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SHORT COMMUNICATION

Anxiety disorders and salivary cortisol levels in older adults: a population-based study

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KEYWORDS
Cortisol;
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Awakening response

Summary
Context: The hypothalamic-pituitary-adrenal (HPA) axis is one of the body's main systems that controls response to stress. It acts through the hormone cortisol. While the dysregulation of cortisol has been associated with anxiety disorders, the evidence is inconsistent. Moreover, only a few small studies have assessed this relationship in older adults.
Objective: To determine whether in adults aged 65 years and over there is a difference in daily cortisol pattern between those with and without an anxiety disorder.
Methods: The study population comprised 1788 older adults from a population-based cohort. The Munich version of the Composite International Diagnostic Interview was used to diagnose anxiety disorders (generalized anxiety disorder, social phobia, specific phobia, agoraphobia and panic disorder). The cortisol awakening response and total cortisol secretion over the day were calculated from cortisol levels in four saliva samples taken over the course of one day (at awakening, 30 min after awakening, at 1700 h, at bedtime).
Results: Older adults with an anxiety disorder ($n = 145$, median duration since first symptoms 41 years) had a lower cortisol awakening response ($p = 0.02$) than those without such a disorder ($n = 1643$). This association was most prominent in those with generalized anxiety disorder ($p = 0.008$), but was not associated with the extent of chronicity of anxiety disorders.
Conclusion: Older adults from the general population with long-lasting anxiety disorders had a lower cortisol awakening response than those without. This is consistent with the notion that chronic anxiety may result in downregulation of HPA-axis activity. Longitudinal studies are needed to confirm this mechanism.

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1. Introduction

The hypothalamic-pituitary-adrenal axis (HPA axis) is one of the body's main systems that controls response to stress. It acts through the hormone cortisol, which is produced in the adrenal cortex and affects many tissues including the brain. Cortisol is secreted in a distinct daily pattern whereby cortisol levels rise rapidly after awakening (the cortisol awakening response or CAR) and decrease slowly thereafter. Deregulation of the CAR, and total cortisol secretion over the day have been associated with various disorders, including anxiety disorders (Vreeburg et al., 2010). In addition, age-dependent HPA-axis dysregulation may increase the vulnerability of older adults for psychiatric disorders (Van Cauter et al., 1996).

Although numerous studies have described the relationship between the HPA axis and anxiety disorders, the evidence is inconsistent and only a few studies assessed this relationship in older adults. While one study reported that older adults (mean age 74, $n = 111$) with generalized anxiety disorder (GAD) had higher cortisol levels than those without GAD (Mantella et al., 2008), another study (mean age 76, $n = 48$) reported no association of cortisol levels with an anxiety symptom score (Heaney et al., 2010). A further study of older people (mean age 73, $n = 201$) also reported no difference in total cortisol levels during the day between people with and without lifetime GAD in a non-stressful condition, but the CAR was not assessed (Chaudieu et al., 2008).

In the current study we assessed not only total cortisol secretion, but also the CAR. We jointly analyzed anxiety disorders, but also present data on GAD, social phobia, specific phobia and agoraphobia separately. Furthermore, this study was no convenience sample, but comprised older adults (aged 65 and over) from the general population to minimize selection effects.

2. Methods

2.1. Study setting

This study was set in the Rotterdam Study, a prospective population-based cohort study of older adults designed to assess risk factors for chronic diseases (Hofman et al., 2011). In 1990, all residents in a district in Rotterdam who were aged 55 years and over were invited to participate. Every four years, participants undergo an extensive home interview and physical examination at a research center. The Medical Ethics Committee of the Erasmus MC approved the Rotterdam Study.

The fourth examination round (2002—2004, $n = 3550$) assessed anxiety disorders and salivary cortisol levels. The study population comprised 1788 people after exclusion of people without a valid anxiety assessment ($n = 287$), people without the first two cortisol measurements ($n = 1152$), people using corticosteroids ($n = 287$), and people with dementia ($n = 36$). Almost two thirds of those who were excluded ($n = 1762$) were female against 56.9% of the study participants. The excluded group was significantly older than the study population (mean age 77.3 versus 74.7) and had a lower education.

