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CHAPTER 7

Human basal cortisol levels are increased in hospital compared to home setting


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Abstract

The impact of study-environment on experimental outcome is mostly not realized and certainly not demonstrated. In the present study, a comparison was made between free salivary cortisol levels in healthy young men in a carefully controlled hospital setting versus a home setting. Cortisol levels during rest were increased in hospital compared to home environment: 2-fold at awakening, 3-fold at the morning peak, and 5-fold late in the evening. Early morning light increased cortisol concentrations only in the home setting, while this effect was absent in the hospital setting. The data of the present study show that study-environment has a major impact on basal HPA-axis activity, which is of particular relevance in future studies in which small changes in HPA-axis activity are subject of study.

Introduction

Human neuroendocrine regulation is often investigated in a laboratory or hospital setting, but the impact of such a study environment on experimental outcome has received little attention. The present study therefore investigates the influence of study environment on cortisol regulation.

Firstly, we compared basal salivary cortisol concentrations found in a carefully controlled hospital setting with those in a home setting. Saliva sampling is a non-invasive and reliable method to investigate free, and therefore bio-active, cortisol [162, 177, 348]. Secondly, we investigated if there was a difference between hospital and home setting in the increase of cortisol concentration by morning light. Thirdly, to explore if the salivary cortisol levels are representative of plasma cortisol and ACTH levels as reported in previous hospital studies, we also analyzed plasma cortisol and ACTH. Finally, we investigated if differences between home and hospital setting were due to changes in sleep quality. Data of the home setting have been published before [290].
All subjects were healthy male volunteers. Before each measurement, subjects had not been exposed to shift-work or transmeridian flights for at least six weeks and did not consume alcohol, caffeinated drinks, or food for at least 24, 4, and 2 h, respectively. In the home setting, fourteen men, 33 ± 1 years of age (mean ± SEM) and with a habitual time of awakening of 06:53 h ± 10 min, were investigated in the morning, twelve of whom were also investigated in the evening (see Fig. 1 for details). In the hospital setting, another group of five men, 24 ± 1 years of age (younger than in the home setting, P < 0.001) and with a habitual time of awakening of 06:51 h ± 16 min, was recruited. In the hospital setting, subjects were habituated to a fixed sleep-wake cycle of getting up within 30 min of 06:30 h and going to bed within 60 min of 22:30 h during the eight days before the experiment. The sleep-wake rhythm was verified by a sleep diary and actigraphy. All subjects gave informed consent and all procedures were approved by the local ethics committee.

The experimental designs are shown in figure 1. All measurements were conducted during supine resting conditions. The habitual (or habituated) time of awakening for each individual was defined 'Zeitgeber Time 0' (ZT0). In the home setting, subjects performed the experiments under 'natural' environmental conditions: i.e., in their own bed, being woken by their own alarm clock at their habitual time of waking (ZT0), at the same time as during the three days before. In the hospital setting, subjects were in the hospital from 18:00 h until 09:00 h on two consecutive days in a sound and light-isolated hospital room. Between 18:00 h and 19:00 h (at least 2 h before measurement), an intravenous catheter was inserted and subjects consumed a light meal. Efforts were made to create a relaxing atmosphere. During all measurements, subjects watched nature-documentaries. In the morning, subjects were woken by their own alarm at the same time as during the eight days before (ZT0). We used techniques for painless blood (VenFlon) and saliva (Salivette, Sarstedt, Nümbrecht, Germany) sampling.

All volunteers were exposed to 1-h darkness and 1-h light on two consecutive days during both the evening and early morning. The subjects were assigned randomly to either darkness on the first and light on the second day or to the reversed order (Fig. 1). In the home setting, light intensity (eye level) was 0 lux during darkness and 800 lux during light exposure, with lights-on within 5 min of awakening and 60 min before the start of the sleep period. In the hospital setting, light intensity was below 2 lux (TV as only light source, 2 m from eyes) during darkness and 1300 lux (Philips TLD 940 fluorescent tubes surrounding TV) during light exposure, with lights-on 50 min after awakening and 60 min before the start of the sleep period.

Saliva sampling and salivary cortisol analysis were performed as described earlier [290]. In the evening, in the home setting, many salivary cortisol concentrations were below detection level. These cortisol concentrations were set at 0.5 ng/ml (=detection level) for further analysis. Eighteen samples analyzed in both the assay of home and hospital set-
A. Protocol for home setting, morning

B. Protocol for home setting, evening

C. Protocol for hospital setting

Fig. 1 Experimental designs (light-dark sequences are as example). A, Home setting, morning. Subjects took 12 salivary samples; 1 sample on both evenings (ZT16, 16h after habitual waking), 4 samples during the first hour after waking (ZT0-1) and 1 sample two hours after waking (ZT2) on both mornings. B, Home setting, evening. The subjects took a total of 8 samples: 4 samples during the last hour before the sleeping-period (ZT15-16) on both evenings. C, Hospital setting. During both evenings and both mornings 6 salivary and 8 plasma samples were taken. The measurements lasted 90 min and started at ZT14:30 in the evening and at ZT00:20 in the morning, 20 min after waking. X-axes indicate periods of two nights and one day. Horizontal black bars, night periods; black vertical bars, dark-exposure; white vertical bars, light-exposure; vertical lines, saliva-sample points; arrows, plasma-sample points.

