The cortisol awakening response - normal values and confounds

Stefan Wüst[1], Jutta Wolf[1], Dirk H. Hellhammer[1], Ilona Federenko[1], Nicole Schommer[1], and Clemens Kirschbaum[2]

[1]*Center for Psychobiological and Psychosomatic Research, University of Trier, Germany*
[2]*Institute of Physiological Psychology II, University of Duesseldorf, Germany*

In several recent investigations it could be demonstrated that the free cortisol response to awakening can serve as an useful index of the adrenocortical activity. When measured with strict reference to the time of awakening the assessment of this endocrine response is able to uncover subtle changes in hypothalamus-pituitary-adrenal (HPA) axis activity, which are, for instance, related to persisting pain, burnout and chronic stress. Furthermore, it has been suggested that the HPA axis might serve as an indicator of allostatic load in subjects exposed to prolonged environmental noise. In the present paper four separate studies with a total of 509 adult subjects were combined in order to provide reliable information on normal values for the free cortisol response to awakening. Corresponding with earlier findings, a mean cortisol increase of about 50% within the first 30 minutes after awakening was observed. The intraindividual stability over time was shown to be remarkably high with correlations up to r=.63 (for the area under the response curve). Furthermore, the cortisol rise after awakening is rather consistent, with responder rates of about 75%. Gender significantly influenced early morning free cortisol levels. Although women showed a virtually identical cortisol increase after awakening compared to men, a significantly delayed decrease was observed. Confirming and extending previous findings, the present study strongly suggests that neither age, nor the use of oral contraceptives, habitual smoking, time of awakening, sleep duration or using / not using an alarm clock have a considerable impact on free cortisol levels after awakening. The cortisol awakening response can be assessed under a wide variety of clinical and field settings, since it is non-invasive, inexpensive and easy-to-employ. The present data provide normal values and information on potential confounds which should facilitate investigations into the endocrine consequences of prolonged exposure to environmental noise.

Keywords: Hypothalamus-pituitary-adrenal axis, cortisol awakening response, saliva

Introduction

Anticipation and exposure to psychological or physical stress frequently causes an activation of the hypothalamus-pituitary-adrenal (HPA) axis. Elevated CRH, ACTH and cortisol levels during stress are interpreted as allostatic (McEwen, 1998) or homeostatic (Munck et al., 1984) responses of the body. Chronic dysregulation of HPA activity seems to be associated with the onset and course of psychosomatic and psychiatric disorders. For instance, HPA hyperactivity was observed in major depression (Carroll et al., 1976, Holsboer et al., 1994, Staner et al., 1994, Pitts et al., 1995) and furthermore seems to be associated with susceptibility to infectious (Mason, 1991) and cardiovascular diseases (McEwen, 1998). On the other hand, HPA hyporeactivity was reported to be related to

autoimmune processes, such as lupus erythematosus (Weiner, 1991), multiple sclerosis (Adams and Victor, 1989), or neurodermitis (Schnyder, 1960, Buske-Kirschbaum et al., 1997). Moreover, with respect to the allostasis concept it was recently suggested that the HPA axis might serve as a read-out system for the sequelae of a prolonged exposure to environmental noise (Kirschbaum and Hellhammer, 1999).

Clinical or basic research on this neuroendocrine system depends on the availability of appropriate markers since HPA activity is characterised by large interindividual differences (Mason, 1968). Frequently, single blood or saliva samples for analysis of total or free cortisol are collected time-locked in the early morning hours and the resulting hormone value is interpreted as an index of unstimulated HPA activity (Gray et al., 1991, Vasankari et al., 1993, Walker et al., 1997). Although easy to assess, this index has a number of weaknesses. Single basal cortisol values measured during this time period are reported to have a rather low intraindividual stability (Schulz and Knabe, 1994, Coste et al., 1994). Moreover, they show large interindividual variation with a significant overlap between healthy individuals and patients with adrenal insufficiency or Cushing's disease (Laudat et al., 1988). Along with other factors these limitations may explain why significant and consistent correlations between single basal cortisol values and psychological variables, e.g. personality measures, cannot be expected. A more reliable index of unstimulated HPA activity is, for instance, a day-time cortisol profile. However, the assessment of this measure requires the collection of numerous saliva or blood samples over a long period of time. This relatively expensive and time-consuming method is of only limited use in studies with large cohorts, e.g. in epidemiological investigations.

