0021-972X/98/$03.00/0
Journal of Clinical Endocrinology and Metabolism

Vol. 83, No. 6
Printed in U.S.A.

Circadian and Ultradian Variations of Leptin in Normal Man under Continuous Enteral Nutrition: Relationship to Sleep and Body Temperature

C. SIMON, C. GRONFIER, J. L. SCHLIENGER, AND G. BRANDENBERGER

Laboratoire des Régulations Physiologiques et des Rythmes Biologiques chez l'Homme 67085 Strasbourg Cedex; and Service de Médecine Interne, CHU Hautepierre, 67098 Strasbourg Cedex, France

ABSTRACT

To determine the influence of circadian rhythmicity and sleep on the 24-h leptin diurnal variations, plasma leptin levels were measured at 10-min intervals over 24 h in seven normal subjects, once during nocturnal sleep, and once after an 8-h shift of sleep. The subjects were submitted to constant conditions (continuous enteral nutrition and bed rest in controlled chambers). Body temperature and plasma glucose and insulin levels were measured simultaneously.

During nighttime sleep, leptin levels increased to a maximum (109.9 ± 2.5% of the 24-h mean) and then decreased to reach a nadir in the late afternoon. The mean diurnal variation was 18.0 ± 3.8% of the 24-h mean. In the daytime sleep condition, leptin levels rose during the night of deprivation to a maximum of 104.7 ± 2.3% of the 24-h mean, decreased to a minimum around 0700 h, and then rose again during diurnal sleep (108.4 ± 3.1% of the 24-h mean); the mean diurnal variation was 13.4 ± 3.6% of the 24-h mean. ANOVA revealed a significant interaction between time of day and sleep effects ($P < 0.05$).

The diurnal and the sleep-related variations of plasma leptin mirrored those of body temperature and roughly paralleled those of plasma glucose and insulin; the amplitudes of the diurnal leptin variations were significantly correlated with the amplitudes of the diurnal body temperature variations ($P < 0.05$). Plasma leptin levels also displayed irregular pulses of low amplitude (mean duration, 70 min) that were not affected by sleep, but were associated with a significant decrease in glucose and insulin levels ($P < 0.01$).

These results demonstrate that under continuous enteral nutrition, plasma leptin levels are modulated by both a slight circadian component and sleep, which interact under normal conditions, and suggest that leptin is implicated in circadian thermoregulatory adjustments. (*J Clin Endocrinol Metab* **83:** 1893–1899, 1998)

CIRCADIAN and ultradian rhythmicities are common characteristics of numerous hormonal systems (1, 2). Recent studies in normal and obese subjects suggest that plasma leptin, the protein product of *ob* gene (3) that is implicated in food intake and energy metabolism regulation (4, 5), also presents diurnal variations with a nocturnal rise (6–8). However, these studies, which were conducted in subjects having diurnal meals and normal nocturnal sleep, do not differentiate the effects of an endogenous circadian rhythmicity and the influence of environmental cues, meals, and sleep. Under normal conditions, the circadian rhythm is synchronized with the feeding and sleep-wake cycles, which also have a 24-h periodicity (9). Therefore, meal ingestion and sleep can contribute to the overall variations and mask the characteristics of an endogenous rhythm. Meal shift has been shown to entrain the diurnal rhythm of leptin (10), suggesting that daytime feeding contributes to the nocturnal leptin increase, a fact that is in accordance with the acute effect of fasting or of insulin on leptin secretion (11–13). On the other hand, a number of physiological variables, including glucose regulation, hormones (2, 14), and body temperature (15), are controlled by both sleep-dependent and circadian processes. Little is known about the sleep influence on leptin levels. Shoeller *et al.* (10) found no evidence for an acute entrainment of plasma leptin levels to the sleep cycle; however, in their study the acute 12-h day/night reversal was associated with a progressive shift in the meal timing over 4 days to simulate jet lag, so that both sleep and circadian effects could have been masked.

To determine the influences of circadian rhythmicity and sleep on the 24-h plasma leptin variations independently of meal ingestion, we used an experimental strategy of acute sleep shift in normal subjects submitted to 24-h periods of constant routine conditions, involving continuous nutrition and bed rest in controlled sound-proof, air-conditioned, and dim light chambers. The 24-h profiles obtained in the same subjects, once under basal conditions with normal nocturnal sleep and once with a night of total sleep deprivation followed by an 8-h daytime sleep, were compared. Caloric and fluid intake were given in the form of a continuous normocaloric enteral nutrition to avoid possible effects of repeated meal ingestion and fasting. The temporal profiles of body temperature, plasma glucose and plasma insulin levels were determined simultaneously.

Plasma leptin levels have also been shown to be pulsatile, with transient pulses superimposed on the diurnal profiles (16, 17). The frequency of the leptin pulses observed is very close to that of the slow oscillations described for plasma glucose and insulin secretion (18, 19), which are best identified during continuous enteral nutrition and are known to

Received December 31, 1997. Revision received February 19, 1998. Accepted February 26, 1998.

Address all correspondence and requests for reprints to: C. Simon, Laboratoire des Régulations Physiologiques et Rythmes Biologiques chez l'Homme Faculté de Médecine, 4 rue Kirschleger, 67085 Strasbourg Cedex, France. E-mail: Chantal.Simon@medecine.u-strasbg.fr.

be affected by sleep (20). A rapid sampling procedure (10-min intervals) was therefore used to assess the influence of sleep on the leptin ultradian pulses and to determine their temporal relationships with the glucose and insulin ultradian rhythms.

