

CLINICAL STUDY

The cortisol awakening response is blunted in patients with active Cushing's disease

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Abstract

Introduction: Cortisol awakening response (CAR) is a rapid increase of cortisol levels within 30–45 min after awakening.

Objective: This study evaluates CAR compared with cortisol circadian rhythm in active and in remission Cushing's disease (CD).

Materials and methods: We evaluated healthy controls (HC, $n=19$), obese (OB, $n=10$), in remission ($n=8$), and active CD patients ($n=10$). Salivary free cortisol (SF) was determined at 0800, 1100, 1700, 2000, and 2300 h on the first day. CAR was obtained the next morning immediately upon awakening and at 15, 30, 45, and 60-min post-wake up.

Results: We observed differences in SF levels throughout the day in HC, OB, and in remission CD (ANOVA $P=0.0001$) but not in active CD ($P=0.2$). We demonstrated SF increment after awakening in HC, OB, and in remission CD (ANOVA $P=0.007$), with no effect of time on SF in active CD. The relative increment of SF obtained at the peak after awakening (CARI%) in the active CD ($67 \pm 57\%$) was lower than in HC ($154 \pm 107\%$), OB ($240 \pm 188\%$), and in remission CD ($186 \pm 184\%$) patients ($P=0.009$). There was a negative correlation between the SF at awakening and the CARI% in HC ($r=-0.8$), OB ($r=-0.78$), and in remission CD ($r=-0.74$) but not in active CD ($r=-0.35$; $P=0.31$).

Conclusion: This study originally described a blunted CAR in active CD in contrast to its presence in HC, OB, and in remission CD. This subtle dysfunction of the hypothalamus–pituitary–adrenal axis may represent a distinct and additional physiopathological phenomenon superimposing the dysregulated cortisol circadian rhythm in this disease.

European Journal of Endocrinology 168 657–664

Introduction

The hypothalamus–pituitary–adrenal (HPA) axis is a major endocrine system adapting the organism to environmental challenges in order to adjust predictive homeostasis. HPA is under regulatory control of circadian oscillators, resulting in 24-h rhythm of cortisol secretion from the adrenal cortex, with several secretory episodes of short duration and high amplitude. Cortisol levels steadily decline throughout the day with lowest levels during the first half of the night (1, 2). Circadian influences on physiological systems are mainly transmitted via the endogenous central pacemaker: the suprachiasmatic nucleus (SCN). The circadian rhythm of the HPA axis is largely controlled by the SCN clock genes, which influences adrenocortical activity via input to the paraventricular nuclei of the hypothalamus (3, 4, 5).

In addition to the well-described cortisol diurnal cycle, there is a distinct phenomenon termed cortisol awakening response (CAR), which is a rapid increase in cortisol

levels of about 50–75% levels within 30–45 min after awakening in the morning. The CAR appears to be a phenomenon superimposing the circadian rhythm of cortisol as it adds a significant incremental effect to the linear trend of increasing cortisol concentrations in the early morning hours (6, 7). These data are supported by studies on continuous polysomnographical recordings and repeated blood sampling in the sleep laboratory. Post-awakening cortisol increase is linked to the process of awakening possibly through activation of memory representations about the self and orientation in time and space that have the potential to stimulate HPA axis activity (7). Both cortisol circadian rhythm and CAR are sensitive to light exposure; therefore, it is possible that the SCN-derived biological clock might be an additional important structure for the CAR (8, 9). Besides the classical neuroendocrine control of the adrenal cortex by the corticotropin-releasing hormone (CRH)/ACTH, projections of the SCN via autonomic nervous system determine the adrenal sensitivity to ACTH. Thus, pre- and post-awakening cortisol secretions are affected by

the combination of the activity of the HPA axis and the sensitivity of the adrenal to ACTH (10).

The CAR has been studied extensively over the past two decades in healthy populations (6, 7, 11, 12). The research on CAR from a physiological perspective has improved the knowledge of this phenomenon and its putative impact on physiopathology of the HPA axis dysregulation. Indeed, altered CAR has been described, mainly in neurological diseases (13, 14), psychiatric disorders (15), such as depression, post-traumatic stress and alcohol dependence, among others (for details see references (8, 11)), but also in hypertension (16), overweight (17), and metabolic syndrome (18). There are no studies on CAR in Cushing's syndrome, as the majority of studies have focused on basal cortisol samples during the morning hours (0745–0900 h) and loss of normal cortisol circadian rhythm but not related to awakening (19, 20). As CAR is considered a reliable measure for the acute reaction to awakening of the HPA axis, in the search of a new biological marker of cortisol secretion, we hypothesized that CAR study could contribute for the detection of a possible subtle dysfunction of the HPA axis in the Cushing's disease (CD). Therefore, this study aims to evaluate CAR in CD in comparison with the cortisol circadian rhythm, the well-known altered parameter of the HPA axis in this disease.