2.2. Assessment of anxiety disorders

Prevalent anxiety disorders were diagnosed during the home interview. Trained lay interviewers conducted a slightly adapted version of the Munich version of the Composite International Diagnostic Interview (M-CIDI, Wittchen et al., 1998). The following anxiety disorders were assessed with a computerized diagnostic algorithm according to DSM-IV criteria: GAD, panic disorder, agoraphobia, social phobia and specific phobia. Age of symptom onset was recorded. Obsessive compulsive disorder and post-traumatic stress disorder (PTSD) were not assessed, because these disorders are relatively rare in the general population.

2.3. Salivary cortisol protocol

Saliva samples were collected on awakening (T1), 30 min after awakening (T2), at 1700 h (T3), and at bedtime (T4). Cortisol levels in these samples were determined as previously described (Dekker et al., 2008). The multiple measures of cortisol were combined in summary measures to provide valid information about the diurnal pattern of cortisol. We calculated the area under the curve with respect to the ground (AUCg) and the cortisol awakening response (CAR). The AUCg summarizes overall diurnal cortisol exposure. The CAR is a measure of the dynamics of the HPA-axis response upon awakening. The CAR and the AUCg are thought to be regulated differently (Clow et al., 2004). The AUCg was calculated as the total area under the curve from the individual cortisol measures on the *Y*-axis and the time between cortisol measures on the *X*-axis. To not measure the effect of the CAR, we did not include T2 in the calculation. We corrected for total time awake and only calculated the AUCg for those with data on all three time points ($n = 1664$). The CAR was calculated as the difference between cortisol levels at T2 and T1 over two ($n = 1788$, Pruessner et al., 2003). Analyses on the CAR were adjusted for the time between measurements.

2.4. Assessment of other variables

Age, sex, marital status, psycholeptics use, psychoanaleptics use, hormonal drug use, usual sleep duration, and alcohol, coffee and tea consumption were recorded. Education was grouped according to the Standard Classification of Education and rated on a scale from primary education (1) to university level (7). Height and weight were measured at the research center to calculate body mass index (BMI). Smoking was coded according to current smoking status. Cognitive capacity was assessed using the Mini Mental State Examination. A cut-off of 26 indicated adequate cognitive capacity versus impaired cognitive capacity. Disability (Activities of Daily Living) was assessed with the Stanford Health Assessment Questionnaire. The standard cut-off of a mean score of 0.5 indicated no disability versus mild to severe disability. Participants were continuously monitored for occurrence of coronary heart disease. International Classification of Diseases, 10th Revision was used to assign diagnoses of myocardial infarction (I21), percutaneous transluminal coronary angioplasty, coronary artery bypass graft and other forms of acute (I24) or chronic ischemic heart disease (I25). Diabetes

Mellitus was diagnosed when a fasting plasma glucose level was at least 7.0 mmol/l, a non-fasting plasma glucose level was at least 11.1 mmol/l, or oral antidiabetes medication or insulin were used, or treated by diet and registered by the general practitioner (GP) as having diabetes. The number of sites with atherosclerotic plaques was identified by ultrasonography of both carotid arteries. Blood pressure was measured at the right brachial artery using a random-zero sphygmomanometer with the participant in sitting position.

In those who screened positive for depressive symptoms with the Center for Epidemiological Studies Depression scale (CES-D, cut-off score of 16), DSM-IV-defined major depression was assessed with a semi-structured clinical interview (Schedules for Clinical Assessment of Neuropsychiatry). The CES-D has a high sensitivity for major depression in older adults (Beekman et al., 1997). Depressive syndromes during follow-up were assessed based on two clinical interviews, self-report depression and GP records. Month of cortisol sampling was categorized in months with less daylight (October—February) and months with more daylight (March—September). All variables, except education, were assessed during the same examination round as anxiety disorders and salivary cortisol.

2.5. Statistical analysis

Baseline characteristics for persons with and without anxiety disorder were compared using chi-square statistics, Student's *t*-tests and the Mann—Whitney *U* test. AUCg and CAR were normally distributed after excluding values above the 99th percentile. Covariates were imputed using the Expectation—Maximization algorithm. All covariates had less than 2% missing values. Differences in AUCg and CAR in persons with and without anxiety disorders were tested using linear regression analyses. Covariates were selected if the effect estimates of the association between cortisol levels and anxiety disorders changed more than 5% in the models. Subtypes of anxiety disorders were analyzed jointly and separately with the exception of panic disorder which was too infrequent. We repeated all analyses excluding people with major depression. Data were analyzed using SPSS PASW version 17.

