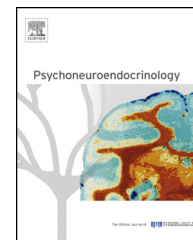


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Salivary cortisol levels and the 2-year course of depressive and anxiety disorders

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Summary

Introduction: Depression and anxiety disorders have been associated with hyperactivity of the hypothalamic-pituitary adrenal (HPA) axis. However, lower cortisol levels have also been observed in depressed patients. Whether cortisol level predicts the course of these disorders has not been examined in detail. We examined whether salivary cortisol indicators predict the 2-year course of depression and anxiety disorders.

Methods: Longitudinal data are obtained from 837 participants of the Netherlands Study of Depression and Anxiety, with a DSM-IV based depressive and/or anxiety disorder at baseline. At baseline, seven saliva samples were obtained, including the 1-h cortisol awakening response, evening cortisol level and a 0.5 mg dexamethasone suppression test. At follow-up, DSM-IV based diagnostic interviews and Life Chart Interview integrating diagnostic and symptom trajectories over 2 years were administered to determine an unfavorable course.

Results: 41.5% of the respondents had a 2-year unfavorable course trajectory without remission longer than 3 months. Adjusted analyses showed that a lower awakening response was associated with an unfavorable course (RR = 0.83, $p = 0.03$). No associations were found between evening cortisol or cortisol suppression after dexamethasone ingestion and an unfavorable course trajectory.

Conclusions: Among patients with depressive or anxiety disorders, a lower cortisol awakening response – which may be indicative of underlying exhaustion of the HPA axis – predicted an unfavorable course trajectory.

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1. Introduction

Depressive and anxiety disorders are prevalent and disabling disorders. The burden is partly due to their course, often chronic or recurrent. In addition, comorbidity of depression and anxiety disorder frequently occurs (Kessler et al., 1996) and is related to an even poorer outcome (Merikangas et al., 2003; Penninx et al., 2011). However, little is known about predictors of the course of these disorders, while such knowledge would greatly improve our understanding of these diseases or could lead to identification of risk groups. Hypothalamic-pituitary-adrenal (HPA) axis dysregulation is believed to play an important role in the pathophysiology of depressive and – to a lesser extent – anxiety disorders. Mostly, hyperactivity of the HPA axis has been observed, as reflected by elevated cortisol levels, in addition to less suppression of cortisol after a dexamethasone suppression test (DST) or Dexamethasone/Corticotropin Releasing Hormone (Dex/CRH) test for depression (Modell et al., 1997; Bhagwagar et al., 2005; Vreeburg et al., 2009a) or panic disorder (Abelson et al., 2007). However, there also appears to be a group of patients showing hypocortisolism (Oldehinkel et al., 2001; Bremmer et al., 2007; Penninx et al., 2007), possibly a sign of exhaustion of the HPA axis, after chronic and recurrent depressive episodes (Oldehinkel et al., 2001; Bremmer et al., 2007). Salivary cortisol measures are increasingly used, since they reflect unbound, active cortisol, and their collection is minimally intrusive (Kirschbaum and Hellhammer, 1989). The cortisol awakening response reflects the natural response of the HPA axis on awakening (Fries et al., 2009), which represents a distinct feature of the HPA axis and has received increasing interest as a promising biomarker in the previous years (Clow et al., 2010). Evening levels reflect basal activity, and the DST provides information on the negative feedback system (Carroll et al., 1981). Using these measures, we observed that persons with a remitted or current Major Depressive Disorder (MDD) showed a higher cortisol awakening curve (Vreeburg et al., 2009a), as did persons with a current Panic Disorder with agoraphobia (Vreeburg et al., 2010b). Persons with other anxiety disorders e.g. social phobia, generalized anxiety disorder and panic disorder without agoraphobia did not show different cortisol levels as compared to controls.

