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Higher cortisol awakening response in young adolescents with persistent anxiety problems

Greaves-Lord K, Ferdinand RF, Oldehinkel AJ, Sondeijker FEPL, Ormel J, Verhulst FC. Higher cortisol awakening response in young adolescents with persistent anxiety problems.

Objective: The aims of the present study were to test the association between current anxiety problems and basal cortisol levels in a large population sample of young preadolescents, and to test if HPA-axis activity differs between individuals with no, only current, or persistent anxiety problems.

Method: Cortisol levels of 10- to 12-year olds ($n = 1768$) from the general population were measured on three time points during the day. A self-report questionnaire (RCADS) was used to assess current anxiety, a parent-report questionnaire (TPBQ) to assess anxiety problems at age 4.

Results: Associations between cortisol levels and current anxiety problems were not found. However, individuals with persistent anxiety problems had higher morning cortisol levels and a higher cortisol awakening response.

Conclusion: Apparently, only persistent, and not current, anxiety problems are associated with higher HPA-axis activity. Alterations in HPA-axis activity might underlie persistent anxiety problems, or result from the stress accompanied by persistent anxiety problems.

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Key words: Hypothalamo-Hypophyseal System (HPA-axis); anxiety; adolescent; population

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Significant outcomes

- No associations were found between basal daytime cortisol levels and current anxiety levels.
- Individuals with persistent anxiety problems had higher morning cortisol levels and a higher cortisol awakening response.

Limitations

- Only retrospective data were used to investigate the persistence of anxiety problems.
- The influence of daily stresses or stressful life-events on HPA-axis activity was not measured.

Introduction

Anxiety problems occur frequently (1, 2), result in considerable suffering and impairment, and tend to persist across time (3–6). Therefore it is important to investigate putative etiological mechanisms.

One of the biological systems that possibly plays a role in anxiety is the hypothalamic-pituitary-adrenal (HPA)-axis. Under stress, the HPA-axis is

activated; the hypothalamic production of corticotropin-releasing hormone (CRH) increases, which stimulates the pituitary release of adrenocorticotropin hormone (ACTH) and, as a consequence, cortisol secretion by the adrenal cortex. The HPA-axis is also active in normal circumstances, showing a diurnal pattern of cortisol levels. In the morning, cortisol levels rise quickly during waking up (cortisol awaking response, 7). Then, approximately half an hour after awakening,

cortisol levels begin to drop, and generally continue to decrease during the day.

Kagan and colleagues have suggested that some children are more vulnerable to anxiety problems, because they have a lower threshold for activation of the HPA-axis through environmental stimuli, which increases cortisol concentrations (8). According to this theory, one would expect anxiety problems to be associated with higher basal cortisol levels. Gunnar and Vazquez hypothesized that stressful influences in early life may provoke frequent elevations in cortisol. These elevations would however eventually lead to downregulation of components of the HPA-axis (9). Based on their theory, and the assumption that stress in early life would also result in future anxiety, anxiety problems would be expected to be associated with lower basal cortisol levels.

One of the most robust findings in biological psychiatry is that of higher basal cortisol levels in adult patients with major depression (9–11). In contrast, evidence has been found for lower cortisol levels in both adult and adolescent patients with posttraumatic stress disorder (PTSD, 12–16). For other anxiety disorders, that are not caused by extreme environmental stressors, findings were inconclusive. For instance, some findings in adults suggested increased HPA-axis activation in panic disorder (e.g. 17) while other findings did not support this hypothesis (e.g. 18).

In the few studies that investigated the association between anxiety problems and cortisol levels in children and adolescents, findings were as inconclusive as in adults. Kagan and colleagues found that basal cortisol levels were higher in inhibited than in uninhibited young children (8). An inhibited temperament overlaps with anxiety; both represent fearful and withdrawn behaviours (e.g. 19). On the other hand, Feder and colleagues found lower nighttime cortisol levels and a slower morning rise in anxious 6- to 12-year-old children, compared to depressed children or controls (20). In another study, Martel and colleagues did not find differences in basal cortisol levels of social phobic adolescent girls versus matched controls (21).

