ORIGINAL ARTICLE

Glucocorticoid Receptor Function and Cognitive Performance in Women With HIV

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ABSTRACT

Objective: Alterations in glucocorticoid receptor (GCR) function may be a risk factor for cognitive complications among older people with human immunodeficiency virus (HIV). We evaluated whether HIV serostatus and age modify the GCR function-cognition association among women.

Methods: Eighty women with HIV ($n = 40$, <40 years of age [younger]; $n = 40$, >50 years of age [older]) and 80 HIV-uninfected women ($n = 40$ older, $n = 40$ younger) enrolled in the Women's Interagency HIV Study completed a comprehensive neuropsychological test battery. Peripheral blood mononuclear cells collected concurrent with neuropsychological testing were assessed for GCR function. Multivariable linear regression analyses were conducted to examine whether a) HIV serostatus and age were associated with GCR function, and b) GCR function-cognition associations are moderated by HIV serostatus and age adjusting for relevant covariates.

Results: Among older women, higher baseline *FKBP5* expression level was associated with lower attention/working memory performance among women with HIV ($B = 6.4$, standard error = 1.7, $p = .0003$) but not in women without HIV infection ($B = -1.7$, standard error = 1.9, $p = .37$). There were no significant HIV serostatus by age interactions on dexamethasone (DEX)-stimulated expression of the genes regulated by the GCR or lipopolysaccharide-stimulated tumor necrosis factor α levels (with or without DEX stimulation; p values > .13). HIV serostatus was associated with GC target genes *PER1* ($p = .006$) and *DUSP1* ($p = .02$), but not *TSC22D3* ($p = .32$), after DEX stimulation.

Conclusions: Collectively, these data suggest that HIV serostatus and age may modify the influence of the GCR, such that the receptor is likely engaged to a similar extent, but the downstream influence of the receptor is altered, potentially through epigenetic modification of target genes.

Key words: HIV, aging, women, glucocorticoids, cognition.

INTRODUCTION

Effective antiretroviral therapy (ART) has changed the face of the human immunodeficiency virus (HIV) epidemic from a disease with increased mortality to a manageable chronic condition. According to the Centers for Disease Control and Prevention, by 2015, more than 50% of people with HIV (PWH) in the United States were 50 years or older, and in 2020, that number was expected to have increased to 70% (1,2). Consequently, the "graying of the HIV epidemic" has led to new challenges including elevated incidence of comorbidities traditionally associated with chronic stress exposure such as cardiovascular disease, type 2 diabetes, cancer, and

ART = antiretroviral therapy, **CV** = coefficient of variation, **DEX** = dexamethasone, **DUSP1** = dual-specificity phosphatase 1, **ELISA** = enzyme-linked immunosorbent assay, **FKBP5** = FK506-binding protein 5, **GC** = glucocorticoid, **GCR** = glucocorticoid receptor, **GILZ** = glucocorticoid-induced leucine zipper, **HIV** = human immunodeficiency virus, **HPA** = hypothalamic-pituitary-adrenal, **LNS** = Letter-Number Sequencing, **LPS** = lipopolysaccharide, **NF-κB** = nuclear factor kappa-light-chain-enchancer of activated B cells, **PBMCs** = peripheral blood mononuclear cells, **PER1** = period circadian regulator 1, **PWH** = people with HIV, **RPL13A** = ribosomal protein L13a, **SE** = standard error, **TNF-α** = tumor necrosis factor α, **TSC22D3** = TSC22 Domain Family Member 3, **WRAT-3** = Wide Range Achievement Test 3, **WIHS** = Women's Interagency HIV Study, **WWH** = women with HIV

neuropsychiatric complications (3,4). PWH are exposed to a higher-than-average stressor burden, and the degree of stress-related comorbidities exceeds similar comorbidities observed in other chronic illnesses (5–8). Perceived stigma has been linked to decreased quality of life among PWH (9), and women living with HIV report higher psychological distress (10). Furthermore, stress-related disorders increase three-fold to fourfold after HIV diagnosis among women (11). In addition to the impact of psychosocial stressors, HIV may directly impact the stress response systems. Cytokines from viral infections stimulate the hypothalamic-pituitary-adrenal (HPA) axis and alter function of cortisol (12). Finally, evidence suggests that PWH have impaired coordination between the immune system and the HPA axis, particularly in women (13). Together, data suggest that HIV uniquely impacts stress physiology.

The glucocorticoid (GC) receptor (GCR) is a prime candidate as a mediator of stress-related diseases and disorders and may be modified by HIV. GCRs are present on nearly every cell in the body and are stimulated by GCs, primarily cortisol in humans. Cortisol is released as the culminating step of activation of the HPA axis, which is engaged daily as part of the circadian rhythm, and robustly activated during responses to physical or psychological stressors. The GCR is both a receptor and a transcription factor. Therefore, stimulation of the GCR produces a robust cascade of changes that are primarily centered on the mobilization of resources to cope with the energetic demands created by the stress response. In addition to functions in the regulation of metabolic processes, the GCR also has potent anti-inflammatory effects through primary changes in gene expression and transrepression of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), a prominent proinflammatory transcription factor. Interactions between the GCR and immune mediators, such as NF-κB, position the GCR as both a potential mechanism of HIV-associated chronic inflammation and a potential biomarker of progression toward inflammation-related comorbidities such as cognitive impairment (14) and related impairments in everyday functioning (15–18). Furthermore, previous studies in animal models demonstrate that HIV-related proteins can independently alter function of the HPA axis and precipitate changes in affective-like behaviors and cognition (19,20).

Previous efforts to examine the role of stress and alterations in stress biology in HIV-associated comorbidities have primarily focused on cortisol and have yielded disparate and equivocal results (21–24). Viruses are known to increase activation of the HPA axis (12,25), which may further limit the interpretation of the overall impact of HIV by the singular metric of circulating cortisol. Although cortisol measurements are an accurate assessment of circulating steroid concentrations, they provide limited information on GCR function. As is the case with many steroids and their associated receptors, the function of cortisol is heavily regulated by binding globulins, chaperones, and co-chaperones. Because of these additional levels of regulation, concentration alone does not necessarily reflect function.

