Increased cortisol awakening response was associated with time to recurrence of major depressive disorder

Florian Hardeveld\textsuperscript{a,*}, Jan Spijker\textsuperscript{a,b,c}, Sophie A. Vreeburg\textsuperscript{d}, Ron De Graaf\textsuperscript{b}, Sanne M. Hendriks\textsuperscript{a}, Carmilla M.M. Licht\textsuperscript{d}, Willem A. Nolen\textsuperscript{e}, Brenda W.J.H. Penninx\textsuperscript{d}, Aartjan T.F. Beekman\textsuperscript{d}

\textsuperscript{a} Pro Persona, Institute for Mental Health Care, P.O. Box 70, 6710 RR Ede, The Netherlands
\textsuperscript{b} Netherlands Institute of Mental Health and Addiction, P.O. Box 725, 3500 AS Utrecht, The Netherlands
\textsuperscript{c} Behavioral Science Institute, Radboud University Nijmegen, P.O. Box 9104, 6500 HE Nijmegen, The Netherlands
\textsuperscript{d} Department of Psychiatry/EMGO Institute for Health and Care/Neuroscience Campus Amsterdam, VU University Medical Center Amsterdam, P.O. Box 7057, 1007 MB Amsterdam, The Netherlands
\textsuperscript{e} Department of Psychiatry, University Medical Center Groningen, University of Groningen, P.O. Box 72, 9700 AB Groningen, The Netherlands

Received 20 March 2014; received in revised form 28 July 2014; accepted 28 July 2014

KEYWORDS
HPA axis recurrence major depressive disorder

Summary
Introduction: Although HPA-axis activity has been studied extensively in relation to depression, there is no consensus whether HPA-axis parameters predicts major depressive disorder (MDD) recurrence. We investigated whether HPA-axis parameters (cortisol awakening response (CAR), the dexamethasone suppression test (DST) and evening cortisol) predict time to recurrence in remitted subjects with a history of MDD and whether childhood trauma and life events interact with HPA-axis parameters in increasing the risk for recurrence.

Method: Data were derived from 549 subjects with a lifetime diagnosis of MDD in remission for at least six months preceding the baseline assessment of the Netherlands Study of Depression and Anxiety (NESDA). Subjects were followed up with two interviews over the course of four years to assess recurrence. DSM-IV based diagnostic interviews were used to assess time to recurrence of MDD. Seven salivary cortisol samples collected at baseline with information on CAR, evening cortisol and the DST. Hazard ratios were calculated using Cox regression analysis, adjusted for covariates.

* Corresponding author at: Pro Persona, P.O. Box 70, 6710 RR Ede, The Netherlands. Tel.: +31 318433400; fax: +31 318433614.
E-mail address: f.hardeveld@propersona.nl (F. Hardeveld).

http://dx.doi.org/10.1016/j.psyneuen.2014.07.027
0306-4530/© 2014 Published by Elsevier Ltd.
1. Introduction

Major depressive disorder (MDD) is often a chronic or recurrent disorder (Judd, 1997) and is one of the most disabling disorders worldwide (World Health Organization, 2008). Prevention of recurrence is therefore an important goal in the management of major depression. To that end, further knowledge on pathogenic mechanisms underlying recurrence of major depression is needed. The hypothalamic pituitary adrenal (HPA) axis is one of the main neuroendocrine systems activated under stress. Hyperactivity of the HPA-axis among depressed patients is a rather consistent research finding (Stetler and Miller, 2011) and alterations of the HPA-axis generally normalizes after full remission of depressive symptoms (Holsboer, 2000; Kaestner et al., 2005; Aihara et al., 2007; Pariante, 2009; McKay and Zakzanis, 2010). However, inconsistent findings have been observed (Bhagwagar et al., 2003; Mannie et al., 2007; Vreeburg et al., 2009b; Lok et al., 2012). Hyperactivity of the HPA-axis often results in hypercortisolism, which is associated with the pathophysiological pathway leading to MDD known as the glucocorticoid cascade hypothesis (Holsboer, 2000). A number of alterations in the HPA-axis found in major depression indicate hyperactivity of the HPA-axis. These are (i) hypercortisolism, resulting in a high evening cortisol (Kirschbaum and Hellhammer, 1989), (ii) an impaired circadian rhythm in terms of cortisol secretion in the first hour after awakening as reflected by an elevated cortisol awakening response (CAR) (Pruessner et al., 1997; Clow et al., 2010), (iii) a reduced negative feedback response to a dexamethasone suppression test (DST) (Ribeiro et al., 1993) and (iv) increased release of adrenocorticotropic hormone (ACTH) and cortisol in response to corticotrophin-releasing hormone (CRH) after administration of 1.5 mg of dexamethasone, known as the combined dexamethasone/corticotrophin releasing hormone test (DEX/CRH test; Nemeroff, 1996; Holsboer, 2000).

