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Cross-Sectional and 35-Year Longitudinal Assessment of Salivary Cortisol and Cognitive Functioning: The Vietnam Era Twin Study of Aging

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Abstract

High levels of cortisol, a sign of potential hypothalamic-pituitary-adrenal (HPA) axis dysregulation, have been associated with poor cognitive outcomes in older adults. Most cortisol research has focused on hippocampal-related abilities such as episodic memory; however, the presence of glucocorticoid receptors in the human prefrontal cortex suggests that cortisol regulation is likely to be associated with prefrontally-mediated executive function abilities. We hypothesized that elevated cortisol levels would be associated with poorer frontal-executive function in addition to episodic memory. We assessed cortisol from 15 saliva samples paralleling individual diurnal rhythms across three non-consecutive days in a group of 778 middle-aged twin men ages 51 to 60. Cognitive domains created from 24 standard measures included: general cognitive ability, verbal and visual-spatial ability, verbal and visual-spatial memory, short-term/immediate memory, working memory, executive function, verbal fluency, abstract reasoning, and psychomotor processing speed. Adjusting for general cognitive ability at age 20, age, race, and multiple health and lifestyle indicators, higher levels of average area-under-the-curve cortisol output across three days were significantly associated with poorer performance in three domains: executive (primarily set-shifting) measures, processing speed, and visual-spatial memory. In a 35-year longitudinal component of the study, we also found that general cognitive ability at age 20 was a significant predictor of midlife cortisol levels. These results possibly support the notion that glucocorticoid exposure is associated with cognitive functions that are mediated by frontal-striatal systems, and is not specific to hippocampal-dependent memory. The results also suggest that the direction of effect is complex.

Keywords

Cortisol; HPA Axis; aging; cognition; cognitive aging; VETSA

Introduction

Hypothalamic-pituitary-adrenal (HPA) axis dysregulation has been associated with poorer cognitive outcomes in aging populations (Lupien et al., 2007; Lupien et al., 2009). Following McEwen's (McEwen et al., 1968) seminal paper, which showed that the highest density of corticosteroid receptors in the rat brain was in the hippocampus, most cognitive and brain research related to the effect of glucocorticoids has focused on the hippocampus and episodic memory (Kirschbaum et al., 1996; Lupien et al., 1994; Lupien and McEwen, 1997; Newcomer et al., 1994; Wolkowitz et al., 1990). However, in addition to the hippocampus, in humans the prefrontal cortex is a major site of glucocorticoid receptors (Lupien and Lepage, 2001; Lupien et al., 1999b; Sánchez et al., 2000; Sarrieau et al., 1986). The high concentration of glucocorticoid receptors in the human prefrontal cortex suggests that HPA axis dysregulation—in particular, elevated levels of cortisol—may have a significant impact on cognitive functions mediated by the prefrontal cortex, in particular executive function abilities. Executive functions are those that require higher levels of cognitive processing (e.g., abstraction, planning, strategic control of cognitive resources

such as those found in task inhibition, set shifting, suppressing interference). Consistent with this suggestion, some associations between cortisol, executive and other non-episodic memory functions have been found (Lee et al., 2007; Li et al., 2006; Lupien et al., 1994; Lupien et al., 1999a; Newcomer et al., 1999; Walder et al., 2000; Young et al., 1999).

Increasing evidence supports the idea that normative aging disproportionately affects frontal-executive systems. Indeed, some researchers have articulated a two-factor model of brain aging which posits that normal aging primarily involves frontal-striatal and associated executive function changes whereas pathological cognitive aging (e.g., Alzheimer's disease) is more strongly associated with hippocampal abnormalities (Buckner, 2004; Hedden and Gabrieli, 2004). Even the pattern of neuropsychological deficits in Cushing's syndrome—a disease resulting in hypercortisolemia—is not consistent with that of prominent hippocampal dysfunction. It has been argued that this endocrinopathy may produce diffuse bilateral brain dysfunction (Whelan et al., 1980); neuropsychological findings in Cushing's patients point to deficits in several cognitive tasks that are thought to be frontally mediated (Forget et al., 2000; Khiat et al., 1999).

Most studies of cortisol and cognitive function have involved limited sets of cognitive tests with little overlap in measures between studies. Many of the samples are small and selected to be very healthy. The MacArthur Studies involved a 2.5-year follow-up of 194 people ages 70–79. Adjusting for multiple health and lifestyle covariates, verbal episodic memory was inversely associated with cross-sectional cortisol levels and with increased cortisol over time (based on 12-hour urinary cortisol), but only in women (Seeman et al., 1997). In further MacArthur follow-ups over 7 years in 538 people, both men and women in the top quartile of baseline cortisol had larger declines on the Short Portable Mental Status Questionnaire compared with the rest of the sample (Karlman et al., 2005). However, in the Rotterdam Study of 169 adults ages 55 to 80, the single measure of morning cortisol was not associated with Mini-Mental Status Examination scores either in cross-sectional analyses or in a 1.9-year follow-up (Kalmijn et al., 1998).

Three studies find support for an association between cortisol levels and processing speed. Three cognitive tests were administered in the Massachusetts Male Aging study (MMA; n=981; ages 48–80); cortisol measures were based on two morning blood samples taken 20 minutes apart. Higher cortisol levels were associated with poorer processing speed (digit symbol) (Fonda et al., 2005). In a prospective study of cortisol and cognitive function in 197 non-depressed elderly men and women age 65 to 90, three saliva samples were collected on each of two consecutive days—one testing day at a hospital, the next day at home (Beluche et al. 2010). Adjusting for age and education, men with higher morning cortisol at the hospital performed more poorly on a measure of verbal fluency and on the Trails B (a measure of executive function when adjusted for processing speed). The Trails B was not adjusted for processing speed (Trails A). In longitudinal analyses, men with a flatter cortisol slope at time 1 performed more poorly on the Trails B, and the Benton visual memory task. Finally, MacLulich et al. examined plasma cortisol and cognition associations in 97 very healthy men age 65–70; blood samples were collected at 09:00 and 14:30h on the same day as cognitive testing (MacLulich et al., 2005b). Adjusting for an estimate of prior intelligence (i.e. the National Adult Reading Test), elevated morning cortisol was associated with poorer performance on two of eight cognitive tests—the digit symbol substitution test and 24 hour delayed paragraph recall, as well as the general cognitive factor created by factor analysis. Visual memory performance was not associated with cortisol levels. Thus there appears to be some effect of cortisol on cognitive functioning in older adults; however findings are inconsistent between studies and only a few types of cognitive processes have been examined. Overall, performance on the association between cortisol regulation and frontal-executive tests has received little attention.

