ORIGINAL ARTICLE

Major Depressive Disorder and Hypothalamic-Pituitary-Adrenal Axis Activity

Results From a Large Cohort Study

Sophie A. Vreeburg, MD; Witte J. G. Hoogendijk, MD, PhD; Johannes van Pelt, PhD; Roel H. DeRijk, PhD; Jolanda C. M. Verhagen, BSc; Richard van Dyck, MD, PhD; Johannes H. Smit, PhD; Frans G. Zitman, MD, PhD; Brenda W. J. H. Penninx, PhD

Context: There is a central belief that depression is associated with hyperactivity of the hypothalamic-pituitary-adrenal axis, resulting in higher cortisol levels. However, results are inconsistent.

Objective: To examine whether there is an association between depression and various cortisol indicators in a large cohort study.

Design, Setting, and Participants: Data are from 1588 participants of the Netherlands Study of Depression and Anxiety who were recruited from the community, general practice care, and specialized mental health care. Three groups were compared: 308 control subjects without psychiatric disorders, 579 persons with remitted (no current) major depressive disorder (MDD), and 701 persons with a current MDD diagnosis, as assessed using the *DSM-IV* Composite International Diagnostic Interview.

Main Outcome Measures: Cortisol levels were measured in 7 saliva samples to determine the 1-hour cortisol awakening response, evening cortisol levels, and cortisol suppression after a 0.5-mg dexamethasone suppression test.

Results: Both the remitted and current MDD groups showed a significantly higher cortisol awakening response compared with control subjects (effect size [Cohen *d*] range, 0.15-0.25). Evening cortisol levels were higher among the current MDD group at 10 PM but not at 11 PM. The postdexamethasone cortisol level did not differ between the MDD groups. Most depression characteristics (severity, chronicity, symptom profile, prior childhood trauma) were not associated with hypothalamic-pituitary-adrenal axis activity except for comorbid anxiety, which tended to be associated with a higher cortisol awakening response. The use of psychoactive medication was generally associated with lower cortisol levels and less cortisol suppression after dexamethasone ingestion.

Conclusions: This large cohort study shows significant, although modest, associations between MDD and specific hypothalamic-pituitary-adrenal axis indicators. Because a higher cortisol awakening response was observed among both subjects with current MDD and subjects with remitted MDD, this may be indicative of an increased biological vulnerability for depression.

Arch Gen Psychiatry. 2009;66(6):617-626

Author Affiliations:
Department of Psychiatry and EMGO Institute (Drs Vreeburg, Hoogendijk, van Dyck, Smit, and Penninx) and Center for Neurogenomics and Cognitive Research and Neurocampus Amsterdam (Drs Hoogendijk and Penninx), VU University Medical Center, Amsterdam, the Netherlands; Departments of Clinical Chemistry (Dr van Pelt and Ms Verhagen) and Psychiatry (Drs DeRijk, Zitman, and Penninx), Leiden University Medical Center, Leiden, the Netherlands; and Department of Psychiatry, University Medical Center Groningen, Groningen, the Netherlands (Dr Penninx).

THERE IS A CENTRAL BELIEF THAT a dysregulated hypothalamic-pituitary-adrenal (HPA) axis plays a role in the pathophysiology of depression.[1,2] Although not always consistent, evidence for hyperactivity of the HPA axis as indicated by higher daytime cortisol levels, more nonsuppression after dexamethasone ingestion, and higher corticotropin-releasing hormone and adrenocorticotropin hormone levels among persons with depression has been reported since the 1960s.[3-7] Dysregulation of the HPA axis could also help to explain unfavorable somatic health consequences, eg, cardiovascular disease or diabetes, observed among depressed persons.[8,9] The facts that the most prominent form of hypercortisolism, Cushing disease, is also associated with both depression and cardiovascular complications and that chronic stress is associated with higher cortisol levels strengthen this hypothesis.[10]

To assess HPA axis activity, salivary cortisol measures are increasingly used to reflect the active, unbound form of cortisol and are considered to be minimally intrusive on HPA axis regulation.[11] The cortisol awakening response (CAR) assesses the natural response of the HPA axis to awakening. Other commonly used cortisol measures include evening cortisol levels, reflecting basal activity, and the dexamethasone suppression test (DST), providing information on the negative feedback system of the HPA axis.[12] Although salivary cortisol measures such as the CAR have been associated with depression,[13,14] there are some conflicting results as well.[15,16] These inconsistencies in results remain largely unexplained but could

be partly owing to the facts that studies differ in adjustment for confounding variables (S.A.V., W.J.G.H., J.v.P., R.H.d.R., R.v.D., J.H.S., F.G.Z., B.W.J.H.P., and Boudewijn P. Kruijtzer, MSc, unpublished data, March 2008) and that study samples differ in terms of type and severity of depression,[1,17,18] use of psychoactive medication,[1,19-21] comorbidity of anxiety disorders,[22] level of neuroticism,[23] or daily hassles.[24] Higher cortisol levels have most frequently been reported among medicated inpatients with more severe melancholic or psychotic depression,[25,26] but studies in outpatient samples have shown few differences in cortisol levels between depressed and nondepressed persons.[17,25,27] Function of the HPA axis has not yet been studied in outpatients while adequately controlling for important covariates and analyzing depression characteristics in a large sample.

Our study among 1588 subjects of the Netherlands Study of Depression and Anxiety is one of the largest studies to examine whether there are differences between depressed subjects and healthy control subjects in various salivary cortisol measures (CAR, evening cortisol level, and cortisol suppression after dexamethasone ingestion), correcting for detailed covariates and additionally analyzing the role of depression characteristics.

METHODS

STUDY SAMPLE

Data are from the Netherlands Study of Depression and Anxiety, a large cohort study on the course of depressive and anxiety disorders. In total, 2981 respondents were recruited from the community, general practice care, and specialized mental health care. The study sample included persons with psychopathological findings as well as control subjects without a psychiatric diagnosis. For objectives and methods of the Netherlands Study of Depression and Anxiety, see the article by Penninx et al.[28] The research protocol was approved by the ethical committee of participating universities, and all of the respondents provided written informed consent.

To investigate whether cortisol levels were different in depressed vs nondepressed respondents taking into account remission of symptoms, 3 groups were created: control subjects, persons with remitted major depressive disorder (MDD), and persons with current MDD. Control subjects were defined as having no lifetime history of anxiety disorder (panic disorder, generalized anxiety disorder, agoraphobia, or social phobia) or depressive disorder (MDD or dysthymia) as assessed by the *DSM-IV* Composite International Diagnostic Interview (CIDI) version 2.1, no family history of depression, and a score below 14 on the Inventory of Depressive Symptoms (IDS). These criteria fit 413 Netherlands Study of Depression and Anxiety respondents. Persons with remitted MDD had a history of MDD but no diagnosis of MDD in the past 6 months as diagnosed by the CIDI; this included 810 respondents. The third group consisted of 1115 participants with a current diagnosis of MDD, ie, in the past 6 months, as assessed by the CIDI (of whom 802 [71.9%] had been diagnosed with MDD in the past month). We subsequently excluded a total of 22 pregnant or breastfeeding women and 137 participants receiving corticosteroids, leaving an initial sample of 2179 respondents. Of these, 1594 (73.2%) (309 control subjects, 581 subjects with remitted MDD, and 704 subjects with current MDD) returned saliva samples (see later). Respondents on saliva collection did not differ from nonrespondents in sex but were older (age, 44.1 vs 38.2 years, respectively; $P<.001$), more educated (12.4 vs 11.6 years of education, respectively; $P<.001$), and less likely to be currently depressed (44.2% vs 57.4%, respectively; $P<.001$).

