

Altered Neuroendocrine Sleep Architecture in Patients with Type 1 Diabetes

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ABSTRACT

Objective: The modulatory influence of nocturnal sleep on neuroendocrine secretory activity is increasingly recognized as a factor critical to health. Disturbances of sleep may arise from and contribute to the disease process in chronically ill patients suffering from type 1 diabetes mellitus (T1DM).

Research Design And Methods: Neuroendocrine sleep architecture was assessed under well-controlled non-hypoglycemic conditions in 14 T1DM patients and 14 healthy controls matched for age, gender, and BMI, using standard polysomnography and repetitive blood sampling.

Results: As expected, plasma glucose ($P = 0.02$) and serum insulin ($P < 0.001$) levels were constantly higher in T1DM patients than in healthy subjects throughout the night. Beside these characteristic alterations of glucose metabolism, T1DM patients displayed higher blood levels of growth hormone ($P = 0.001$) and epinephrine ($P = 0.02$) during the entire night, and higher levels of ACTH ($P = 0.01$) as well as a tendency towards higher cortisol levels ($P = 0.072$) during the first night-half, compared with healthy controls. Patients spent slightly less time in slow wave sleep ($P = 0.09$) during the first night-half (where this sleep stage predominates), and overall exhibited an increased proportion of stage 2 sleep ($P = 0.01$). Correspondingly, assessment of mood and symptoms after sleep revealed that subjective sleep was less restorative in T1DM patients than in healthy subjects.

Conclusions: Our data indicate distinct alterations in the neuroendocrine sleep architecture of patients with T1DM which add to the generally adverse impact of the disease on the patients' health.

The endocrine system is subject to a number of periodic processes reflecting the spectrum of biological rhythms. Apart from the circadian rhythm, nocturnal sleep profoundly influences the release of hormones. Sleep onset is followed by a major burst of growth hormone secretion, a rise in circulating prolactin and leptin levels, and a suppression of hypothalamo-pituitary-adrenal (HPA) secretory activity (1). The late part of night-time sleep is associated with a sharp rise in HPA secretory activity, while release of growth hormone, TSH and prolactin is more or less decreased (1).

Whereas neuroendocrine secretion patterns during night-time sleep have been thoroughly examined in healthy subjects, little is known about the dynamics of hormone release during sleep in patients suffering from type 1 diabetes mellitus (T1DM). Compared with healthy controls, patients with T1DM showed elevated growth hormone levels during the first part of the night-time sleep (2). Also, cortisol levels have been found elevated in T1DM patients in the late evening and slightly lowered in the morning (3). However, these results were obtained in studies that did not control for a most important confounding factor, i.e. nocturnal hypoglycemia, which frequently occurs in T1DM patients (4) and per se increases the secretion of cortisol and growth hormone (5). The present study examined whether the neuroendocrine sleep architecture is changed in T1DM patients under non-hypoglycemic conditions.

RESEARCH DESIGN AND METHODS

Subjects. A total of 14 T1DM patients (7 women) and 14 healthy control subjects (7 women) matched for age (mean \pm SEM: 31.3 \pm 2.6 yrs and 28.9 \pm 1.5 yrs) and body mass index (24.2 \pm 0.8 kg/m² and 23.1 \pm 0.7 kg/m²) participated in the experiments. All subjects had a regular sleep-wake cycle and did not work on night shifts during the four weeks before experiments. Habitual sleep duration, as systematically assessed by a

