# THE RELATIONSHIP BETWEEN BASAL CORTISOL LEVELS AND COGNITIVE FUNCTIONING ACROSS THE ADULT LIFESPAN

A Thesis
Presented to
The Academic Faculty

By

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In Partial Fulfillment
Of the Requirements for the Degree
Masters of Science in Psychology

Georgia Institute of Technology

May 2016

The Relationship between Basal Cortisol Levels and Cognitive Functioning Across the Adult Lifespan

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Date Approved: March 1, 2016

## ACKNOWLEDGEMENTS

I would like to thank Dr. Scott Moffat for his guidance through this process and my committee members for their insight during the production of this document, as well as Dr. Gilda Ennis for her advice and mentoring. This work was funded by the Ruth L. Kirschstein National Research Service Award (NRSA) Institutional Research Training Grant from the National Institutes of Health (National Institute on Aging) T32AG000175 and NIA grant R01-AG028466.

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## LIST OF SYMBOLS ABBREVIATIONS

| | |
|---|---|
| APOE | Apolipoprotein E |
| ATP | Adenosine Triphosphate |
| BLSA | Baltimore Longitudinal Study of Aging |
| BMI | Body mass index |
| BVRT | Benton Visual Retention Task |
| CD | Cushing's Disease |
| CESD | Center for Epidemiologic Studies Depression Scale |
| CKD | Chronic kidney disease |
| Cr | Creatinine |
| CVLT | California Verbal Learning Test |
| DBP | Diastolic blood pressure |
| HPA | Hypothalamic-Pituitary-Adrenal |
| LDL | Low-density lipoprotein |
| MCI | Mild cognitive impairment |
| MMSE | Mini-mental Status Examination |
| RNA | Ribonucleic acid |
| SBP | Systolic blood pressure |
| TMT | Trail Making Test |
| UFC | Urinary free cortisol |
| WAIS | Wechsler Adult Intelligence Scale |
| WAIS-R | Wechsler Adult Intelligence Scale - Revised |
| WMS | Wechsler Memory Scale |
| $\hat{\gamma}$ | Unstandardized regression coefficient |

## SUMMARY

Age-related declines in cognitive functioning have been well documented, however, there are vast individual differences in the age of onset and magnitude of these changes. This observation has spurred the investigation of the potential risk factors for cognitive decline. Chronic elevations of the steroid hormone cortisol have been shown to compromise hippocampal- and frontal cortex- dependent cognitive tasks in rodents, non-human primates and Cushing's disease patients. Several studies have extended these findings to investigate possible associations between cortisol and cognition in aging human populations. However, these previous examinations of the role of cortisol in cognitive aging have been hampered by the predominant use of single time-point measures of cortisol, small sample sizes, limited age ranges and/or constrained cognitive testing batteries. The present cross-sectional study investigated the relationship between basal cortisol levels, indexed by a 24-hr free cortisol to creatinine ratio, and cognitive functioning on twelve cognitive outcomes in a sample of 1,853 non-demented adults aged 18 to 93 years. The results showed that elevated cortisol levels had small but significant negative effects on verbal learning and working memory performance across the lifespan and significant negative effects limited to older age on a measure of speeded processing. Longitudinal investigation is warranted to examine if within-person changes in cortisol level predict cognitive change.

# INTRODUCTION

Despite the ubiquity of studies reporting individual differences in the severity and timing of age-related declines in cognitive functioning (Drachman, 1986; Rapp & Amaral, 1992; Rowe & Kahn, 1987), relatively little is understood about the possible biological mechanisms contributing to these changes. Chronic exposure to elevated glucocorticoid levels, specifically the stress hormone cortisol in humans, has been hypothesized to mediate age-related declines in cognitive functioning by damaging relevant neural structures and producing subsequent dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis (McEwen, 2002; Sapolsky, Krey, & McEwen, 1986).

Cortisol is an end product of HPA axis stimulation and works in concert with several other hormones to restore homeostasis following exposure to a stressor (Cowell & Buckingham, 2001; Vedder, 2008). Under normal conditions, plasma cortisol levels fluctuate between 0.04 and 0.93 μg/dL ("Rochester 2016 Interpretive Handbook," 2016, p. 569) and increase as much as fourfold in response to moderate stressors (Dickerson & Kemeny, 2004; Kirschbaum, Pirke, & Hellhammer, 1993). Due to its liposolubility, cortisol is able to cross the blood-brain barrier (Pardridge & Mietus, 1979) where it binds to a widespread network of receptors and exerts both short and long-term auto-regulatory effects (Fulford & Harbuz, 2005).

Included in this network are the pyramidal neurons of the hippocampus and prefrontal cortex (Klok et al., 2011; Seckl, Dickson, Yates, & Fink, 1991; Watzka et al., 2000; Webster, Knable, O'Grady, Orthmann, & Weickert, 2002). In addition to contributing to the auto-regulatory circuit, these brain regions subserve a number of cognitive functions including memory formation. Cortisol binding affects the functioning of these neurons such that moderate

levels increases primed burst potentiation (a function believed to underlie memory formation) (Bliss & Collingridge, 1993), promote memory consolidation (de Kloet, Oitzl, & Joëls, 1999; Lupien et al., 2002), and may be necessary for working memory function (Mizoguchi, Ishige, Takeda, Aburada, & Tabira, 2004). However, under conditions of prolonged glucocorticoid elevation outside of the normal circulating range (such as can be caused by repetitious exposure to stressors or a disease state) these same neuronal functions may be impaired (Kim, Pellman, & Kim, 2014; Pavlides, Nivón, & McEwen, 2002; Pavlides, Watanabe, & McEwen, 1993).

Chronic elevations of cortisol are believed to contribute to cognitive impairments by temporarily or permanently altering the relevant underlying neural structures. Two relevant pathways of structural change are (1) reversible dendritic retraction and (2) necrotic neuron death due to impaired ability to survive coincident neurological insults (Sapolsky, 2007). Cortisol is central to both of these processes because it impairs cellular transport of glucose by bound pyramidal neurons, thereby creating a deficit in available adenosine triphosphate (ATP) (de Leon et al., 1997; Horner, Packan, & Sapolsky, 1990). The shortage of ATP can result in excitotoxic concentrations of glutamate in the synapses, producing reversible apical dendritic retraction of neurons in the hippocampus and medial prefrontal cortex (mPFC) (McEwen, 2007). Elevations of glutamate can also produce calcium excesses in the post-synaptic neuron capable of triggering protein malfolding, cytoskeletal degradation and oxygen radical generation, processes which, in extreme instances, can collectively result in necrotic cell death (Popoli, Yan, McEwen, & Sanacora, 2012; Rossi, Oshima, & Attwell, 2000; Sapolsky, 1993; Sapolsky, 1999, 2000).

### 1.1 Animal Models of Glucocorticoids and Cognition

The hypothesis that glucocorticoid elevations could contribute to age-related cognitive

changes arose from the rodent literature (Landfield, Waymire, & Lynch, 1978). It was observed that severely limiting glucocorticoid exposure via adrenalectomy in middle-aged rodents prevented structural declines in old age (Landfield, Baskin, & Pitler, 1981), whereas prolonged maintenance of glucocorticoids in the high physiological range via exogenous administration induced structural declines in young rodents analogous to those seen in aged animals (Sapolsky, Krey, & McEwen, 1985). It was subsequently reported that these structural changes had functional consequences. For example, younger rodents with glucocorticoid-induced neural structure changes demonstrated deficits in cognitive performance comparable to those observed in older rodents (Arbel, Kadar, Silbermann, & Levy, 1994). Since these initial observations, decades of research has confirmed that chronic exposure to elevated glucocorticoid levels as a result of stress or exogenous glucocorticoid administration can produce deficits in hippocampal-dependent spatial learning (see Conrad, 2010 for review) mirroring the impairments observed in older rodents (Endo, Nishimura, & Kimura, 1996).

Evidence for the deleterious effects of elevated glucocorticoid exposure on neural structure has been extended to non-human primates. Chronic elevations in cortisol levels as a result of induced stress or exogenous cortisol administration have been demonstrated to produce structural impairments to the hippocampus including cell layer irregularity, dendritic atrophy, cell shrinkage and nuclear pyknosis (Fuchs, Uno, & Flügge, 1995; Magariños, McEwen, Flügge, & Fuchs, 1996; Sapolsky, Uno, Rebert, & Finch, 1990; Uno, Tarara, Else, Suleman, & Sapolsky, 1989; see also Vollmann-Honsdorf, Flügge, & Fuchs, 1997). Notably, only one study reported a reduction in neuron number, but it is assumed that the animals examined in this experiment experienced severe psychosocial stress for a period of months to years prior to death (and presumably concomitantly elevated cortisol levels) (Uno et al., 1989); a paradigm more extreme

than the typical stress-induction paradigm, which fails to produce volume declines detectable by structural magnetic resonance imaging (MRI) (Ohl, Michaelis, Vollmann-Honsdorf, Kirschbaum, & Fuchs, 2000).

Additionally, deficits in both hippocampal and frontal cortex functioning have been demonstrated in non-human primates following exposure to chronic stress or exogenous glucocorticoid treatment (Lyons, Lopez, Yang, & Schatzberg, 2000; Ohl et al., 2000) (cf. Bartolomucci, De Biurrun, Czéh, van Kampen, & Fuchs, 2002; Ohl & Fuchs, 1999). Ohl et al. (2000) reported that seven weeks after the cessation of a four-week psychosocial stress paradigm, male tree shrews were impaired on a hippocampal-dependent spatial memory task and Lyons et al. (2000) reported a negative effect of 28 days of cortisol administration on response inhibition, a frontal-cortex dependent task. The strong evidence from non-human species for glucocorticoid induced structural and cognitive changes has led a number of researchers to examine whether prolonged exposure to elevated cortisol levels in human populations might elicit similar effects in humans.

### 1.2 Glucocorticoids and Cognition in Cushing's Disease

Spontaneous Cushing's disease (CD) patients provide a naturalistic opportunity to examine the effects of chronically elevated cortisol levels in humans. CD is characterized by highly elevated glucocorticoid secretion that ordinarily lasts from months to years prior to diagnosis and subsequent treatment (Castinetti, Morange, Conte-Devolx, & Brue, 2012). As would be predicted from the animal literature, a negative relationship between cortisol levels and hippocampal volume has been reported in CD patients (Starkman, Gebarski, Berent, & Schteingart, 1992). Greater cerebral atrophy than age-matched controls has been observed in CD

patients, albeit only in patients 60 years and older (Bourdeau et al., 2002; Simmons, Do, Lipper, & Laws Jr, 2000).

An early study of 35 CD patients found that performance on several Wechsler Adult Intelligence Scale (WAIS) subscales was impaired in almost half of CD patients (Whelan, 1980). More recent studies of CD patients have consistently reported deficits on a number of WAIS-R and Wechsler Memory Scale (WMS) measures compared to age-matched controls (Dorn & Cerrone, 2000; Forget, Lacroix, Bourdeau, & Cohen, 2016; Forget, Lacroix, Somma, & Cohen, 2000; Martignoni et al., 1992; Mauri et al., 1993; Starkman, Giordani, Berent, Schork, & Schteingart, 2001).

The biochemical and surgical treatments of CD allow for the examination of the long-term impact of glucocorticoid elevations in the absence of elevated circulating levels. To examine this relationship, several researchers have followed CD patients longitudinally prior to- and following treatment to determine if cognitive functioning improves once circulating cortisol levels are normalized. While improvements in cognitive functioning, including performance on the Digit Symbol Substitution Test and Visual Reproduction performance, have been reported at follow-ups 6 to 36 months post-treatment (Forget et al., 2016; Hook et al., 2007; Mauri et al., 1993), this is not true of all patients (Dorn & Cerrone, 2000), and many cognitive domains do not show improvements, even when normal cortisol levels are maintained for a period of months to years. Similar results have been obtained for structural brain changes. Following the normalization of cortisol levels, significant increases in hippocampal volume have been reported (Starkman et al., 1999; Toffanin et al., 2011). Declines in cerebral atrophy, third ventricle diameter and bicaudate diameter were reported for 22 patients following correction of

hypercortisolism, but improvement appears to plateau in less than two years (Andela et al., 2015; Bourdeau et al., 2002).

### 1.3 Glucocorticoids and Cognition in Normal Human Aging

The negative relationship between cortisol indices and cognitive functioning in both the animal literature and CD patients have motivated a number of researchers to investigate the association between cortisol levels and cognitive abilities across the lifespan, particularly within healthy older adults. This discussion focuses on the results of cross-sectional examinations of contemporaneously measured (i.e. within two weeks of each other) cortisol levels and cognitive functioning in non-demented adults. In line with findings from animal research and studies of CD patients, these findings hint at a negative influence of elevated cortisol levels on hippocampal-dependent memory and frontally-mediated executive functioning. However, the literature is far from conclusive, primarily due to difficulties inherent to cortisol measurement, sample size deficiencies and variations in cognitive test selection.

For example, three large studies (Comijs et al., 2010; Fonda, Bertrand, O'Donnell, Longcope, & McKinlay, 2005; Kuningas et al., 2006) reported significant negative relationships between morning plasma cortisol levels and performance on information processing measures but another large study, Schrijvers et al. (2011) did not find any relationship between morning plasma cortisol and Letter-Digit Substitution Task performance. Comijs et al. (2010) further reported a negative relationship between immediate word-list learning performance and morning plasma cortisol levels but Kuningas et al. (2006) did not find such a relationship.

Using salivary measures, two large studies (Gaysina, Gardner, Richards, & Ben-Shlomo, 2014; Gerritsen, Comijs, Deeg, Penninx, & Geerlings, 2011) reported negative associations of

bedtime cortisol levels with immediate and delayed word-list learning (respectively) while Singh-Manoux et al. (2014) and Stawski et al. (2011) did not find such relationships (respectively) using similar sampling techniques. A significant negative relationship between a composite measure of executive functioning and daytime (sampled between 0800 and 1830) salivary cortisol levels was reported by Lee et al. (2007), however, Stawski et al. (2011) reported a significant negative relationship on a similar construct with bedtime cortisol levels, but not with cortisol samples taken in the morning or at lunchtime.

### 1.3.1 Complications of single time-point measures.

Despite the number of well-powered investigations into the relationship between cortisol and cognitive functioning it is difficult to construct a cohesive interpretation of the findings, largely due to the widespread use of single time-point plasma or salivary cortisol measures. Although the sampling and assay techniques have been validated to accurately reflect the level of circulating cortisol at the time of collection (Gatti et al., 2009; Smyth, Hucklebridge, Thorn, Evans, & Clow, 2013), the circadian and ultradian rhythms complicate the interpretation of these values. Further, these measures are poor proxies for individuals' overall cortisol exposure, correlating only loosely ($r = .32$) with 24-hour cortisol production (Yehuda et al., 2003).