SALIVARY CORTISOL MEASUREMENTS

At the baseline interview, respondents were instructed to collect saliva samples at home on a regular (preferably working) day shortly after the interview, which has been shown to be a reliable and minimally intrusive method to assess the active, unbound form of cortisol.[11] The median time between the interview and saliva sampling was 9.0 days (25th-75th percentile, 4-22 days). Instructions prohibited eating, smoking, drinking, or brushing teeth within 15 minutes. Saliva samples were obtained using Salivettes (Sarstedt AG and Co, Nümbrecht, Germany) at 7 time points. The CAR includes 4 sampling points: at awakening (T1) and at 30 (T2), 45 (T3), and 60 (T4) minutes later. The 2 evening values were collected at 10 PM (T5) and 11 PM (T6). Dexamethasone suppression was measured by cortisol sampling the next morning at awakening (T7) after ingestion of 0.5 mg of dexamethasone directly after the saliva sample was taken at 11:00 PM (T6). Samples were stored in refrigerators and returned by mail. After receipt, Salivettes were centrifuged at 2000*g* for 10 minutes, aliquoted, and stored at –80°C. Cortisol analysis was performed by competitive electrochemiluminescence immunoassay (E170; Roche, Basel, Switzerland) as described by van Aken et al.[29] The functional detection limit was 0.07 µg/dL (to convert to nanomoles per liter, multiply by 27.588) and the intra-assay and interassay variability coefficients in the measuring range were less than 10%. Assays were repeated if cortisol levels were very high (>2.90 µg/dL) or very low (<0.04 µg/dL) (n=128). All of the very high samples remained high and the mean of the values was used. In 80% of the very low samples, the repeated cortisol value was within the normal range and used for analysis; otherwise, the mean was used.

Three cortisol indicators were used: the cortisol awakening response, the evening cortisol level, and cortisol suppression on the DST.

Cortisol Awakening Response

In addition to conducting mixed model analyses (see the "Statistical Analysis" section) using all 4 saliva samples that determine the CAR, we calculated the area under the curve (AUC) with respect to the increase (AUCi) and with respect to the ground (AUCg) using the formulas by Pruessner et al.[30] The AUCg is an estimate of the total cortisol secretion and predicts mean cortisol levels throughout the day, and the AUCi is a measure of the dynamic of the CAR, more related to the sensitivity of the system and emphasizing changes over time.[30-33] If samples were collected outside of a margin of 5 minutes before or after the time protocol, values were assigned missing. In data cleaning, we assigned the 61 cortisol values (of 6274 values) that were more than 2 SDs from the mean (range, 2.03-4.74 µg/dL) as missing. The CAR analyses included all persons with at least 2 valid CAR cortisol values (n=1507; 295 control subjects, 548 subjects with remitted MDD, and 664 subjects with current MDD) because linear mixed model (LMM) analyses can adequately interpolate for missing values. For AUC analyses, at least 3 samples had to be available. For those with 1 missing cortisol value (n=87), the missing value was imputed using linear regression analyses including information on the available 3 cortisol levels, sex, age, awakening time, and smoking status.

Evening Cortisol Level

Data cleaning excluded cortisol values more than 2 SDs from the mean (1.59 µg/dL for T5, 2.35 µg/dL for T6), excluding 19

of 3135 cortisol values. Ultimately, data from 1579 subjects were available for evening cortisol level analyses (304 control subjects, 575 subjects with remitted MDD, and 700 subjects with current MDD).

Dexamethasone Suppression Test

Data cleaning was performed by excluding cortisol values more than 2 SDs from the mean for T7 (1.36 µg/dL), designating 12 cortisol values (out of 1532 samples) as missing and leaving 1520 subjects with values for T7. Of these, 1464 subjects (96.3%) (281 control subjects, 529 subjects with remitted MDD, and 654 subjects with current MDD) had taken 0.5 mg of dexamethasone after 11:00 PM on the first sampling day and were available for the DST analyses. In addition to the cortisol level at awakening after dexamethasone ingestion (T7), we used a cortisol suppression ratio: the cortisol value at awakening on the first day (T1) was divided by the cortisol value at awakening the next day (T7) after ingestion of 0.5 mg of dexamethasone the evening before. In addition, to indicate nonsuppressors more clearly, we also used a dichotomized indicator of the T1/T7 ratio, with a ratio below 1.51 (representing 1 SD below the mean) denoting a nonsuppressor.

In sum, 308 control subjects, 579 persons with remitted MDD, and 701 persons with current MDD provided at least 1 usable salivary cortisol variable (CAR, evening cortisol level, or DST result) and constituted the sample for the present analyses.

COVARIATES

We previously described associations between sociodemographics (sex, age, education, Northern European ancestry), sampling factors (awakening time, work status, weekday, season, sleep duration), and health indicators (smoking, physical activity) on salivary cortisol variables in our study (S.A.V., W.J.G.H., J.v.P., R.H.d.R., R.v.D., J.H.S., F.G.Z., B.W.J.H.P., and Boudewijn P. Kruijtzer, MSc, unpublished data, March 2008). These identified determinants were considered covariates.

Respondents reported time of awakening and working status on the sampling day. Sampling date information was used to categorize weekday vs weekend day, and season was categorized by months with less daylight (October-February) and more daylight (March-September). The average sleep duration during the last 4 weeks was dichotomized as 6 or fewer hours per night vs more than 6 hours per night, and smoking status was dichotomized as current smoker vs nonsmoker. Physical activity was assessed using the International Physical Activity Questionnaire[34] and expressed as activity per 1000 MET-minutes (metabolic equivalent of number of calories spent per minute) per week.

DEPRESSION CHARACTERISTICS

In post hoc analyses, several characteristics were explored to evaluate their potential differentiating effect on HPA axis activity. Chronicity of symptoms was considered present when in the past 5 years at least 24 months of depressive symptoms were reported as assessed by the Life Chart method.[35] The occurrence of suicide attempts in the past was examined using the Scale for Suicide Ideation.[36] Comorbid anxiety disorder was assessed through the CIDI and was considered present when panic disorder, generalized anxiety disorder, agoraphobia, or social phobia was diagnosed in the past 6 months. The total score on the 30-item self-report version of the IDS[37] was used as an indicator of depression severity. To analyze whether subtypes of depression exhibit differences in HPA axis activity, atypical and melancholic features were defined using specific IDS items. The atypical subtype was defined according to Novick et al[38] based on *DSM-IV* criteria as having mood reactivity and at least 3 of the following: hyperphagia, hypersomnia, leaden paralysis, and interpersonal rejection sensitivity. The melancholic subtype was defined as lacking mood reactivity and loss of pleasure with at least 3 of the following: distinct quality of mood, mood worsening in the morning, early morning awakening, psychomotor retardation, significant anorexia or weight loss, and excessive guilt. The episode was assessed as first or recurrent during the CIDI. Using the World Health Organization Anatomical Therapeutic Chemical classification,[39] psychoactive medication was categorized into antidepressants (tricyclic antidepressants [N06AA], selective serotonin reuptake inhibitors [N06AB], and other antidepressants [N06AF, N06AG, N06AX]) and benzodiazepines (N03AE, N05BA, N05CD, N05CF). Neuroticism was measured with the 12-item subscale of the NEO 5-Factor Inventory,[40] with scores ranging from 0 (low neuroticism) to 48 (high neuroticism). Experience of daily hassles was measured with the 20-item Daily Hassles Questionnaire,[41] rating the experience of health, family, friends, and environment hassles on a total scale from 0 (no hassles) to 60 (many hassles). Finally, to examine the role of earlier childhood trauma, we constructed a cumulative childhood trauma index using the Netherlands Mental Health Survey and Incidence Study childhood trauma interview.[42] This summarizes the frequency of 4 reported traumas—emotional neglect, psychological abuse, physical abuse, and sexual abuse—before age 16 years, resulting in an index score between 0 and 8.