Materials and methods

Subjects

This study was approved by the Ethics Committee of the School of Medicine of Ribeirao Preto – University of Sao Paulo and, according to the requirements of the Declaration of Helsinki, written informed consent was obtained from all participants.

Eighteen patients with CD were investigated between 2009 and 2012 at the University Hospital of the School of Medicine of Ribeirao Preto, University of Sao Paulo. In addition to the clinical features of chronic hypercortisolism, the diagnosis of CD was established by the lack of circadian variation in salivary cortisol levels, urinary free cortisol (UFC), and other standard tests of pituitary–adrenal function, including plasma ACTH levels, low- and high-dose dexamethasone suppression, CRH tests, inferior petrosal sinus sampling, and image studies (20, 21). All 18 patients had ACTH–pituitary-dependent disease; in whom the diagnosis was later confirmed by transsphenoidal surgery or positive histopathology. Patients were clustered into two different groups: active CD (ten females; age 38.9 ± 11.0 , ranging from 22 to 60 years) and CD after remission of the hypercortisolism (six females/two males; age 34.1 ± 11.7 , ranging from 20 to 55 years). CD was considered in remission if the patient achieved undetectable postoperative serum cortisol levels < 85 nmol/l (3.0 µg/dl) within 1–14 days

after surgery (22, 23). Mean time of late biochemical remission at the study date was 57.3 ± 50.5 months. Patients were not taking medical therapy, were not submitted to radiotherapy, and did not show hypopituitarism. In addition, they also presented suppressed salivary cortisol levels after 1 mg dexamethasone test.

Moreover, we also studied ten obese women (OB) presenting essential hypertension, idiopathic hirsutism, or menstrual disorders (age 34.3 ± 10.2 , ranging from 22 to 56 years). Some patients were on medical treatment for hypertension, diabetes, and dyslipidemia, and two patients were on estrogen treatment. These obese controls had normal salivary cortisol circadian variation and suppressed salivary cortisol levels after 1 mg dexamethasone test. Furthermore, we studied 19 normal, no obese healthy volunteers (13 females/six males, age 34.9 ± 12.0 , ranging from 22 to 63 years). Exclusion criteria from healthy volunteers were previous use of any drugs, including glucocorticoids, and no history of acute or chronic cardiopulmonary, hepatic, renal, neuropsychiatric illness, or sleep disturbance. In addition, none of them were pregnant or a night-shift worker.

Methods

Cortisol circadian rhythm and CAR assessed by

salivary cortisol Salivary free cortisol (SF) samples were collected by Salivette sampling devices (Salivette; Sarstedt, Nuembrecht, Germany) on two consecutive weekdays after instruction by one of the investigators. Participants were asked not to use alcohol or work out two days before the study.

Samples of healthy volunteers were collected at home while samples from CD and obese patients were obtained inpatient at University Hospital. Circadian rhythm was determined at 0800, 1100, 1700, 2000, and 2300 h on the first day. CAR was obtained in the next morning immediately upon awakening and at 15, 30, 45, and 60 min post-wake up. Moreover, specific instructions were given to all participants: they should note the time they went to the bed, the duration of sleep, the wake-up time, and also register the time of food ingestion and the time of each sample collection. Any deviations from the recommended schedule should also be noted. Participants were asked not to eat or drink anything, brush their teeth, or smoke 1 h before collection of the samples. The salivary samples were stored at -20 °C until analysis. Salivary cortisol measurements were performed as previously described by RIA method on 25 µl samples of saliva without prior extraction or chromatography (24). All samples obtained from each subject were analyzed in duplicate in the same assay.

UFC was measured during three consecutive days by liquid chromatography associated with tandem mass spectrometry (LC–MS/MS – normal range of 3–43 µg/24 h) (25) in active CD. The mean of the three measurements was used to perform statistical analysis.

