ORIGINAL ARTICLE

Associations of Salivary Cortisol With Cognitive Function in the Baltimore Memory Study

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Context: The stress responses of the hypothalamic-pituitary-adrenal axis can produce adverse effects on the brain. Previous studies have concluded that an elevated level of cortisol is a risk factor for cognitive dysfunction and decline in aging but have been limited by sex exclusion, restricted cognitive batteries, and small sample sizes.

Objective: To examine associations among salivary cortisol metrics and cognitive domain scores in an urban adult population.

Design, Setting, and Participants: A cross-sectional analysis was conducted using data from a longitudinal study involving 1140 Baltimore, Maryland, residents aged 50 to 70 years. Four salivary cortisol samples were obtained from 967 participants across 1 study visit (before, during, and after cognitive testing as well as at the end of the visit) from which 7 cortisol metrics were created. We examined associations of cortisol metrics with cognitive performance using multiple linear regression.

Main Outcome Measures: Performance on 20 standard cognitive tests was measured and combined to form summary measures in 7 domains (language, processing speed, eye-hand coordination, executive functioning, verbal memory and learning, visual memory, and visuoconstruction).

Results: Higher levels of pretest and mean cortisol as well as the area under the curve of cortisol over the study visit were associated with worse performance ($P<.05$) in 6 domains (language, processing speed, eye-hand coordination, executive functioning, verbal memory and learning, and visual memory). For instance, an interquartile range increase in the area under the curve was equivalent to a decrease in the language score expected from an increase in 5.6 (95% confidence interval, 4.2-7.1) years of age.

Conclusions: Elevated cortisol was associated with poorer cognitive function across a range of domains in this large population-based study. We believe the findings are consistent with the hypothesis that hypothalamic-pituitary-adrenal axis dysregulation may be a risk factor for poorer cognitive performance in older persons.

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THE AGING BRAIN MAY BE VULnerable to the effects of stress, which can be defined as repeated exposure to psychosocial hazards giving rise to a bodily state that can be deleterious to multiple physiological systems. Psychosocial hazards are relatively stable and visually or auditorily perceptible characteristics of the environment that give rise to a heightened state of vigilance, alarm, or threat[1]; examples include loud noise, crime, threatening social situations, or physical incivilities.[2,3] On exposure to psychosocial hazards, the hypothalamic-pituitary-adrenal (HPA) axis triggers the secretion of glucocorticoids to promote an adaptive response.[4] The brain is dense with receptors presenting targets for glucocorticoid-mediated actions that can affect neuronal metabolism, morphology, and survival.[5] It is theorized that with chronic overactivation or underactivation of HPA axis response mechanisms leading to dysregulation, the brain and body incur cumulative wear and tear (termed *allostatic load* by some investigators) that may contribute to the development of adverse health outcomes.[6-10] Multiple neurologic agents subserve the HPA axis response and are potentially harmful to the brain; however, glucocorticoids, particularly cortisol, have been emphasized as primary mediators in studies of adverse health consequences of exposure to psychosocial hazards.

A growing body of evidence in human studies supports the idea that HPA axis dysregulation and excess cortisol may be detrimental to cognition. Elevated basal

cortisol levels have been associated with cognitive impairment and decreased hippocampal volume in persons with Cushing syndrome,[11,12] depression,[13,14] and Alzheimer disease.[15-17] Several observational studies of nondemented elderly persons have linked increased basal cortisol levels with decreased cognitive abilities in hippocampus-dependent learning and memory and reduced hippocampal volume.[18] If the findings of these studies are true, then the implications are clear: prolonged exposure to psychosocial hazards may lead to decreased cognitive function. Studies have been limited, however, by sex exclusion,[19,20] small sample sizes,[21-24] and restricted cognitive test batteries.[20,25,26]

While basal cortisol levels can provide a picture of steady-state HPA axis functioning, assessing cortisol response to a psychosocial challenge can serve as a marker of potential HPA axis dysregulation. A recent study[27] measuring cortisol during cognitive testing indicated that higher HPA axis responses to testing were associated with decreases in memory. However, because a psychosocial challenge can itself alter cognition,[28] these results may have been an artifact of testing anxiety or acute cortisol effects[29] rather than from longer-term exposures. Because assessing HPA axis dysregulation during a study visit can have logistical advantages, large population-based studies that attempt to replicate these results with an extensive cognitive battery and careful control for possible acute stress effects are now needed.

This article describes cross-sectional associations between salivary cortisol metrics and cognitive performance, accounting for covariates, in a sample with diverse sociodemographic characteristics. We obtained 4 saliva samples (before, during, and after cognitive testing as well as at visit completion) across 1 study visit. We hypothesized that differences in cortisol patterns, as denoted by metrics of prechallenge cortisol levels and cortisol response to cognitive testing, would be associated with poorer cognitive performance. After accounting for possible acute stress effects, evaluation of these associations would provide evidence that HPA axis dysregulation may have longer-term adverse effects on cognitive function in older persons.