Subjects and Methods

Subjects

Seven healthy day-active male subjects, aged 21–25 yr, participated in this experiment. All subjects were of normal weight, with an average body mass index of 22.2 ± 0.6 kg/m^2, and had normal routines of work, meals, and sleep. They were selected after medical examination, screening tests, and questionnaires on their usual sleep-wake cycles, work, and light exposure schedules. Subjects with any personal history of obesity or sleep disorders, those who had experienced time shift or sleep deprivation during the previous weeks, as well as smokers, subjects with underlying signs of disease, or subjects taking medication were excluded from the study. They all gave their written informed consent, and the study was approved by the local ethics committee.

Procedure

The experiments were performed in soundproof, air-conditioned sleep chambers communicating with an adjoining room where blood samples and sleep data were collected.

The subjects were studied twice in random order, with a 1-month interval between the experiments; they were admitted to the laboratory for a continuous period of about 48 h, with admission at 2200 h to undergo a night of habituation from 2300–0700 h. They ate standard meals at 0700 and 1200 h; they were then studied once during a normal 24-h sleep-wake cycle, with a nocturnal sleep from 2300–0700 h, and once during a 24-h cycle with an acute 8-h shift in the sleep period obtained by a total sleep deprivation during the night and a daytime recovery sleep from 0700–1500 h. The subjects remained supine for the 4 h preceding blood sampling and throughout the experiment to avoid postural influence. When awake, they were maintained in dim light (<100 lux) and were allowed to read or watch television. During the night of sleep deprivation, they were kept under continuous watch and conversed with the laboratory staff.

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, and 15% protein; 378 kilojoules/h), which began at 1600 h. A catheter was then inserted under local anesthesia in an antecubital vein and was kept patent with heparinized solutions. Electrodes were attached for uninterrupted electrophysiological recordings. Rectal temperature was recorded once per min by a probe (YSI 400, Yellow Springs Instrument Co., Yellow Springs, OH), and values were averaged for each 10-min period.

Blood sampling and hormone assays

Blood samples were taken continuously throughout the 24-h experiments from 2300 h on day 1 to 2300 h on day 2 at 10-min intervals, using a peristaltic pump. Ethylenediamine tetraacetate-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, Mannheim, Germany), with an intraassay coefficient of variation (CV) below 1.3%. Plasma leptin levels were determined by RIA using a commercial kit (Linco Research, St. Charles, MO), with a lower sensitivity of 0.5 ng/mL; the intraassay CV was 4.4% for values below 15 ng/mL and 3.4% for values above 15 ng/mL. Plasma insulin levels were determined by a double antibody RIA (Sorin Biomedica Diagnostics, Milan, Italy) with a lower level of sensitivity of 4 μU/mL. The mean intraassay CV was 6.6% for values below 50 μU/mL and 10.6% for values above 50 μU/mL. 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 (21). On this basis, total sleep time, the total durations of slow wave sleep (SWS) and rapid eye movement (REM) sleep, sleep onset, SWS and REM sleep latencies, and the number and duration of intrasleep awakenings were quantified. The sleep efficiency index was defined as the ratio of total sleep time to time allocated to sleep.

Data analysis

Circadian rhythmicity analysis. To quantify the long term diurnal wave changes in leptin, body temperature (T_{re}), glucose, and insulin independently of the more rapid ultradian variations, a smooth best-fit curve using a robust, locally weighted regression procedure, as proposed by Cleveland (22), was calculated for each individual profile. A window of 2 h was used. The acrophases and nadirs were respectively defined as the times of occurrence of maxima and minima in the best-fit curve. The diurnal mean amplitude of each individual profile was defined as the difference between the nocturnal acrophase (nadir) and the diurnal nadir (acrophase).

Two approaches were used to quantify the long term temporal relationships between the 24-h changes in plasma leptin and those in T_{re}, plasma glucose, and insulin. Firstly, a cross-correlation analysis (Box-Jenkins Time Series Analysis, BMDP Statistical Software, Los Angeles, CA) was realized on the best-fit curves. Cross-correlation coefficients between paired plasma leptin levels and T_{re} (and then plasma glucose and insulin levels, respectively) were calculated at different time lags. For each individual pair of profiles, the highest coefficient of cross-correlation was identified. The individual correlation coefficients were then averaged using Fischer's z transformation to yield an average estimate of the correlation between plasma leptin levels and T_{re} (and then plasma glucose and insulin levels, respectively). This average coefficient was computed after a χ^2 homogeneity test on the individual transformed coefficients (23). Secondly, the different parameters derived from the best-fit curves (mean levels, time and level of acrophases and nadirs, relative and absolute increments associated with sleep, and mean amplitude) were used to quantify the temporal changes in plasma leptin levels and their association with changes in T_{re}, plasma glucose, and plasma insulin levels.

Pulse analysis. The individual 24-h profiles of plasma leptin, glucose, and insulin levels were analyzed for pulse identification using the computer program ULTRA (24). This algorithm eliminates all peaks when either the increment or the decrement does not reach a certain threshold. The threshold for pulse detection was set at twice the CV; moreover, only the pulses that lasted more then 20 min were considered significant. For each significant pulse, the time of occurrence, the increment, the decrement, and the total duration were determined.

