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Research Article

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Entrainment of the Diurnal Rhythm of Plasma Leptin to Meal Timing

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Abstract

To identify the physiologic factor(s) that entrain the diurnal rhythm of plasma leptin, leptin levels were measured hourly after changes in light/dark cycle, sleep/wake cycle, and meal timing. Four young male subjects were studied during each of two protocols, those being a simulated 12-h time zone shift and a 6.5-h meal shift. During the baseline day, plasma leptin demonstrated a strong diurnal rhythm with an amplitude of 21%, zenith at 2400 h, and nadir between 0900 and 1200 h. Acute sleep deprivation did not alter plasma leptin, but day/night reversal (time zone shift) caused a 12±2 h shift ($P < 0.01$) in the timing of the zenith and nadir. When meals were shifted 6.5 h without changing the light or sleep cycles, the plasma leptin rhythm was shifted by 5–7 h ($P < 0.01$). The phase change occurred rapidly when compared with changes in the diurnal rhythm of cortisol, suggesting that leptin levels are not acutely entrained to the circadian clock. The leptin rhythm was altered by meal timing in a manner very similar to the rhythm of de novo cholesterol synthesis. We conclude that the diurnal rhythm of plasma leptin in young males is entrained to meal timing. (*J. Clin. Invest.* 1997. 100:1882–1887.) Key words: hormones • circadian • energy metabolism • adipose tissue

Introduction

Leptin, the product of the *ob* gene, has been shown to play a significant role in the regulation of body mass. A mutation in this gene has been demonstrated in the *ob*/*ob* mouse. This mutation results in an inactive form of leptin and obesity which is reversed when exogenous leptin is administered (1, 2). Further studies in the *ob*/*ob* and *db*/*db* mice have indicated that leptin acts on a receptor in the hypothalamus, producing a satiety signal (3, 4). This and other evidence indicates that leptin acts as a feedback signal from adipose tissue to the hypothalamus for long-term regulation of energy balance (2–5).

The level of expression of leptin mRNA responds to nutritional status in rodents. Short-term starvation results in a decrease in leptin mRNA, and refeeding results in a rapid return to previous levels of expression (6, 7). Even within a day, leptin mRNA expression undergoes cyclic variation, with an increase during the night shortly after initiation of feeding, and a decrease during the day (6). It is not clear which physiologic factor(s) entrain this diurnal variation. Both corticosteroids and insulin have been reported to upregulate leptin mRNA expression, which could indicate either a circadian regulation related to cortisol, or a diurnal regulation related to food consumption (6–9).

A diurnal variation of plasma leptin has been observed in humans (10). Like the rodent, the zenith occurs during the dark cycle, and the nadir during the light. This pattern, however, represents a difference relative to the feeding cycle, as humans are normally daytime feeders. Humans also demonstrate a rapid decrease of plasma leptin levels after a short-term fast, with rapid recovery to a level appropriate for adipose mass after refeeding (11, 12). Other than these acute effects of fasting and feeding, little is known about the physiologic regulation of leptin levels during the day.

The aim of this study was to determine the physiologic factors that entrain the diurnal rhythm of leptin in humans. Two paradigms were used: a simulated jet lag in which the subjects underwent a day/night reversal, (a 12-h time zone shift) to determine if the diurnal rhythm is entrained to the circadian clock, or a 6.5-h shift in meal timing to determine if the rhythm is entrained to meal timing alone.

Methods

Subjects. Six subjects were recruited for two protocols. Two subjects participated in both protocols, while four participated in only one, such that there were four subjects in each of the two protocols. The subjects were healthy volunteers from the university community. All were nonsmokers without a history of endocrine, renal, liver, or other metabolic as well as psychiatric disorders. They were young adult males with an average age of 25 yr (range 23–35), an average BMI of 25 kg/m^2 (range 20–30), and had normal fasting glucose (inclusion range 3.9–6.1 mmol/liter) and triglyceride levels (inclusion range 0.1–1.8 mmol/liter). Subjects were excluded if they had irregular work, sleep, or meal schedules, worked nights, or reported any prescription medications. All procedures were approved by the Institutional Review Board of the University of Chicago, and subjects gave informed written consent.