3. Results

The study population comprised 145 people with an anxiety disorder and 1643 people without an anxiety disorder. Agoraphobia ($n = 71$, 3.9%) and GAD ($n = 39$, 2.1%) were the most prevalent anxiety disorders. Thirty-five people were diagnosed with specific phobia (1.9%), sixteen had social phobia (0.9%) and four had a panic disorder (0.3%). Sixteen people (0.9%) had more than one anxiety disorder. The characteristics of the study population are shown in Table 1. The group with anxiety disorders comprised more females than the group without such a disorder (81% vs. 55%, $p < 0.001$). In addition, those with an anxiety disorder had a lower education ($p = 0.003$) and an eight-fold higher prevalence of depression than those without an anxiety disorder (8% vs. 1%, $p < 0.001$).

Unadjusted cortisol levels at 30 min after awakening and at 1700 h were lower in people with anxiety disorders. Supplementary Fig. 1 shows the cortisol pattern over the day of older adults with and without anxiety disorders. After adjustment for covariates, cortisol level at 30 min after awakening was lower in those with an anxiety disorder (beta = −2.67; 95%CI = −4.44, −0.90; $p = 0.003$), but not cortisol level at awakening (beta = −0.28; 95%CI = −1.79, 1.23; $p = 0.72$), at 1700 h (beta = −0.47; 95%CI = −1.06, 0.12; $p = 0.13$) or at bed time (beta = −0.18; 95%CI = −0.63, 0.28; $p = 0.44$). In addition, those with an anxiety disorder had significantly lower CAR than those without an anxiety disorder (beta = −1.19; $p = 0.02$, Table 2). Total cortisol secretion did not differ. When anxiety disorders were stratified by subtype, the association between GAD and the CAR was significant (beta = −2.50; $p = 0.008$, Table 2). Exclusion of people with major depression did not change these results (all anxiety disorders: beta = −1.18; 95%CI = −2.17, −0.18; $p = 0.021$, GAD: beta = −2.58; 95%CI = −4.66, −0.50; $p = 0.015$).

In a post hoc analysis we tested the influence of chronicity of the anxiety disorders on the CAR by comparing the CAR of people with an anxiety disorder with duration shorter than 5.44 years (lowest quartile) to those with an anxiety disorder that lasted longer than 59.69 years (highest quartile). The groups did not differ significantly, but, if anything, the people with a more chronic anxiety disorder had a higher CAR (beta = 2.52; 95%CI = −0.86, 5.90; $p = 0.14$).

4. Discussion

In this population-based study of older adults, people with an anxiety disorder had a significantly lower CAR than those without anxiety disorder.

Three previous studies assessed the association between anxiety and cortisol levels in older adults. First, Mantella et al. (2008) found higher cortisol levels (CAR as well as total cortisol secretion) in people with GAD than in healthy controls. This is opposite to our finding and may result from recruitment differences. Mantella et al. used a convenience sample recruited via e.g. advertisements and mental health clinician referral that likely included more acute and help-seeking cases while we used a population-based sample with prevalent and chronic cases. Second, Heaney et al. (2010) did not find a difference in cortisol levels between older adults with and without anxiety. While our study included 145 people with anxiety disorders, the study of Heaney et al. comprised 25 older people with anxiety symptoms. This study may not have had enough power to detect small differences in cortisol levels. Third, Chaudieu et al. (2008) assessed total cortisol secretion and not the CAR in a population-based setting. They observed no difference in cortisol secretion between people with and without lifetime GAD in non-stressful conditions, which is compatible with our finding.

In the largest study of anxiety and cortisol in adults (age range 18—65), Vreeburg et al. (2010) observed a higher CAR in people with anxiety disorders. This population was largely recruited from GPs and specialized mental health care institutions. Consequently, the study comprised many acute, help-seeking cases, whereas we included only prevalent, chronic anxiety cases from the general population. This difference is also reflected in the higher comorbidity of major depression (77.8%) and a higher prevalence of panic disorder cases (59.4%) in the sample of Vreeburg et al. Furthermore, the high CAR was driven by those with a panic disorder with agoraphobia and by those with a comorbid depression.

Table 1 Characteristics of the study group.