GCR co-chaperones, in particular, *FKBP5*, are essential for GCR function and can directly alter GCR sensitivity and action (26). *FKBP5* harbors the GCR in the cytosol of the cell, effectively acting as a negative regulator of GC function. Alterations in *FKBP5* are associated with cognitive impairment and altered stress responsivity. For example, in psychiatric disorders, *FKBP5* gene expression is related to memory and function of the hippocampus and prefrontal cortex (27,28). In rodents, increased *FKBP5* is related to reduced GCR translocation and reduced GCR-DNA binding that in turn

is associated with memory impairments (29). Translating that basic science evidence to humans, impaired GCR function could predispose women with HIV (WWH) to cognitive impairment linked to GC resistance defined as reduced efficacy of GCR stimulation. *FKBP5* is altered in postmortem brains of PWH (30,31), but neither the impact of HIV on *FKBP5* in peripheral cells nor the potential predictive value of subthreshold changes in *FKBP5* and GCR function has been considered as an underlying mechanism of cognitive function.

HIV seems to interact with/or accelerate age-related inflammation (32) and possibly exacerbate neuroinflammation within the brain (33). Given substantial sex differences in the immune response (34) and the dearth of neuroHIV studies focused on WWH (35–37), studies in this subgroup require particular attention. Identification of the mechanisms of HIV-associated chronic inflammation will provide the critical framework needed to design therapeutic strategies for the chronic inflammation that affects WWH and consequently may reduce comorbidities such as cognitive impairment that are associated with chronic inflammation (38).

Given that HIV infection and age may independently and synergistically perturb the function of the GCR, we examined the role of chronological aging and HIV serostatus on GCR function in peripheral blood mononuclear cells (PBMCs). We assessed ex vivo GCR function in three ways: a) assessment of co-chaperone expression as an indicator of potential GCR resistance, b) examination of expression of genes responsive to GCR stimulation as a metric of GCR response to ligand presentation, and c) assessment of the anti-inflammatory potential of the GCR. We first examined *FKBP5* in PBMCs and hypothesized that aging and HIV would interact to associate with increased expression of *FKBP5* beyond the independent effects of either HIV or aging. We then examined the stimulatory effects of dexamethasone (DEX) on GCR-mediated gene transcription. Given that *FKBP5* inhibits translocation of the GCR to negatively regulate GCR transcriptional activity, we hypothesized that age and HIV would interact to attenuate the ability of DEX stimulation to engage GCR-mediated gene transcription. We tested this hypothesis by examining GCR-mediated gene transcription by measuring common genes controlled by the transcriptional activity of GCR: *FKBP5, DUSP1, TSC22D3* (encodes for protein: GC-induced leucine zipper [*GILZ*]), and *PER1*. In addition, the anti-inflammatory capacity of GCR stimulation was examined by assessing the ability of DEX stimulation to block inflammatory signaling induced by the inflammatory stimulus lipopolysaccharide (LPS). The prefrontal cortex and hippocampus are particularly prominent sites of GCRs (39–43) and are critical components of neural circuitry underlying verbal and working memory (44,45). We therefore hypothesized that expression of *FKBP5* would be more strongly associated with verbal and working memory difficulties in older WWH compared with younger WWH or HIV-uninfected (HIV−) younger or older women. Given the established role of the GC in the modulation of inflammation and in brain function, an understanding of the associations among HIV, age, and GCR function will provide insight regarding the degree to which GCR dysfunction could contribute to elevated inflammation and poorer cognitive function in WWH.

METHODS

Participants

Participants were enrolled in the Women's Interagency HIV Study (WIHS), an ongoing longitudinal multisite cohort study of the natural and treated

history of WWH and HIV− women. For this cross-sectional, nested case/control study, we used participants enrolled in the first three waves of the study who also completed baseline neuropsychological testing between 2009 and 2011. The waves of study enrollment occurred between October 1994 and November 1995, October 2001 and September 2002, and January 2011 and January 2013, from six sites (Brooklyn, Bronx, Chicago, DC, Los Angeles, and San Francisco). Detailed information regarding recruitment procedures, the core study protocol, and eligibility criteria have been previously published (46–48). All participants provided informed consent, and the WIHS Executive Committee approved the concept for this study.

To determine which cases were selected for analyses, our initial sampling frame was women with one neuropsychological assessment completed between 2009 and 2011 and stored biospecimens (PBMCs) collected and available for analysis concurrent with the neuropsychological assessment ($n = 1595$). Women ($n = 382$) were excluded from case selection for factors that can impact GCR function and/or cognition including: a) use of hormone-mediated contraceptives, pregnant, and/or breastfeeding within the past 6 months (49), d) self-reported use of antipsychotic or Alzheimer's medication within the past 6 months (50), and/or c) current CD4 cell count <400 (51,52). From the remaining 1213 cases, 160 women were selected for the present study to yield four groups of women: a) older WWH (age >50 years), b) younger WWH (age <40 years), c) older HIV− women (age >50 years), and d) younger HIV− women (age <40 years). To balance the four groups on key variables, we first selected 40 older WWH then matched the other three groups as close as possible on key variables including age, race, and education. Because of cell viability, final sample sizes were $n = 40$ younger WWH, $n = 38$ older WWH, $n = 39$ younger HIV− women, and $n = 38$ older HIV− women.