The association between individual pulses of leptin and those of glucose or insulin was tested by means of a lagged coincidence analysis, based on a model of conditional probability deriving from two binomial distributions and leading to an hypergeometric probability density function, as proposed by Veldhuis (25). Coincidence was tested at different lags (*i.e.* peak maxima in leptin series were stipulated to precede or to follow maxima in paired series by a designed number of time units). Coincidence was defined within a window of ± one sample.

Statistical analysis. The results were expressed as the mean ± SEM. The sleep and circadian parameters from normal rhythms and from shifted day-sleep rhythms were compared using bilateral paired *t* tests.

An ANOVA for repeated measures with Greenhouse-Geiser correction and a bilateral paired *t* test with Bonferroni procedure for multiple comparisons were used to assess the statistical differences among the mean levels, the mean number of pulses, and their relative or absolute amplitudes. Three periods were considered: 2300–0700 h, 0700–1500 h, and 1500–2300 h.

Results

Sleep characteristics

Total sleep time and sleep efficiency were similar in both experimental conditions (Table 1). The latency of sleep onset

TABLE 1. Effect of an 8-h delay of the sleep-wake cycle on sleep parameters

Time (min)	Nocturnal sleep	Daytime sleep
Total sleep time (min)	459 ± 5	461 ± 9
Sleep efficiency (%)	85 ± 3	85 ± 2
REM sleep duration (min)	83 ± 12	91 ± 9
SWS duration (min)	66 ± 9	75 ± 5
Sleep onset latency (min)	21 ± 5	4 ± 1[a]
REM sleep latency (min)	154 ± 32	85 ± 18
SWS latency (min)	37 ± 5	26 ± 5

Values are the mean ± SEM (n = 7). Unless indicated, P = NS.
[a] $P < 0.05$.

significantly decreased during daytime sleep compared with that during nighttime sleep, whereas the duration and the latency of REM sleep and SWS were not affected by sleep shift.

24-h leptin profiles

Figure 1 illustrates the mean 24-h profiles of plasma leptin, T_{re}, plasma insulin, and plasma glucose obtained in the two experimental conditions, *i.e.* with a normal nocturnal sleep from 2300–0700 h (*left*) and with a nocturnal sleep deprivation followed by a diurnal recovery sleep from 0700–1500 h (*right*). To eliminate the effects of interindividual variations in mean plasma leptin levels (ranging from 2.4–8.9 ng/mL), individual values of leptin were expressed as percentages of the 24-h mean value.

During normal nighttime sleep, plasma leptin levels increased during the early part of the night, reached a maximum around midsleep, and then decreased progressively and attained a nadir in the late afternoon (2008 h ± 12 min). This progressive decrease was interrupted by a slight increase around midday. The mean nocturnal maximum that was observed in all subjects averaged 109.9 ± 2.5% of the 24-h mean levels. Mean plasma leptin levels were significantly higher during nocturnal sleep compared with those during the remaining 8-h waking periods ($P < 0.05$); the mean amplitude of the diurnal leptin variations, *i.e.* the difference between the nocturnal maximum and the daytime minimum, was 18.0 ± 3.8% of the mean 24-h levels.

In the daytime sleep condition, marked differences were observed (Table 2). During the night of sleep deprivation, plasma leptin levels rose slightly in all subjects, suggesting the existence of an intrinsic circadian rhythm. The maximum level was slightly lower than that observed in the condition of normal nocturnal sleep (104.7 ± 2.3% of the 24-h mean levels *vs.* 109.9 ± 2.5%), but this difference was not significant. This maximum level occurred later in the night ($P < 0.05$) and was followed by an abrupt decrease during the second part of the night, with a minimum level between 0700–0915 h depending on the subject. A second rise was then clearly apparent during the first hours of shifted diurnal sleep with a maximum at midday, 5 h after sleep onset (108.4 ± 3.1% of the 24-h mean levels), followed by a progressive decline with a minimum in the late afternoon. The sleep-associated diurnal maximum was significantly higher than the inconsistent and slight increase observed during the daytime in the absence of sleep ($P < 0.05$). There was no significant difference in mean plasma leptin levels during the three 8-h periods considered; the amplitude of the mean diurnal leptin variations was lower than that during normal nocturnal sleep (13.4 ± 3.6% of the mean 24-h levels), although the difference was not significant.