METHODS

SAMPLE SELECTION AND RECRUITMENT

The Baltimore Memory Study is a multilevel longitudinal study of risk factors for cognitive decline in urban residents. Sampling and recruitment have been previously described.[30] Participants were drawn from 65 contiguous neighborhoods in central and north Baltimore city, Maryland, targeted to ensure heterogeneity of relevant demographic characteristics including socioeconomic status and race/ethnicity. Altogether, 1140 participants were enrolled from the eligible population of 50- to 70-year-old residents of Baltimore neighborhoods who had lived within the greater Baltimore area for at least the previous 5 years. The Committee for Human Research of the Johns Hopkins Bloomberg School of Public Health, Baltimore, approved the study. All of the participants provided written, informed consent prior to entering the study and were paid $50 for participation.

DATA COLLECTION

Trained research assistants (Maggie Maly, AA, Ryan Tracy, BA, Tondalaya McCoy, Patricia Gonzales, BA, James Foreman, BA, Danielle Mariast, BA, Lisa Henry, BS, Megan Weil, MPH, Aleruchi Mpi, Quan Lan Jasmine Lew, BA, and Jolie Susan, BS) performed all of the data and saliva collection and cognitive testing at the Baltimore Memory Study clinic in Baltimore city. Data were collected in the following order: cognitive testing, blood pressure, height, weight, spot urine collection, structured interview, venipuncture, and a satisfaction survey. A structured interview captured self-reported information on demographics, medical history, chronic conditions, current and past medication use, alcohol use, and tobacco use. Race/ethnicity was assessed because the study was funded under the disparities initiative of the National Institutes of Health. Participants provided self-reported race/ethnicity using the 2000 US census method.[30] Depressive symptoms were measured using the Center for Epidemiologic Studies–Depression Scale.[31] Recent exposure to stressful events was assessed using a questionnaire that asked whether each of 25 events had occurred in the previous 7 days.

SALIVA SAMPLING AND CORTISOL MEASUREMENT

In prior studies, nonpharmacologic approaches to assess HPA axis dysregulation have measured cortisol levels either in a steady state (basal or nonchallenge) or in response to a psychosocial challenge such as the Trier Social Stress Test.[32] The large sample size of community-dwelling adults in the present study rendered the steady-state sampling of cortisol over a long period (ie, 12-hour urine or plasma collections) logistically infeasible. In addition, the Trier Social Stress Test is time-consuming to administer and cannot be done in conjunction with cognitive testing because it can produce unwanted acute effects on test scores. We instead took maximal advantage of our large sample size and standardized procedures, and unified aspects of both nonchallenge and challenge assessments of HPA axis function, by using the cognitive battery as a mild psychosocial challenge and measuring salivary cortisol in relation to various aspects of the study visit. An analysis[33] using random-effects growth-curve models to evaluate cortisol levels across the study visit showed a small but significant increase in cortisol during the test session after adjustment for diurnal variation and other covariates.

Four salivary cortisol samples from each participant were obtained across 1 study visit (before, during, and after cognitive testing as well as at visit completion). Although cognitive performance was assessed at 3 yearly study visits, cortisol samples were obtained at only 1 visit, at either the second or third visit. Sample collection involved asking each participant to chew lightly on 1 Salivette swab (Sarstedt Inc, Newton, North Carolina) for 45 seconds. Self-reported subjective distress at the time of each saliva collection was assessed by asking participants to choose a number between 1 (lowest distress) and 10 (highest distress) using a visual analogue scale. Visits were scheduled at all times of the day to accommodate the large sample size. Of the 992 participants who underwent cortisol sampling, 373 (37.6%) submitted a first saliva sample from 08:00 to 09:45, 501 (50.5%) from 09:46 to 14:30, and 118 (11.9%) from 14:31 to 18:30. The mean (SD; range) times between sample collections were as follows: samples 1 to 2, 46.8 (10.5; 10.0-98.0) minutes; samples 1 to 3, 77.7 (13.5; 50.0-148.0) minutes; and samples 1 to 4, 158.7 (25.2; 76.0-387.0) minutes.

Cortisol was measured by the core laboratory of the General Clinical Research Center at the Johns Hopkins Bayview Medical Center campus, Baltimore, using a standard radioim-

munoassay (Diagnostic Systems Laboratories, Inc, Webster, Texas). The limit of detection was 0.1 μg/dL (2.76 nmol/L). For cortisol samples below the limit of detection, values were included as the limit of detection divided by the square root of 2.[34]

COGNITIVE BATTERY

The cognitive battery required approximately 90 minutes to complete and has been described elsewhere.[30] The trained technicians first read a statement to each participant designed to orient the participant to the battery while avoiding any mention of testing as a means of evaluating individual ability; this was done to minimize performance-related distress. Each participant completed 20 standard tests grouped into 7 cognitive domains: language (Boston Naming Test; letter fluency; category fluency), processing speed (simple reaction time), eye-hand coordination (Purdue Pegboard dominant hand, nondominant hand, both hands; Trail-Making Test A), executive functioning (difference scores: Purdue Pegboard assembly minus both hands; Stroop Test C form minus A form; Trail-Making Test B minus A), verbal memory and learning (Rey Auditory Verbal Learning Test immediate recall, delayed recall, recognition), visual memory (Rey Complex Figure delayed recall; symbol digit), and visuoconstruction (Rey Complex Figure copy). The categorization of domains and creation of domain scores have been previously described.[35] The 7 cognitive domain scores, expressed in standard deviation units, were the primary study outcomes.