GCR Function

Cell Culture

Blood was collected as part of a routine WIHS core visit. PBMCs were isolated and cryopreserved in the WIHS repository (Precision Bioservices, Rockville, Maryland). After concept approval by WIHS and sample selection (see the Participants section), PBMCs were shipped to Emory University, where they were maintained in liquid nitrogen until plated. Cells were thawed in an RPMI medium supplemented with 5% fetal bovine serum, 2 mM L-glutamine, and 10/10 pen/strep. Cell viability was confirmed with Trypan blue, and cells were counted and plated at a density of 1×10^6 cells/ml in 12-well cell culture plates (Corning Inc., Corning, New York; 2×10^6 cells/well total). Cell viability from this PBMC thawing protocol has a ~92% viability rate as measured by Trypan blue exclusion. After overnight incubation in a humidified atmosphere at 37°C in 5% CO_2, the cells were stimulated with vehicle (baseline), DEX (Sigma Aldrich, product D-4902), LPS (Sigma Aldrich, product L2880), or concurrent LPS and DEX (LPS + DEX). DEX (concentration of 10^{-8} M) and/or LPS (concentration of 30 ng/ml) was added at 0 hours. Cells were harvested at 6 hours, centrifuged at 1000 rcf for 5 minutes at 4°C, and after a wash with ice-cold phosphate-buffered saline, the cell pellets were lysed for RNA extraction. Cell lysates and cell culture supernatant were stored at −80°C until they were used for RNA extraction and enzyme-linked immunosorbent assay (ELISA). Cell pellets from baseline and DEX conditions were used for quantitative polymerase chain reaction experiments, whereas cell culture supernatant from baseline, LPS, and LPS + DEX conditions were used for ELISA assessments.

Quantitative Polymerase Chain Reaction

RNA was extracted from cell pellets using the RNeasy Mini Kit (Qiagen, Valencia, California) according to the manufacturer's instructions. RNA integrity was assessed by a NanoDrop One spectrophotometer (ThermoScientific, Wilmington, Delaware), and RNA samples were reverse transcribed using the High Capacity RNA to cDNA Kit (Applied Biosystems, Foster City, California). To ensure uniform amounts of total cDNA across groups, cDNA was quantified via the PicoGreen Assay (Invitrogen, Carlsbad,

California) and standardized throughout all samples. The human gene *RPL13A* was determined to be the optimal endogenous control based on an intergroup variance of less than 10% across groups. *FKBP5* was selected for assessment given its established role in GCR resistance, cognition, and stress pathophysiology (19,26,28,49). *DUSP1* (53,54), *TSC22D3* (55,56), and *PER1* (57,58) were selected because they are reliable indicators of GCR engagement. Primers for *FKBP5* (forward: CTTGCTGCCTTTCTGAACCT, reverse: CCCTTGGCTGACTCAAACTC; protein: FKBP5), *DUSP1* (forward: GATACGAAGCGTTTTCGGCT, reverse: GGGCCACCCTGATCGTAGA; protein: dual specificity phosphatase 1), *TSC22D3* (forward: TGGTGGCCATAGACAACAAG, reverse: CTTCAGGGCTCAGACAGGAC; protein: GC-induced leucine zipper, GILZ), *PER1* (forward: GGCAAGGACTCAGAAGGAAC, reverse: CTCCAGCTCCTCCAGGGTAT; protein: period circadian regulator 1), and *RPL13A* (forward: ATGCTGCCTCACAAGACCA, reverse: TAGGCTTCAGACGCACGAC; protein: ribosomal protein L13a) were designed and purchased from Applied Biosystems. The real-time polymerase chain reaction cycling conditions used on the QuantStudio Flex 6 (Applied Biosystems) were as follows: 50°C (2 minutes) and 95°C (2 minutes), followed by 40 cycles of 95°C (15 seconds) and 60°C (1 minute). Samples were run in triplicate and averaged, and the coefficient of variation (CV) within the triplicates was no more than 4%. Baseline gene expression data were calculated and presented as cycle of threshold (C_t) values normalized with regard to the endogenous control gene (*RPL13A*; formula: normalized $C_t = [C_t \text{ of target gene}] − [C_t \text{ of endogenous control}]$), with larger values indicating less gene expression because more cycles are necessary to amplify the gene to the threshold level. Post-DEX C_t values were calculated as [DEX normalized C_t] − [Baseline normalized C_t]. As the value reported approaches zero, this indicates less effective DEX stimulation of GC-related gene expression, with zero being indicative of no effect of DEX on GC function, as measured by GC-related gene expression.

Enzyme-Linked Immunosorbent Assay

Cytokine expression was assessed via ELISA in cell culture supernatant at baseline, after LPS (stimulated), and after LPS + DEX (suppression of LPS-stimulated cytokines by DEX) as additional indices of GC function. Human tumor necrosis factor α (TNF-α) ELISA kits were purchased from R&D Systems (Minneapolis, Minnesota), and assays were performed according to the manufacturer's instructions. The sensitivity of the assay was 0.5 to 5.5 pg/ml, and the average intra-assay CV was 7.56% and the interassay CV was 12.01%. Samples were run in duplicates and averaged, and the CV among the duplicates was less than accepted cutoff of 15%. DEX suppression was calculated for each subject using the following equation:

$$[((TNF\alpha \text{ from LPS condition}) \\ -(TNF\alpha \text{ from LPS + DEX condition})) \\ /(TNF\alpha \text{ from LPS condition})] \times 100$$

Neuropsychological Function

All women completed a comprehensive neuropsychological test battery between 2009 and 2011 and had available biospecimens for GCR function analysis. Neuropsychological domain assessments and outcome measures were as follows: *verbal learning and memory*, Hopkins Verbal Learning Test—Revised (outcomes = total words recalled across trials 1–3, delay free recall); *attention/working memory*, Letter-Number Sequencing (LNS; outcomes = total correct for experimental and control conditions); *executive function*, Trail Making Test Part B (outcome = time to completion) and Stroop Test Trial 3 (outcome = time to completion); *psychomotor speed*, Symbol Digit Modalities Test (outcome = total correct) and Stroop Trial 2 (outcome = time to completion); *fluency*, Letter (outcome = total correct words generated on F, A, S) and semantic (outcome = total correct animals generated); and *motor skills*, Grooved Pegboard (outcome = average time to complete dominant and nondominant hand). A log transformation was used on all timed outcomes to normalize distributions and then reverse scored so that higher scores equated to better performance across all domains.

