Dysregulation of the hypothalamic–pituitary–adrenal (HPA) axis is believed to play a key role in the pathophysiology of depression. Moreover, depression and its course may be partly genetically determined. We previously found a higher cortisol awakening response in people with a current or a remitted major depressive disorder. Together with other reports, this may indicate that HPA axis dysregulation represents a trait rather than a state marker. A similarly elevated cortisol awakening response was observed for current panic disorder with agoraphobia. Together with other reports, this may indicate that HPA axis dysregulation resembles those observed in participants with depression or anxiety disorders.

Method

Data were from the Netherlands Study of Depression and Anxiety. Within the participants without a lifetime diagnoses of depression or anxiety disorders, three groups were distinguished: 180 people without parental history, 114 with self-reported parental history and 74 with CIDI-diagnosed parental history. These groups were additionally compared with people with major depressive disorder or panic disorder with agoraphobia (n = 1262). Salivary cortisol samples were obtained upon awakening, and 30, 45 and 60 min later.

Results

As compared with unaffected participants without parental history, unaffected individuals with diagnosed parental history of depression or anxiety showed a significantly higher cortisol awakening curve (effect size (d) = 0.50), which was similar to that observed in the participants with depression or anxiety disorders. Unaffected people with self-reported parental history did not differ in awakening cortisol levels from unaffected people without parental history.

Conclusions

Unaffected individuals with parental history of depression or anxiety showed a higher cortisol awakening curve, similar to that of the participants with depression or anxiety disorders.

Declaration of interest

None.

Method

Data are from the baseline assessment (September 2004 to February 2007) of the Netherlands Study of Depression and Anxiety (NesDA). Respondents (aged 18–65) were recruited from the community, general practice and specialised mental healthcare services, and included people with and without psychopathology. General exclusion criteria were: a primary diagnosis of psychotic disorder, obsessive–compulsive disorder, bipolar disorder or severe addiction disorder and not being fluent in Dutch. The research protocol was approved by the Ethical Committee of participating universities and all respondents provided written informed consent.

Within the participants with no lifetime history of panic disorder, generalised anxiety disorder, agoraphobia, social phobia, major depressive disorder or dysthymia as assessed by the DSM–IV Composite International Diagnostic Interview (CIDI version 2.1) and no subthreshold symptoms (Beck Anxiety Inventory (BAI) ≤ 20, Inventory of Depressive Symptoms (IDS) ≤ 14) or use of antidepressants or benzodiazepines, three groups were compared.

Participants without parental history

These participants reported no parental history of depression or anxiety disorder, as assessed by the family history inventory (n = 238) on which the respondent reported on the presence of an anxiety or depressive disorder in all individual first-degree relatives.
Participants with self-reported parental history
These participants reported at least one biological parent with depression and/or anxiety (n = 173).

Participants with diagnosed parental history
These participants (n = 122) had previously participated in the Adolescents at Risk of Anxiety and Depression (ARIADNE) study into the development of depression and anxiety disorders among offspring (aged 13–25) of people with psychiatric disorders, approximately 4 years before participating in NESDA. Parents were treated for depression or panic disorder in specialised mental healthcare. Both offspring and parent were interviewed with the CIDI.17

Comparison group
A comparison group of participants consisted of 2010 people who were diagnosed with a remitted or current major depressive disorder and/or current panic disorder with agoraphobia as assessed by the CIDI, for whom we observed the highest cortisol levels as compared to people without psychopathology.6,8

Exclusions and final sample
We subsequently excluded 22 pregnant or breastfeeding women and 140 participants on corticosteroids, leaving 2381 respondents. Of these, 1630 (68.5%) returned sufficient saliva samples: 180 (81.8%) without parental history; 114 (70.4%) with self-reported parental history; 74 (61.7%) with diagnosed parental history and 1262 (67.2%) with a psychiatric diagnosis, P < 0.001.

