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Genetic factors, perceived chronic stress, and the free cortisol response to awakening

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Abstract

Recent studies have demonstrated that the free cortisol response to awakening can serve as a useful index of hypothalamus–pituitary–adrenal axis (HPA) activity. This endocrine marker is rather consistent, shows good intraindividual stability across time and appears to be able to uncover subtle changes in HPA regulation. The present twin study investigated genetic factors as sources of the interindividual variation of the cortisol awakening response. Furthermore, the relationship between psychological variables and morning cortisol levels was studied.

On two consecutive days saliva samples were collected 0, 30, 45 and 60 minutes after awakening in 52 monozygotic and 52 dizygotic twin pairs. Moreover, samples were obtained at 0800, 1100, 1500 and 2000 h. ('short day-time profile'). Additionally, the participants filled out questionnaires assessing chronic stress load, self-esteem and self-efficacy.

Heritability estimates of h^2=0.40 for the mean increase and of h^2=0.48 for the area under the response curve indicate a significant impact of genetic factors on cortisol levels after awakening. However, no genetic influence on the short day-time profile could be observed. Furthermore, several aspects of perceived chronic stress, namely 'worries', 'social stress' and 'lack of social recognition' were significantly associated with the awakening cortisol response.

The evidence for a medium-sized, yet distinct genetic influence on cortisol levels after awakening is discussed with regard to a potential clinical relevance of genetic determinants of HPA (re)activity. In line with several recent studies, the present findings further support the view

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that the cortisol awakening responses is consistently enhanced under chronic stress conditions.
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1. Introduction

The availability of appropriate markers of hypothalamus–pituitary–adrenal axis (HPA) activity can be regarded as a substantial prerequisite for clinical or basic science studies of this neuroendocrine system. Frequently, single blood or saliva samples for analysis of total or free cortisol are collected at a predefined time 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 (Coste et al., 1994; Schulz and Knabe, 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.

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 which provides 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 this response was found to be independent of the time of awakening, sleep duration, sleep quality, physical activity, or morning routines (Pruessner et al., 1997; Schulz et al., 1998; Schmidt-Reinwald et al., 1999; Wüst et al., 2000). Furthermore, although the cortisol awakening response was shown to be altered in subjects who were woken earlier than they expected (Born et al., 1999), in a large cohort the free cortisol response did not differ between subjects who either woke up spontaneously or used an alarm clock (Wüst et al., 2000).

However, a number of factors influence the response magnitude and time course including gender, use of oral contraceptives, persisting pain, burnout or chronic stress (Geiß et al., 1997; Pruessner et al. 1997, 1999; Schulz et al., 1998). Moreover, this HPA index is significantly correlated with the adrenocortical response to $ACTH_{1–24}$ (Schmidt-Reinwald et al., 1999) and with the decrease of secretory immunoglobulin A after awakening (Hucklebridge et al., 1998). The relatively high intraindividual stability of the free cortisol awakening response (mean correlation of r=0.55 across studies) justifies the hypothesis that it can, in part, be regarded as a person trait, which, in turn may be influenced by genetic factors.

Although twin studies constitute a powerful method for identifying genetic influences on human physiological variables, only few attempts were previously made to estimate the impact of genetic factors on the regulation of cortisol levels. While Maxwell et al. (1969) observed a higher resemblance of unstimulated plasma cortisol levels in monozygotic (MZ) twin pairs compared to dizygotic (DZ) twin pairs only in female subjects, Meikle et al. (1988) found evidence for a moderate genetic impact on basal cortisol levels also in male twin pairs. Moreover, some aspects of the 24-h plasma cortisol profile were reported to be affected by genetic factors (Linkowski et al., 1993) while a decided genetic influence on the cortisol response to hCRH and, to a lower degree, to psychosocial stress has been observed by this laboratory (Kirschbaum et al., 1992).

Taking advantage of the unique genetic relationship among twins, the present study investigated genetic factors as potential sources of the large interindividual variation of the free cortisol response to awakening. In addition, the relationship between several psychological variables and early morning cortisol levels was studied.