The circadian rhythm of cortisol is characterized by a morning peak just following waking, followed by a decline throughout the day until the nadir during sleep (Bliss, Sandberg, Nelson, & Eik-Nes, 1953; Horrocks et al., 1990; Weitzman et al., 1971). Deviations from this pattern, especially flattening of the slope across the day, have been associated with poorer health (Jones & Benca, 2015; Reutrakul & Knutson, 2015; Videnovic & Zee, 2015). Thus, direct comparisons between concentrations collected at different times of day (e.g. comparing morning

levels to afternoon levels) is inappropriate because elevated or depreciated cortisol levels at different points across the day do not have the same meaning.

Further, because the circadian rhythm is heavily influenced by an individuals' sleep-wake cycle, between-participant comparisons of samples collected at fixed occasions are subject to large amounts of variance if participants' time of waking is not statistically controlled. Of the seven aforementioned studies examining the relationship between cortisol levels and cognitive functioning, two used fixed sampling occasions (prior to 1000 and prior to 1100) (Comijs et al., 2010; and Kuningas et al., 2006, respectively), but did not statistically control for the time of sampling or participants' time of waking. Two studies using a broader sampling range (between 0800 and 1100 and between 0800 and 1830) statistically controlled for the time of sampling, but not for the participant's time of waking (Lee et al., 2007; Schrijvers et al., 2011). Other approaches to limiting the variance introduced by the circadian rhythm include using the average of two samples collected within four hours of waking (Fonda et al., 2005) and aligning time-point sampling with the participants' bedtime (Gerritsen et al., 2011; Stawski et al., 2011). Only Stawski et al. (2011) explicitly controlled for participants' time of waking. Thus, the results of extant studies have been hampered by uncontrolled circadian variation in corticosteroid levels.

Finally, the ultradian rhythm underlying the circadian rhythm of cortisol introduces even more between- and within-person variation. This rhythm is characterized by pulsatile secretions of cortisol from the adrenal glands at varying frequencies, ranging from 95 to 180 minutes apart (Follenius, Simon, Brandenberger, & Lenzi, 1987). The exact timing of these pulses varies extensively day-to-day, such that the cortisol levels obtained from samples collected at the same time on consecutive mornings from the same individual are only moderately correlated (intraclass correlation coefficient (ICC) = .47) (Viardot et al., 2005).

### 1.3.2 Summary measures of cortisol production.

One approach to improving the validity of single time-point samples as a proxy for cumulative cortisol exposure is to create summary measures calculated from several samples collected at specified times across the day. Using this method, a study of 778 men in their fifth decade of life reported significant negative relationships between the area-under-the-curve (AUC) relative to ground (Pruessner, Kirschbaum, Meinlschmid, & Hellhammer, 2003) averaged across three days and performance on WMS-II delayed visual reproduction, the Delis-Kaplan Executive Function System (D-KEFTS) (Delis, Kaplan, & Kramer, 2001) Trail Making Test (conditions 2, 3, and 4 adjusted for 2) and Verbal Fluency (Franz et al., 2011). Another large study conducted by Singh-Manoux et al. (2014) did not find a relationship between Alice Heim 4-I Test performance (Heim, 1970) and mean cortisol levels from a single day. Neither study found a relationship between AUC cortisol levels and immediate word-list learning performance or Verbal Fluency (category or letter).

Although summary measures calculated from pooling several single time-point measures, such as AUC, provide a measure of total daily output, these measures are still affected by circadian variation. These measures demonstrate little improvement in stability from a single, single time-point measure, such that across three consecutive days of measurement 44.4% of the total variance in AUC is attributable to day-to-day factors (Ross, Murphy, Adam, Chen, & Miller, 2014). However, reliability of these measures can be increased by averaging single time-point samples taken at the same time across multiple days. It is recommended that researchers aiming to detect between-person differences in summary measures created using salivary collection average samples from across three days (Segerstrom, Boggero, Smith, & Sephton, 2014). This procedure reduces the size of the 95% confidence interval for the steady state value

by 43% (Brambilla, O'Donnell, Matsumoto, & McKinlay, 2007). However, of the aforementioned studies, only Franz et al. (2011) and Stawski et al. (2011) averaged across multiple days.

### 1.3.3 Cumulative cortisol indices.

Rather than estimating cumulative cortisol exposure from a few single time-point samples taken across the day, some researchers have opted to collect all urinary cortisol excretion for 12 or 24 hours or sample plasma cortisol hourly for 24 hours. To date, two studies have utilized urinary cortisol measures. The first, Rubinow, Post, Savard, and Gold (1984), reported a null relationship between at least two averaged 24-hr mean cortisol excretion levels and performance on the Halstead Category Test (Reitan & Wolfson, 1985) in 31 cognitively normal adults aged 19 – 64 years. The second, Seeman, McEwen, Singer, Albert, and Rowe (1997), reported a negative relationship between delayed story recall performance and overnight urinary cortisol among 103 women in their seventh decade of life. Notably, this relationship did not emerge for the 88 men of the same age. Of the two studies collecting hourly plasma samples for a consecutive 24 hours, Lupien et al. (1994) reported null relationships between the mean cortisol level and a large cognitive battery among 19 cognitively normal adults aged 60 – 80 and Fiocco, Poirier, Joober, Nair, and Lupien (2008) reported a null relationship between cued recall performance and total cortisol output among 65 cognitively normal older adults.

### 1.3.4 Summary of present state of the literature.

At present, there is a sizable literature examining the relationship between cortisol and cognitive functioning in healthy adults. However, coherent interpretation of this literature is limited by the predominant use of single time-point measures. A few studies have used summary

measures of cortisol, thereby facilitating interpretation across studies, however, these studies are not without their limitations.

For example, one of the largest and most informative studies to date, Franz et al. (2011), included only male subjects and restricted the age range examined to the fifth decade of life, a period that some argue is too early to detect age-related cognitive changes (Nilsson, Sternäng, Rönnlund, & Nyberg, 2009; Rönnlund, Nyberg, Bäckman, & Nilsson, 2005; cf. Salthouse, 2009). The large study conducted by Singh-Manoux et al. (2014) included women as just over 20% of the sample and included a broader age range (approximately 49 to 74 years), but used a limited testing battery. The four studies using measures of cumulative exposure to cortisol (e.g. 24-hr or overnight sampling) (Fiocco et al., 2008; Lupien et al., 1994; Rubinow et al., 1984; Seeman et al., 1997) have been severely limited by small sample sizes and/or limited cognitive testing batteries.

### 1.4 Contributions of the Present Study

The present study circumvents many of the problems in the extant literature by correlating performance on a comprehensive cognitive test battery with 24-hr urinary cortisol levels that minimize the influence of circadian fluctuation in a large (N = 1,853), adult lifespan (aged 18 – 93 years) sample of participants from the Baltimore Longitudinal Study of Aging (BLSA). The present study will also control for the possible influence of a number of demographic, health and lifestyle covariates that could potentially affect individuals' cortisol levels and/or cognitive performance.

### 1.4.1 Predictions.

It is anticipated that age-related declines will be present in all cognitive outcome variables. Gender differences are predicted such that males will demonstrate superior performance on the Card Rotations Test while a female advantage will be present for California Verbal Learning Test (CVLT) and Verbal Fluency outcomes.

In line with previous cross-sectional studies and theoretical models of negative cortisol effects on hippocampally and pre-frontally-mediated cognitive functions it is anticipated that elevated cortisol levels will be associated with poorer performance on the Trail Making Test (TMT) part B, both the learning and delayed recall outcomes of the CVLT, and the BVRT. Further, it is hypothesized that these relationships will withstand controlling for several potential confounding variables.

# METHODS

## 2.1 Participants

The analyses will be conducted using data collected from the BLSA between March 1981 and May 2008. The BLSA consists of volunteers who return to the National Institute of Aging (NIA) in Baltimore biannually where they receive a number of behavioral and medical assessments and provide specimen samples, including a 24-hr urine sample, which are subsequently stored in the BLSA specimen bank. Six thousand and sixty-three of these urine samples contributed by 1,865 individuals were selected for assay on the basis of overlapping cognitive testing.

### 2.1.1 Participant exclusion.

Given that the goal of the current study is to examine the relationship between cortisol exposure and cognitive functioning in healthy individuals, all data from subjects demonstrating cognitive impairment were excluded. Clinical and neuropsychological data from BLSA participants were reviewed at a consensus conference if they screened positive on the Blessed Information Memory Concentration score (Fuld, 1978) (score $\geq 4$), if their Clinical Dementia Rating (Morris, 1993) score was $\geq 0.5$ using subject or informant report, or if concerns were raised about their cognitive status. In addition, all participants were evaluated by case conference upon death or withdrawal. Diagnoses of dementia and Alzheimer's disease were based on DSM-III-R (American Psychiatric Association, 1987) and the National Institute of Neurological and Communication Disorders—Alzheimer's Disease and Related Disorders Association criteria (McKhann et al., 1984), respectively. Mild cognitive impairment (MCI) was based on the Petersen criteria (Petersen et al., 1999) and diagnosed when (1) cognitive impairment was

evident for a single domain (typically memory) or (2) cognitive impairment in multiple domains occurred without significant functional loss in activities of daily living.

Further, participants were excluded from all analyses if they had a history of vascular dementia, depression, Parkinson's disease, cancer, myocardial infarction or congestive heart failure. Cortisol and cognitive data were excluded on a per-visit basis if a participant reported the use of steroid, hypno-sedative or anti-depressive medications resulting in a total of 1,853 cortisol samples and 11,464 scores across 12 cognitive outcomes. Because the BLSA sample has been continuously recruited since 1958, subjects entered the BLSA in varying years and at varying ages, thus the demographics provided in Table 1 reflect participant characteristics at the first visit for which a corresponding cortisol measure and cognitive outcome were obtained.

Table 1: Baseline Demographic Characteristics

| | Value | Min | Max |
|---|---|---|---|
| **N** | 1,853 | | |
| **UFC:Cr** | 19 ± 12 | 2 | 94 |
| **Age, years** | 58 ± 15 | 18 | 93 |
| **APOE ε4 present (%)** [a] | 458 (25%) | | |
| **Alcohol Use [grams/day]** | 11 ± 17 | 0 | 120 |
| **CESD Score** [b] | 6 ± 6 | 0 | 45 |
| **Education [years]** [c] | 17 ± 3 | 6 | 21 |
| **Non-Hispanic white (%)** | 1333 (72%) | | |
| **Females (%)** | 906 (49%) | | |
| **Smoking Status** [d] | | | |
| **Current** | 106 (6%) | | |
| **Former** | 818 (44%) | | |
| **Diastolic Blood Pressure** [e] | 78 ± 12 | 40 | 124 |
| **Systolic Blood Pressure** [e] | 128 ± 21 | 88 | 220 |
| **Body Mass Index** [f] | 27 ± 5 | 17 | 54 |
| **Low Density Lipoprotein** [g] | 115 ± 33 | 31 | 263 |
| **Diuretic Use** | 196 (11%) | | |
| **Sex Hormone Use** | 310 (17%) | | |
| **Statin Drug Use** | 276 (15%) | | |
| **Thyroid Drug Use** | 227 (12%) | | |
| **Cardiac or Vascular Disease** | 494 (27%) | | |
| **Chronic Kidney Disease** | 76 (4%) | | |
| **Diabetes** | 114 (6%) | | |

*Note*. Data presented as Mean ± SD or N (%); UFC:Cr = Urinary free cortisol: Creatinine ratio

[a] N = 1,607. [b] N = 1,621. [c] N = 1,1,815. [d] N = 1,824. [e] N = 1,802 [f] N = 1,848 [g] N = 1,753.

## 2.2 Cognitive Tasks

### 2.2.1 The Benton Visual Retention Test.

The BVRT (Benton, 1945) measures visual memory, perception and constructive abilities (Strauss, Sherman, & Spreen, 2006, p. 691). The BLSA utilized alternate forms C, E and D of the Revised BVRT (Benton, 1974). Participants were first administered form C during their first visit, then E, then D, then form C again on the fourth visit and so forth (Shock et al., 1984). All forms were administered using the standard procedure in which the subject is shown a series of 10 designs, each with 1 – 3 figures. Each design is presented for 10 seconds then withdrawn from the subject and the subject reproduces the design from memory. The total number of errors made in the reproduction of the designs (0 – 25) was used as the outcome measure.

### 2.2.2 The Boston Naming Test.

The Boston Naming Test consists of 60 (Kaplan, Goodglass, & Weintraub, 1978) black and white drawings of common objects (Kaplan, Goodglass, & Weintraub, 1983) presented to participants for naming with increasing difficulty. The test was started with item 30 (harmonica) then proceeded forward. If the subject missed any of the items 30 – 37, the experimenter proceeded backwards from item 29 until eight consecutive preceding items are correctly named without assistance, then the test resumed in the forward direction from the initial missed item. Testing was discontinued when the participant make eight consecutive errors. One point is awarded for each spontaneously named item (for a maximum of 60), with those correctly identifying items 30 – 37 being awarded the 29 points for items 1 – 29 (Strauss et al., 2006, pp. 901-904).

### 2.2.3 The California Verbal Learning Test.

The CVLT (Delis, Kramer, Kaplan, & Ober, 1987) is a multiple-trial list-learning task that measures verbal learning and memory (Strauss et al., 2006, p. 730). Participants were presented with a list of 16 words. Immediately after list presentation, participants were asked to recall as many words from the list as possible. This is repeated for a total of five presentation-recall trials. The total number of words correctly recalled across the five trials (0 – 80) was recorded as the first CVLT outcome measure (*CVLT Learning*). This recall is followed by a 20-minute delay during which non-verbal testing occurs. After 20 minutes participants are again prompted to free-recall words. The number of correctly recalled words (0 – 16) comprised the second CVLT outcome measure (*CVLT Delayed*).

### 2.2.4 Card Rotations Test.

The Card Rotations Test evaluates individuals' ability to mentally rotate objects presented in the picture plane (Ekstrom, French, Harman, & Dermen, 1976, pp. 149-151). Subjects are required to indicate whether each of eight drawings reflects rotated or mirrored versions of the target option. There are two parts of the test, each containing 14 target drawings. Participants were given three minutes to complete each part. The total score on the test is calculated as the total number marked correctly for both parts minus the total number of incorrect responses (maximum score 224) (Ekstrom et al., 1976, pp. 149-151).