Sleep quality test To evaluate sleep quality, we used the Pittsburgh Sleep Quality Index (PSQI). This index is a self-rated questionnaire that assesses sleep quality and disturbances over a 1-month time interval. Nineteen individual items generate seven 'component' scores: subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleeping medication, and daytime dysfunction. The sum of scores for these seven components yields one global score. A global PSQI score >5 yielded a diagnostic sensitivity of 89.6% and specificity of 86.5% in distinguishing good and poor sleepers (26).

Statistical analysis

Data are expressed as mean \pm s.d. To define the normal circadian salivary cortisol rhythm, we adopted the cutoff value of 2300 h salivary cortisol levels of 9.8 nmol/l (or 350 ng/dl) by RIA (27).

To obtain indices for CAR, the absolute increment was obtained by salivary cortisol at the peak after awakening minus salivary cortisol at awakening (zero time). Relative increment was obtained by the absolute increment divided by salivary cortisol at awakening (zero time) (8). A salivary cortisol-relative increment of 50% within the first 45 min after awakening was considered as CAR, according to a previous study in normal subjects (6). Kruskal–Wallis ANOVA was performed for multiple comparisons among different groups and the Dunn's multiple comparison test was used as a *post hoc* test. The Wilcoxon–Mann–Whitney *U* test was used when appropriate. Receiver-operating characteristic (ROC) curves were generated to detect the best CAR-relative increment values associated with discrimination between active CD and a group comprising obese patients and healthy subjects. Data analyses were carried out with the statistical package GraphPad PRISM 4.0 (GraphPad software, Inc., 2003, La Jolla, CA, USA). Significance was assumed when $P < 0.05$.

Results

There were no differences in age and gender among active CD, CD after remission of the hypercortisolism (in remission CD), obese patients (OB), and healthy control (HC) groups. Regarding clinical findings, the BMI (kg/m^2) in the OB group (40.7 ± 4.7) was the highest compared with all other groups ($P < 0.01$). Active CD

group BMI (34.5 ± 4.5) was higher than in remission CD (26.4 ± 4.5 ; $P = 0.004$) and HC (23.6 ± 3.3 ; $P = 0.0001$) groups; there was no difference in BMI between in remission CD and HC groups ($P = 0.14$). Regarding the mean blood pressure (BP; mmHg) levels, HC group (86 ± 2) showed the lowest level ($P = 0.003$) compared with in remission CD (95 ± 7), obese (103 ± 3.5), and active CD (100 ± 3); no difference in mean BP was observed among active CD, in remission CD, and OB groups. There was no difference in years of education among active CD (13.1 ± 2.6), in remission CD (13.1 ± 2.1) and OB (12.7 ± 2.7) groups. However, the HC group had the highest level of education (18.3 ± 3.5 years of education).

All subjects had at least four meals per day. There were no differences in the mealtimes among all groups, but the HC group had dinner 1 h later. All groups went to bed at similar times, from 2300 h to midnight and were woken up at 0500 to 0615 h. There were no differences regarding the duration of sleep among all groups (6–8 h of sleep). A global PSQI score <5 were observed in normal controls (4.3 ± 2.0 ; median of 4.0, range 2–10) and in remission CD (3.9 ± 2.8 ; median of 3.0, range 1–10) indicating good sleepers. However, PSQI scores in active CD (8.2 ± 3.9 , median of 8.5, range 3–14) and OB (7.5 ± 3.9 , median of 7.0, range 3–14) groups were higher than 5, indicating poor sleepers. We observed no difference in the global quality of sleep between HC and in remission CD patients and both groups showed lower PSQI scores than OB and active CD patients ($P = 0.02$). There was no difference in PSQI scores between OB and active CD groups. No abnormal event occurred during the experimental protocol in any group.

Salivary cortisol circadian rhythm and CAR

The mean (\pm s.d.) values of salivary cortisol at 0800, 1100, 1700, 2000, and 2300 h in HC, OB, in remission CD, and active CD groups are shown in Table 1. Figure 1 shows individual salivary cortisol values obtained at 0800 and 2300 h for each group. ANOVA demonstrated significant differences in salivary cortisol levels among 0800, 1100, 1700, 2000, and 2300 h for HC, OB, and in remission CD ($P = 0.0001$) but not in active CD ($P = 0.2$). The salivary cortisol levels of patients with active CD were significantly elevated at 0800, 1100, 1700, 2000, and 2300 h compared with all other groups ($P = 0.01$); however, there were no differences

Table 1 Salivary cortisol circadian profile in healthy controls, obese, in remission Cushing's disease (CD), and active CD patients. Cortisol concentrations in nmol/l; values in $\bar{X} \pm$ s.d.

