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ORIGINAL ARTICLE

Patients with atopy exhibit reduced cortisol awakening response but not cortisol concentrations during the rest of the day

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Abstract

It has been documented that cortisol release in response to acute stressors is reduced in patients with atopic dermatitis, allergic rhinitis, and other atopic diseases compared to that in healthy subjects. We aimed to test the hypothesis that atopic patients exert reduced salivary cortisol awakening response (CAR) in comparison with healthy subjects. The hypothesis was tested on a stressful and a relax day selected subjectively. Moreover, we evaluated the impact of trait anxiety. The sample consisted of 60 subjects, out of which 28 were patients with atopy and 32 healthy volunteers of both sexes. Saliva samples were collected in the morning to evaluate CAR as well as in the early afternoon and evening to look at cortisol concentrations during the rest of the day. The results showed reduced CAR in atopic patients compared to that in healthy subjects. This effect was modulated by sex with a significant difference observed in males. While CAR was reduced, atopic patients had unchanged cortisol concentrations throughout the day. The evening cortisol was even higher in atopic patients. If the subjects were stratified according to the trait anxiety, no significant differences in CAR between high and low anxiety were observed. No differences in cortisol variables including CAR were observed between the stressful and relax day. In conclusion, this study presents evidence on reduced CAR suggesting an insufficient HPA axis reactivity in atopy. Furthermore, the data in atopic patients demonstrate that reduced HPA axis reactivity does not necessarily mean lower cortisol concentrations throughout the day. This might be of relevance to immune system function and the course of the disease.

Keywords Salivary cortisol · Atopy · Trait anxiety · Stress

Abbreviations

AUC_G	Area under the curve relative to ground
CAR	Cortisol awakening response
HPA	Hypothalamic-pituitary-adrenocortical axis
MnInc	Mean increase
PSS	Perceived stress scale
STAI-T	State and trait anxiety inventory-trait version

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Introduction

It has been well documented that cortisol release in response to acute stressors is reduced in patients with atopic diseases compared to that in healthy subjects, more specifically in patients with atopic dermatitis [1, 2] and allergic rhinitis [3], as well as in a combined sample of both diagnoses [4, 5]. This was accompanied by blunted responses of salivary alpha amylase activity and aldosterone concentrations during acute psychosocial stress [5].

Evaluation of cortisol secretion in allergic individuals is an important topic due to the immunosuppressive action of glucocorticoids [6] and their role in suppressing allergic inflammation as well as promoting Th1 to Th2 immune response shift on the longer term [7]. Stress and stress hormones therefore play an important role in the onset and exacerbation of various atopic manifestations. Prominence of psychological factors in atopy is underlined by the fact that atopic diseases are associated with high trait anxiety, and worse emotional regulation [8, 9]. Interestingly, blunted cortisol stress

reactivity was also found in healthy subjects with high trait anxiety [10], which is similar to previously mentioned findings in allergic patients.

One of the main characteristics of the hypothalamic-pituitary-adrenocortical (HPA) axis and particularly cortisol release is the known rhythmicity throughout the day with highest values in the morning and lowest concentrations in the evening [11, 12]. A special feature is the cortisol awakening response (CAR), which has been intensively studied after the measurement of cortisol in saliva became available [13, 14]. CAR is a rapid increase in cortisol secretory activity following morning awakening. In healthy adults, it has a magnitude ranging from 50 to 150% increase in salivary cortisol concentrations [15, 16]. Increased CAR was previously associated with increased daily demands [17] and acute stress [18]; however, blunting of CAR was reported in chronic stress [19], as well as in burnout [20]. CAR was found to be reduced in subjects with high trait anxiety [21, 22]; however, there are also contradicting reports [23].

With respect to patients with atopic diseases and CAR, little information has been obtained so far. A cross-sectional study in asthmatic children failed to establish a link between CAR and further progression of the disease [24]. A cognitive behavioral intervention in adult patients with atopic dermatitis led to reduced CAR; however, no comparisons with healthy subjects were performed [25].

Based on the reduced salivary cortisol responses during acute psychosocial stress situations in patients with atopy, we aimed to test the hypothesis that atopic patients exert reduced salivary CAR in comparison with healthy subjects. Additional samples of saliva for cortisol measurement were collected in the early afternoon and evening to get information on cortisol secretion throughout the day. Purposely, we recruited both patients and healthy subjects with either high or low trait anxiety to be able to distinguish the effects of atopy and high trait anxiety. Moreover, we expected increased CAR on a subjectively selected stressful day compared to a day of leisure.