Demographically adjusted *T* scores were derived for each outcome adjusting for age, years of education, Wide Range Achievement Test 3 (WRAT-3) reading subtest, race (African American versus not), ethnicity (Hispanic versus not), and number of times the test had been administered following methods used by other large-scale HIV cohorts including the WIHS (59,60). For each domain, a composite *T* score was derived by averaging the *T* scores for domains with at least two outcomes. If only one test in a domain was completed, the *T* score for that test was used. The primary outcome measures of interest were verbal memory and attention/working memory.

Covariates

Candidate covariates were based on our previous WIHS publications (selected a priori) (59,60) and are listed in Table 1. These variables (sociodemographic factors, clinical, and behavioral variables) have warranted consideration as covariates in analyses where we have examined cognition as a primary end point in the WIHS. Covariates included in all analyses were selected because they were associated with the independent (HIV, age) and dependent variables (cognitive outcomes) in univariate analyses at *p* < .20. All other variables did not shift our regression coefficients of interest and were not included as covariates in the model. The WRAT-3 reading subtest scores were not included as a covariate because this variable is accounted for in the cognitive domain–specific *T* scores. These variables included the following: depressive symptoms (Center for Epidemiological Studies Depression scale, ≥16 cutoff (61,62)); smoking status (current, former, never); heavy alcohol use (>7 drinks per week or >4 drinks in one sitting); crack, cocaine, and/or heroin use (ever, never); and recent antidepressant medication use. For analyses conducted in WWH only, the following HIV-related clinical characteristics were selected from a larger subset of factors (Table 1) and included the following: CD4 count, prior AIDS diagnosis, years on combination ART, and proportion of total WIHS visits that HIV RNA was undetectable.

Statistical Analyses

For the first aim addressing the role of age and HIV serostatus on GCR function, we conducted a series of multivariable linear regression analyses. Predictor variables initially included in the model were as follows: age (younger, older), HIV serostatus (WWH, HIV−), and the two-way interaction. The two-way interaction was removed from the model if it was not significant (*p* > .05). Outcome variables included baseline *FKBP5* expression levels, reporter gene expression after DEX (*DUSP1, PER1, TSC22D3*), and TNF-α after combined DEX + LPS. See the Covariates section for the covariates included in the models. For the second aim examining whether GCR function-cognition associations are moderated by HIV serostatus and age, we conducted a series of multivariable linear regressions (simple bivariate associations are included in Table S1, Supplemental Digital Content, http://links.lww.com/PSYMED/A864). Predictor variables included age, HIV serostatus, GCR function (baseline *FKBP5* expression levels, reporter gene expression after DEX, or TNF-α after combined DEX + LPS), and the two- and three-way interactions. Unstandardized β (*B*) coefficients, standard errors (SEs), and *p* values were extracted from the models for interpretation of higher-order interactions. If higher-order interactions were not significant (*p* > .05), they were removed from the models. Outcome measures included the cognitive domain *T* scores. Primary outcomes included verbal memory and attention/working memory. All other cognitive domains were considered secondary outcomes. Covariates are listed in the Covariates section. Analyses were conducted in SAS version 9.4, and significance was set at *p* < .05 for all primary outcomes. A false discovery rate correction (Benjamini-Hochberg procedure) was applied for secondary outcome measures to adjust for multiple comparisons.

RESULTS

Table 1 shows the participant characteristics as a function on HIV serostatus and age (younger, older). Among younger women, WWH and HIV-uninfected women were only found to differ on marijuana and crack, cocaine, and/or heroin use; HIV− women were more likely to use these illicit substances in the past 6 months compared with WWH. Among older women, WWH were slightly older (56 versus 53 years), had higher scores on the WRAT-3 reading subtest, had lower depressive symptoms, and were less likely to report heavy alcohol use or smoke.

Does HIV Infection or Age Independently or Synergistically Perturb Function of the GCR (Baseline Levels and Post-DEX Stimulation)?

Baseline Levels

The interaction between HIV serostatus and age on baseline *FKBP5* expression levels was not significant (*p* = .94). After removing the two-way interaction from the model, HIV serostatus and age were also not independently associated with baseline *FKBP5* expression levels (*p* values > .30; Figure 1A). The interaction between HIV serostatus and age was not significant on baseline TNF-α levels (*p* = .20; Table S2, Supplemental Digital Content, http://links.lww.com/PSYMED/A864). After removing the two-way interaction from the model, HIV serostatus (*p* = .07) and age (*p* = .98) were not associated with baseline TNF-α levels.

Post-DEX Stimulation

There were no significant HIV serostatus by age interactions on DEX-stimulated expression of the genes regulated by the GCR or LPS-stimulated TNF-α levels (with or without DEX stimulation; *p* values > .13). After removing the two-way interaction from the models, HIV serostatus was associated with *PER1* (*p* = .006) and *DUSP1* (*p* = .02), but not *TSC22D3* (*p* = .32) after DEX stimulation (Figures 1B–D). Specifically, *PER1* and *DUSP1* reporter gene inductions were lower in WWH compared with HIV-uninfected women irrespective of age. In addition, there was an age difference on *TSC22D3* after DEX stimulation, with older women (mean = −2.87, SE = 0.1) having lower gene expression compared with younger women (mean = −3.0, SE = 0.1; *p* = .03), which remained significant after adjusting for depressive symptoms and other factors that differed between the older women. Age was not associated with *DUSP1* (*p* = .06) or *PER1* (*p* = .13).

Is the Association Between GCR Function and Cognition Modified by HIV Serostatus and Age?