### 2.2.5 WAIS-R: Digit Span.

The Digit Span (forward and backward) are subtests of the WAIS-R (Wechsler, 1981), measuring participants' ability to remember a series of auditorily presented digits. Each subtest consists of 14 trials with digit spans from two to eight with two trials of each span. The experimenter begins with the two-digit span then continues increasing the span load by one digit

every two trials. This continues until the participant incorrectly recalls both trials of a given digit span or correctly recalls both 8 digit spans. The participants' score is the total number of correct trials (0 – 14) (Wechsler, 2008). The same procedure is used to determine the participant's backward digit span, except that participants must recall the digits in the reverse order of presentation.

### 2.2.6 WAIS-R: Similarities.

The Similarities subtest of the WAIS-R is a measure of participant's verbal IQ, specifically their Verbal Comprehension. Participants were required to articulate the similarities between 18 pairs of objects or words, such as "Horse" and "Tiger" or "Poem" and Statue". All items are phrased verbatim in the form: "In what way are (a[n]) ____ and (a[n]) _____ alike?". Participants are awarded a score of 0, 1 or 2 for each response, creating a maximum total score of 36. The test is discontinued if the participant receives three consecutive scores of zero (Wechsler, 1981).

### 2.2.7 Trail Making Test.

The TMT (Partington & Leiter, 1949) is a measure of attention, speed and mental flexibility and is often interpreted as a measure of executive functioning (Strauss et al., 2006, pp. 655-677). The task has been adapted by (Reitan, 1955, 1958) and has been included in a number of test batteries including the Army Individual Test Battery (US Army, 1944) and the Halstead-Reitan Test Battery (Reitan & Wolfson, 1985). Part A consists of 25 encircled numbers randomly arranged on a page. Participants were tasked with connecting the circles in proper numerical order (i.e. 1 – 2 – 3 etc.) using a pencil. Part B consists of 25 encircled alternating numbers and letters arranged in random order. Participants were instructed to connect the encircled numbers and letters in alternating but sequential order (i.e. 1 – A – 2 – B – 3 - C etc.).

If the participant made a mistake, the experimenter brought it to his or her attention immediately and directed the participant back to the last correct item. Prior to the start of either part A or B, a practice exercise containing only 8 enclosed numbers (A) or numbers and letters (B) are administered (Strauss et al., 2006, pp. 655-677). The relevant outcome measures are the time (in seconds) taken to complete each part. If participants did not complete part A in 160 seconds (n =1) or part B in 300 seconds (n = 3) the task was discontinued; these scores were excluded from the present analyses.

### 2.2.8 Verbal fluency.

The Letter and Category fluency tasks (Thurstone & Thurstone, 1943) evaluates one's ability to spontaneously produce words to criteria (Strauss et al., 2006, pp. 499-526). For the Letter Fluency task, participants were given 60 seconds to produce as many words as possible that began with a specified letter (F, A and S). The Category Fluency condition instructed participants to producing as many exemplars of a particular category as possible in 60 seconds (fruits, animals and vegetables). The participant's score is the average number of admissible words produced for all three of the letters or categories. Inadmissible words include proper names, multiple repetitions of the same word (it is only counted once), or variations of the same word (e.g. *eat* and *eating* only count as one production). See Table 2 for baseline cognitive task scores.

Table 2: Baseline Cognitive Task Scores

| Task | Valid N | Mean (SD) |
|---|---|---|
| Benton Visual Retention Task | 1248 | 4.4 (3.5) |
| Boston Naming Test | 616 | 54.1 (6.8) |
| California Verbal Learning Test | | |
| Sum of Learning Trials | 1042 | 55.3 (11.0) |
| Delayed Recall | 1043 | 11.5 (3.2) |
| Card Rotations Test | 1181 | 88.4 (39.0) |
| Digit Span | | |
| Backward | 1079 | 7.3 (2.3) |
| Forward | 1079 | 8.5 (2.2) |
| Similarities | 1012 | 21.1 (3.6) |
| TMT (Seconds) | | |
| A | 788 | 34.2 (13.7) |
| B | 788 | 82.2 (40.5) |
| Verbal Fluency | | |
| Category | 794 | 16.2 (3.5) |
| Letter | 794 | 14.5 (4.4) |

*Note.* TMT = Trail Making Test.

### 2.3 Cortisol Measures

The cortisol measures used in the present study were obtained using the 24-hr urine samples participants provided as part of their biennial assessments. Participants were instructed to void upon waking at approximately 8 am and discard this specimen. Following initial voiding, all urine including the final specimen voided at the end of the 24-hour collection was collected by the subject in containers provided to them. Cortisol levels have been demonstrated to remain stable following 24-hr of storage at room temperature without preservative (Gouarne, Foury, & Duclos, 2004). Subsequently, multiple 20 mL samples of urine were aliquoted from the total pool and stored at -80° C in the BLSA specimen bank. Several studies have indicated that cortisol and creatinine are stable in urine when frozen for long durations (Miki & Sudo, 1998; Soliman, Abdel-Hay, Sulaiman, & Tayeb, 1986).

Assays were conducted by Esoterix Incorporated. Urinary free cortisol (UFC) was measured by liquid chromatography with mass spectrometry after nonpolar solvent extraction. Interassay coefficients of variation for mean values of 0.05, 2.84, 5.53 and 9.41 ug/dl were 13.4%, 6.6%, 4.4% and 5.3% respectively. Creatinine (Cr) concentrations were determined using the Synchron LX System (Beckman Coulter, 1998) by means of the Jaffe rate method (Jaffe, 1886). Interassay coefficients of variation for creatinine mean values of 66.6 ng/mL and 145.8 ng/mL were 1.5% and 1.1% respectively.

The ratio of UFC to Cr (hereafter referred to as UFC:Cr) expressed as μg/g was calculated for each specimen, a practice consistent with prior studies (Kacsoh, 2000; Seeman et al., 1997). Because creatinine secretion is proportional to muscle mass and is a fairly stable parameter in adults, the comparison of 24-hr free cortisol to creatinine excretion allows for the identification of incomplete samples (Barr et al., 2005; Kacsoh, 2000, pp. 116-119; Wu, 2006, p. 316) (cf. De Keyzer et al., 2012). In addition, this practice is particularly important in the study of aging because kidney filtration is reduced with normal aging leading to a reduction in urinary cortisol excretion (Alessio, Berlin, Dell'Orto, Toffoletto, & Ghezzi, 1985; Driver & McAlevy, 1980). Therefore, if urinary cortisol values are not adjusted for creatinine, an artifactual negative relationship between age and cortisol levels may appear (Timiras, 1995, p. 33). UFC:Cr values greater than 5 SD from the mean were excluded from analyses.

**2.4 Statistical Analyses**

The cross-sectional relationships between 24-hr UFC:Cr measures and cognitive functioning will be investigated in IBM SPSS version 21 using the multilevel model framework restricted to the level of fixed effects estimated by full maximum likelihood estimation. Analyses

will be conducted in a manner consistent with several BLSA investigators such that a "minimally adjusted model" will be examined followed by a "fully adjusted model" (Harik-Khan, Wise, & Fozard, 1998; Waldstein, Giggey, Thayer, & Zonderman, 2005).

The minimally adjusted model will regress cognitive task performance on a centered linear UFC:Cr term, a quadratic UFC:Cr ($UFC:Cr^2$) term and a centered age term. The fully adjusted model will include the terms from the minimally adjusted model as well as a number of covariates. The selection of covariates will be determined by conducting partial correlations between potential covariates and task performance controlling for age. Those covariates correlated ($p < .1$) with cognitive task performance will be retained for use in a fully adjusted model. The covariates that will be examined are demographic and lifestyle variables: alcohol use (absolute grams of alcohol per day), APOE ε4 genotype (ε4 allele present/absent) depressive symptoms (measured by the Center for Epidemiologic Studies Depression Scale (CES-D) (Radloff, 1977)), education, race (white/nonwhite), sex (male/female), and smoking status (current, former, never); health indices: blood pressure (diastolic (DBP) and systolic (SBP)), body mass index (BMI), and low density lipoprotein (LDL); medication usage (diuretics, sex hormones, statins, and thyroid); and medical conditions (cardiac or vascular disease (defined as the presence of angina, arterial fibrillation, hypertension, or history of transient ischemic attack (CoVD)), chronic kidney disease (CKD), and diabetes (medication usage or diagnosis). After running the initial fully adjusted model, those variables not significantly contributing to the model ($p > .05$) will be removed and the model will be re-run with only significant variables. This process will be repeated until all variables in the model are significant, producing a "final model".

In addition to the minimally and fully adjusted model, two separate "interaction models" will be tested to probe for interactions of cortisol with age and sex. These models will include a linear UFC:Cr term, the relevant variable term (i.e. age or sex) and the relevant interaction term (i.e. UFC:Cr*Age or UFC:Cr*Sex). In the case of a significant UFC:Cr*Age term, the fully adjusted model including the pre-determined covariates will be re-run split by age group (Young: < 40, Middle: 40 – 65, Older > 65). In the case of a significant UFC:Cr*Sex term, the minimally adjusted model will be re-run split by sex. As before, following the minimally adjusted model, the fully adjusted model will be conducted and subsequently iterated until a final model contains only significant terms.

# RESULTS

## 3.1 UFC:Cr

For the entire sample, mean baseline UFC:Cr levels were 19.2 ± 12.0 μg/g. There was not a significant correlation between UFC:Cr levels and age ($r = -.02$, $p = .301$), nor was there a significant difference in mean UFC:Cr levels between men and women, (means 18.9 and 19.6 μg/g respectively, $t(1,851) = 1.4$, $p = .156$).

## 3.2 Age

Bivariate correlations between age and all cognitive outcomes revealed significant negative relationships between age and cognitive functioning on all tests except for the Boston Naming Test, Similarities and Letter Fluency (results not shown). This same pattern was evident when age was included as a covariate in both the minimally and fully adjusted models (see Tables 3 and 4).

## 3.3 Sex

In the partial correlations controlling for age, a number of cognitive tasks demonstrated an effect of sex such that male sex was positively associated with performance on the BVRT ($r = -.079$, $p = .006$), the Boston Naming Test ($r = .089$, $p = .018$) the Card Rotations Test ($r = .223$, $p < .001$), Digit Span Backward ($r = .126$, $p < .001$) and Digit Span Forward ($r = .126$, $p < .001$), while female sex was positively associated with performance on CVLT Learning and Delayed ($r = -.288$, $p < .001$ and $r = -.180$, $p < .001$, respectively) and Category Fluency ($r = -.181$, $p < .001$).

When entered into the fully adjusted models, the male advantage remained for BVRT performance ($\hat{y} = -0.461$), Card Rotations ($\hat{y} = 14.400$) and Digit Span Forward ($\hat{y} = 0.443$), while the female advantage remained for CVLT Learning ($\hat{y} = -6.282$), CVLT Delayed ($\hat{y} = -$

1.091) and Category Fluency ($\hat{\gamma}$ = -1.485). All p's < .05. The results of the fully adjusted models are displayed in Table 4. All values represent unstandardized regression coefficients ($\hat{\gamma}$).

### 3.4 Other Covariates

Covariates correlated (p < .1) with each cognitive outcome after partialling the effects of age are included in table 5. Those covariates remaining significant (p < .05) in the fully adjusted model for a given cognitive outcome are included in bold font. Participant race significantly predicted performance on all cognitive tasks such that identification as non-white was associated with poorer performance on all tasks. This relationship remained significant in the fully adjusted models for all cognitive tasks. Education remained a significant predictor in the fully adjusted models for four cognitive tasks, though the effect sizes were negligible and in two cases predicted worse performance with higher levels of education. Other covariates affecting cognitive performance included CESD score, alcohol use, DBP, thyroid medication use, a history of cardiac or vascular disease and diabetes diagnosis or medication use. See Table 4.

### 3.5 Minimally Adjusted Models

Performance on all cognitive tasks demonstrated a positive linear relationship with UFC:Cr levels such that higher UFC:Cr was associated with better task performance, with the exception of performance on the Boston Naming Task which did not significantly correlate with the linear UFC:Cr term. Further, a number of tasks (BVRT, Boston Naming, CVLT (Learning and Delayed), Digit Span Forward, Similarities and Verbal Fluency (Category and Letter)) demonstrated significant correlations with the quadratic UFC:Cr term. All quadratic relationships

were negative (with the exception of BVRT which is reverse scored) and reveal an inverted U-shaped quadratic association between UFC:Cr and cognitive performance. See Table 3.

Table 3: Minimally Adjusted Model Outcomes.

| Outcomes | Parameters | | |
|---|---|---|---|
| | UFC:Cr | UFC:Cr$^2$ | Age |
| BVRT | -0.025* | 0.001* | 0.098** |
| Boston Naming | 0.046 | -0.003** | -0.064 |
| CVLT Learning | 0.152** | -0.003** | -0.311** |
| CVLT Delayed Recall | 0.040** | -0.001** | -0.079** |
| Card Rotations | 0.319* | -0.002 | -1.042** |
| Digit Span Backward | 0.021* | -0.000 | -0.013* |
| Digit Span Forward | 0.017* | -0.001** | -0.022** |
| Similarities | 0.043** | -0.001* | -0.009 |
| TMT A | -0.121** | 0.002 | 0.561** |
| TMT B | -0.418** | 0.006 | 1.447** |
| Category Fluency | 0.040** | -0.001* | -0.098** |
| Letter Fluency | 0.046** | -0.001* | -0.006 |

*Note*. *p < .05, **p < .01. Unstandardized regression coefficients ($\hat{\gamma}$). (BVRT): Benton Visual Retention Test; (CVLT): California Verbal Learning Test; (TMT): Trail Making Test

### 3.6 Fully Adjusted Models

The only significant linear relationship between UFC:Cr and cognitive functioning that survived the addition of the covariates was the positive relationship between UFC:Cr and performance on the learning outcome of the CVLT. Quadratic relationships surviving the addition of covariates included the negative quadratic relationships between UFC:Cr and performance on the learning outcome of the CVLT (see Figure 1), Digit Span Forward (see Figure 2) and the Boston Naming Test (see Figure 3). In all cases the quadratic relationships revealed an inverted U-shaped association. See Table 4.