Material and methods

Research sample

Seventy-three volunteers were originally recruited to participate in this naturalistic design study. The sample consisted of students from Comenius University, Bratislava, as well as patients recruited from ambulant allergologists in Slovakia. All subjects were between 18 and 35 years old, with mean age 22.3 years, SD = 2.74. Inclusion criteria to the atopic group were based on diagnosis of atopic dermatitis or allergic rhinitis diagnosed by a clinician, and the occurrence of allergic symptoms for at least 2 years. All participants in the non-atopic condition had no history of atopy in their anamnesis. Exclusion criteria for participation in this study were any other chronic conditions that could seriously influence the results (e.g., cardiovascular, endocrine, psychiatric diseases), use of corticosteroid medication at least a month before the sampling, antihistaminics at the time of the sampling, or any other medication that could be a confounder (e.g., antibiotics).

Besides atopy, the sample was also analyzed based on differences in their trait anxiety. The sample was divided into high and low anxious groups based on their score in state and trait anxiety inventory (STAI-T). Cutoff raw scores of below 40 for low anxiety and above 45 for high anxiety were used similarly to previous studies in Slovak population [10, 26]. People who scored in between these boundaries were not included in the study.

Due to subject dropout during the study, missing or insufficient data, reported significant delays in CAR sampling, and other reasons, 13 subjects were excluded from the study, leaving us with the final sample of 60 subjects (see the characteristics of the participants in Table 1). The study was approved by the Ethics Committee of the Trnava Self-Governing Region, Slovakia. All subjects provided written informed consent prior to participating in the study.

Study design

This study used domestic setting for data acquisition. Subjects performed the saliva sampling at home, kept the samples refrigerated, and handed them to the research team on the following day. The sampling was done during two previously agreed days, one selected by the participant as subjectively stressful, and the other one selected as relax. Here we aimed to look how the subjective anticipation of stress or relax days impacts the CAR.

Collection of salivary samples was performed via sterile salivettes (Sallivette, Sarstedt, UK). On each of the selected days, subjects took five separate saliva samples, first sample (S1) right at the awakening, then 15 min (S2) and 30 min later (S3), as well as in the afternoon at 14:00 (S4) and in the evening at 20:00 (S5).

Subjects were instructed to take the first sample immediately after awakening, to write down the time of the awakening, as well as the times of the later samples. Subjects were also instructed to refrain from behaviors that could influence the cortisol values (such as brushing teeth, eating, chewing a gum etc.) before the first sample, and in between S1 and S3, as well as 15 min before all subsequent samples.

After the collection, samples were frozen at −20 °C and transported to the laboratory for analysis. Cortisol levels in the samples were determined using a commercially available enzyme-linked immunosorbent assay (IBL International, Hamburg, Germany).

Table 1 Characteristics of the research sample (means ± SEM)

Research group	Research sample ($n = 60$)	BMI		Age	
A1/atopy	28 subjects, 11 males, 17 females.	22.51	± 0.60	20.82	± 0.62
A2/healthy	32 subjects, 12 males, 20 females	21.19	± 0.35	20.13	± 0.39
B1/low anxiety	35 subjects, 14 males, 21 females	21.07	± 0.54	20.40	± 0.50
B2/high anxiety	25 subjects, 9 males, 16 females	22.37	± 0.42	20.49	± 0.50

Psychological measures

To determine trait anxiety, the Slovak version of STAI-T [27] was used. The scale consists of 20 self-statements, to which participants respond on a four-point scale. Besides anxiety, experienced stress was also measured and investigated as a covariate. Actual experience during the subjectively selected stressful and relax days was monitored by participant's assessment of their state using stress-related adjectives and enumeration of actual potentially stressful events. More long-term stress experience was measured via perceived stress scale (PSS) which is a ten-item scale that reflects on the experiences of the last few weeks [28].

Quantification of CAR

As for the measures of CAR, we calculated the two most commonly used parameters in literature, namely the mean increase in cortisol (MnInc)[1] that captures the dynamic increase of salivary cortisol from awakening to CAR peak [29], and the area under the curve relative to ground (AUC_G)[2] [16, 30]. Besides CAR, cortisol concentrations in the individual samples throughout the day were also investigated.