Baseline Levels

The test of the primary hypothesis—the three-way HIV serostatus by age by baseline *FKBP5* expression levels—was significant in the domain of attention/working memory (*p* = .009) but not in verbal memory after adjusting for confounding factors (*p* = .84; Table 2). This indicated that the magnitude of the association between baseline *FKBP5* expression levels and attention/working memory varied as a function of HIV serostatus and age. Specifically, HIV serostatus significantly moderated the relationship between baseline *FKBP5* expression and attention/working memory among older (*p* = .002) but not younger women (*p* = .53). Further interpretation of this interaction based on the *B* coefficients, SE, and *p* values from this single model revealed that, among older women, higher expression of baseline *FKBP5* expression levels was associated with lower attention/working memory performance

TABLE 1. Demographics and Other Descriptive Information About Study Participants as a Function of HIV Serostatus (WWH, HIV−) and Age (Younger, <40 Years, Older, >50 Years) at Baseline

	Younger			Older		
	HIV− (n = 40)	WWH (n = 39)	p	HIV− (n = 38)	WWH (n = 38)	p
Age, M (SD), y	33 (4)	35 (3)	.16	53 (3)	56 (5)	<.001
Years of education, M (SD)	13 (2)	12 (3)	.65	12 (3)	13 (2)	.07
WRAT-3 reading subtest, M (SD)	97 (16)	93 (17)	.30	86 (17)	95 (17)	.04
Race/ethnicity, n (%)			.48			.24
Black, non-Hispanic	22 (55)	27 (69)		25 (66)	19 (50)	
White, non-Hispanic	6 (15)	3 (8)		3 (8)	9 (24)	
Hispanic	6 (15)	6 (15)		9 (24)	8 (21)	
Other	6 (15)	3 (8)		1 (3)	2 (5)	
Annual household income ≤12,000/y, n (%)	15 (37)	10 (26)	.26	18 (47)	14 (37)	.35
Elevated depressive symptoms, n (%)	8 (20)	12 (31)	.27	16 (42)	8 (21)	.04
Antidepressant medication use	1 (2)	3 (8)	.29	6 (16)	12 (31)	.11
Hepatitis C RNA positive, n (%)	0 (0)	1 (2)	.31	1 (3)	3 (8)	.31
Smoking status, n (%)			.11			.04
Current	15 (38)	9 (23)		15 (39)	11 (29)	
Former	17 (42)	14 (36)		19 (50)	14 (37)	
Never	8 (20)	16 (41)		4 (11)	13 (34)	
Recent heavy alcohol use, n (%)	14 (35)	9 (23)	.25	12 (31)	3 (7)	.01
Marijuana use, n (%)			.007			.76
Recent	16 (40)	8 (20)		6 (16)	7 (18)	
Former	19 (47)	14 (36)		26 (68)	23 (60)	
Never	5 (13)	17 (44)		6 (16)	8 (21)	
Crack, cocaine, and/or heroin use, n (%)			.02			.20
Recent	4 (10)	0 (0)		3 (8)	2 (5)	
Former	24 (60)	17 (44)		28 (74)	22 (58)	
Never	12 (30)	22 (56)		7 (18)	14 (37)	
HAART medication[a]	—	28 (71)		—	34 (89)	
Nadir CD4 count in WIHS, median (IQR)	—	282 (201)		—	223 (192)	
Current CD4 count, median (IQR)	—	733 (336)		—	820 (312)	
HIV RNA, Median (IQR)	—	48 (2422)		—	48 (27)	
Proportion of WIHS visits, M (SD)						
HIV RNA <500 copies/ml	—	58 (31)		—	54 (22)	
On HAART	—	59 (33)		—	60 (27)	
Adherence (≥95%) to ART, n (%)	—	24 (80)		—	30 (88)	
HAART duration, M (SD), y	—	9 (4)		—	10 (5)	
Previous AIDS diagnosis, n (%)	—	7 (18)		—	18 (47)	

HIV = human immunodeficiency virus; WWH = women with HIV; HIV− = HIV uninfected; M (SD) = mean (standard deviation); WRAT-3 = Wide Range Achievement Test standard score; HAART = highly active antiretroviral therapy; IQR = interquartile range; WIHS = Women's Interagency HIV Study; ART = antiretroviral therapy.

Variables reported as n (%) were analyzed with χ^2 tests. Variables reported as M (SD) were analyzed with analyses of variance. Variables reported as median (IQR) were analyzed with Wilcoxon-Mann-Whitney test. Column percents may not total to 100% because of rounding.

[a] Quantified over the three study visits; current refers to within the past week; recent, refers to within 6 months of the most recent WIHS visit; heavy alcohol use reflects >7 drinks a week or >4 drinks in one sitting.