	Anxiety disorder* (*n* = 145)	No anxiety disorder (*n* = 1643)	*p***
% Women	80.7	54.8	<0.001
Age, years, mean (SD; range)	74.7 (5.3; 66.1–90.0)	74.7 (5.6; 65.1–92.7)	0.93
BMI, kg/m^2, mean (SD; range)	27.7 (4.2; 18.6–41.3)	27.5 (4.0; 14.2–45.7)	0.44
% Current smokers	53.8	58.5	0.29
Alcohol drinking, units/day, median (IQR; range)	0.1 (1.0; 0–4.7)	0.5 (1.2; 0–5.0)	<0.001
Coffee drinking, cups/day, median (IQR; range)	3.0 (2.0; 0–23)	3.0 (2.0; 0–24)	0.14
Tea drinking, cups/day, median (IQR; range)	3.0 (2.1; 0–12)	3.0 (3.0; 0–16)	0.64
Mean sleep duration, hours, mean (SD; range)	6.5 (1.5; 2.5–10)	6.9 (1.2; 2–11)	0.013
Education, mean (SD; range)	2.6 (1.5; 1–6)	3.1 (1.7; 1–7)	0.003
Marital status			0.68
% single	4.1	5.2	
% married	60.7	64.5	
% widowed	29.0	25.3	
% divorced	6.2	5.2	
Cognitive status (MMSE), mean (SD; range)	27.4 (1.8; 19.0–30.0)	27.7 (1.8; 17.0–30.0)	0.09
% Impaired cognitive capacity	14.5	9.0	0.04
Functional disability, median (IQR; range)	0.6 (0.7; 0–2.3)	0.3 (0.5; 0–2.8)	<0.001
% Mild to severe functional disability	64.8	39.0	<0.001
% Major depression	8.3	1.1	<0.001
% Depression during follow-up	46.2	25.6	<0.001
Depressive symptoms (CES-D), median (IQR; range)	9.0 (16.1; 0.0–48.0)	3.0 (6.0; 0.0–44.0)	<0.001
Negative affect (CES-D), median (IQR; range)			
Anxiety duration, years, median (IQR; range)	2.0 (7.0; 0.0–20.0)	0.0 (1.0; 0.0–16.0)	<0.001
% Psycholeptics users	40.6 (54.4; 0.3–73.2)	–	–
% Psychoanaleptics users	25.5	12.4	<0.001
% Hormonal medication users	9.7	3.6	<0.001
	2.1	1.2	0.34
% Diabetes Mellitus	13.1	17.7	0.16
% Coronary Heart Disease	4.8	8.5	0.12
Systolic blood pressure, mean (SD; range)	156.6 (22.6; 106.0–217.0)	152.4 (21.3; 99.5–240.0)	0.02
No. of atherosclerotic plaques, mean (SD; range)	2.7 (1.8; 0–6)	2.9 (1.9; 0–6)	0.13
Cortisol levels, nmol/l, mean (SD; range)			
T1, at awakening	14.1 (7.7; 0.3–35.1)	14.7 (8.6; 0.0–60.6)	0.40
T2, 30 min after awakening	15.9 (8.3; 0.2–41.9)	18.4 (10.2; 0.1–64.2)	0.001
T3, 1700 h	3.7 (2.4; 0.4–13.7)	4.2 (3.4; 0.0–29.3)	0.012
T4, at bedtime	2.2 (3.1; 0.1–26.7)	2.3 (2.4; 0.0–24.3)	0.71
AUCg	6.5 (3.0; 0.3–17.2)	7.0 (3.4; 0.4-26.3)	0.12
CAR	0.9 (5.1; −15.6–14.3)	1.8 (5.5; −27.0–28.0)	0.05
Time of the first sample, mean (SD; range)	0731 h (48 min; 0425–0941 h)	0734 h (55 min; 0305–1245 h)	0.60
% Sampling in months with more daylight	58.6	52.5	0.16

Abbreviations: AUCg, area under the curve with respect to the ground; BMI, body mass index; CAR, cortisol awakening response; CES-D, Center for Epidemiological Studies Depression scale; IQR, inter quartile range; MMSE, mini mental state examination; SD, standard deviation.

* The group of anxiety disorders comprised people with agoraphobia, social phobia, specific phobia, panic disorder and generalized anxiety disorder.