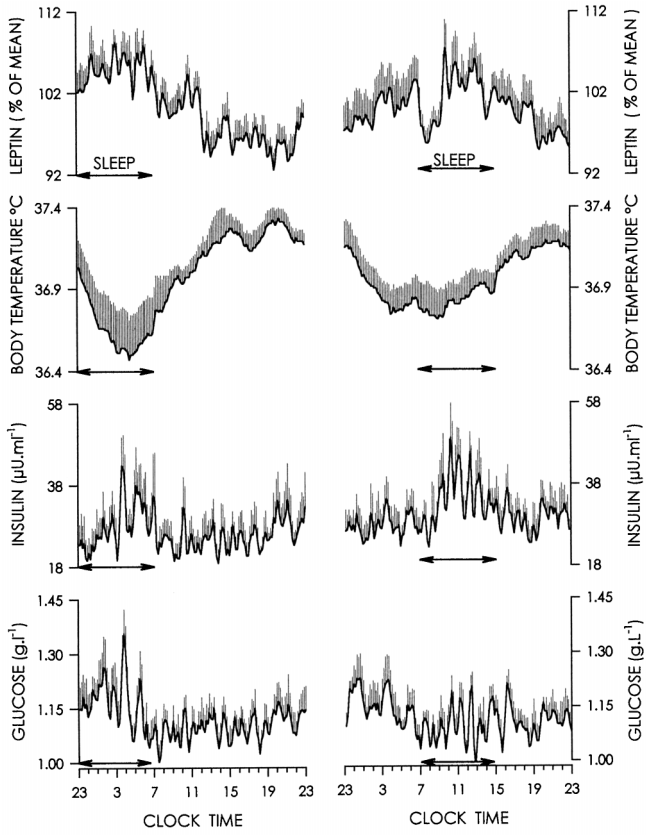

FIG. 1. Mean (±SEM) 24-h profiles of plasma leptin, body temperature, plasma insulin and glucose in the same subjects (n = 7), studied once with a normal nocturnal sleep (*left*) and once with an 8-h shift in sleep obtained by sleep deprivation followed by a diurnal recovery sleep (*right*). For leptin, individual values were expressed as percentages of the 24-h mean value.

Comparison of the plasma leptin levels by means of ANOVA revealed a significant interaction ($P < 0.05$) between daytime and condition (sleep or wakefulness), suggesting that the plasma leptin diurnal variations reflect both an intrinsic component and sleep influence, which are superimposed in normal conditions.

Relationship with body temperature, glucose, and insulin 24-h profiles in nighttime and daytime sleep conditions

In the conditions of normal nocturnal sleep, the mean rectal T_{re} profile mirrored the mean plasma leptin profile with a nocturnal minimum and a significant rise in the late afternoon. In contrast, the profiles of mean plasma glucose and insulin paralleled the mean plasma leptin profile with a significant rise in the second part of the night and a progressive decline throughout the day. The peak of leptin at night preceded the nadir in T_{re} by about 1 h; the cross-correlation analysis showed that the smoothed profiles of plasma leptin and those of T_{re} were significantly and in-

TABLE 2. Quantitative characteristics of the leptin, insulin, glucose, and body temperature 24-h profiles obtained twice in the same subjects (n = 7), once with a normal nocturnal sleep, once after an 8-h shift in the sleep period

	Nighttime (2300–0700 h)		Daytime (0700–1500 h)	
	Nocturnal sleep	Sleep deprivation	Wakefulness	Daytime sleep
Plasma leptin				
Mean level (ng/mL)	4.26 ± 0.93	4.33 ± 0.89	4.03 ± 0.89	4.57 ± 1.02[a]
Time of maximum (h ± min)	0239 ± 42	0429 ± 48[a]	1457 ± 70	1155 ± 74
Maximum level (% of 24-h mean)	109.9 ± 2.5	104.7 ± 2.3	100.8 ± 1.9	108.4 ± 3.1[a]
Plasma glucose				
Mean level (g/L)	1.16 ± 0.05	1.15 ± 0.04	1.09 ± 0.03	1.10 ± 0.03
Time of maximum (h ± min)	0235 ± 33	0030 ± 43[a]	1345 ± 79	1253 ± 73
Maximum level (g/L)	1.21 ± 0.05	1.20 ± 0.04	1.12 ± 0.03	1.13 ± 0.02
Plasma insulin				
Mean level (μU/mL)	28.13 ± 4.72	27.80 ± 3.85	24.12 ± 3.95	34.22 ± 5.89[b]
Time of maximum (h ± min)	0535 ± 31	0212 ± 51[b]	1249 ± 57	1113 ± 50
Maximum level (μU/mL)	31.84 ± 5.21	31.93 ± 3.26	25.76 ± 4.02	38.12 ± 4.77[b]
Body temperature				
Mean level (C)	36.84 ± 0.21	36.87 ± 0.19	37.10 ± 0.12	36.83 ± 0.17[a]
Time of minimum (h ± min)	0400 ± 22	0400 ± 18		0919 ± 77
Minimum level (C)	36.42 ± 0.13	36.64 ± 0.14		36.61 ± 0.17

Values are the mean ± SEM. Unless significant contrast between sleep and wakefulness state for the corresponding time period is indicated, P = NS.

[a] $P < 0.05$.

[b] $P < 0.01$.

versely correlated for six of the seven subjects, with an average cross-correlation coefficient at lag −60 min of −0.68 ($P < 0.001$). The variations in plasma leptin levels were roughly associated with those of glucose. However, the paired cross-correlation analysis between the smoothed profiles of plasma leptin and the smoothed profiles of plasma glucose and plasma insulin failed to reveal any systematic significant relationship among the subjects.