CREATION OF CORTISOL METRICS

In studies with measures of salivary cortisol, no consensus has been achieved about the optimal method of summarizing repeated measures into useful metrics for analysis. Hence, our study took an exploratory approach. Prior to the creation of the cortisol metrics (based on the 4 samples), the distribution of each of the samples was examined. The cortisol samples were severely right skewed; therefore, values were natural log transformed. Initially, 16 metrics were created from the transformed cortisol measurements to represent different aspects of the overall cortisol response. Seven metrics of biological plausibility were then selected for further analysis (**Figure 1**): pretest level (the first sample level), mean level (mean of all samples), area under the curve with respect to zero (AUC),[36] AUC with respect to baseline (AUC_b), variance (variance of all 4 samples), $slope_{12}$ (slope from samples 1-2), and $slope_{34}$ (slope from samples 3-4).

This set of metrics was hypothesized to be sensitive to environmental conditions capable of contributing to HPA axis dysregulation. In an analysis (T.A.G. and B.S.S., unpublished data, 2006) of the metrics and a 12-item scale of objective measures developed using factor analysis to measure neighborhood psychosocial hazards,[1] a consistent and significant J-shaped relationship was found. For example, the mean AUC increased from neighborhoods with the lowest levels of psychosocial hazards (the first quartile) to the third quartile and slightly decreased to the fourth quartile, suggesting a dose-response relationship between cortisol levels and indicators of neighborhood conditions measured independently of study subjects, such as violent crime, abandoned housing, unemployment rates, and street conditions that would lead to differences in HPA axis function. Thus, we believe the metrics may capture the differential impact of long-term exposure of the study participants to psychosocial hazards in the urban environment.

STATISTICAL ANALYSIS

The main objective of the analysis was to evaluate the relationships among the cortisol metrics and the cognitive domain scores, controlling for covariates. In addition, the study explored whether these relationships were modified by age, sex, race/ethnicity, and time of day. Adjusted analyses included a maximum of 967 participants who had complete cortisol and cognitive test data. All of the statistical analyses were performed using the R statistical software package version 2.2.1.[37]

MULTIVARIABLE ANALYSIS USING LINEAR REGRESSION

Multiple linear regression was used to examine the relationships of the cortisol metrics with the cognitive domain scores. The following base covariates were included in model 1 based on a priori knowledge of independent associations with the outcome or if the variable changed the relation of cortisol with the outcome: age (years), sex, race/ethnicity (African American, African American mixed race, and other, with white as the reference group), household wealth (natural log–transformed sum of household income and household assets), educational status (9 levels), study visit, testing technician, and time of day of cortisol sampling (linear and quadratic terms).

Subsequent models were motivated by specific hypotheses to be tested. For increasing control of recent exposures to factors extraneous to the challenge that could influence cortisol levels, model 2 included the covariates of model 1 plus consumption of alcohol in the previous 24 hours (yes or no), number of cigarettes on the day of the visit (5 levels), use of recreational drugs in the past 24 hours (yes or no), and recent stressful events (5 levels). Model 3 included, along with the model 2 covariates, variables for cardiovascular and psychosocial health, including history of stroke, diabetes, cardiovascular disease, and hypertension (yes or no), Center for Epidemiologic Studies–Depression Scale score (continuous), and use of antidepressants, anxiety medications, and hormone replacement therapy (currently prescribed or not). Because self-reported health conditions and medications data were collected at visits 1 and 3, the most current data prior to the visit were analyzed, ie, if a participant's study visit was visit 2, information collected from visit 1 was used. Subjective distress assessed at the time of cortisol collection was also included as a variable to determine whether associations of the metrics with cognitive performance were modified.

Effect modification was assessed using cross-product terms for sex, race/ethnicity, age (ordinal ages <55, 55 to <60, 60-65, and >65 years), and study visit. To evaluate whether time of day influenced associations of the cortisol metrics with cognitive performance, cross-product terms for time of day (late morning to early afternoon, mid-afternoon to late afternoon) were added to model 1. Final models were evaluated for violation of assumptions of linear regression and model fit using standard diagnostic procedures and refit after excluding influential data points.

EVALUATION OF MAGNITUDE OF ASSOCIATION BY COMPARISON WITH AGE

In studies of cognitive function in older populations, one strategy to interpret the magnitude of association of a suspected risk factor with cognitive test scores is to compare an equivalency effect with increase in age.[38] From the linear regression models, we multiplied the β coefficient of a given metric by its interquartile range and divided by the β coefficient for age to obtain an estimate for equivalency effect expressed in terms of age.