standard questionnaire, did not differ between T1DM patients and healthy subjects (7:25 h \pm 7 min [range: 6:75 – 8:25 h] vs. 7:32 h \pm 8 min [range 6:75 – 8:25 h], $P = 0.78$). Also, the usual bed time (22:59 h \pm 6 min vs. 23:07 h \pm 6 min) and the usual awake up time (06:33 h \pm 6 min vs. 06:44 h \pm 7 min) were well comparable between both groups. Patients with T1DM were selected to participate only when they had no clinical evidence of diabetic complications. Mean diabetes duration was 9.3 \pm 1.6 years (range: 1 - 23 years) and mean HbA1c was 7.7 \pm 0.3% (range: 6.0 – 10.0 %; upper limit of the normal range 6.7%). Ten patients were on an intensive conventional therapy (ICT) regime with at least 3 injections of regular insulin and 1 to 2 injections of long-acting insulin per day. The remaining 4 T1DM patients were on continuous subcutaneous insulin infusion (CSII). The mean \pm SEM cumulative insulin dose was 54.9 \pm 3.9 U per day. The study conformed to the Declaration of Helsinki and was approved by the Ethics Committee on Research Involving Humans of the University of Luebeck. All subjects gave written informed consent prior to participation.

Study design and procedure. Following an adaptation night in the sleep laboratory including the placement of two intravenous catheters and electrodes for standard polysomnography, each subject was tested on one experimental night. During the night, spontaneous hypoglycemia (< 3.9 mmol/l) was prevented by infusing 20% glucose solution whenever necessary, which was the case in 5 patients.

On the day of the experimental night, subjects reported to the laboratory at 20:00 h. Two intravenous catheters were inserted, one into a vein in the back of the hand for infusion of glucose and the other one into an antecubital vein of the other arm for blood sampling. Electrodes were attached to the scalp (electroencephalogram, EEG), around the eyes (horizontal and vertical electrooculogram, EOG) and on the chin

(electromyogram, EMG) for polysomnography. Subjects went to bed and lights were turned off at 23:00 h. Wake up time was 06:30 h. The entire bed period time was monitored by the experimenters via video observation. In the case of awakening before lights on, subjects stayed in bed until 06:30 h. Blood was sampled every 30 minutes via long thin tubes from an adjacent room without disturbing the subject's sleep. Mood and tiredness were assessed by an adjective check list in the evening (between 20:30 and 20:50 h) and in the next morning between 6:50 and 7:10 h.

Sleep recordings. Polysomnographic recordings were scored offline according to standard criteria (6). The following sleep parameters were determined for the first and second half of the night (i.e., end of first half – beginning of second half at 3:00 h) as well as for the total nights: total sleep time (TST), time spent in sleep stages (S) 1, 2, 3, 4 and REM sleep between sleep onset and final awakening), time spent awake after sleep onset (WASO), time spent in S1, S2, S3, S4, SWS (defined as the sum of S3 and S4) and REM sleep (in min and % of TST) as well as movements (defined as the sum of movement times and arousals, in % of 30-sec epoch's) and movement times (MT; in % of TST).

Mood. A standardized adjective check list (EWL) was used to assess mood and feelings of fatigue (7). It consists of a total of 123 adjectives describing the subject's actual mood on 14 dimensions: 'activation', 'deactivation', 'fatigue', 'numbness', 'extraversion', 'introversion', 'self-assuredness', 'well-being', 'agitation', 'sensitivity', 'anger', 'anxiousness', 'depressiveness', and 'dreaminess'. For each adjective, the subject indicated whether or not it reflected aspects of his/her current mood. For each dimension, the numbers of adjectives were counted which were marked by the subject to correctly indicate his/her current mood.

Blood assays. Plasma glucose was measured by means of the Glucose Analyzer II (Beckman Instruments, Inc., Palo Alto, CA).