The daytime sleep conditions made it possible to dissociate some of the temporal relationships observed in the normal conditions of nocturnal sleep. In these conditions, a temporal relationship clearly remained between plasma leptin and T_{re}, with a mean temperature profile characterized by two nadirs, one during nocturnal sleep deprivation and the other during daytime sleep. The maximum nocturnal plasma leptin level was concomitant with a minimum in T_{re}, whereas during diurnal sleep, the leptin acrophase followed the T_{re} nadir; the cross-correlation analysis between the paired profiles of plasma leptin and T_{re} revealed significant cross-correlation coefficients for several subjects but not for the others, so that the test of Snedecor indicated the nonhomogeneity of the coefficients within the group. In the sleep-shift condition, the T_{re} minima reached during the night and during diurnal sleep were of similar levels, but both were higher than the minimum noticed during normal nocturnal sleep; this resulted, as observed for plasma leptin, in a diminished, although not significantly, amplitude of the diurnal body temperature variations (1.6 ± 0.2% *vs.* 2.6 ± 0.3% of the mean 24-h levels).

When both conditions, *i.e.* normal nocturnal sleep and daytime shifted sleep, were considered together, the amplitudes of the diurnal plasma leptin and T_{re} variations were significantly correlated ($r = 0.57$; $P < 0.05$). This correlation remained significant after adjustment for body mass index. Thus, the larger the decrease in leptin levels from nocturnal maximum to evening minimum, the higher the T_{re} rise from nocturnal nadir to evening maximum.

As previously described (20), mean plasma glucose and insulin profiles were characterized by a nocturnal decrease followed by a daytime sleep-induced increase, reflecting both circadian and sleep influences. Mean plasma insulin levels were markedly influenced by sleep with only a slight circadian effect, whereas mean nocturnal glucose levels were similar whatever the sleep conditions, suggesting a more potent circadian influence with only a slight sleep effect. Despite this dual circadian and sleep-associated influence on glucose regulation, no systematic quantitative relationship between smoothed plasma leptin and plasma insulin or glucose profiles was observed.

Leptin ultradian pulsatility

Figure 2 gives the individual leptin profiles in two representative subjects in both conditions, *i.e.* with a normal nocturnal sleep from 2300–0700 h (*left*) and with a nocturnal sleep deprivation followed by a diurnal recovery sleep from 0700–1500 h (*right*).

Pulse analysis of the individual profiles revealed an average of 13.4 significant pulses occurring during the 24-h period regardless of the experimental conditions (Table 3). Their mean amplitude was 0.71 ± 0.12 ng/mL (17.4 ± 0.9% when expressed as a percentage of the precedent trough level), and their mean duration was 70.0 ± 4.3 min. The number of pulses was not influenced by either sleep or time of day, but the high variability in the interpeak time indicated the irregular periodicity of the leptin pulses, which contrasted with the relative regularity of the glucose and insulin oscillations.

As expected (18), significant glucose and insulin oscillations were observed throughout the 24-h experiments with mean periodicities of 60.2 ± 2.2 and 59.4 ± 1.6 min, respectively. Their mean amplitudes [0.19 ± 0.01 g/L for glucose (19.9 ± 0.9%) and 18.1 ± 1.6 μU/mL for insulin (98.4 ± 4.2%)] were significantly enhanced during sleep periods ($P < 0.05$),

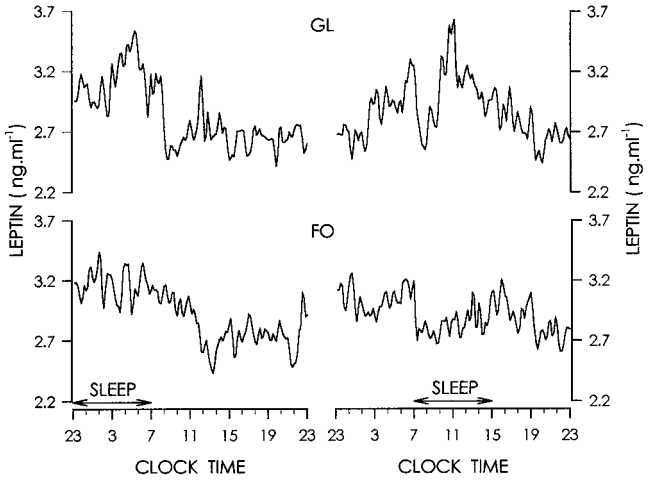

FIG. 2. Individual profiles of plasma leptin in two representative subjects studied once with a normal nocturnal sleep (*left*) and once with an 8-h shift in sleep obtained by sleep deprivation followed by a diurnal recovery sleep (*right*).

whatever the time of day. Coincidence analysis revealed that, on the average, 46% of leptin pulses were preceded by an insulin pulse within a lag of 30 ± 10 min ($P < 0.05$), and 51% were preceded by a glucose pulse within the same lag ($P < 0.02$). Figure 3 illustrates the mean relative variations in plasma leptin levels, aligned by the maximum of the significant pulses (average of 186 individual significant leptin pulses) together with the concomitant plasma insulin levels. The leptin pulses were associated with significant variations in plasma insulin levels ($P < 0.01$) with a minimum at time zero and the highest levels 30–40 min before the leptin peak. Similar results were observed for plasma glucose levels ($P < 0.01$).

Discussion

In the present study, using an acute shift in the normal sleep time and a continuous enteral nutrition, we demonstrated that plasma leptin levels are modulated by both a circadian rhythmicity and sleep and that they present a clear relationship with T_{re} variations. Furthermore, the 10-min interval blood-sampling procedure allowed us to confirm the existence of irregular, ultradian pulses of low amplitude that are superimposed on the long term variations and appear to be partly associated with the glucose and insulin ultradian oscillations.

