Published in final edited form as:

Psychoneuroendocrinology. 2008 July ; 33(6): 773–781. doi:10.1016/j.psychneuen.2008.03.002.

Salivary cortisol is associated with diagnosis and severity of late-life generalized anxiety disorder[☆]

Rose C. Mantella^{a,*}, Meryl A. Butters^a, Janet A. Amico^b, Sati Mazumdar^{a,c}, Bruce L. Rollman^d, Amy E. Begley^a, Charles F. Reynolds^a, and Eric J. Lenze^e

Rose C. Mantella: mantellarc@upmc.edu; Meryl A. Butters: ; Janet A. Amico: ; Sati Mazumdar: ; Bruce L. Rollman: ; Amy E. Begley: ; Charles F. Reynolds: ; Eric J. Lenze: lenzee@psychiatry.wustl.edu

^a Department of Psychiatry, University of Pittsburgh School of Medicine, Pittsburgh, PA 15213, USA

^b Department of Medicine, Division of Endocrinology, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261, USA

^c Department of Biostatistics, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA 15213, USA

^d Division of General Internal Medicine, Center for Research on Health Care, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261, USA

^e Department of Psychiatry, Washington University School of Medicine, St. Louis, MO 63110, USA

Summary

Age-associated alterations in hypothalamic–pituitary–adrenal (HPA) axis functioning may make individuals more susceptible to HPA dysregulation in the context of mood and anxiety disorders. Little to no research has been done to examine HPA axis function in generalized anxiety disorder (GAD), particularly in late-life GAD, the most prevalent anxiety disorder in the elderly. The study sample consisted of 71 GAD subjects and 40 nonanxious comparison subjects over 60 years of age. We examined the hypotheses that elderly individuals with GAD will have elevated salivary cortisol levels compared to nonanxious subjects, and that elevated cortisol levels in GAD will be associated with measures of symptom severity. We report that late-life GAD is characterized by elevated basal salivary cortisol levels, with higher peak cortisol levels and larger areas under the curve, compared to nonanxious subjects. Additionally, severity of GAD as measured by the GAD Severity Scale and the Penn State Worry Questionnaire are positively correlated with cortisol levels. These data demonstrate HPA axis dysfunction in late-life GAD and suggest the need for additional research on the influence of aging on HPA axis function in mood and anxiety disorders.

Keywords

Cortisol; Generalized anxiety disorder; Elderly; Worry; HPA axis; Saliva

[☆]These data were presented in part at the 2007 annual meeting of the International Society of Psychoneuroendocrinology, Madison, Wisconsin, August 19–22, 2007.

*Corresponding author. Tel.: +1 412 246 6863; fax: +1 412 246 6260.

Conflict of interest

Dr. Lenze has received research support from Pfizer, Johnson & Johnson, and Forest Laboratories. Dr. Reynolds has received research support in the form of pharmaceutical supplies from GlaxoSmithKline, Pfizer, Forest Laboratories, Eli Lilly, and Bristol-Myers Squibb. All other authors declare no potential conflicts of interest.

1. Introduction

Estimates of the prevalence of anxiety disorders in community-dwelling older adults range from 1% to 19% (US Department of Health and Human Services, 1999). Generalized anxiety disorder (GAD) is the most common disorder in older adults with a prevalence as high as 7% (Flint, 1994; Beekman et al., 1998). GAD is defined by excessive and uncontrollable anxiety and worry about various life events, producing an important source of subjective distress (American Psychiatric Association, 2000). Persistent or severe distress associated with GAD could result in unremitting activation of the hypothalamic–pituitary–adrenal (HPA) axis. Chronic or repeated stressful events can result in changes to the sensitivity or number of corticotropin-releasing hormone and/or glucocorticoid receptors at the level of the hippocampus, limbic system, and cortical levels (brain areas associated with anxiety disorders), resulting in increased or decreased suppression of HPA function in anxiety disorders. Therefore, it is hypothesized that, like depression and other anxiety disorders, GAD is characterized by HPA axis dysregulation.

Studies of primary anxiety disorders have reported mixed findings regarding HPA axis function, with each classification of anxiety disorder exhibiting distinctive, and sometime conflicting, patterns of HPA impairment. For instance, Yehuda (2006) has reported lower cortisol levels, blunted diurnal rhythm, and increased cortisol suppression in response to dexamethasone suppression in post-traumatic stress disorder (PTSD), while others have found no differences in circadian salivary (Rasmusson et al., 2001; Altemus et al., 2003) and 24 h urinary cortisol (Baker et al., 1999; Rasmusson et al., 2001; Young and Breslau, 2004), or even higher levels of 24 h urinary (Pitman and Orr, 1990; Lemieux and Coe, 1995) and daytime salivary cortisol (Lindley et al., 2004). Similarly, some studies have characterized panic disorder by elevated cortisol concentrations at night (Abelson and Curtis, 1996) and impaired negative feedback (Erhardt et al., 2006), while others have found no differences in mean urinary cortisol levels (Uhde et al., 1988).

Unlike the numerous studies of HPA function in PTSD and panic disorder, few studies have examined HPA axis function in GAD. Initial examinations of HPA axis function of GAD involved the use of the dexamethasone suppression test (DST). Studies reported nonsuppression rates of 27–37% (Avery et al., 1985; Schweizer et al., 1986; Tiller et al., 1988) but failed to show an association with GAD symptoms. Furthermore, more recently the sensitivity of the DST has been brought to question (Heuser et al., 1994; Ising et al., 2005; Watson et al., 2006). Thus, due to methodological limitations at the time these studies were performed we currently have a limited understanding of HPA axis function in GAD.