Salivary cortisol
A minimally invasive way to measure basal cortisol level is through saliva sampling, reflecting the active unbound form of cortisol.18 As described in more detail elsewhere,19 participants through saliva sampling, reflecting the active unbound form of Salivary cortisol formulas.20 The AUCg is an estimate of the total cortisol secretion (AUCi) and with respect to the ground (AUCg) using Pruessner's we calculated the area under the curve with respect to the increase interassay variability coefficients in the measuring range were less luminescence immunoassay (E170 Roche, Switzerland). The Cortisol analysis was performed by competitive electrochemi-

Results
Sample characteristics are presented in Table 1. Participants with diagnosed parental history were younger, had later awakening times, less often slept ≤6 h/night, had a lower trauma index score

Statistical analyses
Baseline characteristics were compared using $\chi^2$ and ANOVA statistics. Area under the curves showed normal distributions, 1-h awakening cortisol levels were slightly skewed and therefore log-transformed for linear mixed models analyses. Back-transformed values were used in the figure. To analyse differences in 1-h awakening cortisol across groups, ANCOVA analyses with AUCi and AUCg were conducted. First, we compared the three unaffected parental history groups. Additionally, parental history groups were compared with the group of participants with a psychiatric diagnosis. Random coefficient analysis of the four morning cortisol levels was performed using linear mixed models, which keeps original values on all four data points, can accommodate for incomplete cases, and takes correlation between repeated measurements into account.24 Parental history groups, time point (T1, T2, T3, T4) and all covariates were entered as fixed factors, participants 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 a group × time interaction term. For significant findings, effect sizes were calculated with Cohen’s d. All analyses were conducted using SPSS version 15.0 for Windows.

Covariates
Previously, we described effects of gender, age, time of awakening, working status, season, sleep duration, physical activity and smoking on salivary cortisol levels,19 which were considered as covariates. Respondents reported time of awakening and working status on the sampling day. Season was categorised into less (October through to February) and more daylight (March through to September) months. Average sleep duration during the past 4 weeks was dichotomised as ≤6 or >6 h/night,25 and smoking status as current versus non-smoker. Physical activity was assessed using the International Physical Activity Questionnaire and expressed in 1000 MET-min (metabolic energy turnover per min) a week.26 Menstrual cycle phase, menopausal status or use of oral contraceptives were not associated with salivary cortisol and were therefore not included as covariates.19

Explanatory factors
To examine whether found effects were influenced by neuroticism, childhood trauma and life events we adjusted all analyses for these factors. Neuroticism was measured with the 12-item subscale of the NEO Five-Factor Inventory (NEO–FFI) Questionnaire ranging from 0 (low neuroticism) to 48 (high neuroticism). In order to examine the role of earlier childhood trauma, we constructed a cumulative childhood trauma index using the NEMESIS childhood trauma interview, which summarises the frequency of four reported traumas before the age of 16 – emotional neglect, psychological abuse, physical abuse and sexual abuse – resulting in an index score between 0 and 8. Finally, negative life events in the past year were assessed by the Brugha questionnaire and included 12 specific events and one ‘other’ category asking about another serious (not specified) negative life event. Since symptom severity scores were not associated with salivary cortisol levels in our previous studies, these were not included as covariates.6,8

In addition to conducting linear mixed models analyses (see statistical analyses section) using all four morning saliva samples, we calculated the area under the curve with respect to the increase (AUCi) and with respect to the ground (AUCg) using Pruessner’s formulas.27 The AUCg is an estimate of the total cortisol secretion over the first hour after awakening, whereas the AUCi is a measure of the dynamic of the cortisol awakening response, more related to the sensitivity of the system, emphasising changes over time after awakening.21–23 If samples were collected outside of a margin of 5 min around the time protocol, values were assigned missing. Morning cortisol analyses included all participants with at least two valid morning cortisol values (n = 1630), since linear mixed models analyses can adequately interpolate for missing data.24 For 1441 participants all four morning samples were available and could therefore be included in the AUC analyses (158 without parental history, 101 with self-reported parental history, 68 with diagnosed parental history and 1114 with a psychiatric diagnosis).
and higher neuroticism scores than other unaffected participants (Table 1). Participants with self-reported parental history reported more childhood trauma and had higher neuroticism scores than unaffected participants without parental history. Compared with parents in the diagnosed parental history group, parents in the self-reported parental history group had more often only anxiety disorders. Diagnosed parents mostly had depression with or without comorbid anxiety disorder.