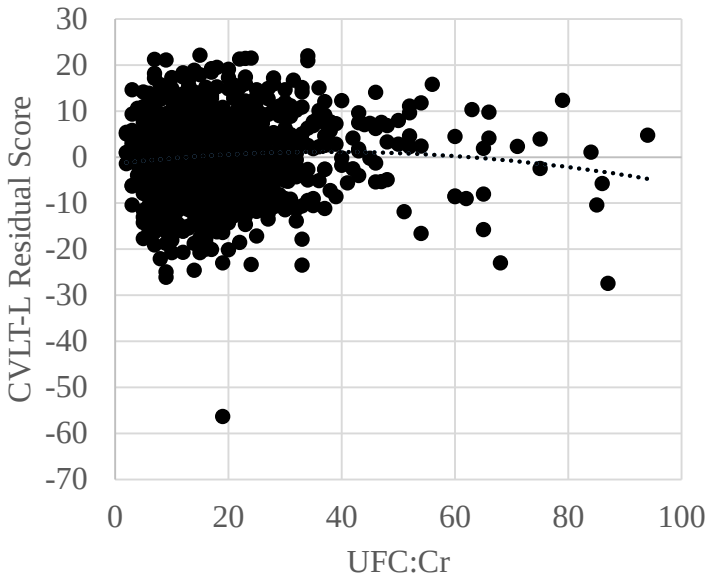


Figure 1: The relationship between UFC:Cr levels and CVLT Learning performance after adjusting for all possible confounders

Table 4: Fully adjusted model outcomes

| | BVRT | BNT | CV-L | CV-D | CRD | DS B | DS F | SIM | TMT A | TMT B | CAT | LET |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| UFC:Cr | -0.002 | -0.012 | **0.082** | 0.019 | 0.169 | 0.006 | 0.010 | 0.126 | -0.033 | -0.172 | 0.024 | 0.015 |
| UFC:Cr$^2$ | 0.000 | **-0.002** | **-0.002** | -0.000 | | | **-0.001** | -0.000 | | | -0.000 | -0.001 |
| Age | **0.092** | **-0.120** | **-0.303** | **-0.079** | **-1.201** | **-0.020** | -0.028 | -0.015 | **0.550** | **1.398** | **-0.087** | -0.007 |
| Alcohol | -0.010 | 0.023 | **0.034** | 0.010 | 0.073 | 0.001 | 0.003 | 0.007 | -0.012 | | | |
| APOE | | | **-0.000** | | | | | | | 0.002 | | |
| CESD | **0.000** | **-0.000** | | **-0.000** | -0.001 | | -0.000 | -0.000 | -0.000 | 0.001 | -0.000 | -0.000 |
| Education | 0.000 | 0.000 | -0.000 | **-0.000** | -0.001 | -0.000 | 0.000 | -0.000 | **0.001** | **0.002** | **-0.000** | -0.000 |
| Race | **1.631** | **-6.292** | **-5.214** | **-1.426** | **-15.661** | **-1.156** | **-0.703** | **-2.065** | **3.927** | **14.402** | **-1.546** | **-1.603** |
| Sex | **-0.461** | 0.000 | **-6.282** | **-1.091** | **14.400** | 0.250 | **0.443** | | | | **-1.485** | |
| Smoking | | 0.000 | | | | | 0.000 | | | 0.000 | | |
| DBP | | | | | | | | | 0.007 | | **0.018** | |
| SBP | | | -0.000 | | | | | | -0.007 | -0.000 | -0.018 | -0.000 |
| BMI | 0.000 | 0.000 | -0.000 | | | -0.000 | -0.000 | -0.000 | | -0.001 | -0.000 | 0.000 |
| LDL | | | | | | | | | | | | -0.000 |
| Diuretics | | | | | | | | | 1.570 | | | |
| SXH | | | -0.795 | 0.441 | -0.511 | | | | | | -0.312 | |
| Statins | 0.352 | | -1.036 | -0.237 | | | | -0.235 | | | | -0.580 |
| Thyroid | | | 1.436 | 0.253 | | | | 0.577 | | **-8.215** | 0.131 | **1.099** |
| CoVD | **0.545** | | -0.352 | -0.109 | | | | -0.489 | 1.555 | 4.809 | | **-0.912** |
| CKD | 0.403 | | | | | | | | | | | -0.659 |
| Diabetes | **1.426** | **-1.896** | -1.709 | -0.449 | | | | -0.310 | | | -0.100 | |

Note. Unstandardized regression coefficients ($\hat{\gamma}$); Bold font indicates $p < .05$; (BVRT) Benton Visual Retention Test; (BNT): Boston Naming Test; (CV-L): California Verbal Learning Test – Learning Outcome; (CV-D): California Verbal Learning Test – Delayed Recall Outcome; (CRD): Card Rotations Test; (DS B): Digit Span Backward; (DS F): Digit Span Forward; (SIM): Similarities; (TMT A): Trail Making Test Part A; (TMT B): Trail Making Test Part B; (CAT): Verbal Fluency: Category; (LET): Verbal Fluency: Letter.

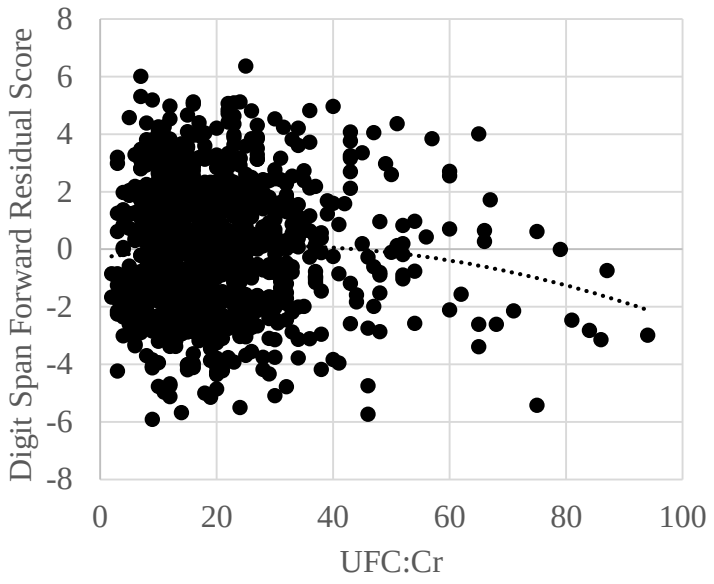


Figure 2: The relationship between UFC:Cr levels and Digit Span Forward performance after adjusting for all possible confounders

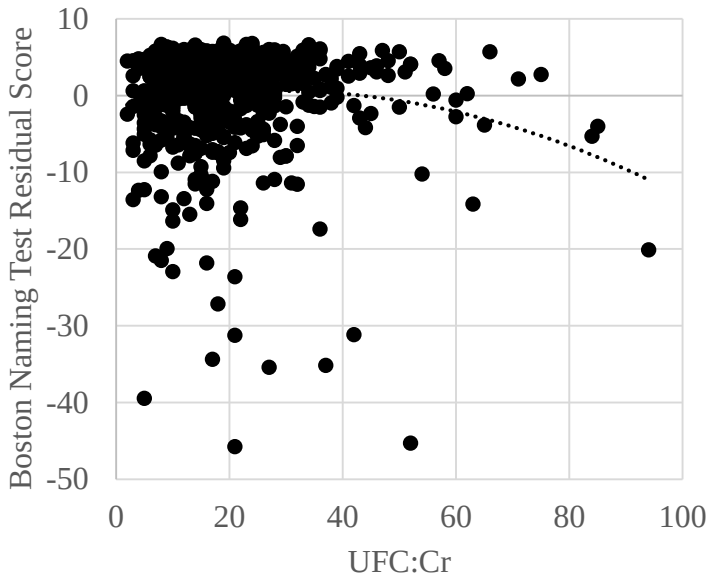


Figure 3: The relationship between UFC:Cr levels and Boston Naming Test performance after adjusting for all possible confounders

### 3.7 Interactions

There was a significant UCF:Cr*Age interaction for TMT part B ($\hat{\gamma}$ = -0.027, p = ,045). When split by age group (Young: < 40, Middle: 40 – 65, Older > 65) the fully adjusted model revealed a negative linear and positive quadratic relationship between UFC:Cr and TMT part B performance in the older adult group only ($\hat{\gamma}$= -.757, p =.002 and $\hat{\gamma}$= 0.015, p = .027 respectively). Subsequent iteration produced a final model containing UFC:Cr ($\hat{\gamma}$= -0.837, p = .001), UFC:$Cr^2$ ($\hat{\gamma}$ = 0.017, p = .015), age ($\hat{\gamma}$ = 2.211, p < .001), APOE ε4 status ($\hat{\gamma}$ = 0.002, p = .001) and race ($\hat{\gamma}$ = 25.559, p < .001). See Figure 4. No other interaction terms reached significance.

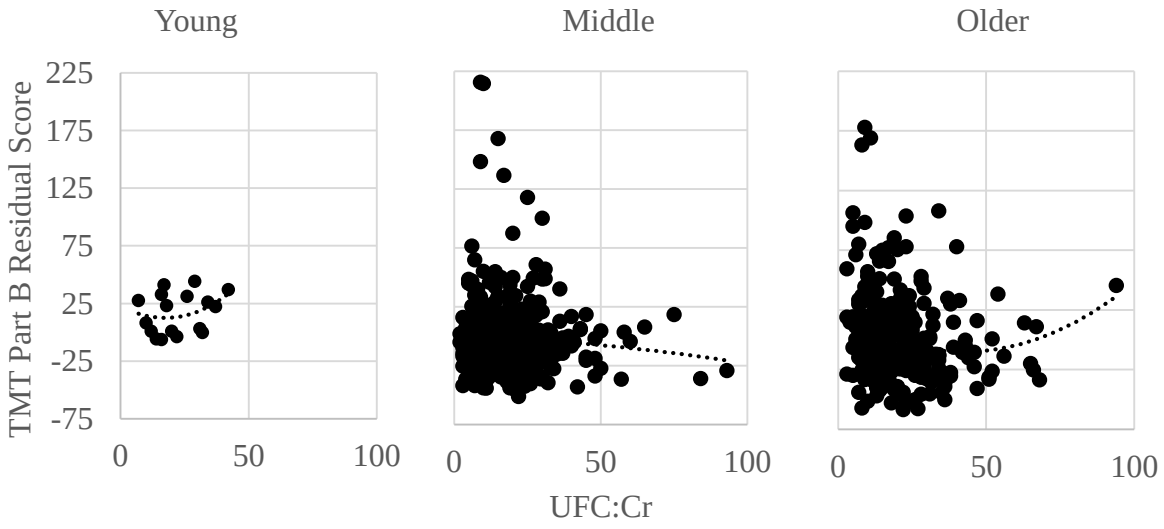


Figure 4: The relationship between UFC:Cr levels and Trail Making Test part B performance in all three age groups (Young: < 40, Middle: 40 – 65, Older > 65) adjusting for all possible confounders.

## DISCUSSION

The present study replicated well-established findings in the cognitive aging literature including negative associations between age and several cognitive domains including episodic

memory, information processing speed, and visual memory. Somewhat contrary to predictions but not without precedent (Heaton, Taylor, & Manly, 2003; Salthouse & Saklofske, 2010; Strauss et al., 2006, p. 504), we did not find negative associations between age and performance on the Similarities subtest, Letter Fluency or Digit Span Forward. As predicted, a male advantage in performance was present for the Card Rotations Test, in line with previous research demonstrating a male advantage on mental rotation (Sanders, Soares, & D'Aquila, 1982). Similarly, a female advantage in performance was present for both CVLT outcomes (Spreen & Strauss, 1998, p. 316) and category fluency (Spreen & Strauss, 1998, pp. 456-457). Contrary to predictions, a male advantage was present for BVRT and Digit Span Forward performance although male advantages are not typically observed for these tasks (Strauss et al., 2006). These advantages cannot be explained by differences in education because education was included as a covariate in all fully adjusted models.

The primary purpose of the present study was to examine the association between cortisol exposure as indexed by UFC:Cr levels and cognitive function in a sample of non-demented adults. Contrary to some previous studies, we observed positive linear relationships between cortisol and performance on all cognitive tasks except for the Boston Naming Test. However, these positive associations were largely eliminated by the inclusion of relevant covariates. This moderation effect suggests that the positive associations may be artifact of not including relevant demographic, health and lifestyle factors that are correlated with cortisol levels or cognitive function.

The main analyses of interest (i.e. the fully adjusted models) revealed negative quadratic associations between UFC:Cr and performance on the CVLT Learning outcome, Digit Span Forward and Boston Naming Test. All of these associations demonstrated an inverted U-shaped

relationship between UFC:Cr and cognition indicating that moderate levels of cortisol may be associated with better cognitive performance. Visual inspection of Figures 2 through 4 suggests that this association may be driven by detriments in performance only in the very high UFC:Cr range. An analogous outcome was observed for TMT part B performance, but only in the older adult group, such that a quadratic relationship between UFC:Cr and performance was present, and was largely driven by poorer performance of those with highly elevated UFC:Cr levels.

The finding that those with highly elevated basal UFC:Cr levels exhibit poorer performance on the hippocampally-mediated CVLT Learning outcome is in line with several other findings of a negative influence of elevated cortisol levels on word-list learning performance in healthy older adults (Comijs et al., 2010; Gaysina et al., 2014; Gerritsen et al., 2011) and is the first study to extend this finding to individuals younger than 60 years of age. The reported association between UFC:Cr and Digit Span Forward performance is unprecedented in healthy older adults, however, of the studies discussed, only two included this measure in the cognitive battery (Franz et al., 2011; Lupien et al., 1994). However, Digit Span Forward performance has been demonstrated to be worse in CD patients than controls (Forget et al., 2000). Further, neuroimaging work provides evidence that the right dorsolateral PFC and anterior cingulate contribute to Digit Span Forward performance (Gerton et al., 2004), regions shown to contain high densities of glucocorticoid receptors in primates (Klok et al., 2011; Patel et al., 2000; Watzka et al., 2000; Webster et al., 2002).

Only one study has investigated the association between cortisol and TMT part B performance, but it was included as one of three measures of an Executive Functioning composite. This study reported a  negative relationship between cortisol and the executive composite (Lee et al., 2007). Slower TMT part B performance has been reported in CD patients

(Forget et al., 2016; Forget et al., 2000) and neuroimaging evidence demonstrates activation of the left dorsolateral and medial PFC during TMT part B completion (Zakzanis, Mraz, & Graham, 2005). Notably, TMT part B was the only cognitive outcome for which the relationship between UFC:Cr and performance was limited to the older adult group (aged 66 to 93 years). A potential explanation for this finding is that TMT part B performance is reliant on a different neural network than the CVLT Learning outcome or Digit Span Forward. That is, CVLT Learning is a hippocampal-dependent task, and differences in glucocorticoid receptor distribution between the hippocampus and frontal cortex have been documented (de Kloet & Reul, 1987; Klok et al., 2011; Watzka et al., 2000; Webster et al., 2002). Further, although TMT part B and Digit Span Forward rely on frontal functioning, TMT part B processing is *left*-lateralized while Digit Span Forward performance is *right*-lateralized. Imaging work suggests the possibility of hemispheric asymmetries in the effects of glucocorticoids (MacLullich et al., 2006).