Older adults may experience increased sensitization of the HPA axis to stress. Many studies have found an age-associated increase in HPA activity (Van Cauter et al., 1996), resulting in elevated cortisol levels (Lupien et al., 1994; Ferrari et al., 1995; Deuschle et al., 1997) and attenuation, or a flattening, of cortisol diurnal rhythm (van Coevorden et al., 1991) suggesting age-dependent HPA axis dysregulation. Additionally, it has long been speculated that chronic elevations of stress-related hormones such as cortisol can weaken multiple physiological systems of older adults resulting in impaired cardiovascular and immune function, as well as contributing to dementia, and mood and anxiety disorders. Recently it has been reported that unlike elderly subjects without psychiatric disorder, elderly subjects with anxiety disorders showed a greater increase in cortisol secretion following exposure to a stressor (Chaudieu et al., 2008). Furthermore, while children and adolescents display elevated cortisol levels to persistent anxiety, associations between cortisol levels and anxiety disorders have not been reported (Greaves-Lord et al., 2007; Kallen et al., 2007). Therefore, age-associated changes in HPA function may leave older adults with GAD more vulnerable to developing and less

responsive to treatment of medical disorders (i.e., hypertension, diabetes, metabolic syndrome, etc.), as well as mood disorders.

The purpose of this study is to evaluate free, unbound cortisol in elderly GAD and comparison subjects to test the hypothesis that elderly patients with GAD would have increased or decreased levels of free cortisol than comparison subjects. Additionally, we predicted that cortisol levels of GAD patients would be associated with the severity of GAD.

2. Methods

2.1. Study participants

GAD and group-matched comparison subjects were community-dwelling adults aged 60 years or older. They were recruited through advertisements, primary care and specialty mental health clinician referral, community agencies, and research registries. All study participants provided informed consent in accordance with the University of Pittsburgh Internal Review Board regulations.

Study participants underwent an initial evaluation, which included collection of demographic information, self-reported current and past medical history and medication use, the Mini-Mental State Exam (MMSE; Folstein et al., 1975), and the Structured Clinical Interview for DSM-IV Axis I Disorders (First et al., 1996). GAD subjects with a secondary diagnosis of comorbid depression, panic disorder, social phobia, or specific phobia were included. GAD subjects were tapered off of antidepressant or antianxiety medications for a minimum of 2 weeks, with the exception of low doses of benzodiazepines, prior to entering this study. Comparison subjects were excluded if they had a history or current psychiatric disorder and/or were on psychiatric medication. All subjects were excluded from this analysis if they showed evidence of dementia (MMSE <24) or had a history of psychosis, unstable medical illness, or alcohol or substance abuse (within the past 6 months). Participants were also excluded if they were currently using corticosteroids and female participants were excluded if they were on hormone replacement therapy. While not a criterion for exclusion from the study, none of the study participants were smokers.

2.2. Clinical measures

In addition to the clinical interview, the Hamilton Anxiety Rating Scale (HRSA; Hamilton, 1959) was used to assess general anxiety symptomology, the Penn State Worry Questionnaire (PSWQ) was used to measure the intensity of pathological worry (Meyer et al., 1990), and the Generalized Anxiety Disorder Severity Scale (GADSS; Shear et al., 2006) was used to measure the severity of GAD-specific symptoms.

Because depression and medical illness could potentially influence basal cortisol levels, the Cumulative Illness Rating Scale for Geriatrics (CIRS-G; Miller et al., 1992), an assessment of medical burden, and the Hamilton Depression Rating Scale (HRSD; Hamilton, 1967), a standard measure of depressive symptoms, were also administered.

2.3. Salivary sampling protocol

At the initial evaluation, the salivary collection protocol was explained to each study participant and they were shown the correct use of the Salivette salivary collection device (Sarstedt, Newton, NC) by a trained research associate. Participants were told not to eat, drink, smoke, brush their teeth, or use mouthwash in the 30 min prior to salivary collection and not to drink alcohol during the 2 days of collection. All subjects also received written instructions. No saliva stimulants were used to encourage salivation. Subjects also received a follow-up phone call prior to the day that they were scheduled to start saliva collection.

Subjects collected saliva using the provided cotton swabs and samples were obtained immediately upon waking, 30 min later, 1200, 1600, 2000 h, and at bedtime for 2 consecutive days. The time points for salivary collection were chosen based on published recommendations made by the MacArthur Research Network on Socioeconomic Status and Health (John and Catherine, 1999) and a report by Ice et al. (2004) which examined salivary cortisol levels every 2 h from 8:00 am to 10:00 pm in elderly subjects. It was expected that these time points would span the peak and nadir of cortisol release during waking hours.

Timex watches with signaling alarms were offered to be used at the convenience of the participant. Participants were instructed to place the salivary samples in a provided plastic bag and store in the freezer immediately after collection. Participants were also asked to keep a log of the exact times that salivary collections were made. In addition, because the amount and quality of sleep may influence morning cortisol levels (Kudielka and Kirschbaum, 2003; Backhaus et al., 2004), we evaluated the amount of sleep, number of times sleep was interrupted, and the times that subjects awakened during the night. Subjects were asked to bring salivary samples and the collection log with them to an ensuing clinic visit.