Groups	0800 h	1100 h	1700 h	2000 h	2300 h
Healthy controls ($n=19$)	49.5 ± 21.7	23.8 ± 14.1	19.1 ± 9.5	12.0 ± 8.2	5.4 ± 3.1
Obese ($n=10$)	38.9 ± 14.1	20.9 ± 9.5	19.4 ± 14.9	11.0 ± 5.7	7.4 ± 5.0
Remission CD ($n=8$)	26.9 ± 5.4	17.2 ± 10.0	11.6 ± 5.2	12.2 ± 8.7	10.0 ± 6.9
Active CD ($n=10$)	70.8 ± 27.7	57.4 ± 22.8	49.3 ± 16.7	58.4 ± 22.1	49.1 ± 23.3

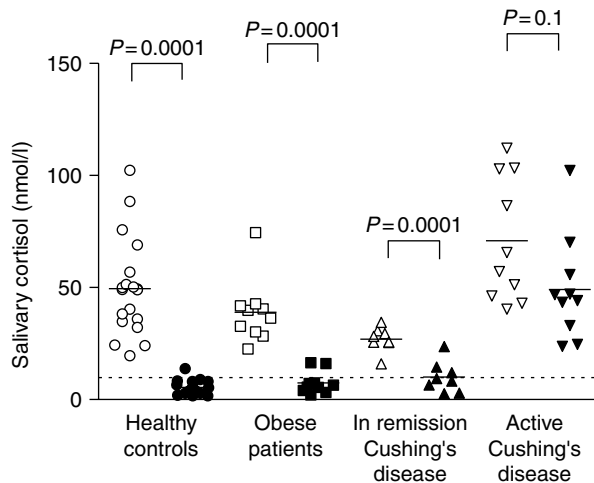


Figure 1 Individual data for salivary cortisol values obtained at 0800 h (open symbols) and 2300 h (closed symbols) for healthy controls (open circle), obese (open square), in remission Cushing's disease (CD) (open triangle), and active CD patients (open down triangle). The horizontal dotted line represents CI 95% salivary cortisol cutoff (9.8 nmol/l) used to discriminate Cushing's syndrome from obese patients.

among the HC, OB, and in remission CD groups. In addition, each patient from the active CD group had elevated UFC, individual values ranging from 48.1 to 235.7 ($X \pm s.d.$; $120 \pm 76 \mu\text{g}/24 \text{ h}$).

Considering the cutoff values of salivary cortisol of 9.8 nmol/l (350 ng/dl), normal cortisol circadian rhythm was characterized in 95% of the healthy subjects, 80% of OB patients, 62% of remission CD patients, and in none of the active CD patients.

Table 2 shows the mean ($\pm s.d.$) values of salivary cortisol in HC, OB, in remission CD, and active CD groups at awaking (time 0) and after 15, 30, 45, and 60 min, as well as the absolute and relative increment of CAR values. ANOVA revealed a significant main effect of time on salivary cortisol in HC ($P=0.0001$), OB ($P=0.001$), and in remission CD ($P=0.007$), with a significant increment at 30 and 45 min after awakening. However, in active CD, we observed no effect of time on salivary cortisol values post-awakening ($P=0.76$, Fig. 2A).

The absolute increment of salivary cortisol obtained at the peak after awakening was not different among all groups. There was also no difference in the relative

increment of salivary cortisol peak among HC ($154 \pm 107\%$), OB ($240 \pm 188\%$), and in remission CD ($186 \pm 184\%$) patients. However, the relative increment of salivary cortisol in the active CD ($67 \pm 57\%$) was lower than HC ($P=0.009$), OB ($P=0.01$), and in remission CD ($P=0.01$) patients. Figure 2B shows the mean values in the percentage of relative increment of salivary cortisol after awakening at 15, 30, 45, and 60 min in all studied groups. CAR was present in HC, obese patients, and in remission CD groups and blunted in active CD as a group. However, considering the individual salivary cortisol-relative increment of 50% after awakening, CAR was observed in 95, 90, 88, and 50% of HC, obese, in remission CD, and active CD patients respectively. Using the ROC curve, the sensitivity and the specificity of the CAR-relative increment of salivary cortisol in identifying active CD compared with healthy subjects and obese patients were 80% when the salivary cortisol-relative increment of 77% was applied.