Simulated jet lag. Subjects were asked to adhere to a regular schedule for 1 wk before the study: meals at 0730, 1230, and 1730 h and sleep between 2300 and 0700 h. Subjects were asked to abstain from caffeine and alcohol. On the final 3 d before the study, the subjects consumed their meals at prescribed times in the Clinical Research Center (CRC)[1] on an outpatient basis. Meals provided energy of 1.80 times measured resting metabolic rate (RMR) with 40, 35, and 25% of energy from carbohydrate, fat, and protein, respectively. On the last night before the study, the subject slept in the CRC after placement of a forearm venous catheter to become customized to the CRC.

The subjects reported back to the CRC at 1600 h and remained there as inpatients for 5 d (see Fig. 1). A venous catheter was inserted and kept patent with a saline drip. The catheter was moved at least

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1. *Abbreviations used in this paper:* CRC, Clinical Research Center; RMR, resting metabolic rate.

every 96 h. Dinner was served at 1730 h. The baseline day began with blood sampling at 1800 h, which continued hourly. A 12-ft catheter was extended into an adjoining room during sleep so that blood samples could be drawn without waking or otherwise disturbing the subjects. Subjects slept from 2300–0700 h. Meals were provided at 0730, 1230, and 1730 h. Each meal provided 33% of total energy intake, and total dietary energy equaled 1.55 × RMR. This value is lower than the energy provided in the outpatient period because of the expected reduction in physical activity under CRC inpatient conditions.

Day 1 was defined as starting at 1800 h after completion of the baseline day. Subjects were deprived of sleep on the night of day 1. Lights were dimmed to minimize stimulation of the light receptors, but the subject was not allowed to sleep or nap. Breakfast was provided at 0730, and lunch at 1030 h. At 1100 h, blackout curtains were drawn, and the subjects were allowed to sleep from 1100–1900 h. At 1900 (day 2), the subjects were awakened, and bright lights were placed 4 ft. from the subjects to simulate daylight (2500–2750 lux at 4 ft.). Meals were provided at 1930, 0030, and 0530 h, or exactly 12 h later than on the baseline day. This pattern was repeated on days 3 and 4 of the simulated jet lag.

Meal shift. The week before the testing was identical to the jet lag protocol, except that the meal times were at 0700, 1150, and 1640 h. Subjects were admitted to the CRC at 2200 h and remained there for 4 d. All meals were provided at fixed times. The energy content was 1.55 × RMR with 35% of energy from fat, 25% from protein, and 40% from carbohydrate. The three meals were isocaloric. Subjects were allowed 20–30 min to consume their meals and were not allowed any snacks or caloric beverages at other times. On the baseline day, blood draws began at 0800 h and continued hourly using a catheter kept patent with saline. Breakfast was eaten at 0700 h, lunch at 1130 h, and dinner at 1640 h (see Fig. 1). Subjects slept from 2330 to 0700 h, and were not permitted to nap. No lighting other than that provided by the windows and room lights was used.

On day 1, the meal timing was changed, but the content of the meals was identical to baseline. Breakfast was eaten at 1330 h, lunch at 1820 h, and dinner at 2310 h, which are 6.5 h later than on the baseline day. This pattern was repeated on days 2 and 3. Blood was sampled hourly on days 1 and 3. A 12-ft. catheter was used during the sleep period such that blood samples could be collected in an adjoining room so as not to awaken the subject.

Leptin assay. Blood was collected, and plasma was removed and frozen at −18°C. Plasma (100 μL) leptin levels were assayed using a Linco RIA kit (Linco Research, St. Charles, MO).

Other assays. As reported elsewhere (13, 14), plasma levels of cortisol as well as de novo cholesterol synthesis were also measured in these subjects. In brief, cortisol was measured by radioimmunoassay (Diagnostic Products Corp., Los Angeles, CA). De novo cholesterol fractional synthetic rate was measured by the rate of appearance of deuterium in plasma total cholesterol during a prime-constant administration of deuterium oxide.

Statistical analysis. To test for diurnal variation, the function [M + A sine (0.262t + p)] was fit to the plasma leptin concentration where M is the mean 24-h leptin level (ng/mliter), A is the amplitude (ng/mliter), t is the clock time, and p is phase. With 20–24 samples per day, a correlation between the predicted and measured leptin levels of 0.42–0.38 was required for significance.

The time of nadir and zenith were taken as the time at which leptin levels were lowest or highest in each individual during each 24-h period, and the mean and standard deviation calculated using standard formula. The amplitude (half the range of oscillation) was expressed as a percentage of the 24-h level. The observed average r^2 for the baseline days was 0.55 ($r = 0.74$), indicating that we had a power of 0.8 to detect ($P = 0.05$) a diurnal variation in leptin levels with an amplitude as small as 11%.