Although HPA-axis activity has been studied extensively in relation to depression (Stetler and Miller, 2011), there is no consensus on whether HPA-axis parameters have predictive value for recurrence of MDD. HPA-axis alterations may represent an underlying active disease process in depression and may predict risk for recurrence. Although a number of studies have examined this issue (Ribeiro et al., 1993; Zobel et al., 1999; Harris et al., 2000; Zobel et al., 2001; Hatzinger et al., 2002; Appelhof et al., 2006; Aubry et al., 2007; Bhagwagar and Cowen, 2008; Pintor et al., 2009; Rao et al., 2010; Bockting et al., 2012; Vrshek-Schallhorn et al., 2013), different HPA-axis measurements were used. Of the above mentioned studies, one study found that higher evening cortisol levels predicted recurrence (Rao et al., 2010), a literature review suggested that non suppression on the DST was related to relapse/recurrence (Ribeiro et al., 1993), and five studies found an association between non-response on the DEX/CRH test and recurrence (Zobel et al., 1999, 2001; Hatzinger et al., 2002; Appelhof et al., 2006; Pintor et al., 2009). However, also inconsistent findings have been reported, e.g. a higher CAR predicted MDD recurrence in two studies (Harris et al., 2000; Vrshek-Schallhorn et al., 2013) whereas a lower CAR was found to do so in another study (Bockting et al., 2012).

It has also been suggested that early-life stress can induce persistent changes in the response of the HPA axis, which becomes especially visible when persons are exposed to psychosocial stressors in adulthood (Baes et al., 2012). A possible mechanism is reduction of glucocorticoid receptor function leading to a decrease in inhibitory feedback resulting in hypercortisolism. Rao et al. (2010) observed that the risk for recurrence was higher among those with elevated cortisol levels and recent life events. Therefore, when investigating the predictive value of HPA-axis parameters on recurrence of MDD, it is important to take a potential interaction effect of recent stressors and childhood trauma into account (Adam et al., 2010). However, the literature is inconsistent, e.g. two other studies did not find any interactions (Bockting et al., 2012; Vrshek-Schallhorn et al., 2013).

Since results are inconsistent and different HPA-axis parameters were measured, which makes previous studies difficult to compare, there is a need for further research. To our knowledge, large-scale prospective studies that examine different HPA-axis parameters simultaneously along with the interaction of HPA axis parameters with childhood trauma and life events are scarce and some of these studies only targeted adolescents (Rao et al., 2010; Vrshek-Schallhorn et al., 2013). We assessed whether HPA axis parameters predict recurrence in remitted adult MDD subjects and whether stress-related factors (childhood trauma, life events) interact with HPA-axis parameters in predicting recurrence. Since hyperactivity as well as hypo-activity have been found to be associated with recurrence of MDD, we will examine potential non-linear associations with recurrence.

2. Methods

2.1. Study sample

Data were drawn from the Netherlands Study of Depression and Anxiety (NESDA), an ongoing longitudinal cohort study on the long-term course of depressive and anxiety disorders in different health care settings and illness phases. For the present study, we used the baseline assessment and follow-up assessments at two and four years. The study protocol was approved by the Ethical Review Committee of the Academic Medical Center.
The Dutch cortisol preceding jects (mean depressive disorder, the fer line of psychiatric disorder, obsessive—compulsive disorder, bipolar disorder, severe addiction disorder, and those not fluent in Dutch were excluded.

For the present study, we selected subjects with a lifetime history of major depressive disorder who did not fulfill the criteria for major depressive disorder in the six months preceding the baseline assessment, as recommended in a previous study (Furukawa et al., 2008). An advantage of including people with remitted depression at baseline was that the measurement of life events, childhood trauma and cortisol levels was not influenced by the presence of a major depressive episode. This definition of MDD was based on the DSM-IV based Composite International Diagnostic Interview (CIDI), Lifetime Version 2.1 (World Health Organization Lifetime Version 2.1, 1997). 810 participants met the criteria for a remitted MDD diagnosis. We excluded pregnant and breastfeeding women (n = 6) as they may have altered cortisol levels. We did not take into account the timing of the menstrual cycle because in a previous study performed with NESDA data (Vreeburg et al., 2009a) this was shown not to be associated to cortisol levels. None of the subjects used corticosteroid derivatives. Furthermore, 34 subjects were excluded because their diagnosis was changed to bipolar disorder at follow-up, which puts them at a different recurrence risk level. Of the 770 subjects who met the criteria, 702 were re-interviewed at least once, either at the two- or four-year follow-up assessment. After attrition, the sample ‘at risk’ for recurrence of MDD consisted of 702 subjects. Of these, 549 (78.2%) subjects had usable saliva samples to contribute at least one of the saliva cortisol analyses and they constitute the present study sample.

