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Low Neuroactive Steroids Identifies a Biological Subtype of Depression in Adults with Human Immunodeficiency Virus on Suppressive Antiretroviral Therapy

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Background. The prevalence and mortality risk of depression in people with human immunodeficiency virus (HIV) infection receiving antiretroviral therapy (ART) is higher than in the general population, yet biomarkers for therapeutic targeting are unknown. In the current study, we aimed to identify plasma metabolites associated with depressive symptoms in people with HIV receiving ART.

Methods. This is a prospective study of ART-treated HIV-infected adults with or without depressive symptoms assessed using longitudinal Beck Depression Inventory scores. Plasma metabolite profiling was performed in 2 independent cohorts (total n = 99) using liquid and gas chromatography and tandem mass spectrometry.

Results. Participants with depressive symptoms had lower neuroactive steroids (dehydroepiandrosterone sulfate [DHEA-S], androstenediols, and pregnenolone sulfate) compared with those without depressive symptoms. The cortisol/DHEA-S ratio, an indicator of hypothalamic-pituitary-adrenal axis imbalance, was associated with depressive symptoms ($P < .01$) because of low DHEA-S levels, whereas cortisol was similar between groups. The odds of having depressive symptoms increased with higher cortisol/DHEA-S ratios (adjusted odds ratio, 2.5 per 1-unit increase in z score; 95% confidence interval, 1.3–4.7), independent of age and sex. The kynurenine-to-tryptophan ratio showed no significant associations.

Conclusions. These findings suggest that altered neuroactive steroid metabolism may contribute to the pathophysiological mechanisms of depression in ART-treated HIV-infected adults, representing a potential biological pathway for therapeutic targeting.

Keywords. depression; HIV; neuroactive steroids; acylcarnitines; metabolomics; HPA axis; DHEA.

Depression is a major challenge for people with human immunodeficiency virus (HIV) infection (PWH), with prevalence estimates of 20%–45% [1–5]. A study of patients receiving clinical care in the United States from 2009 to 2014 showed that 27% of HIV-infected adults prescribed antiretroviral therapy (ART) are diagnosed with current depression, which is 3-fold higher than the prevalence in the general population [1, 4]. Depressive symptoms are associated with delayed ART initiation, worse adherence, and less virological suppression in HIV-infected cohorts [3, 5–8]. Depressive symptoms are also associated with higher mortality rates in adults with HIV infection [6–8]. Thus, understanding psychosocial and biological determinants that underlie depression during suppressive ART is critical to optimizing clinical care for PWH.

Depression is phenotypically heterogeneous, and its pathophysiological mechanisms remain poorly defined. Current first-line antidepressant therapies target the monoamine system, but evidence suggests that inflammation, hypothalamic-pituitary-adrenal (HPA) axis dysregulation, and stress responses also contribute to depressive symptoms [3, 7]. A biological pathway previously implicated in depression among PWH involves induction of indoleamine 2,3-dioxygenase-1, which leads to shunting of tryptophan away from serotonin synthesis and toward kynurenine production. The kynurenine-to-tryptophan (K/T) ratio, an indicator of increased activity of the kynurenine pathway and tryptophan catabolism, is associated with depression in cancer [9], inflammatory diseases [10], postpartum status in women [11], and HIV infection [12–15]. Depressive symptoms improve after ART initiation, an effect partially mediated by an increase in tryptophan levels and decrease in K/T

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ratio [13]. However, the relationship between the kynurenine pathway and depressive symptoms in chronic HIV infection is mixed, with some studies showing high K/T ratio [12, 14, 15] and others finding no statistically significant association with K/T ratio [16] in virally suppressed individuals with depression.

Dysregulation of the HPA axis is common in PWH [17–19] and has been implicated in the pathophysiological mechanisms of depression [20–23]. Dehydroepiandrosterone (3β-hydroxy-5-androstene-17-one; DHEA) and its sulfated metabolite (DHEA-S) are abundant endogenous steroids regulated by HPA axis signaling [24]. DHEA, DHEA-S, and other HPA axis steroid metabolites may contribute to depression through mechanisms regulating neurotransmitter receptors and are considered neuroactive steroids because they influence neuronal excitability and have anxiolytic, analgesic, and other neuropsychological effects [24–26].

In untreated HIV infection, DHEA levels correlate inversely with disease progression [17, 27], independent of $CD4^+$ T-cell counts [28]. Levels of sulfated neuroactive steroids (DHEA-S, pregnenolone sulfate, and androstene sulfate) are also lower in HIV-infected patients than in uninfected controls [29]. Preclinical evidence suggests that targeting neuroactive steroids is effective in models of anxiolysis (reviewed in [30]) and depressed mood in the general population [25, 30]. Importantly, an intravenous formulation of the neuroactive steroid allopregnanolone was recently approved in the United States to treat postpartum depression and may have implications for other biological subtypes of depression [31].

The use of reliable biomarkers in depression is critical to improving diagnosis and can provide mechanistic insight and potential targets for intervention. A prior untargeted metabolomics study identified alterations in monoamines, acylcarnitines, and K/T ratio in a mixed population of ART-treated and untreated adults with or without depressive symptoms [14]. In the current study, we used untargeted metabolite profiling in 99 plasma samples across 2 independent cohorts to identify metabolic pathways contributing to chronic depression in virally suppressed PWH on ART.