The significant relationship between UFC:Cr levels and performance on the Boston Naming Test was unexpected. Lee et al. (2007) used the Boston naming as one of three outcomes of a Language composite score. They found no association between cortisol and the language composite. Further, it appears that Boston Naming Test performance has not been examined in CD patients. One potential explanation for this unexpected finding is that while picture-naming is typically attributed to temporal or parietal regions (Baldo, Arévalo, Patterson, & Dronkers, 2013), performance has also been shown to positively correlate with left hippocampal volume in a sample of healthy older adults (Petersen et al., 2000). Therefore, it is possible that the observed elevations in UFC:Cr levels affected the hippocampal contribution to Boston Naming Task performance manifesting as the small negative relationship observed.

Although the present study reports significant quadratic relationships between UFC:Cr levels and performance on some cognitive tasks hypothesized and shown empirically to relate to cortisol levels in previous literature, the effect sizes in the present study are small compared to most findings in the extant literature. Specifically, the standardized effect sizes for the significant quadratic relationships for CVLT Learning, Digit Span Forward and Boston Naming Test were .091, .193 and .127 respectively. The author argues that the comparably small effects observed in the present study represent the true influence of basal cortisol levels on cognitive functioning in healthy adults and offers several explanations for the discrepant findings in the literature.

First, some of the most convincing evidence for a relationship between cortisol and cognitive functioning in humans, specifically on measures of speeded processing as well as verbal and visual memory, comes from studies of CD patients. However, CD patients experience drastically elevated levels of cortisol, such that one study reported mean 24-hr UFC:Cr levels to be more than 16 times higher in CD patients than normal controls (Viardot et al., 2005). Further, CD patients typically experience these significant elevations for months to years before diagnosis and subsequent treatment. Given that even in these extreme cases, glucocorticoid-induced cognitive impairment is not always observed, it is not surprising that such small effects would appear in a healthy population whose cortisol elevations do not approach the diagnostic threshold for CD.

Second, to my knowledge, this is the first study to report significant quadratic relationships between cortisol levels and cognitive functioning. This inverted U-shaped relationship with performance is fairly common in hormonal and pharmacologic research, suggesting an optimal level of performance (Celec, Ostatniková, Putz, & Kudela, 2002; Gouchie & Kimura, 1991; Moffat & Hampson, 1996; Neave, Menaged, & Weightman, 1999; Shute,

Pellegrino, Hubert, & Reynolds, 1983). This relationship is also present for cortisol and primed burst potentiation (Diamond, Bennett, Fleshner, & Rose, 1992) and excitability (Joëls & de Kloet, 1990, 1991). However, only two (Comijs et al., 2010; Schrijvers et al., 2011) of the ten aforementioned large studies reported testing for non-linear effects. If, as the present results suggest, the negative influence of cortisol levels on cognitive functioning is limited to sufficiently high basal levels, investigations of normal healthy populations limited to linear terms are likely to miss this relationship.

Third, although some studies have reported up to moderate effects of cortisol on cognitive functioning, the literature is decidedly mixed. These discrepancies are unsurprising if the true effects of cortisol are small, as suggested by the present results. Further, the presence of a true, but small effect of cortisol on cognitive functioning could explain why there appears to be no obvious pattern in the assay methodology (e.g. saliva versus plasma), time of day (e.g. morning versus evening) or indices used (e.g. AUC versus mean) that determines whether a study will find a significant cortisol/cognitive functioning relationship.

From a more theoretical perspective, the findings that elevated UFC:Cr levels specifically may impair functions dependent on the hippocampus and frontal cortex are in agreement with predictions made by the Allostatic Load Hypothesis (McEwen, 1998). The Allostatic Load Hypothesis posits that when presented with adversity, a number of body systems are forced to adapt, entering into an "allostatic state" in order to restore homeostasis. The cost of this ability to adapt is the induced wear and tear on the body and brain, or "allostatic load". Frequent or prolonged exposure to stressors can force the body into an allostatic state for an extended time, manifesting in chronically elevated glucocorticoid levels, possibly causing dendritic atrophy.

Although the accumulation of allostatic load is not restricted to older age, the Allostatic Load Hypothesis has been most consistently applied to explain age-related brain changes because the aged organism is thought to be more vulnerable to negative outcomes produced by glucocorticoid elevations (McEwen, 1992, 1999, 2002). For example, although glucocorticoid-induced dendritic retraction is reversible, Bloss, Janssen, McEwen, and Morrison (2010) reported that mid-aged and old rats demonstrated impaired dendritic recovery following the cessation of a chronic stressor compared to young rats. Further, Kerr, Campbell, Applegate, Brodish, and Landfield (1991) reported that while chronic stress induced neurophysiologic changes in all tested rodents, gross cell loss was limited to the aged rodents. Similarly, Bodnoff et al. (1995) reported that spatial memory deficits following glucocorticoid treatment did not appear in young rodents and instead were limited to the mid-aged rats. Given what appears to be preferential vulnerability of aged animals to glucocorticoid-induced damage and cognitive impairments, it is often hypothesized that glucocorticoid-induced cognitive changes will be restricted to older age. However, in the present study, this was only true for performance on the Trail Making Test part B. The author argues that while older adults' neural structure may be more vulnerable to cortisol elevations, such elevations are not limited to older age. That is, highly elevated cortisol levels may be a manifestation of extreme stress or illness that can be present at any age. Given that there was no relationship between UFC:Cr levels and age in the present sample, there is no evidence to suggest that older individuals were systematically experiencing greater levels of stress than their younger counterparts. Therefore it is unsurprising that the cortisol-cognition relationships were not limited to older age.

### 4.1 Contributions of the Present Study

The present study represents the largest investigation to date utilizing a 24-hr collection of cortisol to investigate the relationship between basal cortisol levels and cognitive functioning. In addition to allowing for adequate statistical control of a number of potentially confounding covariates, the large sample size enabled the detection of small quadratic effects that have not been identified previously in the literature. The variety in the cognitive testing battery was also advantageous in this study because it allowed for the examination of the domain-specific effects of cortisol elevations. Finally, the use of a 24-hr cortisol index collected with medical supervision and measured via the most sophisticated assay techniques available provided accurate assessments of individuals' basal cortisol levels.

As previously noted, earlier investigations using 24-hr cortisol collections have been limited by small sample sizes. In addition to prohibiting these studies from detecting small effect sizes, the small sample sizes impede the ability to adequately control for a number of demographic and health covariates. Increased cortisol levels and pro-inflammatory cytokines have been observed in a number of age-associated metabolic, somatic and psychiatric conditions and may act on multiple levels of the HPA system (Silverman & Sternberg, 2012). The importance of controlling for potential confounding variables has been demonstrated several times in this literature. For example, Comijs et al. (2010) reported that prior to adjustment for health and life-style covariates, there was a significant negative relationship between morning plasma cortisol levels and retention performance on a word-list learning task, but this relationship was eliminated with the addition of the relevant covariates. Similarly, Lee et al. (2007) reported that prior to the inclusion of health and lifestyle covariates (e.g. diabetes, cardiovascular disease, hypertension, depression as indexed by CESD and hormone replacement therapy), significant negative relationships were present between daytime salivary cortisol levels

and performance on composite scores of language, visual memory and verbal memory and learning. These relationships were reduced to non-significance with the addition of covariates. Likewise, in the present study, the addition of covariates largely eliminated the positive linear relationships found in the minimally adjusted models. These results suggest that the failure to adequately control for potentially confounding covariates can potentially produce spurious relationships between cortisol levels and cognitive functioning.

Another benefit of the present study is that it employed an extensive cognitive testing battery. Although comprehensive testing batteries have been commonly employed in many of the large single time-point cortisol studies, those studies of 24-hr cortisol measures have not included such expanded batteries. The breadth of cognitive tasks is important for this literature because theories of the relationship between cortisol and cognitive functioning, for example the Allostatic Load Hypothesis, predict specific cognitive effects as a result of cortisol elevations. Specifically, cortisol-induced cognitive effects may be limited to functions subserved by those brain regions with the highest densities of extra-hypothalamic corticosteroid receptors, namely the hippocampus and frontal cortex. Notably, the findings of the present study support this hypothesis, as elevated cortisol levels were significantly associated with performance on the hippocampal-dependent CVLT Learning outcome and the frontal-dependent TMT part B in older adults.

Finally, although the use of 24-hr UFC:Cr measures is not entirely novel (see Seeman et al., 1997), they are the optimal assay for examining the relationship between basal cortisol levels and cognitive functioning. In addition to minimizing the influence of circadian fluctuations, thereby providing a more reliable index than single time-point measures (Rosmalen et al., 2014), 24-hr urinary measures are indicative of total daily cortisol exposure. Given that the theories

relating elevated cortisol levels and cognitive functioning are predicated on cortisol elevations being present for extended periods, 24-hr collections are a better reflection of typical exposure than single time-point measures are.

Despite the advantages of using 24-hr urinary cortisol measures, poor participant compliance is typically prohibitive of their use. However, the design of the present study addressed this in two ways. First, participants stayed overnight at the NIA allowing them to be supervised by nurses for the 24-hr collection. Second, 24-hr creatinine levels were assayed to insure that urine samples were complete (Barr et al., 2005; cf De Keyzer et al., 2012; Kacsoh, 2000, pp. 116-119; Wu, 2006, p. 316) and to control for age-related reductions in kidney functioning (Alessio et al., 1985; Timiras, 1995). Following collection, the urine samples were assayed using liquid chromatography with mass spectrometry, a method which is more accurate than previous immunoassay measures for urinary free cortisol because it is not artificially elevated due to interference from cortisol metabolites in urine (Wood et al., 2008). This assay technique increases measurement accuracy and reliability, thereby reducing the error variance in the present analyses.

### 4.2 Limitations of the Present Study

Although 24-hr UFC:Cr levels are informative about an individual's daily cumulative exposure to cortisol, this measure does have several shortcomings. First, while 24-hr cortisol measures have been demonstrated to have good day-to-day reliability (Rosmalen et al., 2014), they are only representative of a single day. As previously noted, theories relating elevated glucocorticoid levels to declines in cognitive functioning predicate this relationship on extended

elevations. Thus, while 24-hr measures provide better estimates of typical basal levels than single time-point estimates, there is still a great deal of variation that is not accounted for.

Second, the use of 24-hr measures masks information regarding the diurnal variation in circulating cortisol levels. Even though the present study aimed to address the relationship between basal cortisol levels and cognitive functioning hypothesized to be mediated through such processes as dendritic reorganization in the hippocampus, acute effects of circulating cortisol levels on cognition have been extensively documented (Lupien & McEwen, 1997), and the effects of these acute fluctuations cannot be account or controlled for by the 24-hr measure.

Additionally, a number of researchers have investigated the possibility that HPA axis dysregulation in older age manifests as a flatter diurnal slope from the morning peak to the quiescent nadir without necessarily producing an overall increase in total cortisol production (Fiocco, Wan, Weekes, Pim, & Lupien, 2006; Nater, Hoppmann, & Scott, 2013; van Cauter, Leproult, & Kupfer, 1996). This flattened pattern is associated with poorer self-reported health (Dmitrieva, Almeida, Dmitrieva, Loken, & Pieper, 2013) and some evidence suggests that attenuated diurnal slopes may be predictive of poorer cognitive performance in older adults (Beluche, Carrière, Ritchie, & Ancelin, 2010; Fiocco et al., 2006).

The design of the present study introduced several other limitations. First, all participants did not necessarily receive all constituent tests, meaning that the analyses of different cognitive tests consisted of different individuals, at least to some extent. Given that cortisol is predicted to affect specific cognitive domains, having identical participants across tests would have provided a more powerful test of this specificity. Second, the cognitive battery did not include a task that is directly analogous to the Symbol-Digit Substitution-type tasks used by a number of other authors to index information processing speed. Given that this domain was demonstrated to be

influenced by cortisol elevations in several studies of healthy adult populations, an analogous measure would have been useful for direct comparison of this study to previous studies. Third, there were no surveys administered addressing individuals' perceived or objective stress levels. Cortisol is frequently referred to as the stress hormone and reliably elevates in response to stressors. It follows that information about individuals' stress experience would contribute to our understanding of their basal cortisol levels. Notably however, perceived stress does not always significantly predict observed cortisol levels (van Eck, Berkhof, Nicolson, & Sulon, 1996), and a large number of other factors can contribute to observed cortisol levels on a day-to-day basis. Fourth, although 24-hr cortisol collection during the visit to NIA allowed for supervision by a nursing staff, it is unclear how representative the observed cortisol levels are of participants' typical days because it is possible that participants perceived the visit itself as a stressor. Finally, this study only includes a single occasion of concurrent cortisol and cognitive testing. This does not allow for examinations of how changes in cortisol levels over time could affect subsequent cognitive changes. Thus, longitudinal study of repeated cortisol and cognitive measures is warranted.

### 4.3 Summary

The findings of the present study suggest a small, but significant, quadratic relationship between basal cortisol levels and hippocampal- and frontally-mediated domains of cognitive functioning. This association was observed in a large, healthy lifespan adult sample and was independent of a number of demographic, lifestyle and health covariates. These relationships appear to be driven primarily by individuals with highly elevated cortisol levels. These findings are in line with the predictions of the Allostatic Load hypothesis and corroborate several

previous reports of elevated cortisol levels being associated with poorer performance on cognitive tasks subserved by those brain regions with the highest densities of extra-hypothalamic glucocorticoid receptors. The longitudinal study of the relationship between cortisol elevations and cognition is the next critical step in investigating whether cortisol is a useful biomarker for predicting or mediating age-related cognitive changes.

## REFERENCES


Alessio, L., Berlin, A., Dell'Orto, A., Toffoletto, F., & Ghezzi, I. (1985). Reliability of urinary creatinine as a parameter used to adjust values of urinary biological indicators. *International Archives of Occupational and Environmental Health, 55*(2), 99-106. doi: 10.1007/BF00378371

American Psychiatric Association. (1987). *Diagnostic and Statistical Manual of Mental Disorders, DSM-III-R*. Washington, D.C.: American Psychiatric Association.