In total 68.5% of the respondents showed an increase in cortisol levels within 1 h of waking up (65.0% in the group without parental history, 68.4% in the self-reported parental history group, 77.0% in the diagnosed parental history group, \( P = 0.17 \)). Parental history groups showed significant differences in AUCg (\( F = 3.47, \text{df.} = 2/316, P = 0.03 \)), but not in AUCi (\( F = 1.73, P = 0.18 \)). The group with diagnosed parental history showed higher overall adjusted cortisol levels than participants without parental history, reflected by a larger AUCg (\( P = 0.01 \), effect size (Cohen’s \( d \)) = 0.50, Table 2) and confirmed by a borderline significant direct effect in linear mixed models analyses (\( F = 3.39, P = 0.07 \), data not shown). There was a trend towards a larger AUCi (\( P = 0.08, d = 0.35 \), Table 2) for the diagnosed parental history group, but the interaction with time was not significant (\( F = 1.09, P = 0.35 \)). The group with self-reported parental history did not show significant differences in morning cortisol compared with the group without parental history (Table 2, linear mixed models analyses: direct effect: \( F = 0.05, P = 0.82 \); interaction with time: \( F = 0.85, P = 0.47 \)).

### Table 1 Sample characteristics

<table>
<thead>
<tr>
<th></th>
<th>Participants without lifetime depression/anxiety</th>
<th>Participants with major depressive disorder and/or panic disorder with agoraphobia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No parental history (( n = 180 ))</td>
<td>Self-reported parental history (( n = 114 ))</td>
</tr>
<tr>
<td>Female, %</td>
<td>57.8</td>
<td>57.0</td>
</tr>
<tr>
<td>Age, years: mean (s.d.)</td>
<td>47.9 (11.8)</td>
<td>47.8 (11.9)</td>
</tr>
<tr>
<td>Time of awakening, mean (s.d.)</td>
<td>07.00 (1 h 11)</td>
<td>07.22 (1 h 02)</td>
</tr>
<tr>
<td>Working on day of sampling, %</td>
<td>65.9</td>
<td>65.8</td>
</tr>
<tr>
<td>Sampling in month with more daylight, %</td>
<td>56.0</td>
<td>48.2</td>
</tr>
<tr>
<td>&lt; 6 h of sleep, %</td>
<td>18.1</td>
<td>15.8</td>
</tr>
<tr>
<td>Smoker, %</td>
<td>21.1</td>
<td>22.8</td>
</tr>
<tr>
<td>Physical activity, 1000 MET-min/week: mean (s.d.)</td>
<td>4.0 (3.2)</td>
<td>3.8 (2.9)</td>
</tr>
<tr>
<td>Life event in past year, %</td>
<td>30.0</td>
<td>29.8</td>
</tr>
<tr>
<td>Trauma index, mean (s.d.)</td>
<td>0.4 (1.1)</td>
<td>0.9 (1.7)**</td>
</tr>
<tr>
<td>Neuroticism, mean (s.d.)</td>
<td>23.8 (3.7)</td>
<td>25.0 (5.7)**</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Type of parental disorder</th>
<th>Anxiety only, %</th>
<th>Depression only, %</th>
<th>Comorbidity, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>No parental history</td>
<td>NA</td>
<td>28.1</td>
<td>NA</td>
</tr>
<tr>
<td>Self-reported parental history</td>
<td>40.4</td>
<td>52.9</td>
<td>NA</td>
</tr>
<tr>
<td>Diagnosed parental history</td>
<td>31.6</td>
<td>41.2</td>
<td>NA</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>MET, metabolic energy turnover, NA, not applicable.</th>
</tr>
</thead>
<tbody>
<tr>
<td>( ^* P &lt; 0.10, ^** P &lt; 0.05 ) compared with unaffected participants with no parental history.</td>
</tr>
<tr>
<td>a. March through to September.</td>
</tr>
</tbody>
</table>

### Table 2 Results of age-adjusted and fully adjusted ANCOVA analyses associating parental history with the cortisol awakening curve*

