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Twenty-four-hour rhythms of plasma glucose and insulin secretion rate in regular night workers

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Simon, C., L. Weibel, and G. Brandenberger. Twenty-four-hour rhythms of plasma glucose and insulin secretion rate in regular night workers. *Am. J. Physiol. Endocrinol. Metab.* 278: E413–E420, 2000.—To determine whether the ultradian and circadian rhythms of glucose and insulin secretion rate (ISR) are adapted to their permanent nocturnal schedule, eight night workers were studied during their usual 24-h cycle with continuous enteral nutrition and a 10-min blood sampling procedure and were compared with 8 day-active subjects studied once with nocturnal sleep and once with an acute 8-h-shifted sleep. The mean 24-h glucose and ISR levels were similar in the three experiments. The duration and the number of the ultradian oscillations were influenced neither by the time of day nor by the sleep condition or its shift, but their mean amplitude increased during sleep whenever it occurred. In day-active subjects, glucose and ISR levels were high during nighttime sleep and then decreased to a minimum in the afternoon. After the acute sleep shift, the glucose and ISR rhythms were split in a biphasic pattern with a slight increase during the night of deprivation and another during daytime sleep. In night workers, the glucose and ISR peak levels exhibited an 8-h shift in accordance with the sleep shift, but the onset of the glucose rise underwent a shift of only 6 h and the sleep-related amplification of the glucose and ISR oscillations did not occur simultaneously. These results demonstrate that despite a predominant influence of sleep, the 24-h glucose and ISR rhythms are only partially adapted in permanent night workers.

ultradian and circadian rhythms; sleep

IN NORMAL HUMANS, INSULIN secretion rate (ISR) presents a pattern characterized by slow, ultradian oscillations with a periodicity of 50 to 150 min coexisting with rapid and small fluctuations recurring every 8 to 15 min (8, 10, 24, 25, 28). The ultradian, circhoral ISR oscillations, which are closely associated with similar changes in plasma glucose, have been shown to be of functional significance; abnormalities in their pattern are observed in noninsulin-dependent diabetes (26, 30) and also in conditions of minimal impaired glucose tolerance (16, 21). In these situations, the ISR oscillations are less regular, have a reduced amplitude, and the tight coupling with the glucose oscillations is altered.

We previously demonstrated that the 24-h ISR and plasma glucose profiles arise, at least partly, from a modulation of the ultradian oscillations (27). In normal subjects receiving enteral nutrition at a constant rate and studied in a recumbent position, the amplitude of both the ISR and glucose oscillations increases during nocturnal sleep, yielding an elevation of the ISR and of the plasma glucose mean levels. The oscillation amplification, which occurs without any change in oscillation frequency, is also observed during daytime sleep, after an abrupt delay in the sleep period, suggesting a primarily sleep-dependent effect. However, studies involving an 8- to 12-h acute shift of the sleep-wake period in subjects receiving constant enteral nutrition (27) or intravenous glucose infusion (32) have shown modest increases of ISR and plasma glucose during the night of sleep deprivation and a nadir at about 0700, indicating the presence of a weak circadian modulation of glucose regulation interacting with the sleep processes to produce the overall 24-h pattern. The higher glucose and insulin responses to oral glucose (9) or to mixed meals (23) in the evening rather than in the morning further support the existence of a circadian influence on glucose metabolism. In conditions of usual sleep, the effects of sleep and of circadian rhythmicity are superimposed and synchronized.

The ISR and plasma glucose rhythms have not been studied after a prolonged shift of the sleep-wake cycle. We recently demonstrated that the circadian system of permanent night workers, even with high work satisfaction, is only partially adapted to their active night schedule, resulting in an internal dissociation of the markers of different 24-h rhythms, such as melatonin, cortisol, thyrotropin (TSH), and temperature (36, 38, 39). The sleep-wake cycle and the night-day alternance, which act as conflicting synchronizers, have also been shown to affect growth hormone (GH) secretion (37), mainly dependent on sleep processes. The aim of this study was to define the ultradian and the circadian rhythmicities of ISR and plasma glucose in night workers with four to six consecutive night shifts per week for at least 2 yr and to evaluate their degree of adaptation to the nocturnal schedule. To avoid the effects of discontinuous food ingestion and of physical activity on the rhythms, subjects were studied during continuous enteral nutrition and were in recumbent position throughout the 24 h. They were compared with day-active subjects studied in the same conditions once

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with usual nocturnal sleep and once after an acute 8-h shift of the sleep period.

SUBJECTS AND METHODOLOGY

Subjects

Eight healthy regular night workers (24–35 yr) who had been working on permanent shifts for at least 2 yr and eight healthy male day-active subjects (23–30 yr) participated in this experiment. The night workers selected worked four to six consecutive night shifts per week and claimed that no naps were allowed during their night work. During days off, they did not revert to a usual lifestyle but maintained a partial shift in their activity cycle. All were in good health with a high work satisfaction, and no complaints of permanent fatigue, sleep disturbance, or alteration of mood were noted.

The day-active subjects had normal routines of work, meals, and sleep. They were selected after screening tests and questionnaires on their usual sleep-wake cycle and their work and light-exposure schedule. Subjects with any personal history of sleep disorders or having experienced time-shift or sleep deprivation during the previous weeks were excluded from the study.

All individuals were selected after medical examination and were of normal body weight (<25 kg/m^2). None had a personal or family history of metabolic or endocrine disorders. Subjects with underlying signs of disease, subjects taking medication, and smokers were excluded. Subjects all gave their written informed consent, and the study was approved by the local Ethics Committee.