METHODS

Study Population and Depressive Symptom Classification

Longitudinal clinical data, Beck Depression Inventory (BDI) scores, and plasma samples from HIV-infected adults were obtained from the National NeuroAIDS Tissue Consortium (NNTC) and the HIV Neurobehavioral Research Center. All study procedures were approved by each site's institutional review board, and all participants provided written informed consent. The inclusion criteria were HIV infection, current ART, and age >35 years (to reduce confounding by age). All participants had ≥2 BDI scores, including ≥1 assessment with metabolite profiling within 12 months. Participants with BDI scores ≤13 at all assessments were classified as having low depressive symptoms, and those with BDI scores >20 as having high depressive symptoms [32]. Participants whose BDI scores were not consistently ≤13 or >20 were excluded. Additional exclusion criteria were plasma viral load ≥1000 copies/mL within 90 days of metabolite profile (93 participants [94%] had a viral load <200 copies/mL) and estimated glomerular filtration rate <60 mL/min (calculated using the Modification of Diet in Renal Disease equation [33]), because kidney function strongly influences levels of many circulating metabolites (Figure 1). Cardiovascular disease (CVD) and/or stroke were included in descriptive tables if reported before the metabolite profile.

Metabolite Profiling, Data Processing, and Statistical Analysis

Plasma metabolite profiles obtained from 55 NNTC participants comprised the discovery set (calendar period 2000–2013), whereas profiles from an additional 44 NNTC or HIV Neurobehavioral Research Center participants (calendar period 2006–2016) were used to replicate the accuracy of models. Metabolite profiles were obtained as part of ongoing studies on HIV-associated comorbid conditions, and profiling was performed using ultra-high-performance liquid chromatography and tandem mass spectrometry and gas chromatography/mass spectrometry (Metabolon, Inc., [Durham, NC]; Supplementary Methods) [14, 29]. Data sets were assayed in separate experimental batches, and assayed by research personnel blinded to depressive symptoms.

A subset of participants (72 of 99 [73%]) had >1 available metabolite profile within 12 months of a BDI score. We used the metabolite profile closest to the highest BDI score when the participant was classified as having high depressive symptoms, and the profile closest to the lowest BDI score when the participant was classified as having low depressive symptoms. One metabolite profile per participant was used in analyses. We used the initial data set of 55 participants to identify metabolites that differentiated between high and low depressive symptoms (discovery) and the subsequent 44 participant data set to replicate findings (validation). A total of 1016 and 1442 metabolites were identified in the discovery and validation cohort, respectively (Figure 1). After quality control, data extraction, and normalization steps (Supplementary Methods and Supplementary Table 1) [14, 29], a total of 501 (discovery) and 561 (validation) metabolites were considered for inclusion; of these, 447 metabolites were identified in both data sets.

To standardize values between data sets assayed in separate batches, a z score for metabolites was calculated using means and pooled standard deviations. R (version 3.5.1) [34] and SAS (version 9.4; SAS Institute) software was used for data manipulation and analyses. Additional statistical tools are described in

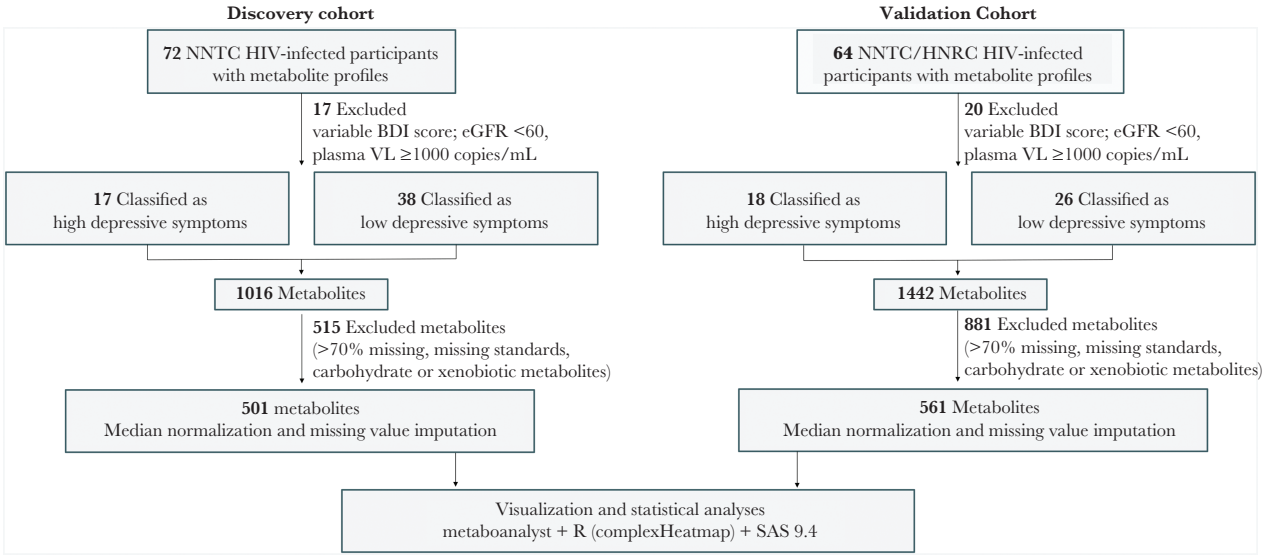

Figure 1. Schematic of the workflow used to define study populations and identify metabolites altered in human immunodeficiency virus (HIV)–infected cohorts with or without high depressive symptoms, in discovery and validation cohorts. Abbreviations: BDI, Beck Depression Inventory; eGFR, estimated glomerular filtration rate; HNRC, HIV Neurobehavioral Research Center; NNTC, National NeuroAIDS Tissue Consortium; VL, viral load.