among WWH ($B = 6.4$, SE = 1.7, $p = .0003$) but not HIV-uninfected women ($B = −1.7$, SE = 1.9, $p = .37$; Figure 2). To ensure that the finding among older WWH was not driven by HIV-related clinical characteristics, we conducted a subanalysis among this group only. The association remained significant among older WWH after further adjusting for HIV-related clinical characteristics including current CD4 count, the proportion of virally suppressed visits in WIHS, and the number of years on combination ART ($B = 7.1$, SE = 1.9, $p = .001$). Exploratory analyses examining the pattern of performance for each outcome measure contributing to the attention/working memory domain indicated the same three-way interaction on the number correct for the working memory condition on LNS ($p = .004$) and missed statistical significance on the attention condition on LNS ($p = .09$). On verbal memory, there were no significant
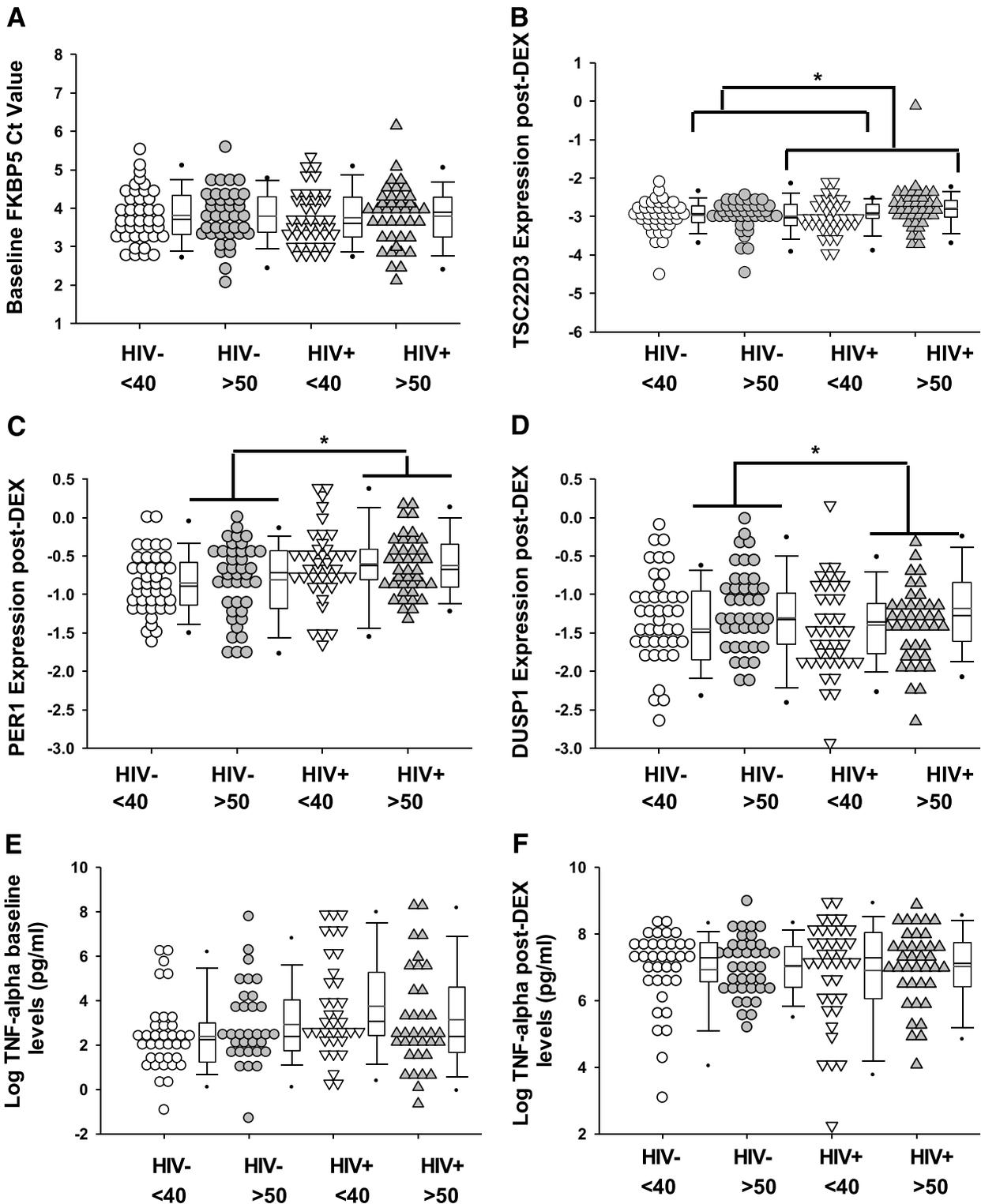
FIGURE 1. Baseline *FKBP5* Ct value (A), *TSC22D3* (GILZ) expression levels post-DEX (B). *PER1* expression levels post-DEX (C). *DUSP1* expression levels post-DEX (D), TNF-α baseline levels ((E), and TNF-α levels after LPS + DEX (F) as a function of HIV serostatus and age category (<40 and >50 years). Baseline gene expression data are presented as normalized cycle of threshold (C_t) values, with larger values indicating less gene expression (A, B). Post-DEX C_t values were calculated as [DEX normalized C_t] − [Baseline normalized C_t]. As the value reported on the *y* axis approaches zero, this indicates less effective DEX stimulation of GC-related gene expression, with zero being indicative of no effect of DEX on GC function, as measured by GC-related gene expression (C, D). DEX = dexamethasone; GC = glucocorticoid; HIV = human immunodeficiency virus; LPS = lipopolysaccharide; TNF-α = tumor necrosis factor α.

TABLE 2. Adjusted Association Between Baseline *FKBP5* Expression and Cognition as a Function of HIV Serostatus (WWH, HIV−) and Age (Younger, <40 Years, Older, >50 Years)

	Younger			Older			HIV by Age FKBP5 Interaction, *p* Value
Outcomes	HIV−, *B* (SE)	WWH, *B* (SE)	WWH Versus HIV−, *p* Value	HIV−, *B* (SE)	WWH, *B* (SE)	WWH Versus HIV−, *p* Value	
Primary							
Verbal memory	0.2 (2.0)	−3.0 (2.0)	.25	0.4 (2.0)	−2.0 (1.8)	.37	.88
Attention/working memory	2.6 (1.9)	0.9 (1.9)	.53	−1.7 (1.9)	6.4 (1.7)***	.002	**.009**
Secondary							
Verbal learning	−1.6 (2.1)	−3.2 (2.2)	.60	0.9 (2.2)	−0.3 (2.0)	.67	.94
Executive function	0.2 (2.1)	−0.5 (2.0)	.82	−0.7 (2.1)	−0.3 (1.8)	.89	.78
Speed	1.1 (1.9)	−0.0 (1.9)	.66	1.6 (1.9)	2.4 (1.7)	.76	.58
Fluency	−1.4 (1.9)	0.9 (1.9)	.39	−3.0 (2.0)	1.3 (1.8)	.11	.55
Motor skills	1.3 (2.0)	−3.2 (2.0)	.11	−0.5 (2.0)	0.1 (1.8)	.87	.18

HIV = human immunodeficiency virus; WWH = women with HIV; HIV− = HIV uninfected; M (SD) = mean (standard deviation); *B* = unstandardized *β* weight; SE = standard error.

Each model included HIV serostatus, age, baseline *FKBP5* expression, and all two- and three-way interactions between these factors. All models adjusted for depressive symptoms (Center for Epidemiological Studies Depression scale, score ≥16 cutoff); smoking status (current, former, never); heavy alcohol use (>7 drinks a week or >4 drinks in one sitting); crack, cocaine, and/or heroin use (ever, never); and recent antidepressant medication use. For each outcome, the *B* (SE) and *p* values are from a single model.