These 549 subjects were older in comparison with the 261 subjects who were excluded from the present study sample (mean age 45.0 versus 40.5, p < 0.001), but they did not differ in sex, educational attainment or number of previous episodes of MDD.

2.2. Time to recurrence of MDD

Recurrence of MDD was assessed prospectively at two and four year follow-up using the CIDI, which measured the 1-month, 6-month, 1-year and 2-year prevalence of depression. To assess time to recurrence, the average within a certain period of prevalence was taken. Time from baseline to recurrence was calculated on the basis of this data. So, eight time intervals were made (12, 18, 21, 23.5, 36, 42, 45, 47.5 months). For example, if the respondent had a 1-month prevalence of MDD after two years, it was estimated that the time to recurrence from baseline was 23.5 months (24 – 0.5 months). This represents the time to the first new major depressive episode (MDE). If the respondent had multiple episodes during follow-up the time from baseline to the first recurrence was taken.

2.3. Cortisol assessments

Cortisol measurements have been described previously (Vreeburg et al., 2009a). In short, cortisol was measured through saliva sampling, reflecting the active unbound form of cortisol (Kirschbaum and Hellhammer, 1989). Subjects were instructed to collect saliva samples at home shortly after the interview. Saliva samples were obtained using cotton salivettes (Srastied, Germany) seven times during the day. The cortisol awakening response includes four sampling points; upon awakening (t1), and 30 (t2), 45 (t3) and 60 (t4) min thereafter. Morning cortisol values were collected at 22:00 h (t5) and 23:00 h (t6). Dexamethasone suppression was measured using cortisol sampling the next morning upon awakening (t7) after ingestion of 0.5 mg dexamethasone directly after the saliva sample of 23:00 h (t6). Samples were centrifuged at 2000 × g for 10 min, aliquoted and stored at –80 °C. The analysis of cortisol was performed using competitive eleetrochemiluminescence immunoassay (E170; Roche, Basel, Switzerland) as described by Van Aken et al. (2003). 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%. Assays were repeated if cortisol levels were very high (>80 nmol/l) or very low (<1 nmol/l). Data cleaning was performed by excluding cortisol values higher than two standard deviations (SD) from the mean (47 of 3813 values). Additionally for CAR analysis, morning cortisol samples were excluded which were collected more than 5 min before or after the right protocol time (132 of 2170 values).

Three cortisol indicators were calculated: the CAR, the evening cortisol level, and the DST. The CAR was assessed by calculating the area under the curve with reference to the increase (AUCI) and the ground/zero (AUCg) using Pruessner’s formulas (Pruessner et al., 2003). The AUCg measures total cortisol secretion during the first hour after awakening and estimates total body exposure to cortisol. The AUCI measures cortisol increase with respect to awakening and is therefore a measure of the dynamics of the CAR, related to the sensitivity of the system and change in cortisol exposure over time (Clow et al., 2010). As the two evening cortisol values are strongly correlated (Spearman’s rho = 0.71, p < 0.001), we used the mean of both cortisol levels as a measure of evening cortisol, which reflects basal activity. The DST provides information on the negative feedback system of the HPA-axis, since dexamethasone reduces cortisol levels by acting on the pituitary (Carroll et al., 1981). We calculated a cortisol suppression ratio by dividing the cortisol value at T1 by the value at T7 the following morning.
2.4. Covariates

Based on previous studies (Burcusa and Iacono, 2007; Vreeburg et al., 2009a; Hardeveld et al., 2010), various factors with the potential to predict MDD recurrence and salivary cortisol levels were assessed at baseline.

*Socio-demographic factors:* sex; age.

*Clinical factors:* number of previous episodes (MDEs), categorized into single versus recurrent episodes. Anxiety disorders (social phobia, panic disorder with/without agoraphobia, agoraphobia, and generalized anxiety disorder) in the six months preceding baseline assessment were deemed to constitute relevant comorbid disorders. Severity of residual symptoms of depression was measured with the inventory of depressive symptoms (IDS; Rush et al., 1996); because cortisol levels could be state dependent (Stetler and Miller, 2011), we adjusted for residual depressive symptoms. History of depression in first-degree family members was assessed using a family tree inventory (Fyer and Weissman, 1999), categorized into 'yes' and 'no'.