<table>
<thead>
<tr>
<th></th>
<th>No parental history (( n = 183 )) Mean (s.e.)</th>
<th>Self-reported parental history (( n = 120 )) Mean (s.e.)</th>
<th>Self-reported v. no parental history ( P )</th>
<th>Diagnosed parental history (( n = 73 )) Mean (s.e.)</th>
<th>Diagnosed v. no parental history ( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUCg, nmol/l/hc</td>
<td>17.5 (0.5)</td>
<td>17.6 (0.7)</td>
<td>0.91</td>
<td>19.9 (1.0)</td>
<td>0.07</td>
</tr>
<tr>
<td>AUCg1, nmol/l/hc</td>
<td>17.4 (0.5)</td>
<td>17.5 (0.6)</td>
<td>0.90</td>
<td>20.6 (1.0)</td>
<td>0.01</td>
</tr>
<tr>
<td>AUCg2, nmol/l/hc</td>
<td>1.0 (0.5)</td>
<td>1.7 (0.6)</td>
<td>0.38</td>
<td>3.1 (1.0)</td>
<td>0.09</td>
</tr>
<tr>
<td>AUCg3, nmol/l/hc</td>
<td>1.0 (0.5)</td>
<td>1.8 (0.6)</td>
<td>0.30</td>
<td>3.1 (1.0)</td>
<td>0.08</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>AUCg, nmol/l/hc</th>
<th>AUCg1, nmol/l/hc</th>
<th>AUCg2, nmol/l/hc</th>
<th>AUCg3, nmol/l/hc</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.5 + 0.25) + (((T3 + T4)/2)</td>
<td>0.5 + 0.25 + (((T1 + T2)/2) + 0.5))</td>
<td>(((T1 + T2)/2) + 0.25) + (((T3 + T4)/2) + 0.25))</td>
<td></td>
</tr>
</tbody>
</table>

*Area under the morning curve with respect to the ground (AUCg) = ((((T + T + T + T)/2) + 0.5) + 0.5) + 0.5). Area under the morning curve with respect to the increase (AUCc) = AUCg − (T (0.5 + 0.25 + 0.25)).

b. Adjusted for age.

c. Additionally adjusted for gender, working status, time of awakening, season, sleep, physical activity and smoking.
AUCg in the self-reported parental history group (mean factor and reflects an underlying biological vulnerability marker. 

suggest that a higher cortisol awakening curve represents a trait 

patients with a CIDI diagnosis treated in specialised mental 

sizes (parental history was medium (14.5 (s.e. = 1.5) nmol/l/h, 

history (17.7 (s.e. = 1.7) nmol/l/h, 

self-reported parental history group (17.7 (s.e. = 1.7) nmol/l/h, P = 0.13, 

d = 0.50, n = 13) compared with participants without parental 

history (14.5 (s.e. = 1.5) nmol/l/h, n = 20). 

Since the self-reported parental history group had relatively 

more parents with anxiety compared with depression or 

comorbidity, we reanalysed differences only including participants 

with self-reported parental depression or comorbid disorders. 

Results showed a higher AUCg in the diagnosed parental history 

group (mean AUCg = 17.3 (s.e. = 0.8), P = 0.96) as compared with controls 

(mean AUCg = 17.3 (s.e. = 0.5) nmol/l/h).

Main findings

This study shows that cortisol awakening levels in unaffected 

participants with diagnosed parental history of depression or 

anxiety were significantly higher than in unaffected participants 

without parental history, and similar to those in participants with 

major depressive disorder or panic disorder with agoraphobia.6,8 

In fact, the effect size for unaffected participants with diagnosed 

parental history was medium (d = 0.50) and higher than the effect 

sizes (d = 0.15–0.32) previously described for major depressive 

disorder and panic disorder with agoraphobia.6,8 Our results 

suggest that a higher cortisol awakening curve represents a trait 

factor and reflects an underlying biological vulnerability marker. 