Analysis of variance for repeat measures and post-hoc *t* test were used to test for differences between days in the 24-h mean levels, time of nadir and zenith, and amplitude. A Bonferroni adjustment for multiple comparisons was used. Correlations between the diurnal rhythm of leptin and other rhythms were performed using linear regression between the hourly values. The values were offset in 1-h increments until the highest level of correlation was identified. Results are expressed as mean±SD, except as noted in the figures. $P < 0.05$ was required for significance.

Results

Baseline leptin. Leptin demonstrated a diurnal variation during the baseline day in each subject enrolled in the two studies. The hourly leptin levels were significantly correlated with a sine function for each of the individual days. The average *r* was 0.74 ($P < 0.001$) with a range from 0.54 ($P < 0.05$) to 0.94 ($P < 0.001$). Moreover, the temporal patterns did not differ markedly between subjects or between the two studies (Tables I and II). The zenith occurred near midnight, and the nadir between 0900 h and noon. The fasting leptin levels at 0800 h were 3.1±0.9 ng/mliter, which was 15±11% lower than the 24-h average levels ($P < 0.01$).

Simulated jet lag. Acute sleep deprivation followed by sleep recovery beginning the period of day/night reversal had no effect on the diurnal rhythm of plasma leptin (Table I), ex-

Table I. Summary of Diurnal Variations During the Simulated Jet Lag Study

		Baseline	Day 1	Day 2	Day 4
Leptin	Zenith (h)	2400±210	2330±250	1010±30‡	1350±140‡
	Nadir (h)	1140±120	1010±100	2050±100‡	2430±120‡
	24-h mean (ng/mliter)	3.5±1.0	3.6±0.7	4.5±0.9	4.4±1.2
	Amplitude (%)	21±8	19±13	23±8	20±7
Cortisol§	Zenith (h)	0630±120	0700±50	0640±100	1000±110‡
	Nadir (h)	2440±40	2340±110	2330±30	0300±240
	24-h mean (mM/liter)	158±20	181±24	188±28	232±57
	Amplitude (%)	112±15	105±14	105±18	68±32*
Cholesterol synthesis§	Zenith (h)	2350±110	2150±100	0510±30‡	0800±220*
	Nadir (h)	0900±150	0910±140	1440±310	2330±100*
	24-h mean (pool/d)	0.040±0.013	0.050±0.007	0.042±0.002	0.036±0.007
	Amplitude (%)	78±48	74±31	95±24	99±42

Results are expressed±SD. *$P < 0.05$ or ‡$P < 0.01$ relative to baseline; §from reference 13.

Table II. Summary of Diurnal Variations During the 6.5-h Meal Study

		Baseline	Day 1	Day 3
Leptin	Zenith (h)	2440±130	0340±140	0530±40‡
	Nadir (h)	0910±100	1550±120‡	1600±50‡
	24-h mean (ng/mliter)	4.0±1.0	3.8±0.9	4.0±0.7
	Amplitude (%)	20±2	34±11	22±6
Cortisol§	Zenith (h)	0630±120	0720±30	0630±30
	Nadir (h)	2410±100	2220±60	2440±50
	24-h mean (mM/liter)	213±24	212±30	230±49
	Amplitude (%)	121±36	89±5	106±21
Cholesterol synthesis§	Zenith (h)	2200±320	0400±150*	0640±40‡
	Nadir (h)	1130±140	1000±200	1800±230‡
	24-h mean (pool/d)	0.042±0.006	0.050±0.004	0.033±0.010
	Amplitude (%)	109±30	122±18	233±68*

Results are expressed±SD. *$P < 0.05$ or ‡$P < 0.01$ relative to baseline; §from reference 14.

cept that the nocturnal rise during the recovery sleep beginning at 1100 h was greater than that during the baseline day for the same clock time ($P < 0.01$), but did not exceed the baseline zenith (Fig. 1).

On day 2 of the simulated jet lag, the diurnal rhythm phase shifted relative to the baseline day (Table I). The nadir advanced by 9.3±2.0 h ($P < 0.01$), and the zenith advanced by 12.5±1 h ($P < 0.01$). The average 24-h plasma leptin level tended to increase (NS vs. baseline), and the amplitude was unchanged.