Previous studies were all confined to small, clinical samples, thereby limiting the generalizability of their findings. Furthermore, earlier work mostly concerned the association between HPA-axis activity and current anxiety problems. It can be argued however that, in those with an inborn lower threshold for activation of the HPA-axis (8), chronically elevated anxiety levels can be found. Therefore, alterations in HPA-axis activity might be most evident in individuals with a long lasting history of anxiety problems. Finally, it is worth

while mentioning that it is important to adjust for comorbid symptoms of major depression. As symptoms of major depression have been associated with alterations in HPA-axis activity, such associations might explain or interfere with the associations with anxiety problems that are investigated.

In conclusion, data on the association of HPA-axis activity and anxiety problems in children or adolescents are hardly available and inconclusive. Further, studies that investigated the association between persistence of anxiety problems and HPA-axis activity in a large population sample, adjusting for comorbid major depression, are lacking.

Aims of study

- i) To test the association between current anxiety problems and basal cortisol levels in a large population sample of preadolescents.
- ii) To test if HPA-axis activity differs between preadolescents with no, only current, or persistent anxiety problems.

Material and methods

Sample

All participants took part in the TRacking Adolescents' Individual Lives Survey (TRAILS), a general population study of Dutch early adolescents aged 10–12 years, who were assessed from March 2001 to July 2002. The TRAILS target sample consisted of young adolescents from five municipalities in the North of the Netherlands, including both urban and rural areas. Of all individuals who were approached ($n = 3145$), 6.7% were excluded. Of the remaining 2935 young adolescents, 76.0% participated in the study ($n = 2230$, mean age 11.09 years, SD 0.55, with 50.8% girls). Responders and non-responders did not differ with respect to the proportion of single parent families, or the prevalence of teacher-rated problem behaviour. Furthermore, no differences between responders and non-responders were found regarding associations between socio-demographic variables and mental health outcomes (22).

Cortisol was obtained in 1768 participants (79.3% of all TRAILS participants). There was no difference in Total Anxiety scores (RCADS, see below) between participants who provided saliva samples versus those who did not ($t = 0.287$; $P = 0.774$). Also, there were no differences in terms of gender (49.4% male vs. 48.5% male, $\chi^2(df = 1) = 0.132$; $P = 0.7$). The participants that

did not provide saliva samples were slightly older (11.16 years vs. 11.08 years, $t = -3.084$; $P = 0.002$). Written consent was obtained from the young adolescent's parents after the procedure had been fully explained.

Questionnaires

RCADS The Revised Child Anxiety and Depression Scale (RCADS, 23, 24) is a revision of the Spence Children's Anxiety Scale (SCAS; 25). The RCADS is a self-report questionnaire with 47 items, that are scored on a 4-point scale (0 = never, 1 = sometimes, 2 = often, 3 = always). The questionnaire covers six of the DSM-IV dimensions of anxiety disorders and depressive disorder: separation anxiety disorder, generalized anxiety disorder, social phobia, panic disorder, obsessive-compulsive disorder and major depressive disorder (MDD). In this study, a Total Anxiety Scale was computed by summing the scores on the separate anxiety items and dividing this by the number of items that were completed. Similarly, a MDD scale was computed, and was used to adjust for comorbid depressive problems.

The psychometric properties of the RCADS in the TRAILS sample were reported elsewhere (26).

TPBQ The TRAILS Preschool-Behaviour Questionnaire (TPBQ) is a parent-report questionnaire developed by TRAILS to measure behaviours at preschool age (± 4 years old) retrospectively. Parents were asked to compare their child's behaviour with the behaviour of other children. The questionnaire contains 17 items about the occurrence of (problem) behaviours that are scored on a 5-point scale (1 = a lot less, 2 = less, 3 = as much, 4 = more, 5 = a lot more) and covers scales of different behaviours (e.g. aggressive, social). The subscale that covers anxiety problems was used for analyses. The items of this scale were: i) anxious, ii) shy, iii) gloomy, and iv) afraid to go to school. The internal consistency of this subscale in the TRAILS sample was 0.76.

Cortisol

TRAILS participants collected three samples of saliva, by using the Salivette sampling device. Collection of salivary cortisol is a relatively stress-free approach that avoids confounding by stress responses, e.g. as induced by venipuncture (27). According to several authors correlations between saliva cortisol levels and serum cortisol concentrations are high (e.g. 28, 29).