We are aware of only one large study with an extensive neurocognitive test battery that would enable direct comparison of the relationship of cortisol levels to a wide variety of different cognitive domains. The Baltimore Memory Study is a large-scale study (n=967; ages 50–74) that used a multi-domain neuropsychological test battery so that the pattern of cognitive strengths and weaknesses associated with a variety of indicators of cortisol levels could be assessed (Lee et al., 2007). Cortisol measures were based on four saliva samples taken just before and during the 90-minute testing session; differences in time of day were accounted for statistically. Higher levels of diurnal cortisol secretion were significantly associated with poorer executive function (set shifting), verbal fluency, verbal and visual memory and processing speed¹—even after adjusting for age and a variety of potential confounders (e.g., race, gender, educational level, household wealth, testing technician, visit condition, time of day). This pattern is consistent with effects on frontal-striatal and hippocampal systems. Interestingly, after adjusting for additional health-related confounders (e.g., alcohol consumption, smoking, drug use, stressful events, history of stroke, diabetes, cardiovascular disease, hypertension, use of specific medications), neither verbal nor visual memory was significantly associated with cortisol levels. Thus evidence suggests that individual differences in HPA activity are likely to be associated with prefrontally-mediated executive functions as well as hippocampal systems in middle-aged and older adults (Buckner, 2004; Hedden and Gabrieli, 2004; Pugh and Lipsitz, 2002). Utilizing an extensive neurocognitive test battery allows for some assessment of the specificity of this association.

In the Vietnam Era Twin Study of Aging (VETSA), we utilized a comprehensive test battery covering multiple domains of cognitive function. We included tests that are unlikely to have ceiling effects in community-dwelling samples so that they would be more sensitive to change in future assessments. It is well known that cortisol levels generally follow a predictable diurnal variation but most of the previous large-scale studies measured cortisol levels only in the morning, on single days, or at different times for different people. In VETSA we collected five saliva samples per day, at comparable times, in order to capture the diurnal rhythm. Data were collected on three non-consecutive days—two days at home and one day in the laboratory. In many studies of cognition in older adults, education is used as an indicator of prior cognitive ability because no measure of prior cognitive ability is available. In the VETSA, we had a measure of general cognitive ability from age 20, assessed 35 years prior to the salivary cortisol collection. This provided a rare opportunity to address the question of directionality of influences over a very long interval.

The present study examined cross-sectional and longitudinal relationships between cognition and cortisol in middle-aged men. First, we examined whether cognitive ability at age 20 predicted cortisol levels 35 years later at midlife. Second, we examined cross-sectional associations between cortisol levels and performance in specific cognitive domains at midlife, adjusting for prior cognitive ability. We predicted that elevated cortisol would be associated concurrently with poorer frontal-executive function as well as poorer episodic memory. Finally, because the executive function domain is multidimensional, we examined whether cortisol levels were associated with specific types of frontal-executive functions.

¹Our labeling of neuropsychological domains is slightly different than those used in the Baltimore study. For example, we refer to their language domain as verbal fluency because two of the three measures comprising it were verbal—letter and category—fluency measures. We refer to their eye-hand coordination domain—which consisted of Trails A and Purdue Pegboard—as processing/psychomotor speed.

Methods

Sample

The VETSA baseline assessment comprises 1237 male twins (614 pairs and 9 unpaired twins) ages 51–60 (Kremen et al., 2006). Twins had to be between ages 51 and 59 at the time of recruitment, and both members of a pair had to agree to participate. We recruited participants randomly selected from the Vietnam Era Twin Registry sample of male-male monozygotic (MZ) and dizygotic (DZ) twin pairs who had participated in a 1992 study of psychological health (Tsuang et al., 2001). The twin registry was established in the early 1980s; to be part of the registry, both twins served in the United States military at some time during the Vietnam era (1965 to 1975). The majority of participants did not serve in combat or in Vietnam (72 percent) (Eisen et al., 1987; Henderson et al., 1990). A combination of DNA testing, questionnaire and blood group methods was used to determine zygosity (Eisen et al., 1989); comparisons of the genotype-based and questionnaire-based zygosity measures indicated 95% accuracy. Demographic comparisons indicate that VETSA participants were largely representative of the Registry sample and of middle-aged American men (Kremen et al., 2006; National Health and Nutrition Examination Survey (NHANES III), 1999–2004). We began to assess HPA axis regulation in the third year of the VETSA project, which included the last 795 participants.

Procedures

Twins traveled either to the University of California, San Diego or to Boston University for a day-long assessment involving an extensive neuropsychological test battery, a medical history interview, and functional assessments. When a participant could not or did not wish to travel ($n = 33$ individuals, 2.6%), research assistants conducted assessments at a facility close to the twin's home. IRB approval was obtained at all sites, and all participants provided signed informed consent. Details of the derivation of the test battery and sample have been described elsewhere (Kremen et al., 2006). A month prior to the in-laboratory day of testing (DOT), participants completed a packet of psychosocial and demographic measures at home. On the DOT, participants arrived at 800 h, had a structured medical history interview and then underwent a standardized protocol that included neurocognitive testing and functional assessments until approximately 1600 h, with a scheduled hour break at lunch and breaks as needed.