STATISTICAL ANALYSIS

All of the cortisol values showed normal distributions except for the cortisol suppression ratio, which was log transformed for analyses. Baseline characteristics of the 3 MDD groups were compared using χ^2 and analysis of variance statistics. To analyze differences in CAR across groups, analyses of covariance with AUCi and AUCg were conducted. In addition, random coefficient analysis of the 4 morning cortisol data points was performed using LMM analyses. This keeps original values on all of the 4 data points, can accommodate for incomplete cases, and takes correlation between repeated data into account.[43] The MDD group, time point (T1, T2, T3, or T4), and all of the covariates were entered as fixed factors, subjects were treated as a random effect, and a random intercept was estimated. To examine whether the course of cortisol level after awakening was different across groups, we added an MDD group × time interaction term. Analyses of covariance were used to examine differences across MDD groups in evening cortisol level (cortisol level at 10 PM and 11 PM) and cortisol suppression after dexamethasone ingestion (cortisol at T7 and the log ratio of T1/T7). A multivariable logistic regression analysis examined MDD group status on the dichotomous DST nonsuppression indicator. Additional analyses among depressed subjects were performed to evaluate the role of depression characteristics using multivariable linear regression analyses and LMM analyses. For significant findings, effect sizes were calculated with Cohen *d*.[44] All of the analyses were conducted using SPSS version 15.0 statistical software (SPSS Inc, Chicago, Illinois).

RESULTS

Characteristics across MDD groups are presented in **Table 1**. Subjects with remitted or current MDD were more often female, younger, less educated, less likely to work, more likely to sleep fewer hours, and more likely to smoke. Subjects with current MDD had the least fa-

Table 1. Subject Characteristics

Characteristic	Control Subjects (n = 308)	Subjects With Remitted MDD (n = 579)	Subjects With Current MDD (n = 701)	*P* Value
Demographic				
Female, %	56.8	70.5	65.3	<.001
Age, mean (SD), y	47.8 (11.7)	44.7 (12.4)	42.0 (12.0)	<.001
Education, mean (SD), y	13.5 (3.3)	12.4 (3.3)	12.0 (3.2)	<.001
Northern European ancestry, %	96.4	97.1	93.6	.008
Time of awakening, mean (SD)	7:18 AM (1 h, 7 min)	7:28 AM (1 h, 9 min)	7:30 AM (1 h, 14 min)	.05
Working on day of sampling, %	66.1	60.7	52.2	<.001
Sampling on a weekday, %	90.1	93.8	89.5	.03
Sampling in month with more daylight, %	52.6	58.8	51.4	.03
≤6 h of sleep, %	16.4	26.3	37.5	<.001
Smoking, %	21.4	36.1	38.5	<.001
Physical activity/1000 MET-min/wk, mean (SD)	3.8 (3.0)	3.7 (3.0)	3.4 (3.3)	.17
Depression				
>2 y of symptoms, %	NA	14.7	32.4	<.001
Suicide attempts in past, %	1.3	11.0	19.1	<.001
Comorbid anxiety disorder, %	NA	35.9	63.2	<.001
IDS score, mean (SD)	5.4 (3.6)	17.5 (10.1)	31.4 (12.2)	<.001
Subtype of depression, %				
Melancholic features	NA	0.3	12.4	<.001
Atypical features	NA	5.2	20.8	<.001
Recurrent episode, %	NA	49.9	55.2	.06
No psychoactive medication use, %[a]	NA	76.0	54.2	<.001
Antidepressant use, %	NA	23.1	42.1	<.001
TCA	NA	2.8	3.7	.35
SSRI	NA	16.4	27.8	<.001
Other	NA	4.5	10.6	<.001
Benzodiazepine use, %	NA	3.8	12.4	<.001
Childhood trauma index score, mean (SD)	0.6 (1.4)	1.7 (2.1)	2.1 (2.3)	<.001
Neuroticism score, mean (SD)	24.2 (5.7)	35.7 (8.1)	41.9 (6.8)	<.001
Daily hassles score, mean (SD)	24.5 (4.0)	30.4 (6.8)	36.1 (8.3)	<.001
Cortisol				
CAR, mean (SD), μg/dL				
T1, at awakening	0.61 (0.25)	0.60 (0.24)	0.62 (0.25)	.28
T2, 30 min after awakening	0.72 (0.33)	0.77 (0.32)	0.78 (0.36)	.06
T3, 45 min after awakening	0.65 (0.31)	0.74 (0.36)	0.73 (0.37)	.002
T4, 60 min after awakening	0.58 (0.32)	0.64 (0.33)	0.66 (0.38)	.01
Evening cortisol level, mean (SD), μg/dL				
T5, at 10 PM	0.18 (0.13)	0.20 (0.13)	0.21 (0.16)	.005
T6, at 11 PM	0.19 (0.17)	0.20 (0.16)	0.20 (0.16)	.87
Dexamethasone suppression test				
Cortisol level at T7, at awakening, mean (SD), μg/dL	0.25 (0.14)	0.26 (0.12)	0.26 (0.13)	.92
Cortisol suppression ratio, mean (SD)[b]	0.39 (0.22)	0.38 (0.20)	0.40 (0.21)	.23
Nonsuppression, %	14.9	13.8	11.0	.18

Abbreviations: CAR, cortisol awakening response; IDS, Inventory of Depressive Symptoms; MDD, major depressive disorder; MET-min, metabolic equivalent of number of calories spent per minute; NA, not applicable; SSRI, selective serotonin reuptake inhibitor; T1-T7, time points; TCA, tricyclic antidepressant.

SI conversion factor: To convert CAR, evening cortisol level, and cortisol level at T7 to nanomoles per liter, multiply by 27.588.

[a] No psychoactive medication use indicates no frequent use of benzodiazepines or antidepressants.

[b] Cortisol suppression ratio = log(salivary cortisol level at T1/salivary cortisol level at T7).

vorable scores on all of the depression characteristics. Of the subjects with current MDD, 198 (28.2%) were severely depressed as defined by an IDS score of 39 or higher, corresponding to a score of 20 or higher on the 17-item Hamilton Depression Scale.[45]

Among the respondents, 1101 of 1507 (73.1%) showed an increase in the cortisol level in the first hour after awakening, with a mean increase of 0.38 μg/dL (or 79.3%). Unadjusted cortisol levels did not differ between groups at awakening but became significantly higher in depressed subjects during the hour after awakening (Table 1). Also, the evening cortisol level at 10 PM was significantly higher in subjects with current MDD, but no differences across groups were found at 11 PM or for the DST results.

CORTISOL AWAKENING RESPONSE

With respect to the CAR, 2 elements can be distinguished. First, a direct effect on overall morning cortisol values is indicated by a larger AUCg and/or a significant (direct) effect in LMM analyses. Second, a difference in the course over time (or shape of the CAR) is reflected by a difference in the AUCi and/or a significant interaction between group and time in LMM analyses. Adjusted CAR results illustrate that consistently after

awakening, the current MDD group shows higher overall cortisol levels but a time course that is similar to that of the control group (**Figure 1**). This is reflected by the significant group effect (current MDD group vs control group, $P<.001$) but nonsignificant current MDD group × time interaction ($P=.46$) in LMM analyses and by a significantly higher AUCg ($P=.001$; effect size [Cohen *d*] = 0.25) but a nonsignificant effect on the AUCi ($P=.28$) (**Table 2**). The remitted MDD group shows a higher CAR compared with the control group as illustrated by a significant group effect (remitted MDD group vs control group, $P=.007$), a significant remitted MDD group × time interaction ($P=.008$), and a higher AUCg ($P=.02$; effect size = 0.18) and AUCi ($P=.04$; effect size = 0.15) (Table 2). The remitted MDD group did not differ significantly from the current MDD group (AUCg, $P=.26$; AUCi, $P=.25$; LMM analyses: group, $P=.25$; group × time, $P=.15$).