the Supplementary Methods. Correction for multiple hypotheses testing was performed by calculating the false discovery rate (FDR), and a threshold was set at $P < .05$ and FDR < 0.15 [35, 36].

RESULTS

Clinical Characteristics

The discovery and validation cohorts included 55 participants (median age [interquartile range (IQR)], 50 [47–58] years; 75% male) and 44 participants (49 [47–58] years; 84% male), respectively (Supplementary Table 2). Their median (IQR) $CD4^+$ T-cell counts (373/μL [195–550] vs 451/μL [246–707/μL], respectively; $P = .23$) and $CD4^+$ T-cell nadirs (66/μL [22–154/μL] vs 28/μL [I9–152/μL]; $P = .17$) and their mean (standard deviation) durations of HIV infection (16 [7] years vs 17 [7] years; $P = .53$) did not differ significantly between cohorts.

Seventeen and 38 participants were classified as having high (median [IQR] BDI, 32 [25–35]) or low (2.5 [0–6]) depressive symptoms, respectively, in the discovery cohort; median BDI scores were similar in the validation data set (Table 1). In participants with high depressive symptoms, 47% (discovery) and 44% (validation) reported use of antidepressants. Although participants with high versus low depressive symptoms were more likely to be prescribed efavirenz and have higher total blood cholesterol in the validation cohort ($P = .03$ for both), these differences were not observed in the discovery cohort. In addition, the numbers of participants with HIV-associated neurocognitive disorders or CVD did not different significantly between groups in both data sets (Table 1 and Supplementary Table 2).

Low Neuroactive Steroid Levels Associated With Depressive Symptoms in HIV-Infected Adults

In the discovery cohort, the most common metabolite categories were lipids (244 of 447 [55%]) and amino acids (171 of 447 [38%]). Fifty-eight plasma metabolites exhibited significant mean differences between participants with high versus low depressive symptoms, of which 59% were related to lipids or fatty acid metabolism (Figure 2A). Eleven metabolites with significant differences in the discovery cohort also had significant mean differences in the validation set ($P < .05$; t test) (Figure 2A, boldface font), and were down-regulated in participants with high compared with low depressive symptoms in both cohorts. These included 3 neuroactive steroids (pregnenolone sulfate, pregnanediol-3-glucuronide, and DHEA sulfate [DHEA-S]), 5 direct DHEA metabolites (androstenediol [3β,17β] monosulfate [1 and 2] and disulfate [1 and 2] and 5α-androstane-3β,17β-diol monosulfate), and 3 acylcarnitines (acetylcarnitine, octanoylcarnitine, and palmitoylcarnitine). Box plots for 9 of 11 metabolites with the largest mean differences between groups are shown in Figure 2B.

Given prior reports that the kynurenine pathway is associated with depression, we tested the relationship between depressive symptoms and kynurenine, tryptophan, and K/T ratio. Contrary to expectations, we did not detect differences between these metabolites, K/T ratio, and depressive symptoms in either cohort (Supplementary Figure 1 and Supplementary Table 3).

Elevated Cortisol/DHEA-S Ratio and Low DHEA-S Levels in Participants With Depressive Symptoms

The cortisol/DHEA-S ratio is a proposed indicator of HPA axis imbalance [21–23]. Therefore, we generated metabolite ratios of cortisol to pregnenolone sulfate, DHEA-S, or androstenediol