Saliva collection—Of the 795 available VETSA participants, nine eligible twins declined participation in the cortisol data collection, saliva samples from 3 twins were lost or spilled, and five participants were missing more than one assay from a single day leaving 778 individuals in the cortisol sample (Franz et al., 2010). In brief, the protocol included five saliva samples paralleling individual circadian patterns on each of two non-consecutive work days at home two to three weeks prior to traveling to the test site, and five equivalent samples on the DOT. Nonconsecutive days were chosen to offset the possibility of something unusual about a given single day that may bias samples provided on the next day. All saliva collection materials used by the participants that could come into contact with saliva (e.g., vials, gum, straws) were tested in advance by one of the investigators (SPM) to ensure that they did not influence cortisol assay results.

Participants provided passive drool saliva samples according to their usual daily schedule corresponding to: immediately upon awakening, 30 minutes after awakening, 1000 h, 1500 h, and bedtime. They were provided with a saliva kit that included all supplies: labeled 4.5 ml Cryotube spit vials, a pre-set reminder watch, a daily log, instructions, straws to facilitate drooling into each vial, Trident original sugarless gum (to be used only if needed), and a storage container with a track cap. They were contacted in advance in order to individualize

the saliva kit information and to set times on reminder watches. Reminder calls ensured that instructions were understood and that the participant placed the kit by his bed for the morning sample. Participants were reminded to provide the awakening sample while they were still in bed and to not consume caffeine between the awakening and the awake-plus-30 minutes sample. If participants were acutely ill or experiencing unexpected stress, they were asked to call us to modify their schedule.

For the DOT, participants arrived the day before testing started and received their saliva kit supplies when they arrived at the hotel. As they did at home, on the DOT twins provided samples as soon as they woke up, then half an hour after awakening. The 1000 h and 1500 h saliva samples were collected in the laboratory; bedtime samples were provided back at the hotel. DOT samples were collected between specific tests (close to 1000 h and 1500 h) rather than at exact times so that the collection of saliva samples was standardized across participants. Test day protocols were standardized across sites. Immediately following each saliva sample, participants completed a written log indicating their mood, food and drink intake, medications taken, alcohol use, and their activities during the previous hour. Saliva samples were shipped over-night to the University of California, Davis to be assayed. The majority of participants (98%) reported diurnal cycles that typically involved awakening in the early morning (i.e., 0800 h or earlier); the average awakening time occurred at 0631 h (SD=2.25) (Franz et al., 2010a).

Cortisol assays—Prior to conducting the assays, samples were centrifuged at 3000 rpm for 20 min to separate the aqueous component from mucins and other suspended particles. Salivary concentrations of cortisol were estimated in duplicate using commercial radioimmunoassay kits (Siemens Medical Solutions Diagnostics, Los Angeles, CA). Assay procedures were modified to accommodate overall lower levels of cortisol in human saliva relative to plasma as follows: 1) standards were diluted to concentrations ranging from 2.76 to 345 nanomols per liter (nmol/L); 2) sample volume was increased to 200 μ l, and 3) incubation times were extended to 3 h. Serial dilution of samples indicates that the modified assay displays a linearity of 0.98 and an assay sensitivity (least detectable dose) of 1.3854 nmol/L. Intra- and inter-assay coefficients of variation are 3.962% and 5.662%. Of the 13,311 possible saliva samples, 149 (1%) samples were missing due to participant lapses or technical problems. Participants with more than one assay missing from a day were omitted from these analyses (n=5; N=778).

All samples from a participant were analyzed in the same assay batch; one to three individuals were included in the same assay batch. Batch numbers were retained in order to adjust for possible batch-specific effects. Cortisol assays were performed without knowledge of the zygosity of the participant. If salivary cortisol concentrations exceeded 50 nmol/L, the value was set to missing based on findings of Hellhammer et al. (Hellhammer et al., 2009); in our sample, this value also corresponds with cortisol concentrations three standard deviations above the average awakening mean. Scores were imputed for missing values only if the participant had no more than one missing value on a day. In order to impute missing data, we first calculated the full sample mean cortisol change between the time point with the missing value and the adjacent time point; for all time points except awakening, we used the time point prior to the missing value. We then added (or subtracted) the mean cortisol change for those two points from the individual participant's non-missing time point to obtain the imputed value for the missing time point in question. For example, if a participant was missing a cortisol value for 1500 h, the full sample mean change cortisol from 1000 h to 1500 h was calculated. This value was then subtracted from the participant's 1000 h value to obtain the 1500 h value. Cortisol values were natural log transformed prior to data analysis in order to normalize the distributions.

Cortisol Indices—Two cortisol indices were created to reflect HPA axis regulation. The area-under-the-curve (AUC) cortisol with respect to ground uses values from all five time points in a day and accounts for minor differences in the amount of time between cortisol samples by adjusting for the actual times of the cortisol samples. AUC cortisol is considered a measure of total hormonal output across the day (Pruessner et al., 2003). A second index was created to measure the amount of increase in cortisol from awakening to 30 minutes after awakening (when levels typically peak). This index is the cortisol awakening response (CAR) and reflects responsivity of morning cortisol (Doane et al., 2010; Hellhammer et al., 2009; Hellhammer et al., 2007). Total (three day) scores for AUC Cortisol and CAR were created by averaging values across all three days for each type of indicator. We also examined mean cortisol levels corresponding to each of the AUC measures. The pattern of results for the mean cortisol levels was similar to those for the AUC values, but associations for the mean cortisol levels tended to be slightly lower in significance. Therefore, we present the AUC and CAR results.

Measures

Cognitive Measures—General cognitive ability was assessed with the Armed Forces Qualification Test (AFQT Form 7A), a 50-minute paper-and-pencil test with 100 multiple-choice items. The same version of the AFQT was administered to the participants 35 years previously (prior to military induction) with the same standardized instructions. Scores for the AFQT Form 7A at age 20 were acquired from military records and archived at the twin registry (Orme et al., 2001; Uhlaner, 1952). The AFQT is highly correlated ($r=0.84$) with measures of general cognitive ability such as the Wechsler Adult Intelligence Scale (Wechsler, 1997a). In this sample, AFQT scores were correlated .74 across 35 years (Lyons et al., 2009).