Statistical modeling

To investigate both between and within subject effects, mixed regression modeling was used. This approach is appropriate for multiple repeated measures with both fixed and random parameters [31]. Investigated models included binary factors of sex, atopy, trait anxiety, and their interactions, while the covariates of awakening time and phase of the menstrual cycle were also checked consistently with CAR methodological recommendations [29]. Data from two research days were included as repeated measures with compound symmetry covariance structure. Statistical analyses were performed in IBM SPSS 20 software.

As the salivary cortisol concentrations in individual samples and CAR variables were positively skewed, data were transformed by log10 to get normal distribution before the statistical modeling.

[1] $\mathrm{MnInc} = \left(\frac{S2+S3}{2} - S1\right)$

[2] $\mathrm{CARAUC_G} = \left(\frac{S2+S1}{2} + \frac{S3+S2}{2}\right)$

Results

Experienced stress on adjective scale and PSS scale as evaluated by related samples t test differed significantly between the two measurement days—subjectively selected stressful and relax day. However, when looked at the cortisol concentrations, we found no differences between the "stressful" and "relax" days in any of the salivary cortisol variables including CAR (data not shown). Moreover, experienced stress (PSS score) did not correlate with any of the salivary cortisol variables (data not shown).

In the CAR MnInc, significant interactions between atopy and subject's sex explained the largest portion of the variance ($F_{(1,\ 54)} = 5.92$, $p < 0.05$). Main effect of atopy was also significant ($F_{(1,\ 54)} = 5.65$, $p < 0.05$; parameter estimate = − 0.33; $t = -3.04$, $p < 0.01$), with lower MnInc observed in allergic subjects. Similar, but non-significant trend was present in trait anxiety; with interaction of trait anxiety and sex ($F_{(1,\ 56)} = 3.54$, $p = 0.07$), and main effect of trait anxiety ($F_{(1,\ 56)} = 2.49$, $p = 0.12$; parameter estimate = − 0.25; $t = -2.17$, $p < 0.05$). Main effect of sex was not significant ($p = 0.10$).

As there were significant interactions between atopy and participant's sex, we also looked at the effects of atopy in men and women separately. In women, there were no differences explainable by atopy ($p = 0.95$), nor trait anxiety ($p = 0.78$). However, in men, the differences due to atopy ($F_{(1,\ 24)} = 6.91$, $p < 0.05$) were present. Similar, but non-significant, effect was observed also for trait anxiety ($F_{(1,\ 24)} = 3.75$, $p = 0.07$) (see Fig. 1).

In a different model, which used CAR AUC_G as outcome measure, there were no significant main effects of atopy ($p = 0.56$), trait anxiety ($p = 0.23$), nor their interactions with participant's sex (data not shown). Similarly, no main effect of sex on CAR AUC_G was found ($p = 0.27$).

Next to CAR, the course of cortisol changes throughout the day, as measured in all time points (S1–S5), were evaluated. In the mixed modeling with the data from all cortisol samples, we found main effect of time ($F_{(4,\ 126)} = 157.32$, $p < 0.01$), confirming the daily cortisol rhythm (Fig. 2).

With respect to individual time points, no significant difference explainable by atopy or trait anxiety was observed in the awakening sample (S1) and afternoon sample (S4). In the evening sample (S5), cortisol concentrations were higher in atopic subjects, but this effect was only significant in log-transformed values ($F_{(1,\ 53)} = 4.01$, $p < 0.05$) (Fig. 2a).