Although cross-sectional associations have been established, it remains largely unclear whether HPA-axis dysregulation predicts the course of depression and anxiety disorders. Longitudinal studies investigating such temporal associations are important, since they learn us more about the underlying interaction of HPA-axis dysregulation and depression and anxiety disorders. In addition, they provide insights into the clinical implications of HPA-axis dysregulation as they may point out whether hypo- and/or hyperactivity of the HPA-axis does identify patients with a more unfavorable course trajectory e.g. due to HPA-axis dysregulation negatively impacting mood recovery processes or limiting treatment efficacy. There is some evidence that increased cortisol responses to the Dex/CRH test or DST predict relapse in remitted outpatients with depressive disorder (Ribeiro et al., 1993; Appelhof et al., 2006; Aubry et al., 2007; Pintor et al., 2009), panic disorder (Coryell et al., 1989), or in depressed inpatients (Zobel et al., 1999; Ising et al.,

2007). Baseline salivary cortisol, cortisol responses on the DST or Dex/CRH test, however, were not related to the treatment response or outcome of depression or panic disorder (Coryell and Noyes, 1988; Ribeiro et al., 1993; Hatzinger et al., 2002; Schule et al., 2003; Brouwer et al., 2006; Papakostas et al., 2010). However, for panic disorder, abnormal DST results (Coryell et al., 1991) or elevated 24-h cortisol levels (Abelson and Curtis, 1996) were also associated with more anxiety, phobias and disability 2–4 years later.

Overall, the above described studies do provide some inconsistent indications that HPA-axis activity may predict the course trajectory of depression and anxiety disorders. Several of these studies were rather small-scaled and not always checked whether the predicting value of HPA-axis indicators is due to or independent of correlated clinical characteristics, such as baseline symptom severity. Large-scale longitudinal studies examining the role of HPA-axis indicators in the naturalistic course trajectory of patients with a current depressive or anxiety disorder remain to be conducted. This study examines whether various salivary cortisol measures (cortisol awakening response, evening level and suppression after dexamethasone ingestion) predict the 2-year course trajectory in 837 subjects with baseline depression or anxiety disorders, correcting for detailed demographic and clinical covariates.

2. Methods

2.1. Study sample

Data are from the Netherlands Study of Depression and Anxiety (NESDA), a large cohort study on the course of depressive and anxiety disorders among 2981 adults (18–65 years). Respondents were recruited from the community, in primary care through a screening procedure conducted among 65 general practitioners, and in specialized mental health care when newly enrolled at one of the 17 participating mental health organization locations. The overall study sample included persons with psychopathology as well as controls without a psychiatric diagnosis. General exclusion criteria – determined through physicians' records as well as through screening questions to the respondents during the screening phone interview – were: a primary diagnosis of psychotic disorder, obsessive compulsive disorder, bipolar disorder, or severe addiction disorder and not being fluent in Dutch. For objectives and methods of NESDA see Penninx et al. (2008) The research protocol was approved by the Ethical Committee of participating universities and all respondents provided written informed consent.

Baseline data were obtained from September 2004 to February 2007. After 2 years (October 2006 to April 2009), a face-to-face follow-up assessment was conducted with a response of 87.1% (2596 of the 2981 respondents participated). Non-response was significantly higher among those with younger age, lower educational level, non-North European ancestry and those with major depressive disorder, but was not associated with sex or the presence of anxiety disorder (Lamers et al., 2012).

The presence of depressive (Major Depressive Disorder, Dysthymia) or anxiety (Panic Disorder, Social Phobia, Generalized Anxiety Disorder, Agoraphobia) disorders was

established using the Composite Interview Diagnostic Instrument (CIDI) according to DSM-IV criteria (American Psychiatric Association, 2001). For the present analysis, only subjects who were symptomatic in the month before baseline were included. Consequently, we restricted the sample to the 1456 subjects with a 6-month depressive or anxiety diagnosis who confirmed symptoms in the month prior to baseline at either the CIDI recency questions or the Life Chart Interview (see below). Of these 1456 subjects, 1185 (81.4%) participated in the 2-year follow-up assessment (median in-between time = 24 months) and had complete data on outcome indicators. We subsequently excluded a total of 7 pregnant or breastfeeding women and 77 participants on corticosteroids, leaving an initial sample of 1101 respondents.

Of these, 837 (76.0%) returned sufficient saliva samples to contribute to at least one of the saliva cortisol analyses (1-h awakening values, evening value or DST) and therefore constitute the study sample. These 837 persons did not significantly differ from the 264 respondents with missing cortisol indicators in sex, or course outcome, but were older (42.8 versus 38.7 years, $p < 0.001$), more educated (12.0 versus 11.3 years, $p = 0.005$) and differed in baseline psychiatric status (22.2% depression, 42.8% anxiety disorder and 35.0% comorbidity versus 21.2% depression, 34.8% anxiety disorder and 43.9% comorbidity, $p = 0.02$).