On day 4 of simulated jet lag the diurnal rhythm was reversed from baseline day. The nadir was advanced by 12.2±2.2 h ($P < 0.01$) while the zenith was advanced 11.2±1.9 h ($P < 0.01$). The amplitude and 24-h average leptin levels did not differ from baseline, but the variance increased (Fig. 2). The increased variance in the relative leptin levels did not reflect a between-subject variation in the time of the nadir or zenith, but rather a variation in the average 24-h leptin level relative to the baseline value that was used for normalization. Specifically, the average 24-h leptin levels of two of the subjects increased by 58 and 59%, while those of the other two subjects increased 14% or decreased 11% relative to the baseline day. When the results were expressed as the percent of the individual's average 24-h leptin level on a day-by-day basis, the standard error on day 4 was reduced twofold, and was comparable to that of the baseline day (Fig. 2, inset).

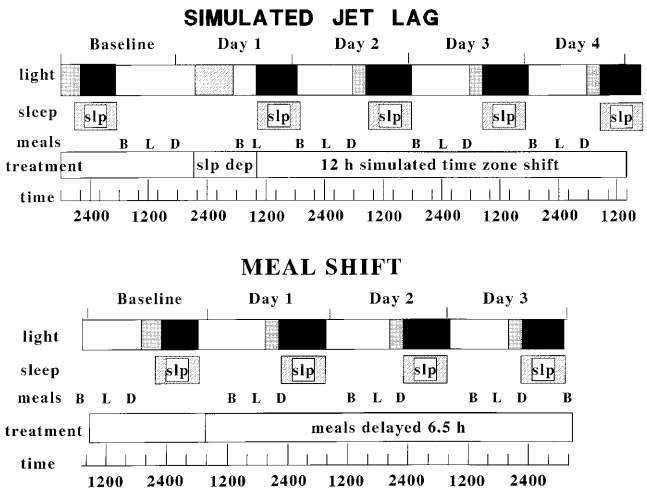

Figure 1. Schematic presentation of the simulated jet lag and meal shift protocols. Meals are indicated by B (breakfast), L (lunch), and D (dinner); lighting level is indicated by shading of the bar for dark (*black*), dim (*gray*), and light (*white*), and sleep time, shown in row 2, is indicated by the cross-stippled bar. Time is central standard time.

Plasma cortisol and de novo cholesterol synthesis values from these subjects are published elsewhere (13, 14). The diurnal rhythm of plasma leptin was highly correlated with that of cortisol at baseline ($r = 0.73$) with the leptin rhythm leading cortisol by 8±3 h (Table I). On day 1, the correlation was 0.64 and the phase difference was still 8±4 h, but on day 2, there was a phase shift in the leptin rhythm, but none in the cortisol rhythm. Thus, the phase difference decreased to 0±2 h ($P < 0.05$ vs. baseline) with no change in the correlation indicating that the leptin rhythm underwent a phase shift, but that the marker of circadian rhythm was unchanged. The correlation decreased on day 4 ($r = 0.48$) due to the bimodal pattern in cortisol that occurred on day 4 (13). Cortisol demonstrated a 2-h phase shift in the zenith ($P < 0.01$) and a trend towards a delay such that cortisol led leptin by 4±5 h.

In contrast, the leptin rhythm and cholesterol synthesis rhythms stayed in phase (Table I). Leptin and cholesterol synthesis rhythms correlated well throughout the study ($r = 0.63$–0.53). Cholesterol synthesis led the leptin rhythm by 2±6 h at baseline, by 1±3 and 1±4 h on days 1 and 2, and by 4±9 h on day 4 (all NS) because cholesterol synthesis was undergoing a phase shift quite similar to that of leptin.

6.5-h meal shift. The plasma leptin levels on the baseline day were similar to those on the baseline day of the simulated jet lag (Fig. 2 and Table II). Changes relative to baseline in the diurnal rhythm occurred the first day of the meal shift. The nadir was delayed by 6.8±1.9 h ($P < 0.01$). The plasma leptin level at 0900 h was similar to that observed on the baseline day, but continued to decrease for 6–7 h, reaching a nadir of 2.7±0.88 ng/liter which was less than the nadir on the baseline day ($P < 0.05$). The zenith tended to be delayed by 3.0±2.9 h (NS). On day 3 of shifted meals, both the nadir and zenith were delayed 4.8±1.5 ($P < 0.01$) and 6.8±1.7 ($P < 0.01$) h rel-