Andela, C. D., van Haalen, F. M., Ragnarsson, O., Papakokkinou, E., Johannsson, G., Santos, A., . . . Pereira, A. M. (2015). Cushing's syndrome causes irreversible effects on the human brain: A systematic review of structural and functional magnetic resonance imaging studies. *European Journal of Endocrinology, 173*(1), R1-R14. doi: 10.1530/eje-14-1101

Arbel, I., Kadar, T., Silbermann, M., & Levy, A. (1994). The effects of long-term corticosterone administration on hippocampal morphology and cognitive performance of middle-aged rats. *Brain Research, 657*(1–2), 227-235. doi: 10.1016/0006-8993(94)90972-5

Baldo, J. V., Arévalo, A., Patterson, J. P., & Dronkers, N. F. (2013). Grey and white matter correlates of picture naming: Evidence from a voxel-based lesion analysis of the Boston Naming Test. *Cortex, 49*(3), 658-667. doi: http://dx.doi.org/10.1016/j.cortex.2012.03.001

Barr, D. B., Wilder, L. C., Caudill, S. P., Gonzalez, A. J., Needham, L. L., & Pirkle, J. L. (2005). Urinary creatinine concentrations in the U.S. Population: Implications for urinary biologic monitoring measurements. *Environmental Health Perspectives, 113*(2), 192-200. doi: 10.2307/3436465

Bartolomucci, A., De Biurrun, G., Czéh, B., van Kampen, M., & Fuchs, E. (2002). Selective enhancement of spatial learning under chronic psychosocial stress. *European Journal of Neuroscience, 15*(11), 1863-1866. doi: 10.1046/j.1460-9568.2002.02043.x

Beckman Coulter, I. (1998). *Synchron LX Systems Chemistry Information*.

Beluche, I., Carrière, I., Ritchie, K., & Ancelin, M. L. (2010). A prospective study of diurnal cortisol and cognitive function in community-dwelling elderly people. *Psychological Medicine, 40*(6), 1039-1049. doi: 10.1017/S0033291709991103

Benton, A. L. (1945). A visual retention test for clinical use. *Archives of Neurology & Psychiatry, 54*(3), 212-216. doi: 10.1001/archneurpsyc.1945.02300090051008

Benton, A. L. (1974). *Revised Visual Retention Test*. New York, NY: Psychological Corporation.

Bliss, E. L., Sandberg, A. A., Nelson, D. H., & Eik-Nes, K. (1953). The normal levels of 17-hydroxycorticosteroids in the peripheral blood of man. *Journal of Clinical Investigation, 32*(9), 818-823. doi: 10.1172/JCI102798

Bliss, T. V. P., & Collingridge, G. L. (1993). A synaptic model of memory: Long-term potentiation in the hippocampus. *Nature, 361*(6407), 31-39. doi: 10.1038/361031a0

Bloss, E. B., Janssen, W. G., McEwen, B. S., & Morrison, J. H. (2010). Interactive effects of stress and aging on structural plasticity in the prefrontal cortex. *The Journal of Neuroscience, 30*(19), 6726-6731. doi: 10.1523/jneurosci.0759-10.2010

Bodnoff, S. R., Humphreys, A. G., Lehman, J. C., Diamond, D. M., Rose, G. M., & Meaney, M. J. (1995). Enduring effects of chronic corticosterone treatment on spatial learning, synaptic plasticity, and hippocampal neuropathology in young and mid-aged rats. *Journal of Neuroscience, 15*(1), 61-69.

Bourdeau, I., Bard, C., Noël, B., Leclerc, I., Cordeau, M.-P., Bélair, M., . . . Lacroix, A. (2002). Loss of brain volume in endogenous Cushing's syndrome and its reversibility after correction of hypercortisolism. *The Journal of Clinical Endocrinology & Metabolism, 87*(5), 1949-1954. doi: 10.1210/jcem.87.5.8493

Brambilla, D. J., O'Donnell, A. B., Matsumoto, A. M., & McKinlay, J. B. (2007). Intraindividual variation in levels of serum testosterone and other reproductive and adrenal hormones in men. *Clinical Endocrinology, 67*(6), 853-862. doi: 10.1111/j.1365-2265.2007.02976.x

Castinetti, F., Morange, I., Conte-Devolx, B., & Brue, T. (2012). Cushing's disease. *Orphanet Journal of Rare Diseases, 7*, 41. doi: 10.1186/1750-1172-7-41

Celec, P., Ostatniková, D., Putz, Z., & Kudela, M. (2002). The circalunar cycle of salivary testosterone and the visual-spatial performance. *Bratislava Medical Journal, 103*(2), 59-69.

Comijs, H. C., Gerritsen, L., Penninx, B. W. J. H., Bremmer, M. A., Deeg, D. J. H., & Geerlings, M. I. (2010). The association between serum cortisol and cognitive decline in older persons. *The American Journal of Geriatric Psychiatry, 18*(1), 42-50. doi: 10.1097/JGP.0b013e3181b970ae

Conrad, C. D. (2010). A critical review of chronic stress effects on spatial learning and memory. *Progress in Neuro-Psychopharmacology & Biological Psychiatry, 34*(5), 742-755. doi: 10.1016/j.pnpbp.2009.11.003

Cowell, A. M., & Buckingham, J. C. (2001). Glucocorticoids and the HPA axis. In N. J. Goulding & R. J. Flower (Eds.), *Glucocorticoids* (pp. 129-145). Basel, Switzerland: Birkhäuser Verlag.

De Keyzer, W., Huybrechts, I., Dekkers, A. L. M., Geelen, A., Crispim, S., Hulshof, P. J. M., . . . De Henauw, S. (2012). Predicting urinary creatinine excretion and its usefulness to identify incomplete 24 h urine collections. *British Journal of Nutrition, 108*(06), 1118-1125. doi: 10.1017/S0007114511006295

de Kloet, E. R., Oitzl, M. S., & Joëls, M. (1999). Stress and cognition: Are corticosteroids good or bad guys? *Trends in Neurosciences, 22*(10), 422-426. doi: 10.1016/S0166-2236(99)01438-1

de Kloet, E. R., & Reul, J. M. H. M. (1987). Feedback action and tonic influence of corticosteroids on brain function: A concept arising from the heterogeneity of brain receptor systems. *Psychoneuroendocrinology, 12*(2), 83-105. doi: 10.1016/0306-4530(87)90040-0

de Leon, M. J., McRae, T., Rusinek, H., Convit, A., De Santi, S., Tarshish, C., . . . McEwen, B. S. (1997). Cortisol reduces hippocampal glucose metabolism in normal elderly, but not in Alzheimer's disease. *Journal of Clinical Endocrinology & Metabolism, 82*(10), 3251-3259. doi: 10.1210/jcem.82.10.4305

Delis, D. C., Kaplan, E., & Kramer, J. H. (2001). *Delis-Kaplan Executive Function System technical manual*. San Antonio TX: US: The Psychological Corporation.

Delis, D. C., Kramer, J. H., Kaplan, E., & Ober, B. A. (1987). *California Verbal Learning Test, Research Edition*. New York, NY: The Psychological Corporation.

Diamond, D. M., Bennett, M. C., Fleshner, M., & Rose, G. M. (1992). Inverted-U relationship between the level of peripheral corticosterone and the magnitude of hippocampal primed burst potentiation. *Hippocampus, 2*(4), 421-430. doi: 10.1002/hipo.450020409

Dickerson, S. S., & Kemeny, M. E. (2004). Acute Stressors and Cortisol Responses: A Theoretical Integration and Synthesis of Laboratory Research. *Psychological Bulletin, 130*(3), 355-391. doi: 10.1037/0033-2909.130.3.355

Dmitrieva, N. O., Almeida, D. M., Dmitrieva, J., Loken, E., & Pieper, C. F. (2013). A day-centered approach to modeling cortisol: Diurnal cortisol profiles and their associations among U.S. adults. *Psychoneuroendocrinology, 38*(10), 2354-2365. doi: http://dx.doi.org/10.1016/j.psyneuen.2013.05.003

Dorn, L. D., & Cerrone, P. (2000). Cognitive function in patients with Cushing syndrome: A longitudinal perspective. *Clinical Nursing Research, 9*(4), 420-440. doi: 10.1177/10547730022158672

Drachman, D. A. (1986). Memory and cognitive function in normal aging. *Developmental Neuropsychology, 2*(4), 277-285. doi: 10.1080/87565648609540349

Driver, A. G., & McAlevy, M. T. (1980). Creatinine height index as a function of age. *The American journal of clinical nutrition, 33*(9), 2057-2057.

Ekstrom, R. B., French, J. W., Harman, H. H., & Dermen, D. (1976). *Manual for kit of factor referenced cognitive tests*. Princeton, NJ: Educational Testing Service.

Endo, Y., Nishimura, J.-I., & Kimura, F. (1996). Impairment of maze learning in rats following long-term glucocorticoid treatments. *Neuroscience Letters, 203*(3), 199-202. doi: 10.1016/0304-3940(95)12296-6

Fiocco, A. J., Poirier, J., Joober, R., Nair, N. P. V., & Lupien, S. J. (2008). Acute and long-term associations between ApoE genetic polymorphism, cortisol levels, and declarative memory performance in older adults. *Psychoneuroendocrinology, 33*(5), 625-633. doi: 10.1016/j.psyneuen.2008.02.002

Fiocco, A. J., Wan, N., Weekes, N., Pim, H., & Lupien, S. J. (2006). Diurnal cycle of salivary cortisol in older adult men and women with subjective complaints of memory deficits and/or depressive symptoms: Relation to cognitive functioning. *Stress, 9*(3), 143-152. doi: 10.1080/10253890600965674

Follenius, M., Simon, C., Brandenberger, G., & Lenzi, P. (1987). Ultradian plasma corticotropin and cortisol rhythms: Time-series analyses. *Journal of Endocrinological Investigation, 10*(3), 261-266. doi: 10.1007/bf03348128

Fonda, S. J., Bertrand, R., O'Donnell, A., Longcope, C., & McKinlay, J. B. (2005). Age, hormones, and cognitive functioning among middle-aged and elderly men: Cross-sectional evidence from the Massachusetts male aging study. *Journals of Gerontology: Series A, 60*(3), 385-390. doi: 10.1093/gerona/60.3.385

Forget, H., Lacroix, A., Bourdeau, I., & Cohen, H. (2016). Long-term cognitive effects of glucocorticoid excess in Cushing's syndrome. *Psychoneuroendocrinology, 65*, 26-33. doi: 10.1016/j.psyneuen.2015.11.020

Forget, H., Lacroix, A., Somma, M., & Cohen, H. (2000). Cognitive decline in patients with Cushing's syndrome. *Journal of the International Neuropsychological Society, 6*(1), 20-29. doi: 10.1017/s1355617700611037

Franz, C. E., O'Brien, R. C., Hauger, R. L., Mendoza, S. P., Panizzon, M. S., Prom-Wormley, E., . . . Kremen, W. S. (2011). Cross-sectional and 35-year longitudinal assessment of salivary cortisol and cognitive functioning: The Vietnam Era Twin Study of Aging. *Psychoneuroendocrinology, 36*(7), 1040-1052. doi: 10.1016/j.psyneuen.2011.01.002

Fuchs, E., Uno, H., & Flügge, G. (1995). Chronic psychosocial stress induces morphological alterations in hippocampal pyramidal neurons of the tree shrew. *Brain Research, 673*(2), 275-282. doi: 10.1016/0006-8993(94)01424-G

Fuld, P. A. (1978). Psychological testing in the differential diagnosis of the dementias. In R. Katzman, R. D. Terry, & K. L. Bock (Eds.), *Alzheimer's Disease: Senile dementia and related disorders* (Vol. 7, pp. 185-193). New York: NY: Raven Press.

Fulford, A. J., & Harbuz, M. S. (2005). An introduction the the HPA axis; Part 1: The Neurobiology of Stress. In T. Steckler, N. H. Kalin, & J. M. H. M. Reul (Eds.), *Handbook of Stress and the Brain* (Vol. 15, pp. 43-66). Amsterdam, The Netherlands: Elsevier, V.B.

Gatti, R., Antonelli, G., Prearo, M., Spinella, P., Cappellin, E., & De Palo, E. F. (2009). Cortisol assays and diagnostic laboratory procedures in human biological fluids. *Clinical Biochemistry, 42*(12), 1205-1217. doi: 10.1016/j.clinbiochem.2009.04.011

Gaysina, D., Gardner, M. P., Richards, M., & Ben-Shlomo, Y. (2014). Cortisol and cognitive function in midlife: The role of childhood cognition and educational attainment. *Psychoneuroendocrinology, 47*, 189-198. doi: 10.1016/j.psyneuen.2014.05.018

Gerritsen, L., Comijs, H. C., Deeg, D. J. H., Penninx, B. W. J. H., & Geerlings, M. I. (2011). Salivary cortisol, APOE-ε4 allele and cognitive decline in a prospective study of older persons. *Neurobiology of Aging, 32*(9), 1615-1625. doi: 10.1016/j.neurobiolaging.2009.09.007

Gerton, B. K., Brown, T. T., Meyer-Lindenberg, A., Kohn, P., Holt, J. L., Olsen, R. K., & Berman, K. F. (2004). Shared and distinct neurophysiological components of the digits forward and backward tasks as revealed by functional neuroimaging. *Neuropsychologia, 42*(13), 1781-1787. doi: 10.1016/j.neuropsychologia.2004.04.023

Gouarne, C., Foury, A., & Duclos, M. (2004). Critical study of common conditions of storage of glucocorticoids and catecholamines in 24-h urine collected during resting and exercising conditions. *Clinica Chimica Acta, 348*(1–2), 207-214. doi: 10.1016/j.cccn.2004.05.023

Gouchie, C., & Kimura, D. (1991). The relationship between testosterone levels and cognitive ability patterns. *Psychoneuroendocrinology, 16*(4), 323-334. doi: 10.1016/0306-4530(91)90018-O

Harik-Khan, R., Wise, R., & Fozard, J. (1998). Determinants of maximal inspiratory pressure. *American Journal of Respiratory and Critical Care Medicine, 158*(5), 1459-1464. doi: 10.1164/ajrccm.158.5.9712006

Heaton, R. K., Taylor, M. J., & Manly, J. J. (2003). Demographic effects and use of demographically corrected norms with the WAIS-III and WMS-III. In R. K. Tulsky, R. K. Saklofske, G. Heaton, G. Chelune, R. A. Ivnik, R. A. Bornstein, A. Prifitera, & M. Ledbetter (Eds.), *Clinical interpretation of the WAIS-III and WMS-III* (pp. 183-210). San Diego, CA: Academic Press.

Heim, A. W. (1970). *AH 4 Group Test of General Intelligence*. Windsor, UH: NFER-Nelson Publishing Company Ltd.