2. Methods

2.1. Subjects

All twin pairs in the age range 8–65 yrs listed in the residents registration office of the city of Trier were contacted by mail and invited to participate in the study. A total of 104 pairs with a mean age of 19.6 yrs (8–64 yrs) signed up. As confirmed by later DNA fingerprint analysis (see below), this group comprised 52 monozygotic (MZ) and 52 dizygotic (DZ) pairs. The MZ group consisted of 62 females and 42 males, while in the DZ group 52 females and 52 males were investigated. The latter group (coincidentally) included 26 same-sex and 26 opposite-sex twin pairs. Twin pairs older than 15 yrs (n=51, mean age: 27.5 yrs) were considered ‘adult pairs’, those aged 15 years and younger (n=53, mean age: 12 yrs) were considered ‘children pairs’. All subjects were medication free except for use of oral contraceptives and reported to be in good health.

2.2. Saliva collection

Early morning salivary cortisol levels were assessed at home on two consecutive weekend 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. In order to compare the cortisol awakening response with a second index of unstimulated adrenocortical activity with respect to the impact of genetic factors, a shortened version of a day-time cortisol profile (sampling at 0800, 1100, 1500 and 2000 h) was also assessed on both days. Food intake 10 minutes before

saliva sampling as well as smoking on test days 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.

2.3. Psychological and sociodemographic assessment

Perceived chronic stress, self-esteem as well as self-efficacy were assessed in order to investigate the relationship between these psychological variables and the free cortisol response to awakening. Since the employed questionnaires are not suitable for children they were only administered to adult twin pairs.

Perceived stress was assessed by the 'Trier Inventory for the Assessment of Chronic Stress (TICS)', which was recently developed in this laboratory and is described in detail elsewhere (Schulz and Schlotz, 1999). Subjects have to indicate how often the described stressful situations were experienced during the past year. The TICS comprises six subscales, namely 'work overload', 'work discontent', 'social stress', 'lack of social recognition', 'worries', and 'intrusive memories'. Internal consistency ranges between 0.76 and 0.91 (Cronbach's α), and split-half-reliability between 0.79 and 0.89 (Guttman). The 'Rosenberg Self-Esteem Scale' (RSE) is a widely used measure of global self-esteem adapted for use in German by Ferring and Filipp (1996). It assesses the attitude of a person toward him- or herself. On each item participants have to indicate whether a given positive or negative aspect of one's self-esteem applies to them or not. Cronbach's α of the scale ranges from 0.81 to 0.88, and split-half-reliability was shown to range between 0.81 and 0.84 (Guttman). The scale 'Generalized self-efficacy' (GS, Schwarzer, 1994a,b) assesses the subject's estimation of her/his own capabilities to cope with demands and difficult situations. Subjects are required to state whether a given situation involving the aspect of self-efficacy is true for them. Cronbach's α for this scale varies between 0.74 and 0.93, depending on the sample, retest-reliability is 0.47 for men and 0.63 for woman after two years.

Moreover, information on sex, age, weight, smoking habits and chronic diseases were assessed. 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.

2.4. Cortisol analysis

Salivary cortisol was analyzed 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%, respectively.

2.5. Zygosity testing

If no clear evidence for dizygosity was apparent (e.g. discordant eye and/or hair color), a blood sample was drawn from both siblings and zygosity was determined by DNA fingerprint analysis employing the Multiplex–PCR–System 'DNA–Profiler–Kit' (Applied Biosystems, Weiterstadt). Briefly, 100 μg DNA are amplified in a Multiplex PCR, containing a total of nine different primer pairs for nine STR (short–tandem–repeat) loci. With the use of fluorescent primer labels, fragment lengths can be determined by capillary electrophoresis and laser-induced fluorescence. While fragment lengths in MZ twin pairs are identical, different fragment lengths between pairs indicate dizygosity. The probability of an incorrect identification of zygosity with this technique is less than 0.1%.