*Stress related factors:* negative life events over the past year were assessed with the Brugha questionnaire (Brugha et al., 1985) which included 12 specific events and one 'other' category asking about other serious negative life events (sum score ranging from 0 to 5). The number of life events in the past year was calculated. In order to examine the role of childhood trauma, a cumulative childhood index using the NEMESIS childhood trauma interview was constructed (De Graaf et al., 2004; Wiersma et al., 2009; Hovens et al., 2010). Participants were asked four questions regarding childhood experiences of emotional neglect, and emotional, physical and sexual abuse. A cumulative index was calculated as the sum of the number and frequency of the four types of abuse for each participant (sum score ranging from 0 to 8) in line with earlier studies (Wiersma et al., 2009; Hovens et al., 2010).

*Sampling factors associated with cortisol levels:* Vreeburg et al. (2009a) established sampling factors relevant for HPA-axis indicators in our study: smoking (current or nonsmoker); awakening time; working day (yes, no). The presence of cardiovascular disease was established with an algorithm based on self-report and medication use, categorized into ‘yes’ and ‘no’.

*Treatment:* pharmacological treatment was assessed based on inspection of the medication boxes used in the past month and coded using the WHO Anatomical Therapeutic Chemical (ATC) classification (REF to URL). Regular use of antidepressants was categorized into selective serotonin reuptake inhibitors (ATC-code N06AB), tricyclic antidepressants (ATC-code N06AA) or other antidepressants (ATC-code N06AF/N06AX).

Sociodemographic, clinical, sampling factors and use of antidepressants were set as potential confounders. The stress related factors (childhood trauma and number of life events in past year) were set as potential effect modifiers (Shea et al., 2005; Rao et al., 2010; Morris et al., 2012). In a previous study performed with NESDA data (Holleman et al., 2012) stress related factors were found not to be associated to HPA-axis parameters and they were therefore not further considered potential confounders in our study.

2.5. Statistical analyses

T-tests and $\chi^2$-square tests were performed to compare recurrent and non-recurrent subjects on baseline characteristics. The associations between HPA-axis parameters and risk for MDD recurrence over the course of the four year follow-up were analyzed using Cox regression analyses. The independent variables were the HPA-axis parameters, time to recurrence of depression was the dependent variable which was expressed in months and based on the CIDI. Hazard ratios and their 95% confidence intervals (95% CI) were calculated, adjusted for covariates. The covariates were entered in blocks; sociodemographic factors, sampling factors and treatment with antidepressants in the first block, and clinical factors in the second block. As previous studies identified both hyperactivity and hypoactivity as predictors of MDD recurrence, we checked for potential non-linear associations by dividing the HPA axis parameters into quintiles. Linear mixed model analyses (LMM) were performed for the four morning cortisol measurements. LMM analyses can accommodate for incomplete cases and takes correlations between repeated measurements into account (Gueorguieva and Krystal, 2004). Therefore, LMM analysis included all subjects with at least two valid CAR values. In this analysis, recurrence of MDD, morning cortisol moments and all covariates were entered as fixed factors. Recurrence of MDD during follow-up was operationalized dichotomously (yes/no). Subjects were treated as a random effect and a random intercept was estimated. Recurrence of depression was the independent variable and the various morning cortisol levels were entered as the dependent variables. This analysis was used as a confirmation of the results of the Cox regression analysis. Furthermore, we checked whether interactions between stress-related factors (childhood trauma, number of life events in past year) and the HPA-axis parameters (CAR, DST, evening cortisol) were associated with the risk for MDD recurrence. Interaction terms were calculated by multiplying the stress-related factors by the different HPA-axis parameters which were centered because they were continuous. So, eight interaction terms were assessed. $p$ Values $\leq0.05$ were deemed to be statistically significant for main effects and $p$ values $\leq0.10$ for interaction terms.