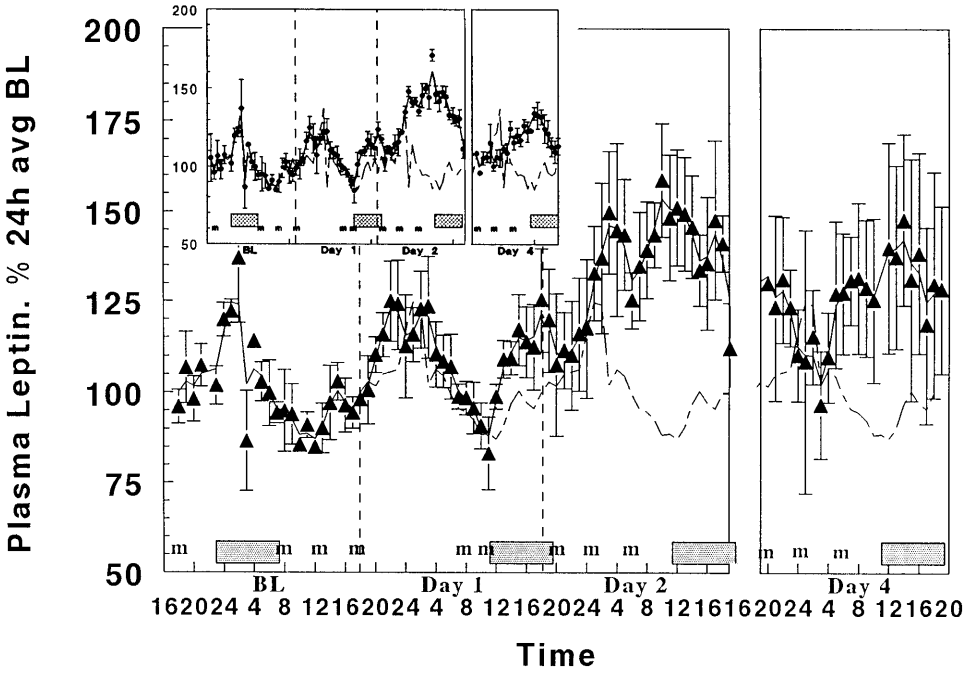

Figure 2. Diurnal rhythm of plasma leptin during the simulated jet lag study. Plasma levels (mean±SEM) are expressed as the percentage of each subject's average 24-h leptin level on the baseline day (*BL*). The *dashed line* represents the rhythm observed on the baseline day for visual comparison. The inset expresses the leptin level as the percentage of each day's 24-h average level (mean±SEM).

ative to the baseline day, respectively. The 24-h average leptin level and the amplitude were not different than baseline.

Similar to the results of the jet lag study, the diurnal rhythm of leptin correlated well with cortisol ($r = 0.70$) and led cortisol by 11±1 h on the baseline day. The correlation tended to increase on days 1 and 3 ($r = 0.72$ and 0.78) with no change in phase on day 1 (leptin leading by 6±2 h, NS), but a shift in the phase of the leptin rhythm relative to cortisol was observed on day 3; i.e., leptin leading cortisol by 4±1 h, $P < 0.01$. This shift was due to a phase shift in the leptin rhythm with no change in cortisol (Table II), indicating that the diurnal rhythm of plasma leptin again underwent a phase shift in the absences of a shift in the marker of circadian rhythm. The leptin rhythm was highly correlated with cholesterol synthesis throughout the meal shift study ($r = 0.84$, 0.76, and 0.68 at baseline, day 1, and day 3). No phase shift relative to cholesterol synthesis was observed as both shifted in the same direction (Table II). Cholesterol synthesis led leptin by 2±1 h at baseline, 2±1 h on day 1, and trailed by 1±2 h on day 2.

Discussion

The results of this investigation indicate that the diurnal rhythm of plasma leptin is entrained to meal pattern. Although there was a strong correlation between plasma leptin levels with cortisol levels during the baseline day during both protocols, the diurnal rhythm of plasma leptin demonstrated a very rapid phase shift relative to plasma cortisol during the simulated jet lag. Because cortisol is a marker of circadian time (15), it is unlikely that leptin is entrained to the circadian clock. This fact also indicates that plasma leptin levels are not acutely regulated by cortisol, although corticosteroids have been shown to upregulate leptin mRNA in cultured adipocytes in vitro (6, 9). Strong evidence for physiological regulation by meal pattern was provided by the meal shift protocol. Leptin demonstrated an acute phase shift that directly corresponded to the shift in meal timing, while plasma cortisol did not change at all.