Table 1. Clinical Characteristics Stratified by Depressive Symptoms

Characteristic	Discovery Cohort		Validation Cohort	
	Low Depressive Symptoms (n = 38)	High Depressive Symptoms (n = 17)	Low Depressive Symptoms (n = 26)	High Depressive Symptoms (n = 18)
Demographic				
Age, median (IQR), y	52 (48–61)	53 (47–60)	49 (46–57)	49 (47–56)
Race, no. (%)				
Black	9 (24)	5 (30)	7 (27)	6 (33)
White	17 (45)	9 (53)	15 (58)	10 (57)
Male sex, no. (%)	28 (74)	13 (77)	23 (89)	14 (78)
Educational level, median grade (IQR), y	12 (11–13)	13 (12–16)	12 (12–14)	13 (12–14)
HIV-specific parameters				
T-cell count, median (IQR), cells/μL				
$CD4^+$	360 (219–525)	399 (168–603)	362 (217–653)	617 (384–726)
$CD8^+$	805 (572–1329)	934 (705–1126)	899 (695–1104)	973 (747–1147)
$CD4^+$ nadir	74 (22–154)	66 (24–114)	14 (6–45)[c]	142 (33–215)[c]
Plasma VL, median (IQR) copies/mL[a]	40 (40–40)	40 (40–103)	40 (40–40)	40 (40–40)
CNS penetration effectiveness score, mean (SD)	8 (2)	9 (2)	9 (2)[c]	7 (1)[c]
Efavirenz use, no. (%)	8 (21)	2 (12)	3 (12)[c]	8 (44)[c]
Estimated duration of HIV, mean (SD), y	16 (7)	17 (6)	17 (6)	17 (8)
Neurological characteristics				
BDI score, median (IQR)	3 (0–6)[c]	32 (25–35)[c]	1 (0–5)[c]	23 (22–31)[c]
Current antidepressant use, no. (%)	9 (24)	8 (47)	4 (15)	8 (44)
SSRI or SNRI use, no. (%)	7 (18)	7 (41)	3 (12)	6 (33)
Current HAND diagnosis, no. (%)[b]	10 (26)	6 (35)	6 (23)	7 (39)
Current MND or HAD, no. (%)	5 (13)	5 (29)	1 (3.8)	3 (16)
Other clinical characteristics				
BMI, mean (SD), kg/m^2	27 (6)	23 (4)	25 (4)	24 (6)
CVD, no. (%)	10 (26)	4 (24)	1 (4)	4 (22)
Current hyperlipidemia, no. (%)	12 (32)	3 (18)	6 (23)	6 (33)
Mean level (SD), mg/dL				
Total cholesterol	182 (44)	174 (41)	165 (34)[c]	189 (22)[c]
LDL cholesterol	104 (40)	100 (19)	87 (28)	106 (29)
HDL cholesterol	42 (12)	52 (10)	46 (18)	51 (19)
Triglycerides	237 (133)	216 (102)	165 (97)	159 (67)
Statin use, no. (%)	8 (21)	2 (12)	3 (12)	6 (33)
HCV Ab and RNA positive, no. (%)	5 (13)	1 (6)	1 (4)	3 (17)
eGFR, mean (SD)[d]	80 (23)	76 (22)	86 (27)	88 (19)
Current cocaine use, no. (%)	5 (13)	4 (24)	5 (19)	2 (11)
Current smoker, no. (%)	22 (58)	9 (53)	13 (50)	12 (67)

Abbreviations: Ab, antibody; BDI, Beck Depression Inventory; BMI, body mass index; CNS, central nervous system; CVD, cardiovascular disease; eGFR, estimated glomerular filtration rate; HAD, HIV-associated dementia; HAND, HIV-associated neurocognitive disorders; HCV, hepatitis C virus; HDL, high-density lipoprotein; HIV, human immunodeficiency virus; LDL, low-density lipoprotein; MND, mild neurocognitive disorder; SD, standard deviation; SNRI, serotonin and norepinephrine reuptake inhibitor; SSRI, selective serotonin reuptake inhibitor; VL, viral load.

[a]The VL was <200 copies/mL in 51 participants (93%) and 42 participants (95%) in the discovery and validation cohort, respectively.

[b]HAND diagnosis includes asymptomatic neurocognitive impairment, MND, or HAD.

[c]*P* value < .05 for difference between low and high depressive symptoms within a cohort.

[d]eGFR calculated using the Modification of Diet in Renal Disease Study equation [33].

(3β,17β) monosulfate (1) (androstene) to examine the association between HPA axis imbalance and depressive symptoms in the 2 cohorts. As highlighted in Figure 3A, significantly down-regulated metabolites (log_2 fold-change, ≤ −1.2; $P < .05$; FDR, <0.15) in participants with high versus low depressive symptoms included DHEA-S ($P < .001$ for both discovery and validation cohorts) and androstene neuroactive steroids ($P < .001$ for both cohorts). In contrast, cortisol/DHEA-S and cortisol/androstene ratios were markedly elevated in the high depressive symptom group (log_2 fold-change ≥ 1.2; $P < .05$; FDR, <0.15); a similar trend was observed for the cortisol/pregnenolone sulfate ratio (Supplementary Table 4).

High cortisol levels have been associated with depressive symptoms and contribute to high cortisol/DHEA-S ratio in HIV-uninfected adults with depression [21, 23]. In our findings, mean cortisol levels were similar between groups in the discovery and validation data set ($P > .5$ for both cohorts; Figure 3B), suggesting that high cortisol/DHEA-S ratio (and other ratios) primarily reflect

Figure 2. Low neuroactive steroids and acylcarnitines distinguish between human immunodeficiency virus (HIV)–infected adults with high versus low depressive symptoms. *A,* Metabolites significantly altered ($P < .05$; *t* test) between participants with high versus low depressive symptoms in the 2 cohorts (right). Arrows indicate direction of mean differences; boldface font denotes 11 metabolites significantly altered in both cohorts. *B,* Box plots show mean differences for 9 of the 11 overlapping metabolites altered in participants with high versus low depressive symptoms. Dashed horizontal bars represent medians; error bars, interquartile ranges. Two overlapping metabolites (5α-androstan-3β,17β-diol monosulfate [2] and androstenediol [3β,17β] disulfate [2]) are not shown. These metabolites have the same chemical formulas and expected mass as metabolites shown with (1), with minor differences in the mass-to-charge ratio. Abbreviation: DHEA-S, dehydroepiandrosterone sulfate. *P* values are displayed for each comparison.

differences in neuroactive steroids and not cortisol levels. To further evaluate the ability of cortisol/DHEA-S ratio to distinguish between subjects with high and low depressive symptoms, we calculated *z* scores to standardize values between cohorts and computed the area under the receiver operating characteristic curve (AUC) from logistic regression models. Cortisol/DHEA-S *z* scores discriminated between participants with high versus low depressive symptoms with an AUC of 0.70 ($P = .03$) and 0.80 ($P = .02$) for the discovery and validation cohorts, respectively (Figure 3C). When the cortisol/DHEA-S *z* score was coupled with androstene and pregnenolone sulfate metabolites in regression models, discrimination improved, with AUCs of 0.81 and 0.85, respectively.