In the VETSA neurocognitive battery we also administered multiple tests to assess 10 specific cognitive domains: Verbal Ability, Visual-Spatial Ability, Verbal Memory, Visual Spatial Memory, Short-Term Memory, Working Memory, Executive Functions, Verbal Fluency, Abstract Reasoning, and Processing Speed. Individual test scores were standardized and averaged in order to create the 10 cognitive domain scores. Analyses were conducted with domain scores first. For sake of comparison with other studies, results for all of the individual tests are shown.

The Verbal Domain was assessed with the Vocabulary subtest from the Wechsler Abbreviated Scale of Intelligence (WASI) (Wechsler, 1997a). The Visual-Spatial Ability domain included Thurstone's adaptation of the Gottschaldt Hidden Figures Test (GHFT) (Thurstone, 1944), and the Card Rotation test (Ekstrom et al., 1976). The GHFT requires that the person identify figures that are embedded in more complex geometric figures. The Card Rotation is a mental rotation test in which participants determine whether rotated figures are the same as a target figure. Both Hidden Figures and the Card Rotation tests are timed tests.

Verbal Memory was assessed with the California Verbal Learning Test-Version 2 (CVLT-2) (Delis et al., 2000), and the Logical Memories Test from the Wechsler Memory Scale-III (WMS-III) (Wechsler, 1997b). For the CVLT, participants repeat all words they can remember from a 16-item list on five learning trials followed by a different list, and then short- and long-delay free and cued recall conditions, and a recognition condition. CVLT scores included in our verbal memory domain were the immediate free recall, delayed free recall, and delayed free recall adjusted for the immediate free recall score. Logical Memories consists of two stories read to participants for immediate and delayed free recall. In our administration, stories were presented only once.

The Visual-Spatial Memory domain was measured using the WMS-III Visual Reproductions tests (Wechsler, 1997b). Five designs are each viewed for 10 seconds and then drawn from memory for immediate and delayed recall. The Short-Term Memory domain was based on scores from WMS-III Digit Span (forward condition) and Spatial Span (forward condition; Wechsler, 1997b). The Working Memory domain included three tests from the WMS-III: Digit Span backward condition adjusted for Digit Span forward; Spatial Span backward adjusted for Spatial Span forward; and Letter-Number Sequencing adjusted for Digit Span forward (Wechsler, 1997b). In this classification, short-term memory involves only maintenance of information, whereas working memory involves processing or manipulation of information (tapped by regressing out the variance due to simple maintenance of information).

Measures in the Executive Function domain included two time scores from the Delis-Kaplan Executive Function System (D-KEFS; Delis et al., 2001) Trail Making Test: number-letter switching (Condition 4) adjusted for number sequencing (Condition 2); and number-letter switching (Condition 4) adjusted for letter sequencing (Condition 3). The time score for the Trails switching condition was adjusted for processing speed (as assessed by the Trails number sequencing (Condition 2) and Trails letter sequencing (Condition 3) in order to isolate the executive function switching component. Scores on the category switching component of the D-KEFS Verbal Fluency test (alternating between saying fruit words and furniture words) were adjusted for scores on the category (animal) fluency test. Both the Trails and Category Switching indices were created to isolate the set-shifting component of the tests. Another Executive measure was the Stroop (Golden, 2003; Stroop, 1935). We used the Stroop interference condition score adjusted for Stroop word reading performance in order to isolate the cognitive inhibition component.

The Verbal Fluency domain included the total correct on the D-KEFS Letter (F,A,S) and Category (animals, boys' names) Fluency tests (Delis et al., 2001). The Abstract Reasoning domain used scores from the Matrix Reasoning subtest of the WASI (Wechsler, 1997a). The Processing Speed domain included three measures: D-KEFS Trails number sequencing; D-KEFS Trails letter sequencing; and the Stroop word reading condition. For the sake of clarity in interpreting data, all scores presented in the tables are modified so that higher scores indicate better performance.

Statistical analyses

Using SAS 9.2, we performed generalized linear mixed models (Proc Mixed). In order to address the first hypothesis, cognitive ability at age 20 was the independent variable (IV) of focus, cortisol at age 55 the dependent variable (DV). For the second hypothesis, we conducted separate analyses for each cognitive domain. Cognitive domains were the DVs, and cortisol at age 55 the IV. For the sake of comparison with other studies, we also conducted the cross-sectional analyses with each cognitive test as the DV and cortisol as the IV. Our primary analyses focused on the cognitive domain scores as the DVs and cortisol as the IV of interest. Although the results of each individual test score are presented, as a check on multiple testing we interpret or discuss individual measures only if the cortisol measure was significantly associated with the cognitive domain to which the cognitive domain belonged. To adjust for the effect of non-independence, the family identifier (i.e., a number shared by the two twins who are nested in the same family), and a batch identifier (i.e., a number shared by all individuals assayed in the same batch) were entered as random effects in the models.

We conducted three sets of statistical models, with each subsequent model adding covariates or potential confounders that were not in the previous models. For the cross-sectional analyses in which we examined whether cortisol (IV) was associated with various types of

cognitive performance, Model 1 adjusted for age, as well as for batch and family random effects. Model 2 additionally adjusted for general cognitive ability (AFQT) at age 20. Model 3 additionally adjusted for health, social, and lifestyle influences (described in the next section). Results are reported for the Type III tests of fixed effects for the cortisol measures. These indicate the unique association of cortisol (AUC or awakening response) with cognitive performance, adjusting for other variables in the model.