Participants and their parents were instructed to collect saliva at three time points during the day; directly after waking up (while still lying in bed, Cort 1), half an hour later (Cort 2), and at 8.00 P.M. (Cort 3). Parents of all the participants received both written and oral instructions. First, they received a letter, containing information about the purpose of saliva collection and some background information about diurnal basal cortisol levels. Then, a member of our team visited them at home and gave further instructions. It was stressed that it was important to collect saliva on a normal day, during a normal week, without special events or stressful circumstances. Also, parents were told that their child should not be ill, have a cold, be menstruating, or take any medication at the day of saliva collection. With regard to the actual saliva sampling, it was stressed that participants should always rinse their mouth with tap water before sampling saliva, and not consume sour products or brush their teeth before sampling. Parents were encouraged to place the first salivette next to their child's bed, so that he or she could collect the first sample directly after waking up. Participants were told to not fall asleep and stay awake between providing the first sample and the second sample, and to provide the second sample exactly half an hour later. Finally, it was told that the salivettes should be placed in a freezer directly after saliva collection. All these instructions were also handed over on an instruction form. If any of the requirements were not met, parents could note this down on an accompanying form. Twenty-two preadolescents were excluded because of use of antibiotics or corticosteroids. Furthermore, a number of participants were excluded because of extreme cortisol values ($> 3SD$), which eventually yielded $n = 1666$, $n = 1683$, and $n = 1689$ for Cort 1, Cort 2, and Cort 3 respectively. As the schools that participated in TRAILS started at approximately the same time, the sampling-time variation of the morning samples among the children was limited and the estimated corresponding times are 0700 h for the first sample (Cort 1) and 0730 h for the second sample (Cort 2).

The saliva samples were stored at -20°C until analysis. Competitive solid phase time-resolved fluorescence immunoassays with fluorometric end point detection (DELFA = dissociation-enhanced lanthanide fluorescent immunoassays) were used to determine cortisol concentrations in the saliva samples. The intra-assay coefficient of variation was between 4.0% and 6.7%, and the corresponding inter-assay coefficients of variation between 7.1% and 9.0% (details about the procedure in (30)).

Other individual characteristics

Given the possible confounding effects, information on gender, age, pubertal stage, medication, smoking habits, disruptive behaviours, height and weight (Body Mass Index, BMI) and perinatal variables, like pregnancy duration and birth weight, were also assessed. Pubertal stage was assessed using schematic drawings of secondary sex characteristics associated with the five standard Tanner stages of pubertal development (31, 32). In a parent interview, the parent (usually the mother) was provided with gender-appropriate sketches, and asked which of the sketches 'looked most like their child'. These ratings have been widely used and have demonstrated good reliability and validity (34). Information on medication and the perinatal variables was also assessed during this parent-interview. Smoking habits and disruptive behaviours were assessed in a self-report questionnaire of which the anonymity was stressed.

Statistical analyses

Scores on the RCADS Total Anxiety scale, RCADS MDD scale and the TPBQ Anxiety scale were calculated by computing the sum of the separate items of the scale and dividing this sum by the number of items of that scale that were completed. If more than 33% of the items were missing, the scale score was coded as missing. To approximate a normal distribution, all anxiety and depression scales and all cortisol measures were root-transformed.

To obtain an index for the cortisol awakening response (CAR, 7, 35), we calculated the CAR measure using the following formula:

$$CAR = \frac{Cort1 + Cort2}{2}$$

The cortisol awakening response is a useful index of HPA-axis activity, which is rather consistent, shows good intraindividual stability across time and appears to be useful for assessing subtle changes in HPA-axis activity (35, 36). Previous research provided evidence for a significant genetic influence on the cortisol awakening response and this response was found to be independent of the time of awakening, 'manner of awakening' (spontaneously or by an alarm clock), sleep duration, sleep quality, physical activity, or morning routines (35, 36, 37). Furthermore, the cortisol awakening response has proven to be a good index to uncover associations between HPA-axis activity and stress-related

problems, such as worrying, social stress, persisting pain, and burnout (e.g. 7, 35, 38–40).

Linear regressions To investigate the association between current anxiety problems and cortisol levels, several linear regression analyses were performed with scores on the RCADS Total Anxiety scale as the predictor and respectively, Cort 1, Cort 2, Cort 3, and CAR as dependent variables.