2.2. Course of depressive and anxiety disorders

As described elsewhere (Penninx et al., 2011), course of depressive and anxiety disorders was determined using two main sources of data collected during the 2-year follow-up assessment: (1) the CIDI interview and the (2) Life Chart Interview (LCI). The CIDI interview determined the presence of DSM-IV classified depressive and anxiety disorders during the time between baseline assessment and 2-year follow-up assessment. Organic exclusion rules were used in defining diagnoses, and hierarchy-free diagnoses were made to allow for research into comorbidity. For all persons with detected depressive or anxiety symptoms in the CIDI interview, the LCI was completed. This assessment uses a calendar method to determine life events during the 2-year follow-up period to refresh memory, and then assessed separately the presence of depressive and anxiety symptoms at each month during this period (Lyketsos et al., 1994). In addition, for each month with reported symptoms, the severity of symptoms was assessed ranging from no or minimal, mild, moderate, severe, or very severe symptoms. Symptoms on LCI were only considered to be present when at least of mild severity. Using both the CIDI and LCI data, distinction was made between those with *remission of symptoms* – defined as the occurrence of a time-point during follow-up at which no symptoms of the index disorder was reported for three consecutive months – versus those with a *chronic course* – defined as no remission during the 2-year follow-up. For instance, remission of an anxiety disorder was considered present when no anxiety symptoms at all existed for three consecutive months during follow-up, and likewise a comorbid condition was considered remitted when no symptoms at all of both anxiety or depression existed for three consecutive months. This three-month criterion is in line with that used before (Spijker et al., 2002) and was uniformly applied

across all disorders to allow comparison of outcomes. No distinction was made between remission and recovery because the data did not allow for such precision.

2.3. Salivary cortisol

As described in more detail elsewhere (Vreeburg et al., 2009b), respondents were instructed to collect saliva samples at home on a regular (working) day shortly after the interview at baseline. Instructions concerning saliva sampling prohibited eating, drinking tea or coffee or brushing teeth within 15 min before sampling. Furthermore, no dental work 24 h prior to sampling was allowed. Saliva samples were obtained using Salivettes (Sarstedt, Germany) at seven time points covering 1-h awakening cortisol levels, evening levels and a dexamethasone suppression test. 1-h awakening cortisol includes four sampling points; at awakening (T1) and 30 (T2), 45 (T3) and 60 (T4) minutes later. The two evening values were collected at 2200 h (T5) and 2300 h (T6). Dexamethasone suppression is measured by cortisol sampling the next morning at awakening (T7) after ingestion of 0.5 mg dexamethasone directly after the saliva sample at 2300 h (T6). Respondents were instructed to write down the exact sampling times and time of ingestion of dexamethasone, so that non-compliance could be detected. Samples were stored in refrigerators and returned by mail. After receipt, Salivettes were centrifuged at $2000 \times g$ for 10 min, aliquoted and stored at -80°C . Cortisol analysis was performed by competitive electrochemiluminescence immunoassay (E170 Roche, Switzerland). The functional detection limit was 2.0 nmol/l and the intra- and inter-assay variability coefficients in the measuring range were less than 10%. Data cleaning excluded values >2 SD above the mean.

One-hour awakening cortisol. We calculated the area under the curve with respect to the increase (AUC_i) and with respect to the ground (AUC_g) using Pruessner's formulas (Pruessner et al., 2003). The AUC_g is an estimate of the total cortisol secretion over the first hour after awakening, whereas the AUC_i is a measure of the dynamic of the cortisol awakening response (CAR), more related to the sensitivity of the system, emphasizing changes over time after awakening (Schmidt-Reinwald et al., 1999; Edwards et al., 2001; Fekedulegn et al., 2007). If samples were collected outside of a margin of five minutes around the time protocol, values were assigned missing. All persons for whom all four morning samples were available ($n = 706$) could be included in the AUC analyses. In addition, we conducted Linear Mixed Models (LMM) analyses (see Section 2.6) using all four morning saliva samples, which keeps original values on all four data points, can accommodate for incomplete cases, and takes correlation between repeated measurements into account (Gueorguieva and Krystal, 2004). All persons with at least two valid morning cortisol values ($n = 793$) could be included in LMM analyses, thereby reducing the effects of missing values and including more patients in the analyses.

Evening cortisol. Since cortisol levels at 22h00 and 23h00 were strongly correlated (Spearman's $\rho = 0.8$, $p < 0.01$), we used the mean of both cortisol levels. Data from 832 subjects were available for evening cortisol analyses.