Recently, it has been reported that cortisol levels rapidly increase after awakening (Späth-Schwalbe et al., 1992, Linkowski et al., 1993, Van Cauter et al., 1994). Studies from this and other laboratories suggest that repeated assessment of the awakening cortisol response can serve as a more useful index of adrenocortical activity. It can provide important information on the (re)activity of the HPA axis in addition to challenge tests like stimulation with hCRH or $ACTH_{1-24}$. Within the first 30 minutes after awakening, free cortisol levels rise by 50-60 % and remain elevated for at least 60 minutes (Pruessner et al., 1997, Schulz et al., 1998, Schmidt-Reinwald et al., 1999). This response was found to be independent of the time of awakening, total time slept, sleep quality, physical activity, or morning routines. Furthermore, the free cortisol response to awakening appears to be able to uncover subtle changes in HPA activity. The response magnitude and time course was shown to be significantly influenced by gender, persisting pain, burnout and chronic stress (Geiß et al., 1997, Pruessner et al., 1997, Pruessner et al., 1999, Schulz et al., 1998). In a recent study a further significant impact of genetic factors as well as birth weight and duration of pregnancy on the cortisol awakening response could be observed (unpublished data). Moreover, this HPA index is significantly correlated with the adrenocortical response to $ACTH_{1-24}$ (Schmidt-Reinwald et al., 1999) and with a decrease of secretory immunoglobulin A after awakening (Hucklebridge et al., 1998). Early morning free cortisol levels, measured with strict reference to the time of awakening, are easy and inexpensive to assess, consistent and show relatively good intraindividual stability across days and weeks.

Based on these encouraging findings the present paper aims to provide normal values and other basic methodological information on the free

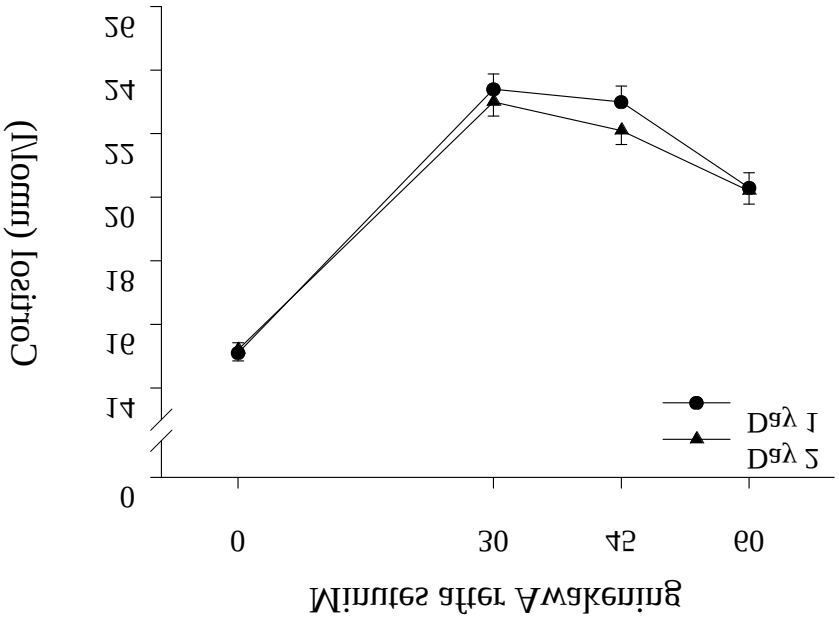


Figure 1. Mean salivary cortisol levels (SE) after morning awakening on two consecutive sampling days (n=509)

cortisol response to awakening for researchers who are interested in employing this novel index of adrenocortical activity.