Hook, J. N., Giordani, B., Schteingart, D. E., Guire, K., Giles, J., Ryan, K., . . . Starkman, M. N. (2007). Patterns of cognitive change over time and relationship to age following

successful treatment of Cushing's disease. *Journal of the International Neuropsychological Society, 13*(01), 21-29. doi: 10.1017/S1355617707070051

Horner, H. C., Packan, D. R., & Sapolsky, R. M. (1990). Glucocorticoids inhibit glucose transport in cultured hippocampal neurons and glia. *Neuroendocrinology, 52*(1), 57-64. doi: 10.1159/000125539

Horrocks, P. M., Jones, A. F., Ratcliffe, W. A., Holder, G., White, A., Holder, R., . . . London, D. R. (1990). Patterns of ACTH and cortisol pulsatility over twenty-four hours in normal males and females. *Clinical Endocrinology, 32*(1), 127-134. doi: 10.1111/j.1365-2265.1990.tb03758.x

Jaffe, M. (1886). Ueber den Niederschlag, welchen Pikrinsäure im normalen Harn erzeugt, und über eine neue Reaction des Kreatinins. *Zeitschrift für Physiologische Chemie, 10*, 391-400.

Joëls, M., & de Kloet, E. R. (1990). Mineralocorticoid receptor-mediated changes in membrane properties of rat CA1 pyramidal neurons in vitro. *Proceedings of the National Academy of Sciences, 87*(12), 4495-4498. doi: 10.1073/pnas.87.12.4495

Joëls, M., & de Kloet, E. R. (1991). Effect of corticosteroid hormones on electrical activity in rat hippocampus. *Journal of Steroid Biochemistry and Molecular Biology, 40*(1–3), 83-86. doi: 10.1016/0960-0760(91)90170-A

Jones, S. G., & Benca, R. M. (2015). Circadian Disruption in Psychiatric Disorders. *Sleep Medicine Clinics, 10*(4), 481-493. doi: 10.1016/j.jsmc.2015.07.004

Kacsoh, B. (2000). *Endocrine Physiology*. New York, NY: McGraw-Hill.

Kaplan, E. F., Goodglass, H., & Weintraub, S. (1978). *The Boston Naming Test: Experimental edition*. Boston: MA: Kaplan & Goodglass or Veterans Administrations Hospital.

Kaplan, E. F., Goodglass, H., & Weintraub, S. (1983). *Boston Naming Test*. Philadelphia, PA: Lea & Febiger.

Kerr, D. S., Campbell, L. W., Applegate, M. D., Brodish, A., & Landfield, P. W. (1991). Chronic stress-induced acceleration of electrophysiologic and morphometric biomarkers of hippocampal aging. *Journal of Neuroscience, 11*(5), 1316-1324.

Kim, J. J., Pellman, A. B., & Kim, J. E. (2014). Neural-cognitive effects of stress in the hippocampus. In M. Popoli, D. Diamond, & G. Sanacora (Eds.), *Synaptic Stress and Pathogenesis of Neuropsychiatric Disorders* (pp. 151-165). New York, NY: Springer New York.

Kirschbaum, C., Pirke, K. M., & Hellhammer, D. H. (1993). The 'Trier Social Stress Test' – A Tool for Investigating Psychobiological Stress Responses in a Laboratory Setting. *Neuropsychobiology, 28*(1-2), 76-81. doi: 10.1159/000119004

Klok, M. D., Alt, S. R., Lafitte, A. J. M. I., Turner, J. D., Lakke, E. A. J. F., Huitinga, I., . . . DeRijk, R. H. (2011). Decreased expression of mineralocorticoid receptor mRNA and its splice variants in postmortem brain regions of patients with major depressive disorder. *Journal of Psychiatric Research, 45*(7), 871-878. doi: 10.1016/j.jpsychires.2010.12.002

Kuningas, M., de Rijk, R. H., Westendorp, R. G. J., Jolles, J., Eline Slagboom, P., & van Heemst, D. (2006). Mental performance in old age dependent on cortisol and genetic variance in the mineralocorticoid and glucocorticoid receptors. *Neuropsychopharmacology, 32*(6), 1295-1301. doi: 10.1038/sj.npp.1301260

Landfield, P. W., Baskin, R. K., & Pitler, T. A. (1981). Brain aging correlates: Retardation by hormonal-pharmacological treatments. *Science, 214*(4520), 581-584. doi: 10.1126/science.6270791

Landfield, P. W., Waymire, J. C., & Lynch, G. (1978). Hippocampal aging and adrenocorticoids: Quantitative correlations. *Science, 202*(4372), 1098-1102. doi: 10.2307/1747852

Lee, B. K., Glass, T. A., McAtee, M. J., Wand, G. S., Bandeen-Roche, K., Bolla, K. I., & Schwartz, B. S. (2007). Associations of salivary cortisol with cognitive function in the Baltimore Memory Study. *Archives of General Psychiatry, 64*(7), 810-818. doi: 10.1001/archpsyc.64.7.810

Lupien, S. J., Lecours, A. R., Lussier, I., Schwartz, G., Nair, N. P. V., & Meaney, M. J. (1994). Basal cortisol levels and cognitive deficits in human aging. *Journal of Neuroscience, 14*(5), 2893-2903.

Lupien, S. J., & McEwen, B. S. (1997). The acute effects of corticosteroids on cognition: Integration of animal and human model studies. *Brain Research Reviews, 24*(1), 1-27. doi: 10.1016/S0165-0173(97)00004-0

Lupien, S. J., Wilkinson, C. W., Brière, S., Ménard, C., Ying Kin, N., & Nair, N. P. V. (2002). The modulatory effects of corticosteroids on cognition: Studies in young human populations. *Psychoneuroendocrinology, 27*(3), 401-416. doi: 10.1016/S0306-4530(01)00061-0

Lyons, D. M., Lopez, J. M., Yang, C., & Schatzberg, A. F. (2000). Stress-level cortisol treatment impairs inhibitory control of behavior in monkeys. *Journal of Neuroscience, 20*(20), 7816-7821.

MacLullich, A. M. J., Ferguson, K. J., Wardlaw, J. M., Starr, J. M., Deary, I. J., & Seckl, J. R. (2006). Smaller left anterior cingulate cortex volumes are associated with impaired

hypothalamic-pituitary-adrenal axis regulation in healthy elderly men. *The Journal of Clinical Endocrinology & Metabolism, 91*(4), 1591-1594. doi: 10.1210/jc.2005-2610

Magariños, A. M., McEwen, B. S., Flügge, G., & Fuchs, E. (1996). Chronic psychosocial stress causes apical dendritic atrophy of hippocampal CA3 pyramidal neurons in subordinate tree shrews. *Journal of Neuroscience, 16*(10), 3534-3540.

Martignoni, E., Costa, A., Sinforiani, E., Liuzzi, A., Chiodini, P., Mauri, M., . . . Nappi, G. (1992). The brain as a target for adrenocortical steroids: Cognitive implications. *Psychoneuroendocrinology, 17*(4), 343-354. doi: 10.1016/0306-4530(92)90040-E

Mauri, M., Sinforiani, E., Bono, G., Vignati, F., Berselli, M. E., Attanasio, R., & Nappi, G. (1993). Memory impairment in Cushing's disease. *Acta Neurologica Scandinavica, 87*(1), 52-55. doi: 10.1111/j.1600-0404.1993.tb04075.x

McEwen, B. S. (1992). Re-examination of the glucocorticoid hypothesis of stress and aging. In D. Swaab, M. Hofman, R. Mirmiran, F. Ravid, & F. van Leeuwen (Eds.), *Progress in Brain Research* (Vol. 93, pp. 365-383): Elsevier.

McEwen, B. S. (1998). Stress, adaptation, and disease: Allostasis and allostatic load. *Annals of the New York Academy of Sciences, 840*(1), 33-44. doi: 10.1111/j.1749-6632.1998.tb09546.x

McEwen, B. S. (1999). Stress and the aging hippocampus. *Frontiers in Neuroendocrinology, 20*(1), 49-70. doi: 10.1006/frne.1998.0173

McEwen, B. S. (2002). Sex, stress and the hippocampus: Allostasis, allostatic load and the aging process. *Neurobiology of Aging, 23*(5), 921-939. doi: 10.1016/S0197-4580(02)00027-1

McEwen, B. S. (2007). Physiology and neurobiology of stress and adaptation: Central role of the brain. *Physiological Reviews, 87*(3), 873-904. doi: 10.1152/physrev.00041.2006

McKhann, G., Drachman, D., Folstein, M., Katzman, R., Price, D., & Stadlan, E. M. (1984). Clinical diagnosis of Alzheimer's Disease: Report of the NINCDS-ADRDA Work Group* under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. *Neurology, 34*(7), 939-944. doi: 10.1212/wnl.34.7.939

Miki, K., & Sudo, A. (1998). Effect of urine pH, storage time, and temperature on stability of catecholamines, cortisol, and creatinine. *Clinical Chemistry, 44*(8), 1759-1762.

Mizoguchi, K., Ishige, A., Takeda, S., Aburada, M., & Tabira, T. (2004). Endogenous glucocorticoids are essential for maintaining prefrontal cortical cognitive function. *Journal of Neuroscience, 24*(24), 5492-5499. doi: 10.1523/jneurosci.0086-04.2004

Moffat, S. D., & Hampson, E. (1996). A curvilinear relationship between testosterone and spatial cognition in humans: Possible influence of hand preference. *Psychoneuroendocrinology, 21*(3), 323-337. doi: 10.1016/0306-4530(95)00051-8

Morris, J. C. (1993). The Clinical Dementia Rating (CDR): Current version and scoring rules. *Neurology, 43*(11), 2412-2414.

Nater, U. M., Hoppmann, C. A., & Scott, S. B. (2013). Diurnal profiles of salivary cortisol and alpha-amylase change across the adult lifespan: Evidence from repeated daily life assessments. *Psychoneuroendocrinology, 38*(12), 3167-3171. doi: 10.1016/j.psyneuen.2013.09.008

Neave, N., Menaged, M., & Weightman, D. R. (1999). Sex differences in cognition: The role of testosterone and sexual orientation. *Brain and Cognition, 41*(3), 245-262. doi: 10.1006/brcg.1999.1125

Nilsson, L.-G., Sternäng, O., Rönnlund, M., & Nyberg, L. (2009). Challenging the notion of an early-onset of cognitive decline. *Neurobiology of Aging, 30*(4), 521-524. doi: 10.1016/j.neurobiolaging.2008.11.013

Ohl, F., & Fuchs, E. (1999). Differential effects of chronic stress on memory processes in the tree shrew. *Cognitive Brain Research, 7*(3), 379-387. doi: 10.1016/S0926-6410(98)00042-1

Ohl, F., Michaelis, T., Vollmann-Honsdorf, G. K., Kirschbaum, C., & Fuchs, E. (2000). Effect of chronic psychosocial stress and long-term cortisol treatment on hippocampus-mediated memory and hippocampal volume: A pilot-study in tree shrews. *Psychoneuroendocrinology, 25*(4), 357-363. doi: 10.1016/S0306-4530(99)00062-1

Pardridge, W. M., & Mietus, L. J. (1979). Transport of steroid hormones through the rat blood-brain barrier: Primary role of albumin-bound hormone. *Journal of Clinical Investigation, 64*(1), 145-154. doi: 10.1172/JCI109433

Partington, J. E., & Leiter, R. G. (1949). Partington's Pathways Test. *Psychological Service Center Journal, 1*, 11-20.

Patel, P. D., Lopez, J. F., Lyons, D. M., Burke, S., Wallace, M., & Schatzberg, A. F. (2000). Glucocorticoid and mineralocorticoid receptor mRNA expression in squirrel monkey brain. *Journal of Psychiatric Research, 34*(6), 383-392. doi: 10.1016/S0022-3956(00)00035-2

Pavlides, C., Nivón, L. G., & McEwen, B. S. (2002). Effects of chronic stress on hippocampal long-term potentiation. *Hippocampus, 12*(2), 245-257. doi: 10.1002/hipo.1116

Pavlides, C., Watanabe, Y., & McEwen, B. S. (1993). Effects of glucocorticoids on hippocampal long-term potentiation. *Hippocampus, 3*(2), 183-192. doi: 10.1002/hipo.450030210

Petersen, R. C., Jack, C. R., Xu, Y.-C., Waring, S. C., O'Brien, P. C., Smith, G. E., . . . Kokmen, E. (2000). Memory and MRI-based hippocampal volumes in aging and AD. *Neurology, 54*(3), 581. doi: 10.1212/wnl.54.3.581

Petersen, R. C., Smith, G. E., Waring, S. C., Ivnik, R. J., Tangalos, E. G., & Kokmen, E. (1999). Mild cognitive impairment: Clinical characterization and outcome. *Archives of Neurology, 56*(3), 303-308. doi: 10.1001/archneur.56.3.303

Popoli, M., Yan, Z., McEwen, B. S., & Sanacora, G. (2012). The stressed synapse: the impact of stress and glucocorticoids on glutamate transmission. *Nature Reviews Neuroscience, 13*(1), 22-37. doi: 10.1038/nrn3138

Pruessner, J. C., Kirschbaum, C., Meinlschmid, G., & Hellhammer, D. H. (2003). Two formulas for computation of the area under the curve represent measures of total hormone concentration versus time-dependent change. *Psychoneuroendocrinology, 28*(7), 916-931. doi: 10.1016/S0306-4530(02)00108-7

Radloff, L. S. (1977). The CES-D scale: A self-report depression scale for research in the general population. *Applied Psychological Measurement, 1*(3), 385-401. doi: 10.1177/014662167700100306

Rapp, P. R., & Amaral, D. G. (1992). Individual differences in the cognitive and neurobiological consequences of normal aging. *Trends in Neurosciences, 15*(9), 340-345. doi: 10.1016/0166-2236(92)90051-9

Reitan, R. M. (1955). The relationship of the Trail Making Test to organic brain damage. *Journal of Consulting Psychology, 19*(5), 393-394. doi: 10.1037/h0044509

Reitan, R. M. (1958). Validity of the Trail Making Test as an indicator of organic brain damage. *Perceptual and Motor Skills, 8*(3), 271-276. doi: 10.2466/pms.1958.8.3.271

Reitan, R. M., & Wolfson, D. (1985). *The Halstead-Reitan neuropsychological test battery*. Tuscon, AZ: Neuropsychology Press.

Reutrakul, S., & Knutson, K. L. (2015). Consequences of Circadian Disruption on Cardiometabolic Health. *Sleep Medicine Clinics, 10*(4), 455-468. doi: 10.1016/j.jsmc.2015.07.005

. Rochester 2016 Interpretive Handbook. (2016): Mayo Foundation for Medical Education and Research.