2.4. Cortisol assay protocol

Samples were stored at -80°C before laboratory centrifugation and assayed for salivary cortisol with a commercially available enzyme immunoassay kit (Salimetrics, State College, PA). Assay results were considered acceptable only if the coefficient of variation for the duplicate measure of a sample was $<10\%$. Assay sensitivity was 0.03 ng/ml. Intra-assay coefficients of variation of low and high controls were averaged 5.82% and 5.95%, respectively. The mean values of the low and high assay controls were 1.05 and 10.47 ng/ml, respectively. The inter-assay coefficients of variation for low, medium, and high controls were 9.6%, 8.4%, and 5.7%, respectively. The mean values of the low, medium, and high assay controls were 0.52, 3.40, 9.41 ng/ml, respectively.

2.5. Statistical analysis

Demographic, clinical, and sleep-related characteristics were compared between GAD and nonanxious control subjects. *t*-Tests were conducted for continuous variables and χ^2 tests were conducted for categorical variables. Repeated measures mixed model analysis of variance (ANOVA) was used to compare salivary cortisol levels in the two groups over the six time points (waking, 30 min after waking, 1200, 1600, 2000 h, and at bedtime). Time was used as a class variable to compare discrete time points. Post hoc tests were performed using the model to examine values at all time points. Area under the concentration time curve (AUC) for the total waking day was compared using a *t*-test. We used the formula for the AUC with respect to ground as defined by Pruessner et al. (2003a), which represents total hormone concentration over a given time.

Because of the high comorbidity of GAD with depression and other anxiety disorders the repeated measures mixed model ANOVA was repeated in the GAD group to examine differences in cortisol concentrations within the GAD subgroups: GAD alone, GAD with comorbid anxiety disorder (secondary diagnosis of panic disorder, social phobia, and/or specific phobia), and GAD with comorbid depression (secondary diagnosis of major depressive disorder). It was also used to examine whether cortisol concentrations of GAD subjects taking benzodiazepines differed from GAD subjects not taking benzodiazepines. Pearson correlations were calculated to test the hypothesis that GAD severity is associated with cortisol concentrations in GAD subjects. Three measures of mean cortisol response, identified *a priori*, were used to investigate the relationship with GAD symptom severity in the current study: waking, 30 min after waking, and the AUC for the total waking day. Cortisol levels at awakening were included because they have been shown to be a reliable marker of HPA

regulation (Pruessner et al., 1997). Lastly, age of GAD onset and lifetime duration of GAD was examined to see whether they were correlated with cortisol levels or severity of anxiety as measured by the HRSA, PSWQ, and the GADSS.

3. Results

3.1. Descriptive analysis

Seventy-five GAD and 42 group-matched comparison subjects equated by age, sex, race, and medical burden were recruited into this study. At the time of cortisol assay it was determined that the sample of one comparison subject had a low pH (acidic) and was unsuitable for assay, two anxious and one comparison subject did not collect salivary samples for analysis, and two anxious subjects collected too few samples for use in the analysis. Therefore, the data presented include 71 GAD and 40 group-matched comparison subjects.

The anxious and comparison groups did not differ in age, sex, race, education, or medical burden (Table 1). As expected, anxious subjects had significantly higher HRSD scores, HRSA scores, PSWQ scores, and GADSS scores. Anxious and comparison subjects did not differ in their waking time, number of hours spent sleeping, or number of times sleep was interrupted.

3.2. Salivary cortisol

As hypothesized, subjects diagnosed with GAD had increased salivary cortisol concentration levels (ANOVA, $F_{(1,109)} = 23.49, p < 0.0001$; Figure 1) and AUC values (31.8+14.4 and 21.4+8.3, GAD and comparison subjects, respectively; $t = 4.80, p < 0.0001$) than comparison subjects. Both groups showed the expected diurnal curve with peak cortisol concentrations shortly after waking and cortisol levels decreasing during the evening hours. Morning (waking, $p < 0.0001$; waking+30 min, $p < 0.0001$; 1200 h, $p < 0.02$), unlike late afternoon, and evening, cortisol concentrations were significantly elevated in GAD compared to comparison subjects.

3.3. Association of cortisol concentrations and GAD severity

We explored whether comorbid diagnoses leading to possible greater GAD severity influenced cortisol levels. No significant differences in cortisol concentrations within the GAD group in subjects with comorbid depressive ($n = 28$) or anxiety disorders ($n = 9$) or without comorbid disorders ($n = 34$) were found (ANOVA, $F_{(2,68)} = 1.49, p = 0.23$). Furthermore, post hoc analysis revealed that cortisol levels of GAD subjects taking benzodiazepines ($n = 15$), which have been shown to potentially decrease cortisol levels, did not differ GAD subjects not taking benzodiazepines ($n = 56$; ANOVA, $F_{(2,68)} = 0.07, p = 0.80$).

Pearson correlations revealed a statistically significant association of PSWQ scores of GAD subjects, but not comparison subjects, with cortisol levels (Figure 2). Increased cortisol concentrations were related to increased PSWQ scores at waking ($r = 0.36, p = 0.002, n = 70$) and 30 min afterwards ($r = 0.44, p < 0.0001, n = 71$), as well as increased AUC ($r = 0.52, p < 0.0001, n = 71$). We also found a similar association between waking cortisol levels ($r = 0.25, p = 0.04, n = 70$) and AUC ($r = 0.27, p = 0.02, n = 71$) with GADSS scores of GAD subjects. Cortisol concentrations and HRSA and HRSD were not significantly correlated. To ensure that the findings were not biased by the inclusion of subjects with comorbid depression or anxiety disorders, Pearson correlations were also conducted in subjects with “pure” GAD ($n = 34$). The analyses revealed similar associations of PSWQ scores of “pure” GAD subjects ($n = 34$) with cortisol concentrations at waking ($r = 0.42, p = 0.01$), 30 min afterwards ($r = 0.52, p < 0.001$), and AUC ($r = 0.53, p < 0.001$), as well as associations of GADSS scores with waking cortisol levels ($r = 0.24, p = 0.04$) and AUC ($r = 0.54, p < 0.001$). Lastly, neither age of GAD onset nor lifetime duration of GAD was associated with cortisol levels or severity of anxiety as measured by the HRSA, PSWQ, and GADSS.