The putative impact of confounders was also investigated, by adding these variables to the regression model. Given gender influences on both anxiety levels and cortisol levels, first gender was taken into account as a confounding factor. In addition, because we found some correlations between pubertal stage and anxiety levels and between age and both anxiety levels and evening cortisol levels, we decided to take pubertal stage and age into account as possible confounders. As stated above, possible confounding effects of depressive problems (MDD) were also taken into account. The effects of perinatal variables, like pregnancy duration and birth weight, and of BMI were not taken into account, since Rosmalen and colleagues found no significant confounding effects of these variables on the cortisol measures in the TRAILS sample (30). Moreover, disruptive behaviours were not taken into account, because no or very weak associations were found between these problems and cortisol levels in the TRAILS sample (33). Given the age-range of our sample (10–12 years old), there was no use of oral contraceptives (0.0%) and a very low number of smokers (0.9% of our sample had smoked on occasion). Therefore these variables were not included in the analyses.

For each index of HPA-axis activity (Cort 1, Cort 2, Cort 3, and CAR) separately multiple linear regression analyses were conducted in which first, RCADS Total Anxiety was entered into the model as a predictor. Subsequently the following possible confounding predictors were added: i) gender, ii) pubertal stage iii) age, and iv) MDD.

One-way analysis of covariance (ANCOVA) To investigate if HPA-axis functioning differed between individuals with no, only current, or persistent anxiety problems, three groups were composed. The first group, with no anxiety problems, consisted of those participants who scored below the 50th percentile of the cumulative frequency distribution of the TPBQ Anxiety scale (< P50) and who scored < P50 on the RCADS Total Anxiety scale. The second group consisted of participants with only current anxiety problems. These participants scored < P50 on the TPBQ Anxiety scale, but

above the 90th percentile of the cumulative frequency distribution ($>P90$) of the RCADS Total Anxiety scale. The third group, of participants with more persistent anxiety problems scored $>P90$ on both the TPBQ Anxiety scale and the RCADS Total Anxiety scale.

Mean daytime cortisol levels (Cort 1, Cort 2, Cort 3) were computed for each group to compose a figure of daytime cortisol levels. Then, differences between the groups on all cortisol measures (Cort 1, Cort 2, Cort 3 and CAR) were calculated using four ANOVAS, with gender, pubertal stage, age and MDD as covariates. *Post-hoc* Bonferroni tests were computed to detect between-group differences.

Results

Linear regressions

There were no significant associations between RCADS Total Anxiety scores and any of the cortisol measures (Cort 1; $\beta = .020$, $P = \text{n.s.}$ Cort 2; $\beta = -.022$, $P = \text{n.s.}$ Cort 3; $\beta = -.026$, $P = \text{n.s.}$ CAR; $\beta = -.008$, $P = \text{n.s.}$). The addition of gender, pubertal stage, age and MDD to the regression models improved the fit of the models, but the effect of the Total Anxiety scores on the cortisol measures remained insignificant.

One-way ANCOVA's

In Fig. 1 mean daytime cortisol levels for each group are presented.

The three groups differed significantly with respect to Cort 1 levels [$F = 4.316$, $P = 0.014$, effect size (R^2) = 5.3%]. According to Cohen (41)

Fig. 1. Day time cortisol levels (in nmol/l) for participants with no anxiety problems ($n = 324$), only current anxiety problems ($n = 52$), or persistent anxiety problems ($n = 30$): Note: 1 = cortisol directly after awakening; 2 = cortisol half an hour after awakening; 3 = cortisol at 8.00 p.m.; The cortisol awakening response is the increase in cortisol levels between 1 and 2.

effect sizes $\geq 13.8\%$ are large, between 5.9% and 13.8% medium, and between 1.0% and 5.9% small. *Post-hoc* analyses indicated that this effect was due to higher Cort 1 levels in individuals with persistent anxiety problems versus only current anxiety problems ($P = .049$, $R^2 = 1.4\%$). The groups did not differ with respect to Cort 2 levels ($F = 2.622$, $P = 0.074$), although participants with persistent anxiety problems had non-significantly higher Cort 2 levels than participants with no anxiety problems. No significant group differences were found either for evening cortisol levels (Cort 3; $F = 0.496$, $P = 0.609$).

The cortisol awakening response, CAR, was significantly higher in preadolescents with persistent anxiety problems than in preadolescents with no, or only current anxiety problems ($F = 6.678$, $P = 0.001$, $R^2 = 5.8\%$). Participants with persistent anxiety problems differed significantly from those with no anxiety problems ($P = 0.007$, $R^2 = 2.8\%$) and from those with only current anxiety problems ($P = 0.003$, $R^2 = 2.8\%$). Participants with no, or only current anxiety problems did not differ from each other ($P = 0.773$).