Procedure

Night workers were admitted to the laboratory immediately following their last night shift and slept from 0700 to 1500, which represents their normal sleep period, to undergo an habituation session. They were then studied during their normal 24-h sleep-wake cycle with daytime sleep from 0700 to 1500 and left the laboratory at 2300 on *day 2*. The normally day-active subjects were studied twice in random order with 1-mo interval between the experiments. They were admitted to the laboratory for a continuous 48-h period, with admission at 2200 to undergo a night of habituation from 2300–0700. They were then studied once during a normal 24-h sleep-wake cycle, with nocturnal sleep from 2300 to 0700, and once during a 24-h cycle, with an acute 8-h shift in the sleep period obtained by total sleep deprivation during the night and a daytime recovery sleep from 0700 to 1500. They left the laboratory at 2300 on *day 2*.

The experiments were performed in soundproof, air-conditioned sleep chambers communicating with an adjoining room where blood samples and sleep data were collected. To avoid the influence of repeated meal ingestion and fasting, the subjects received continuous enteral nutrition (Sondalis ISO; Sopharga Puteaux, France; 50% carbohydrate, 35% fat, 15% protein, 378 kJ/h), which began at 1700 and lasted 30 h. The subjects did not consume any additional calories until the end of the study. A catheter was then inserted under local anesthesia into an antecubital vein and was kept patent with heparinized solutions. Electrodes were attached for uninterrupted electrophysiological recordings. The subjects then remained supine for the hours preceding blood sampling and throughout the experiment to avoid postural influence. During the wakefulness period they were maintained in dim light (<100 lux) and were kept under continuous supervision; they conversed with the laboratory staff and were allowed to read or watch television. During the sleep period, lights were switched off.

Blood Sampling and Hormone Assays

Blood samples were taken continuously, at 10-min intervals, throughout the 24-h experiments from 2300 on *day 1* to 2300 on *day 2* using a peristaltic pump. EDTA-K_2-treated tubes (1 mg/ml) were used. Blood samples were immediately centrifuged at 4°C, and plasma was stored at −25°C until assay. Plasma glucose levels were measured using a glucose oxidase method (Boehringer, Mannheim, Germany) with an intra-assay coefficient of variation (CV) below 1.3%. Plasma C-peptide levels were determined by radioimmunoassay (Byk-Sangtec Diagnostica, Dietzenbach, Germany), with a detection limit of 0.05 ng/ml. The mean intra-assay CV was 4.8% for values below 1.2 ng/ml, 4.1% between 1.2 and 4.5 ng/ml, and 3.0% for values above. All samples from one individual were analyzed in a single assay.

Sleep Recording and Analysis

Polygraphic sleep recordings included two electroencephalographic derivations, two electrooculograms, one electromyogram, and one electrocardiogram. Sleep stages were scored at 30-s intervals according to the Rechtshaffen and Kales criteria (19). On this basis, total sleep time, the total duration of slow-wave sleep (SWS) and of rapid-eye-movement (REM) sleep, sleep onset, SWS and REM sleep latencies, and the number and duration of intra-sleep awakenings were quantified. The sleep efficiency index was defined as the ratio of total sleep time to time allowed to sleep, i.e., when the lights were off from 2300 to 0700.

Data Analysis

Determination of ISR. For each subject, the ISR during each 10-min interval was mathematically derived, as previously described (27), from the plasma C-peptide levels using a two-compartment model. This deconvolution method is based on the fact that insulin and C-peptide are cosecreted in equimolar concentrations and that C-peptide, unlike insulin, is not significantly extracted by the liver and has constant metabolism at different times of the day (2) and in different nutritional circumstances (12). The kinetic parameters for C-peptide distribution and metabolism were obtained from published data adjusted for sex, age, and body surface area (33). No assumption was made for the shape of the secretory pulses. Statistical error propagation of the uncertainty in C-peptide measurements was taken into account in the determination of the secretory profiles, and the standard deviation associated with each estimated secretory rate was calculated.

Circadian rhythmicity. To quantify the long-term diurnal wave changes in plasma glucose and ISR, independently of the ultradian variations, a smooth best-fit curve using a robust locally weighted regression procedure, as proposed by Cleveland (4), was calculated for each individual profile, with a window of 2 h. The times of occurrence of maxima (peaks) and of minima (nadirs) in the best-fit curve were determined. The diurnal amplitude of each individual 24-h profile was defined as the difference between the values at peak and at nadir. The oscillations of plasma glucose and ISR create an uncertainty in the determination of the nadir. Therefore, the onset and the offset of the glucose and ISR sleep-associated rises were additionally used as markers of the 24-h rhythms, as has been proposed for melatonin (11) or cortisol (13). Considering the great pulsatility of glucose and ISR profiles the procedure was adapted to our high-frequency blood

sampling. The onset (offset) of the plasma glucose and ISR rise was defined as the time when the value of the best-fit curve exceeded (undercut) the value of the nadir plus 30% of the diurnal amplitude in at least 10 consecutive samples.

Ultradian rhythmicity analysis. The individual 24-h plasma glucose and ISR profiles were analyzed for pulse identification using the computer program ULTRA (31). The threshold for pulse detection was set at two times the intra-assay CV in the relevant range of concentration for glucose and at three times the standard deviation associated with the estimated secretory rate for ISR. For each significant pulse, the time of occurrence, the increment, the decrement, and the total duration were determined.