4. Discussion

These data demonstrate a 40–50% increase in basal cortisol levels of elderly GAD versus matched comparison subjects. GAD subjects had elevated cortisol levels, specifically during early morning hours when cortisol levels normally peak. In addition, we report increased free cortisol concentration levels during waking hours (AUC) in GAD subjects. The reported elevations in cortisol concentration and morning salivary cortisol levels are also associated with symptoms associated with GAD, which has not been reported before.

The characteristic rise in cortisol levels that occurs after morning awakening is increasingly used as an indicator of adrenocortical activity and is associated with different pathologies. Studies of patients with depression (Bhagwagar et al., 2003), PTSD (Wessa et al., 2006), or chronic stress (Wust et al., 2000; Pruessner et al., 2003b) have indicated that the cortisol awakening response is distinctly altered suggesting that the post-awakening activity of the HPA axis is a promising marker of mood and anxiety disorders in psychiatry. We report similar findings in our study with GAD subjects showing an exaggerated increase in morning cortisol levels.

It is possible that the amount and quality of sleep may influence morning cortisol levels (Kudielka and Kirschbaum, 2003; Backhaus et al., 2004). Since altered sleep–wake cycles are common in older adults (Carskadon et al., 1982; Bliwise, 1993), we evaluated the amount of sleep, number of times sleep was interrupted, and the times that subjects awakened during the night. We did not find between group differences in any of these measures. We also examined the relationship between the waking time and amount of time slept with waking and waking +30 cortisol levels and did not find a correlation. Thus, the between-group differences in morning cortisol levels do not appear to be secondary to differences in sleep pattern.

Age-dependent changes in neuroendocrine functioning, specifically elevated glucocorticoid levels, could make elderly individuals more susceptible to negative sequelae of mood and anxiety disorders. This premise stems from the “glucocorticoid cascade hypothesis” (Sapolsky et al., 1986), which suggests that there is a significant relationship between cumulative exposure to high levels of glucocorticoids and atrophy (e.g. hippocampus) or impaired region-specific brain functioning of glucocorticoid receptor (GR)-rich brain areas (e.g. amygdala, prefrontal cortex) that are also involved in regulating mood and anxiety disorders. More recently, Urry et al. (2006) have reported that successful downregulation of negative affect, as evidenced by higher medial prefrontal cortex and lower amygdala activation, is associated with a more adaptive diurnal rhythm of circulating free cortisol secretion in older adults. Therefore, it is possible to hypothesize that elevated free cortisol levels in elderly individuals with GAD are associated with maladaptive function of the prefrontal cortex and the amygdala.

On the other hand, because 42% of GAD subjects in this study reported late-onset GAD (60 +years), is also possible that age-associated atrophy, aging-related abnormalities in prefrontal cortex and amygdala function, and/or dementia may be responsible for hypercortisolemia and GAD. Since findings in this field have reported a bi-directional relationship, longitudinal and interventional research is needed to further investigate this association.

A point in the data that requires further comment is the lack of difference in cortisol concentration in subjects with comorbid diagnoses, especially because comorbidity is generally associated with greater severity and HPA axis dysfunction has been linked to severity (Kumar et al., 1986; Dratcu and Calil, 1989). Young et al. (2004) have reported greater ACTH and cortisol responses to a stressor in depressed subjects with comorbid anxiety disorders as compared to those with depression or anxiety alone (Young et al., 2004). In this study, subjects’ depression was either in remission or was a secondary diagnosis to GAD, which may have led to our findings. The exclusion of persons with more severe depression may have biased against

finding an effect of comorbidity on cortisol concentration. Further studies comparing HPA axis function in depressed and anxious individuals with and without comorbid mood disorders would be necessary to evaluate the specificity of HPA dysregulation in GAD, or to determine whether HPA dysfunction is a merely a marker of severe psychological distress that transcends diagnostic boundaries.

Studies that have used cortisol levels of GAD patients as an indicator for treatment response supports the theory that exaggerated cortisol levels in GAD are state dependent. For instance, GAD patients who underwent cognitive behavioral therapy showed reductions in anxiety symptoms and cortisol levels after 24 weeks compared with GAD patients who did not receive treatment (Tafet et al., 2005), and elderly adults with GAD also showed reductions in anxiety symptoms and plasma cortisol levels following acute and chronic diazepam treatment (Pomara et al., 2005). These studies suggest that elevated cortisol levels may reflect an anxious state and can be attenuated with treatment. Similarly, we report that GAD symptoms, as measured by the PSWQ and the GADSS, are associated with elevated morning cortisol levels and daytime cortisol concentrations of GAD subjects. However, we did not find an association with the age of onset or lifetime duration of GAD with cortisol levels. Thus, our findings also suggest that elevated cortisol levels reflect an anxious state and are not dependent upon the duration of the anxiety disorder. As reported by Tafet et al. (2005) and Pomara et al. (2005) we expect that with successful treatment of GAD, cortisol levels will normalize and GAD-associated symptoms will attenuate.