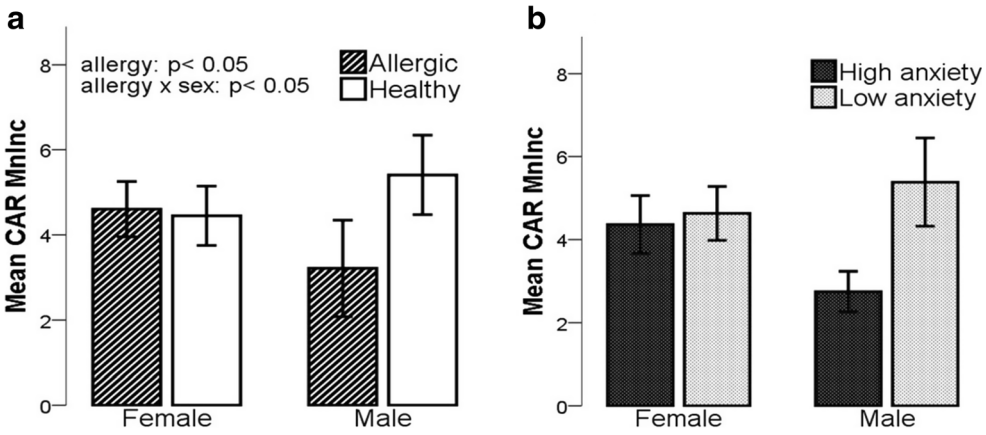

Fig. 1 Differences in cortisol awakening response (MnInc) in male and female subjects stratified according to atopy (**a**) and trait anxiety (**b**). Statistical significance as revealed by linear mixed modeling. *$p < 0.05$. Data are given as means ± SEM

Among the investigated covariates, the awakening time did not show any statistical significant effect in MnInc ($p = 0.96$) nor in S1 ($p = 0.68$). Mean awakening time in atopic subjects was 7:41 a.m. (SEM = 8.75 min) and 7:44 a.m. (SEM = 8.51 min) in healthy subjects. The difference between the awakening times evaluated by *t* test was not significant. The same applied to sex differences in awakening time. Similarly, the phase of the menstrual cycle in women was not significant for CAR or any of the salivary cortisol measures (data not shown).

Discussion

The present results show reduced CAR in atopic patients compared to that in healthy subjects. This effect was modulated by subject sex with a clear difference observed in males. While CAR was reduced, atopic patients did not show reduced overall cortisol secretion throughout the day. The differences in CAR between persons with high and low trait anxiety were not statistically significant, though the concentrations were lower in anxious subjects. There were no differences in CAR due to the subjectively evaluated stressfulness of the sampling day.

Present finding of reduced CAR (MnInc) in atopic subjects in comparison to healthy controls point towards a difference in HPA axis responsiveness in atopy. To our knowledge, there are no reports focused on CAR in patients with atopy. Buske-Kirschbaum et al. [3] reported no significant differences in morning cortisol values; however, no further information on the CAR evaluation was included. A part of the TRAILS study (Tracking adolescents' individual lives survey) [24] failed to establish a link between CAR and asthma progression. However, the measurements were done only on 1 day in children (11 years old), together with assessment of asthma symptoms at that time and in the follow-ups 3 and 5 years later. Another study by Schut et al. [25] measured the effects of cognitive behavioral intervention on CAR in atopic

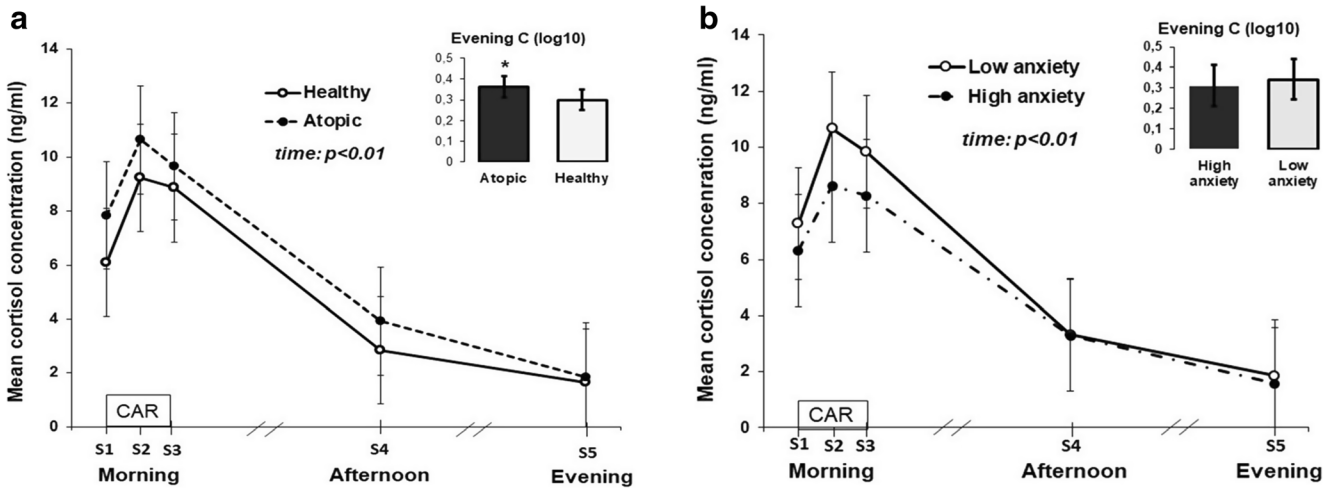

Fig. 2 Changes in salivary cortisol concentrations throughout the day in subjects stratified according to atopy (**a**) and trait anxiety (**b**). Bar charts in the top right corner express the difference in the log-transformed evening cortisol concentrations. Statistical significance as revealed by linear mixed modeling. *$p < 0.05$. Data are given as means ± SEM