Confounders/Covariates: Health, lifestyle, and social influences were included as covariates because of their associations with cognitive aging, particularly in the context of cortisol (Barnes et al., 2006; Elwood et al., 1999; Paul et al., 2008). Participants were coded as having hypertension (yes/no) if their average blood pressure across four measures taken in the morning and afternoon on the day of testing was greater than or equal to 140 systolic and/or 90 diastolic, or if they currently took medication for hypertension (Chobanian et al., 2003). The cardiovascular index (yes/no) indicated the presence or absence of having had a heart attack, heart failure, peripheral vascular disease, stroke, heart surgery, catheterization, or angioplasty (Carmelli et al., 1994). Participants were coded as diabetic (yes/no) if they were taking medication for diabetes and said they had been given a diagnosis of diabetes. Presence of these conditions was based on self report that a physician had told the person that he had the diagnosis. Current smoking was coded as 1=yes and 0=no. Current alcohol consumption was coded as follows based on consumption during the previous two weeks: 0= never drank or did not drink alcohol (beer, wine or hard liquor) in previous 14 days, 1= one or fewer drinks per day, 2= more than one and up to two drinks per day; 3= more than two drinks per day (Paul et al., 2008). Depressive symptoms were assessed using the Center for Epidemiologic Studies Depression Scale (CES-D; Radloff, 1977). CES-D scores were log transformed to normalize the data.

Results

Descriptive statistics (Table 1)

Participants in the VETSA cortisol study were predominantly non-Hispanic Caucasian (629; 85%). The average age at testing was 55.9 (SD=2.6, range 51–60). Average education was 13.8 (SD=2.1) years. The health status of VETSA cortisol participants is fairly consistent with that of men from the United States in the same age group in the 2004–2007 National Health Interview Survey by the Centers for Disease Control and Prevention (Schoeneborn and Heyman, 2009). Rates of hypertension and cardiovascular disease were almost identical in the two samples, with other measures varying only slightly. Overall, the typical VETSA participant's blood pressure was in the pre-hypertension range (Chobanian et al., 2003); average blood pressure was 133.6 mmHg systolic and 83 mmHg diastolic. Rates of diabetes were low in the VETSA sample (Table 1) as compared with the general population (15.5%). This may be due to the fact that only healthy young adults (without Type 1 diabetes or major chronic health problems) were accepted into the military 35 years previously. Less than one percent of the sample self-reported autoimmune diseases such as multiple chemical sensitivity, chronic fatigue, or fibromyalgia; about 9% had asthma. Participants taking glucocorticoids (N=35; 4.5%) were omitted from analyses because of the influence of glucocorticoids on cortisol levels; this left a sample of 743 participants. Of the 743 participants 367 were monozygotic twin brothers (174 pairs, 19 unpaired) and 376 were dizygotic (173 pairs, 30 unpaired).

Unadjusted correlations among cognitive domains and covariates

We examined the extent of collinearity among the predictor variables. Major health problems (e.g., hypertension, diabetes, cardiovascular problems) commonly associated with cognitive performance in older adults for the most part were not associated with cognitive

performance or AUC cortisol. A table of intercorrelations is available from the first author. Current smoking was associated with poorer performance on tests of processing speed ($r=-.12$, $p=.0007$), verbal memory ($r=-.08$, $p=.0336$), visual spatial memory ($r=-.07$, $p=.0448$), verbal ability ($r=-.12$, $p=.0009$), visual spatial ability ($r=-.09$, $p=.0142$), and abstract reasoning ($r=-.12$, $p=.0013$). Smoking was also associated with elevated AUC cortisol ($r=.16$, $p<.0001$) and CAR ($r=.10$, $p=.0063$ respectively). As previously reported, depressive symptoms and age were significantly associated with poorer cognitive performance (Franz et al. 2010b). AUC cortisol and cortisol awakening response were correlated $r=.46$ ($p<.0001$). Diagnostics conducted in SAS (i.e. condition index, tolerance, and variance inflation factors) indicated very weak collinearity among the predictors/covariates.

As expected, there were moderately high intercorrelations among the cognitive domain measures. Correlation coefficients ranged from $r=.16$ ($p<.0001$) between short-term memory with verbal memory to $r=.46$ ($p<.0001$) between executive function and visual-spatial memory. Associations between age 20 general cognitive ability and age 55 cognitive domains ranged from $r=.31$ to $r=.54$ (all $ps<.0001$). When conducting mixed models we did not include all of the cognitive measures simultaneously, therefore the correlations among the different cognitive measures do not cause collinearity in these models.

Longitudinal analysis of age 20 AFQT and current cortisol levels

Age 20 cognitive ability (AFQT) was correlated with AUC cortisol ($r=-.14$, $p=.0002$) and CAR ($r=-.11$, $p=.0039$) across 35 years. After adjusting for covariates, the age 20 AFQT score was still a significant predictor of age 55 AUC cortisol ($t=-2.85$, $df=238$, $p=.005$). The association between CAR and the AFQT was no longer significant after adjusting for overall level of cortisol.

Multivariate concurrent analyses of AUC cortisol and cognitive domains

In Model 1, adjusting for age, assay batch, and family, higher cortisol AUC was associated with poorer performance on all of the cognitive domains except verbal fluency (Table 2, Model 1). After additionally adjusting for AFQT at age 20, AUC Cortisol was significantly associated with AFQT at age 55 and the visual-spatial memory, executive function, abstract reasoning, and processing speed domains (Table 2, Model 2). Addition of the health and lifestyle covariates to the model did not substantially change the results from Model 2 except that abstract reasoning and AFQT at age 55 were no longer significant (Table 2, Model 3). Thus in the final model (Model 3), elevated AUC cortisol was significantly associated with poorer visual-spatial memory, poorer executive function, and slower processing speed. The significant correlations in Model 1 ranged from $-.08$ to $-.17$; after adjusting for health and lifestyle covariates in Model 3, the correlations ranged from $-.07$ to $-.13$ (Table 2).

It is informative to more thoroughly examine associations between individual cognitive measures and AUC cortisol for significant domains in the final model. For the visual-spatial memory domain, higher levels of cortisol were associated with delayed recall but not immediate recall on the visual reproductions test. For the executive function domain, elevated cortisol was associated with individual executive measures involving inhibition (the adjusted Trails and category switching measures) but not interference (adjusted Stroop color-word interference). For the processing speed domain, higher AUC cortisol was associated with poorer performance on the tasks involving manual/motor abilities (i.e., Trails connecting numbers or letters) but not on the Stroop (i.e., word or color reading).