Methods

Subjects and geral experimental outline

In four independent studies a total of 509 adult subjects with a mean age of 37.3 yrs. (18-71 yrs., SD=13.63) was investigated for early morning free cortisol levels after awakening. The group consisted of 319 females and 190 males and all subjects reported to be in good health. Twenty-three percent of the participants were smokers and 28.8% of the female subjects used oral contraceptives (OC). In one of the four studies (n=81) smokers as well as OC users were excluded. Moreover, on days of saliva sampling, a state questionnaire provided information on time of going to bed, total time slept, time of awakening, physical activity and self reports of health status and acute stress. Subjects completed the questionnaires at home and returned them to the laboratory along with the saliva samples.

Saliva collection

Early morning salivary cortisol levels were assessed at home on two consecutive days. Using the Salivette sampling device (Sarstedt, Rommelsdorf, Germany), subjects obtained saliva samples 0, 30, 45 and 60 minutes after awakening, on each day. Subjects were instructed not to brush their teeth before completing saliva sampling to avoid contamination of saliva with blood caused by micro-injuries in the oral cavity. Food intake 10 minutes before saliva sampling as well as smoking during the sampling period was not allowed. Besides these restrictions, subjects were free to follow their normal daily routines on the sampling days. Subjects stored the saliva samples in their freezers until completing the experimental protocol and then returned the samples to the laboratory.

Cortisol analysis

Salivary cortisol was analysed with a time-resolved immunoassay with fluorescence detection (DELFIA) as described in detail elsewhere (Dressendörfer et al., 1992). Intra- and

interassay variability of the assay was less than 10 and 12 percent, respectively.

Statistical analysis

Three-way analyses of variance (ANOVAs) with repeated measures (group by day by time) were computed to test for possible effects of several variables (e.g. sex, OC use, smoking) on the free cortisol response to awakening. Adjustments of degrees of freedom were employed according to Greenhouse-Geisser where appropriate and effect sizes were computed when ANOVA procedures revealed significant results. In order to receive indices for the cortisol response to awakening, areas under the curve (AUC) as well as 'mean increases' (MnInc: (Awakening $cortisol_{30min}$+AC_{45}+AC_{60})/3-AC_0) were calculated. Pearson correlations were computed between cortisol levels across the two sampling days in order to assess the stability of the cortisol awakening response over time. Furthermore, Pearson correlations were calculated between AUC as well as MnInc values and the subjects' age, total hours slept and awakening time.

Results

Corresponding with earlier findings, a significant increase of salivary cortisol levels after awakening could be observed ($F=223.08$, $p<.0001$). The effect size for the cortisol response was $f^2=.33$, explaining 25% of the variation of cortisol levels during the first hour after awakening. (Figure 1).

The mean cortisol levels as well as mean AUC and MnInc values across the two sampling days are shown in Table 1. Within the first 30 minutes, cortisol levels rose by approximately. 50% (7.84

Table 1. Normal values and retest reliability coefficients of salivary cortisol levels (SE) after morning awakening (n=509)

	Mean (nmol/l)	**SD**	**Minimum** (nmol/l)	**Maximum** (nmol/l)	$r_{day1 \times day2}$ *
$AC_{0\ min}$	15.12	6.25	1.35	44.78	0.37
$AC_{30\ min}$	22.95	9.13	1.00	60.30	0.51
$AC_{45\ min}$	22.31	9.33	0.80	74.25	0.62
$AC_{60\ min}$	20.23	8.89	1.14	67.49	0.66
MnInc	6.77	8.25	-24.33	43.42	0.47
AUC (arbitrary units)	1233.41	435.82	62.78	3295.50	0.63

(**$AC_{"x"min}$** = cortisol level "x" minutes after awakening, **MnInc** = mean increase *{AC_{30}+AC_{45} +AC_{60}}/3-AC_0}*, **AUC** = area under the curve {AC_0x30+{{AC_{30}-AC_0}x30}/2}+{AC_{30}x15+ {{AC_{45}-AC_{30}}x15}/2}+{AC_{45}x15+{{AC_{60}-AC_{45}}x15}/2}; * all correlations $p<0.001$)

Figure 2. Mean salivary cortisol levels (SE) after awakening in women (n=390) compared to men (n=119)

nmol/l) and started to decrease thereafter, without reaching baseline levels at the end of the sampling interval. A mean cortisol increase of 34% was still observed 60 minutes after awakening. Cortisol levels showed the expected marked interindividual variation, as indicated by the large standard deviations as well as the large differences between minimum and maximum values.