3. Results

Characteristics of the 549 subjects are presented in Table 1. Of these, 392 subjects (71.4%) were female and the mean age was 45.0 years. The mean number of previous episodes of MDD was 2.9; 484 subjects (88.2%) remitted more than 12 months ago and 227 (41.3%) had no depressive symptoms in the five years preceding baseline which was assessed with the life chart interview (Lyketsos et al., 1994). During the four year follow-up, 131 subjects (23.9%) experienced a recurrence of MDD over the first two years, and 193 (35.2%) over the course of the four year follow-up. Mean time to recurrence was 27.4 months (sd = 12.1). Subjects who experienced a recurrence ($n = 193$) during the 4-year follow-up were more often younger, more likely to have a history of more than one episode of depression at baseline, more often had a 6-month comorbid anxiety disorder at baseline, had
more severe depressive symptoms at baseline, a higher number of traumatic youth experiences, used antidepressants more frequently, and had a higher AUCi. There were no differences between recurrent and non-recurrent subjects in sex, number of negative life events in the past year, family history of depression and covariates related to cortisol levels.

A Cox regression analysis was performed, adjusted for covariates. Fully adjusted results illustrate that a higher AUCi is associated with time to recurrence of MDD (HR 1.03, 95%CI 1.003–1.060, p = 0.03; Table 2). These results were also confirmed by LMM analyses (direct effect: p = 0.10, interaction with time: p = 0.05; Fig. 1). Evening cortisol levels and cortisol suppression after dexamethasone ingestion were not related to time to recurrence. The hazard ratios were constant over time. This was checked by calculating the different interaction terms (HPA-axis parameter × time) which were not statistically significant.

To analyze whether higher as well as lower cortisol levels were related to time to recurrence, we divided the HPA-axis parameters into quintiles, resulting in five categories. The middle quintile was the reference category. Table 2 shows that the highest AUCi, fully adjusted for covariates, was associated with recurrence (HR 1.81, 95% CI 1.02–3.21, p = 0.04). AUCg, evening cortisol and DST were also divided into quintiles. No difference in risk for MDD between low and high cortisol levels was found for these HPA-axis parameters. In the fully adjusted Cox regression model, we checked whether the stress related factors (childhood trauma and number of life events in past year) and the HPA axis parameters (AUCi, AUCg, DST, mean evening cortisol) interacted by entering the interaction term separately into the model (8 interactions). However, no statistically significant interaction terms were found (p interaction > 0.10).

4. Discussion

The aim of this study was to examine whether HPA-axis parameters are related to risk for MDD recurrence among subjects who had recovered from a previous episode of MDD. We found that a higher cortisol awakening response was associated with time to recurrence of MDD. Hypocortisolism was not related to recurrence and we did not find any associations with other HPA-axis parameters (DST and evening cortisol). Furthermore, no significant interactions between HPA axis parameters and stress related factors were found. This indicates that the increased risk for a recurrence of depression due to a high CAR is not dependent on recent life events or childhood trauma.
Table 2  Recurrence of MDD across various salivary cortisol indicators adjusted for covariates.  