EVENING CORTISOL LEVEL

Evening cortisol levels at 10:00 PM were significantly higher in the MDD groups; this did not apply to the remitted MDD group after correction for lifestyle factors (Table 2) but remained significant for the current MDD group ($P=.008$; effect size = 0.22). There was a trend toward higher cortisol levels in the current MDD group compared with the remitted MDD group ($P=.07$). No significant differences across MDD groups were seen for the sample at 11 PM. Significant differences in intragroup variance ([cortisol level at T5 – cortisol level at T6]/cortisol level at T5) were found between depressed groups and the control group (mean [SD], –0.09 [0.03] for the remitted MDD group vs –0.19 [0.04] for the control group, $P=.01$; mean [SD], –0.06 [0.02] for the current MDD group vs –0.19 [0.04] for the control group, $P=.002$), indicating greater variance in the evening cortisol levels among control subjects.

DEXAMETHASONE SUPPRESSION TEST

Depression groups hardly differed in cortisol level at awakening after dexamethasone ingestion the night before. A significant finding was present for the current MDD group ($P=.03$; effect size = 0.17) when using the cortisol suppression ratio, indicating more cortisol suppression (Table 2). This was confirmed by logistic regression analysis using the dichotomous indicator (odds ratio for nonsuppression = 0.51; 95% confidence interval, 0.33-0.81; $P=.004$). The current MDD group also showed more cortisol suppression than the remitted MDD group ($P=.02$).

DEPRESSION CHARACTERISTICS

Table 3 reports additional analyses exploring specific associations with depression characteristics among the (combined) depressed groups (n = 579 + 701 = 1280). For the CAR, no effects were found for depression characteristics (eg, severity, symptom profile, chronicity, or childhood trauma) except for the presence of comorbid anxiety disorder and the use of psychoactive medication. Depressed subjects with comorbid anxiety showed

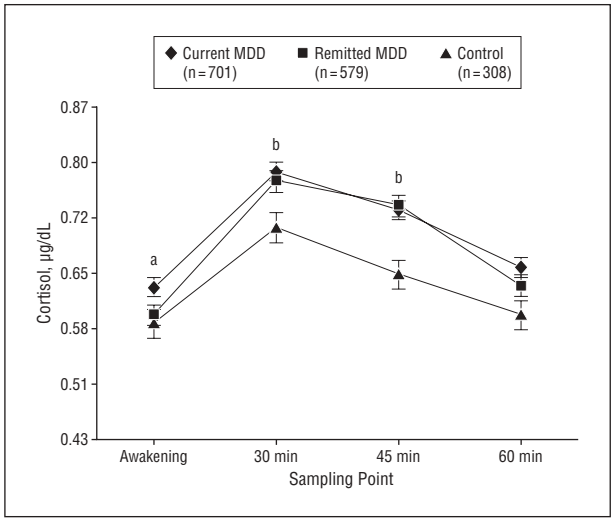

Figure 1. Mean salivary cortisol levels of the cortisol awakening response for control subjects, subjects with remitted major depressive disorder (MDD), and subjects with current MDD. All results are adjusted for sex, age, education, Northern European ancestry, working, weekday, time of awakening, sleep, month with more daylight, smoking, and physical activity. Error bars indicate SE. To convert cortisol to nanomoles per liter, multiply by 27.588. [a]For subjects with current MDD vs control subjects, $P \leq .01$. [b]For both MDD groups vs control subjects, $P<.01$. Results of mixed model analyses are as follows: for subjects with current MDD vs control subjects, $P<.001$, interaction with time, $P=.46$; for subjects with remitted MD vs control subjects, $P=.007$, interaction with time, $P=.008$.

a significant interaction with time in LMM analyses ($P=.009$) and a trend for a higher AUCg ($P=.06$). These findings are illustrated in **Figure 2**, showing a higher CAR among depressed subjects with comorbid anxiety. Analysis of variance revealed an AUCg of 0.70 (SE = 0.01) µg/dL/h for MDD without comorbid anxiety compared with 0.66 (SE = 0.02) µg/dL/h for control subjects ($P=.02$; effect size = 0.16) and an AUCg of 0.73 (SE = 0.01) µg/dL/h for MDD with comorbid anxiety compared with 0.66 (SE = 0.02) µg/dL/h for control subjects ($P<.001$; effect size = 0.27) ($P=.06$ compared with MDD without anxiety). The other CAR determinant was the use of tricyclic antidepressants, which was associated with a significantly lower AUCi ($b=-0.085$; $P=.03$) and confirmed by a time interaction in LMM analyses ($P=.05$). The use of benzodiazepines was associated with lower evening cortisol levels, especially at 10 PM (Table 3), and recurrent episodes tended to be associated with a higher evening cortisol level at 11 PM. Finally, the postdexamethasone cortisol level was significantly higher in subjects using selective serotonin reuptake inhibitors or tricyclic antidepressants and tended to be higher in depressed subjects with a recurrent episode. Results of logistic regression with the nonsuppression indicator confirmed only the association with selective serotonin reuptake inhibitor use (odds ratio of nonsuppression = 1.58; 95% confidence interval, 1.05-2.40; $P=.03$). Taking the use of antidepressants into account did not change any of the observed differences across MDD groups for any of the cortisol indicators.

Saliva collection took place on average 9 days after interviewing. To check whether a large in-between period could have obscured associations, analyses were repeated among those for whom saliva was collected within

Table 2. Results of Analyses of Covariance Associating the 3 Control or Depression Groups With Salivary Cortisol Indicators

Salivary Cortisol Indicator	Control Subjects, Mean (SE) (n = 308)	Subjects With Remitted MDD, Mean (SE) (n = 579)	Subjects With Remitted MDD vs Control Subjects, *P* Value	Subjects With Current MDD, Mean (SE) (n = 701)	Subjects With Current MDD vs Control Subjects, *P* Value
AUC, µg/dL/h					
AUCg					
Unadjusted	0.66 (0.01)	0.71 (0.01)	.03	0.72 (0.01)	.004
Basic adjustment[a]	0.65 (0.01)	0.70 (0.01)	.004	0.72 (0.01)	<.001
Full adjustment[b]	0.66 (0.01)	0.70 (0.01)	.02	0.72 (0.01)	.001
AUCi					
Unadjusted	0.05 (0.01)	0.10 (0.01)	.007	0.09 (0.01)	.03
Basic adjustment[a]	0.06 (0.01)	0.11 (0.01)	.02	0.09 (0.01)	.10
Full adjustment[b]	0.07 (0.01)	0.11 (0.01)	.04	0.09 (0.01)	.28
Evening cortisol level, µg/dL					
T5					
Unadjusted	0.18 (0.01)	0.20 (0.01)	.06	0.21 (0.004)	.001
Basic adjustment[a]	0.18 (0.01)	0.20 (0.01)	.04	0.21 (0.01)	.001
Full adjustment[b]	0.18 (0.01)	0.20 (0.01)	.23	0.21 (0.004)	.008
T6					
Unadjusted	0.19 (0.01)	0.20 (0.01)	.67	0.20 (0.01)	.61
Basic adjustment[a]	0.20 (0.01)	0.20 (0.01)	.73	0.20 (0.01)	.70
Full adjustment[b]	0.20 (0.01)	0.20 (0.01)	.64	0.20 (0.004)	.69
Dexamethasone suppression test					
T7, µg/dL					
Unadjusted	0.25 (0.01)	0.26 (0.01)	.68	0.26 (0.004)	.75
Basic adjustment[a]	0.25 (0.01)	0.26 (0.01)	.51	0.26 (0.004)	.67
Full adjustment[b]	0.26 (0.01)	0.26 (0.01)	.89	0.26 (0.004)	.95
Cortisol suppression ratio[c]					
Unadjusted	0.39 (0.01)	0.38 (0.01)	.56	0.40 (0.01)	.43
Basic adjustment[a]	0.38 (0.01)	0.37 (0.01)	.75	0.41 (0.01)	.10
Full adjustment[b]	0.37 (0.01)	0.38 (0.01)	.88	0.41 (0.01)	.03

Abbreviations: AUC, area under the curve; AUCg, area under the morning curve with respect to the ground, AUCi, area under the morning curve with respect to the increase; MDD, major depressive disorder; T5-T7, time points.

