as data on repeated measurements of these metabolites over time are limited. In a diet intervention trial, Yu et al [38] found that plasma levels of tryptophan and related metabolites changed after participants had been on a Mediterranean diet for 1 year. Furthermore, increases in tryptophan after 1-year diet intervention were associated with a lower risk of CVD. Thus, the possible utility of tryptophan and related metabolites as potential targets for interventions deserves further study. Indeed, in HIV-infected individuals, ART and niacin treatment have been found to reduce tryptophan catabolism and increase tryptophan levels [39], although it is unknown whether these changes are associated with CVD risk.

Our study has several limitations. Plasma kynurenine and several other downstream metabolites could not be measured using

our liquid chromatography–mass spectrometry in the positive ion mode. However, it has been suggested that kynurenic acid, not kynurenine, may reflect tryptophan consumption and promote atherosclerosis [16]; kynurenic acid along with other downstream kynurenines showed consistent associations with CVD risk [26]. We did not have a case group with incident clinical CVD events, but our subclinical measure of carotid artery atherosclerosis has been validated as a surrogate outcome of clinical CVD events [20]. While our results were consistent in 2 cohorts of women and men with HIV infection, replication of our findings in other HIV cohorts would provide further validation. Although there was little evidence of metabolite–HIV interactions, our study did not have enough power to determine effect modification by HIV serostatus. Given the current sample size, we had 80% power to detect interaction RRs of 2.0, 2.3, and 2.2 for carotid artery plaque per SD increment in tryptophan, kynurenic acid, and KYNA/TRP ratio, respectively, with the statistical significance of 0.05. The observed differences in RRs of metabolites on carotid artery plaque between individuals with and without HIV infection were much smaller than those estimated in power calculation.

In summary, in this study from 2 HIV cohorts, we found that lower plasma tryptophan and higher levels of kynurenic acid and KYNA/TRP ratio were associated with progression of carotid artery atherosclerosis, suggesting a potential role of tryptophan catabolism and kynurenine pathway metabolites in atherogenesis and the development of CVD. Further studies are warranted to clarify underlying mechanisms and examine whether interventions that target these metabolites may help prevent CVD in HIV-infected individuals.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Acknowledgments. Data for this article were collected as part of the WIHS.

Disclaimer. The contents of this article are solely the responsibility of the authors and do not represent the official views of the National Institutes of Health (NIH).

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