Journal of Neuroendocrinology, 2003, Vol. 15, 851–854

Sleep Loss Reduces Diurnal Rhythm Amplitude of Leptin in Healthy Men

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Key words: sleep deprivation, leptin, diurnal rhythm.

Abstract

The aim of the current study was to investigate the effects of sleep loss on the diurnal rhythm of circulating leptin levels. An indwelling forearm catheter was used to sample blood at 90-min intervals for a total of 120 h, which included 88 h of sustained sleeplessness, in 10 healthy men. The diurnal amplitude of leptin was reduced during total sleep deprivation and returned toward normal during the period of recovery sleep. This finding provides evidence that sleep influences the nocturnal leptin profile, and may have implications for the understanding of the role of sleep in metabolic regulation and the aetiologies of obesity and the night eating syndrome.

Leptin is an adipocyte-derived hormone that reflects the amount of energy stored in adipose tissue, and has been implicated in the regulation of food intake and metabolism (1). Circulating levels of leptin are highly organized and have a distinct diurnal and circadian rhythm, with minimum values during daytime and a nocturnal rise with maximum values during early to mid sleep (2–7). The amplitude of the circadian variation averages approximately 30% and is higher in men than in women (6). It has been suggested that the nocturnal rise in leptin serves to suppress appetite during the overnight period of fast and sleep (6).

Studies involving shifting meal schedules have shown that the timing of the daily maximum leptin levels is dependent on the timing of the meals (8). However, the circadian rhythm of leptin is not simply a response to meal patterns because this rhythm persists in subjects on continuous enteral feeding (9). An experiment that involved an abrupt shift of sleeping period (delay of 8 h) resulted in one nocturnal leptin peak, as well as a second peak following the onset of daytime recovery sleep (9). These data suggest that both an intrinsic circadian rhythmicity and sleep–wake homeostasis influence the release and circadian rhythm of leptin. However, no previous studies have examined the effect of acute prolonged sleep deprivation on the diurnal variation of leptin.

We conducted a study to investigate the effects of acute sleep deprivation on serum leptin levels. In this study, subjects who normally slept between 7 h and 9 h per night were sleep deprived for 88 h, and blood was drawn at 90-min intervals for 5 days.

Materials and methods

Subjects

Ten healthy men [mean age 27.2 years, range 22–37; mean body mass index (BMI) 26.1 kg/m^2, range 20–34.5] provided their informed consent to participate in a study that was approved by the Institutional Review Board (IRB) of the University of Pennsylvania. Subjects underwent a complete medical history and physical screening to rule out hepatitis, cancer, or other serious medical conditions. Clinical chemistry and urine tests were performed to ensure subjects were free of active infection, medications or drugs. Normal sleep wake rhythms and average sleep duration (between 7 h and 9 h of sleep per night, with morning wake time between 06.00 h and 09.00 h) were verified by sleep logs and actigraphy for a period of at least 1 week before participation in the study.

A 10-day protocol was carried out in the NIH General Clinical Research Center (GCRC) of the Hospital of the University of Pennsylvania. As participants in the control arm of a double-blind placebo controlled study on the effects of sustained low-dose caffeine as a countermeasure to sleepiness during sleep deprivation, subjects were first permitted 3 nights of scheduled sleep between 11.30 h and 07.30 h, followed by 88 continuous hours of vigil. Subjects were not permitted visitors and were kept in constant dim ambient light of $\leq$50 lux. At the completion of the sleep deprivation period, subjects were given 3 nights of recovery sleep for either 7 h or 14 h per night. Throughout the protocol, subjects were required to remain in a standard double hospital room with another study participant. Subjects were supervised during wakefulness by a monitor who was to keep the subjects awake by talking, watching videos and playing board games. Maintenance of wakefulness outside of scheduled sleep periods was verified using ambulatory recordings of electroencephalography, electrooculography and electromyography (MR-95, Oxford Medical, Oxford, UK). Subjects were tested every 2 h for approximately 30 min per test, for performance ability, mood and subjective symptoms throughout the study.

Subjects received three meals per day (08.40 h, 12.40 h and 06.40 h) plus an optional evening snack during baseline and recovery, and a scheduled late evening

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snack during the deprivation (10.40 h). Subjects were not required to eat all of the food offered them, but meals were designed to maintain body weight. Subjects were sedentary throughout the study, and weight remained stable from start to end of the protocol; Mean (±SE) BMI on entry into the GCRC was 26.1 (1.9) kg and, at the end of their stay, was 26.5 (±1.7) kg. No alcohol, caffeine, tobacco, or other drugs were permitted. Water was available *ad libitum*.