Bold indicates *p* value.

***$p < .001$, association between baseline *FKBP5* and lower attention/working memory performance among older WWH.

two-way interactions (HIV serostatus by baseline *FKBP5* expression levels; age by baseline *FKBP5* expression levels) or general associations between baseline *FKBP5* expression levels and performance. On the secondary outcome measures (using the false discovery rate correction), there were no significant two- or three-way interactions or a significant association of baseline *FKBP5* expression levels with performance.

With respect to immune function, all two- and three-way interactions between HIV serostatus, age, and baseline TNF-α concentrations were not significant (*p* values > .05). After removing the interactions from the model, higher basal TNF-α concentrations were associated with poorer psychomotor speed ($B = −0.72$, SE = 0.35, $p = .04$).

Post-DEX Stimulation
In an adjusted model, the three-way HIV serostatus by age by *DUSP1* expression post-DEX was significant in the domain of attention/working memory ($p < .0001$). This indicated that the magnitude of the association between *DUSP1* expression post-DEX and attention/working memory varied as a function of HIV serostatus and age. HIV serostatus significantly moderated the relationship between *DUSP1* induction after DEX and attention/working memory among both younger and older women ($p = .003$ and $p = .008$, respectively). Further interpretation of this interaction based on the *B* coefficients, SE, and *p* values from this single model revealed that, among older women, a less robust induction of *DUSP1* after DEX stimulation was associated with better attention/working memory performance among WWH ($B = 8.1$, SE = 2.6, $p = .003$) but not HIV− women ($B = −1.2$, SE = 2.8, $p = .67$; Figure 2). The association remained significant among older WWH after further adjusting for HIV-related clinical characteristics including current CD4 count, the proportion of virally suppressed visits in WIHS, and the number of years on highly active antiretroviral therapy

($B = 8.7$, SE = 3.0, $p = .007$). This three-way interaction was not observed for either *PER1* ($p = .38$) or *TSC22D3* ($p = .84$) after DEX stimulation on attention/working memory. After removing all nonsignificant interactions, less responsive transcription of *PER1* after DEX stimulation was associated with higher attention/working memory ($p = .04$).

There were no other significant three-way interactions on any other cognitive domain. After removing nonsignificant three-way interactions, there was a significant HIV by *TSC22D3* interaction on learning and memory ($p = .04$). Less *TSC22D3* induction after DEX associated with better learning and memory among WWH but not HIV-uninfected women. This again reflects the pattern of a more robust GC response to DEX predicting impaired domain-specific cognitive function. A similar pattern was noted on memory. On motor, there was a significant age by *PER1* ($p = .003$) interaction. Higher *PER1* induction was associated with worse motor function among older but not younger women. There were no other significant associations between any expression levels of genes after DEX and cognition.

With respect to GC suppression of LPS-stimulated inflammation, higher TNF-α concentrations after LPS + DEX were associated with poorer attention/working memory ($B = −2.53$, SE = 0.66, $p = .0002$). However, after the removal of two bivariate outliers, the association was no longer significant ($B = 3.90$, SE = 2.43, $p = .11$).

DISCUSSION
The data presented here demonstrate that HIV serostatus and chronological age interact in a cohort of women to alter metrics of GCR function and the relationship between GCR metrics and certain cognitive domains. In this cross-sectional study, elevated baseline *FKBP5*, a negative regulator of GCR function, was associated with poorer attention/working memory in older
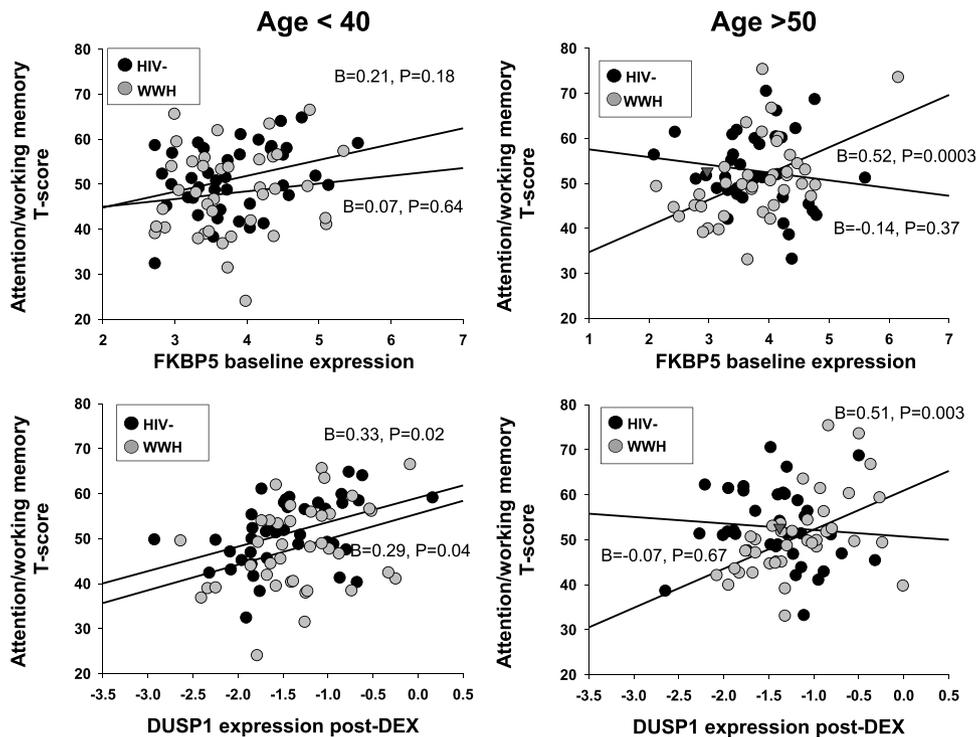
FIGURE 2. Associations between baseline *FKBP5* expression (top; one model) and *DUSP1* expression post-DEX (bottom; one model) and attention/working memory as a function of age category (<40 years, left; >50 years, right) and HIV serostatus (women with HIV [WWH]; HIV−). For *FKBP5* panels, gene expression data are presented as normalized cycle of threshold (C_t) values, with larger values indicating less gene expression. Post-DEX C_t values were calculated as [DEX normalized C_t] − [Baseline normalized C_t]. As the value reported on the *x* axis approaches zero, this indicates less effective DEX stimulation of GC-related gene expression, with zero being indicative of no effect of DEX on GC function, as measured by GC-related gene expression of *DUSP1*. *B* = standardized β weights from the multivariable linear regression models. Each model included HIV serostatus, age (baseline *FKBP5* expression or *DUSP1* expression post-DEX), and all two- and three-way interactions between these factors. All models adjusted for depressive symptoms (Center for Epidemiological Studies Depression scale, CES-D ≥16 cutoff), smoking status (current, former, never), heavy alcohol use (>7 drinks a week or >4 drinks in one sitting); crack, cocaine, and/or heroin use (ever, never); and recent antidepressant medication use. For each outcome, the *B* (SE) and *p* values are from a single model. CES-D = Center for Epidemiological Studies Depression scale; DEX = dexamethasone; GC = glucocorticoid; HIV = human immunodeficiency virus; SE = standard error; WWH = women with HIV.