To investigate the regularity of the glucose and ISR oscillations and to identify the dominating frequency underlying them, spectral analysis was performed (BMDP Statistical Software, Berkeley, CA) on the plasma glucose and ISR individual profiles. First a difference filter was used to remove low frequency components. Then discrete fast Fourier transform algorithm and a cosine-shaped smoothing window with a bandwidth of 160 min were used on the filtered data to assess the spectral density function. The period having the highest spectral density was identified, and its significance was tested by rejecting the null hypothesis of no periodicity at 0.05 level using Hartley's test based on the distribution of each periodogram, which is proportional to χ^2 (18). The normalized spectral density functions were then averaged according to group and experimental conditions.

The association between individual pulses of plasma glucose and those of ISR was tested by means of a lagged coincidence analysis, based on a model of conditional probability derived from two binomial distributions and leading to a hypergeometric probability density function, as proposed by Veldhuis et al. (35). Two pulses were considered to be concomitant if their peaks occurred within ±10 min of each other.

Statistical analysis. The results are expressed as means ± SE. The parameters for day-active subjects sleeping at night and at daytime were compared using bilateral paired Student's *t*-tests, and comparisons of parameters between day-active subjects sleeping at night and night workers were performed using Student's *t*-tests for unpaired groups. A repeated measures ANOVA with Greenhouse-Geiser correction and bilateral paired *t*-test with Bonferroni procedure for multiple comparisons were used to assess the statistical differences between the different time periods in each experimental rhythm. Depending on the parameters, three periods of 8 h (2300–0700, 0700–1500, and 1500–2300) or six periods of 4 h (2300–0300, 0300–0700, 0700–1100, 1100–1500, 1500–1900, 1900–2300) were considered.

RESULTS

Sleep Characteristics

Sleep characteristics are summarized in Table 1. The sleep parameters were similar for night workers and the day-active subjects sleeping at night. In day-active subjects, total sleep time and sleep efficiency (ratio of total sleep time to time allowed to sleep) were not affected by the shift in sleep. However, as previously reported, the sleep onset latency and the SWS latency significantly decreased ($P < 0.05$) during daytime sleep in comparison with nighttime sleep. In addition the duration of SWS was significantly enhanced ($P < 0.05$), whereas the latency and the duration of REM sleep were not significantly affected by the sleep shift.

Table 1. *Comparison of sleep parameters*

	Day-Active Subjects With Nocturnal Sleep	Day-Active Subjects With Daytime Sleep	Night Workers With Daytime Sleep
Total sleep time, min	403 ± 16	421 ± 10	378 ± 28
Sleep efficiency, %	84 ± 4	88 ± 2	79 ± 6
Sleep onset latency, min	23 ± 5	4 ± 1*	20 ± 4
SWS latency, min	40 ± 7	16 ± 2†	54 ± 14
SWS sleep duration, min	74 ± 11	95 ± 12*	59 ± 12
REM latency, min	146 ± 26	77 ± 16	132 ± 24
REM duration, min	83 ± 10	82 ± 6	71 ± 12

Values are means ± SE; n = 8 night workers and 8 day-active subjects. SWS, slow-wave sleep; REM, rapid eye movement. Differences between day-active subjects when sleeping at day and at night: *$P < 0.05$ and †$P < 0.01$. Differences between regular night workers and day-active subjects sleeping at night all not significant (NS).

Mean 24-h Glucose and ISR Profiles

Figure 1 illustrates the mean 24-h plasma glucose and ISR profiles obtained in eight day-active subjects sleeping once at night and once at daytime and in eight night workers sleeping as usual at daytime. The parameters derived from the regression curves, which quantify the 24-h temporal changes, are detailed in Table 2. The mean 24-h glucose and ISR levels were similar in the three experiments.

As previously shown (27), the profiles observed in day-active subjects in the two sleep conditions indicated the presence of modulatory effects of both sleep and a circadian component. During normal nocturnal sleep, plasma glucose and ISR increased in the first part of the night to a maximum around mid sleep and then decreased rapidly with a nadir in the morning. Mean plasma glucose and ISR were significantly higher during nocturnal sleep than during the waking periods ($P < 0.01$).

In the daytime sleep conditions, the mean plasma glucose and ISR profiles were split in a biphasic pattern. After a slight increase during the night of sleep deprivation, plasma glucose decreased to a minimum around 0700 and then rose slightly to a maximum around mid sleep. There was no significant difference in the mean plasma glucose levels between the sleep and waking periods. Similarly, a slight increase in ISR was observed during the night of sleep deprivation, followed by a nadir around 0700. The sleep-associated ISR increase that followed was lower but occurred at the same time, after lights off, as during usual nocturnal sleep.

In the night workers, the permanent delay of 8 h in the sleep period induced an 8-h shift of the glucose and ISR peaks to daytime, which remained located at mid sleep as in day-active subjects, with no modification in the levels. Mean plasma glucose and ISR were significantly higher than during the waking periods ($P < 0.01$). Neither the glucose and ISR peak and nadir levels, nor the amplitude of the 24-h variations was significantly different from day-active subjects sleeping at night. However the onset of the sleep-associated rise in plasma glucose was shifted by only 6 h, and because