2.6. Statistical analysis

Three-way analyses of variance (ANOVAs) with repeated measures (group by day by time) were computed to test for possible effects of psychological variables 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 $\text{cortisol}_{30\text{min}}+AC_{45}+AC_{60})/3–AC_0$) were calculated. The respective indices for the shortened version of the day-time cortisol profile were (a) the sum of all four cortisol measurements (SUM) and (b) the difference between the 0800 h and 2000 h cortisol levels (DIF). Pearson correlations were computed between indices for the cortisol response to awakening and individual questionnaire scores.

The possible impact of genetic factors on cortisol variation was estimated for the mean levels of both sampling days for the variables AC_0, AUC, MnInc, CP_{0800h}, SUM and DIF. The rationale for this data aggregation was to enhance the proportion of variance due to the trait component of cortisol secretion by minimizing the influence of situational factors. The contribution of genetic factors to total phenotypic variance was estimated following the standard approach of 'heritability in the broad sense', which includes the additive genetic component as well as variance components due to genetic dominance and epistasis. This concept assumes no assortative mating and neither covariance nor interaction between genetic and environmental factors (for details see Vogel and Motulsky, 1982). Intrapair correlation coefficients (r_i) were calculated for each variable in monozygotic and dizygotic twin pairs. They take into account the variance within pairs (V_W) as well as between pairs (V_B) and were calculated as follows: $r_i=V_B/(V_B+V_W)$. This procedure results in higher intrapair correlation coefficients in very heterogeneous groups of twin pairs (i.e. in samples with high between pairs variance)since not only the genetic resemblance but also other similarities within a pair can contribute to a given correlation. In order to estimate the impact of this bias on the observed coefficients, additional intrapair correlations were calculated in a group of 'random' pairs who were matched for age

and sex but had no above-average genetic similarity. Significant correlations would indicate an effect of the matched variables on the intrapair coefficients in MZ and DZ pairs.

The resulting coefficients were tested for statistical difference with the formula from Bortz (1989, p. 264). In order to receive heritability indices the proportion of variance due to genetic factors was estimated by doubling the difference in r_i coefficients between MZ and DZ twins (h^2, Falconer, 1960). The contribution of shared environmental factors (c^2) was determined by subtracting the resulting h^2 from r_{MZ} and finally, the contribution of nonshared environmental factors and measurement error (e^2) was estimated by subtracting r_{MZ} from 1. (e.g. Loehlin and Nichols, 1976; McGue and Bouchard, 1998).

3. Results

Corresponding with earlier findings, a significant increase of salivary cortisol levels after awakening could be observed with a F-value of 58.97 for the main effect of awakening ($P<0.0001$). Within the first 30 minutes, cortisol levels showed a 38.5% (i.e. 4.47 nmol/l) mean elevation and then started to decrease. The effect size for the cortisol response was $f^2=0.22$, explaining 18% of the variation of cortisol levels during the first hour after awakening. The short day-time cortisol profile showed the expected pattern with cortisol levels continuously declining from 0800 h to 2000 h ($F=283.04$, $P<0.0001$, $f^2=1.06$, $\omega^2=0.51$) (Fig. 1a and b).

The focus of the present study was to estimate the impact of genetic factors on salivary cortisol levels after awakening and the results of the corresponding analysis are shown in Table 1. In monozygotic twins (MZ) the intrapair correlation coefficient for the single cortisol value AC_0 was $r_i=0.39$, whereas the similarity was higher for the indices MnInc and AUC, which included all four measurements of the morning cortisol response, with correlations of $r_i=0.58$ and $r_i=0.63$, respectively. Although the morning cortisol response shows good intraindividual stability over time, thus suggesting that this endocrine response partly reflects a personal trait, it is still influ-

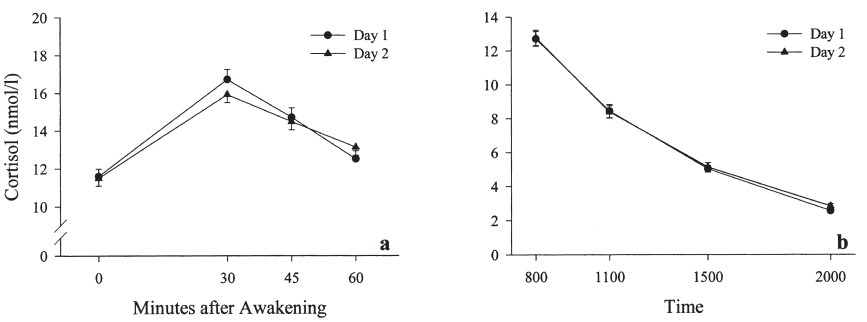


Fig. 1. Mean salivary cortisol levels (±SE) on two consecutive sampling days (a) after morning awakening (n=208) and (b) at 0800, 1100, 1500 and 2000 h. ('short day-time cortisol profile', n=208).