Estimating age equivalency effects—Finally, one approach to examining the effect of a risk factor in cross-sectional aging studies is to estimate an equivalency effect with

increase in age, as implemented by Schafer et al (2005) and Lee et al .(2007). Thus, for a given cognitive domain the parameter estimate from the model is multiplied by the AUC cortisol interquartile range (difference between the 75th and 25th percentiles) and divided by the parameter estimate for age. The parameter estimates in these models are akin to β weights in a multiple regression analysis. We used Model 2 for these analyses because it is most comparable to the models that were examined in this way in the Baltimore Memory Study (Lee et al., 2007). For the significant domains in Model 2, we calculated the age equivalent change in cognitive ability associated with having low AUC cortisol (e.g., 25th percentile) compared with having high cortisol (e.g. in the 75th percentile). For the age 55 AFQT, this corresponded to an expected increase in age equivalent to 1.98 years. Corresponding age increases for the other significant domains were as follows: visual-spatial memory (3.53 years); executive function (2.03 years); abstract reasoning (2.10 years); and processing speed (1.76 years). Given that ages in the VETSA sample ranged only from age 51 to 60, these age equivalents suggest clinically meaningful effects of elevated cortisol on performance in these domains.

Multivariate concurrent analyses of cortisol awakening response (CAR) and cognitive domains

Cortisol awakening response (CAR) was significantly correlated with visual spatial memory ($r=-.13$, $p=.0004$), working memory ($r=-.10$, $p=.0066$); it was associated at a trend level with visual spatial ability ($r=-.07$, $p=.0570$) and short term memory ($-.07$, $p=.0633$). After including the covariates in mixed models run separately for each cognitive domain, only visual-spatial memory remained significant ($r=-.08$, $p=.0256$). Because AUC cortisol and CAR were moderately correlated ($r=.46$, $p<.0001$), in post-hoc analyses we re-analyzed the association with visual-spatial memory but included AUC cortisol in the model. In these analyses, CAR was no longer associated with visual-spatial memory ($r=-.03$, $p=.3758$); however, the association between AUC cortisol and visual-spatial memory remained significant ($r=-.11$, $p=.0046$). These results suggest that it may be the overall high concentration of cortisol rather than the morning responsivity of cortisol that is related to poorer performance on these measures.

We also examined whether AUC cortisol on the DOT was more strongly associated with performance on the cognitive tests. The DOT results paralleled the results based on the average AUC cortisol across all three days (data not shown). Therefore, we have shown only the results for the average of all three days.

Discussion

We found small, but fairly widespread associations between cortisol levels and cognitive performance. Overall, higher cortisol levels were associated with poorer cognitive performance. After adjusting for a variety of potential confounders (Model 3), three of the cognitive domains examined remained significantly associated with cortisol AUC: visual-spatial memory, executive functions, and processing speed. Thus, our hypothesis of an association between cortisol level and frontal-executive function was supported. There was also evidence of a relationship with hippocampal-dependent memory, but it was restricted to visual-spatial and not verbal memory. Executive function is a heterogeneous construct, and our results indicate that it was set-shifting (whether on Trails or category fluency) and not cognitive inhibition (Stroop interference) that was associated with cortisol. Within the visual memory domain, only delayed recall (both adjusted and unadjusted for immediate recall) was significantly associated with cortisol. It is also noteworthy that general visual-spatial ability was not associated with cortisol, supporting the inference of some specificity for visual-spatial memory. Verbal memory has been examined far more than visual memory in studies of aging, but in the VETSA sample, it was visual memory that was significantly

associated with cortisol level. Indeed, visual memory showed the strongest association of the different cognitive domains examined. For the processing speed domain, it was only the Trails measures and not Stroop word that were associated with cortisol level. This pattern may suggest that processing speed involving more manual-motor abilities is associated with cortisol level. Morning responsivity (CAR) did not appear to contribute meaningfully to the association between cortisol and cognitive ability, once the association with AUC cortisol was accounted for.

These results are fairly consistent with other studies. Several studies have now found associations between processing speed measures and cortisol (Fonda et al., 2005; Lee et al., 2007; MacLulich et al., 2005a). Consistent with our measures, their processing speed measures all included manual-motor functions. Beluche et al. also found associations between Trails B and cortisol; however, their result confounded speed and executive functions since they did not adjust the Trails B measure for processing speed (Beluche et al., 2010). As did two other studies, we found support for an association between cortisol and executive function (Beluche et al., 2010; Lee et al., 2007); very few studies have included measures of executive function in their test batteries. Lee et al.'s (2007) executive domain included both set-shifting and cognitive inhibition measures, but they provided only the overall domain results; Beluche et al (2010) used only a single indicator of executive function (Trails B). However, given our differential findings for set-shifting and cognitive inhibition, our results suggest that important information can be gained by unpacking domain variables and examining individual scores.

On the other hand, other studies find evidence for associations between cortisol and verbal fluency and verbal memory; but verbal fluency, verbal memory, and verbal ability had no relationship with cortisol in our study. Verbal fluency was not a cognitive ability for which we hypothesized an association with cortisol level, but it is one that obviously warrants further study given the discrepant results. The Baltimore study also included a visual memory domain, but neither their verbal nor visual memory was significantly associated with cortisol level after adjusting for confounders in that study. Finally, consistent with Beluche et al (2010), we found significant associations between visual spatial memory and cortisol; in MacLulich et al., however, this association was not significant (MacLulich et al., 2005a).