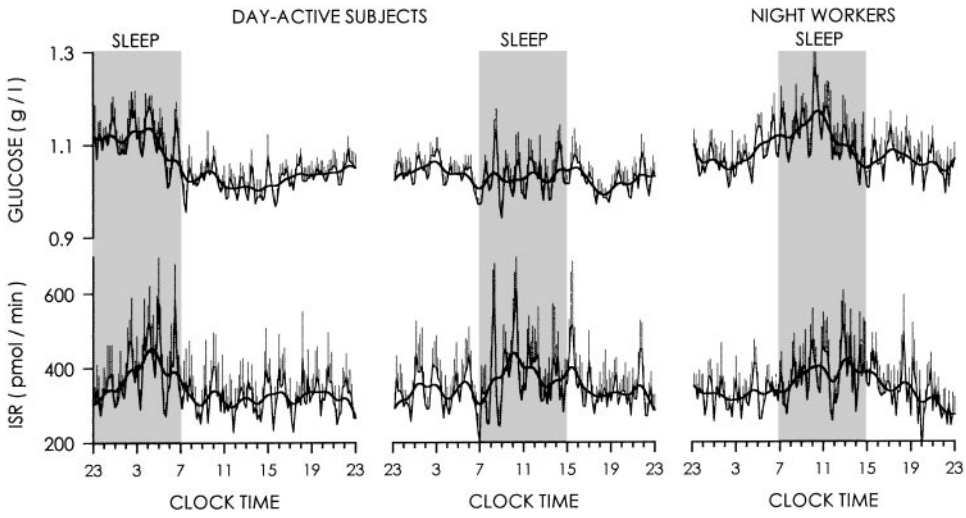

Fig. 1. Mean ± SE 24-h profiles of plasma glucose and insulin secretion rate (ISR) in 8 day-active subjects, sleeping once at night (left) and once at daytime after an acute shift in sleep (middle), and in 8 night workers sleeping as usual at day (right). Continuous lines illustrate best-fit curve quantifying waveshape of rhythms.

of a later offset time the ISR rise was more prolonged ($P < 0.01$) than in day-active subjects.

Ultradian Glucose and ISR Rhythmicity

As illustrated for individual subjects in Fig. 2, in which the sleep period has been placed in the middle of each graph, high-amplitude, synchronous oscillations in plasma glucose and ISR were observed throughout the 24 h in day-active subjects as well as in night workers. Coincidence analysis revealed that on average 76% of ISR oscillations were preceded by a glucose oscillation within a lag of ±10 min, a concordance that was similar in the three experimental series. The characteristics of the oscillations are given in Table 3.

The mean oscillation count, oscillation duration, and interpulse interval were similar in the three experimental series. The low interpulse interval variability indicated the regularity of the ultradian rhythm. Spectral analysis of the 24-h individual profiles confirmed the strength of the ultradian cyclic component, with significant periodic components between 65 and 130 min for ISR and between 60 and 120 min for plasma glucose. The mean normalized spectral density function was affected by neither the acute nor the permanent shift of sleep (Fig. 3).

The mean absolute and relative amplitudes of the plasma glucose and ISR oscillations were similar in the three experimental series. As previously reported in day-active subjects (27), increased amplitude of the plasma glucose and ISR oscillations was observed during the sleep period, independently of the time of day and irrespective of the experimental conditions with no modification of their duration. The sleep-associated increase in amplitude, compared with the 16-h waking period, was similar in the three experimental series. However, closer examination of the 24-h profiles revealed some differences, as seen in Fig. 4, which displays the mean amplitude of the plasma glucose and ISR oscillations for the six consecutive 4-h periods of nycthemeron. Here again, the sleep period was placed in the middle of each graph, so that conclusions were easier to evaluate. In the permanent night workers, the glucose oscillation increase began earlier than in the day-active subjects studied with their usual nocturnal sleep. The mean amplitude of the oscillations was higher during the 4-h period preceding sleep onset than during the earlier waking periods ($P < 0.02$). Their increase was more progressive during sleep than

Table 2. *Characteristics of 24-h mean plasma glucose and ISR profiles*

	Day-Active Subjects With Nocturnal Sleep	Day-Active Subjects With Daytime Sleep	Night Workers With Daytime Sleep
Plasma glucose			
Mean 24-h level, g/l	1.05 ± 0.02	1.03 ± 0.02	1.08 ± 0.02
Sleep-associated mean increase, % of waking period	107.9 ± 1.8	100.0 ± 0.5†	104.0 ± 1.2
Peak level, g/l	1.20 ± 0.05	1.08 ± 0.02* 1.10 ± 0.02*	1.21 ± 0.03
Time of sleep-associated maximum	0421 ± 34	0411 ± 38	0410 ± 39
Onset of sleep-associated rise	−0129 ± 37	0052 ± 43*	−0327 ± 34‡
Offset of sleep-associated rise	0937 ± 43	0556 ± 35*	1010 ± 60
Insulin secretion rate			
Mean 24-h level, pmol/min	341.9 ± 51.0	352.6 ± 50.0	353.4 ± 35.6
Sleep-associated mean increase, % of waking period	120 ± 3.2	112.8 ± 2.8*	115.9 ± 4.2
Peak level, pmol/min	488.7 ± 71	402.0 ± 60.2† 490.2 ± 75.1	473.5 ± 56.3
Time of sleep-associated maximum	0457 ± 32	0425 ± 47	0458 ± 53
Onset of sleep-associated rise	0111 ± 29	0026 ± 25	−0018 ± 41
Offset of sleep-associated rise	0910 ± 41	0756 ± 33	1215 ± 33§

Values are means ± SE; n = 8 night workers and 8 day-active subjects. Times are expressed in reference to lights off. ISR, insulin secretion rate. Differences between day-active subjects when sleeping at day and at night: *$P < 0.05$; †$P < 0.01$. Differences between regular night workers and day-active subjects sleeping at night: ‡$P < 0.05$; §$P < 0.01$. NS otherwise.