In order to estimate the consistency of the cortisol rise after awakening, responder rates were assessed. A cortisol response was defined as an increase of salivary cortisol levels of at least 2.5 nmol/l above individual baseline. This criterion was chosen according to Weitzman et al. (Weitzman et al., 1971) who viewed an increase of at least 55.2 nmol/l in total plasma cortisol measures as a secretory episode. Since in saliva only about two to five percent of total plasma cortisol levels are found this criterion appears to be rather strict. According to this criterion a cortisol response to awakening was observed in 76.8% of the subjects (mean across day 1 and 2).

The correlation between AUC values, which included the basal cortisol levels (AC_0) and MnInc values, which indicates the average change from AC_0 was r=0.52 ($p<0.001$). In order to assess the relationship between baseline levels and the magnitude of the cortisol awakening rise, a Pearson correlation between AC_0 and MnInc levels was calculated. The resulting coefficient of r=-.34 ($p<0.001$) indicates that high baseline levels are followed by smaller cortisol awakening responses. Next, the stability of the cortisol awakening response was estimated and the corresponding intercorrelations (day 1 x day 2) are also shown in Table 1. The resulting coefficients for MnInc and AUC were r=0.47 and r=0.63, respectively (both $p<0.001$). Thus, the explained variance for these correlations varied between 22 and 40 percent, indicating a moderate to high stability of the cortisol awakening response across days.

In the present investigation, which included only adult subjects, no significant impact of age on free cortisol levels after awakening could be observed. Correlations between age and AUC (r=-0.07) as well as MnInc levels (r=0.01) did virtually not exceed zero (both p>.10). Corresponding results were obtained with a median-split (<36 yrs. vs. ≥36 yrs.) and a subsequent ANOVA. The main effect as well as the time by age interaction did not reach statistical significance (both p>0.10).

As previously described (Pruessner et al., 1997) woman showed significantly larger increases of early morning free cortisol levels after awakening compared to men (interaction effect: F=18.35, p< 0.001; Figure 2). However, the size of this time by gender effect was rather small (f^2=0.03), thus explaining three percent of variability in cortisol levels after awakening. Next, the influence of oral contraceptive (OC) usage on the cortisol awakening response was tested and ANOVA did neither reveal a significant main effect (F=0.78, p>0.10) nor a significant time by OC interaction (F=2.14, p>0.05).

In smokers a slightly attenuated cortisol rise after awakening compared to non-smokers was observed. However, even though the time by smoking interaction reached statistical significance (F=3.22, p<0.05), the corresponding effect size of f^2=0.003 indicates that this effect explains less than one percent of variability in early morning cortisol levels and therefore it appears to be virtually negligible.

An effect of similar magnitude was observed when the impact of sleep duration on the cortisol awakening response was tested. On one hand this variable significantly influenced free cortisol levels after awakening. The correlation between sleep duration (mean=7.3 hrs., SD=0.99) and MnInc levels was r=-0.16 (p<0.05),suggesting a slightly larger cortisol awakening response in subjects who reported a shorter sleep length. And correspondingly, ANOVA revealed a significant time by sleep duration interaction after a median-split had assigned subjects to a 'short sleep' group and a 'long sleep' group, respectively (F=6.89; p<0.001). But on the other hand, this effect also can be almost ignored, since it explains less than one percent of variability in free cortisol levels after awakening (f^2=0.008). Moreover, no significant correlation between the cortisol awakening response and the individual awakening time (mean=7:45 AM, SD=1.06) could be observed, with correlation coefficients of r=0.04 (awakening time x MnInc) and r=-0.05 (awakening time x AUC; both p>0.10), respectively.