The higher cortisol levels were only found in offspring of 

patients with a CIDI diagnosis treated in specialised mental 

healthcare and not in offspring with affected parents based on 

self-report. Although often used to assess parental history, our 

findings may indicate that self-report of parental psychopathology 

is of insufficient reliability. Alternatively, the parents reported on 

by their offspring may represent the milder cases and be associated 

with lower familial loading, especially since it was not asked 

whether parents were treated, in contrast to the diagnosed parents 

of the ARIADNE sample for whom referral status was required for 

enrolment in the study. Higher familial loading in the diagnosed 

parental history group might also be reflected by the higher 

neuroticism scores in this group relative to the self-reported 

parental history group. In addition, when excluding participants 

older than 30 years, effect sizes were higher in both parental 

history groups relative to the group without parental history, also 

possibly reflecting the importance of familial loading. Presumably, 

and consistent with the idea that cortisol elevation represents a 

liability for later onset of anxiety and depression, part of the 

younger parental history group may not have developed 

depression or anxiety yet. However, the older group, being still 

unaffected, may never develop depression or anxiety disorder. 

The larger proportion of anxiety disorder without comorbid 

depression in the self-reported parental history group did not 

explain the results, since additional analysis excluding anxiety 

disorder without comorbid depression generated similar results. 

Our results are in line with one study reporting higher 

morning cortisol,9 but in contrast with studies reporting no 

association with morning cortisol in unaffected people with 

parental history.10,11 However, the latter two studies measured 

the morning curve with only one and two saliva samples, 

respectively. Participants with diagnosed parental history showed 

overall higher cortisol levels (AUCg) and a trend towards a higher 

increase (AUCi), indicating that especially the total cortisol 

regulation was elevated. This emphasises the importance of collecting multiple morning 

samples. The increased AUCg is possibly reflective of basal 

hypofunction of the HPA axis, since it is related to cortisol levels 

during the rest of the day, whereas the AUCg is less dependent 

on the diurnal cortisol rhythm and is regarded an indicator of 

reactivity of the HPA axis.21–23 It has been hypothesised that the 

AUCg is particularly suited to measure trait effects, whereas the 

AUCg is better suited for state effects.29 

An important finding was that cortisol levels were 

comparable with those found in participants with a psychiatric 

diagnosis. Previous studies did not compare morning cortisol 

levels of individuals with familial history with people with 

depression/anxiety. However, reports on cortisol release on the 

dexamethasone/corticotropin-releasing hormone test showed 

cortisol levels in between controls and patients.30 

Genetic factors

Genetic factors could account for our findings.3,31 The cortisol 

awakening response has a heritability of 32–48%32,33 and depression 

has a heritability of 37%.3 It could be that the same genes underlie 

both depression and morning cortisol regulation, for example the 

serotonin transporter gene,31 mineralocorticoid or glucocorticoid 

receptor genes.2 Epigenetic factors such as histone modification or 

methylation of deoxyribonucleic acid (DNA) as a result of early 

life stress could also play a role.8,34 

Also, early childhood trauma and adversity could be more 

prevalent in people growing up in families with psychiatric 

problems, which may exert direct effects on inflammatory and 

glucocorticoid signalling.35,36 However, adjustment for childhood 

trauma, including emotional abuse, did not change our results, 

since these variables were not associated with saliva cortisol levels.
Strengths and limitations

Our study had several strengths, including offspring from parents with confirmed diagnoses, the use of four morning cortisol samples and an additional comparison group of participants with a psychiatric diagnosis. Moreover, we studied an adult sample, whereas most studies included children, showing that HPA axis dysregulations persist into adulthood. Some limitations have to be acknowledged. First, non-adherence to the sampling instructions could have resulted in a blunted cortisol response after awakening. However, even when awakening is closely monitored at least 15% of all participants still do not respond with a cortisol rise. Moreover, we have no reason to believe that possible non-adherence is unevenly distributed among our groups. Second, since the majority of parents with a diagnosis had a depressive disorder, our results are mostly restricted to depression with or without comorbid anxiety. Future studies are warranted to specifically examine HPA axis activity and parental history of anxiety disorders.

Implications

Our study adds to the evidence that HPA axis alterations in depression and anxiety represent a trait factor that may indicate a biological vulnerability for the development of these disorders. Therefore, the cortisol awakening curve may constitute an important endophenotype of depression (and anxiety) for genetic research. Although the clinical relevance of the difference in 1-hour awakening cortisol needs to be explored in further large-scale research, there is some evidence that morning cortisol levels predict unfavourable metabolic and mental health outcomes.

References