There was a negative correlation between the salivary cortisol at zero time (at awakening) and the relative increment of salivary cortisol at the peak after awakening in healthy subjects ($r=-0.8$; $P<0.0001$), OB ($r=-0.78$; $P=0.007$), and in remission CD ($r=-0.74$; $P=0.04$) but not in active CD ($r=-0.35$, NS). Figure 3 shows the correlation between salivary cortisol at awakening and the relative increment of salivary cortisol at the peak after awakening in all subjects ($r=-0.73$; $P=0.0001$). There was a trend of association between the relative increment of salivary cortisol at the peak after awakening and the 2300 h salivary cortisol levels ($r=-0.25$; $P=0.07$) as well as with the duration of symptoms ($r=-0.55$; $P=0.1$). We observed in active CD neither correlation between the relative increment of salivary cortisol at the peak after awakening and the mean concentration of salivary cortisol during the day nor the UFC ($r=0.11$ and $r=-0.13$ respectively). There was a negative correlation between the PSQI score and the relative increment of salivary cortisol at the peak after awakening ($r=-0.4$; $P=0.04$).

Discussion

In this study, CAR was evaluated, for the first time, in patients with active CD. We observed no effect of time on salivary cortisol values post-awakening in these

Table 2 Salivary cortisol awakening response in healthy controls, obese, in remission Cushing's disease (CD), and active CD patients. Cortisol concentrations in nmol/l; values in $X \pm s.d.$; absolute increment means salivary cortisol at the peak after awakening minus salivary cortisol at awakening (zero time). Relative increment means the absolute increment divided by salivary cortisol at awakening (zero time).

Groups	0 min	15 min	30 min	45 min	60 min	Absolute increment	Relative increment (%)
Healthy controls	31.7 ± 13.0	49.3 ± 17.9	58.3 ± 19.4	62.6 ± 20.2	57.7 ± 20.2	39.1 ± 17.4	154 ± 107
Obese	25.7 ± 12.3	43.2 ± 21.3	61.3 ± 17.1	61.6 ± 25.8	48.3 ± 14.3	45.6 ± 22.9	240 ± 188
Remission CD	21.3 ± 8.1	34.9 ± 13.2	48.0 ± 17.3	42.1 ± 11.4	43.7 ± 7.8	29.8 ± 14.9	186 ± 184
Active CD	48.5 ± 21.6	62.1 ± 29.0	66.4 ± 33.5	67.5 ± 36.8	59.1 ± 30.1	28.0 ± 25.8	67 ± 57