In the present study the subjects were free to wake up either spontaneously or to use an alarm clock. Therefore, the possible impact of this difference on the cortisol awakening response was tested. Combining both sampling days 47.5% of the participants reported that they woke up spontaneously and 52.5% used an alarm clock. As tested by ANOVA, neither the corresponding main effect (F=.06) nor the time by group interaction (F=1.00) turned out to be statistically significant (both p>0.10).

Discussion

Appropriate markers of HPA activity, which is characterised by both large inter- as well as marked intraindividual variability, are a substantial prerequisite for research on this neuroendocrine system. Several recent studies could demonstrate that the free cortisol response to awakening can serve as an useful index in psychobiological studies. The assessment of the cortisol awakening response is able to uncover subtle changes in HPA activity, which are, for instance, related to persisting pain, burnout and chronic stress (Geiß et al., 1997, Schulz et al., 1998, Pruessner et al., 1999).

In the present paper data from 509 adult subjects were combined in order to replicate recent findings and to provide further information on the free cortisol response to awakening for researchers who consider to include this index of adrenocortical activity in future studies.

Summarising the present data, waking up in the morning is a potent stimulus for the HPA axis. Corresponding with earlier findings, the magnitude of the cortisol increase within the first 30 minutes after awakening was about 50 % over individual baselines (i.e., at the time of awakening). An important characteristic of this endocrine response is the observed intraindividual stability over time, which was shown to be remarkably high for a basal cortisol measure. It varied between r=0.47 for the mean increase and r=0.63 for the area under the curve, respectively. In addition to the stability across days reported here, Pruessner et al., (1997) found correlation coefficients of comparable size even with a one week interval between sampling days. This intraindividual stability enhances the chance of finding consistent relations between basal HPA activity and psychological variables, e.g. personality measures or individual stress load.

Furthermore, the cortisol rise after awakening is rather consistent, with responder rates of about 77%. The finding that about 23% of the subjects do not show an increase of cortisol levels after awakening again documents the above mentioned marked interindividual variability of HPA activity. On the other hand, however, one could speculate that at least in some of the subjects the missing of a cortisol response is an artifact. For instance, the observed endocrine pattern would probably be significantly altered, if participants awake early in the morning, doze for a short period of time and fall asleep again for 30 minutes before they start to collect the first saliva sample. Nevertheless, preliminary findings from a study in shift-workers (unpublished data) suggest that 'real' non-responders do exist. Although all subjects in this study used an alarm clock and awoke between 4:00 AM and 5:00 AM, some participants did not show an cortisol increase after awakening.

In this study only data from adult subjects are presented, but recent findings from this laboratory suggest, that the cortisol awakening response is also useful in children. Pruessner et al. (1997) reported virtually identical retest reliability coefficients in a group of 42 children compared with adults and similar mean AUC levels in both age groups.

The previous finding that the cortisol awakening response is a rather robust phenomenon could be replicated in the present study. The data strongly suggest that neither age, nor the use of oral contraceptives, habitual smoking, time of awakening, total time slept or using / not using an alarm clock have a considerable impact on free cortisol levels after awakening. Not being forced to carefully control these variables can be regarded as a further advantage of this index.. Concerning the awakening time this conclusion has to be qualified by mentioning that in all reported studies the mean awakening time was in a normal range. Preliminary findings from the above mentioned study in shift-workers (unpublished) suggest an enhanced cortisol awakening response in subjects who awoke between 4:00 AM and 5:00 AM while a diminished response was found in participants who awoke between 11.30 AM and 2.30 PM.

A sex difference was observed in early morning salivary cortisol levels with woman showing larger responses compared to men. For the first 30 minutes after awakening, cortisol increases in woman were similar to those in men. However, while cortisol concentrations clearly decreased in men thereafter, women showed a delayed decrease resulting in larger AUC values compared to men. This result supports similar

findings in a previous study (Pruessner et al., 1997) suggestive of a rather consistent sex difference. It should be kept in mind, however, that only 3% of the total variability in cortisol levels are explained by the subject´s gender. The difference in cortisol levels 60 minutes after awakening between women and men was about 3 nmol/l. Future studies have to clarify whether or not such differences are of functional relevance for target tissues. Since large intra- as well as interindividual variability is a well known characteristic of HPA (re)activity, it can generally be assumed that most effects on the cortisol awakening response are relatively small. This assumption is consistent with previously uncovered effects with comparable sizes, for instance the impact of several aspects of chronic stress on the cortisol rise after awakening (Schulz et al., 1998, Wüst et al., unpublished).