Independent Association Between Cortisol/DHEA-S Ratio and High Depressive Symptoms

DHEA-S levels decline with age and differ between men and women [24]. Age was correlated weakly with cortisol/DHEA-S ratio when we analyzed a combined discovery and validation data set ($r = 0.23$; $P = .02$), with stronger correlations seen among the subset of participants with high depressive symptoms ($r = 0.42$; $P = .02$) (Supplementary Figure 2). In regression models, female sex was associated with a trend toward higher cortisol/DHEA-S ratios in the combined cohort ($\beta = 0.42$; $P = .10$); a test of interaction between sex and depressive symptoms was not statistically significant ($P = .34$). Given that prior studies identified associations between HPA axis activity and antidepressants, we tested the relationship between cortisol/DHEA-S levels and antidepressant use in participants with or without depressive symptoms. Mean cortisol/DHEA-S levels were highest among participants with high depressive symptoms off antidepressants, lower among those taking antidepressants, and lowest in those without depressive symptoms and no antidepressants ($P < .01$; analysis of variance) (Supplementary Figure 3). There was no evidence of a difference in cortisol/DHEA-S levels between

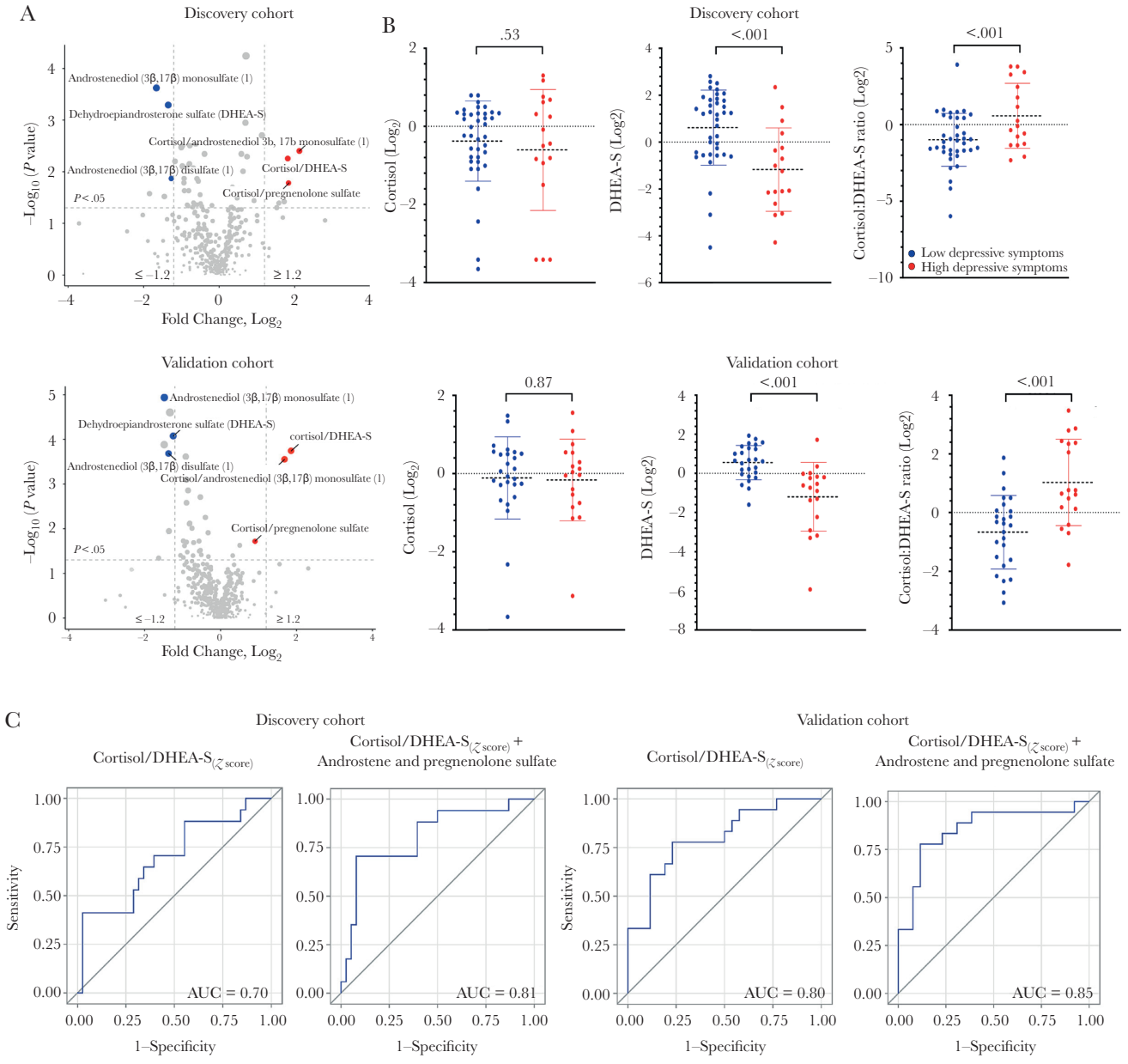

Figure 3. High cortisol/dehydroepiandrosterone sulfate (DHEA-S) ratio is associated with high depressive symptoms in human immunodeficiency virus (HIV)–infected adults receiving antiretroviral therapy. *A,* Volcano plots in the discovery (*top*; n = 55) and validation (*bottom*; n = 44) sets, illustrating statistical significance of the fold change for neuroactive steroid metabolites (*blue data points*) and cortisol/steroid ratios (*red data points*) in participants with high versus low depressive symptoms. *B,* Beeswarm plots illustrating individual participant levels of cortisol (*left panels*), DHEA-S (*middle panels*), and cortisol/DHEA-S ratios (*right panels*) in participants with low versus high depressive symptoms in discovery (*top panels*) and validation (*bottom panels*) cohorts. DHEA-S levels are lower in participants with high versus low depressive symptoms, while cortisol levels are similar. Low DHEA-S accounts for high cortisol/DHEA-S ratios observed in participants with high depressive symptoms ($P < .01$). Horizontal bars represent means; error bars, standard deviations. Significance was calculated using unpaired *t* tests, and *P* values are shown for comparisons. *C,* Receiver operating characteristic (ROC) curve from logistic regression models assessing classification of participants with low versus high depressive symptoms using cortisol/DHEA-S ratio (*z* score) or cortisol/DHEA-S ratio with androstenediol (3β,17β) monosulfate (1) (androstene) and pregnenolone sulfate in the discovery (*left panels*) and validation (*right panels*) cohorts. Abbreviation: AUC, area under the ROC curve.

participants taking antidepressants with high compared with low depressive symptoms ($P = .66$; post hoc Tukey test). The relationship between cortisol/DHEA-S levels and ritonavir use was tested in view of data on ritonavir and HPA axis dysregulation [17, 29], and no significant correlations were identified (data not shown).