Dexamethasone suppression test (DST). This test provides information on the negative feedback system of the HPA axis, since dexamethasone reduces cortisol level by acting on the

pituitary (Carroll et al., 1981). Of the 800 persons with T1 and T7, 768 (96.0%) subjects had taken the 0.5 mg dexamethasone (indicated by self-report) and were available for the DST analyses. We calculated a cortisol suppression ratio by dividing the cortisol value at T1 by the value at T7 the next morning.

2.4. Covariates

Sociodemographics (sex, age, years of education), sampling factors (awakening time and work status on the sampling day) and health indicators (smoking status (current versus no), cardiovascular disease) with effects on salivary cortisol variables in our study (Vreeburg et al., 2009b) and possible effects on the course of psychopathology were included as covariates. All covariates were assessed at the baseline measurement.

2.5. Clinical characteristics

Several clinical baseline characteristics were taken into account, because they showed to be predictive of subsequent 2-year course of depression and/or anxiety disorders in the NESDA study (Penninx et al., 2011). Severity of depressive symptoms was measured with the 30-item Inventory of Depressive Symptomatology (Rush et al., 1996). Severity of anxiety symptoms was measured using the 15-item Fear Questionnaire (Marks and Mathews, 1979). Information on duration of symptoms prior to baseline was derived from the Life Chart Interview (LCI) (Lyketsos et al., 1994) conducted at baseline, which assessed the percent of time the patient spent with depressive and/or anxiety symptoms in the 4 years prior to baseline. Age of onset of the index disorder was assessed in the CIDI interview, and earliest age was used for those with comorbid disorders. Finally, baseline disorder status was included (Anxiety disorder, Depression, Comorbid disorders). In the NESDA study, we did not observe that additional information on subtype of disorder (such as panic disorder versus social phobia versus generalized anxiety disorder, or dysthymia versus major depressive disorder) was not differentially associated with 2-year course outcomes, therefore, we did not include subtype as additional covariates (Penninx et al., 2011).

2.6. Statistical analyses

The associations between salivary cortisol indicators (AUC_g, AUC_i, evening cortisol and cortisol suppression ratio) and 2-year course outcome (unfavorable course versus remission) were analyzed using multiple logistic regression analyses. In addition, random coefficient analysis of the four morning cortisol levels was performed using LMM analyses comparing morning cortisol levels between persons with and without an unfavorable course. Two-year outcome (unfavorable course yes/no), time point (1, 2, 3, 4) and all 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 for persons with a unfavorable course versus remission, we added a group by time interaction term.

3. Results

Baseline characteristics are presented in Table 1. In our sample, 65.1% were women and the mean age at baseline was 42.8 years. 186 (22.2%) had a pure depressive disorder ('Dep'), 358 (42.8%) had a pure anxiety disorder ('Anx') and 293 (35.0%) had comorbid depressive and anxiety disorders ('Comorbid') at baseline. 347 (41.5%) developed an unfavorable course compared to 490 (58.5%) persons with remission during the 2 years of follow-up. 73.5% of respondents showed an increase in cortisol in the first hour after awakening, with a mean increase of 10.4 nmol/l (or 80.3%). Of the 837 persons, 157 (18.8%) had dysthymia, 512 (61.2%) had a major depressive disorder, 361 (43.1%) had social phobia, 224 (26.8%) had a panic disorder with agoraphobia, 115 (13.7%) had a panic disorder without agoraphobia, 104 (12.4%) had agoraphobia without a panic disorder, and 222 (26.5%) had a generalized anxiety disorder CIDI diagnosis in the past 6 months (not shown in Table) (Table 2).

Fully adjusted results illustrated that a lower AUC_g as well as a lower AUC_i were associated with the risk of an unfavorable course (RR = 0.85, $p = 0.06$ and RR = 0.83, $p = 0.03$, respectively), as confirmed by LMM results (direct effect: $F = 1.88$, $p = 0.17$, interaction with time: $F = 3.48$, $p = 0.02$, Fig. 1). Evening cortisol and cortisol suppression after

Table 1 Baseline characteristics.