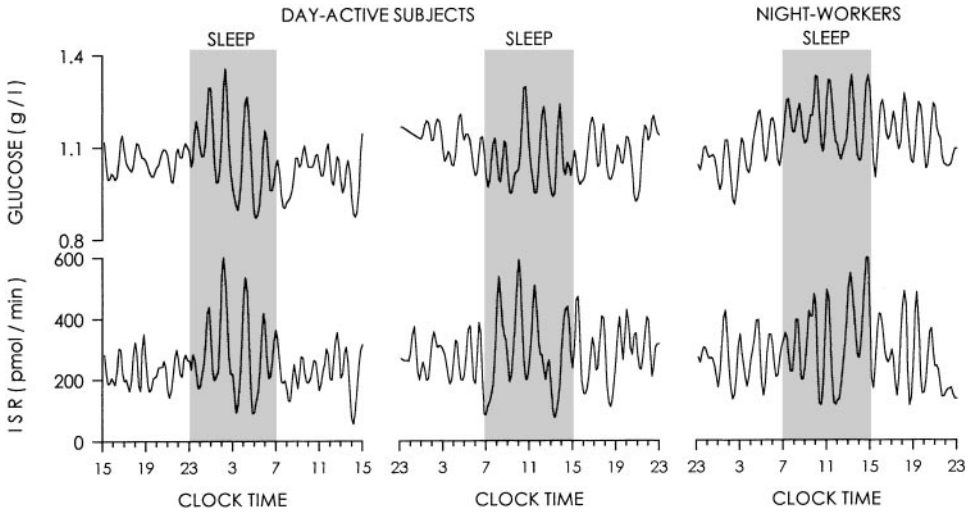

Fig. 2. Individual 24-h profiles of plasma glucose levels and of ISR in 1 representative day-active subject sleeping once at night (left) and once at daytime after an acute shift in sleep (middle), and in 1 representative night worker sleeping as usual at daytime (right). Sleep period was placed in middle of each graph.

in the day-active subjects, resulting in higher amplitudes during late sleep than during early sleep ($P < 0.05$), and their decrease in the late part of sleep was slower. Contrasting with this early glucose oscillation increase and taking into account the timing of the shift period, the increase in the ISR oscillations was quite similar to that observed in the day-active subjects. It began generally after sleep onset, with a progressive increase throughout sleep and a decrease in the daytime leading to a desynchronization of the glucose and ISR rhythms.

Table 3. *Characteristics of the glucose and ISR oscillations*

	Day-Active Subjects With Nocturnal Sleep	Day-Active Subjects With Daytime Sleep	Night Workers With Daytime Sleep
Plasma glucose oscillations			
Number/24 h	20.1 ± 1.1	20.5 ± 0.9	22.5 ± 1.2
Duration, min	66.1 ± 3.1	64.5 ± 2.9	60.0 ± 2.6
Interpulse interval, min	71.7 ± 3.6	69.3 ± 3.0	64.0 ± 3.0
Absolute amplitude, g/l	0.19 ± 0.01	0.18 ± 0.01	0.20 ± 0.02
Relative amplitude, % of 24-h mean	17.8 ± 0.8	17.2 ± 1.5	18.5 ± 1.6
Sleep-associated oscillation amplitude increase, % of waking mean amplitude	150.8 ± 14.1	186.2 ± 15.2	157.7 ± 15.2
Insulin secretion rate oscillations			
Number/24 h	19.1 ± 0.8	19.0 ± 0.8	19.6 ± 1.1
Absolute amplitude, pmol/min	348.3 ± 44.5	346.1 ± 42.5	325.6 ± 40.1
Duration, min	64.9 ± 2.5	65.4 ± 1.5	62.6 ± 2.7
Interpulse interval, min	73.4 ± 3.1	74.0 ± 2.2	70.9 ± 3.5
Relative amplitude, % of 24-h mean	105.7 ± 7.3	101.5 ± 4.0	97.1 ± 5.5
Sleep-associated oscillation amplitude increase, % of waking mean amplitude	145.1 ± 11.7	148.2 ± 11.8	130.0 ± 9.7

Values are means ± SE; n = 8 night workers and 8 day-active subjects. Differences between day-active subjects when sleeping at day and at night and differences between regular night workers and day-active subjects sleeping at night all NS.

DISCUSSION

The metabolic consequences of night-shift work have been poorly explored. In the present study, we demonstrated that even regular night workers, the subgroup of shift workers that is the most prone to adjust its circadian system to its nocturnal work schedule, only partially adapted their 24-h rhythms of plasma glucose and ISR.

By replacing the normal caloric intake with a constant enteral nutrition, our experimental conditions avoided the confounding effects of discontinuous food ingestion and of prolonged fasting. An habituation session minimized stress effects due to laboratory procedures, and because the subjects remained supine

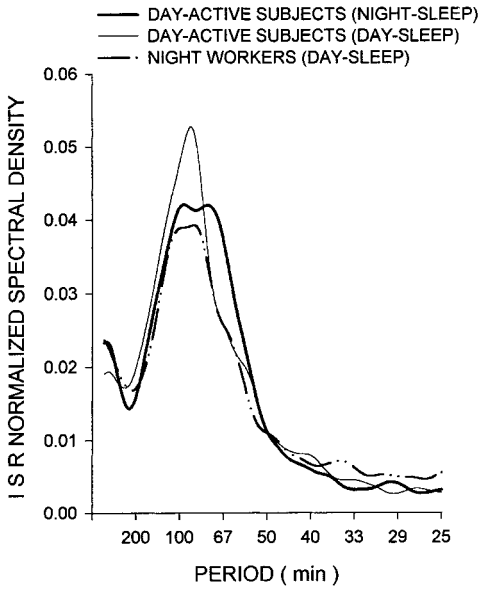

Fig. 3. Mean normalized spectral density function of 24-h ISR.