SI conversion factor: To convert AUC to nanomoles per liter per hour and to convert evening cortisol level and dexamethasone suppression test cortisol levels at T7 to nanomoles per liter, multiply by 27.588.

[a] Adjusted for sex, age, education, Northern European ancestry, working, weekday, time of awakening, sleep, and month with more daylight.

[b] Additionally adjusted for smoking and physical activity.

[c] Cortisol suppression ratio = log(salivary cortisol level at T1/salivary cortisol level at T7), after 0.5 mg of dexamethasone ingestion.

1 week after interviewing. Also, we repeated analyses only considering subjects to be currently depressed when they fulfilled 1-month (instead of 6-month) diagnosis criteria. These analyses revealed very similar results.

COMMENT

This study, to our knowledge one of the largest of its kind, described some significant—although modest—differences in specific indicators of HPA axis activity between depressed persons and healthy control subjects. First, higher cortisol values in the hour after awakening were found for both subjects with current MDD and subjects with remitted MDD compared with control subjects. Second, although not confirmed with data from 11:00 PM, significantly higher evening cortisol levels were found at 10:00 PM for the current MDD group. However, the MDD groups did not show more cortisol nonsuppression after ingestion of 0.5 mg of dexamethasone, and current MDD was even associated with more suppression. Additional analyses on several depression characteristics revealed no significant associations for most characteristics (eg, chronicity, symptom severity, childhood trauma) except for a higher CAR among depressed subjects with comorbid anxiety. In addition, the use of psychoactive medication was generally associated with decreased cortisol levels and less cortisol suppression after dexamethasone ingestion.

Literature on the role of the HPA axis in depression is extensive, but results are conflicting. The CAR has most frequently been associated with depression.[13,14] In our depressed sample, in line with most literature,[1,13,14,46,47] we found an association with the CAR for both AUCi and AUCg variables, indicating that the dynamic of the CAR as well as total cortisol secretion is increased in subjects with MDD compared with control subjects.[31,33] Remarkably, this was present for both subjects with current MDD and subjects with remitted MDD, indicating that an increased CAR likely represents a trait rather than a state marker, reflecting either a specific biological depression vulnerability or a biological scar as shown before.[48] Interestingly, recent studies have found increased cortisol levels in subjects at familial risk for depression, suggesting a biological vulnerability for depression.[49,50] Considering this observation, it is not very surprising that various other depression characteristics (eg, severity, chronicity, atypical symptoms) did not further differ in the CAR as described before.[13,17] However, it should be

Table 3. Results of Multivariable Analyses Associating Depression Characteristics With Various Cortisol Indicators Among Subjects With Remitted or Current Major Depressive Disorder[a]

Depression Characteristic	AUCg, µg/dL/h (n = 1132)		AUCi, µg/dL/h (n = 1132)		Mixed Model Analyses (n = 1212)		Evening Cortisol Level, µg/dL				Postdexamethasone Cortisol Level, µg/dL (n = 1183)	
					Direct Effect	Interaction With Time	T5 (n = 1256)		T6 (n = 1261)			
	b	*P* Value	*b*	*P* Value	*P* Value	*P* Value	*b*	*P* Value	*b*	*P* Value	*b*	*P* Value
>2 y of symptoms, yes vs no	−0.018	.36	0.007	.70	.57	.92	−0.013	.20	−0.012	.20	−0.010	.25
Suicide attempt in past, yes vs no	0.003	.89	−0.019	.35	.44	.35	−0.016	.15	0.005	.64	−0.011	.27
Comorbid anxiety disorder, yes vs no	0.029	.06	0.000	.98	.25	.009	0.003	.70	0.004	.61	0.003	.69
Depression severity per IDS score increase	0.000	.80	0.000	.91	.48	.99	0.000	.93	0.000	.73	0.000	.39
Subtype of symptoms, yes vs no												
Melancholic features	−0.004	.91	0.011	.71	.87	.85	−0.003	.85	−0.015	.32	−0.008	.59
Atypical features	−0.015	.50	0.016	.44	.34	.81	−0.016	.16	−0.016	.14	−0.002	.87
Recurrent episode, yes vs no	0.014	.35	−0.007	.63	.26	.82	0.009	.27	0.013	.07	0.012	.10
Medication, yes vs no												
TCA	−0.062	.15	−0.085	.03	.21	.05	0.005	.80	0.020	.34	0.064	.001
SSRI	−0.004	.84	0.001	.95	.88	.55	0.012	.20	0.011	.21	0.022	.01
Other antidepressant	−0.034	.23	−0.017	.52	.28	.58	0.012	.41	0.015	.27	0.018	.17
Benzodiazepine	0.017	.57	−0.017	.54	.92	.83	−0.034	.02	−0.024	.08	0.005	.72
Childhood trauma index per score increase	0.003	.50	0.001	.85	.78	.87	0.000	.87	−0.001	.47	0.000	.94
Neuroticism per score increase	0.000	.80	0.000	.80	.18	.27	0.000	.61	0.000	.98	−0.001	.13
Daily hassles per score increase	0.001	.18	0.000	.97	.06	.80	0.000	.58	0.000	.39	−0.001	.14

Abbreviations: AUCg, area under the morning curve with respect to the ground; AUCi, area under the morning curve with respect to the increase; IDS, Inventory of Depressive Symptoms; SSRI, selective serotonin reuptake inhibitor; T5 and T6, time points; TCA, tricyclic antidepressant.

SI conversion factor: To convert regression coefficients for AUCg and AUCi to nanomoles per liter per hour and to convert regression coefficients for evening cortisol level and postdexamethasone cortisol level to nanomoles per liter, multiply by 27.588.

[a] Analyses are adjusted for sex, age, education, Northern European ancestry, working, weekday, time of awakening, sleep, month with more daylight, smoking, and physical activity.

noted that the literature on the importance of severity, chronicity, and type of depressive symptoms on HPA axis function conflicts with both present[1,17,18] and absent[13,17,26,51,52] associations. We also observed that the CAR was especially increased among—although not restricted to—depressed subjects with comorbid anxiety, which is in line with some earlier findings that adrenocorticotropin hormone and cortisol response to stress appeared to be mainly due to the presence of comorbid anxiety among the depressed subjects[22]; these findings were suggested to be due to interacting roles of the HPA axis and noradrenergic systems, which enhance each other, because there is evidence for an association of both anxiety and MDD with activation of the HPA axis as well as noradrenergic systems.

In addition to an increased CAR, we also found a higher evening cortisol level for the current MDD group, although only at 10 PM and not at 11 PM. Increased evening activation of the HPA axis has been described before.[53] Nevertheless, our inconsistency across 2 time points is difficult to explain. First, it should be noted that evening cortisol values are usually low; consequently, their assessment is affected by measurement error (eg, higher variance due to more cortisol values below the functional detection limit of 0.07 µg/dL) as may be illustrated by the higher intragroup variance among control subjects. However, after exclusion of very low samples (<0.07 µg/dL), we found similar results. Second, because even modest hassles influence HPA axis activity in healthy subjects,[54] we can also hypothesize—although posteriori—that some individuals may have had increased cortisol levels owing to postponing their usual bedtime as a consequence of the collection protocol or owing to experiencing anticipatory stress for subsequent dexamethasone ingestion. This will then especially affect the cortisol measurement at 11 PM and could contribute to the inconsistent evening cortisol level findings. Our intriguing result for higher cortisol levels at 10 PM among currently depressed subjects, however, should stimulate further confirmative studies.