The 24-h profiles observed in normal nocturnal sleep conditions confirm and extend the findings of previous studies indicating a nocturnal increase in plasma leptin levels in normal and obese subjects (6–8, 10), with an amplitude of about 40–50% of the 24-h mean. In our experimental conditions, confusing or masking effects of food ingestion or prolonged fasting were avoided by replacing the normal caloric intake by constant enteral nutrition. Moreover, the potential influences of posture and physical activity were eliminated because the subjects remained supine before and throughout the experiment. A 24-h habituation session minimized stress effects due to laboratory procedures. In these conditions, the diurnal variations in plasma leptin levels remained clearly apparent, although their amplitude (18% of the 24-h mean) was reduced, thus confirming that in free living conditions the variations are amplified by food intake, daily energy expenditure, and posture.

Individual subjects, who were asked to comply with a regular schedule of sleep during the week preceding the experiment to optimize interindividual synchronization, were studied twice during 24-h experiments, once with an 8-h normal nocturnal sleep and once with an acute 8-h shift in the sleep period. This methodology allowed us to study the effects of the time of day independently of those of sleep as well as the effects of sleep at an unusual time. The profiles observed in these conditions clearly indicate that circadian rhythmicity and sleep both influence plasma leptin levels. Indeed, during the night, plasma leptin levels increased, whatever the sleep conditions (normal nocturnal sleep or sleep deprivation) and then decreased. The persistence of a slight nocturnal increase despite the 8-h sleep shift and in the absence of variation of any environmental condition such as external temperature, light, physical activity, or diurnal food intake indicates that the diurnal variations are at least partly controlled by a robust endogenous circadian pacemaker, which explains variations with an amplitude of about 13.8% of the 24-h mean. On the other hand, after a night of sleep deprivation, a significant increase in plasma leptin levels was observed after morning sleep onset, which gave evidence of sleep influence whatever the time when it occurs. Such a dual influence is a common finding for numerous biological processes (14), and most of the 24-h endocrine rhythms have been shown to be under multifactorial control, combining a circadian oscillator, located in the suprachiasmatic nuclei of the hypothalamus, and environmental or sleep-related influences. Even hormonal systems, which are markedly modulated by a circadian rhythm such as cortisol or TSH, are influenced by sleep (14, 26, 27). Similarly, the existence of a weak endogenous circadian rhythmicity has been revealed in clearly defined sleep-related rhythms, such as that of PRL or GH, which are known to display a major pulse concomitant to sleep onset (28).

In humans, core body temperature is also characterized by a circadian rhythmicity, which is partly explained by the hypothermic properties of melatonin. However only about 40% of the amplitude of the body temperature rhythm can be accounted for by the endogenous peak of melatonin (29). In the present study, body temperature variations mirrored those of leptin in both experimental conditions. Furthermore, the amplitude of the diurnal variations in plasma leptin levels were significantly correlated with the amplitude of the diurnal body temperature variations, suggesting that leptin could be involved in the acute circadian metabolic adjustments independently of its action on food intake. Indeed, it has been shown that in *ob/ob* mice, an acute injection of leptin has a profound stimulatory effect on energy expenditure, which is particularly obvious during the period of minimal metabolic rate (30); similarly, administration of leptin twice a day leads to the disappearance of normal body temperature and metabolic rate circadian cycles (31). Such metabolic effects, which have been related to a sympathetic activation of thermogenesis, remain to be demonstrated in man. They would fit in, however, with recent studies indicating that

TABLE 3. Effect of an 8-h delay of the sleep-wake cycle on the leptin pulses

	24-h period	2300–0700 h	0700–1500 h	1500–2300 h
No.				
Nighttime sleep	14.2 ± 1.2	5.3 ± 0.9	4.8 ± 0.6	4.0 ± 0.5
Daytime sleep	12.7 ± 3.0	3.7 ± 0.7	3.8 ± 1.1	5.2 ± 1.1
Relative amplitude (%)				
Nighttime sleep	17.8 ± 0.9	16.9 ± 1.3	17.2 ± 1.7	19.2 ± 3.4
Daytime sleep	16.9 ± 1.4	18.2 ± 2.1	16.9 ± 2.4	15.8 ± 0.9

Values are the mean ± SEM (n = 7). P = NS for each.

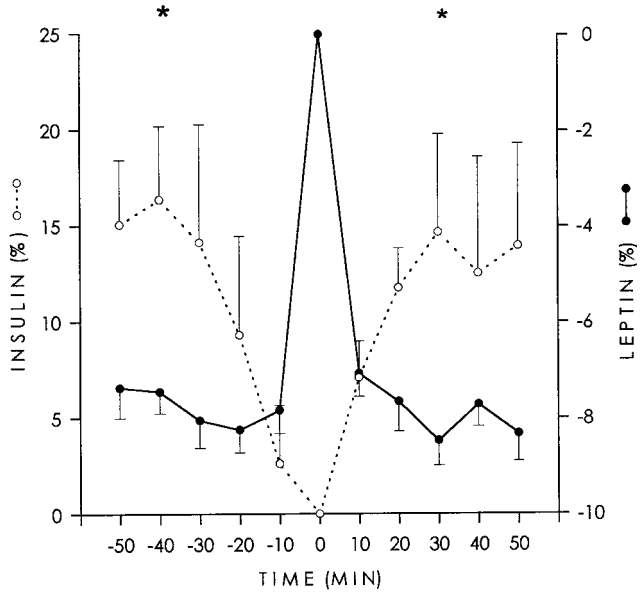

FIG. 3. Mean relative variations in plasma leptin levels, aligned by the maximum of the significant pulses (average of 186 significant leptin pulses), together with the concomitant plasma insulin levels. Values were expressed as percentages of the value at time zero. *, $P < 0.01$ *vs.* insulin levels at time zero.