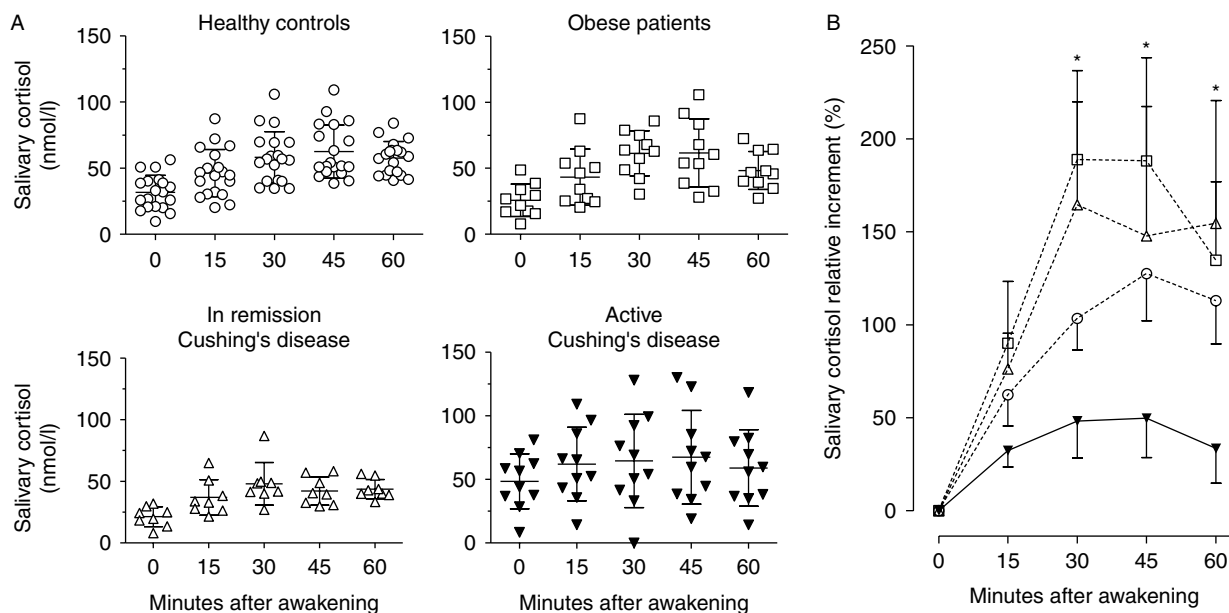


Figure 2 (A) Individual salivary cortisol levels at awakening (0), 15, 30, 45, and 60 min after awakening in healthy controls (open circle), obese (open square), in remission Cushing's disease (CD) (open triangle), and in active CD patients (closed down triangle). Healthy controls, obese, and in remission CD patients show significant salivary cortisol increment at 30 and 45 min after awakening ($P < 0.01$). No effect of time on salivary cortisol values post-awakening was observed in active CD ($P = 0.76$). (B) Mean (\pm s.e.m.) percentage increment of salivary cortisol after morning awakening. * $P < 0.05$ between active CD patients and the other three groups. No difference was observed among HC, OB, and in remission CD.

patients, in contrast to the presence of CAR in HC, obese patients, and in remission CD patients. In addition, the relative increment of salivary cortisol at the peak after awakening was negatively correlated with salivary cortisol at awakening in all subjects. Our results suggest that the blunted CAR in active CD patients would be related to the hypercortisolism at awakening.

The introduction of salivary cortisol assessment, both in inpatient and outpatient settings, has allowed the study of HPA axis response patterns without the need of repeated blood samples (19, 20, 28). In this study, we evaluated the salivary cortisol levels of patients with active CD compared with HC, obese, and in remission CD groups. As expected, active CD patients showed elevated cortisol levels throughout the day, characterizing a disrupted cortisol circadian rhythm, independently of the adopted criteria.

CAR represents a distinct phenomenon of the HPA axis regulation and has never been described in active or in remission CD. We demonstrated that CAR is blunted in this series of active CD patients, as there was no effect of time on salivary cortisol values post-awakening, whereas it was present in CD after remission as well as in obese patients and HC. It is important to point out the novelty of this data; however, further CAR studies need to be performed in a large number of active CD patients in order to confirm our data. In this study, we evaluated three different control groups as altered CAR has been associated with demographic aspects, such as: age and gender, BMI, and food ingestion; menstrual cycle phase,

oral contraceptive intake, and smoking habit; physical factors; psychological or psychiatric diseases; and sleep-related factors including waking time, sleep duration, and sleep quality (11).

Obesity and metabolic syndrome share several clinical features with Cushing's syndrome (18). Therefore, to avoid potential influences of metabolic syndrome

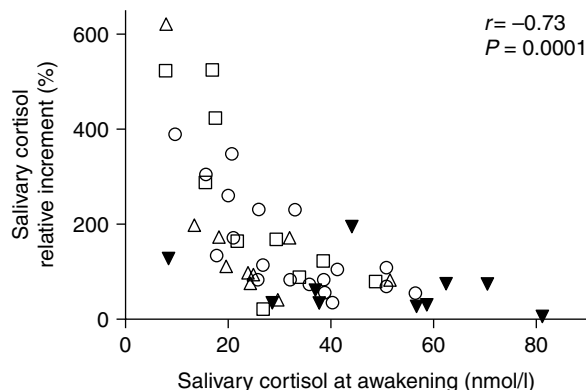


Figure 3 Pooled data obtained from the correlation between salivary cortisol at zero time (at awakening) and relative increment of salivary cortisol at the peak after awakening in healthy controls (open circle), obese (open square), in remission Cushing's disease (CD) (open triangle), and active CD (closed down triangle). Each group showed a negative correlation between the salivary cortisol at awakening and the relative increment at the peak: healthy subjects ($r = -0.8$, $P < 0.0001$), obese ($r = -0.78$, $P = 0.007$), and in remission CD ($r = -0.74$, $P = 0.04$), but active CD ($r = -0.35$, NS).

components in salivary cortisol on circadian rhythm and awakening response evaluation, we included patients with obesity, hypertension, and menstrual disorders. We found that although this group showed BMI and BP similar to the active CD patients, they showed normal cortisol rhythm and CAR. Our data are in agreement with that recently described by DeSantis *et al.* (29), demonstrating no association of metabolic syndrome components with the magnitude of CAR.