We did not find higher rates of cortisol nonsuppression in the depressed groups, and the subjects with current MDD were even more likely to be suppressors. Most earlier studies that found more nonsuppression after dexamethasone ingestion among depressed subjects were conducted among more severely depressed inpatients with melancholic, psychotic, or bipolar depression.[26,55] It could be that significant dysregulation of the negative HPA axis

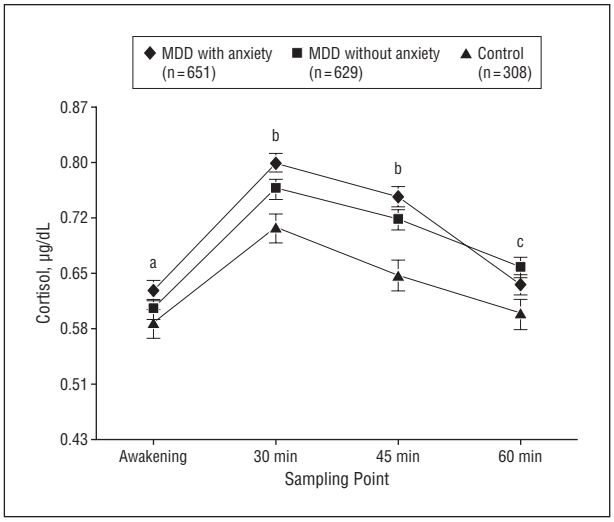

Figure 2. Mean salivary cortisol levels of the cortisol awakening response for control subjects, subjects with major depressive disorder (MDD) without comorbid anxiety, and subjects with MDD with comorbid anxiety. All results are adjusted for sex, age, education, Northern European ancestry, working, weekday, time of awakening, sleep, month with more daylight, smoking, and physical activity. Error bars indicate SE. To convert cortisol to nanomoles per liter, multiply by 27.588. [a]For subjects with MDD with anxiety vs control subjects, $P=.02$. [b]For subjects with MDD with anxiety vs control subjects, $P<.001$; for subjects with MDD without anxiety vs control subjects, $P\leq.02$. [c]For subjects with MDD without anxiety vs control subjects, $P=.03$. Results of mixed model analyses are as follows: for subjects with MDD with anxiety vs control subjects, $P<.001$, interaction with time, $P=.01$; for subjects with MDD without anxiety vs control subjects, $P=.006$, interaction with time, $P=.11$.

feedback system is confined to severe (psychotic) depression such as that in inpatients. Indeed, a meta-analysis on cortisol nonsuppression after dexamethasone ingestion showed that psychotic depression was most clearly associated with prominent nonsuppression, whereas the nonsuppression rate in nonmelancholic outpatients was low.[26] It has been postulated that hypercortisolemia could contribute to psychosis by enhancing dopamine activity possibly through effects on tyrosine hydroxylase.[56] Alternatively, maybe differences in the negative feedback system of the HPA axis become more pronounced when we use the more sensitive dexamethasone and corticotropin-releasing hormone test.[57] Finally, it could be that HPA axis dysregulation is more pronounced when subjects are exposed to acute stress because studies have reported blunted stress reactivity and impaired stress recovery in depressed subjects in response to a psychological stressor.[58]

In line with other studies,[1,20,21] we found an association between psychoactive medication use and cortisol levels, resulting overall in decreased cortisol levels and less cortisol suppression after dexamethasone ingestion. It has been postulated that long-term use of antidepressants up-regulates the glucocorticoid and mineralocorticoid receptors in the brain, which may underlie normalization of the HPA axis and could contribute to the clinical efficacy of antidepressant treatment.[59] Also, benzodiazepines seem to mediate inhibitory effects of γ-aminobutyric acid on the secretion of corticotropin-releasing hormone.[19]

Our study had some limitations. First, because these analyses were cross-sectional, our results do not indicate any causal directions of the associations found. Second, when relying on saliva sampling in an ambulatory setting, compliance with the sampling instructions is essential. This is true especially for the CAR because it is a very characteristic curve within the first hour of awakening and largely dependent on the awakening sample. Noncompliance with instructions could have resulted in measurement error and may explain why some persons (406 of 1507 subjects [26.9%]) did not demonstrate a cortisol increase at all after awakening. However, it should be noted that even when awakening is closely monitored, still at least 15% of all persons do not respond with a cortisol increase.[60] For the DST, it is impossible to guarantee that all persons indeed ingested the dexamethasone pill. However, when we measured dexamethasone levels with a radioimmunoassay using the antidexamethasone antibody (IgG Corp, Nashville, Tennessee; the functional detection limit is 0.01 µg/dL and the reported cross-reactivity for cortisol is 0.04%[61]) among 47 respondents with a T1/T7 ratio less than 1.5 (indicative of cortisol nonsuppression) who reported dexamethasone ingestion, we found detectable dexamethasone levels (>0.01 µg/dL) in the T7 saliva samples among 42 subjects (89.4%), indicating that noncompliance with dexamethasone ingestion is not likely to be frequent. Third, although we assumed that depressed subjects in our study had generally higher levels of stress and mood symptoms—as illustrated by higher IDS scores, neuroticism, and daily hassles scores—compared with control subjects, day-to-day variation that we could not further address could play an additional role in HPA axis function. Finally, some depressed subjects may have (unnoticed) bipolar disorder, which may be associated with more pronounced HPA axis regulation[55] and therefore may have caused a slight overestimation of the association between MDD and cortisol levels.

Despite these limitations, our study had many strong aspects, including the large sample size with clearly distinct depression groups, the inclusion of multiple cortisol measures indicative of different aspects of HPA axis activity, the exploration of depression characteristics, and the adjustment of various covariates. Smoking is an especially important covariate because it is associated with depression as well as higher cortisol levels (we found effect sizes up to 0.38 for smoking [S.A.V., W.J.G.H., J.v.P., R.H.d.R., R.v.D., J.H.S., F.G.Z., B.W.J.H.P., and Boudewijn P. Kruijtzer, MSc, unpublished data, March 2008]). Indeed, correcting for lifestyle indicators did affect our findings (Table 2). Nevertheless, even after adjustment for covariates, our study found significant differences in HPA axis activity indicators among depressed subjects when compared with healthy control subjects. Effect sizes for the CAR and evening cortisol level differences were in the range of 0.15 to 0.25, indicating modest effects. Although the clinical relevance of such differences needs to be explored in further large-scale research, there is some evidence that such salivary cortisol level differences may result in a higher risk of relapse[62] and unfavorable somatic outcomes such as atherosclerotic plaques[63] and progression of intima media thickness.[64]

Submitted for Publication: July 11, 2008; final revision received October 20, 2008; accepted December 2, 2008.
Correspondence: Brenda W. J. H. Penninx, PhD, De-

partment of Psychiatry, VU University Medical Center, A. J. Ernststraat 887, 1081 HL Amsterdam, the Netherlands (b.penninx@vumc.nl).

Financial Disclosure: None reported.

Funding/Support: The infrastructure for the Netherlands Study of Depression and Anxiety (NESDA) (http://www.nesda.nl) is funded through the Geestkracht program of the Netherlands Organisation for Health Research and Development (Zon-Mw grant 10-000-1002) and is supported by participating universities and mental health care organizations (VU University Medical Center, GGZ inGeest, Arkin, Leiden University Medical Center, GGZ Rivierduinen, University Medical Center Groningen, Lentis, GGZ Friesland, GGZ Drenthe, IQ Healthcare, Netherlands Institute for Health Services Research [NIVEL], and Netherlands Institute of Mental Health and Addiction [Trimbos]).