	N = 837
Sociodemographics	
% female	65.1
Age (mean in years, SD)	42.8 (12.3)
Educational level (mean in years, SD)	12.0 (3.3)
Health indicators	
% smoking	37.5
% cardiovascular disease	6.6
Baseline diagnosis	
% Depression only	22.2
% Anxiety disorder only	42.8
% Comorbid disorder	35.0
Age of onset (mean, SD)	21.1 (12.7)
Symptom duration (mean %, SD)	57.2 (35.7)
Depression severity (mean IDS, SD)	29.4 (11.8)
Fear score (mean, SD)	33.3 (19.7)
Sampling factors	
Awakening time (mean, SD)	7h32 (1h15)
% working on sampling day	58.7
Salivary cortisol levels	
<i>One-hour awakening cortisol^a:</i>	
AUC _g (mean in nmol/l/h, SD)	19.4 (7.2)
AUC _i (mean in nmol/l/h, SD)	2.7 (6.5)
Mean evening level (mean in nmol/l, SD)	5.4 (3.3)
<i>Dexamethasone suppression test^a:</i>	
Cortisol suppression ratio ^b (mean, SD)	2.8 (1.7)

Abbreviations: SD, standard deviation; IDS, inventory of depressive symptoms; AUC_g/i, area under the curve with respect to the ground/increase.

^a $N = 706$ for AUCs, $N = 832$ for evening cortisol and $N = 768$ for DST.

^b Cortisol suppression ratio = cortisol T1/cortisol T7.

Table 2 The adjusted risk of having a 2-year chronic course across various salivary cortisol indicators.

	2-Year chronic course			
	RR (95% CI) ^a	<i>p</i>	RR (95% CI) ^b	<i>p</i>
AUC _g (nmol/l/h) ^c	0.87 (0.74–1.02)	0.09	0.85 (0.71–1.01)	0.06
AUC _i (nmol/l/h) ^c	0.89 (0.76–1.05)	0.16	0.83 (0.70–0.98)	0.03
Evening cortisol (nmol/l) ^c	1.06 (0.91–1.23)	0.47	1.05 (0.89–1.24)	0.56
Cortisol suppression ratio ^c	0.97 (0.84–1.12)	0.68	0.97 (0.83–1.14)	0.71

Abbreviations: RR, relative risk; CI, confidence interval; AUC_g/i, area under the curve with respect to the ground/increase.

^a Adjusted for sex, age, education, smoking, working, awakening time and cardiovascular disease.

^b Additionally adjusted for IDS score, fear score, age of onset, symptom duration and type of baseline disorder (Dep, Anx, Comorbid).

^c Relative risks for continuous measures are given per SD increase. AUC_g: SD = 7.2; AUC_i: SD = 6.5; evening cortisol: SD = 3.3, cortisol suppression ratio: SD = 1.7.

dexamethasone ingestion were not related to a 2-year unfavorable course. When we tested for quadratic relations, no *p*-values below 0.10 were obtained for all the quadratic terms, indicating that no additional non-linear associations could be confirmed.

To further graphically explore the relationship between the AUC_g and AUC_i with an unfavorable course, we created quintiles of these cortisol measures. Fig. 2 depicts the relationship between the cortisol awakening curve and the risk of unfavorable course across quintiles. Where quintiles of the AUC_g were not significantly associated with the risk of unfavorable course, the lowest quintile of the AUC_i was associated with an increased risk of unfavorable course as compared to the middle quintile (Fig. 2). The quintiles did not appear to be consistently associated with risk of unfavorable course, the risk was only increased for the lowest quintile of AUC_i (lowest quintile versus all higher quintiles together: RR = 1.90, 95% CI = 1.24–2.90, *p* = 0.003).

Additional analyses were conducted on the association of AUC_i and unfavorable course to examine whether the found association was consistent across disorders. We tested the presence of an interaction between AUC_i and baseline disorder status (Anx, Dep, Comorbid), but this was not significant (*p* > 0.6), suggesting that the finding appears to be

robust across baseline disorder groups. Although the receipt of medication treatment at baseline was not associated with course outcome in multivariable analyses (Penninx et al., 2011) and only tricyclic antidepressant (TCA) use was associated with baseline CAR levels (Vreeburg et al., 2009a), we conducted additional analyses in which we adjusted for use of TCAs, selective serotonin reuptake inhibitors and other antidepressants. These analyses indeed yielded similar results.