Multiple factors could contribute to the higher cortisol levels found in elderly GAD subjects. For instance, changes in CRH or CRH receptor function may contribute to this phenomenon. Patients with major depression usually have a blunted ACTH response to the administration of CRH, which is believed to be secondary to desensitized CRH receptors (Holsboer, 2000). Treatment of depressed patients with pyrazolopyrimidine (CRH receptor antagonist) have shown reductions in clinician and patient-reported depression and anxiety scores (Zobel et al., 2000), suggesting not only therapeutic potential, but also implying that normalization of HPA axis function and subsequent treatment of mood and anxiety disorders are dependent on CRH and CRH receptor function.

Elevations in peak cortisol levels of GAD subjects could also suggest impaired corticosteroid receptor function. Continued or repeated cortisol hypersecretion leads to over-exposure of corticosteroid receptors, which in turn may be downregulated. This premise has been the source of a number of studies evaluating the efficacy of glucocorticoid inhibitors (Belanoff et al., 2001, 2002; Jahn et al., 2004; Flores et al., 2006) and glucocorticoid receptor antagonists (Bachmann et al., 2003; Johnson et al., 2007) as anti-depressants. Thus, it is possible that there are differences in the autoregulatory feedback of GAD and comparison subjects due to corticosteroid receptor performance.

In conclusion, this study demonstrated elevated free cortisol levels in elderly GAD versus matched comparison subjects during morning hours, as well as an overall elevation of cortisol concentration. These findings were independent of history or secondary diagnoses of anxiety or depression. Additionally, exaggerated cortisol levels in elderly GAD subjects were associated with greater worry and GAD symptoms. These data suggest the need for further research on the influence of age, as well as the implications of treatment on HPA function in GAD. Since elderly persons are expected to be increasingly sensitive to alterations HPA axis function and the subsequent negative effects on health and brain function, understanding more about the underlying pathophysiology of GAD might aid in the design of new treatments and inform the course of GAD in aging.

Acknowledgments

The authors thank Caroline Ciliberti, Michelle Costantino, Krissy Koenig, Sara Snyder, and Rebecca Zoretich for assistance with recruiting and data collection. We also wish to acknowledge the efforts of Ann Black with the data management.

Role of the funding source

Funding for this study was provided by NIMH Grants R01 MH070547 (Lenze), R01 MH072947 (Butters), P30 MH071944 (Reynolds), T32 MH019986 (Reynolds), the John A. Hartford Foundation Center of Excellence in Geriatric Psychiatry, and the University of Pittsburgh Medical Center Endowment in Geriatric Psychiatry. Funding sources had no further role in study design; in the collection, analysis, and interpretation of the data; in the writing of the report; and in the decision to submit the paper for publication.

References

- Abelson JL, Curtis GC. Hypothalamic–pituitary–adrenal axis activity in panic disorder. 24-h secretion of corticotropin and cortisol. *Arch Gen Psychiatry* 1996;53:323–331. [PubMed: 8634010]
- Altemus M, Cloitre M, Dhabhar FS. Enhanced cellular immune response in women with PTSD related to childhood abuse. *Am J Psychiatry* 2003;160:1705–1707. [PubMed: 12944352]
- American Psychiatric Association. Dignostic and Statistical Manual of Mental Disorders. Vol. 4. American Psychiatric Association; Washington, DC: 2000. text revised
- Avery DH, Osgood TB, Ishiki DM, Wilson LG, Kenny M, Dunner DL. The DST in psychiatric outpatients with generalized anxiety disorder, panic disorder, or primary affective disorder. *Am J Psychiatry* 1985;142:844–848. [PubMed: 4014507]
- Bachmann CG, Linthorst AC, Holsboer F, Reul JM. Effect of chronic administration of selective glucocorticoid receptor antagonists on the rat hypothalamic–pituitary–adrenocortical axis. *Neuropsychopharmacology* 2003;28:1056–1067. [PubMed: 12700716]
- Backhaus J, Junghanns K, Hohagen F. Sleep disturbances are correlated with decreased morning awakening salivary cortisol. *Psychoneuroendocrinology* 2004;29:1184–1191. [PubMed: 15219642]
- Baker DG, West SA, Nicholson WE, Ekhtator NN, Kasckow JW, Hill KK, Bruce AB, Orth DN, Geraciotti TD Jr. Serial CSF corticotropin-releasing hormone levels and adrenocortical activity in combat veterans with posttraumatic stress disorder. *Am J Psychiatry* 1999;156:585–588. [PubMed: 10200738]
- Beekman AT, Bremmer MA, Deeg DJ, van Balkom AJ, Smit JH, de Beurs E, van Dyck R, van Tilburg W. Anxiety disorders in later life: a report from the Longitudinal Aging Study Amsterdam. *Int J Geriatr Psychiatry* 1998;13:717–726. [PubMed: 9818308]
- Belanoff JK, Flores BH, Kalezhan M, Sund B, Schatzberg AF. Rapid reversal of psychotic depression using mifepristone. *J Clin Psychopharmacol* 2001;21:516–521. [PubMed: 11593077]
- Belanoff JK, Rothschild AJ, Cassidy F, DeBattista C, Baulieu EE, Schold C, Schatzberg AF. An open label trial of C-1073 (mifepristone) for psychotic major depression. *Biol Psychiatry* 2002;52:386–392. [PubMed: 12242054]
- Bhagwagar Z, Hafizi S, Cowen PJ. Increase in concentration of waking salivary cortisol in recovered patients with depression. *Am J Psychiatry* 2003;160:1890–1891. [PubMed: 14514508]
- Bliwise DL. Sleep in normal aging and dementia. *Sleep* 1993;16:40–81. [PubMed: 8456235]
- Carskadon MA, Brown ED, Dement WC. Sleep fragmentation in the elderly: relationship to daytime sleep tendency. *Neurobiol Aging* 1982;3:321–327. [PubMed: 7170049]
- Chaudieu I, Beluche I, Norton J, Boulenger JP, Ritchie K, Ancelin ML. Abnormal reactions to environmental stress in elderly persons with anxiety disorders: evidence from a population study of diurnal cortisol changes. *J Affect Disord* 2008;106:307–313. [PubMed: 17727959]
- Deuschle M, Gotthardt U, Schweiger U, Weber B, Korner A, Schmider J, Standhardt H, Lammers CH, Heuser I. With aging in humans the activity of the hypothalamus–pituitary–adrenal system increases and its diurnal amplitude flattens. *Life Sci* 1997;61:2239–2246. [PubMed: 9393943]
- Dratcu L, Calil HM. The dexamethasone suppression test: its relationship to diagnoses, severity of depression and response to treatment. *Prog Neuropsychopharmacol Biol Psychiatry* 1989;13:99–117. [PubMed: 2748868]