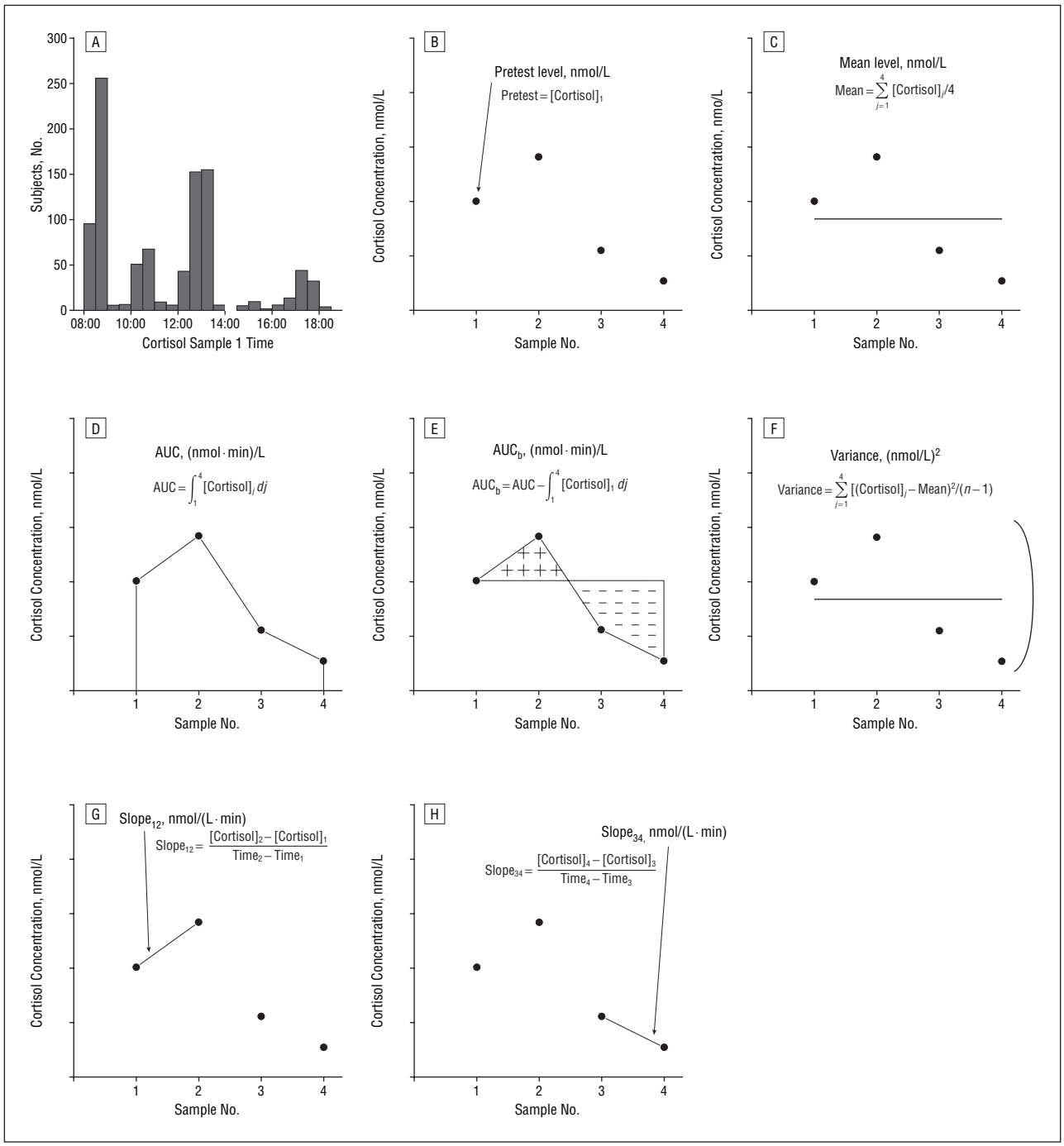

Figure 1. Time distribution of the first cortisol sample (A) and schematic figures (B-H) summarizing cortisol metrics created from 4 saliva samples collected during a study visit for each individual. Individuals were participating in the Baltimore Memory Study from May 30, 2001, to April 19, 2005. Formulas for metrics are included (AUC indicates area under the curve; AUC_b, area under the curve with respect to baseline).

RESULTS

The 967 study participants comprised 642 women (66.4%) and 325 men (33.6%) with a mean (SD) age of 61.1 (6.0) years at the time of the visit (**Table 1**). In comparing persons with and without cortisol measurements (n=992 and n=151, respectively), the individuals in whom cortisol measurements were not obtained were more likely to be less educated ($P=.001$) and African American ($P=.11$). No statistically significant differences were present by sex ($P=.19$) and age ($P=.40$). The 3 metrics of pretest level, mean level, and AUC were highly correlated with each other (**Table 2**). The AUC_b was negatively correlated with pretest and variance but positively correlated with $slope_{12}$. $Slope_{34}$ was negatively correlated with variance. Cognitive domain scores were also correlated, ranging from a low Pearson *r* of 0.29 (processing speed with visual memory) to a high Pearson *r* of 0.64 (executive functioning and eye-hand coordination). Subjective distress ratings increased from the pretest sample