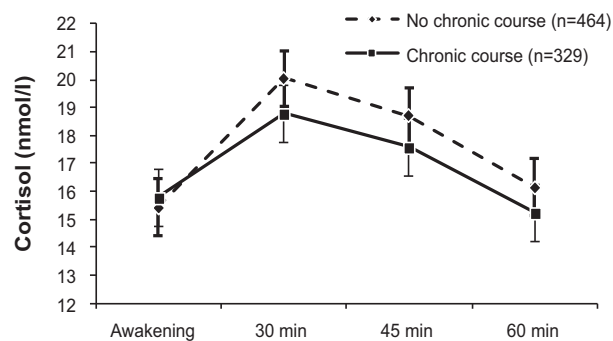


Figure 1 Baseline 1-h cortisol awakening levels for persons with and without a chronic course after two years. Analyses are adjusted for sex, age, education, smoking, working, awakening time, cardiovascular disease, IDS score, Fear score, age of onset, symptom duration and type of baseline disorder (Dep, Anx, Comorbid). LMM results: direct effect: $F = 1.88$, $p = 0.17$, interaction with time: $F = 3.48$, $p = 0.02$. Per time-point: Awakening: $p = 0.48$, 30 min: $p = 0.07$, 45 min: $p = 0.08$ and 60 min: $p = 0.11$.

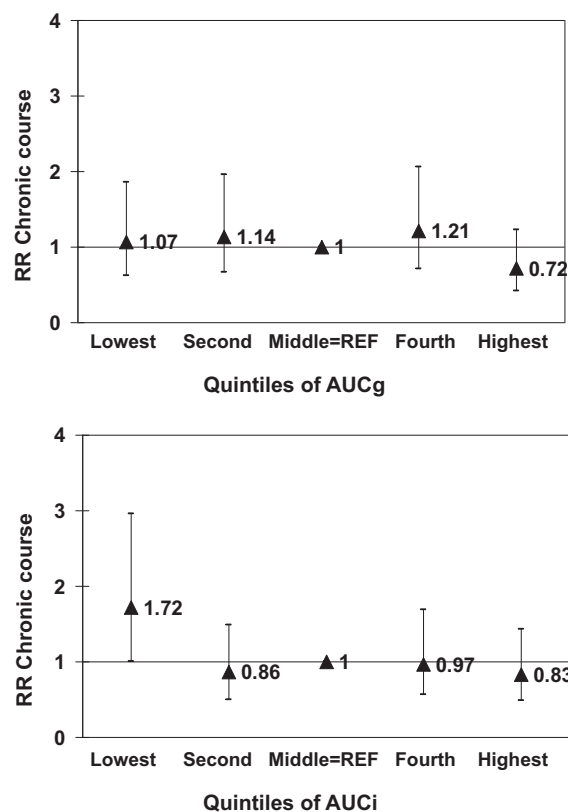


Figure 2 Quintiles of the cortisol awakening response (AUC_g and AUC_i) and risk of chronic course. Analyses are adjusted for sex, age, education, smoking, working, awakening time, cardiovascular disease, IDS score, Fear score, age of onset, symptom duration and type of baseline disorder (Dep, Anx, Comorbid). *P*-values compared to the middle quintile: AUC_g: $p = 0.81$, $p = 0.64$, $p = 0.48$ and $p = 0.22$, respectively, AUC_i: $p = 0.05$, $p = 0.58$, $p = 0.92$ and $p = 0.50$, respectively.

We have described that the use of benzodiazepines (Manthey et al., 2010) and childhood trauma exposure (Holleman et al., 2012) in our study were not associated with HPA-axis indicators. Consequently, we did not further conduct analyses with these additional covariates.

4. Discussion

The present study is the first to examine extensive salivary cortisol measures and 2-year course of depressive and anxiety disorders. Results indicate that a lower cortisol awakening response is associated with an increased risk of an unfavorable, chronic course trajectory of depression and/or anxiety disorders over 2 years. Evening cortisol and cortisol suppression after dexamethasone ingestion were not associated with 2-year course of depression and anxiety.