- Erhardt A, Ising M, Unschuld PG, Kern N, Lucae S, Putz B, Uhr M, Binder EB, Holsboer F, Keck ME. Regulation of the hypothalamic–pituitary–adrenocortical system in patients with panic disorder. *Neuropsychopharmacology* 2006;31:2515–2522. [PubMed: 16841071]
- Ferrari E, Magri F, Dori D, Migliorati G, Nescis T, Molla G, Fioravanti M, Solerte SB. Neuroendocrine correlates of the aging brain in humans. *Neuroendocrinology* 1995;61:464–470. [PubMed: 7783860]
- First, MB.; Spitzer, RL.; Gibbon, M.; Williams, JBW. Structured Clinical Interview for DSM-IV Axis I Disorders (SCID), Clinician Version: Administration Booklet. American Psychiatric Press; Washington, DC: 1996.
- Flint AJ. Epidemiology and comorbidity of anxiety disorders in the elderly. *Am J Psychiatry* 1994;151:640–649. [PubMed: 8166303]
- Flores BH, Kenna H, Keller J, Solvason HB, Schatzberg AF. Clinical and biological effects of mifepristone treatment for psychotic depression. *Neuropsychopharmacology* 2006;31:628–636. [PubMed: 16160710]
- Folstein MF, Folstein SE, McHugh PR. Mini-mental state. A practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res* 1975;12:189–198. [PubMed: 1202204]
- Greaves-Lord K, Ferdinand RF, Oldehinkel AJ, Sondeijker FE, Ormel J, Verhulst FC. Higher cortisol awakening response in young adolescents with persistent anxiety problems. *Acta Psychiatr Scand* 2007;116:137–144. [PubMed: 17650276]
- Hamilton M. The assessment of anxiety states by rating. *Br J Med Psychol* 1959;32:50–55. [PubMed: 13638508]
- Hamilton M. Development of a rating scale for primary depressive illness. *Br J Soc Clin Psychol* 1967;6:278–296. [PubMed: 6080235]
- Heuser I, Yassouridis A, Holsboer F. The combined dexamethasone/CRH test: a refined laboratory test for psychiatric disorders. *J Psychiatr Res* 1994;28:341–356. [PubMed: 7877114]
- Holsboer F. The corticosteroid receptor hypothesis of depression. *Neuropsychopharmacology* 2000;23:477–501. [PubMed: 11027914]
- Ice GH, Katz-Stein A, Himes J, Kane RL. Diurnal cycles of salivary cortisol in older adults. *Psychoneuroendocrinology* 2004;29:355–370. [PubMed: 14644066]
- Ising M, Kunzel HE, Binder EB, Nickel T, Modell S, Holsboer F. The combined dexamethasone/CRH test as a potential surrogate marker in depression. *Prog Neuropsychopharmacol Biol Psychiatry* 2005;29:1085–1093. [PubMed: 15950349]
- Jahn H, Schick M, Kiefer F, Kellner M, Yassouridis A, Wiedemann K. Metyrapone as additive treatment in major depression: a double-blind and placebo-controlled trial. *Arch Gen Psychiatry* 2004;61:1235–1244. [PubMed: 15583115]
- John, D.; Catherine, T. MacArthur Research Network on Socioeconomic Status and Health [Internet]. Rockefeller University: MacArthur Research Network on SES and Health; c. Salivary cortisol measurement. 1999 Dec. 2000. Available from <http://www.macses.ucsf.edu/Research/Allostatic/notebook/salivary/cort.html>
- Johnson DA, Grant EJ, Ingram CD, Gartside SE. Glucocorticoid receptor antagonists hasten and augment neurochemical responses to a selective serotonin reuptake inhibitor antidepressant. *Biol Psychiatry*. 2007
- Kallen VL, Tulen JH, Utens EM, Treffers PD, De Jong FH, Ferdinand RF. Associations between HPA axis functioning and level of anxiety in children and adolescents with an anxiety disorder. *Depress Anxiety*. 2007
- Kudielka BM, Kirschbaum C. Awakening cortisol responses are influenced by health status and awakening time but not by menstrual cycle phase. *Psychoneuroendocrinology* 2003;28:35–47. [PubMed: 12445835]
- Kumar A, Alcser K, Grunhaus L, Greden JF. Relationships of the dexamethasone suppression test to clinical severity and degree of melancholia. *Biol Psychiatry* 1986;21:436–444. [PubMed: 3697434]
- Lemieux AM, Coe CL. Abuse-related posttraumatic stress disorder: evidence for chronic neuroendocrine activation in women. *Psychosom Med* 1995;57:105–115. [PubMed: 7792368]
- Lindley SE, Carlson EB, Benoit M. Basal and dexamethasone suppressed salivary cortisol concentrations in a community sample of patients with posttraumatic stress disorder. *Biol Psychiatry* 2004;55:940–945. [PubMed: 15110738]