Table 1. Characteristics of the Study Population at the Time of the Study Visit for the Baltimore Memory Study, May 30, 2001, to April 19, 2005

Characteristic	Value
Subjects in analysis, No. (%)	967 (100)
Visit 2, saliva sample and cognitive testing	589 (60.9)
Visit 3, saliva sample and cognitive testing	378 (39.1)
Age, mean (range), y	61.1 (50.0-73.7)
Female, No. (%)	642 (66.4)
Race/ethnicity, No. (%)	
Non-Hispanic white	534 (55.2)
Non-Hispanic African American	392 (40.5)
African American mixed	26 (2.7)
Other	15 (1.6)
Education level, No. (%)	
< High school diploma or trade school	104 (10.8)
Completed high school or trade school	387 (40.0)
Some college or associate's degree	59 (6.1)
≥ College degree	420 (43.4)
Pretest cortisol level, median (IQR), nmol/L[a]	
Aged 50-54 y	9.3 (5.7-14.9)
Aged 55-59 y	8.4 (5.0-13.5)
Aged 60-64 y	8.8 (5.4-14.2)
Aged 65-73 y	10.5 (6.9-15.7)
History of chronic disease, No. (%)	
Cerebrovascular accident	32 (3.3)
Myocardial infarction	72 (7.4)
Hypertension	508 (52.5)
Diabetes	186 (19.2)
CES-D Scale score ≥ 16, No. (%)	141 (14.6)
Current use of medications, No. (%)	
Anxiety	53 (5.5)
Antidepressants	87 (9.0)
Hormone replacement	216 (22.3)

Abbreviations: CES-D, Center for Epidemiologic Study–Depression; IQR, interquartile range.

[a] To convert nanomoles per liter to micrograms per deciliter, divide by 27.588.

(sample 1) to sample 2 obtained just after the Stroop Test (mean [SD] rating, 2.6 [2.2] to 3.3 [2.1], respectively; *t* test, $P<.001$), supporting the idea that the cognitive testing acted as a mild psychosocial stressor.

MULTIVARIABLE ANALYSIS USING LINEAR REGRESSION

In adjusted models, analysis revealed that increases in the pretest level, mean level, and AUC were associated with performance deficits in the cognitive domains of language, processing speed, eye-hand coordination, executive functioning, verbal memory and learning, and visual memory. In model 1 (adjusted for age, sex, race/ethnicity, wealth, education, visit, technician, and time of day), higher pretest values were associated ($P<.05$) with worse performance in processing speed, eye-hand coordination, and executive functioning (**Table 3**). Similarly, the mean level was inversely associated with language and executive functioning ($P<.05$) and with processing speed ($P<.10$). The AUC demonstrated significant associations with nearly all of the cognitive domains except visuoconstruction. In contrast, the AUC_b, variance, $slope_{12}$, and $slope_{34}$ were not associated consistently with cognitive domain scores in the adjusted models. Associations of pretest level, mean level, and AUC with executive functioning, adjusted for covariates, are graphically displayed in **Figure 2**.

Increasing control of potential confounders in model 2 and of chronic conditions and medications in model 3 did not appreciably alter associations across all of the cortisol metrics (results for AUC shown in **Table 4**; other results not shown). The addition of subjective distress variables to all of the models did not appreciably alter results and were thus excluded from further analysis.

The metrics associations with cognitive performance were of a magnitude that appears important in both the clinical and public health context. For example, for domains that were associated with both AUC and age in model 1, an increase of AUC from the 25th to the 75th percentile was equivalent to an increase (95% confidence interval) in years of age as follows: language, 5.6 (4.2-7.1) years; eye-hand coordination, 4.0 (3.2-4.7) years; executive functioning, 4.8 (3.8-5.6) years; verbal memory and learning, 3.2 (2.0-4.5) years; and visual memory, 2.7 (2.0-3.4) years.

EVALUATION OF EFFECT MODIFICATION

Effect modification of the associations of the metrics with cognitive domain scores was next evaluated. There was no consistent evidence that the associations of the cortisol metrics with cognitive domains were modified by age, sex, race/ethnicity, or study visit (data not shown). Importantly, associations also did not differ by the time of day that the cortisol was sampled, suggesting that diurnal variation in HPA axis activity was unlikely to have accounted for the observed associations.

COMMENT

We observed consistent associations between higher levels of 3 of our 7 cortisol metrics and worse performance in multiple cognitive domains. These associations were independent of age, sex, education, race/ethnicity, time of day, and other covariates. The magnitudes of the associations were large and comparable with the effects of age on cognitive performance. The study had many design strengths including random selection of participants from an urban population displaying diversity by socioeconomic status and race/ethnicity, an extensive cognitive test battery, assessment and control of a broad set of potential confounders and effect modifiers, and a large sample size. Of note, using a population-based sample of community-dwelling participants to augment generalizability, we have demonstrated these associations in a relatively younger population of adults in which cognitive decline may not be as readily evident.