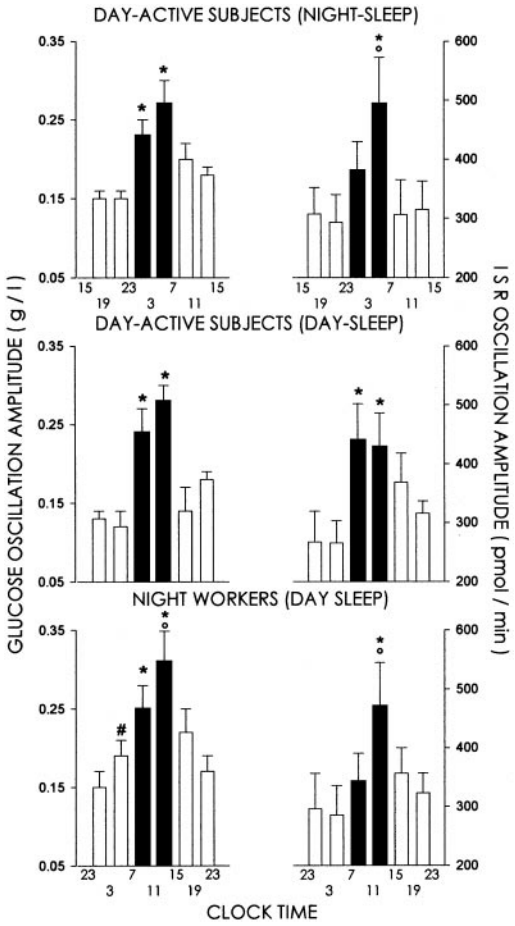

Fig. 4. Mean glucose (left) and ISR (right) oscillation amplitude in 6 successive 4-h periods of nycthemeron for 3 experiments (sleep period in middle of each graph). Intra-group comparisons were made. *$P <$ 0.05 vs. waking 4-h periods. °$P < 0.05$ vs. early-sleep periods. #$P <$ 0.02 vs. preceding wake periods.

throughout the experiment, the influence of posture and of physical activity was minimal and constant. Comparing, in these conditions, the night workers to day-active subjects sleeping either during the night or after an acute shift of their normal sleep time, we confirmed that sleep has a major influence on the driving of the glucose and ISR rhythms. Despite the maintenance of a constant caloric input, sleep onset was associated, irrespective of the time of day, with a diminished glucose tolerance revealed in an amplification in the glucose and ISR oscillations, and an increase in mean plasma glucose levels, followed by a significant increase in mean ISR. Our results in day-active subjects sleeping at daytime also indicate the existence of a weak circadian influence with an increase of plasma glucose levels and, to a lesser extent, of ISR during the night of acute sleep deprivation and a nadir in the morning, which is in agreement with previous works (15, 27, 32). The dissociation of the circadian and sleep modulatory effects observed after an acute shift of the sleep period was only partially reversed in night workers, leading to some desynchronization of the glucose and ISR variations. Contrasting with the reduced amplitude noted after an acute shift of the sleep period, no modification of the amplitude of the plasma glucose and ISR 24-h rhythms was observed; moreover the plasma glucose and ISR peak levels, which underwent a shift of about 8 h, were well adapted to the night-activity schedule. In contrast, the onset of the sleep-associated rise in plasma glucose levels underwent a shift of only 6 h, whereas the sleep-associated ISR rise was lower and of longer duration.

These results are in accordance with our findings concerning cortisol (38) and TSH (36), indicating that the regular night-active routine induces an internal dissociation between some chronobiological markers of these two hormones, leading to a distortion of their 24-h profiles with no modification of their amplitudes. Cortisol and TSH peaks exhibited a shift of about 6.5 h in the permanent night workers, whereas the return of TSH to baseline values and the beginning of the quiescent period of cortisol secretion underwent a shift of only 3 h.

The incomplete adaptation of glucose and insulin rhythms in permanent night workers and the subsequent dissociation of the two rhythms confirm the hypothesis that sleep and circadian effects have different underlying causal mechanisms. Diminished brain glucose utilization (3) and decreased peripheral glucose uptake due to diminished muscle tone during deep sleep are thought to partly explain the sleep-associated increase in plasma glucose; in fact, the latter has been shown to partially reflect the predominance of SWS in early sleep and the greater proportion of waking and REM stages in late sleep (20). However the disturbances in the plasma glucose and ISR profiles observed in regular night workers during sleep are not explained by any difference in the sleep parameters; they thus more probably reflect an incomplete adjustment of other normally sleep-associated modifications, such as the release of GH, known to induce a rapid decrease in muscular glucose uptake (14). Our data demonstrating that GH secretory episodes that mainly occur during early sleep in day-active subjects are more randomly distributed throughout the 24 h in night workers (37) would support this hypothesis. The circadian variations of cortisol secretion are likely to be a substantial contributive factor to the circadian glucose tolerance variations, due to the delayed adverse effect of cortisol on hepatic and peripheral insulin sensitivity. The normally nocturnal rise in cortisol also results in enhanced lipolysis and gluconeogenesis (5), although in our experimental conditions of continuous nutrition these latter were almost entirely inhibited. The phase-advanced rise and the shorter quiescent phase of cortisol secretion that we previously described in night workers studied in the same conditions (38) may explain the early plasma glucose rise onset in these subjects and contribute to the more prolonged increase of ISR that persisted after awakening. Other hormones, such as leptin, which has been shown to display both sleep-associated and circadian variations (29) and to interact with insulin secretion (17) and insulin sensitivity (22), are also likely to play a substantial role.