Sampling resulted in the same salivary cortisol values without a systematic difference. Blood samples were obtained via an intravenous catheter. Blood samples for cortisol analysis were collected in heparin tubes and stored at -20 °C until assay. Blood samples for ACTH analysis were collected in EDTA tubes on ice, centrifuged and stored at -20 °C until assay. Plasma cortisol was measured by a fluorescence polarization immunoassay on a TDx analyser (Abbott Laboratories, North Chicago, IL, USA; detection limit: 50 nmol/l, intra- and inter-assay variance: 4.4 % and 9.5 %). Plasma ACTH was determined by an immunoluminometric assay (Nichols Institute Diagnostics, San Juan Capistrano, LA,
USA; detection limit 1.0 ng/l, intra- and inter-assay variance: 5.0 % and 7.5 %). In the hospital experiment, subjects wore an actigraph (Actiwatch, Cambridge Neurotechnology Ltd, Cambridge, UK) on the non-dominant wrist continuously from eight days before until the end of the hospital measurements, for actigraphy recordings at 1 min intervals. To estimate sleep duration and sleep percentage objectively, automatic sleep/wake scoring was performed with Actiwatch Sleep Analysis 98 (Cambridge Neurotechnology Ltd, V4.15) on the data between “to bed time” and “getting up time” derived from the subjective diaries [143, 175].

Because not all variables were normally distributed (Shapiro’s-Wilk’s W Test), non-parametric tests were used as required. For comparison between values in home and hospital setting in the dark, similar time-points relative to waking and to bedtime were compared using (rank-transformed[65]) two-way analyses of variances (ANOVA) for repeated measures and student’s t-test or Mann-Whitney U test for single time-points. An effect of light was tested with (rank-transformed[65]) two-way ANOVAs for repeated measures. In the hospital setting, a correlation between salivary and plasma cortisol was determined for the evening and morning baseline samples (20 min after start of measurement). For the hospital setting, the averaged sleep duration and sleep percentage between the first and second night in the hospital, and between the average over the eight nights at home and the two nights at the hospital, were compared with a paired student’s t-test. All values are mean ± SEM. A value of P < 0.05 was taken as significant.

Baseline salivary cortisol concentrations were two to five times higher in the hospital setting compared to the home setting, depending on time-of-day. In the morning, there was a significant effect of experimental setting (P < 0.001), time (P < 0.001) and interaction (P = 0.01), with 0 min and 40 min after waking as repeated measures (Fig. 2). At awakening, salivary cortisol concentrations were two times higher in the hospital (5.6 ± 1.7 ng/ml) compared to the home (3.0 ± 0.4 ng/ml) setting (P < 0.05). Forty min after waking and in the dark, salivary cortisol concentrations were three times higher in hospital (11 ± 1.2 ng/ml) compared to home (4.1 ± 0.6 ng/ml) setting (P < 0.001). In the evening, salivary cortisol levels were five times higher in the hospital setting (mean ZT15-16: 2.9 ± 0.6 ng/ml) than in the home setting (mean ZT15-16: 0.6 ± 0.02 ng/ml) (P < 0.001) (Fig. 2). In the home setting, there was a significant effect of light on salivary cortisol 20 and 40 min after lights-on in the morning and no effect in the evening (Fig. 2), as published before [290]. In the hospital setting, however, there was no effect of light on salivary cortisol, plasma cortisol, or plasma ACTH concentrations in morning and evening. In the morning, plasma cortisol and ACTH levels decreased from 452 ± 55 nmol/l and 36 ± 3.5 ng/l at 40 min after waking to 310 ± 35 nmol/l and 26 ± 3.4 ng/l at 110 min after waking, respectively. In the evening, there was no effect of time on plasma cortisol or ACTH levels, and levels just before sleeping were 95 ± 36 nmol/l and 9.3 ± 1.4 ng/l, respectively. There was a strong correlation between baseline plasma and salivary corti-
sol \( (r = 0.91, P < 0.001) \), with the linear correlation-line almost going through zero (salivary cortisol \( \text{ng/ml} \) = 0.074 + 0.0025 x plasma cortisol \( \text{nmol/l} \)). In the hospital setting, there was no difference in sleep duration between the two nights in the hospital (06:54 h ± 14 min) and the eight nights at home (06:57 h ± 21 min) \( (P = 0.81) \). Sleep percentage during the two nights in hospital (94 ± 2 %) was higher than during the eight previous nights at home (91 ± 3 %) \( (P = 0.02) \).