For the metrics of pretest level, mean level, and AUC, regression coefficients were negative for relationships with all of the 7 cognitive outcomes. Moreover, among the 6 cognitive domains found to be associated with pretest level, mean level, and AUC, 4 of the domains (language, processing speed, eye-hand coordination, and executive functioning) were associated with at least 2 of the 3 metrics ($P<.05$). We believe that the internal consistency displayed within these metrics and cognitive domain scores is unlikely to be due to chance.

Table 2. Spearman Rank Correlation Coefficients of Cortisol Metrics for the Baltimore Memory Study, May 30, 2001, to April 19, 2005

	Spearman Rank Correlation Coefficient						
Metric	Pretest Level	Mean Level	AUC	AUC_b	Variance	$Slope_{12}$	$Slope_{34}$
Pretest level	1.00						
Mean level	0.85	1.00					
AUC	0.77	0.90	1.00				
AUC_b	–0.46	0.00	–0.04	1.00			
Variance	0.29	0.09	0.11	–0.50	1.00		
$Slope_{12}$	–0.35	0.06	0.08	0.80	–0.25	1.00	
$Slope_{34}$	–0.20	–0.16	–0.15	0.10	–0.49	–0.15	1.00

Abbreviations: AUC, area under the curve; AUC_b, area under the curve with respect to baseline; $slope_{12}$, slope from samples 1 to 2; $slope_{34}$, slope from samples 3 to 4.

Table 3. Adjusted Associations Between Cortisol Metrics and Standardized Cognitive Domain Summary Scores for the Baltimore Memory Study, May 30, 2001, to April 19, 2005[a]

	Cortisol Metric													
	Pretest Level[b]		Mean Level[b]		AUC[c]		AUC_b[c]		Variance[b]		$Slope_{12}$		$Slope_{34}$	
Cognitive Domain	β	SE	β	SE	β	SE	β	SE	β	SE	β	SE	β	SE
Language	–0.404	0.318	–0.710[d]	0.359	–1.034[e]	0.196	–0.213	0.323	0.660	1.094	–3.296	2.056	0.539	3.027
Processing speed	–1.022[d]	0.459	–0.867[f]	0.468	–1.089[e]	0.245	0.505	0.468	0.614	1.583	3.565	2.976	–1.917	4.380
Eye-hand coordination	–0.867[d]	0.344	–0.605	0.390	–1.058[e]	0.210	0.999[g]	0.354	–1.035	1.202	4.078[f]	2.260	–0.778	3.329
Executive functioning	–0.789[d]	0.331	–0.777[d]	0.374	–1.175[e]	0.201	0.547	0.335	–1.744	1.135	0.557	2.145	2.695	3.138
Verbal memory and learning	–0.159	0.402	–0.531	0.454	–0.497[d]	0.246	–0.381	0.409	–1.415	1.382	–4.539[f]	2.598	7.907[d]	3.817
Visual memory	–0.346	0.376	–0.498	0.425	–0.578[d]	0.230	0.011	0.383	–0.247	1.294	–1.462	2.435	–2.560	3.580
Visuoconstruction	–0.058	0.378	–0.208	0.428	–0.308	0.232	–0.095	0.384	–0.420	1.299	–2.683	2.444	–1.903	3.595

Abbreviations: AUC, area under the curve; AUC_b, area under the curve with respect to baseline; $slope_{12}$, slope from samples 1 to 2; $slope_{34}$, slope from samples 3 to 4.

[a] Model 1 was adjusted for age, race/ethnicity, sex, educational level, household wealth, cognitive testing technician, visit, and time of day.

[b] For ease of interpretation, β coefficients and standard errors are multiplied by 10.

[c] For ease of interpretation, β coefficients and standard errors are multiplied by 1000.

[d] $P < .05$.

[e] $P < .001$.

[f] $P < .10$.

[g] $P < .01$.

The AUC of cortisol level over elapsed time over the study visit is mathematically composed of intensity (cortisol concentration) and duration (time) components.[36] We were concerned that the duration component of the AUC, the time from samples 1 to 4 ($time_{1\text{-}4}$, the entire study visit time), was highly correlated (Pearson $r = 0.68$) with the time from samples 1 to 3 ($time_{1\text{-}3}$, the time for cognitive testing). Correlations of $time_{1\text{-}3}$ with cognitive domain scores (Pearson r ranged from –0.30 to –0.61) indicated that $time_{1\text{-}3}$ was a surrogate for cognitive function in that better performers are likely to complete testing in less time. Thus, the AUC could be associated with cognitive domain scores simply because $time_{1\text{-}3}$ is associated with both AUC and cognitive performance. We conducted 3 analyses to address this concern: (1) we added $time_{1\text{-}4}$ as a covariate to linear regression models; (2) we performed stratified analysis of associations of AUC with cognitive domain scores in quartiles of $time_{1\text{-}4}$; and (3) we modeled AUC as its components, mean level and $time_{1\text{-}4}$, and the cross product of the two. The addition of $time_{1\text{-}4}$ to models decreased the β coefficients for AUC on average 50.1%, although there still was suggestion of an association (4 of 7 P values were $< .10$). The stratified analysis demonstrated that within quartiles of $time_{1\text{-}4}$, there were still statistically significant relationships ($P < .05$) between AUC and cognitive performance, especially within the highest quartile. In the third analysis, 2 associations with the mean level were $P < .10$ while adjusting for $time_{1\text{-}4}$ and the cross product. We believe that these additional analyses support an association between AUC and cognitive domain scores, but the third analysis suggests that this may be owing to the association of the mean component of AUC with domain scores.