Table 1
Heritability analysis for the free cortisol response to awakening and for the shortened version of the day-time cortisol profile[a]

	MZ Pairs r_i	DZ Pairs r_i	$z_{MZ\ vs\ DZ}$	h^2	c^2	e^2	RG Pairs r_i[ii]
Cortisol after awakening							
AC_{0min}	0.39	0.26	1.04	0.26	0.13	0.61	0 (−0.03)[i]
MnInc	0.58	0.38	1.86*	0.40	0.18	0.42	0 (−0.02)[i]
AUC	0.63	0.39	2.34**	0.48	0.15	0.37	0.16
Cortisol profile							
CP_{0800h}	0.47	0.50	−0.28	0 (−0.06)[i]	0.47	0.53	0.16
SUM	0.41	0.40	0.09	0.01	0.40	0.59	0.12
DIF	0.62	0.52	1.06	0.20	0.42	0.38	0.11

[a] (AC_{0min}=cortisol level 0 minutes after awakening, MnInc=mean increase, AUC=area under the curve, CP_{0800h}=cortisol level at 0800 hrs., MZ=monozygotic (n=52 pairs), DZ=dizygotic (n=52 pairs), RG='random group' (n=100 pairs), r_i=intrapair correlation coefficient, $z_{MZ\ vs\ DZ}$=z statistics of r_i comparisons between MZ and DZ pairs, h^2=estimated heritability, c^2=estimated impact of shared environmental factors, e^2=estimated impact of nonshared environmental factors and measurement error, *$P<0.05$, **$P<0.01$, [i]h^2 values as well as intrapair coefficients <0 are not defined and were interpreted as 0), [ii]all $P>0.1$.

enced by situational factors to a considerable degree. Therefore, the magnitude of r_i for MnInc and AUC, can be regarded as being relatively high. In dizygotic pairs (DZ) all intrapair correlations were lower, ranging from r_i=0.26 for AC_0 to r_i=0.39 for AUC. Opposite-sex DZ pairs were not excluded from further analyses since correlations in this subgroup were comparable with corresponding coefficients in same-sex twins (opposite-sex: r_{AC0}=0.21, r_{MnInc}=0.40, r_{AUC}=0.38; same-sex: r_{AC0}=0.30, r_{MnInc}=0.35, r_{AUC}=0.39). In the 'random group' (RG), the correlations did not exceed 0.16 ($P>0.1$). Testing the MZ and DZ intrapair correlations for differences between the two groups revealed that the coefficients for MnInc and AUC were significantly higher in the MZ group ($P<0.05$ and $P<0.01$, respectively), while the AC_0 coefficients were not significantly different ($P>0.1$). The resulting heritability estimates for MnInc (h^2=0.40) and AUC (h^2=0.48) indicate a medium-sized, yet distinct impact of genetic factors on free cortisol levels after awakening. Shared environmental factors (c^2) seem to have only a minor influence, whereas nonshared environmental factors and measurement errors (such as situational factors) further determine a substantial part of the variation of early morning salivary cortisol levels. Since similarity of AC_0 values was not significantly higher in monozygotic pairs, the corresponding h^2 of 0.26 cannot be interpreted as evidence for heredity.