Most prior studies reported no relationship between baseline HPA-axis indicators and treatment response or outcome among patients with depression or panic disorder (Coryell and Noyes, 1988; Ribeiro et al., 1993; Hatzinger et al., 2002; Brouwer et al., 2006; Papakostas et al., 2010). However, none of these studies, examining HPA-axis activity as predictor for course outcome, included the CAR. We observed that a lower CAR was associated with the risk of a chronic course, independent of important covariates including important clinical course predictors. The CAR showed to be unrelated to diurnal cortisol rhythm and to be under genetic control, which is in contrast to the other diurnal cortisol measures (Wust et al., 2000; Edwards et al., 2001). The CAR is more than an index of the HPA axis, since it is probably closely linked to the supra-chiasmatic nucleus and there is evidence that the CAR can vary in response to the anticipated demands of the upcoming day in order to meet those demands, both physically and mentally (Clow et al., 2010). There is increasing interest over the years in the CAR as a promising biomarker because it represents a distinct feature of the HPA axis and because it is easily collected. An attenuated as well as an increased CAR have been associated with adverse psychosocial factors and psychopathology (e.g. Chida and Steptoe, 2009).

How can we explain our finding of a lower CAR to be predictive of an unfavorable 2-year course? A low CAR is potentially indicative of hypocortisolism. It has been hypothesized that after long periods of (psychological or physical) stress, the HPA axis becomes less responsive through down-regulation of the receptors, resulting in low cortisol levels (Heim et al., 2000; Fries et al., 2005). Results supporting this theory come from studies in depressed older persons where hypocortisolism in depression showed to be associated with chronic and recurrent depressive episodes (Bremner et al., 2007) and physical frailty (Penninx et al., 2007). In addition, Miller et al. (2007) conducted a meta-analysis on chronic stress and HPA-axis activity and reported that morning cortisol is especially lower when time has passed since the occurrence of the stressor and the stressor is no longer present, and when the stressor concerns an uncontrollable threat to the physical self, or traumatic stressor. However, these results were mainly based on cross-sectional studies examining one morning cortisol sample. Therefore, longitudinal studies are warranted to examine the temporal associations between chronic stress and HPA-axis activity, including the CAR.

Down-regulation after chronic stress could also be reflected in the CAR, since several studies have reported a relationship between chronic or severe stress exposure and a blunted CAR (Meinlschmidt and Heim, 2005; O'Connor et al., 2009). Although the predictive effect of the CAR for the course of depressive and anxiety disorders in our sample was independent of symptom duration at baseline and childhood trauma, chronic stress exposure could be of importance, since we did not actually assess chronic stress and only captured symptoms of the few years before baseline and not lifetime exposure.

A possible mechanism for hypocortisolism is down-regulation of corticotrophin-releasing-factor (CRH) receptors in the pituitary, following a longer period of stress-induced hypothalamic CRH secretion, resulting in lower adrenocorticotrophic hormone (ACTH) and reduced cortisol levels (Heim et al., 2000). Alternatively, reduced biosynthesis or depletion of CRH, ACTH and/or cortisol or increased sensitivity of the HPA-axis to negative feedback (Heim et al., 2000; Fries et al., 2005) could play a role in hypocortisolism. This latter mechanism might be less important in explaining our findings, since we did not observe an association between the DST and an unfavorable course.

However, it is unclear whether these mechanisms also apply to the CAR, since this is a distinct feature of HPA-axis activity. It has been suggested that the CAR is under regulatory control of the hippocampus (Fries et al., 2009). Although the hippocampus has an inhibitory effect on HPA-axis activity in general, the integrity of the hippocampus appears to be necessary for the CAR (Buchanan et al., 2004; Pruessner et al., 2007; Fries et al., 2009). A reduced hippocampus found especially among the chronically depressed (McKinnon et al., 2009) – has been associated with a blunted CAR (Buchanan et al., 2004; Pruessner et al., 2007). Therefore, the hippocampus might play an important role in explaining our result. A reduced hippocampus may contribute to a chronic course through a permanent vulnerability, possibly mediated by lower cortisol levels. However, longitudinal studies are warranted to entangle the relationship between hippocampus, HPA-axis activity and the onset and course of depressive and anxiety disorders. Taken together, chronic stress exposure may have led to down-regulation of the receptors of the HPA-axis system and reduction of hippocampal volume, thereby resulting in a lower CAR. In addition, depression is associated with inactivity or a lack of interest and anxiety is associated with avoidance behavior. These characteristics may lead to patient's perceptions that there is no reason to get active after awakening, which may make the CAR superfluous. In support of this is that an increase of behavioral activity in depressed people leads to a decrease of symptoms (e.g. Lejuez et al., 2001) and affects hippocampal neurogenesis (Nithianantharajah and Hannan, 2009).