Associations with cognitive performance were not observed for AUC_b, variance, $slope_{12}$, and $slope_{34}$. The random error inherent in each of the 4 cortisol samples could

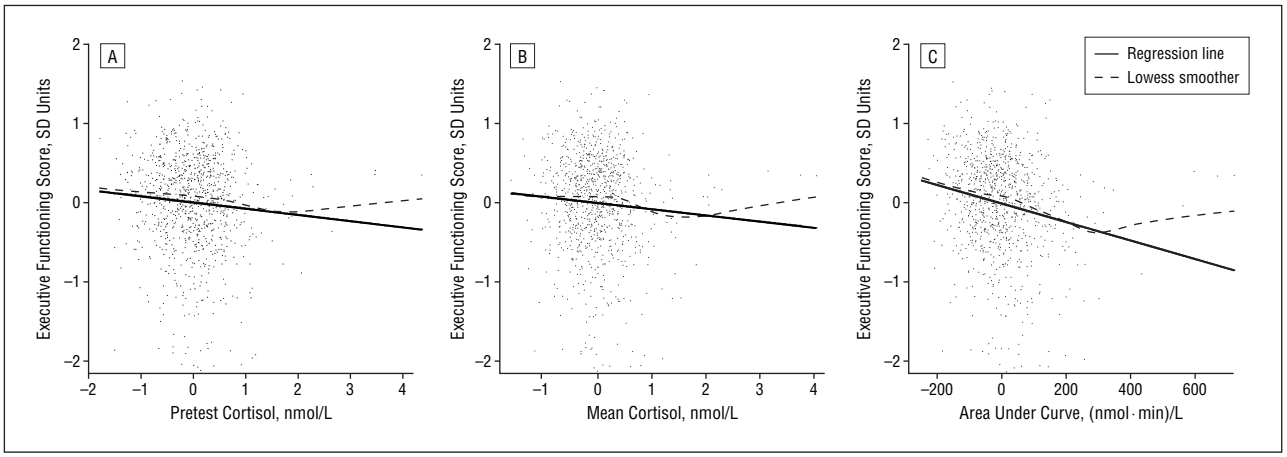

Figure 2. Adjusted variable plots of selected cortisol metrics of pretest level (A), mean level (B), and area under the curve (C) and executive functioning cognitive domain scores in 967 subjects in the Baltimore Memory Study, May 30, 2001, to April 19, 2005. The plots are derived from the linear regression models displayed in Table 3. Metrics were constructed from natural log transformations of 4 cortisol samples (see the "Methods" section). Fitted linear regression lines and lowess curves are overlaid. Lowess lines with smoother span = 0.8 were estimated using R statistical software version 2.2.1.

Table 4. Adjusted Associations Between Cortisol Metric Area Under the Curve and Standardized Cognitive Domain Summary Scores for the Baltimore Memory Study, May 30, 2001, to April 19, 2005[a]

	Model 2 AUC[b]		Model 3 AUC[c]	
Cognitive Domain	β	SE	β	SE
Language	−0.952[d]	0.194	−0.844[d]	0.198
Processing speed	−1.350[d]	0.280	−1.033[d]	0.283
Eye-hand coordination	−1.170[d]	0.212	−1.011[d]	0.219
Executive functioning	−1.138[d]	0.201	−0.967[d]	0.205
Verbal memory and learning	−0.465[e]	0.248	−0.383	0.254
Visual memory	−0.563[f]	0.231	−0.505[e]	0.236
Visuoconstruction	−0.300	0.234	−0.310	0.246

Abbreviation: AUC, area under the curve.

[a] For ease of interpretation, β coefficients and standard errors are multiplied by 1000.

[b] Model 2 was adjusted for covariates in model 1 (described in Table 3) and the following covariates: alcohol consumption in the previous 24 hours (yes or no), number of cigarettes on the day of the visit (5 levels), use of recreational drug in the past 24 hours (yes or no), and recent stressful events (5 levels).

[c] Model 3 was adjusted for covariates in model 2 and the following covariates: history of stroke, diabetes, cardiovascular disease, and hypertension (yes or no), Center for Epidemiologic Studies–Depression Scale score (continuous), and use of antidepressants, anxiety medications, and hormone replacement therapy (currently prescribed or not).

[d] $P < .001$.

[e] $P < .10$.