REFERENCES

1. Holsboer F, Barden N. Antidepressants and hypothalamic-pituitary-adrenocortical regulation. *Endocr Rev*. 1996;17(2):187-205.
2. Nemeroff CB, Vale WW. The neurobiology of depression: inroads to treatment and new drug discovery. *J Clin Psychiatry*. 2005;66(suppl 7):5-13.
3. Carroll BJ, Curtis GC, Mendels J. Neuroendocrine regulation in depression, I: limbic system-adrenocortical dysfunction. *Arch Gen Psychiatry*. 1976;33(9):1039-1044.
4. Gold PW, Licinio J, Wong ML, Chrousos GP. Corticotropin releasing hormone in the pathophysiology of melancholic and atypical depression and in the mechanism of action of antidepressant drugs. *Ann N Y Acad Sci*. 1995;771:716-729.
5. Pfohl B, Sherman B, Schlechte J, Winokur G. Differences in plasma ACTH and cortisol between depressed patients and normal controls. *Biol Psychiatry*. 1985; 20(10):1055-1072.
6. Sachar EJ, Hellman L, Roffwarg HP, Halpern FS, Fukushima DK, Gallagher TF. Disrupted 24-hour patterns of cortisol secretion in psychotic depression. *Arch Gen Psychiatry*. 1973;28(1):19-24.
7. Stokes PE, Stoll PM, Koslow SH, Maas JW, Davis JM, Swann AC, Robins E. Pretreatment DST and hypothalamic-pituitary-adrenocortical function in depressed patients and comparison groups: a multicenter study. *Arch Gen Psychiatry*. 1984;41(3):257-267.
8. Knol MJ, Twisk JW, Beekman AT, Heine RJ, Snoek FJ, Pouwer F. Depression as a risk factor for the onset of type 2 diabetes mellitus: a meta-analysis. *Diabetologia*. 2006;49(5):837-845.
9. Penninx BW, Beekman AT, Honig A, Deeg DJ, Schoevers RA, van Eijk JT, van Tilburg W. Depression and cardiac mortality: results from a community-based longitudinal study. *Arch Gen Psychiatry*. 2001;58(3):221-227.
10. Schulz P, Kirschbaum C, Pruessner JC, Hellhammer D. Increased free cortisol secretion after awakening in chronically stressed individuals due to work overload. *Stress Med*. 1998;14(2):91-97.
11. Kirschbaum C, Hellhammer DH. Salivary cortisol in psychoneuroendocrine research: recent developments and applications. *Psychoneuroendocrinology*. 1994; 19(4):313-333.
12. Carroll BJ, Feinberg M, Greden JF, Tarika J, Albala AA, Haskett RF, James NM, Kronfol Z, Lohr N, Steiner M, de Vigne JP, Young E. A specific laboratory test for the diagnosis of melancholia: standardization, validation, and clinical utility. *Arch Gen Psychiatry*. 1981;38(1):15-22.
13. Bhagwagar Z, Hafizi S, Cowen PJ. Increased salivary cortisol after waking in depression. *Psychopharmacology (Berl)*. 2005;182(1):54-57.
14. 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*. 2003;65(1):92-99.
15. Huber TJ, Issa K, Schik G, Wolf OT. The cortisol awakening response is blunted in psychotherapy inpatients suffering from depression. *Psychoneuroendocrinology*. 2006;31(7):900-904.
16. Stetler C, Miller GE. Blunted cortisol response to awakening in mild to moderate depression: regulatory influences of sleep patterns and social contacts. *J Abnorm Psychol*. 2005;114(4):697-705.
17. Brouwer JP, Appelhof BC, Hoogendijk WJ, Huyser J, Endert E, Zuketto C, Schene AH, Tijssen JG, Van Dyck R, Wiersinga WM, Fliers E. Thyroid and adrenal axis in major depression: a controlled study in outpatients. *Eur J Endocrinol*. 2005; 152(2):185-191.
18. Oldehinkel AJ, van den Berg MD, Flentge F, Bouhuys AL, ter Horst GJ, Ormel J. Urinary free cortisol excretion in elderly persons with minor and major depression. *Psychiatry Res*. 2001;104(1):39-47.
19. Kalogeras KT, Calogero AE, Kuribayiashi T, Khan I, Gallucci WT, Kling MA, Chrousos GP, Gold PW. In vitro and in vivo effects of the triazolobenzodiazepine alprazolam on hypothalamic-pituitary-adrenal function: pharmacological and clinical implications. *J Clin Endocrinol Metab*. 1990;70(5):1462-1471.
20. Michelson D, Galliven E, Hill L, Demitrack M, Chrousos G, Gold P. Chronic imipramine is associated with diminished hypothalamic-pituitary-adrenal axis responsivity in healthy humans. *J Clin Endocrinol Metab*. 1997;82(8):2601-2606.
21. Schule C, Baghai T, Rackwitz C, Laakmann G. Influence of mirtazapine on urinary free cortisol excretion in depressed patients. *Psychiatry Res*. 2003;120 (3):257-264.
22. 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(2):113-120.
23. Portella MJ, Harmer CJ, Flint J, Cowen P, Goodwin GM. Enhanced early morning salivary cortisol in neuroticism. *Am J Psychiatry*. 2005;162(4):807-809.
24. Sher L. Daily hassles, cortisol, and the pathogenesis of depression. *Med Hypotheses*. 2004;62(2):198-202.
25. Maes M, Calabrese J, Meltzer HY. The relevance of the in- vs outpatient status for studies on HPA-axis in depression: spontaneous hypercortisolism is a feature of major depressed inpatients and not of major depression per se. *Prog Neuropsychopharmacol Biol Psychiatry*. 1994;18(3):503-517.
26. Nelson JC, Davis JM. DST studies in psychotic depression: a meta-analysis. *Am J Psychiatry*. 1997;154(11):1497-1503.
27. Strickland P, Morriss R, Wearden A, Deakin B. A comparison of salivary cortisol in chronic fatigue syndrome, community depression and healthy controls. *J Affect Disord*. 1998;47(1-3):191-194.
28. Penninx BW, Beekman AT, Smit JH, Zitman FG, Nolen WA, Spinhoven P, Cuijpers P, De Jong PJ, Van Marwijk HW, Assendelft WJ, Van Der Meer K, Verhaak P, Wensing M, De Graaf R, Hoogendijk WJ, Ormel J, Van Dyck R; NESDA Research Consortium. The Netherlands Study of Depression and Anxiety (NESDA): rationale, objectives and methods. *Int J Methods Psychiatr Res*. 2008;17(3):121-140.
29. van Aken MO, Romijn JA, Miltenburg JA, Lentjes EG. Automated measurement of salivary cortisol. *Clin Chem*. 2003;49(8):1408-1409.
30. Pruessner JC, Kirschbaum C, Meinlschmid G, Hellhammer DH. Two formulas for computation of the area under the curve represent measures of total hormone concentration vs time-dependent change. *Psychoneuroendocrinology*. 2003; 28(7):916-931.
31. Edwards S, Clow A, Evans P, Hucklebridge F. Exploration of the awakening cortisol response in relation to diurnal cortisol secretory activity. *Life Sci*. 2001; 68(18):2093-2103.
32. Fekedulegn DB, Andrew ME, Burchfiel CM, Violanti JM, Hartley TA, Charles LE, Miller DB. Area under the curve and other summary indicators of repeated waking cortisol measurements. *Psychosom Med*. 2007;69(7):651-659.
33. Schmidt-Reinwald A, Pruessner JC, Hellhammer DH, Federenko I, Rohleder N, Schürmeyer TH, Kirschbaum C. The cortisol response to awakening in relation to different challenge tests and a 12-hour cortisol rhythm. *Life Sci*. 1999;64 (18):1653-1660.
34. Craig CL, Marshall AL, Sjostrom M, Bauman AE, Booth ML, Ainsworth BE, Pratt M, Ekelund U, Yngve A, Sallis JF, Oja P. International physical activity questionnaire: 12-country reliability and validity. *Med Sci Sports Exerc*. 2003;35(8):1381-1395.
35. Lyketsos CG, Nestadt G, Cwi J, Heithoff K, Eaton WW. The Life Chart Interview: a standardized method to describe the course of psychopathology. *Int J Methods Psychiatr Res*. 1994;(4):143-155.
36. Beck AT, Kovacs M, Weissman A. Assessment of suicidal intention: the Scale for Suicide Ideation. *J Consult Clin Psychol*. 1979;47(2):343-352.
37. Rush AJ, Gullion CM, Basco MR, Jarrett RB, Trivedi MH. The Inventory of Depressive Symptomatology (IDS): psychometric properties. *Psychol Med*. 1996; 26(3):477-486.
38. Novick JS, Stewart JW, Wisniewski SR, Cook IA, Manev R, Nierenberg AA, Rosenbaum JF, Shores-Wilson K, Balasubramani GK, Biggs MM, Zisook S, Rush AJ; STAR*D Investigators. Clinical and demographic features of atypical depression in outpatients with major depressive disorder: preliminary findings from STAR*D. *J Clin Psychiatry*. 2005;66(8):1002-1011.
39. World Health Organization Collaboration Centre for Drug Statistics Methodology. *Anatomical Therapeutic Chemical (ATC) Classification System*. Oslo, Norway: World Health Organization Collaboration Centre for Drug Statistics Methodology; 2007.
40. Costa PT Jr, McCrae RR. Domains and facets: hierarchical personality assessment using the revised NEO personality inventory. *J Pers Assess*. 1995;64(1):21-50.
41. Kanner AD, Coyne JC, Schaefer C, Lazarus RS. Comparison of two modes of stress measurement: daily hassles and uplifts vs major life events. *J Behav Med*. 1981; 4(1):1-39.