The following assays were used to measure circulating hormone levels: serum insulin - radioimmunoassay (Pharmacia Insulin RIA 100, Pharmacia Diagnostics, Uppsala, Sweden) with an inter-assay coefficient of variation (CV) <5.8% and an intra-assay CV <5.4%; plasma ACTH - luminescence immunoassay (Lumitest, Brahms Diagnostica, Berlin, Germany), inter-assay CV <5.1%, intra-assay CV <3.2%; serum cortisol - enzyme immunoassay (Enzymun-Test Cortisol, Roche Diagnostics, Mannheim, Germany), inter-assay CV <3.9%, intra-assay CV <2.0%; serum growth hormone - radioimmunoassay (HGH, Diagnostic Products Corporation, Los Angeles, USA), inter-assay CV <3.4%, intra-assay CV <1.6%; plasma epinephrine and norepinephrine - standard high performance liquid chromatography, inter-assay and intra-assay CV: epinephrine <5.6% and <2.9%; norepinephrine <6.1% and <2.6%; serum prolactin (Immulate, Diagnostic Products Corporation, Los Angeles, USA), inter-assay CV <6.4%, intra-assay CV <5.7%; serum leptin (Immulate, Diagnostic Products Corporation, Los Angeles, USA), inter-assay CV <3.3%, intra-assay CV <3.0%; serum TSH (Immulate, Diagnostic Products Corporation, Los Angeles, USA), inter-assay CV <10.0%, intra-assay CV <6.2%.

Statistical analysis. Values are presented as mean \pm SEM. Hormonal data were analyzed by analysis of variance (ANOVA) including the repeated measures factors 'time' for multiple measurements during the night and 'group' for differences between T1DM patients and healthy controls. Area under the curve (AUC) measures calculated for the entire night as well as separately for the first (23:30 - 03:00h) and the second (03:00 - 06:30h) half of the night were compared between groups using unpaired Student's t-tests. The same test was used to compare sleep data between groups. Data from the adjective check list were analyzed by ANOVA including a repeated measures factor 'time' (evening vs. morning) and 'group' (T1DM patients vs. controls).

Pearson correlation analyses were performed to examine the relationships between sleep stages and AUC for growth hormone, cortisol, and ACTH. A P -value < 0.05 was considered significant.

RESULTS

Sleep. Table 1 summarizes polysomnographical data. Total sleep time (TST; 395 ± 15 vs. 404 ± 10 min; $P = 0.93$) as well as sleep onset latency (11 ± 1 vs. 12 ± 1 min, $P = 0.50$) did not differ between T1DM patients and healthy subjects. Interestingly, REM sleep latency, i.e. the first time REM sleep occurred after falling asleep, was significantly longer in the T1DM patients than in healthy control subjects (132 ± 15 min vs. 98 ± 8 min, $P = 0.05$). In both T1DM patients and healthy subjects, slow wave sleep (SWS) predominated during the first night-half whereas REM sleep was more prevalent during the second half, reflecting the typical pattern of nocturnal sleep architecture. Compared with the controls, T1DM patients tended to spend less time in SWS during the first night-half ($P = 0.09$). During both the first ($P = 0.04$) and the second ($P = 0.04$) half of the night, T1DM patients spent significantly more time in sleep stage 2 than the healthy subjects. Sleep parameters of the 5 T1DM patients requiring glucose infusion to prevent spontaneous hypoglycemia did not differ from those in the 9 patients who did not require glucose infusion (TST: 412.6 ± 12.8 vs. 397.6 ± 14.5 min; $P = 0.51$; WASO: 2.0 ± 1.0 vs. 1.3 ± 0.6 %, $P = 0.57$; S1: 12.5 ± 1.2 vs. 14.2 ± 2.6 %, $P = 0.64$; S2: 55.0 ± 3.2 vs. 58.3 ± 2.8 %, $P = 0.48$; SWS: 15.5 ± 4.6 vs. 12.5 ± 2.9 %, $P = 0.58$; REM: 14.8 ± 2.7 vs. 13.4 ± 2.2 %, $P = 0.69$; movements: 10.5 ± 3.0 vs. 12.8 ± 2.5 %, $P = 0.58$; MT: 0.7 ± 0.2 vs. 0.6 ± 0.2 %, $P = 0.68$).