<table>
<thead>
<tr>
<th></th>
<th>HR (95% CI)</th>
<th>p</th>
<th>HR (95% CI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>AUCg (nmol/l/h) (n = 399)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Continuous</td>
<td>1.02 (1.00–1.05)</td>
<td>0.08</td>
<td>1.03 (1.00–1.05)</td>
<td>0.08</td>
</tr>
<tr>
<td>&lt;13.68</td>
<td>1.19 (0.68–2.09)</td>
<td>0.55</td>
<td>1.27 (0.72–2.26)</td>
<td>0.41</td>
</tr>
<tr>
<td>13.68–16.63</td>
<td>0.94 (0.51–1.70)</td>
<td>0.85</td>
<td>0.90 (0.50–1.62)</td>
<td>0.72</td>
</tr>
<tr>
<td>16.64–19.73</td>
<td>ref</td>
<td>ref</td>
<td>ref</td>
<td>ref</td>
</tr>
<tr>
<td>19.74–24.30</td>
<td>1.52 (0.88–2.63)</td>
<td>0.13</td>
<td>1.49 (0.86–2.58)</td>
<td>0.15</td>
</tr>
<tr>
<td>&gt;24.30</td>
<td>1.46 (0.80–2.47)</td>
<td>0.24</td>
<td>1.40 (0.79–2.47)</td>
<td>0.25</td>
</tr>
<tr>
<td><strong>AUCi (nmol/l/h) (n = 399)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Continuous</td>
<td>1.03 (1.003–1.060)</td>
<td>0.03</td>
<td>1.03 (1.004–1.059)</td>
<td>0.03</td>
</tr>
<tr>
<td>&lt;2.15</td>
<td>1.14 (0.61–2.20)</td>
<td>0.69</td>
<td>1.03 (0.55–1.92)</td>
<td>0.94</td>
</tr>
<tr>
<td>2.15–0.95</td>
<td>1.34 (0.75–2.40)</td>
<td>0.32</td>
<td>1.38 (0.76–2.50)</td>
<td>0.29</td>
</tr>
<tr>
<td>0.96–3.84</td>
<td>ref</td>
<td>ref</td>
<td>ref</td>
<td>ref</td>
</tr>
<tr>
<td>3.85–7.00</td>
<td>1.33 (0.75–2.36)</td>
<td>0.34</td>
<td>1.27 (0.71–2.27)</td>
<td>0.43</td>
</tr>
<tr>
<td>&gt;7.00</td>
<td>1.90 (1.08–3.33)</td>
<td>0.03</td>
<td>1.81 (1.02–3.21)</td>
<td>0.04</td>
</tr>
<tr>
<td><strong>DST</strong> (n = 345)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Continuous</td>
<td>0.92 (0.79–1.07)</td>
<td>0.28</td>
<td>0.93 (0.80–1.08)</td>
<td>0.32</td>
</tr>
<tr>
<td>&lt;1.69</td>
<td>1.19 (0.68–2.08)</td>
<td>0.54</td>
<td>1.18 (0.67–2.08)</td>
<td>0.56</td>
</tr>
<tr>
<td>1.69–2.21</td>
<td>0.67 (0.37–1.24)</td>
<td>0.20</td>
<td>0.79 (0.43–1.46)</td>
<td>0.45</td>
</tr>
<tr>
<td>2.22–2.65</td>
<td>ref</td>
<td>ref</td>
<td>ref</td>
<td>ref</td>
</tr>
<tr>
<td>2.66–3.27</td>
<td>1.19 (0.69–2.05)</td>
<td>0.54</td>
<td>1.24 (0.72–2.16)</td>
<td>0.44</td>
</tr>
<tr>
<td>&gt;3.27</td>
<td>0.71 (0.39–1.30)</td>
<td>0.27</td>
<td>0.79 (0.43–1.44)</td>
<td>0.43</td>
</tr>
<tr>
<td><strong>Mean evening cortisol</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Continuous</td>
<td>0.99 (0.96–1.03)</td>
<td>0.67</td>
<td>0.99 (0.96–1.02)</td>
<td>0.46</td>
</tr>
<tr>
<td>&lt;3.10</td>
<td>0.82 (0.52–1.29)</td>
<td>0.39</td>
<td>0.81 (0.52–1.27)</td>
<td>0.35</td>
</tr>
<tr>
<td>3.10–4.42</td>
<td>0.78 (0.50–1.23)</td>
<td>0.29</td>
<td>0.73 (0.46–1.16)</td>
<td>0.19</td>
</tr>
<tr>
<td>4.43–5.57</td>
<td>ref</td>
<td>ref</td>
<td>ref</td>
<td>ref</td>
</tr>
<tr>
<td>5.58–7.11</td>
<td>0.95 (0.61–1.47)</td>
<td>0.81</td>
<td>0.94 (0.60–1.47)</td>
<td>0.78</td>
</tr>
<tr>
<td>&gt;7.11</td>
<td>0.88 (0.56–1.39)</td>
<td>0.59</td>
<td>0.83 (0.52–1.31)</td>
<td>0.42</td>
</tr>
</tbody>
</table>

Abbreviations: HR, hazard ratio; CI, confidence interval; AUCi/AUCg area under de curve with respect to the increase/ground; DST, dexamethasone suppression test; ref, reference category. In bold: statistically significant.

a Based on Cox’s survival analyses and adjusted for gender, age, smoking, time of awakening, cardiovascular disease, working day, use of antidepressants.

b Based on Cox’s survival analyses and adjusted for gender, age, smoking, time of awakening, cardiovascular disease, working day, use of antidepressants, history of major depressive episodes, 6-month prevalence of anxiety disorder, severity of depressive symptoms and family history of depression.

c DST = salivary cortisol at T1/cortisol level at T7 after 0.5 mg of dexamethasone ingestion

d Mean evening cortisol = mean cortisol at T5 and T6.