We further examined the relationship between depressive symptoms and cortisol/DHEA-S ratio using logistic regression analyses, accounting for age and sex. In this regression model, increasing cortisol/DHEA-S level was associated with increased odds of high depressive symptoms (adjusted odds ratio, 2.5 [95% confidence interval, 1.3–4.7] per 1-unit increase in *z*

score), whereas age and sex were not associated with depressive symptoms (Table 2 and Supplementary Figure 4). In addition, increasing cortisol/DHEA-S levels were associated with a trend in increased odds of high depressive symptoms (adjusted odds ratio: 1.9 [95% confidence interval, .95–3.94] per 1-unit increase in z score; P = .07) with adjustment for androstene, pregnenolone sulfate, age, and sex.

Interrelationships Between Neuroactive Steroids, Acylcarnitines, and K/T Ratio

Inflammatory conditions can suppress normal HPA function and lead to low DHEA-S levels, whereas HPA axis imbalance can increase immune dysfunction and tryptophan catabolism [10, 37, 38]. In the current study, cortisol/DHEA-S ratio was weakly correlated with K/T ratio (r = 0.20; P = .02; data not shown). To further understand interrelationships between neuroactive steroids and acylcarnitines that showed group differences in both cohorts, cortisol/DHEA-S ratio, and K/T ratio, we used unsupervised hierarchical clustering in the combined cohort (Figure 4A). The top-level clusters based on metabolite profiles grouped most participants with high depressive symptoms in cluster 1 (30 of 35 [86%]), and cluster 2 predominantly included participants with low depressive symptoms (38 of 43 [88%]). A defining feature of cluster 2 was low cortisol/DHEA-S ratio and high levels of neuroactive steroids and acylcarnitines. In contrast, participants in cluster 1 exhibited high cortisol/DHEA-S ratio, low levels of neuroactive steroids, and variable levels of acylcarnitines and represented a heterogeneous population with or without depressive symptoms. K/T ratio did not have clear associations with either cluster.

An overview of altered metabolites and the HPA axis biochemical pathway is shown in Figure 4B. Given that DHEA-S induces peroxisome proliferation and increases expression of peroxisome proliferator-activated receptor α (PPARα), which is involved in fatty acid β-oxidation [24, 39, 40], Figure 4B shows a schematic model that could explain a relationship between DHEA and acylcarnitines via mechanisms involving PPARα-mediated activity and fatty acid β-oxidation.

Table 2. Factors Associated With High Depressive Symptoms in Logistic Regression Models

	OR (95% CI; *P* Value)[a]	
Variable (n = 99)	Univariable	Multivariable
Age (y)	0.99 (.94–1.03; .59)	0.96 (.91–1.01; .17)
Female sex	1.16 (.5–2.68; .77)	0.83 (.33–2.10; .74)
Cortisol/DHEA-S ratio (*z* score)	2.19 (1.24–3.8; .02)	2.46 (1.29–4.67; .02)

Abbreviations: CI, confidence interval; DHEA-S, dehydroepiandrosterone sulfate; OR, odds ratio.