Mood. Data from the adjective check list (summarized in Table 2) revealed that feelings of numbness increased across the night in T1DM patients, but not so in healthy subjects ($P < 0.001$ for group x time interaction). Also, while fatigue decreased

across the night in healthy subjects, it increased in the T1DM patients ($P = 0.05$ for group x time).

Plasma glucose and insulin. Plasma glucose ($P = 0.02$; Fig.1 A) and serum insulin ($P < 0.001$; Fig. 1 B) levels were higher in T1DM patients than in healthy subjects throughout the night. Although plasma glucose levels appeared to decline during the night, this change was not statistically confirmed due to its great variability ($P > 0.32$ for respective main and interaction effects). Serum insulin concentration decreased across the night ($P < 0.001$, for time main effect) with a distinctly more pronounced decrease in the T1DM patients ($P = 0.009$ for group x time).

ACTH, cortisol, growth hormone. Plasma ACTH (Fig. 1 C) and serum cortisol (Fig. 1 D) levels increased in both T1DM patients and healthy subjects during the night ($P < 0.001$ and $P = 0.002$ for time main effects, respectively). Overall, plasma ACTH levels were higher in T1DM patients than in healthy controls ($P = 0.05$), and a similar pattern was observed for average serum cortisol levels although this difference was not significant ($P = 0.16$). During the first night-half, the $AUC_{23:30-03:00h}$ for ACTH was significantly higher (256 ± 39 vs. 163 ± 13 pmol/l*min; $P = 0.01$) and the $AUC_{23:30-03:00h}$ for cortisol tended to be higher ($14,624 \pm 3,603$ vs. $8,123 \pm 1,230$ nmol/l*min; $P = 0.07$) in T1DM patients than in healthy controls. During the second night-half, $AUC_{03:00-06:30h}$ for ACTH (509 ± 53 vs. 392 ± 62 pmol/l*min; $P = 0.16$) and cortisol ($29,571 \pm 4,306$ vs. $23,797 \pm 4,055$ nmol/l*min; $P = 0.36$) did not differ between groups.

Serum growth hormone levels showed a maximum in the beginning of the night at about 00:30h and continuously decreased thereafter in both T1DM patients and healthy subjects ($P = 0.001$ for time main effect; Fig. 1 E). Overall, growth hormone levels were significantly higher in T1DM patients than in controls ($P = 0.05$ for group main effect). $AUC_{23:30-06:30h}$ for the entire

night (951 ± 173 vs. 640 ± 89 $\mu\text{g/l}^*\text{min}$; $P = 0.03$) as well as $\text{AUC}_{03:00-06:30\text{h}}$ for the second night-half (291 ± 72 vs. 126 ± 14 $\mu\text{g/l}^*\text{min}$; $P = 0.016$) were higher in T1DM patients than controls.

Catecholamines. Overall, plasma epinephrine levels tended to be higher in T1DM patients than in controls ($P = 0.05$ for group main effect; Fig. 1 F). Comparison of AUCs revealed significantly higher epinephrine levels in T1DM patients, compared with controls, during the entire night ($17,443 \pm 2,077$ vs. $13,669 \pm 663$ $\mu\text{g/l}^*\text{min}$; $P = 0.02$) and also during the first night-half ($9,070 \pm 1,061$ vs. $6,903 \pm 429$ $\mu\text{g/l}^*\text{min}$; $P = 0.04$). In contrast, there was no evidence for any group difference in plasma norepinephrine concentrations ($P > 0.6$ for all comparisons; Fig. 1 G).

Prolactin, leptin, TSH. Serum prolactin ($P < 0.001$ for time main effect; Fig. 1 H) and serum leptin concentrations ($P = 0.022$; Fig. 1 I) increased in both T1DM patients and healthy subjects during the night, without any significant difference in this increase between groups ($P > 0.1$ for all comparisons). AUCs for prolactin and leptin revealed similar results with no difference between groups ($P > 0.2$). Serum TSH levels decreased during the night ($P < 0.001$ for time main effect; Fig. 1 J) but also did not differ between T1DM patients and healthy subjects (all $P > 0.32$). There was also no difference in AUCs for TSH between groups (all $P > 0.25$).