[f] $P < .05$.

be propagated in creation of the metrics and affect to a greater extent the slope and variance metrics, making it less likely to observe associations. In addition, the lack of associations of these 4 metrics may help explain whether the observed decrements in performance were a result of acute or chronic changes in cortisol levels. One recent study[27] examined whether salivary cortisol response to a cognitive challenge was associated with cognitive performance in persons aged 65 to 80 years. The study found that the difference between the pretest and peak cortisol levels during a cognitive test session, but not the pretest cortisol level itself, was linked with lower scores in 2 verbal memory tasks. The study also found that after adjusting for subjective distress, cortisol response remained significantly associated with only 1 of the 2 tasks, suggesting that test-related anxiety may have in part explained these results. In our study, we found that the rate of initial response ($slope_{12}$) was not associated with cognitive domain scores, indicating that acute changes in cortisol levels did not influence cognitive function in our sample. In addition, we observed that the pretest level was negatively associated with cognitive scores. It is possible that the pretest metric was not representative of basal cortisol levels because the laboratory environment can cause acute elevations in cortisol in older persons due to anxiety about the visit. However, adjustment for self-reported subjective distress at the time of sampling did not weaken the associations of the metrics with cognitive test scores, supporting the conclusions that the metric was more a measure of chronic HPA axis dysregulation and that the associations were more likely a result of longer-term exposure to elevated cortisol.

Our study results must be interpreted in the context of the study design. One limitation is the scheduling of participant visits at all times of the day, as diurnal variation in cortisol levels and differential HPA axis sensitiv-

ity by time of day can affect study results.[39] However, an analysis of 5 independent laboratory studies suggests that comparable HPA axis responses to psychosocial challenges can be reliably measured in the morning and afternoon.[40] Furthermore, we believe that effects of the time of day were minimized through the use of a large sample size to reduce variance, control of important potential confounding variables using linear regression, and evaluation of potential effect modification by time of day.

The cross-sectional nature of our analysis does not allow us to examine cortisol exposure history, which is important to disentangle the issues of long-term vs short-term exposure to cortisol as the instigating factor for worse cognitive performance. Inferential challenges are also presented by the fact that HPA axis dysregulation may occur bidirectionally through either hyperfunction or hypofunction. However, examination of lowess plots revealed no evidence of an inverted U-shaped relationship between the metrics and cognitive domain scores.

It is possible that observed associations are owing to uncontrolled confounding. Determinants of cortisol response to psychosocial hazards include factors such as pharmacological treatments,[41] personality,[42] and mood disorders,[13] and such factors may affect cognition as well. However, our results are biologically plausible and many of these additional "confounders" may actually be mediators or moderators of these relationships; thus, inclusion in our models may not have been appropriate.

Our results are consistent with those of other studies that have explored the cross-sectional and longitudinal associations of elevated cortisol with cognitive function and predominantly found deficits in hippocampally mediated cognitive domains of language and verbal memory.[19-23,25,43,44] We also extend the extant literature by demonstrating the associations of cortisol with eye-hand coordination, executive functioning, and visual memory, which involve brain areas outside of the hippocampus. Given that glucocorticoid receptors are well distributed throughout the primate brain, including the hippocampus, neocortex, and cerebellum,[45] it is not surprising that the harmful effects of elevated cortisol levels may extend to nonhippocampal areas as well. However, the domain-specific associations may be difficult to interpret. Few studies have attempted to map the nature and distribution of glucocorticoid receptors within the primate brain. Compounding this lack of knowledge is the problem that the localization of performance on our cognitive tests to brain structure cannot be achieved with high specificity or reliability. However, because the glucocorticoid receptor is a low-affinity receptor that modulates termination of the stress response, whereas the mineralocorticoid receptor is a high-affinity receptor modulating cortisol feedback at much lower concentrations of glucocorticoids (ie, circadian rhythm),[46] we would speculate that the brain areas involved in this association are those enriched in glucocorticoid receptor and thus injured by long-term exposure to moderate to high cortisol levels.

The recent popularity of studies concerning cortisol and cognition reflect a growing concern over the harmful effects of chronic stress in the context of aging. Extensive literature indicates that the social environment can influence HPA axis function, potentially leading to structural and functional damage of the brain. Repeated exposure to psychosocial hazards such as restraint or subordinate hierarchical status can cause, through glucocorticoid-induced toxic effects, dendritic atrophy and synaptic loss in the hippocampus and the prefrontal cortex as well as suppression of adult neurogenesis in hippocampal formation.[5,47] In accordance with the allostatic load hypothesis[10] that links repeated exposure to environmental stressors with disease through wear and tear of neuroendocrine systems, exposure to psychosocial hazards may result in cognitive impairment via increased cortisol levels in the brain.

In summary, our data demonstrate a dose-response relationship indicating that exposure to higher levels of cortisol in persons aged 50 to 70 years is associated with worse performance in a variety of cognitive domains in the hippocampus and throughout the neocortex. These associations do not differ by age, sex, race/ethnicity, or time of day. The observed lower cognitive performance attributable to higher cortisol levels is not insignificant and is of a magnitude directly comparable to aging. Thus, given that our cortisol metrics may capture dysregulation in the HPA axis and that such dysregulation could be a result of exposure to chronic stress, it is plausible that decrements in cognitive function with aging may be due, at least in part, to long-term exposure to hazards in the psychosocial environment.

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