WWH. This association may be related to the high density of GCRs in brain regions associated with working memory, such as the prefrontal cortex (63,64). *FKBP5* expression did not predict cognitive function in HIV-uninfected women, suggesting that a combination of increased chronological age and HIV infection may increase the influence of *FKBP5* on functional metrics of the GCR.

To provide a metric of GCR function, we assessed GCR-mediated gene expression and the anti-inflammatory efficacy of GCR stimulation in PBMCs. Although the ability of GCR stimulation to inhibit TNF-α concentrations was not associated with age group or HIV serostatus, GCR-mediated gene expression was associated with HIV serostatus or age in a gene-specific manner. Stimulation of the GCR with DEX caused blunted upregulation of *PER1*, a circadian-related gene regulated by GCs (58), in WWH compared with HIV-uninfected women. This association was independent of age group. In addition, the expression of the GCR-regulated gene *DUSP1*, which has an established role in mediating the anti-inflammatory effects of GCs (54), was reduced with DEX induction in WWH compared with HIV-uninfected women. The third GCR-regulated gene that was assessed, *TSC22D3*, which encodes for *GILZ*, a regulator of sensitivity to proinflammatory

cytokines (55,65), was unaltered by HIV serostatus but was differentially expressed by age group with older women exhibiting lower induction of *TSC22D3* after DEX stimulation. The divergent association of HIV serostatus and age with GC-receptor function may reflect alterations in the epigenetic regulation of each specific gene (53,57,66) or changes in the proteins that interact with the GC-receptor and modulate its function. The function of the GCR is modulated by other hormone receptors that also serve as transcription factors including estrogen receptor (67) and inflammation-related transcription factors such as NF-κB (68). Although beyond the limits of the current study, important next steps in understanding the relationship between GCR function and HIV-related outcomes will be to examine GCR function within specific subsets of cells that constitute PBMCs and to conduct broader scale genomic analysis to enable pathway assessments.

Collectively, these data demonstrate that neither HIV serostatus nor age was associated with reduced global GCR function because a global change in GCR function would be anticipated to alter the anti-inflammatory properties of GCR stimulation (69,70) and cause a uniform induction or suppression of GCR-mediated gene expression (71,72). On the contrary, these data suggest that HIV

serostatus and age may modify the influence of the GCR, such that the receptor is likely engaged to a similar extent, but the downstream influence of the receptor is altered, potentially through epigenetic modification of target genes. Such a change could drive individual variability in the effects of GCR stimulation, which is consistent with a potentially differential predictive value of GCR function metrics on cognition as a function of age and HIV serostatus.

In general, higher expression of genes that are indicators of GCR function after DEX-stimulation was associated with poorer attention/working memory. However, the resulting association between higher *PER1* expression after DEX stimulation and poorer attention and working memory was unexpected because overexpression of *PER1* has previously been demonstrated to improve memory in aging mice (57). *DUSP1* interrupts proinflammatory signaling and promotes the anti-inflammatory actions of GCs (54). Therefore, we would have expected increases in *DUSP1* to provide a more protective effect on attention and working memory. In addition, in the context of cognition, lower *TSC22D3* expression in WWH was associated with better learning and memory compared with HIV-uninfected women. These findings were absent among women with lower *TSC22D3* expression regardless of HIV serostatus or age, showing no difference in cognitive function.

Taken together, these data suggest that HIV serostatus interacts with age to alter GCR function. Counter to previous reports of GC resistance predicting cognitive impairment (73), here we demonstrate that, in WWH, an inverse relationship exists such that a more robust response of the GCR to stimulation is associated with poorer cognitive function. The contrast in the relationship between GCR function and cognition between this study and previous works may indicate that WWH occupy a different positionality on the Yerkes-Dodson relationship between stress and cognition (74,75). The present study has a number of limitations including the use of a cross-sectional design, which precludes the ability to address causality, and the limited assessment of peripheral metrics of GCR function. Sample size also limited the statistical power of any subgroup testing (e.g., depression). Larger sample sizes are needed to determine if our preliminary findings are reproducible. Future studies should include longitudinal metrics, cell-specific pathway assessments, and assessments of potential epigenetic alterations that may drive differential GCR influence. In a recent study, activation of the GCR via DEX was sufficient to reduce basal expression of HIV in microglia, suggesting a possible target for therapy (76). However, our findings in PBMCs suggest that DEX-mediated activation of GCR may have unanticipated negative associations with cognition, specifically in WWH older than 50 years, suggesting a divergence in downstream functions of GCR with age. These novel findings reinforce the need for further investigation of the interactions of age and HIV serostatus in mediating GCR actions.

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