In contrast, no genetic influence on the day-time cortisol profile (shortened version) could be observed. In MZ siblings the estimated intrapair correlations (r_i=0.47 for the 0800 h cortisol level — CP_{0800h} —, r_i=0.41 for SUM, and r_i=0.62 for DIF) were comparable to the corresponding correlations for indices of the awakening cortisol response. However, DZ twin pairs showed almost identical similarities for CP_{0800h} (r_i=0.50) as well as for SUM (r_i=0.40) and only a marginally lower correlation for DIF (r_i=0.52). Again, intrapair correlations in the 'random group' were

very low and not significant, with coefficients ranging between r_i=0.11 (DIF) and r_i=0.16 (CP_{0800h}), indicating a virtually complete lack of concordance. Neither intrapair correlations for CP_{0800h} nor the corresponding coefficients for SUM or DIF were significantly higher in the MZ group compared with the DZ group. Accordingly, heritabilities of h^2=0.0 for the 0800 h cortisol level, h^2=0.01 for SUM and h^2=0.20 for DIF as well do not support the assumption of a considerable genetic impact on this index of basal adrenocortical activity. The estimated c^2 values of 0.47 for CP_{0800h}, 0.40 for SUM and 0.42 for DIF were higher compared to the corresponding coefficients for the cortisol response to awakening, indicating a substantial contribution of shared environmental factors. Nonshared environmental factors and measurement errors as well seem to have an important impact on this type of day-time cortisol profile as suggested by e^2 coefficients ranging between 0.38 (DIF) and 0.59 (SUM).

In the present study neither an impact of self-esteem, as assessed with the RSE, nor an impact of self-efficacy, as estimated with the 'Generalized Self–Efficacy'–scale, on free cortisol levels after awakening could be observed. Correlations between AUC as well as MnInc cortisol levels and both psychological variables failed to reach statistical significance (RSE×AUC: r=−0.01; RSE×MnInc: r=−0.16; self-efficacy×AUC: r=−0.06; self-efficacy×MnInc: r=−0.19; all P>0.1). Corresponding results were obtained with a median-split (low vs. high self-esteem and low vs. high self-efficacy, respectively) and a subsequent ANOVA. Both main effects as well as the interactions (time by RSE, time by self-efficacy) did not reach statistical significance (all P>0.1).

In contrast, the perceived chronic stress load did alter the cortisol response to awakening. As shown in Fig. 2, three out of six scales of the 'Trier Inventory for the Assessment of Chronic Stress', namely 'worries', 'social stress' and 'lack of social recognition', were significantly associated with early morning free cortisol levels. ANOVA revealed a significant time by worries interaction after a median-split had assigned subjects to a 'low worries load' group and a 'high worries load' group, respectively (F=2.93; P<0.05). The effect size for this interaction on the cortisol awakening response was rather small (f^2=0.03), thus explaining only 3% of variability in early morning cortisol levels. Subjects reporting a high worries load showed lower mean cortisol baseline levels but a higher increase within the first 30 minutes after awakening compared with the low worries load group. After 60 minutes mean cortisol values were lower in the latter group, suggesting a slower decrease of early morning free cortisol levels in subjects who perceive chronic stress due to worries (Fig. 2a).

Furthermore, the degree of social stress had a distinct impact on the variation of salivary cortisol levels in the first hour after awakening. The mean hormone response was significantly higher in subjects who scored high on this TICS scale (group 'high social stress', assignment by median-split) in comparison with volunteers from the low social stress group. The respective F-value for the (time by social stress) interaction was 4.73 (P<0.01) with an effect size of, again, f^2=0.03, indicating that this interaction explains 3% of variability in the awakening cortisol response (Fig. 2b).

Finally, a similar result was yielded when the group was divided at the median into a subgroup of volunteers reporting a high lack of social recognition and a sub-