In sum, the awakening cortisol response fulfils a number of important criteria required for the study of the HPA axis in larger cohorts. With respect to the impact of environmental noise on health, a repeated assessment of cortisol responses to awakening might prove to be a valuable tool for uncovering even subtle changes in HPA function resulting from noise exposure. In our view the morning cortisol rise qualifies as an easy yet potent method to evaluate the health consequences of environmental noise in epidemiological studies.

Correspondence Address
Stefan. Wüst
Center for Psychobiological and Psychosomatic Research, University of Trier, Dietrichstrasse 10-11, 54290 Trier, Germany.
Phone: +49-651-9758634
Fax: +49-651-9758640
e-mail: wuests@fpp.uni-trier.de

References

Adams, R. D. and Victor, M. (1989) Multiple Sclerosis and Allied Demyelinative Diseases. New York, McGraw-Hill

Buske-Kirschbaum, A., Jobst, S., Wustmans, A., Kirschbaum, C., Rauh, W., and Hellhammer, D. H. (1997) Attenuated free cortisol response to psychosocial stress in children with atopic dermatitis. *Psychosom. Med*. 59: 419-426

Carroll, B. J., Curtis, G. C., and Mendels, J. (1976) Neuroendocrine regulation in depression. *Arch. Gen. Psychiatry* 33: 1039-1044

Coste, J., Strauch, G., Letrath, M., and Bertagna, X. (1994) Reliability of hormonal levels for assessing the hypothalamic-pituitary-adrenocortical system in clinical pharmacology. *Br. J. Pharmacol.* 38: 474-479

Dressendörfer, R. A., Kirschbaum, C., Rohde, W., Stahl, F., and Strasburger, C. J. (1992) Synthesis of a cortisol-biotin conjugate and evaluation as a tracer in an immunoassay for salivary cortisol measurement. *J. Steroid. Biochem. Mol. Biol*. 43: 683-692

Geiß, A., Varadi, E., Steinbach, K., Bauer, H. W., and Anton, F. (1997) Psychoneuroimmunological correlates of persisting sciatic pain in patients who underwent discectomy. *Neurosci. Lett.* 237: 65-68

Gray, A., Feldman, H. A., McKinlay, J. B., and Longcope, C. (1991) Age, disease, and changing sex hormone levels in middle-aged men: results of the Massachusetts male aging study. *J. Clin. Endocrin. Metab*. 73: 1017-1025

Holsboer, F., Grasser, A., Friess, E., and Wiedemann, K. (1994) Steroid effects on central neurons and implications for psychiatric and neurological disorders. *Ann. N Y Acad. Sci.* 746: 345-359

Hucklebridge, F., Clow, A., and Evans, P. (1998) The relationship between salivary secretory immunoglobulin A and cortisol: neuroendocrine response to awakening and the diurnal cycle. *Int. J. Psychophysiol*. 31: 69-76

Kirschbaum, C. and Hellhammer, D. H. (1999) Salivary cortisol as a non-invasive measure of allostatic load. *Noise & Health* 4: 57-65

Laudat, H. M., Cerdas, S., Fournier, C., Guiban, D., Guilhaume, B., and Luton, J. P. (1988) Salivary cortisol measurement: a practical approach to assess pituitary adrenal function. *J. Clin. Endocrin. Metab*. 66: 343-348

Linkowski, P., Van Onderbergen, A., Kerkhofs, M., Bosson, D., Mendlewicz, J., and Van Cauter, E. (1993) Twin study of the 24-h cortisol profile: evidence for genetic control of the human circadian clock. *Am. J. Physiol.* 264: E173-E181

Mason, D. (1991) Genetic variation in the stress response: susceptibility to experimental allergic encephalomyelitis and implications for human inflammatory disease. *Immunol. Today* 12: 57-60