It appears that within the normal range—as reflected in community-based samples—the associations between cortisol level and cognitive function are reliable, but small. Although small, we agree with Lee et al. (2007) that the estimated equivalency effect with respect to age indicates meaningful effects from a public health perspective. The results in the Baltimore study suggested that a cortisol AUC increase from the 25th to 75th percentile was comparable to an increase in age from 2.7 to 5.6 years, depending on the cognitive function. Our results suggested 1.76 to 3.53 years. Only two of the VETSA cortisol study participants were 60 years old; thus, our age range was primarily between 51 and 59. Given our very narrow age range and relatively young sample, it is expectable that these relationships would be smaller in the VETSA sample. It also seems reasonable to conclude that the relative lack of findings of associations between cortisol level and non-episodic memory functions (e.g., executive function, processing speed) in other studies may be due primarily to the lack of emphasis on those other cognitive functions rather than a selective memory effect.

There are multiple theoretical explanations for the effect of aging on cortisol and cognition. Sapolsky's glucocorticoid/neurotoxicity cascade hypothesis predicts that lifetime cumulative glucocorticoid exposure reduces the resistance of neurons to insults, increasing damage (especially to the hippocampus), thus influencing cognition (Kudielka et al., 2009; Lupien et al., 2009b). If neurotoxic effects are strongest to the hippocampus and prefrontal cortex, then

chronically elevated cortisol may affect some specific cognitive abilities more than others. However, if elevated cortisol is associated with other damage—for instance, mitochondrial injury—then there is no reason to expect specificity (Du et al., 2009). In contrast to the neurotoxicity hypothesis, the vulnerability hypothesis suggests that the cortisol levels and associated cognitive changes are due to pre-existing risk factors that are likely to be genetically-mediated. For example, one pre-existing risk factor identified in this study is cognitive ability. Of course, both processes (neurotoxicity and vulnerability) may be involved, individually or interactively, across the lifecycle. Finally, the corticosteroid receptor balance theory would suggest that elevated cortisol is likely to have only minor influence on cognitive performance because of the resilience of the HPA axis: despite aging, homeostatic control of cortisol can be maintained via an altered balance between GR and MR receptors (De Kloet et al., 1991; de Kloet et al., 1998).

In this study, the fact that having lower cognitive ability at age 20 was associated with increased risk for elevated salivary cortisol 35 years later appears to provide some support to the vulnerability hypothesis. It could be that individuals with lower early adult cognitive ability have less adaptive coping skills and gain fewer resources, leading them to experience greater subjective stress and an increased glucocorticoid cascade over the course of their adult lives. It may also be that the relatively small associations between cortisol and cognitive performance in this middle-aged sample reflect the resilience of the HPA axis. These middle-aged men may still be able to compensate in various ways, thus reducing the negative influence of corticosteroids on cognitive processes. Regardless, it is a potentially serious concern to find elevated corticosteroids associated with executive function abilities this early in aging. Executive function abilities, in particular, have been shown to have long-term implications for poorer outcomes across the life course (Alexopoulos et al., 2005; Butters et al., 2008; Cui et al., 2007). Among older adults, executive function deficits are associated with lower functional status, making it harder for older adults to maintain their independent activities of daily living (Kiosses et al., 2001).

Some limitations of the study need to be considered. The Vietnam Era Twin Registry only includes male twins, since very few females were in the military at the time of the creation of the registry. Thus, these results may not generalize to women. Given that the VETSA has only small groups of different racial/ethnic groups, we hesitate to make generalizations about racial/ethnic minorities as well. Finally, the CAR analyses had weaker results than those for AUC cortisol; it is unclear if morning arousal is less predictive of cognitive performance or if the weaker results reflect greater measurement error in the CAR measure. Although we made a major effort to collect the morning awakening and awake+30 minute samples as accurately as possible by using reminder alarms and track caps, it is challenging to obtain these samples reliably (Hellhammer et al., 2007; Kraemer et al., 2006). Accurate CAR measurement is highly dependent on the participants providing the first saliva sample as soon as they wake up and providing the second sample 30 minutes later; deviations from this timing will affect the cortisol slope. Individuals may have different interpretations of when they are awake in the morning and must be relied on to provide the second saliva sample half an hour later without having eaten, brushed their teeth, taken medications, smoked or had a caffeinated beverage.

Strengths of the study included having: a large, community-dwelling sample that is representative of men in the United States in their age group, an extensive cognitive battery, multiple cortisol measures at similar times across multiple days, and a 35-year longitudinal component. The results indicated that elevated cortisol was associated with poorer cognitive function, most strongly in visual-spatial memory, executive function, and processing speed. These findings contribute to the literature which suggests that the association between cortisol and cognition is not hippocampal-specific. The effects were small, but it is worth

noting that several small physiological dysregulations can summate to clinically meaningful dysfunction. Moreover, the results do suggest that cognitive indicators of HPA axis dysregulation are detectable in relatively young, middle-aged individuals. Cognitive ability at age 20 was a predictor of cortisol AUC at age 55, suggesting that the direction of effects is complex. Ongoing longitudinal assessment will be important for understanding individual differences in HPA axis regulation as it relates to cognitive and brain aging.

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Table 1

Descriptive characteristics of the Vietnam Era Twin Study of Aging (VETSA) participants.

Characteristic	Value
Chronic Health Problems, N, (%)	
Cardiovascular Disease	135 (18.2%)
Hypertension	393 (52.9%)
Diabetes	59 (7.9%)
Current smoking, N, (%)	183 (24.7%)
Alcohol Consumption, N, (%)	
None	300 (40.4%)
≤ 1 drink per day for past 2 weeks	317 (42.7%)
>1≤2 drinks per day for past 2 weeks	59 (7.9%)
> 2 drinks per day for past 2 weeks	67 (9.0%)
Center for Epidemiologic Studies	
Depression Scale, mean, (SD), range	8.06 (7.95), 0–52

Table 2

Associations between area-under-the-curve (AUC) cortisol averaged across three days, cognitive domains and individual cognitive measures.