** *t*-tests, chi square tests or Mann–Whitney *U* tests were applied to test for difference.

Numerous studies have indicated that stress activates the HPA axis, raising total cortisol levels and leading to a higher CAR. However, it has been hypothesized that the HPA axis reacts to stress with temporal hyperactivity, but when stress persists the HPA axis becomes hypoactive (Fries et al., 2005). This mechanism has also been observed for depression. Oldehinkel et al. (2001) observed lower urinary cortisol levels in chronically depressed older adults, not in more acutely depressed people. Thus while cortisol may be raised during acute anxiety, the HPA axis reacts to a chronic disorder with reduced cortisol levels. This change from hyperactivity of the HPA axis to hypoactivity is likely mediated through increased sensitivity to negative feedback from circulating cortisol (Houshyar et al., 2001). In our study, we observed no association between extent of chronicity of the anxiety disorder and cortisol levels. However, acute and recent onset cases are rare in community-dwelling older adults, and even the group with the shortest duration of symptoms (lowest quartile) had an average duration of more than 3 years.

Table 2 Associations between anxiety disorders and cortisol summary measures.

	Cortisol summary measures					
	Area under the curve (AUCg)			Cortisol awakening response (CAR)		
	n	Beta (95%CI)	*p*	*n*	Beta (95%CI)	*p*
No anxiety disorder	1533	Reference		1643	Reference	
All anxiety disorders*	131	−0.39 (−1.02, 0.24)	0.23	145	−1.19 (−2.15, −0.23)	**0.02**
Subtypes of anxiety disorders**						
GAD	36	−0.02 (−1.22, 1.18)	0.97	39	−2.50 (−4.34, −0.65)	**0.008**
Social phobia	15	0.68 (−1.10, 2.44)	0.46	16	−1.48 (−4.19, 1.24)	0.29
Specific phobia	31	−1.26 (−2.48, −0.03)	**0.04**	35	−1.14 (−2.98, 0.70)	0.22
Agoraphobia	63	−0.62 (−1.48, 0.25)	0.16	71	−0.30 (−1.60, 1.01)	0.66

Abbreviations: CI, confidence interval; GAD, generalized anxiety disorder.
All analyses were adjusted for age, gender, education, time of the first sample, months with more daylight, psychoanaleptic drug use, total sleep duration, disability (yes/no), major depression, logarithm of negative affect sum score. Analyses of the CAR were additionally adjusted for the time between the first and the second sample.
* All anxiety disorders comprised GAD, social phobia, specific phobia, agoraphobia and panic disorder.
** As some people have comorbid anxiety disorders, the numbers for GAD, social phobia, specific phobia and agoraphobia do not add up to the number for all anxiety disorders. The number of panic disorders was too low to assess separately. In bold: *p*-values that are significant at the 0.05 level.

In addition, we analyzed the association between cortisol and subtypes of anxiety disorders. A lower CAR was observed for all subtypes, but only GAD was significantly associated with a lower CAR. This could not be attributed to impaired cognitive capacity or depression comorbidity. GAD is generally characterized by a chronic course and is closely related to depression for which similar findings were reported (Oldehinkel et al., 2001).

Older people are known to have a lower CAR (Kudielka and Kirschbaum, 2003; Heaney et al., 2010). The exact function of the CAR is unknown, but it has been suggested to play a role in e.g. memory function, and the immune system. A low CAR has been associated with cardiovascular and autoimmune disorders (Kudielka and Kirschbaum, 2003), which in turn have been linked to anxiety disorders (Strine et al., 2008). Older adults with (chronic) anxiety disorders may thus have an increased vulnerability to a wide range of disorders.

Our findings need to be discussed in light of some limitations. First, non-compliance to the cortisol sampling protocol could explain the observed lower CAR, if more common in those with anxiety disorders (Kudielka et al., 2003; Kudielka and Kirschbaum, 2003). Second, cortisol samples were collected on only one day. This may have resulted in less precise estimates and reduced study power. Third, because this study was performed in a population-based setting, our results cannot easily be generalized to a clinical patient group. Fourth, we did not assess PTSD or traumatic experiences, which are associated with blunted cortisol levels. However, PTSD has a relatively low prevalence and this effect is therefore not likely to explain our findings. Fifth, information on any remitted anxiety disorders or parental history was not available. Last, because this study was cross-sectional, we could not infer the causality of the associations we observed.

In conclusion, older adults with an anxiety disorder in this population-based study had a lower CAR than those without such a disorder. This is compatible with the notion that chronic anxiety may result in down regulation of HPA-axis activity. Longitudinal studies are needed to confirm this mechanism.

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The funders had no role in the study design or data collection and analysis.

Conflict of interest

The authors have no competing interests.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.psyneuen.2012.06.006.

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