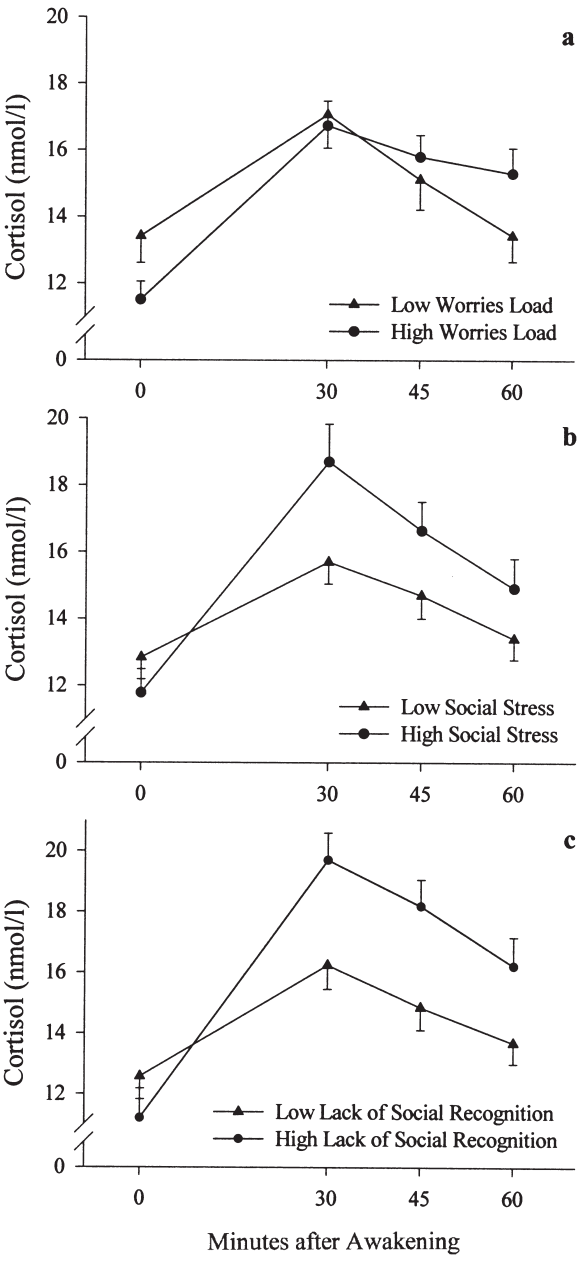


Fig. 2. Mean salivary cortisol levels (±SE) after awakening in adult subjects scoring either high or low on one out of three scales of the 'Trier Inventory for the Assessment of Chronic Stress' (assignment by median-split). (a) worries (low load: n=48; high load: n=54), (b) social stress (low: n=44; high: n=58), (c) lack of social recognition (low: n=46; high: n=56).

group with low lack of social recognition, respectively. ANOVA revealed a significant time by group interaction (F=4.78; $P<0.01$) with higher mean free cortisol levels after awakening in subjects with high lack of social recognition. With an f^2 of 0.03 the effect size of this interaction was identical with the effects of worries and social stress on early morning cortisol secretion (Fig. 2c).

4. Discussion

Several studies could demonstrate that the free cortisol response to awakening can serve as a useful index, which is rather consistent, shows good intraindividual stability across time and appears to be able to uncover subtle changes in HPA activity (Geiß et al., 1997; Pruessner et al. 1997, 1999; Hucklebridge et al., 1998; Schulz et al., 1998; Schmidt-Reinwald et al., 1999).

To these findings, the present study adds strong evidence for a significant genetic influence on the morning cortisol response to awakening in healthy individuals. A significant, medium-sized genetic impact on the two indices which include all four measurements of the morning cortisol response (+0,+30,+45 and +60 min.) could be observed (h^2 for MnInc:0.40; for AUC:0.48). The corresponding intrapair correlations can be regarded as relatively high, when the trait component reducing influence of situational factors on cortisol secretion is considered. A similar genetic effect on AC_0 was not observed, suggesting that the endocrine response to awakening, but not the (still unstimulated) cortisol level immediately after awakening is under detectable genetic control. The comparison with the intrapair coefficients in the 'random group', i.e. in pairs of subjects with neither above-average genetic similarity nor above-average shared environmental factors, indicates that even in dizygotic siblings the morning cortisol response shows a noteworthy concordance.