dermatitis; however, while the authors described significant effect of the intervention, no comparison of atopic and healthy individuals was provided. On the other hand, the present finding of blunted CAR in atopy is consistent with the previously shown blunted cortisol responses to stressors [2, 3, 5].

Reduced CAR in atopy was dependent on subject's sex, as the interaction between sex and atopy explained the most variance in the model. When modeling MnInc separately in males and females, we found significantly blunted CAR in atopic males only. In healthy people, women were initially reported to have higher CAR when compared to men [14, 20]; however, there were problems with replication of these findings [32]. A detailed review on factors influencing CAR [15] concluded that the impact of sex on CAR is likely to by small. The present results in the whole sample are consistent with this conclusion. The blunted CAR in atopic patients observed in the present study allows the suggestion that sex differences may occur under pathological conditions.

Interestingly, reduced cortisol response at awakening was not accompanied by lower cortisol concentrations throughout the day. Moreover, evening cortisol concentrations (after logarithmic transformation) were higher in atopic subjects compared to healthy subjects. So far, salivary cortisol concentrations in atopy have been investigated mainly in context with acute stress responses [3] with less attention given to basal cortisol secretion. Despite lower activation at the time of awakening, the subjects with atopy did not show reduced cortisol concentrations during the rest of the day. This might be of relevance to immune system function and the course of the disease.

With respect to trait anxiety, we did not find any statistically significant differences in CAR. Only a trend to lower MnInc was observed in males with high trait anxiety when males were analyzed separately. This is in agreement with reports on blunted CAR in anxious persons [21, 22], as well as the findings of blunted cortisol stress reactivity in men with high trait anxiety [10, 26].

The data on neurobiological mechanisms of reduced CAR in atopic patients are not available. Based on animal studies showing altered glucocorticoid and cytokine response to an immune challenge in rats with low and high anxiety [33], we may hypothesize that immune alterations in atopic patients exhibiting higher anxiety may contribute to the lower CAR observed. With respect to other neuroendocrine parameters involved, an overexpression of vasopressin in the paraventricular nucleus of the hypothalamus has been suggested to contribute to the inhibition of the HPA axis during social defeat in rats with high anxiety behavior [34]. Thus, the mechanisms responsible for the lower CAR in patients with atopy are likely to involve both immune and neuroendocrine parameters.

It should be noted that all differences in CAR described above were observed in MnInc and no significant effects were found in the AUC_G measure of CAR. This may be due to lower sensitivity of AUC_G to change, as it measures more overall cortisol secretion following awakening, while MnInc reflects a more dynamic aspect of CAR [30, 35].

When comparing the subjectively selected stressful and relax days, we found no differences in CAR or cortisol concentrations in any of the time intervals studied. Previous investigations have shown that cortisol concentrations do not necessarily correspond with subjective experience [26, 36]. According to the results of Duncko et al. [26], there is an observable discrepancy between subjectively reported stress and neuroendocrine reactivity. We also have to consider that only potent stressors, mainly those associated with uncontrollable and social-evaluative elements of the situation, lead to observable changes in cortisol secretion [37, 38].

A limitation of the present study is that awakening time and sample times were recorded via subject's self-assessment. Another limitation of the study is relatively small and unequal number of subjects in the groups based on atopy, trait anxiety, and sex.

In conclusion, this study presents evidence on reduced CAR in people suffering from atopic diseases. This novel finding is consistent with reduced cortisol responses during psychosocial stress and allow suggesting an insufficient HPA axis reactivity in atopy. In atopic patients, significant sex differences were revealed showing reduced CAR in male subjects only. The reduced CAR was not accompanied by lower cortisol concentrations during the day. No relationship between CAR or any of the cortisol measures and stressfulness of the day, or more long-term stress perception (PSS) were present in the whole sample. The present data in atopic patients demonstrate that reduced HPA axis reactivity does not necessarily mean lower cortisol concentrations throughout the day.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interests.