Whatever the mechanisms underlying the 24-h glucose and ISR variations, our study demonstrates that, although mainly sleep dependent, the plasma glucose and ISR rhythms are only partially adapted in night workers. Because diminished insulin sensitivity and even slight elevation of glucose have been shown to be associated with higher cardiovascular risk, these abnormalities may be of clinical relevance. One can expect that the metabolic consequences of a sleep shift may be even greater in subjects less well adapted to their night activity or in workers in fast-rotating shifts.

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REFERENCES

1. **Boden, G., X. Chen, and J. L. Urbain.** Evidence for a circadian rhythm of insulin sensitivity in patients with NIDDM caused by cyclic changes in hepatic glucose production. *Diabetes* 45: 1044–1050, 1996.
2. **Boden, G., J. Ruiz, J. L. Urbain, and X. Chen.** Evidence for a circadian rhythm of insulin secretion. *Am. J. Physiol. Endocrinol. Metab.* 271: E246–E252, 1996.
3. **Boyle, P. J., J. C. Scott, A. J. Krentz, R. J. Nagy, E. Comstock, and C. Hoffman.** Diminished brain glucose metabolism is a significant determinant for falling rates of systemic glucose utilization during sleep in normal humans. *J. Clin. Invest.* 93: 529–535, 1994.
4. **Cleveland W.** Robust locally weighted regression and smoothing scatterplots. *J. Am. Stat. Assoc.* 74: 829–836, 1979.
5. **Dinneen, S., A. Alzaid, J. Miles, and R. Rizza.** Effects of the normal nocturnal rise in cortisol on carbohydrates and fat metabolism in IDDM. *Am. J. Physiol. Endocrinol. Metab.* 268: E595–E603, 1995.
6. **Eaton, R. P., R. C. Allen, D. S. Schade, K. M. Erickson, and J. Standefer.** Prehepatic insulin production in man: kinetic analysis using peripheral connecting peptide behavior. *J. Clin. Endocrinol. Metab.* 51: 520–528, 1980.
7. **Frank, S., D. C. Roland, J. Sturis, M. M. Byrne, J. P. Spire, S. Refetoff, K. S. Polonsky, and E. van Cauter.** Effects of aging on glucose regulation during wakefulness and sleep. *Am. J. Physiol. Endocrinol. Metab.* 269: E1006–E1016, 1995.
8. **Hansen K. M.** Investigations on the blood sugar in man-condition of oscillations, rise and distribution. *Acta Med. Scand.* 4, *Suppl.*: 1–8, 1923.
9. **Jarrett, R.** Rhythms in insulin and glucose. In: *Endocrine Rhythms,* edited by D. Krieger. New-York: Raven, 1979, p. 247–258.
10. **Lang, D. A., D. R. Matthews, J. Peto, and R. C. Turner.** Cyclic oscillations of basal plasma glucose and insulin concentrations in human beings. *N. Engl. J. Med.* 301: 1023–1027, 1979.
11. **Lewy, A. J., S. Ahmed, J. M. Latham Jackson, and R. L. Sack.** Melatonin shifts human circadian rhythms according to a phase-response curve. *Chronobiol. Int.* 9: 380–392, 1992.
12. **Licinio-Paixo, J., K. S. Polonsky, B. D. Given, W. Pugh, D. Ostrega, B. F. Frank, and A. H. Rubenstein.** Ingestion of a mixed meal does not affect the metabolic clearance rate of biosynthetic human C-peptide. *J. Clin. Endocrinol. Metab.* 63: 401–403, 1986.
13. **Linkowski, P., J. Mendlewicz, R. Leclercq, H. Brasseur, P. Hubain, J. Goldstein, G. Copinschi, and E. van Cauter.** The 24-h profile of adrenocorticotropin and cortisol in major depressive illness. *J. Clin. Endocrinol. Metab.* 61: 429–438, 1985.
14. **Moller, N., P. C. Butler, M. A. Antsiferov, and K. G. M. M. Alberti.** Effects of growth hormone on insulin sensitivity and forearm metabolism in normal man. *Diabetologia* 32: 105–110, 1989.
15. **Morgan, L., J. Arendt, D. Owens, S. Folkard, S. Hampton, S. Deacon, J. English, D. Ribeiro, and K. Taylor.** Effect of the endogenous clock and sleep time on melatonin, insulin, glucose and lipid metabolism. *J. Endocrinol.* 157: 443–451, 1998.
16. **O'Meara, N. M., J. Sturis, E. van Cauter, and K. S. Polonsky.** Lack of control by glucose of ultradian insulin secretory oscillations in impaired glucose tolerance and in noninsulin-dependent diabetes mellitus. *J. Clin. Invest.* 92: 262–271, 1993.
17. **Ookuma, M., K. Ookuma, and D. A. York.** Effects of leptin on insulin secretion from isolated rat pancreatic islets. *Diabetes* 47: 219–223, 1998.
18. **Priestly, M. B.** *Spectral Analysis and Time Series*. London: Academic Press, 1981.
19. **Rechtschaffen, A., and A. Kales.** *A Manual of Standardized Terminology, Techniques and Scoring System for Sleep Stages of Human Subjects.* Washington DC: US Government Printing Office, 1968.
20. **Scheen A. J., M. Byrne, L. Plat, R. Leproult, and E. van Cauter.** Relationships between sleep quality and glucose regulation in normal man. *Am. J. Physiol. Endocrinol. Metab.* 271: E261–E270, 1996.
21. **Scheen, A. J., J. Sturis, K. S. Polonsky, and E. van Cauter.** Alterations in the ultradian oscillations of insulin secretion and plasma glucose in aging. *Diabetologia* 39: 564–572, 1996.
22. **Segal, K. R., M. Landt, and S. Klein.** Relationship between insulin sensitivity and plasma leptin concentration in lean and obese men. *Diabetes* 45: 988–991, 1996.
23. **Service, F. J., L. D. Hall, R. E. Westland, P. C. O'Brien, V. L. W. Go, M. W. Haymond, and R. A. Rizza.** Effects of size, time of day and sequence of meal ingestion on carbohydrate tolerance in normal subjects. *Diabetologia* 12: 61–67, 1983.
24. **Shapiro, E. T., H. Tillil, K. S. Polonsky, V. S. Fang, A. H. Rubenstein, and E. van Cauter.** Oscillations in insulin secretion during constant glucose infusion in normal man: relationship to changes in plasma glucose. *J. Clin. Endocrinol. Metab.* 67: 307–314, 1988.
25. **Simon, C., G. Brandenberger, and M. Follenius.** Ultradian oscillations of plasma glucose, insulin and C-peptide in man during continuous enteral nutrition. *J. Clin. Endocrinol. Metab.* 64: 669–674, 1987.
26. **Simon, C., G. Brandenberger, M. Follenius, and J. L. Schlienger.** Alteration in the temporal organisation of insulin secretion in type 2 (noninsulin-dependent) diabetic patients under continuous enteral nutrition. *Diabetologia* 34: 435–440, 1991.
27. **Simon, C., G. Brandenberger, J. Saini, J. Ehrhart, and M. Follenius.** Slow oscillations of plasma glucose and insulin secretion rate are amplified during sleep in humans under continuous enteral nutrition. *Sleep* 17: 333–338, 1994.
28. **Simon, C., M. Follenius, and G. Brandenberger** Postprandial oscillations of plasma glucose, insulin and C-peptide in man. *Diabetologia* 30: 769–773, 1987.
29. **Simon, C., G. Gronfier, J. L. Schlienger, and G. Brandenberger.** Circadian and ultradian variations of leptin in normal man under continuous enteral nutrition: relationship to sleep and body temperature *J. Clin. Endocrinol. Metab.* 83: 1893–1899, 1998.
30. **Sturis, J., K. S. Polonsky, E. T. Shapiro, J. D. Blackman, N. M. O'Meara, and E. van Cauter.** Abnormalities in the ultradian oscillations of insulin secretion and glucose levels in type 2 (noninsulin-dependent) diabetic patients. *Diabetologia* 35: 681–689, 1992.
31. **Van Cauter, E.** Quantitative methods for the analysis of circadian and episodic hormone fluctuations. In: *Human Pituitary Hormones: Circadian and Episodic Variations,* edited by E. van Cauter and G. Copinschi. The Hague: Nijhoff, 1981, p. 1–25.
32. **Van Cauter, E., J. D. Blackman, D. Roland, J. P. Spire, S. Refetoff, and K. S. Polonsky.** Modulation of glucose regulation