Blood draws were performed through an indwelling superficial forearm catheter at 90-min intervals, starting at 09.00 h on the evening of the final baseline night of sleep, through the 3 days of total sleep deprivation and recovery sleep day, for a total of 120 h of blood sampling time. Samples were drawn into polypropylene tubes containing Na-EDTA (1 mg/ml) and Aprotinin (300 kallikrein inhibiting units/ml blood), centrifuged, aliquotted and frozen at −80 °C until analysis. Samples were assayed for leptin, cortisol, a subset for inflammatory mediators (10), and for norepinephrine. Only leptin results are presented here; analysis of cortisol and norepinephrine is currently in progress.

Leptin measurements

Serum leptin levels were measured by a commercially available radioimmunoassay kit (Linco Research, St Louis, MO, USA). The sensitivity limit was 0.5 ng/ml, and the intra-assay coefficient of variation was 6%. All samples and standards were assayed in duplicate within the same assay.

Statistical analysis

A 24-h cosinor (11) was fit to each individual day for each subject and resulting mesor and phase were checked for stability before analysing for amplitude (12). Repeated measures analysis of variance (SPSS Statistical Software, version 9.0, SPSS Inc, Chicago, IL, USA) was used to analyse the within-subjects change across time for phase, mesor, and amplitude data. Paired t-tests were used to assess the differences between individual days for significant repeated measures ANOVA effects.

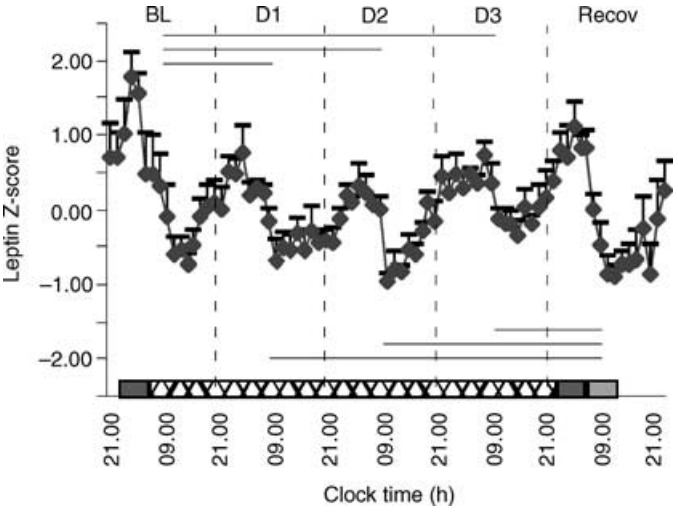

FIG. 1. Z-transformed leptin values are plotted across time, beginning at 21.00 h on the pre-deprivation baseline day and running through to 21.00 h on the evening following recovery sleep. BL and Recov indicate baseline and recovery days, respectively; D1, D2 and D3 indicate days 1, 2 and 3 of sleep deprivation. Horizontal lines on the upper left of the figure indicate significant comparisons with baseline amplitude and, on the lower right, significant comparisons with amplitude of recovery. The cross-hatched bar indicates the period of vigil, and the shaded bars the baseline and recovery (7 h or 14 h) sleep periods, respectively.

Results

The daytime mesor, or the mean (±SE) value of circulating leptin during the day, was 4.6 ± 1.1 ng/ml. The mean (±SE) mesor for each day was: baseline (BL) = 5.18 (1.37), day 1 = 4.78 (1.25), day 2 = 4.53 (1.09), day 3 = 5.03 (1.19) and Recovery = 4.81 (1.26). Repeated measures analysis of variance found no significant effect for mesor across days [$F(4,36) = 1.88$; $P = 0.18$]. There was a small nonsignificant phase shift from the first to last day of 1 ± 0.4 h (mean ± SE) [$F(4,36) = 0.24$; $P = 0.91$].

The amplitude of leptin was found to drop from baseline through the days of sleep loss and then to rise again during the day of recovery sleep [$F(4,36) = 7.78$, $P < 0.001$]. Because leptin values vary considerably even within the ranges of normal body weight, for plotting purposes, values were Z-transformed across days for each subject before averaging (Fig. 1). The amplitude dropped precipitously and remained reduced throughout the sleep deprivation period. Paired t-tests showed that the differences in amplitude were significant when comparing the baseline day with each of the sleep deprivation days [deprivation day 1, $t(9) = 3.35$, $P < 0.01$; deprivation day 2, $t(9) = 4.2$, $P < 0.01$; and deprivation day 3, $t(9) = 4.05$, $P < 0.01$]. In addition, the leptin amplitude on the recovery day was significantly elevated over the second [$t(9) = 2.88$, $P < 0.05$] and third [$t(9) = 3.02$, $P < 0.05$] days of sleep deprivation. There was no difference between amplitude of leptin on baseline versus recovery days.