The results of the present study demonstrate that, compared to a home setting, a hospital setting causes a 2- to 5-fold increase in salivary free cortisol levels, depending on the time of day: 2 times at awakening, 3 times 40 min after awakening, and at least 5 times in the evening. In addition, a hospital setting may obscure a further increase in cortisol levels by an experimental stimulus.

Salivary cortisol analysis provide a reliable estimate of free plasma cortisol levels and is unaffected by salivary flow rate \([162, 177, 348]\). Indeed, as shown previously \([162, 348]\), we find a strong linear correlation between salivary free and plasma total cortisol concentration. In previous studies using a hospital setting to measure basal cortisol levels \([33, 109, 182, 183, 339]\), plasma cortisol levels were 80-110 nmol/l late in the evening and 450-600 nmol/l about 40 min after waking, similar to our 95 ± 36 nmol/l and 452 ± 55 nmol/l, respectively. In hospital studies set out to establish reference range for salivary

**Fig. 2** Effect of setting on salivary cortisol levels. In the late evening, cortisol levels were at least five times higher in the hospital \( (n = 5) \) compared to the home \( (n = 12) \) setting, without influence of light (A). In the early morning, cortisol levels were two to three times higher in the hospital \( (n = 5) \) compared to the home \( (n = 14) \) environment (B). Plasma cortisol levels in hospital setting in evening (C) and morning (D). *\( P < 0.05 \) comparing hospital with home setting. Closed symbols, dark assessment; open symbols, light assessment; squares, hospital setting; circles, home setting.
cortisol levels together with plasma levels \[1, 177\], salivary concentrations were 1.4-2.2 ng/ml in the evening and 1.3-9.8 ng/ml in the early morning, comparable to our 2.9 ng/ml and 5.6 ng/ml, respectively. This indicates that the present hospital experiment is representative of other laboratory and hospital studies including blood sampling.

Higher cortisol concentrations in the hospital setting do not seem due to an impaired sleep quality as a result of hospitalization, since sleep duration was similar and sleep percentage even higher in hospital than at home. The latter may be the result of complete darkness and sound isolation in the hospital room. Surprisingly, salivary cortisol levels measured at awakening were already twice as high as during the home setting. Indeed, the possibility of unconscious modulation of HPA-axis activity during sleep has been demonstrated by Born and co-workers [31]. Although increased cortisol levels are generally thought to correlate with disrupted sleep, this is not supported by the present study and other studies in young healthy subjects [108, 347].

Morning light was unable to further increase cortisol levels in the hospital, in contrast to the home setting. However, since the population size was smaller in the hospital setting, we cannot exclude that the lack of effect was due to a low number of subjects in the hospital. The differential effect of light is only suggested by our data and should be replicated in larger samples. On the other hand, the absence of the cortisol increase by morning light in the hospital setting could be due to the strong impact on cortisol levels of the experimental setting compared to the relatively modest impact of light, although the small difference in circadian phase for lights-on between home and hospital may also contribute. A difference in time of lights-on relative to waking was unlikely to explain this difference between home and hospital setting, because light has a clear effect independent of awakening [182]. The importance of the cortisol level before light exposure is in agreement with findings of Leproult et al [182], who indicated that light does increase cortisol levels in a hospital setting when provided at night when cortisol levels are lower than the levels assessed in the morning. In the Leproult study, light 2.5h before the habitual time of awakening doubled the low cortisol levels (± 220 nmol/l) to levels (± 425 nmol/l) comparable to those in the dark at the habitual time of awakening in that study (± 440 nmol/l) and to those in the present study just before lights-on (452 ± 55 nmol/l).

We propose that, in everyday life, light exposure shortly after waking in the morning increases cortisol concentrations. Although this increase by light exposure is relatively modest, it occurs daily. Furthermore, we propose that in a hospital setting, light can stimulate cortisol levels only at an early circadian phase, when cortisol levels are still low, and that a hospital setting may obscure such stimulation during the later cortisol morning peak.

Although the experiments in the home and hospital setting were conducted in two separate groups, subjects of both groups were quite comparable, consisting of healthy young male volunteers with the same habitual time of awakening. Although the ages of
the subjects differed (hospital: 24 years and home: 33 years), the difference was small, and if anything, cortisol levels would be expected to be lower in the younger men in the hospital setting [333]. The small number of subjects in the hospital group is a limitation. However, because of the strict experimental conditions both in the home and hospital setting, variation was reduced as much as possible. Indeed, these restrictions allowed the clear and repeated demonstration of higher levels of salivary cortisol in the hospital compared to the home setting.

In conclusion, a hospital or laboratory setting including blood sampling has a major impact on basal HPA-axis activity, which is of specific importance for future studies in which small changes of the HPA-axis is subject of study.