[a]To standardize values between cohorts, *z* scores were calculated using means and pooled standard deviations.

DISCUSSION

Depressive disorders among PWH are heterogeneous, with underlying pathophysiological mechanisms that include relationships between HPA axis dysfunction, immune regulation, and comorbid diseases, in addition to social and economic vulnerabilities [3, 7]. Data presented here represents one of the largest studies of the plasma metabolome in HIV-infected adults with or without depressive symptoms on ART, most of whom have been living with HIV for >15 years (Supplementary Table 2) and are at high risk for a diagnosis of depression [4]. We showed that alterations in neuroactive steroids and fatty acid β-oxidation discriminate between HIV-infected adults with chronic high versus low depressive symptoms across independent cohorts. Levels of neuroactive steroids that include DHEA-S and androstenediols were reduced in participants with high depressive symptoms, whereas there were no significant differences in kynurenine, tryptophan, or K/T ratio. The cortisol/DHEA-S ratio, in combination with other neuroactive steroid metabolites, distinguished between participants with high and those with low depressive symptoms with good accuracy (AUC, 0.81 and 0.85 in discovery and validation cohorts, respectively), and high cortisol/DHEA-S ratios were independently associated with depressive symptoms after adjustment for age and sex. Collectively, these data show that HPA axis imbalance contributes to chronic depression in ART-treated HIV infection and may represent a biological subtype ("biotype") of depression.

Neuroactive steroids are cholesterol-derived compounds synthesized in the adrenal glands, gastrointestinal tract, and gonads [24–26] and can be transported across the blood-brain or blood–cerebrospinal fluid barrier or produced de novo in the brain [26]. Low DHEA levels have been previously shown in PWH, but earlier studies were conducted primarily in patients with advanced disease ($CD4^+$ T-cell count <100/μL) and incomplete viral suppression [17–19]. In this context, a pattern of high cortisol and low DHEA synthesis was thought to be driven by increased conversion of pregnenolone to cortisol via the 17-OH progesterone pathway, limiting the bioavailability of pregnenolone. Alternative mechanisms included reduced DHEA biosynthesis due to 17,20 lyase dysfunction [17]. Although we observed low DHEA-S and androstenediol levels, we found no significant differences in cortisol levels. This is in contrast to findings of published studies suggesting that high cortisol levels are associated with depression in the general population [22, 42].

It is possible that DHEA biosynthesis is affected and has an impact on depressive symptoms in a subset of HIV-infected adults receiving ART, whereas shunting toward cortisol synthesis is not a predominant factor. In small randomized double-blind placebo-controlled trials, exogenous administration of oral DHEA for treatment of depression has proved beneficial

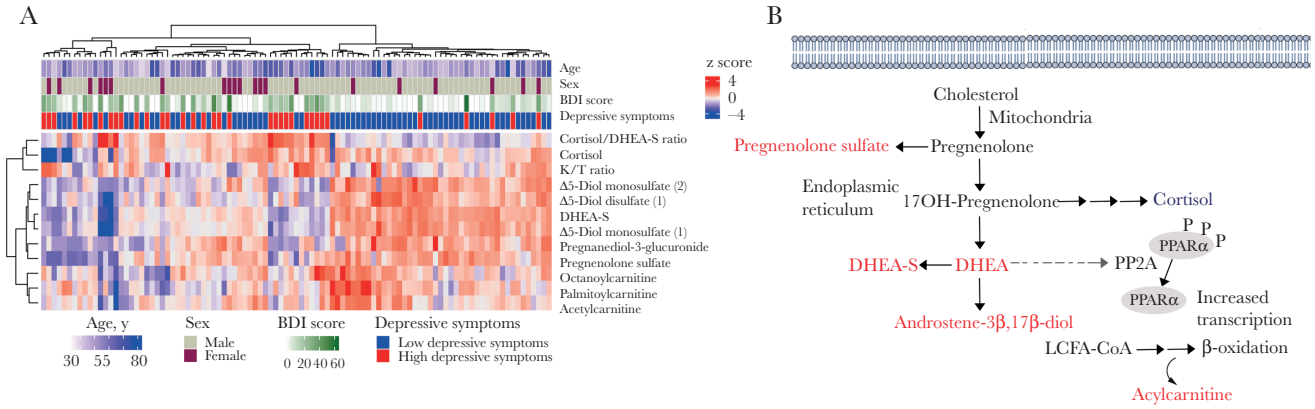

Figure 4. Interrelationships between neuroactive steroids, acylcarnitines, and the kynurenine-to-tryptophan (K/T) ratio in human immunodeficiency virus (HIV)–infected adults with or without depressive symptoms. *A,* Heat map from unsupervised hierarchical clustering analysis of the combined cohort (n = 99). Included metabolites were altered in discovery and validation cohorts in participants with high versus low depressive symptoms ($P < .05$), K/T ratio, and cortisol. Columns represent individual participants; rows, metabolites. Color scale indicates relative expression levels of metabolites using *z* score transformation across all samples. Dendrograms indicate sample cluster (*top*) and metabolite cluster (*left*). Age, sex, Beck Depression Inventory (BDI) score, and depression classification are annotated above the heat map (*top bars, columns*) according to the legend. *B,* Schematic model of the hypothalamic-pituitary-adrenal axis biochemical pathway with metabolites that had significant differences between participants with high versus low depressive symptoms shown in red (*decreased*) and blue (*no change*). In vitro, DHEA activates protein phosphatase 2a (PP2A) (*dashed gray arrow*), leading to increased proliferator-activated receptor α (PPARα)–mediated gene transcription and downstream fatty acid β-oxidation. Long chain acylcarnitines are converted to large chain fatty acids with acyl coenzyme A (LCFA-coA), which then enters the β-oxidation cycle to yield free carnitine and, under certain conditions, acylcarnitines [41].