Mason, J. W. (1968) A review of psychoendocrine research on the pituitary-adrenal cortical system. *Psychosom. Med*. 30: 576-607

McEwen, B. S. (1998) Protective and damaging effects of stress mediators. *N. Engl. J Med.* 338: 171-179

Munck, A., Guyre, P. M., and Holbrook, N. J. (1984) Physiological functions of glucocorticoids in stress and their relation to pharmacological actions. *Endocr. Rev.* 5: 25-44

Pitts, A. F., Samuelson, S. D., Meller, W. H., Bissette, G., Nemeroff, C. B., and Kathol, R. G. (1995) Cerebrospinal fluid corticotropin-releasing hormone, vasopressin, and oxytocin concentrations in treated patients with major depression and controls. *Biol. Psychiatry* 38: 330-335

Pruessner, J. C., Hellhammer, D. H., and Kirschbaum, C. (1999) Burnout, perceived stress, and cortisol responses to awakening. *Psychosom. Med*. 61: 197-204

Pruessner, J. C., Wolf, O. T., Hellhammer, D. H., Buske-Kirschbaum, A. B., von Auer, K., Jobst, S., Kaspers, F., and Kirschbaum, C. (1997) Free cortisol levels after awakening: a reliable biological marker for the assessment of adrenocortical acitvity. *Life. Sci*. 61: 2539-2549

Schmidt-Reinwald, A., Pruessner, J. C., Hellhammer, D. H., Federenko, I., Rohleder, N., Schürmeyer, T. H., and Kirschbaum, C. (1999) The cortisol response to awakening in relation to different challenge tests and a 12-hour cortisol rhythm. *Life. Sci*. 64: 1653-60

Schnyder, U. W. (1960) Neurodermitis, Asthma, Rhinitis.Basel, Karger

Schulz, P., Kirschbaum, C., Pruessner, J., and Hellhammer, D. H. (1998) Increased free cortisol secretion after awakening in chronically stressed individuals due to work overload. *Stress Med*. 14: 91-97

Schulz, P. and Knabe, R. (1994) Biological uniqueness and the definition of normality. Part 2--The endocrine 'fingerprint' of healthy adults. *Med. Hypotheses* 42: 63-68

Späth-Schwalbe, E., Scholler, T., Kern, W., Fehm, H. L., and Born, J. (1992) Nocturnal adrenocorticotropin and cortisol secretion depends on sleep duration and decreases in association with spontaneous awakening in the morning. *J. Clin. Endocrin. Metab*. 75: 1431-1435

Staner, L., Linkowski, P., and Mendlewicz, J. (1994) Biological markers as classifiers for depression: a multivariate study. *Prog Neuropsychopharmacol Biol Psychiatry* 18: 899-914

Van Cauter, E. V., Polonsky, K. S., Blackman, J. D., Roland, D., Sturis, J., Byrne, M. M., and Scheen, A. J. (1994) Abnormal temporal patterns of glucose tolerance in obesity: relationship to sleep-related growth hormone secretion and circadian cortisol rhythmicity. *J. Clin. Endocrin. Metab.* 79: 1797-1805

Vasankari, T. J., Kujala, U. M., Heinonen, O. J., and Huhtaniemi, I. T. (1993) Effects of endurance training on hormonal responses to prolonged physical exercise in males. *Acta Endocrinol.* (Copenh.) 129: 109-113

Walker, B. R., Best, R., Noon, J. P., Watt, G. C. M., and Webb, D. J. (1997) Seasonal variation in glucocorticoid activity in healthy men. *J. Clin. Endocrin. Metab.* 82: 4015-4019

Weiner, H. (1991) In Psychoneuroimmunology. Ader, R., Felten, D. L. & Cohen, N., eds. Academic Press, San Diego.

Weitzman, E. D., Fukushima, D., Nogeire, C., Roffwarg, H., Gallagher, T. F., and Hellman, L. (1971) Twenty-four hour pattern of the episodic secretion of cortisol in normal subjects. *J. Clin. Endocrin. Metab*. 33: 14-22