References

1. Buske-Kirschbaum A, Jobst S, Wustmans A, Kirschbaum C, Rauh W, Hellhammer D. Attenuated free cortisol response to psychosocial stress in children with atopic dermatitis. Psychosom Med. 1999;59(4):419–26.
2. Buske-Kirschbaum A, Geiben A, Höllig H, Morschhäuser E, Hellhammer D. Altered responsiveness of the hypothalamus-

pituitary-adrenal Axis and the sympathetic Adrenomedullary system to stress in patients with atopic dermatitis. J Clin Endocrinol Metabol. 2002;87(9):4245–51.
3. Buske-Kirschbaum A, Ebrecht M, Hellhammer DH. Blunted HPA axis responsiveness to stress in atopic patients is associated with the acuity and severeness of allergic inflammation. Brain Behav Immun. 2010;24(8):1347–53.
4. Wamboldt MZ, Laudenslager M, Wamboldt FS, Kelsay K, Hewitt J. Adolescents with atopic disorders have an attenuated cortisol response to laboratory stress. J Allergy Clin Immunol. 2003;111(3):509–14.
5. Hlavacova N, Solarikova P, Marko M, Brezina I, Jezova D. Blunted cortisol response to psychosocial stress in atopic patients is associated with decrease in salivary alpha-amylase and aldosterone: focus on sex and menstrual cycle phase. Psychoneuroendocrinology. 2017;78(1):31–8.
6. Chrousos GP, Kino T. Glucocorticoid action networks and complex psychiatric and/or somatic disorders. Stress. 2007;10(2):213–9.
7. Segerstrom SC, Miller GE. Psychological stress and the human immune system: a meta-analytic study of 30 years of inquiry. Psychol Bull. 2004;130(4):601–30.
8. Hashizume H, Takigawa M. Anxiety in allergy and atopic dermatitis. Curr Opin Allergy Clin Immunol. 2006;6(5):335–9.
9. Buske-Kirschbaum A, Ebrecht M, Kern S, Gierens A, Hellhammer DH. Personality characteristics in chronic and non-chronic allergic conditions. Brain Behav Immun. 2008;22(5):762–8.
10. Jezova D, Makatsori A, Duncko R, Moncek F, Jakubek M. High trait anxiety in healthy subjects is associated with low neuroendocrine activity during psychosocial stress. Prog Neuro-Psychopharmacol Biol Psychiatry. 2004;28(8):1331–6.
11. Krieger DT. Rhythms of ACTH and corticosteroid secretion in health and disease, and their experimental modification. J Steroid Biochem. 1975;6(5):785–91.
12. Jezova D, Vigas M. Apomorphine injection stimulates beta-endorphin, adrenocorticotropin, and cortisol release in healthy man. Psychoneuroendocrinology. 1988;13(6):479–85.
13. Spath-Schwalbe E, Scholler T, Kern W, Fehm HL, Born J. Nocturnal adrenocorticotropin and cortisol secretion depends on sleep duration and decreases in association with spontaneous awakening in the morning. J Clin Endocrinol Metab. 1992;75:1431–5.
14. Wust S, Federenko I, Hellhammer DH, Kirschbaum C. Genetic factors, perceived chronic stress, and the free cortisol response to awakening. Psychoneuroendocrinology. 2000;25(7):707–20.
15. Fries E, Dettenborn L, Kirschbaum C. The cortisol awakening response (CAR): facts and future directions. Int J Psychophysiol. 2009;72(1):67–73.
16. Clow A, Hucklebridge F, Stalder T, Evans P, Thorn L. The cortisol awakening response: more than a measure of HPA axis function. Neurosci Biobehav Rev. 2010;35(1):97–103.
17. Thorn L, Hucklebridge F, Evans P, Clow A. Suspected non-adherence and weekend versus week day differences in the awakening cortisol response. Psychoneuroendocrinology. 2006;31(8):1009–18.
18. Hellhammer J, Fries E, Schweisthal OW, Schlotz W, Stone AA, Hagemann D. Several daily measurements are necessary to reliably assess the cortisol rise after awakening: state- and trait components. Psychoneuroendocrinology. 2007;32(1):80–6.
19. O'Connor DB, Hendrickx H, Dadd T, Elliman TD, Willis TA, Talbot D, et al. Cortisol awakening rise in middle-aged women in relation to psychological stress. Psychoneuroendocrinology. 2009;34(10):1486–94.