Correlation analyses. In pooled data of both the T1DM patients and the healthy control subjects, $\text{AUC}_{23:30-03:00\text{h}}$ for ACTH was correlated with plasma glucose levels ($r = 0.378$; $P = 0.047$), while $\text{AUC}_{23:30-03:00\text{h}}$ for cortisol was correlated with serum insulin levels ($r = 0.463$; $P = 0.013$). Cortisol levels during the first part of the night were inversely correlated with the amount of SWS in healthy control subjects ($r = -0.569$, $P = 0.03$), but not in T1DM patients ($r = -0.126$, $P = 0.67$). There were no other significant correlations between sleep stages and hormone levels.

In patients with T1DM, $\text{AUC}_{23:30-03:00\text{h}}$ for cortisol was correlated with HbA1c levels ($r = 0.562$, $P = 0.04$), while total night $\text{AUC}_{23:30-06:30\text{h}}$ for cortisol was correlated with the duration of T1DM ($r = 0.585$, $P = 0.03$). There were no other significant correlations between HbA1c, disease duration, sleep stages, and hormonal levels in T1DM patients (all $P > 0.122$).

CONCLUSIONS

Our data show discrete alterations in the neuroendocrine sleep architecture of T1DM patients. Whereas growth hormone and epinephrine levels were increased during the whole night in T1DM patients, concentrations of HPA hormones were elevated mainly during the first night half. The hormonal changes were accompanied by a tendency towards more shallow sleep in T1DM patients with decreased amounts of SWS during the first night half and an overall increased proportion of stage 2 sleep throughout the night. Also, increased numbness and fatigue point to a weakened restorative effect of sleep in T1DM patients.

Our findings of increased levels of HPA hormones and growth hormone in T1DM patients are in line with previous studies (2,3). However, only one of these studies assessed sleep architecture via polysomnographic recordings and none included an approach to prevent nocturnal hypoglycemia representing a major confounder of hormonal activity. Carefully controlling for glucose levels in the patient group, our study shows that increased HPA secretory activity and enhanced growth hormone levels in T1DM patients in combination with lighter sleep do not depend on the occurrence of hypoglycemic episodes.

Our data do not allow conclusions regarding cause-effect relationships. Nevertheless, it is tempting to speculate that the increase in HPA secretory activity during the first night-half is a result of diminished SWS and increased sleep stage 2 during this time, because SWS has been demonstrated to actively inhibit HPA

secretory activity (8). However, reduced SWS in T1DM patients should be associated with decreased rather than increased growth hormone levels, since the release of growth hormone during sleep is closely connected to slow wave sleep and EEG synchronization (9). Also, in light of the moderate size of the reduction in SWS, it is unlikely that the observed changes in neuroendocrine activity in T1DM patients are a mere result of alterations in central nervous sleep regulation.

As demonstrated in our study, patients with T1DM frequently display elevated levels of plasma glucose and insulin due to the fact that even sophisticated regimens of insulin substitution do not perfectly mimic the physiological regulation of plasma glucose and insulin secretion. Apart from discrete hypoglycemic episodes that were prevented in our experiments, slightly but persistently elevated concentrations of glucose and insulin can likewise stimulate HPA activity and the release of epinephrine. Previous studies in healthy subjects indeed showed a stimulatory influence of insulin on HPA secretory activity (10) and circulating epinephrine (11) during daytime wakefulness. Also, both type 1 and type 2 diabetic patients display an overall increased HPA activity during spontaneous activity as well as in stimulation and suppression tests (3,12-14). Some of those studies suggest that increased HPA secretory activity depends on the strength of glycemic control, i.e. the degree of hyperglycemia (3,12-14). Epinephrine levels have also been found to be elevated in T1DM (15-17). However, the influence of hyperglycemia and hyperinsulinemia on neuroendocrine sleep architecture has not been directly assessed so far. Comparing effects of human and porcine insulin on sleep under non-hypoglycemic conditions in T1DM subjects, Roth et al. (18) observed reduced power in the 14-Hz spindle frequency range when the patients were switched from porcine to human insulin. Also, patients with human insulin felt less relaxed after sleep. However this study did

not include a control in healthy subjects without insulin administration which hampers the interpretation of the results.