A recent study suggested that higher educational level was associated with greater cortisol levels post-awakening (30). Although the healthy subjects of our study showed the highest level of education compared with all other groups, they did not show greater CAR.

Sleep duration and disturbance were self-reported by subjects using a well-validated scale (26). The sleep schedule and sleep duration were similar among all groups, although the PSQI sleep quality indicated that active CD and obese patients were poor sleepers, with no difference in PSQI scores between both groups. Therefore, it is unlikely that the quality of sleep would be responsible for the blunted CAR observed in active CD patients. Alteration of parameters of sleep, such as self-reported short sleep duration in an English cohort, has been associated with both increased CAR and shallow slope in diurnal cortisol secretion (31). However, in agreement with our data, another previous study in healthy women showed that the morning CAR after disturbed sleep did not differ from the morning CAR following undisturbed sleep (32). Altogether, these data indicate that sleep disturbance *per se* might not have a significant influence on CAR.

Other parameters directly related to the dysregulation of the HPA axis status would be responsible for the blunted CAR observed in active CD patients. The most recommended parameters to characterize the CAR are the first waking sample (zero time) and the relative increment, which means the relative change in cortisol secretion following awakening (10). We observed a negative correlation between the salivary cortisol at awakening and the relative increment of salivary cortisol at the peak after awakening in healthy subjects, obese, and in remission CD patients, indicating that high levels of awakening cortisol are associated with attenuated CAR in these three control groups. Our data in controls are in agreement with previous studies also performed in healthy individuals (7, 33, 34). In addition, we expanded this knowledge to obese and in remission CD patients, in which groups a similar negative correlation was observed between the salivary cortisol at awakening and its relative increment at the peak after awakening. Moreover, we described for the first time that this relationship was lost in active CD patients, indicating clearly that the hypercortisolism at awakening influences the relative amplitude of CAR.

It is interesting to note that pre- and post-awakening cortisol secretions are under different regulatory controllers. Cortisol at zero time represents the

immediate pre-awakening period, which seems to be associated with decreased adrenal sensitivity to ACTH (10, 11). Crucial brain regions implicated in this process include the hippocampus (35), which plays a major role in inhibiting the HPA axis before awakening (36, 37, 38). The zero time awakening salivary cortisol is elevated in CD patients; this phenomenon could mask CAR. However, we cannot rule out that the blunted CAR could be due to the reduction in SCN-mediated 'fine tuning' of adrenal sensitivity as the pre-awakening hippocampus activation might be impaired in active CD. Indeed, a reduced hippocampal volume in patients with Cushing's syndrome and in other conditions associated with hypercortisolism has been described (39, 40). In addition, these changes are reversible, at least in part, following treatment of CD (41).

Another possible explanation for the blunted CAR in active CD patients would be the severity of hypercortisolism evaluated by the mean concentration of salivary cortisol and UFC. This possibility cannot be supported by our data. On the other hand, we observed a trend of association between more attenuated CAR and the duration of symptoms in active CD and also with 2300 h salivary cortisol levels. Further studies with large series of CD patients are necessary to completely rule out the role of sustained hypercortisolism in blunted CAR observed in active CD patients. Moreover, other studies are also important to increase the number of patients in order to establish the putative use of CAR as a biomarker of hypercortisolism in the differential diagnosis of Cushing's syndrome. Even so, the sensitivity and specificity of a single outpatient 2300 h salivary cortisol level above the 90th percentile of the obese patients' values (93.3%/93.3%) (19, 20) are superior to CAR (80%/80%). At the present time, CAR evaluation is not suitable for diagnostic use in CD.

In summary, this study originally described a blunted CAR on active CD. This subtle dysfunction of the HPA axis may represent a distinct and additional physiopathological phenomenon superimposing the dysregulated cortisol circadian rhythm in this disease.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding

This work was supported by grants from Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP 07/58365-3; 10/03039-7) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq 314279/2009-1).

Acknowledgements

The authors would like to thank the expert technical support of José Roberto da Silva, Lucimara Bueno, and Adriana Rossi.

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Received 8 November 2012

Revised version received 1 February 2013

Accepted 7 February 2013