Cognitive Domains and Tests ^a	Model 1 ^b				Model 2 ^c				Model 3 ^d			
	df	r	t-test	p	df	r	t-test	p	df	r	t-test	p
General Cognitive Ability (AFQT @ age 55)	253	-.16	-4.30	.0001	244	-.10	-2.61	.0097	237	-.07	-1.95	.0519
Verbal Ability (WASI Vocabulary)	248	--	-1.67	.0956	239	--	0.03	.9791	232	--	0.38	.7009
Visual Spatial Ability	253	-.10	-2.77	.0006	244	--	-1.61	.1096	236	--	-1.12	.2622
Hidden Figures	250	-.13	-3.57	.0004	241	-.09	-2.35	.0194	234	--	-1.79	.0748
Card/Mental Rotation	251	--	-1.44	.1522	242	--	-0.52	.6044	234	--	-0.32	.7493
Verbal Memory	254	-.08	-2.05	.0416	245	--	-1.06	.2909	237	--	-0.88	.3775
Logical Memory immediate	251	--	-0.38	.7008	242	--	0.88	.3816	235	--	1.03	.3024
Logical Memory delayed	249	--	-0.91	.3652	240	--	0.13	.8994	233	--	0.49	.6249
Logical Memory delayed adjusted for Logical Memory immediate	248	--	-1.05	.2931	239	--	-1.22	.2222	232	--	-0.72	.4733
CVLT Short Delay Free Recall	250	--	-0.99	.3213	241	--	-0.16	.8736	233	--	-0.43	.6673
CVLT Long Delay Free Recall	249	-.07	-2.03	.0437	240	--	-1.04	.2983	232	--	-0.95	.3430
CVLT Long Delay adjusted for Short Delay	248	--	-1.74	.0833	239	--	-1.12	.2627	231	--	-0.63	.5323
Visual Spatial Memory	250	-.17	-4.67	<.0001	241	-.14	-3.79	.0002	234	-.13	-3.54	.0005
Visual Reproduction immediate	250	-.11	-2.95	.0035	241	--	-1.96	.0507	234	--	-1.54	.1246
Visual Reproduction delayed	250	-.17	-4.67	<.0001	241	-.14	-3.81	.0002	234	-.13	-3.59	.0004
Visual Reproduction delayed adjusted for Visual Reproduction immed.	249	-.13	-3.64	.0003	240	-.12	-3.35	.0009	233	-.12	-3.38	.0009
Short Term Memory	253	-.09	-2.46	.0147	244	--	-1.54	.1248	237	--	-1.56	.1189
Spatial Span Forwards	251	--	-1.51	.1330	242	--	-0.89	.3744	235	--	-0.77	.4438
Digit Span Forwards	250	-.09	-2.32	.0211	241	--	-1.46	.1458	234	--	-1.48	.1405
Working Memory	252	-.08	-2.16	.0316	243	--	-1.13	.2584	236	--	-1.14	.2558
Spatial span Backward adjusted for Spatial Span Forward	250	-.09	-2.43	.0157	241	--	-1.47	.1418	234	--	-1.32	.1897
Digit span Backward adjusted for Digit Span Forward	248	--	-1.03	.3017	239	--	0.54	.5920	232	--	-0.76	.4502
Letter-Number Sequencing adjusted for Digit Span Forward	246	--	-1.45	.1483	237	--	-0.77	.4404	230	--	-0.87	.3836
Executive Functions	251	-.14	-3.75	.0002	242	-.09	-2.53	.0123	235	-.08	-2.25	.0257
Trails Switching adjusted for Trails 2	247	-.12	-3.18	.0016	238	-.08	-2.19	.0295	231	-.09	-2.08	.0384
Trails Switching adjusted for Trails 3	247	-.11	-3.10	.0022	238	-.09	-2.15	.0329	231	--	-1.87	.0624
Stroop Color-Word Interference adjusted for Stroop Word	243	--	-1.45	.1478	234	--	-0.56	.5752	227	--	-0.40	.6917

		Model 1 ^b			Model 2 ^c			Model 3 ^d				
D-KEFS Verbal Fluency Category Switching adjusted for Animal Fluency	249	-.12	-3.35	.0009	240	-1.10	-2.82	.0052	233	-.09	-2.47	.0142
Verbal Fluency	250	--	-1.07	.2876	241	--	-0.12	.9047	234	--	-0.02	.9806
D-KEFS Letter Fluency	250	--	-0.79	.4300	241	--	0.12	.9055	234	--	0.12	.9061
D-KEFS Category Fluency	250	--	-1.22	.2221	241	--	-0.41	.6801	233	--	-0.23	.8161
Abstract Reasoning (Matrix Reasoning)	251	-.14	-3.77	.0002	242	-.09	-2.31	.0215	234	--	-1.57	.1180
Processing Speed	251	-.13	-3.51	.0005	242	-.10	-2.75	.0064	235	-.07	-2.00	.0468
Trails Number Sequencing (Trails 2)	250	-.13	-3.54	.0005	241	-.11	-2.95	.0034	234	-.08	-2.12	.0349
Trails Letter Sequencing (Trails 3)	250	-.13	-3.42	.0007	241	-.12	-3.18	.0017	234	-.10	-2.64	.0088
Stroop Word	244	--	-1.19	.2370	235	--	-0.49	.6220	228	--	-0.15	.8823

Notes:

^a Shown are Type III fixed effects for the association between AUC cortisol (IV) and cognitive measures (DVs). For all associations with cognitive domain scores, negative values indicate worse performance. r =correlation adjusted for covariates in that model; AFQT=Armed Forces Qualification Test; CVLT=California Verbal Learning Test; Trails 2=Number sequencing; Trails 3=Letter sequencing; p = significance level. Tests shown under each domain are component tests of that domain. Component tests for each cognitive domain were standardized and averaged to create the domain score.

^b Model 1 was adjusted for age; assay batch, and family ID are included as random effects;

^c Model 2: includes covariates from model 1 plus cognitive ability (AFQT) at age 20;

^d Model 3: includes covariates from models 1 and 2 plus: race/ethnicity (0=non-Hispanic white/1=non-white); cardiovascular problems (0=none/1=any); hypertension, diabetes, currently smokes (0=no/1=yes); Alcohol Consumption (0=none or some in the past 14 days/1=more than two drinks a day in past 14 days), and Center for Epidemiologic Depression Scale scores.