Our study supports the predictive role of a higher CAR in recurrence of depression, in line with previous studies which also found that hyperactivity was related to recurrence of MDD (Ribeiro et al., 1993; Zobel et al., 1999; Harris et al., 2000; Zobel et al., 2001; Hatzinger et al., 2002; Appelhof et al., 2006; Aubry et al., 2007; Bhagwagar and Cowen, 2008; Pintor et al., 2009; Vrshek-Schallhorn et al., 2013). The study by Vrshek-Schallhorn et al. (2013) is the most similar to our study and also found a higher CAR to be related to recurrence. One study (Bockting et al., 2012) found decreased mean morning cortisol levels to be predic- tive of recurrence. In line with Bockting et al. (2012) a recent NESDA study (Vreeburg et al., 2013) found that a lower CAR in persons with a current depressive and/or anxiety disorder was associated with an unfavorable prognosis for cases without remission longer than 3 months. Such discrepancies may be explained by the fact that we did not study subjects with multiple episodes in the recent past but those who had an major depressive episode further in the past. Bockting et al. (2012) included subjects with at least two major depressive episodes in the last five years. In our study sample, 41.3% had no depressive symptoms in the five years preceding baseline and only 51.4% of the subjects had one MDE in the past, indicating that subjects in our study sample did not have a chronic recurrent course in the recent past. Thus, while our data suggest that a higher CAR is associated with MDD recurrence in remitted depressed patients, previous studies also based on NESDA data postulate that a lower CAR is associated with chronicity of MDD in currently depressed patients data (Vreeburg et al., 2013). A possible explanation for such discrepancies is that chronic stress or duration of symptoms of depression may lead initially to HPA axis hyperactivity and over time to down-regulation of glucocorticoid and mineralocorticoid receptors, resulting in hypocortisolism (Buchanan et al., 2004; Pruessner et al., 2007). It has been argued that a higher CAR
represents a ‘trait’ marker for recurrence of depression whereas a lower CAR can be identified as a ‘scar’ marker for current depression (Vreeburg et al., 2013). Thus, a high AUCi may also represent a trait marker for recurrence of depression. This is in line with previous studies. A recent study (Vrshek-Schallhorn et al., 2013) concluded that a higher CAR also predicted first onset. Furthermore, the CAR is higher in young people who have not been depressed themselves but have a family history of depression (Mannie et al., 2007; Vreeburg et al., 2010) and a recent study also using data of NESDA did suggest that cortisol levels were not convincingly associated with childhood trauma (Holleman et al., 2012). Besides, Lok et al. (2012) found that remitted highly recurrent MDD patients had higher cortisol concentrations than controls which was not influenced by MDD-episodes during follow-up and HPA axis activity had no association with daily hassles or childhood life events. These data could suggest a genetic vulnerability trait. A previous study (Wüst et al., 2000) also concluded that there is a significant genetic influence on the CAR. It could be that in those in remission for a long time who are not exposed to high stress levels the HPA-axis activity returns to the original level prior to the depression. Hyperactivity of the HPA-axis could function as a risk factor as before a first episode. Interestingly, the study by Vrshek-Schallhorn et al. (2013) found that the CAR was a time-limited risk factor and predicted recurrences of depression with greater strength than it predicted a first episode. However, in our study the number of prior major depressive episodes experienced did not interact with CAR to predict time to recurrence.

Despite a large number of studies on the CAR, the exact function of the sharp cortisol increase after awakening is still unknown. Fries et al. (2009) hypothesized that ‘the cortisol rise after awakening may accompany an activation of prospective memory representations at awakening enabling individual’s orientation about the self in time and space as well as anticipation of demands of the upcoming day, with an important role for the hippocampus’'. In our study, the AUCi was associated with recurrence of depression which is a measure of the dynamics of the CAR, related to the sensitivity of the system (Clow et al., 2010). Therefore, our data provides some indications that the sensitivity of the HPA-axis could play a role in the risk for recurrence, whereas the total amount of cortisol during the day or the negative feedback mechanism as evening cortisol and DST were not related to recurrence. An alternative hypothesis is that the CAR is more sensitive to moderate degrees of depression than the DST, e.g. failure to suppress to dexamethasone is associated

Figure 1  Baseline 1-h cortisol awakening levels for subjects with and without a recurrence of depression after four years based on linear mixed model analyses. Error bars illustrate 95% CI. Analyses are adjusted for gender, age, smoking, time of awakening, cardiovascular disease, working day, use of antidepressants, history of major depressive episodes, 6-month prevalence of anxiety disorder, severity of depressive symptoms and family history of depression.
with more severe depression such as melancholia and inpatient status (Nelson and Davis, 1997). Although there is no use of the CAR as a diagnostic tool or biomarker in a clinical setting so far, it is a valuable instrument in research on stress-related disorders such as MDD. It remains to be seen whether a higher AUC$_{I}$ is an epiphenomenon or plays a substantial role in the onset or course of a depressive episode. A hypothesis could be that a higher CAR reflects a marker for increased sensitivity for (psychosocial) stress which is associated with an increased risk for a recurrence. Though, our analyses did not support this assumption as the CAR did not interact with stressful life events in increasing the risk for a recurrence. However, it is important to note that the stressful life events measures were assessed for the time period prior to baseline assessment.