These underlying mechanisms could explain why a lower CAR was found to be associated with an unfavorable, chronic course trajectory. Another explanation for our findings could be that underlying chronic conditions are associated with a low CAR which further determines poor course trajectory. For instance, a blunted CAR has been observed in post-traumatic stress disorder (Wessa et al., 2006) and chronic fatigue syndrome (Roberts et al., 2004). Alternatively, these disorders may share a common pathophysiology with chronic depression.

We previously observed that a higher CAR was associated with the presence of MDD and panic disorder with agoraphobia (Vreeburg et al., 2009a,b). In contrast, among current patients it appeared to be a low CAR that was predictive of an unfavorable course over time. Possibly, a higher cortisol awakening curve is associated with the onset of depressive disorder and/or panic disorder with agoraphobia and a lower CAR with the unfavorable course of these disorders. The observations that a higher cortisol awakening curve is associated with parental history of depression or anxiety in unaffected offspring (Mannie et al., 2007; Vreeburg et al., 2010a) and with the incidence of depression in adolescents, (Adam et al., 2010) support the idea that a higher cortisol awakening curve is associated with the onset of depression. Possibly, there are two distinct pathways: (1) a (genetic) biological vulnerability of depressive or anxiety disorders via high (morning) cortisol levels; and (2) a 'scar' effect as a result of high allostatic load during life resulting in a lower CAR and chronicity of depression. Future longitudinal studies are warranted to entangle these pathways. Possibly, these different pathways can explain some of the inconsistencies reported in the previous literature on HPA-axis activity in depressive and anxiety disorders.

Most previous studies reported no relationship between basal HPA-axis indicators or the DST and outcome of depression (Coryell and Noyes, 1988; Ribeiro et al., 1993; Hatzinger et al., 2002; Brouwer et al., 2006; Papakostas et al., 2010). Although no studies specifically examined the development of an unfavorable course over 2 years, previous results appear to be in concordance with our negative findings with evening cortisol and the DST. Possibly, the diurnal rhythm and negative feedback system are not of importance in the development of an unfavorable course. However, since associations were present in e.g. panic disorder studies (Coryell et al., 1991; Abelson and Curtis, 1996), it could be that in specific subgroups these HPA-axis indicators are associated with course trajectory.

Our study had several strengths, including a large representative sample of outpatients who were longitudinally examined for 2 years. In addition, we obtained several salivary cortisol measures at baseline, and were able to take several important covariates as well as clinical characteristics into account. Also some limitations have to be acknowledged. The effect we found was similar across different types of baseline disorder (depression only, anxiety disorder only and comorbid disorders), since interaction terms were not significant. However, our subsamples of specific depressive or anxiety disorders, such as social phobia or GAD, were not sufficiently large to examine the role of salivary cortisol measures in their course. In our previous study, we observed that of the subtypes of anxiety disorders only panic disorder with agoraphobia was associated with higher cortisol values (Vreeburg et al., 2010b). However, in Penninx et al. (2011) we reported that subtype of anxiety disorder (panic versus social phobia versus generalized anxiety disorder) was not differentially associated with course trajectory. Therefore, it is unlikely that in our sample, results would have been very different if we would include subtypes as a specific covariate. Restricted analyses on specific subgroups is also very difficult given the large comorbidity of depression and anxiety disorders. Persons who provided saliva samples were older,

more educated and less likely to have anxiety disorders or comorbidity. The latter disorders had a higher risk of an unfavorable course than depression only (Penninx et al., 2011). Since we are looking at within-person associations (baseline levels correlated with longitudinal course outcome within a person), we do not believe a small sample selection can impact on our overall results. In addition, since we still have a substantial number of persons with comorbidity in our sample (35.0%), we do not believe that the generalizability of our results is very much affected. Non-compliance with the sampling instructions could have resulted in a measurement error and could have confounded our results. However, it should be noted that even when closely monitoring awakening, still at least 15% of all persons are not responding with a morning cortisol rise (Dockray et al., 2008). Finally, salivary cortisol samples were only measured on one day. Sampling on multiple days could have increased the reliability of the measurements (Hellhammer et al., 2007). However, the large sample size of our study may have (partly) compensated for this.

To conclude, this study is the first to longitudinally examine the relationship between (salivary) cortisol measures and an unfavorable course trajectory of depressive and/or anxiety disorders. Findings further add to the importance of the CAR in mental health, since we found a lower CAR among depression and anxiety patients to be predictive of an unfavorable course over two years.

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Conflict of interest

None declared.

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