[43–46], including in PWH and those with nonmajor depression [20]. However, baseline DHEA-S levels failed to distinguish between responders and nonresponders to DHEA treatment [20, 43, 45], suggesting that mechanisms underlying DHEA antidepressant effects are more complex than simple correction of a deficient state [43]. Future longitudinal cohort studies will help determine the utility of low neuroactive steroids or high cortisol/DHEA-S in predicting chronic depression and therapeutic response in HIV infection.

DHEA and androstenediols have multiple putative mechanisms of action including activation of PPARs, such as PPARα [24, 47]. PPARα is a ligand-activated transcription factor and critical regulator of genes involved in mitochondrial and peroxisomal β-fatty acid oxidation and lipid homeostasis [40, 48]. High doses of DHEA-S increase the number and size of peroxisomes in animal models [39]. In addition, DHEA decreases PPARα phosphorylation via effects on the protein phosphatase 2A, which leads to increased PPARα transcriptional activity in vitro (Figure 4B) [24, 47]. Although the current study does not provide direct mechanistic evidence that DHEA-S affects PPAR activation, low DHEA-S levels may decrease peroxisomal enzymes, leading to aberrant acylcarnitine metabolism and fatty acid β-oxidation.

This study did not show a relationship between kynurenine, tryptophan, or K/T ratio and depressive symptoms, in contrast to several published reports [13, 14]. In a prior cohort investigating the metabolome of HIV-infected adults, K/T ratio was associated with depression and severity of depressive symptoms [14]. That study included participants not receiving ART, and the majority were HCV coinfected, in contrast to data presented here. In a separate study of treatment-naive HIV-infected adults starting ART, there were modest correlations between K/T ratio and depressive symptoms, as the authors noted [13]. One possibility for the lack of association between K/T ratio and depressive symptoms in our study is that the power to detect all but the largest associations was low, given the relatively small sample size. Alternatively, tryptophan catabolism may contribute to depressive symptoms in untreated or newly HIV-infected individuals, whereas biological factors other than tryptophan levels and indoleamine 2,3-dioxygenase-1 induction may have a greater influence on depression among HIV-infected adults receiving long-term ART [13].

Some aspects of our study design limit our interpretation. Although low levels of DHEA-S and DHEA-related metabolites are associated with chronic depressive symptoms, we cannot reach conclusions about causality. We suspect that low neuroactive steroid levels can lead to chronic depressive symptoms, but it is also conceivable that they are a consequence of depression and/or that there is a bidirectional relationship. We focused on longitudinal evidence of depressive symptoms, irrespective of antidepressant use, and did not use a formal diagnosis of a depressive disorder, so some misclassification of depression is possible. A 2019 epidemiological study showed that depressive symptoms and not depression diagnosis are associated with mortality risk in HIV infection [7], suggesting that depressive symptoms remain an important factor in HIV-related outcomes. Although there were small differences in cortisol/DHEA-S levels between men and women and no significant associations between high depressive symptoms and female sex in logistic regression analyses, there were only 8

women in the high depressive symptom group, compared with 27 men. Given that recent studies in suppressed HIV cohorts show a higher prevalence of depression in women than in men [2], larger biomarker studies that include more women with chronic depressive symptoms are critical to providing a more definitive understanding of biotypes of depression. CVD is associated with depressive symptoms in HIV infection [49], but the number of participants with vascular disease in this cohort was too low to investigate associations with neurosteroids. Finally, these data reflect the plasma metabolome of individuals primarily during midlife (ages 40–60 years) with long durations of HIV infection and may not fully reflect biochemical alterations among older PWH (age >60 years) or those receiving diagnoses in the more recent era of immediate ART initiation.

In summary, low plasma neuroactive steroid levels distinguished between participants with high and those with low depressive symptoms across independent cohorts of virally suppressed HIV-infected adults on ART. These findings suggest that DHEA-S and its direct downstream metabolites have a significant role in mechanisms underlying chronic depressive symptoms in HIV infection on ART and may identify a biological subtype of depression. Future prospective longitudinal studies will be crucial to validating these findings in larger cohorts of HIV-infected individuals and evaluating the predictive performance of low neuroactive steroids in real-world clinical settings. Given limited personalized therapeutic approaches in depression, particularly treatment-resistant depression, integrated approaches targeting neuroactive steroids and HPA axis dysregulation may offer alternative solutions to alleviate chronic depressive symptoms in a subset of PWH receiving ART.

Supplementary Data

Supplementary materials are available at *The Journal of 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

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