While our study for the first time points to an alteration in neuroendocrine sleep architecture in patients with T1DM under non-hypoglycemic conditions, it does not allow any conclusion about its underlying pathophysiological mechanisms. Here, further studies carefully controlling for glycemia as well as for insulinemia (possibly by applying hyperinsulinemic-euglycemic clamp technique) are required to dissociate a potential influence of these factors from the influence of T1DM itself. However, from the clinical point of view our results appear to be of particular interest since they were obtained under conditions that, with exception of the systematic prevention of hypoglycemia, resemble real life conditions in T1DM patients being characterized by various degrees of hyperglycemia and hyperinsulinemia. The elevation in blood concentrations of epinephrine, ACTH, cortisol, and growth hormone observed here in T1DM patients during sleep hints at a generally increased activity of neuroendocrine stress systems in these patients. Besides the presence of hyperglycemic and hyperinsulinemic conditions, psychological factors such as the burden of the disease could contribute to activating stress systems. However, assessment of mood did not indicate any alterations in well-being and self-confidence in T1DM patients and patients also did not show any signs of sleep disruption such as increased WASO, stage 1 or movements, which renders this possibility unlikely.

Our results are of clinical relevance for patients with T1DM. Both chronic disturbances of sleep (19-22) and activity of neuroendocrine stress systems (23,24) have been implicated in increased morbidity and mortality. On this background, we suggest that some of the adverse effects of T1DM on general health are mediated by changes in sleep and associated neuroendocrine activity which, hence, might become

another target for therapeutical interventions in T1DM patients.

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FIGURE LEGENDS

FIGURE 1. Mean \pm SEM levels of glucose (A), insulin (B), ACTH (C), cortisol (D), growth hormone (E), epinephrine (F) norepinephrine (G), prolactin (H), leptin (I), and TSH (J) in T1DM patients (black circles) and 14 healthy controls matched for sex, age, and BMI (open circles) during a 7-h night-time sleep period. Grey horizontal bars indicate intervals (first or second night-half or entire night) of significantly ($P < 0.05$, as derived from ANOVA) increased hormone levels in T1DM patients as compared with controls.