under controlled constant routine, heat production exhibits a significant circadian rhythmicity that parallels heart rate variations and precedes body temperature variations (32). The exact temporal relationships linking plasma leptin and body temperature circadian variations in man have yet to be determined. The inverse relationship between plasma leptin and body temperature could reflect a phase relationship of 8–12 h, with leptin variations leading those in body temperature, so that the diurnal leptin decrease would be related to the subsequent nocturnal body temperature decrease. Alternatively, plasma leptin variations may be due to a counterregulatory negative feedback loop among thermogenesis, body temperature, and leptin secretion.

The significant increase in plasma leptin levels associated with sleep onset, even if it occurred at an abnormal time of the day, was concomitant with the well documented sleep-associated plasma insulin and glucose increases and the body temperature decrease. Changes in plasma insulin and glucose levels associated with sleep reflect changes in glucose utilization during sleep due to diminished brain glucose metabolism and diminished muscle tone (33). Similarly, decreased body temperature is the consequence of a diminished metabolism rate, partially induced by a diminution of sympathetic tone (34). As a consequence, one can expect an adaptive increase in leptin levels due to a stimulatory effect of an increase in plasma insulin or to the diminution of the sympathetic, inhibitory control. Other sleep-related hormonal releases, such as that of GH, may also be implicated.

Concerning leptin pulsatility, we found an average of 13.5 pulses during the 24-h experiments. This number, obtained in man under continuous enteral nutrition, is lower than that observed by Licinio (17), who reported a mean pulse frequency of 32 pulses/24 h. The relative conservative threshold used in our study and the higher blood-sampling frequency used in their study (every 7 min) may partly explain this difference. In addition, the different experimental conditions, such as daytime repeated meal intake or diurnal posture variations, may have influenced in their experiment not only the circadian but also the ultradian rhythmicity. The frequency of the pulses may have been affected or their amplitude, thus making them more difficult to detect. Similarly, the use of a constant glucose infusion associated with a less frequent blood-sampling procedure (every 15 min) probably explains the lower pulse frequency reported by Sinha (16).

In our experimental conditions, the number and the amplitude of the pulses were not affected by either sleep or time of day. However, leptin pulses were associated with a significant decrease in plasma glucose and insulin levels, and a slight, but significant concordance of about 50% was observed between leptin pulses and those of glucose or insulin, with the glucose and insulin pulses leading the leptin pulses. These results suggest that the glucose and insulin ultradian oscillations may affect the subsequent leptin levels, but they also indicate that the concordance is not systematic and that other mechanisms are probably implicated.

In conclusion, our study demonstrates that circadian and ultradian rhythmicities are two components of the plasma leptin pattern in normal man; this may be of physiological significance and should be taken into account when studying leptin in pathological states. Conversely, we also demonstrate that sleep is a physiological regulator of plasma leptin levels; it can, therefore, be suggested that chronic sleep disturbances such as those occurring in night workers or in sleep apnea, situations known to be frequently associated with overweight, may disturb the normal leptin 24-h pattern. Further studies are necessary to elucidate the exact relationship with body temperature regulation and insulin secretion.

Acknowledgments

The authors are indebted to M. Simeoni and B. Reinhardt for RIA analysis and experimental assistance.

