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Chronotype and Cortisol Awakening Response (CAR) The Influence of the Chronotype on the Awakening Response of Cortisol in the Morning

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ABSTRACT

Background: **The chronotype describes the behavioral daytime preference. According to an inherent but interindividually strongly varying biological clock, humans try to best adapt to their environment by tuning their internal clock and therefore their sleep-wake cycle to the social clock, which is reflected by work schedules etc. The chronotype seems to be basically associated with the timing and controlling of the circadian rhythms of biological and psychological parameters. In general, morning types show earlier acrophases and maximum values of biological factors compared to evening types. Like most physiological parameters cortisol follows a strong circadian rhythm, with a peak immediately after awakening, the so-called cortisol awakening response (CAR). Since glucocorticoids in general are assumed to play a key role in the timing and synchronization of the internal clock and the regulation of the transcription in the DNA, a well-tuned CAR might be crucial for the synchronization of one's own organism to the environment. *Purpose:* Since a stable circadian rhythm in general seems to be health-protective, we aimed to determine the association between the chronotype and the CAR in 25 healthy men. *Results:* Our results suggest that evening types show a lower total amount of cortisol, but a significantly prolonged phase of cortisol increase within the first hour after awakening. *Conclusion:* Our data might suggest that an inadequate synchronization between inert chronotype and environment results in an extenuated CAR.**

Key words: chronotype, cortisol, awakening response, circadian rhythm, morningness/ eveningness

INTRODUCTION

Humans show large interindividual differences in the organization of their behavior within the course of a 24h-day. This can be specifically stated in terms of the structure of the sleep-wake cycle and can be explained by the interindividually varying adjustment of the internal biological clock to the external daytime and nighttime. Accordingly, morning types show earlier bedtimes and waking-up times compared to evening types [1]. The chronotypical preference depends on several factors, such as the intensity of light irradiation and entrainment [2] or polymorphisms of the so-called clock genes [3]. Evening types in particular often experience the problem of a deficient fit between their inherent biological clock and the social clock (e.g. work schedules etc.). The discrepancy between working days and free days

might therefore result in a sleep deficit during the week, which is compensated at weekends. The health-related aftermaths of such "social jetlags" are not yet sufficiently clear [4].

Like most biological and psychological parameters, cortisol too is subject to a robust circadian rhythm, peaking in the morning immediately after an individual's awakening and decreasing as the day progresses [5]. The synthesis of cortisol is induced by the activation of the hypothalamus-pituitary-adrenal (HPA) axis. The corticotropin-releasing hormone (CRH) is released from the paraventricular nucleus (PVN) of the hypothalamus, inducing the synthesis of adrenocorticotropin (ACTH). The circadian rhythm of cortisol is most probably controlled by the suprachiasmatic nucleus (SCN) via a neuroendocrine pathway, since the absence of the SCN results in a rhythmic deficiency of cortisol [6]. Furthermore, data from human studies implicate an additional autonomous pathway between the SCN and the adrenal glands, which might be crucial for the synchronization of the body's peripheral cells [7,8]. It is known that glucocorticoids can bind to specific sites called glucocorticoid-sensitive elements on the DNA and induce or repress the transcription of genes, which implies an important role of glucocorticoids in the regulation of gene expression. This is also the case for some so-called clock genes – especially for *Per1* [9] – which among other things are responsible for the tuning of the internal clock and therefore for the control of the circadian rhythm [10-12].

About 77% of healthy subjects show a relatively stable "cortisol awakening response" (CAR) over a series of assessed days. According to the definition of Wust and colleagues, an increase of at least 2.5 nmol/l above the individual level of cortisol might identify "responders" to the naturally occurring process of awakening [13]. Generally, the CAR can be understood as a characteristic unit that can be interpreted as an index for adrenocortical activity [14]. The chronotype, in turn, seems to be closely associated with the controlling and timing of the pulsatile and rhythmic secretion of cortisol [15-17]. So far, data support that morning types display higher cortisol concentrations after awakening compared to evening types [18,19].

A well-functioning circadian rhythm seems to be essential for physiological as well as mental health. In our study, we therefore aspired to investigate the association between chronotype and CAR. Based on previous research [18,19], we assumed specific associations between the CAR and the chronotypes. Our aim was to further elucidate the course of the CAR according to different chronotypes.

METHOD

Study Population, Recruitment Criteria and Prestudy Conditions

Subjects were recruited at the University of Zurich. Exclusion criteria were jetlag, medication intake, sleep disorders, intake of psychotropic substances, psychological or physical illness, hospitalization, smoking and shift work within four months prior to the data acquisition. Data were collected from 31 mentally and physiologically healthy men aged 20 to 30. Due to the fact that sleep habits might change with age [20,21], this range was chosen in order to better control for this effect. The study participants were asked to adhere to a strict routine for one week before participating in the investigation, including regular eating and sleeping times, which they had to record in a diary and fill in a chronotype questionnaire.