* = $P < 0.05$ derived from unpaired Student's t-test

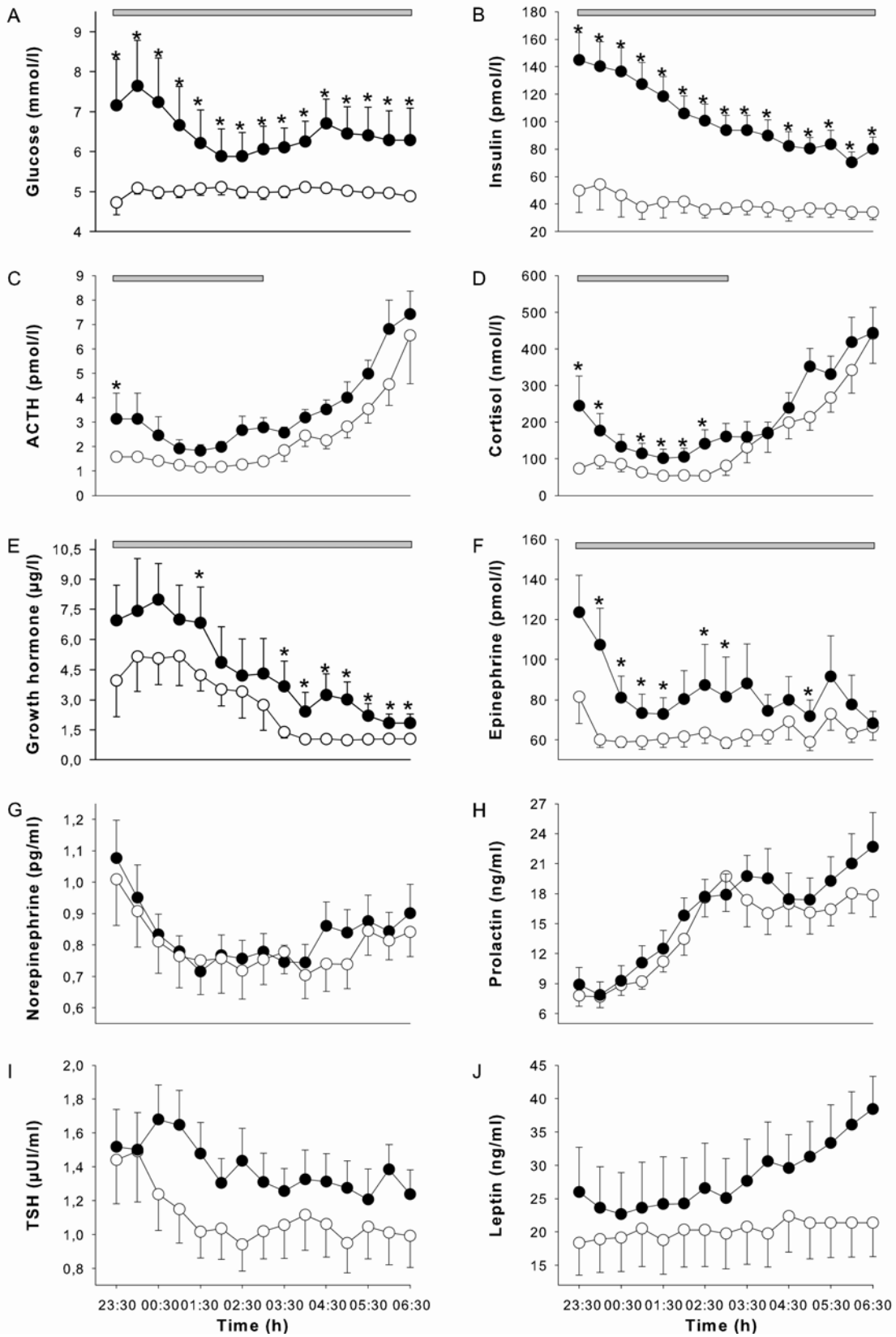


FIGURE 1

TABLE 1. Sleep parameters during the first and the second halves of the night
Data are means \pm SEM. Percent values refer to total sleep time (TST).

	T1DM patients	Healthy subjects	<i>P</i> -value
First night-half			
TST (min)	202.8 \pm 0.8	201.7 \pm 1.6	0.83
WASO (%)	0.7 \pm 0.3	1.5 \pm 0.8	0.25
S1 (%)	11.9 \pm 1.7	17.8 \pm 3.3	0.59
S2 (%)	58.6 \pm 3.1	47.5 \pm 4.0	0.04
SWS (%)	21.3 \pm 3.7	24.7 \pm 3.3	0.09
REM (%)	7.5 \pm 1.5	8.6 \pm 1.8	0.38
Movement (%)	12.3 \pm 2.0	12.7 \pm 2.2	0.33
MT (%)	0.7 \pm 0.2	0.5 \pm 0.2	0.52
Second night-half			
TST (min)	194.6 \pm 9.1	208.4 \pm 7.4	0.69
WASO (%)	3.1 \pm 1.6	3.9 \pm 2.2	0.21
S1 (%)	16.3 \pm 2.4	20.3 \pm 4.9	0.27
S2 (%)	52.6 \pm 3.0	46.6 \pm 5.4	0.04
SWS (%)	8.3 \pm 2.3	7.0 \pm 2.0	0.66
REM (%)	20.0 \pm 2.3	22.2 \pm 2.5	0.27
Movement (%)	13.0 \pm 1.7	17.8 \pm 3.4	0.68
MT (%)	0.6 \pm 0.2	0.5 \pm 0.2	0.54
Entire night			
TST (min)	395 \pm 15	404 \pm 10	0.93
WASO (%)	1.8 \pm 0.8	2.6 \pm 1.1	0.52
S1 (%)	14.2 \pm 1.7	19.2 \pm 3.3	0.34
S2 (%)	55.6 \pm 2.4	47.2 \pm 4.1	0.01
SWS (%)	14.7 \pm 2.6	14.9 \pm 2.3	0.75
REM (%)	13.9 \pm 1.5	15.5 \pm 1.3	0.58
Movement (%)	12.4 \pm 1.7	15.1 \pm 2.3	0.23
MT (%)	0.6 \pm 0.1	0.5 \pm 0.1	0.50