- Lupien S, Lecours AR, Lussier I, Schwartz G, Nair NP, Meaney MJ. Basal cortisol levels and cognitive deficits in human aging. *J Neurosci* 1994;14:2893–2903. [PubMed: 8182446]
- Meyer TJ, Miller ML, Metzger RL, Borkovec TD. Development and validation of the Penn State Worry Questionnaire. *Behav Res Ther* 1990;28:487–495. [PubMed: 2076086]
- Miller MD, Paradis CF, Houck PR, Mazumdar S, Stack JA, Rifai AH, Mulsant B, Reynolds CF III. Rating chronic medical illness burden in geropsychiatric practice and research: application of the cumulative illness rating scale. *Psychiatry Res* 1992;41:237–248. [PubMed: 1594710]
- Pitman RK, Orr SP. Twenty-four hour urinary cortisol and catecholamine excretion in combat-related posttraumatic stress disorder. *Biol Psychiatry* 1990;27:245–247. [PubMed: 2294983]
- Pomara N, Willoughby LM, Sidtis JJ, Cooper TB, Greenblatt DJ. Cortisol response to diazepam: its relationship to age, dose, duration of treatment, and presence of generalized anxiety disorder. *Psychopharmacology (Berl)* 2005;178:1–8. [PubMed: 15338100]
- Pruessner JC, Kirschbaum C, Meinlschmid G, Hellhammer DH. Two formulas for computation of the area under the curve represent measures of total hormone concentration versus time-dependent change. *Psychoneuroendocrinology* 2003a;28:916–931. [PubMed: 12892658]
- Pruessner JC, Wolf OT, Hellhammer DH, Buske-Kirschbaum A, von Auer K, Jobst S, Kaspers F, Kirschbaum C. Free cortisol levels after awakening: a reliable biological marker for the assessment of adrenocortical activity. *Life Sci* 1997;61:2539–2549. [PubMed: 9416776]
- Pruessner M, Hellhammer DH, Pruessner JC, Lupien SJ. Self-reported depressive symptoms and stress levels in healthy young men: associations with the cortisol response to awakening. *Psychosom Med* 2003b;65:92–99. [PubMed: 12554820]
- Rasmusson AM, Lipschitz DS, Wang S, Hu S, Vojvoda D, Bremner JD, Southwick SM, Charney DS. Increased pituitary and adrenal reactivity in premenopausal women with posttraumatic stress disorder. *Biol Psychiatry* 2001;50:965–977. [PubMed: 11750893]
- Sapolsky RM, Krey LC, McEwen BS. The neuroendocrinology of stress and aging: the glucocorticoid cascade hypothesis. *Endocr Rev* 1986;7:284–301. [PubMed: 3527687]
- Schweizer EE, Swenson CM, Winokur A, Rickels K, Maislin G. The dexamethasone suppression test in generalised anxiety disorder. *Br J Psychiatry* 1986;149:320–322. [PubMed: 3779298]
- Shear K, Belnap BH, Mazumdar S, Houck P, Rollman BL. Generalized Anxiety Disorder Severity Scale (GADSS): a preliminary validation study. *Depress Anxiety* 2006;23:77–82. [PubMed: 16411185]
- Tafet GE, Feder DJ, Abulafia DP, Roffman SS. Regulation of hypothalamic–pituitary–adrenal activity in response to cognitive therapy in patients with generalized anxiety disorder. *Cogn Affect Behav Neurosci* 2005;5:37–40. [PubMed: 15913006]
- Tiller JW, Biddle N, Maguire KP, Davies BM. The dexamethasone suppression test and plasma dexamethasone in generalized anxiety disorder. *Biol Psychiatry* 1988;23:261–270. [PubMed: 3337861]
- Uhe TW, Joffe RT, Jimerson DC, Post RM. Normal urinary free cortisol and plasma MHPG in panic disorder: clinical and theoretical implications. *Biol Psychiatry* 1988;23:575–585. [PubMed: 2833321]
- Urry HL, van Reekum CM, Johnstone T, Kalin NH, Thurow ME, Schaefer HS, Jackson CA, Frye CJ, Greischar LL, Alexander AL, Davidson RJ. Amygdala and ventromedial prefrontal cortex are inversely coupled during regulation of negative affect and predict the diurnal pattern of cortisol secretion among older adults. *J Neurosci* 2006;26:4415–4425. [PubMed: 16624961]
- US Department of Health and Human Services. Mental Health: A Report of the Surgeon General. US Department of Health and Human Services; Rockville: 1999.
- Van Cauter E, Leproult R, Kupfer DJ. Effects of gender and age on the levels and circadian rhythmicity of plasma cortisol. *J Clin Endocrinol Metab* 1996;81:2468–2473. [PubMed: 8675562]
- van Coevorden A, Mockel J, Laurent E, Kerkhofs M, L'Hermite-Baleriaux M, Decoster C, Neve P, Van Cauter E. Neuroendocrine rhythms and sleep in aging men. *Am J Physiol* 1991;260:E651–E661. [PubMed: 2018128]
- Watson S, Gallagher P, Smith MS, Ferrier IN, Young AH. The dex/CRH test—is it better than the DST? *Psychoneuroendocrinology* 2006;31:889–894. [PubMed: 16701957]
- Wessa M, Rohleder N, Kirschbaum C, Flor H. Altered cortisol-awakening response in posttraumatic stress disorder. *Psychoneuroendocrinology* 2006;31:209–215. [PubMed: 16154709]