Discussion

Our study is the first to examine serum leptin levels under conditions of prolonged total sleep deprivation. The reduction in leptin amplitude was rapid and sustained through the total sleep deprivation period, with the acrophase maintaining its occurrence approximately one-third to half way through the time of the normal sleep period. The suppression of leptin amplitude by sleep deprivation was reversed during the first night of recovery sleep, without signs of rebound. Thus, these data provide the first evidence that sleep may play an important role in the control of the nocturnal rise of leptin. The mechanisms for this modulation remain to be explored.

Although a single meal does not affect leptin levels (13), reduced appetite at night is consistent with the known nocturnal elevation of leptin (2–7), which may signal positive energy status to the brain during the nocturnal sleep associated fast. Our findings reveal that nocturnal elevations are due to more than circadian influence, and that sleep itself plays a role. During the first night of recovery sleep, the leptin rhythm showed a resumption of the normal diurnal amplitude, without signs of rebound. This is consistent with other studies that failed to find a rebound in leptin following suppression caused by fasting (14).

Leptin is directly influenced by insulin at the level of its production in fat cells (15), and there is evidence that insulin withdrawl leads to a decrease in leptin messenger RNA in fat cells (16). Furthermore, plasma glucose and insulin secretion rate show patterns of slow oscillation (co-occurring at 60–150-min intervals) throughout the day, but the amplitude of these oscillations increases by approximately one and a half times during sleep, although integrated levels do not change (17). Glucose and insulin are known to be involved in leptin signalling in the central nervous system, and it is possible that the absence of sleep, and the consequent reduction of both these signal amplitudes, leads to the dampening of the nocturnal peak in circulating leptin. However, there have been no studies investigating the effects of insulin and glucose on nocturnal leptin profiles.

Hypothalamic-pituitary-axis output is affected by stress, and several studies demonstrated an increase in cortisol, particularly at the nadir, during sleep deprivation (18, 19). Analysis of the diurnal rhythm of cortisol during sleep deprivation is currently being analysed, but preliminary data suggest that there is an increase in the nadir of cortisol in this study as well (20). Although there have been negative reports (21), sympathetic activity (norepinephrine, blood pressure) was shown to increase during acute sleep deprivation (22, 23). Sympathetic activity, as measured by heart rate variability, also increases in partial sleep deprivation (24). The physiological stress of sustained wakefulness may have been a factor in decreasing the leptin amplitude during sleep deprivation in our study. It is known that adrenergic receptor activation is suppressive of leptin production in mice (25), and that leptin is reduced in response to epinephrine infusion in women (26). Although preliminary analyses of cortisol (10) and norepinephrine (27) did not find overall elevations in the current study, diurnal analyses are underway to examine the effects of 88 h of sleep loss on these markers of stress, with and without caffeine as a countermeasure to sleepiness and performance decrement.

First described in 1955, the night-eating syndrome is characterized by morning anorexia, evening hyperphagia and insomnia (28). Interestingly, night-eaters have been reported to show a significant attenuation of the nocturnal rise in leptin levels (29). The attenuation of this rise may prevent adequate suppression of appetite during the night, thus resulting in nocturnal binging (29). Further evidence for this was provided by a study in healthy adolescent females which found that gain in body fat was inversely related with the nocturnal rise in leptin, suggesting that insufficient nocturnal leptin levels may contribute to the development of obesity (30).

It is not surprising that sleep is important for metabolic regulation, and acute (31) and partial sleep deprivation slow glucose metabolism (23). However, in spite of its obvious importance, the mechanisms are not well understood. Our findings are clear in demonstraing that sleep deprivation alters the diurnal pattern of release of leptin into peripheral circulation, and are consistent with the hypothesis that attenuation of the nocturnal rise of leptin may prevent adequate suppression of appetite during the night, which could result in nocturnal eating disorder. Further research is required to investigate the role of sleep in energy balance, together with the mechanisms involved.

Acknowledgements

This work was supported by AFOSR grants F49620-1-0388 and F49620-00-1-0266 (D.F.D.) and NIH RR 00040 to the GCRC at the Hospital of the University of Pennsylvania; and in part by NIH grants MH60641 (J.M.M.), NIDDK RO1-58785 (C.S.M.) and HL70154 (H.V.D.).

Accepted 10 June 2003

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