References

1. **Van Cauter E, Refetoff S.** 1985 Multifactorial control of the 24-hour secretory profiles of pituitary hormones. J Endocrinol Invest. 8:381–391.
2. **Brandenberger G.** 1992 Endocrine ultradian rhythms during sleep and wakefulness. In: Lloyd D, Rossi EL, eds. Ultradian rhythms in life process. London: Springer Verlag; 123–138.
3. **Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM.** 1994 Positional cloning of the mouse obese gene and its human homologue. Nature. 372:425–432.
4. **Pelleymounter MA, Cullen MJ, Baker MB, et al.** 1995 Effects of the obese gene product on body weight regulation in *ob/ob* mice. Science. 269:540–543.
5. **Halaas JL, Gajiwala KS, Maffei M, et al.** 1995 Weight-reducing effects of the plasma protein encoded by the obese gene. Science. 269:543–546.
6. **Sinha MK, Ohannesian JP, Heiman ML, et al.** 1996 Nocturnal rise of leptin in lean, obese, and non-insulin-dependent diabetes mellitus subjects. J Clin Invest. 97:1344–1347.
7. **Laughlin GA, Yen SSC.** 1997 Hypoleptinemia in women athletes: absence of a diurnal rhythm with amenorrhea. J Clin Endocrinol Metab. 82:318–321.
8. **Pombo M, Herrera-Justiniano E, Considine RV, et al.** 1997 Nocturnal rise of leptin in normal prepubertal and pubertal children and in patients with perinatal stalk-transection syndrome. J Clin Endocrinol Metab. 82:2751–2753.
9. **Czeisler CA, Weitzman ED, Moore-Ede MC, Zimmerman JC, Knauer RS.** 1980 Human sleep: its duration and organization depend on its circadian phase. Science. 210:1264–1267.
10. **Schoeller DA, Cella LK, Sinha MK, Caro JF.** 1997 Entrainment of the diurnal rhythm of plasma leptin to meal timing. J Clin Invest. 100:1882–1887.
11. **Rentsch J, Chiesi M.** 1996 Regulation of *ob* gene mRNA levels in cultured adipocytes. FEBS Lett. 379:55–59.
12. **Segal KR, Landt M, Klein S.** 1996 Relationship between insulin sensitivity and plasma leptin concentration in lean and obese men. Diabetes. 45:988–991.
13. **Kolaczynski JW, Nyce MR, Considine RV, et al.** 1996 Acute and chronic effects of insulin on leptin production in humans: studies *in vivo* and *in vitro*. Diabetes 45:699.
14. **Van Cauter E, van Coevorden A, Blackman JD.** 1990 Modulation of neuroendocrine release by sleep and circadian rhythmicity. In: Yen SSC, Vaales WW, eds. Neuroendocrine regulation of reproduction. Norwell: Serono Symposia; 113–122.
15. **Aschoff J.** 1983 Circadian control of body temperature. J Therm Biol. 8:143–147.
16. **Sinha MK, Sturis J, Ohannesian J, et al.** 1996 Ultradian oscillations of leptin secretion in humans. Biochem Biophys Res Commun. 228:733–738.
17. **Licinio J, Mantzoros C, Negrao AB, et al.** 1997 Human leptin levels are pulsatile and inversely related to pituitary-adrenal function. Nat Med. 3:575–579.
18. **Simon C, Brandenberger G, Follenius M.** 1987 Ultradian oscillations of plasma glucose, insulin and C-peptide in man during continuous enteral nutrition. J Clin Endocrinol Metab. 64:669–674.
19. **Shapiro ET, Tillil H, Polonsky KS, Fang VS, Rubenstein AH, Van Cauter E.** 1988 Oscillations in insulin secretion during constant glucose infusion in normal man: relationship to changes in plasma glucose. J Clin Endocrinol Metab. 67:307–314.
20. **Simon C, Brandenberger G, Saini J, Ehrhart J, Follenius M.** 1994 Slow oscillations of plasma glucose and insulin secretion rate are amplified during sleep in humans under continuous enteral nutrition. Sleep. 17:333–338.
21. **Rechtshaffen A, Kales A.** 1968 A manual of standardized terminology, techniques and scoring system for sleep stages of human subjects. Washington DC: U.S. Government Printing Office.
22. **Cleveland W.** 1979 Robust locally weighted regression and smoothing scatterplots. J Am Stat Assoc. 74:829–836.
23. **Edwards AL.** 1957 Experimental design in psychological research. New York: Rinehart.
24. **Van Cauter E.** 1981 Quantitative methods for the analysis of circadian and episodic hormone fluctuations. In: Van Cauter E, Copinschi G, eds. Human pituitary hormones: circadian and episodic variations. The Hague: Martinus Nijhof; 1–25.
25. **Veldhuis JD, Johnson ML, Seneta E.** 1991 Analysis of the copulsatility of anterior pituitary hormones. J Clin Endocrinol Metab. 73:569–576.
26. **Follenius M, Brandenberger G, Bandensapt JJ, Libert JP, Ehrhart J.** 1992 Nocturnal cortisol release in relation to sleep structure. Sleep. 15:21–27.
27. **Goichot B, Brandenberger G, Saini J, Wittersheim G, Follenius M.** 1992 Nocturnal plasma thyrotropin variations are related to slow-wave sleep. J Sleep Res. 1:186–190.
28. **Goldstein J, Van Cauter E, Désir D, et al.** 1983 Effect of 'jet lag' on hormonal patterns. IV. Time shifts increase growth hormone release. J Clin Endocrinol Metab. 56:433–440.
29. **Cagnacci A, Elliot JA, Yen SSC.** 1992 Melatonin: a major regulator of the circadian rhythm of core temperature in humans. J Clin Endocrinol Metab. 75:447–452.
30. **Hwa JJ, Fawzi AB, Graziano MP, et al.** 1997 Leptin increases energy expenditure and selectively promotes fat metabolism in *ob/ob* mice. Am J Physiol. 272:R1204–R1209.
31. **Stehling O, Döring H, Ertl J, Preibisch G, Schmitt I.** 1996 Leptin reduces juvenile stores by altering the circadian cycle of energy expenditure. Am J Physiol. 271:R1770–R1774.
32. **Krauchi K, Wirtz-Justice A.** 1994 Circadian rhythm of heat production, heart rate, and skin and core temperature under unmasking conditions in men. Am J Physiol. 267:R819–R829.
33. **Boyle PJ, Scott JC, Krentz AJ, Nagy RJ, Comstock E, Hoffman C.** 1994 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.
34. **Rietveld WJ, Minors DS, Waterhouse JM.** 1993 Circadian rhythms and masking: an overview. Chronobiol Int. 10:306–312.