WASO = time spent awake after sleep onset; S1 = sleep stage 1; S2 = sleep stage 2; SWS = slow wave sleep; REM = rapid eye movement sleep P-values derive from unpaired Student's t-test

TABLE 2: Mood assessed by an adjective check list on 14 dimensions (left, translated from German) before and after the night-time sleep

	T1DM patients		Healthy subjects		P-value		
	Evening	Morning	Evening	Morning	Time	Group	Time x Group
Activation	0.40 ± 0.08	0.25 ± 0.08	0.40 ± 0.08	0.40 ± 0.09	0.18	0.45	0.13
Deactivation	0.22 ± 0.06	0.28 ± 0.08	0.20 ± 0.06	0.22 ± 0.07	0.38	0.64	0.62
Fatigue	0.28 ± 0.07	0.38 ± 0.06	0.32 ± 0.07	0.21 ± 0.04	0.87	0.35	0.05
Numbness	0.07 ± 0.04	0.34 ± 0.05	0.13 ± 0.04	0.13 ± 0.06	0.001	0.18	< 0.001
Extroversion	0.63 ± 0.05	0.59 ± 0.08	0.57 ± 0.08	0.54 ± 0.08	0.51	0.54	0.85
Introversion	0.17 ± 0.08	0.16 ± 0.08	0.13 ± 0.05	0.11 ± 0.05	0.77	0.62	0.91
Self- Assuredness	0.59 ± 0.07	0.56 ± 0.09	0.51 ± 0.08	0.54 ± 0.08	0.91	0.61	0.63
Well-Being	0.54 ± 0.09	0.49 ± 0.09	0.65 ± 0.08	0.70 ± 0.07	0.91	0.16	0.25
Agitation	0.18 ± 0.05	0.09 ± 0.04	0.19 ± 0.07	0.10 ± 0.03	0.01	0.91	0.90
Sensibility	0.10 ± 0.06	0.04 ± 0.03	0.04 ± 0.02	0.00 ± 0.00	0.13	0.89	0.98
Anger	0.08 ± 0.04	0.07 ± 0.05	0.05 ± 0.04	0.01 ± 0.01	0.14	0.22	0.82
Anxiousness	0.08 ± 0.04	0.07 ± 0.05	0.05 ± 0.04	0.01 ± 0.01	0.252	0.38	0.45
Depressiveness	0.11 ± 0.06	0.08 ± 0.07	0.04 ± 0.02	0.01 ± 0.01	0.07	0.29	0.81
Dreaminess	0.18 ± 0.07	0.33 ± 0.07	0.20 ± 0.07	0.25 ± 0.06	0.41	0.29	0.14

Data are means ± SEM. P-values are derived from ANOVA including a repeated measures factor 'time' (evening vs. morning) and a factor 'group' (T1DM patients vs. healthy controls).