In contrast, the presented data do not support the assumption of a genetic impact on any index of the short day-time cortisol profile. Heritability estimates varied between h^2=0 and h^2=0.20. However, in identical twins the overall similarity of the day-time cortisol profile was not lower than the similarity of the awakening cortisol response. Nevertheless, a genetic effect could not be demonstrated since fraternal twin pairs as well showed intrapair correlations of a similar magnitude. As indicated by relatively high c^2 values, this finding suggests that shared environmental factors have a great impact on this type of day-time cortisol profile. One could speculate that past experiences in a broad sense as well as activities and demands on the sampling days have a more pronounced influence on the cortisol profile during the day in comparison to the awakening response. Furthermore, DZ twins could be as similar as monozygotic pairs with regard this factors. Linkowski et al. (1993) characterized the circadian rhythmicity of cortisol secretion by several measures, including amplitude, phase, and overall waveshape. Their report of a genetic impact on the 24-h cortisol profile appears to be inconsistent with the present findings. However, at closer inspection these discrepancies disappear, since Linkowski et al. observed a significant genetic control only for the timing of the nocturnal nadir and for the proportion of overall temporal variability of the cortisol pulses (both indices were

not assessed in the present study and are presumably not significantly associated with the measures presented here).

Evidence from both animal as well as clinical studies emphasize the relevance of studies on the heritability of HPA regulation. A hereditary HPA hyporeactivity in Lewis (LEW/N) rats was shown to be associated with an increased vulnerability for experimentally induced chronic inflammatory processes, whereas in rats with a highly responsive HPA axis (Fischer, F344/N rats) a relative resistance to these processes was observed. (Sternberg et al., 1989a,b; Calogero et al., 1992). On the other hand, a genetically determined HPA hyperreactivity in mice seems to be related to an elevated frequency of infectious disease symptoms and cancer (BALB/C vs. C57 animals, Mason, 1991).

Further results suggesting a clinical relevance of genetically determined differences in HPA responsiveness were obtained in human studies on mutations of the glucocorticoid receptor (GR). In patients with generalized inherited glucocorticoid resistence (GIGR, Karl and Chrousos, 1993; Chrousos et al., 1996; Werner and Brönnegard, 1996 for overview) an above chance occurrence frequency of point mutations in the ligand-binding domain of the GR was observed (Hurley et al., 1991; Malchoff et al., 1993; Karl et al., 1995). However, polymorphisms of the GR locus are not only associated with the rare GIGR syndrome but rather could be an important source of the above mentioned general large interindividual variability of HPA activity. For instance, clinical studies have suggested that a common polymorphism of the GR locus (BclI RFLP, Murray et al., 1987) is linked to altered GR function. The occurrence frequency of the larger GR allele was reported to be elevated in a group genetically predisposed to develop hypertension (Watt et al., 1992), in obese hyperinsulinemic women (Weaver et al., 1992) and in male and female subjects with a large abdominal visceral fat area (Buemann et al., 1997). Thus, several lines of evidence support the view that genetic determinants of the HPA reactivity may constitute either risk or protective factors for the onset and course of stress-related disorders.

Regarding psychosocial measures, high scores on three out of six TICS scales, namely ‘worries’, ‘social stress’, and ‘lack of social recognition’, were significantly associated with enhanced early morning free cortisol levels. Intercorrelations ranged between 0.48 and 0.53, indicating that these three scales assessed, at least in part, different aspects of chronic stress. Although these effects were found to be rather small (f^2=0.03 for each effect), this finding is remarkable since similar relations between chronic stress, as measured with the TICS, and the cortisol awakening response could be demonstrated in two further recent studies. Schulz et al. (1998) reported an increased free cortisol secretion after awakening in subjects perceiving high work overload while Wüst et al. (unpublished data) found a similar relation to social stress in a sample of 383 healthy adults. Corresponding with studies employing the TICS, an association between prolonged job strain and early morning cortisol elevations was observed by Steptoe et al. (2000). Thus the association between enhanced cortisol awakening responses and high chronic stress can be regarded as rather consistent.

Moreover, in the sample studied in the present investigation the mean scores were in the normal range for all TICS scales and even in the ‘high stress’ groups the

scores did not exceed mean normal values plus one standard deviation. One could speculate that the reported effects would be enhanced in subjects under more pronounced chronic stress. Future studies have to clarify whether or not the observed cortisol increase differences are of functional relevance for target tissues.

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