The study participants were instructed to collect their morning cortisol on two consecutive days in the form of three saliva samples and bring them to the laboratory. The samples were collected immediately after, 30 minutes after and 60 minutes after awakening. At 0800h they arrived in our laboratory and handed in their saliva samples. This early time was chosen in order to simulate a regular work day after a week during which the students lived after their own inherent circadian rhythm. In order to increase the reliability of the data, the mean of the two CARs was used for statistical analyses.

However, six subjects showed a flattened CAR and did not reach an increase of 2.5nmol/l on at least one of the study days. It could not be determined whether these flat cycles indicated non-responders or whether they resulted from a lack of compliance. A flattened CAR might suggest an abnormal HPA-axis activity [13, 22-24]. As a precaution, we therefore decided to exclude these subjects from the calculations, which led to a sample size of n=25.

Ethics

This study was conducted according to the declaration of Helsinki. The study protocol was approved by the ethics committee of the canton of Zurich (Department of Internal Medicine, University Hospital of Zurich) and all participants provided written informed consent regarding participation in the study.

Data Collection and Analysis for Biological as well as Psychometric Parameters

Cortisol samples were collected using Salivettes (Sarstedt, Sevelen, Switzerland) and subsequently frozen at -20°C. Saliva samples were assayed in the Laboratory of Biopsychology of the Technical University of Dresden, Germany (Luminescence Immunoassay, IBL).

The assessment of the chronotype was conducted using the "Munich Chronotype Questionnaire" [1] consisting of 12 questions concerning preferences of sleep habits. One of the advantages of the MCTQ is the calculation of the midsleep of working as well as free days, which considers the correction of a possible social jetlag in subjects [25]. As a consequence of the small sample size, the original seven categories of chronotypes were summarized into three main categories: morning type, normal type and evening type according to the scale of the MCTQ.

Statistics

Statistics were calculated using SPSS 19.0 for Mac. To compare the total amount of cortisol in the morning, areas under the curve were calculated using the formula: $AUCg = ((m_1+m_2)/2 \times t_{1-2}) + ((m_2+m_3)/2 \times t_{2-3}) + ((m_3+m_4)/2 \times t_{3-4}) + \ldots + ((m_x+m_y)/2 \times t_{x-y})$. The total amount of increase of cortisol was determined using the formula: $AUCi = AUCg - m_1 \times t_{total}$ [26]. Percentage change was calculated using the formula: change in % from m1 to m2 = $((m_2-m_1)/m_1)x100$.

According to the Kolmogorov-Smirnov test, the calculated dependent variables were normally distributed, with the exception of the percentage change between measurement time points one and two. Therefore, in this case, a possible difference between chronotypes was calculated using the Kruskal-Wallis test for non-parametric data. The other examinations considering possible differences between the chronotypes were calculated using the general linear model for univariate analysis of variance and for repeated measures, respectively, in order to control for the influence of time (ANOVA). Post-hoc tests between the groups were calculated with t-tests for independent samples and corrected according to Bonferroni (for parametric data). Where sphericity could not be assumed, the calculation was corrected according to Greenhouse-Geisser.

RESULTS

As described above, the subjects were classified into three groups according to their score on the MCTQ, resulting in nine subjects of the morningness type (36%), 12 subjects of the normal type (48%) and four subjects of the eveningness type (16%). According to their sleep diaries, the three types differed in their waking-up time ($F=14.102$; $p<0.000$) and bedtime ($F=19.808$; $p<0.000$), but not in their sleep duration ($F=0.641$; $p=0.537$; see table 1).

Table 1: waking up, sleeping time and sleep duration according to chronotypes Waking up and sleeping times differ significantly between the chronotypes (post hoc tests adjusted to Bonferroni for waking up time show at least p<0.002 for all comparisons; for sleeping time all comparisons p<0.000). Sleep duration did not differ significantly between the chronotypes.

chronotype	n	waking up time	sleeping time	sleep duration
morning type	9	0715h	2320h	7h 58 min
normal type	12	0759h	2341h	8h 17 min
evening type	4	0942h	0136h	8h 15 min

n=number of subjects; waking up and sleeping time (free running) as well as sleep duration are data from the sleep diary; times averaged over 7 days and 6 nights.

Univariate analysis of variance for repeated measures showed that the factor time had a significant influence on the three measurement time points (F=16.720; p<0.000), indicating the morning peak of cortisol. The mean increase from the first to the second time point amounted to 123%, while the mean decrease between time points two and three was -9%. As can be seen from figure 1, the cortisol concentration immediately after awakening is the lowest in evening types, while the morning as well as the normal types display higher cortisol levels (F=1.944; p=0.167). The courses of the cortisol concentration within the first hour of awakening depict that evening types also show the lowest concentrations 30 minutes after awakening, followed by morning types, while the normal types show the highest peak (F=4.387; p=0.025; post hoc: normal type > evening type, p=0.022). Normal types also show the highest concentrations 60 minutes after awakening, while evening types show the lowest concentrations and morning types are in between (F=0.608; p=0.553). However, the area under the curve of the total amount of cortisol within the first hour after awakening narrowly misses a significant difference between the chronotypes (F=3.346; p=0.054), but does indicate a lower total amount of cortisol of the CAR in evening types compared to normal types (post hoc: normal type > evening type: p=0.052).