- Wust S, Federenko I, Hellhammer DH, Kirschbaum C. Genetic factors, perceived chronic stress, and the free cortisol response to awakening. *Psychoneuroendocrinology* 2000;25:707–720. [PubMed: 10938450]
- Yehuda R. Advances in understanding neuroendocrine alterations in PTSD and their therapeutic implications. *Ann NY Acad Sci* 2006;1071:137–166. [PubMed: 16891568]
- Young EA, Abelson JL, Cameron OG. Effect of comorbid anxiety disorders on the hypothalamic–pituitary–adrenal axis response to a social stressor in major depression. *Biol Psychiatry* 2004;56:113–120. [PubMed: 15231443]
- Young EA, Breslau N. Saliva cortisol in posttraumatic stress disorder: a community epidemiologic study. *Biol Psychiatry* 2004;56:205–209. [PubMed: 15271590]
- Zobel AW, Nickel T, Kunzel HE, Ackl N, Sonntag A, Ising M, Holsboer F. Effects of the high-affinity corticotropin-releasing hormone receptor 1 antagonist R121919 in major depression: the first 20 patients treated. *J Psychiatr Res* 2000;34:171–181. [PubMed: 10867111]

Figure 1.

Salivary cortisol levels of generalized anxiety disorder and comparison subjects. Comparison of salivary cortisol levels in generalized anxiety disorder (GAD) and comparison subjects at awakening, 30 min after awakening, 1200, 1600, 2000 h, and bedtime. Significant differences in post hoc comparisons are indicated (* $p < 0.0001$, ** $p < 0.02$).

Figure 2.

Relationship between cortisol and Penn State Worry Questionnaire Scores of generalized anxiety disorder subjects. Scatterplots showing the relationship between salivary cortisol levels at awakening, 30 min after awakening, and the area under the concentration–time curve of cortisol with Penn State Worry Questionnaire scores of GAD subjects.

Table 1
Comparison of demographic and clinical characteristics of generalized anxiety disorder (GAD) and comparison participants.

	Comparison subjects (<i>n</i> = 40)	GAD (<i>n</i> = 71)	Test statistics
Demographics			
Age, year (SD)	75.0 (63) range 62–88 years	73.3 (8.3) range 60–99 years	$t = -1.10$, $df = 109$, $p = 0.28$
Female, no (%)	26 (65.0)	50 (70.4)	$\chi^2 = 0.35$, $df = 1$, $p = 0.56$
White (%)	37 (92.5)	59 (83.1)	$\chi^2 = 1.93$, $df = 1$, $p = 0.16$
CIRS-G (SD) [†]	7.8 (2.9)	7.7 (4.3)	$t = -0.04$, $df = 109$, $p = 0.97$
Education, year (SD)	14.6(3.1) range 6–20 years	13.6 (2.4) range 9–18 years	$t = -1.81$, $df = 109$, $p = 0.07$
Mini-Mental State exam (SD)	28.9 (1.4)	28.5 (1.6)	$t = -1.20$, $df = 109$, $p = 0.23$
Clinical characteristics			
Age of first GAD episode, year (SD)		43.2 (28.7) range 5–86 years	
Lifetime duration GAD, year (SD)		30.1 (28.5) range 1–94 years	
HRSA score (SD) ^{*,†}	4.7 (3.6)	22.2 (3.5)	$t = 14.71$, $df = 40.9$, $p < 0.0001$
Generalized Anxiety Disorder Severity Scale (SD) [†]	1.4 (2.0)	11.7 (2.9)	$t = 22.19$, $df = 105$, $p < 0.0001$
Penn State Worry Questionnaire (SD) [†]	27.4 (6.7)	54.7 (12.2)	$t = 14.78$, $df = 101$, $p < 0.0001$
HRSD score (SD) [†]	1.7 (2.0)	11.2 (3.5)	$t = 18.05$, $df = 109$, $p < 0.0001$
Psychiatric comorbidities			
Depressive disorder/Dysthymia, No		28	
Anxiety disorders, No		9	
Sleep-related characteristics			
Wake-up time	7:00 (66 min)	7:06 (72 min)	$t = 0.49$, $df = 108$, $p = 0.63$
Hours slept	7.2 (1.1)	7.2 (1.3)	$t = -0.05$, $df = 108$, $p = 0.96$
Awakened from sleep, no. of times	1.6 (1.2)	1.8 (1.1)	$t = 0.94$, $df = 108$, $p = 0.35$

Characteristics with statistically significant between group differences are shown in bold.

Note: Raw scores are reported as means (standard deviation) unless otherwise stated.

* Natural log transformation used in the analysis.

[†] Satterthwaite Method for unequal variance.