and insulin secretion by circadian rhythmicity and sleep. *J. Clin. Invest.* 88: 934–942, 1991.
33. **Van Cauter, E., F. Mestrez, J. Sturis, and K. S. Polonsky.** Estimation of insulin secretion rates from C-peptide levels: comparison of individual and standard kinetic parameters for C-peptide clearance. *Diabetes* 41: 368–377, 1992.
34. **Van Cauter, E., K. S. Polonsky, and A. J. Scheen.** Roles of circadian rhythmicity and sleep in human glucose regulation. *Endocr. Rev.* 18: 716–738, 1997.
35. **Veldhuis, J. D., M. L. Johnson, and E. Seneta.** Analysis of the copulsatility of anterior pituitary hormones. *J. Clin. Endocrinol. Metab.* 73: 569–576, 1991.
36. **Weibel, L., G. Brandenberger, B. Goichot, K. Spiegel, J. Ehrhart, and M. Follenius.** The circadian thyrotropin rhythm is delayed in regular night workers. *Neurosci. Lett.* 187: 83–86, 1995.
37. **Weibel, L., M. Follenius, K. Spiegel, C. Gronfier, and G. Brandenberger.** Growth hormone secretion in night workers. *Chronobiology Int.* 14: 49–60, 1997.
38. **Weibel, L., K. Spiegel, M. Follenius, J. Ehrhart, and G. Brandenberger.** Internal dissociation of the circadian markers of the cortisol rhythm in night workers. *Am. J. Physiol. Endocrinol. Metab.* 270: E608–E613, 1996.
39. **Weibel, L., K. Spiegel, C. Gronfier, M. Follenius, and G. Brandenberger.** Twenty-four-hour melatonin and core body temperature rhythms: their adaptation in night workers. *Am. J. Physiol. Regulatory Integrative Comp. Physiol.* 272: R948–R954, 1997.