Rönnlund, M., Nyberg, L., Bäckman, L., & Nilsson, L.-G. (2005). Stability, growth, and decline in adult life span development of declarative memory: cross-sectional and longitudinal data from a population-based study. *Psychology and Aging, 20*(1), 3-18. doi: 10.1037/0882-7974.20.1.3

Rosmalen, J. G. M., Kema, I. P., Wüst, S., van der Ley, C., Visser, S. T., Snieder, H., & Bakker, S. J. L. (2014). 24 h urinary free cortisol in large-scale epidemiological studies: Short-term and long-term stability and sources of variability. *Psychoneuroendocrinology, 47,* 10-16. doi: 10.1016/j.psyneuen.2014.04.018

Ross, K. M., Murphy, M. L. M., Adam, E. K., Chen, E., & Miller, G. E. (2014). How stable are diurnal cortisol activity indices in healthy individuals? Evidence from three multi-wave studies. *Psychoneuroendocrinology, 39,* 184-193. doi: 10.1016/j.psyneuen.2013.09.016

Rossi, D. J., Oshima, T., & Attwell, D. (2000). Glutamate release in severe brain ischaemia is mainly by reversed uptake. *Nature, 403*(6767), 316-321. doi: 10.1038/35002090

Rowe, J. W., & Kahn, R. L. (1987). Human aging: Usual and successful. *Science, 237*(4811), 143-149. doi: 10.1126/science.3299702

Rubinow, D. R., Post, R. M., Savard, R., & Gold, P. W. (1984). Cortisol hypersecretion and cognitive impairment in depression. *Archives of General Psychiatry, 41*(3), 279-283. doi: 10.1001/archpsyc.1984.01790140069008

Salthouse, T. A. (2009). When does age-related cognitive decline begin? *Neurobiology of Aging, 30*(4), 507-514. doi: 10.1016/j.neurobiolaging.2008.09.023

Salthouse, T. A., & Saklofske, D. H. (2010). Do the WAIS-IV tests measure the same aspects of cognitive functioning in adults under and over 65. In L. G. Weiss, D. H. Saklofske, D. L. Coalson, & S. E. Raiford (Eds.), *WAIS-IV. Clinical use and interpretation* (pp. 217-235). Burlington, MA: Elsevier Inc.

Sanders, B., Soares, M. P., & D'Aquila, J. M. (1982). The Sex Difference on One Test of Spatial Visualization: A Nontrivial Difference. *Child Development, 53*(4), 1106-1110. doi: 10.2307/1129153

Sapolsky, R. M. (1993). Potential behavioral modification of glucocorticoid damage to the hippocampus. *Behavioural Brain Research, 57*(2), 175-182. doi: 10.1016/0166-4328(93)90133-B

Sapolsky, R. M. (1999). Glucocorticoids, stress, and their adverse neurological effects: Relevance to aging. *Experimental Gerontology, 34*(6), 721-732. doi: 10.1016/S0531-5565(99)00047-9

Sapolsky, R. M. (2000). The possibility of neurotoxicity in the hippocampus in major depression: A primer on neuron death. *Biological Psychiatry, 48*(8), 755-765. doi: 10.1016/S0006-3223(00)00971-9

Sapolsky, R. M. (2007). Glucocorticoids – Adverse Effects on the Nervous System. In G. Fink (Ed.), *Encyclopedia of Stress (Second Edition)* (pp. 185-190). New York: Academic Press.

Sapolsky, R. M., Krey, L. C., & McEwen, B. S. (1985). Prolonged glucocorticoid exposure reduces hippocampal neuron number: Implications for aging. *Journal of Neuroscience, 5*(5), 1222-1227.

Sapolsky, R. M., Krey, L. C., & McEwen, B. S. (1986). The neuroendocrinology of stress and aging: The glucocorticoid cascade hypothesis. *Endocrine Reviews, 7*(3), 284-301. doi: 10.1210/edrv-7-3-284

Sapolsky, R. M., Uno, H., Rebert, C. S., & Finch, C. E. (1990). Hippocampal damage associated with prolonged glucocorticoid exposure in primates. *Journal of Neuroscience, 10*(9), 2897-2902.

Schrijvers, E. M. C., Direk, N., Koudstaal, P. J., Kirschbaum, C., Hofman, A., Tiemeier, H., & Breteler, M. M. B. (2011). Associations of serum cortisol with cognitive function and dementia: The Rotterdam Study. *Journal Of Alzheimer's Disease, 25*(4), 671-677. doi: 10.3233/jad-2011-110224

Seckl, J. R., Dickson, K. L., Yates, C., & Fink, G. (1991). Distribution of glucocorticoid and mineralocorticoid receptor messenger RNA expression in human postmortem hippocampus. *Brain Research, 561*(2), 332-337. doi: 10.1016/0006-8993(91)91612-5

Seeman, T. E., McEwen, B. S., Singer, B. H., Albert, M. S., & Rowe, J. W. (1997). Increase in urinary cortisol excretion and memory declines: Macarthur studies of successful aging. *Journal of Clinical Endocrinology & Metabolism, 82*(8), 2458-2465. doi: 10.1210/jcem.82.8.4173

Segerstrom, S. C., Boggero, I. A., Smith, G. T., & Sephton, S. E. (2014). Variability and reliability of diurnal cortisol in younger and older adults: Implications for design decisions. *Psychoneuroendocrinology, 49*, 299-309. doi: 10.1016/j.psyneuen.2014.07.022

Shock, N. W., Greulich, R. C., Costa, P. T., Andres, R., Lakatta, E. G., Arenberg, D., & Tobin, J. D. (1984). *Normal human aging: The Baltimore Longitudinal Study of Aging*. (NIH Publication No. 84-2450). Washington, DC: US Government Printing Office Retrieved from http://eric.ed.gov/?id=ED292030.

Shute, V. J., Pellegrino, J. W., Hubert, L., & Reynolds, R. W. (1983). The relationship between androgen levels and human spatial abilities. *Bulletin of the Psychonomic Society, 21*(6), 465-468. doi: 10.3758/BF03330010

Silverman, M. N., & Sternberg, E. M. (2012). Glucocorticoid regulation of inflammation and its functional correlates: from HPA axis to glucocorticoid receptor dysfunction. *Annals of*

*the New York Academy of Sciences, 1261*(1), 55-63. doi: 10.1111/j.1749-6632.2012.06633.x

Simmons, N. E., Do, H. M., Lipper, M. H., & Laws Jr, E. R. (2000). Cerebral atrophy in Cushing's disease. *Surgical Neurology, 53*(1), 72-76. doi: 10.1016/S0090-3019(99)00197-4

Singh-Manoux, A., Dugravot, A., Elbaz, A., Shipley, M., Kivimaki, M., & Kumari, M. (2014). No evidence of a longitudinal association between diurnal cortisol patterns and cognition. *Neurobiology of Aging, 35*(10), 2239-2245. doi: 10.1016/j.neurobiolaging.2014.03.015

Smyth, N., Hucklebridge, F., Thorn, L., Evans, P., & Clow, A. (2013). Salivary cortisol as a biomarker in social science research. *Social and Personality Psychology Compass, 7*(9), 605-625. doi: 10.1111/spc3.12057

Soliman, S. A., Abdel-Hay, M. H., Sulaiman, M. I., & Tayeb, O. S. (1986). Stability of creatinine, urea and uric acid in urine stored under various conditions. *Clinica Chimica Acta, 160*(3), 319-326. doi: 10.1016/0009-8981(86)90200-7

Spreen, O., & Strauss, E. (1998). *A Compendium of Neuropsychological Tets: Administration, Norms, and Commentary* (2nd ed.). New York, NY: Oxford University Press Inc.

Starkman, M. N., Gebarski, S. S., Berent, S., & Schteingart, D. E. (1992). Hippocampal formation volume, memory dysfunction, and cortisol levels in patients with Cushing's syndrome. *Biological Psychiatry, 32*(9), 756-765. doi: 10.1016/0006-3223(92)90079-F

Starkman, M. N., Giordani, B., Berent, S., Schork, M. A., & Schteingart, D. E. (2001). Elevated cortisol levels in Cushing's disease are associated with cognitive decrements. *Psychosomatic Medicine, 63*(6), 985-993. doi: 10.1097/00006842-200111000-00018

Starkman, M. N., Giordani, B., Gebarski, S. S., Berent, S., Schork, M. A., & Schteingart, D. E. (1999). Decrease in cortisol reverses human hippocampal atrophy following treatment of Cushing's disease. *Biological Psychiatry, 46*(12), 1595-1602. doi: 10.1016/S0006-3223(99)00203-6

Stawski, R. S., Almeida, D. M., Lachman, M. E., Tun, P. A., Rosnick, C. B., & Seeman, T. (2011). Associations between cognitive function and naturally occurring daily cortisol during middle adulthood: Timing is everything. *The Journals of Gerontology Series B: Psychological Sciences and Social Sciences, 66B*(Suppl. 1), i71-i81. doi: 10.1093/geronb/gbq094

Strauss, E., Sherman, E. M. S., & Spreen, O. (2006). *A compendium of neuropsychological tests: Administration, norms, and commentary* (3 ed.). New York: NY: Oxford University Press.

Thurstone, L. L., & Thurstone, T. G. (1943). *Chicago Tests of Primary Mental Abilities*. Oxford, England: Science Research Associates.

Timiras, P. S. (1995). Steroid-secreting endocrines: Adrenal, ovary, testis. In P. S. Timiras, W. D. Quay, & A. Vernadakis (Eds.), *Hormones and Aging* (pp. 25-44). USA: CRC Press, Inc.

Toffanin, T., Nifosì, F., Follador, H., Passamani, A., Zonta, F., Ferri, G., . . . Perini, G. I. (2011). Volumetric MRI analysis of hippocampal subregions in Cushing's disease: A model for glucocorticoid neural modulation. *European Psychiatry, 26*(1), 64-67. doi: 10.1016/j.eurpsy.2010.09.003

Uno, H., Tarara, R., Else, J. G., Suleman, M. A., & Sapolsky, R. M. (1989). Hippocampal damage associated with prolonged and fatal stress in primates. *Journal of Neuroscience, 9*(5), 1705-1711.

US Army. (1944). *Army Individual Test Battery: Manual of directions and scoring*. Washington, DC: War Department, Adjutant General's Office.

van Cauter, E., Leproult, R., & Kupfer, D. J. (1996). Effects of gender and age on the levels and circadian rhythmicity of plasma cortisol. *Journal of Clinical Endocrinology & Metabolism, 81*(7), 2468-2473. doi: 10.1210/jcem.81.7.8675562

van Eck, M., Berkhof, H., Nicolson, N., & Sulon, J. (1996). The effects of perceived stress, traits, mood states, and stressful daily events on salivary cortisol. *Psychosomatic Medicine, 58*(5), 447-458.

Vedder, H. (2008). Physiology of the hypothalamic-pituitary-adrenocortical axis. In A. del Rey, G. P. Chrousos, & H. O. Besedovsky (Eds.), *The Hypothalamus-Pituitary-Adrenal Axis* (Vol. 7, pp. 17-32). Oxford, UK: Elsevier.

Viardot, A., Huber, P., Puder, J. J., Zulewski, H., Keller, U., & Müller, B. (2005). Reproducibility of nighttime salivary cortisol and its use in the diagnosis of hypercortisolism compared with urinary free cortisol and overnight dexamethasone suppression test. *The Journal of Clinical Endocrinology & Metabolism, 90*(10), 5730-5736. doi: 10.1210/jc.2004-2264

Videnovic, A., & Zee, P. C. (2015). Consequences of Circadian Disruption on Neurologic Health. *Sleep Medicine Clinics, 10*(4), 469-480. doi: 10.1016/j.jsmc.2015.08.004

Vollmann-Honsdorf, G. K., Flügge, G., & Fuchs, E. (1997). Chronic psychosocial stress does not affect the number of pyramidal neurons in tree shrew hippocampus. *Neuroscience Letters, 233*(2–3), 121-124. doi: 10.1016/S0304-3940(97)00647-2

Waldstein, S. R., Giggey, P. P., Thayer, J. F., & Zonderman, A. B. (2005). Nonlinear relations of blood pressure to cognitive function: The Baltimore longitudinal study of aging. *Hypertension, 45*(3), 374-379. doi: 10.1161/01.hyp.0000156744.44218.74

Watzka, M., Bidlingmaier, F., Beyenburg, S., Henke, R. T., Clusmann, H., Elger, C. E., . . . Stoffel-Wagner, B. (2000). Corticosteroid receptor mRNA expression in the brains of patients with epilepsy. *Steroids, 65*(12), 895-901. doi: 10.1016/S0039-128X(00)00205-1

Webster, M. J., Knable, M. B., O'Grady, J., Orthmann, J., & Weickert, C. S. (2002). Regional specificity of brain glucocorticoid receptor mRNA alterations in subjects with Schizophrenia and mood disorders. *Molecular Psychiatry, 7*(9), 985-994. doi: 10.1038/sj.mp.4001139

Wechsler, D. (1981). *Wechsler Adult Intelligence Scale - Revised*. New York, NY: Psychological Corporation.

Wechsler, D. (2008). *Wechsler Adult Intelligence Scale - Fourth Edition: Administration and scoring manual*. San Antonio, TX: Pearson.

Weitzman, E. D., Fukushima, D., Nogeire, C., Roffwarg, H., Gallagher, T. F., & Hellman, L. (1971). Twenty-four hour pattern of the episodic secretion of cortisol in normal subjects. *The Journal of Clinical Endocrinology & Metabolism, 33*(1), 14-22. doi: 10.1210/jcem-33-1-14

Wood, L., Ducroq, D. H., Fraser, H. L., Gillingwater, S., Evans, C., Pickett, A. J., . . . Turkes, A. (2008). Measurement of urinary free cortisol by tandem mass spectrometry and comparison with results obtained by gas chromatography-mass spectrometry and two commercial immunoassays. *Annals of Clinical Biochemistry, 45*(4), 380-388. doi: 10.1258/acb.2007.007119

Wu, A. H. B. (2006). *Tietz clinical guide to laboratory tests*. St. Louis, Mo.: Saunders/Elsevier.

Yehuda, R., Halligan, S. L., Yang, R. K., Guo, L. S., Makotkine, I., Singh, B., & Pickholtz, D. (2003). Relationship between 24-hour urinary-free cortisol excretion and salivary cortisol levels sampled from awakening to bedtime in healthy subjects. *Life Sciences, 73*(3), 349-358. doi: 10.1016/S0024-3205(03)00286-8

Zakzanis, K. K., Mraz, R., & Graham, S. J. (2005). An fMRI study of the trail making test. *Neuropsychologia, 43*(13), 1878-1886. doi: 10.1016/j.neuropsychologia.2005.03.013