20. Pruessner JC, Wolf OT, Hellhammer DH, Buske-Kirschbaum A, Von Auer K, Jobst S, et al. Free cortisol levels after awakening: a reliable biological marker for the assessment of adrenocortical activity. Life Sci. 1997;61(26):2539–49.
21. Therrien F, Drapeau V, Lupien SJ, Beaulieu S, Doré J, Tremblay A, et al. Awakening cortisol response in relation to psychosocial profiles and eating behaviors. Physiol Behav. 2008;93(1–2):282–8.
22. Walker S, O'Connor DB, Schaefer A, Talbot D, Hendrickx H. The cortisol awakening response: associations with trait anxiety and stress reactivity. Personal Individ Differ. 2011;51(2):123–7.
23. Portella MJ, Harmer CJ, Flint J, Cowen P, Goodwin GM. Enhanced early morning salivary cortisol in neuroticism. Am J Psychiatr. 2005;162(4):807–9.
24. Vink NM, Boezen HM, Postma DS, Rosmalen JG. Basal or stress-induced cortisol and asthma development: the TRAILS study. Eur Respir J. 2013;41(4):846–52.
25. Schut C, Weik U, Tews N, Gieler U, Deinzer R, Kupfer J. Psychophysiological effects of stress management in patients with atopic dermatitis: a randomized controlled trial. Acta Derm Venereol. 2013;93(1):57–61.
26. Duncko R, Makatsori A, Fickova E, Selko D, Jezova D. Altered coordination of the neuroendocrine response during psychosocial stress in subjects with high trait anxiety. Prog Neuro-Psychopharmacol Biol Psychiatry. 2006;30(6):1058–66.
27. Mullner J, Ruisel I, Farkas G. Príručka pre administráciu, interpretáciu a vyhodnocovanie dotazníka na meranie úzkosti a úzkostlivosti. Bratislava: Psychodiagnostické a didaktické testy; 1980.
28. Cohen S, Kamarck T, Mermelstein R. A global measure of perceived stress. J Health Soc Behav. 1983;24(4):385–96.
29. Stalder T, Kirschbaum C, Kudielka BM, Adam EK, Pruessner JC, Wüst S, et al. Assessment of the cortisol awakening response: expert consensus guidelines. Psychoneuroendocrinology. 2016;63:414–32.
30. Pruessner JC, Kirschbaum C, Meinlschmid G, Hellhammer DH. Two formulas for computation of the area under the curve represent measures of total hormone concentration versus time-dependent change. Psychoneuroendocrinology. 2003;28(7):916–31.
31. Blackwell E, Carlos F, Mendes De Leon F, Miller G. Applying mixed regression models to the analysis of repeated- measures data in psychosomatic medicine. Psychosom Med. 2006;68:870–8.
32. Clow A, Thorn L, Evans P, Hucklebridge F. The awakening cortisol response: methodological issues and significance. Stress. 2004;7(1):29–37.
33. Salome N, Tasiemski A, Dutriez I, Wigger A, Landgraf R, Viltart O. Immune challenge induces differential corticosterone and interleukin-6 responsiveness in rats bred for extremes in anxiety-related behavior. Neuroscience. 2008;151(4):1112–8.
34. Frank E, Salchner P, Aldag JM, Salomé N, Singewald N, Landgraf R, et al. Genetic predisposition to anxiety-related behavior determines coping style, neuroendocrine responses, and neuronal activation during social defeat. Behav Neurosci. 2006;120(1):60–71.
35. Clow A, Edwards S, Owen G, Evans G, Evans P, Hucklebridge F, et al. Post-awakening cortisol secretion during basic military training. Int J Psychophysiol. 2006;60(1):88–94.
36. De Rooij SR, Schene AH, Phillips DI, Roseboom TJ. Depression and anxiety: associations with biological and perceived stress reactivity to a psychological stress protocol in a middle-aged population. Psychoneuroendocrinology. 2010;35(6):866–77.
37. Dickerson SS, Kemeny ME. Acute stressors and cortisol responses: a theoretical integration and synthesis of laboratory research. Psychol Bull. 2004;130(3):355–91.
38. Jezova D, Hlavacova N. Endocrine factors in stress and psychiatric disorders: focus on anxiety and salivary steroids. Ann N Y Acad Sci. 2008;1148(1):495–503.