The strengths of our study are that we were able to examine several HPA axis parameters and take into account important covariates in a large representative sample using standardized instruments to determine diagnosis and course. It should be mentioned that our results are restricted to those without a chronic recurrent course of depression in the recent past as 41.3% of our study sample had no depressive symptoms in the five years preceding baseline. Furthermore, the HPA-axis has a diurnal rhythm, and although we took the morning awakening response into account, by creating the area under curve to the ground and to the increase and measured evening cortisol levels we could not determine a more precise diurnal rhythm during the day since we did not collect mid-day cortisol levels. We could have created diurnal rhythm, by extracting the evening curve levels from the morning curve levels. However, the resulting diurnal variable is highly correlated to the morning curve levels. This is due to the fact that the evening levels are generally much lower than the morning curve levels, causing the height of the morning curve levels to heavily determine the diurnal rhythm variable. We also had missing data in our study sample largely because we had no information on cortisol values at baseline of these subjects. Selection was not at random as the study sample was older in comparison with the subjects who were excluded from the study (mean age 45.0 versus 40.5, $p < 0.001$). They did however not differ in sex, educational attainment or number of previous episodes of MDD. Nevertheless, our sample seems representative for an outpatient treated group. When interpreting the results of this study, its limitations should also be taken into account. First, time to recurrence of depression was measured during follow-up with the use of the prevalence rates at 2- and 4 years. The estimated time to recurrence was measured with the averages of these prevalence rates (see Section 2) which may not be completely accurate to assess time to recurrence. Time to recurrence could therefore be overestimated. Also, lifetime diagnoses of depression were assessed retrospectively and could be affected by recall bias. Second, the subjects’ state of depressive symptoms may lead to unreliable results of HPA-axis values. Although they were all in remission for at least six months some subjects may be in a recurrence for a rather short period, others for a more extended period of time. Although subjects were in remission as confirmed by the CIDI psychiatric interview, subjects could experience residual symptoms at baseline. The mean IDS score in our study sample was 17.2 (sd = 9.9). The IDS could affect HPA-axis parameters. We, however, adjusted for the IDS score in the final model which did change the results only slightly and examined possible interaction effects of IDS with HPA-axis parameters which were not found. Also, physical diseases could affect the HPA-axis values which we did not exclude specifically. We, however, excluded pregnant and breastfeeding women, those who used corticosteroid derivate and adjusted for health indicators which in a previous NESDA study (Vreeburg et al., 2009a) have been found to be associated with HPA-axis parameters. We also excluded those with cortisol values two standard deviations above the mean. Furthermore, we did not measure current stress levels or state effects (such as variations in mood, sleep, etc.) on the exact day of saliva sampling. Although we instructed subjects to collect the samples on a representative day without unusual amounts of stress, state effects could have played a role. Sampling on multiple days would have increased the reliability of the measurements (Hellhammer et al., 2007). However, the large sample size of our study may have partly of fully compensated for this. Finally, our study was based on observational data, so no causal association between cortisol levels and recurrence of MDD can be drawn.

In conclusion, this study shows that a higher cortisol awakening response, related to the sensitivity of the HPA-axis, increases vulnerability to new depression episodes, even if a subject has been in remission for a long period. Neither the DST or evening cortisol levels were associated with recurrence and the higher risk for recurrence related to a high CAR was not dependent on stressful life events. Further research should investigate the CAR in association with the course of depression over time to distinguish possible depression trajectories in association with the HPA-axis.

Role of the funding source

The sponsors have not had any role in the conducted analyses, writing the manuscript and the decision to publish these results.

Conflict of interest statement

Dr. Nolen has received speaking fees from Astra Zeneca, Eli Lilly, Pfizer, Servier, Wyeth; unrestricted grants from The Netherlands Organization for Health Research and Development, the European Union, the Stanley Medical Research Institute, Astra Zeneca, Eli Lilly, GlaxoSmithKline, Wyeth; and served on advisory boards for Astra Zeneca, Cyberonics, Pfizer, and Servier.

Dr. Spijker has received speaking fees from Astra Zeneca, Wyeth, Servier, Eli Lilly and GlaxoSmithKline.

Dr. Beekman has received unrestricted research grants from Eli Lilly, Astra Zeneca, Janssen and Lundbeck and as a speaker from Lundbeck and Eli Lilly.

Other authors do no have a conflict of interest.

Acknowledgements

The infrastructure for the NESDA study (www.nesda.nl) is funded through the Geestkracht program of the Netherlands
References