42. de Graaf R, Bijl RV, Ten Have M, Beekman AT, Vollebergh WA. Pathways to comorbidity: the transition of pure mood, anxiety and substance use disorders into comorbid conditions in a longitudinal population-based study. *J Affect Disord*. 2004;82(3):461-467.
43. Gueorguieva R, Krystal JH. Move over ANOVA: progress in analyzing repeated-measures data and its reflection in papers published in the *Archives of General Psychiatry*. *Arch Gen Psychiatry*. 2004;61(3):310-317.
44. Cohen J. *Statistical Power Analysis of the Behavioral Sciences*. Hillsdale, NJ: Lawrence Erlbaum Associates; 1988.
45. Rush AJ, Trivedi MH, Ibrahim HM, Carmody TJ, Arnow B, Klein DN, Markowitz JC, Ninan PT, Kornstein S, Manber R, Thase ME, Kocsis JH, Keller MB. The 16-item quick inventory of depressive symptomatology (QIDS), clinician rating (QIDS-C), and self-report (QIDS-SR): a psychometric evaluation in patients with chronic major depression [published correction appears in *Biol Psychiatry*. 2003;54(5):585]. *Biol Psychiatry*. 2003;54(5):573-583.
46. Carroll BJ, Cassidy F, Naftolowitz D, Tatham NE, Wilson WH, Iranmanesh A, Liu PY, Veldhuis JD. Pathophysiology of hypercortisolism in depression. *Acta Psychiatr Scand Suppl*. 2007;(433):90-103.
47. De Kloet ER. Hormones and the stressed brain. *Ann N Y Acad Sci*. 2004;1018:1-15.
48. Bhagwagar Z, Hafizi S, Cowen PJ. Increase in concentration of waking salivary cortisol in recovered patients with depression. *Am J Psychiatry*. 2003;160(10):1890-1891.
49. Mannie ZN, Harmer CJ, Cowen PJ. Increased waking salivary cortisol levels in young people at familial risk of depression. *Am J Psychiatry*. 2007;164(4):617-621.
50. Wichers MC, Myin-Germeys I, Jacobs N, Kenis G, Derom C, Vlietinck R, Delespaul P, Mengelers R, Peeters F, Nicolson N, Van Os J. Susceptibility to depression expressed as alterations in cortisol day curve: a cross-twin, cross-trait study. *Psychosom Med*. 2008;70(3):314-318.
51. Marques-Deak AH, Neto FL, Dominguez WV, Solis AC, Kurcgant D, Sato F, Ross JM, Prado EB. Cytokine profiles in women with different subtypes of major depressive disorder. *J Psychiatr Res*. 2007;41(1-2):152-159.
52. Watson S, Gallagher P, Del-Estal D, Hearn A, Ferrier IN, Young AH. Hypothalamic-pituitary-adrenal axis function in patients with chronic depression. *Psychol Med*. 2002;32(6):1021-1028.
53. Young EA, Haskett RF, Grunhaus L, Pande A, Weinberg VM, Watson SJ, Akil H. Increased evening activation of the hypothalamic-pituitary-adrenal axis in depressed patients. *Arch Gen Psychiatry*. 1994;51(9):701-707.
54. Peeters F, Nicholson NA, Berkhof J. Cortisol responses to daily events in major depressive disorder. *Psychosom Med*. 2003;65(5):836-841.
55. Daban C, Vieta E, Mackin P, Young AH. Hypothalamic-pituitary-adrenal axis and bipolar disorder. *Psychiatr Clin North Am*. 2005;28(2):469-480.
56. Schatzberg AF, Rothschild AJ. The roles of glucocorticoid and dopaminergic systems in delusional (psychotic) depression. *Ann N Y Acad Sci*. 1988;537:462-471.
57. Watson S, Gallagher P, Smith MS, Ferrier IN, Young AH. The dex/CRH test: is it better than the DST? *Psychoneuroendocrinology*. 2006;31(7):889-894.
58. Burke HM, Davis MC, Otte C, Mohr DC. Depression and cortisol responses to psychological stress: a meta-analysis. *Psychoneuroendocrinology*. 2005;30(9):846-856.
59. de Kloet ER, DeRijk RH, Meijer OC. Therapy Insight: is there an imbalanced response of mineralocorticoid and glucocorticoid receptors in depression? *Nat Clin Pract Endocrinol Metab*. 2007;3(2):168-179.
60. Dockray S, Bhattacharyya MR, Molloy GJ, Steptoe A. The cortisol awakening response in relation to objective and subjective measures of waking in the morning. *Psychoneuroendocrinology*. 2008;33(1):77-82.
61. Weijtens O, van der Sluijs FA, Schoemaker RC, Lentjes EG, Cohen AF, Romijn FP, van Meurs JC. Peribulbar corticosteroid injection: vitreal and serum concentrations after dexamethasone disodium phosphate injection. *Am J Ophthalmol*. 1997;123(3):358-363.
62. Appelhof BC, Huyser J, Verweij M, Brouwer JP, van Dyck R, Fliers E, Hoogendijk WJ, Tijssen JG, Wiersinga WM, Schene AH. Glucocorticoids and relapse of major depression (dexamethasone/corticotropin-releasing hormone test in relation to relapse of major depression). *Biol Psychiatry*. 2006;59(8):696-701.
63. Dekker MJ, Koper JW, van Aken MO, Pols HA, Hofman A, de Jong FH, Kirschbaum C, Witteman JC, Lamberts SW, Tiemeier H. Salivary cortisol is related to atherosclerosis of carotid arteries. *J Clin Endocrinol Metab*. 2008;93(10):3741-3747.
64. Eller NH, Netterstrom B, Allerup P. Progression in intima media thickness: the significance of hormonal biomarkers of chronic stress. *Psychoneuroendocrinology*. 2005;30(8):715-723.