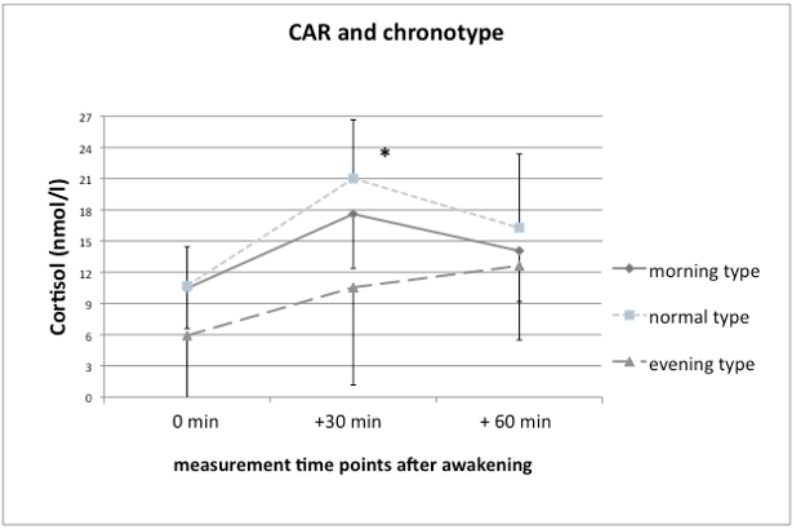

Figure 1: The cortisol awakening response according to chronotypes

The cortisol awakening response is shown according to the chronotypes. Values are given in means and with standard deviations. Evening types show a smaller area under the curve with respect to the ground and therefore a smaller total amount of cortisol within the first hour after awakening. Furthermore, they show a prolonged increase phase compared to normal and morning types, resulting in a less accentuated cortisol peak.

This effect could not be seen when the area under the curve was measured with respect to the first assessment (AUCi: F=0.888; p=0.426), suggesting a comparable increase of cortisol, which is independent of the initial cortisol level.

When comparing the chronotypes concerning the increase (between minute 0 and 30) and the decrease (between minute 30 and 60), a difference can be discerned regarding the percentage change in cortisol: Within the half hour immediately after awakening, all chronotypes show an increase of cortisol levels and no significant difference between the chronotypes (Chi-square=0.486; p=0.784). While in normal and morning types, there is a percentage decrease and consequently also a decrease of the cortisol concentration between minutes 30 and 60 after awakening, the evening types still show an increase of cortisol, indicating a prolonged increase of the cortisol concentration (F=9.228; p=0.001; post hoc: morning type - evening type: p=0.004; normal type - evening type: p=0.001).

DISCUSSION

The data show the hypothesized differences in the cortisol awakening response between the different chronotypes, indicating a lower total CAR in evening types compared to normal and morning types after forced waking-up times. This result is in line with previous [18,19]. Moreover, a significant difference can be discerned in the time course of the CAR: Within the second half hour after awakening (between minute 30 and 60), there is a decrease of the percentage change in cortisol and therefore a decrease of the cortisol concentration in the morning types and normal types. The contrary is the case for evening types, who still show an increase of cortisol levels, pointing to a delayed and less accentuated cortisol peak in the morning after they had lived according to their inherent circadian rhythm for a week prior to the measurement.

Since our sample included subjects who were able to choose to some extent their waking up and sleeping times as well as the center of their day, this is perfectly reflected in their sleep diaries: The subjective recording of waking-up time and sleeping times as well as the sleep duration over one week prior to the data collection of the biological parameters depicts approximate sleep habits under free-running conditions in the morning and therefore reflects the differences of the chronotypes in waking-up times and sleeping times, but no differences concerning the sleep duration [17,27]. In contrast, all the subjects had to come to the laboratory on the two mornings of the measurments, which therefore was a non-free-running condition and simulated a regular condition on workdays for many people. Particularly the subjects describing themselves as evening chronotype had to get up relatively early considering their internal biological clock on the two consecutive days of the data collection, which might be equivalent to the timing of a regular working day in the larger part of the total population. The fact that they show lower CAR with a prolonged increase phase could indicate a crucial problem in society concerning the lack of tuning between the inherent biological clock and regular work schedules.

Shortcomings of our study can definitely be seen in the small sample size, which did not allow to differentiate in more detail the morning and evening types. And as the evening types showed a prolonged increase of cortisol in the morning, it would have been interesting to follow this course for at least another 30 minutes. These considerations should be addressed in future research.

Since the disruption of the circadian clock seems to be heavily associated with health problems, it might also be politically relevant to draw more attention to this problem. Considering the fact that – amongst other parameters - cortisol plays a key role in the communication pathways of the biological clock [28,12], the interaction of chronotypes and height as well as course of the CARs should be further and specifically explored.

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