Discussion

The present study investigated associations between level of anxiety problems and basal cortisol levels in a population sample of 1768 preadolescents. The sample was large and not influenced by referral biases, which provided the opportunity to detect very small effects in a representative sample. Merely studying associations between cortisol levels and current anxiety problems did not yield significant effects. However, even though the effect sizes were small, individuals with high current anxiety levels and a history of anxiety problems early in life showed higher morning cortisol levels and a higher cortisol awakening response. Apparently, only the more persistent anxiety problems were associated with higher HPA-axis activity.

A possible explanation for the association between persistent anxiety problems and cortisol levels may be that inborn alterations in HPA-axis activity result in anxiety problems. Evidence is available indicating that HPA-axis functioning is at least partially determined by genetic (35, 42–44) and prenatal factors (10). These factors may influence CRH secretion patterns (45), feedback effects of cortisol on central glucocorticoid receptors (46), or both (44). In this way, the sensitivity of an individual to stressful stimuli may be determined, which may influence risk for anxiety problems (8).

Another explanation for our findings could be that anxiety problems influence HPA-axis functioning. It is likely that persistent anxiety problems are accompanied by high levels of stress, resulting in elevated cortisol concentrations. Prolonged stress-induced elevations in cortisol levels may influence HPA-axis functioning. These elevations could induce downregulation of components of the HPA-axis, as suggested by Gunnar and Vazquez (9). However, this would lead to lower cortisol levels which is not in accordance with the present study's findings. On the other hand, elevations in cortisol levels that persist across time could also tune HPA-axis activity to a higher level. For instance, elevated cortisol levels could result in damage of the hippocampal glucocorticoid receptors or even a loss of hippocampal neurons (47), which would affect the glucocorticoid feedback inhibition of CRH secretion (48) and would result in higher CRH and cortisol concentrations.

Interestingly, individuals with persistent anxiety problems had both higher basal morning cortisol levels (Cort 1) and higher awakening response levels (CAR). This points to a generally higher activated stress hormone release as well as a higher dynamic responsivity. In other words, not only basal HPA-axis functioning, but also the responsiveness of the HPA-axis is different in individuals with persistent anxiety problems. A higher cortisol awakening response in individuals with persistent anxiety problems indicates a stronger physiological response to the process of awakening, but might also indicate a more general stronger physiological responsivity, that could result in a different stress reaction in these individuals. A possible explanation might lie in exposure to adversities early in life which may have altered HPA-axis functioning. In the present study only the relation between cortisol levels and anxiety was investigated. In future research, it would be interesting to also investigate cortisol responses to daily stresses, psychological stress tasks, or to stressful life-events.

The results of this study did not reveal a linear association between anxiety scores and cortisol levels. Kagan et al. (8) did show an association between anxiety-related temperamental characteristics and cortisol, however, this study had a small sample size and only investigated children with extreme scores, limiting the generalizability of the findings. Our finding of no association between current anxiety scores and cortisol levels in a large population sample shows that cortisol levels are not related to current anxiety levels, which is of importance for the development of theories on the etiology of anxiety. As many anxiety problems

develop later in life, it is possible that cortisol levels predict future anxiety problems. Future prospective longitudinal studies will have to address this topic.

In the current study, only retrospective data have been used to investigate the persistence of anxiety problems. Often this is regarded as a shortcoming, as it could be argued that parents of children with high levels of current anxiety may be more likely to indicate that their children were anxious as well earlier in life, known as 'recall bias'. However, the results of our study indicated that, within the group of all preadolescents with high current anxiety levels, only those with a parent-reported history of anxiety showed higher cortisol levels. We argue that recall bias based on presence of current anxiety is equally likely to be present in both individuals with only current anxiety problems as well as in individuals who also have a parent-reported history of anxiety, especially since both groups did not differ in the severity of the current anxiety problems ($F = 0.005$, $P = 0.945$). Therefore, it is unlikely that recall bias has influenced the results of this study.

The present study indicates that HPA-axis functioning could be one of the factors that determine anxiety. It could be informative to measure HPA-axis functioning together with other important biological and environmental factors at a younger age than we did now, to investigate their role in the etiology of anxiety problems. Longitudinal research is needed to gain more insight in the direction of the causal chain between HPA-axis functioning and anxiety problems.

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