This study also provides evidence for an absence of acute entrainment to the sleep cycle because sleep deprivation did not alter the leptin rhythm. Thus, growth hormone and other sleep-related hormones (16, 17) are not likely to have significant roles in acute regulation of the leptin.

The effects of changes in meal timing were surprisingly acute for a hormone that is believed to be involved in long-term maintenance of energy balance. Although a given meal did not lead to an obvious intraural peak in plasma leptin levels, it was evident that breakfast consumption was the factor that resulted in cessation of the late-night, early-morning de-

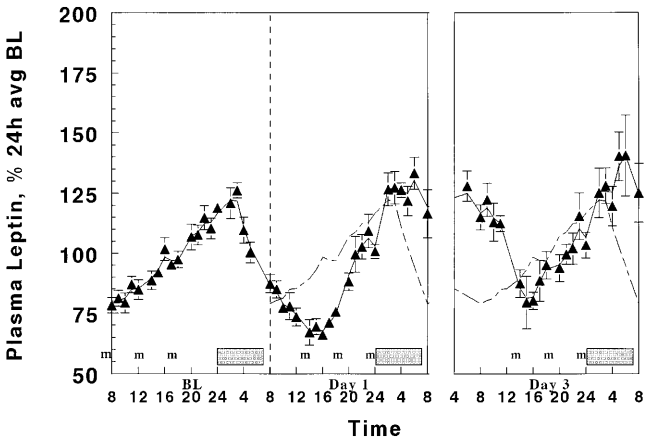

Figure 3. Diurnal rhythm of plasma leptin levels during the meal shift protocol. Plasma levels (mean±SEM) are expressed as the percentage of each subject's average 24-h leptin level on the baseline day (*BL*). The *dashed line* represents the leptin level that was observed on the baseline day for visual comparison.

crease in plasma leptin levels because leptin levels continued their overnight decrease for an additional 6–7 h when breakfast was delayed for 6.5 h on day 1 of the meal shift protocol.

After breakfast, leptin levels began a slow rise, which became significant after lunch and continued through and beyond dinner. This pattern differed from that reported by Sinha et al. (10), who found that leptin levels plateaued during the daylight hours, and only began rising toward their zenith in the late afternoon or early evening. This difference probably reflects the difference in meal patterns of the two studies. Although the 24-h dietary energy levels and macronutrient compositions were similar, the distribution of energy across meals differed. In this study, each of the three meals during the day were isocaloric, and thus provided 33% of the daily energy intake. In the study reported by Sinha et al. (10), the breakfast, lunch, and dinner provided 20, 30, and 40% of daily energy intake with 10% for a snack. Thus, our study provided more dietary energy earlier in the day, which may have caused the earlier rise in leptin levels.

The dramatic increase in the mean 24-h leptin levels observed in two subjects in the meal shift protocol cannot be explained on the basis of the data we collected, but we speculate that this increase may reflect a change in energy balance. Moreover, leptin levels have been shown to rise and fall during periods of positive and negative energy balance, respectively (18, 19). It may be that the change to an inpatient setting resulted in a more positive energy balance in these two individuals, despite our supplying a smaller dietary energy level.

The finding that leptin rhythm is entrained to the meal pattern is similar to our recent report regarding the entrainment of de novo cholesterol synthesis to meal pattern (13, 14). Herein we report that these two diurnal rhythms are highly correlated with no detectable difference in phase, although due to the small number of subjects, we only had a power of 0.8 to detect a difference between the estimated cholesterol phase changes of 8 h in the simulated jet lag study, and 2 h in the meal shift study. Furthermore, the acute changes in phase of the two rhythms are the same in that they both shift relative to cortisol, but not relative to each other. This suggests that the production of leptin and de novo synthesis of cholesterol have some common acute regulatory process(es).

Although this study identifies meal pattern as the physiologic factor that entrains the diurnal rhythm of leptin levels, it does not provide direct evidence regarding the signals that do regulate leptin levels. Previous studies provide insight in this regard. It has been demonstrated that leptin gene expression occurs only in fat tissue in rodents (2, 5), and presumably in humans, although data is not as extensive (12). In humans, morning plasma leptin levels are highly correlated with fat mass (12, 20), although levels in females are higher than in males after adjustment for fat mass (20). Klein et al. (21) measured arterio–venous differences in leptin concentration across the subcutaneous adipose tissue in the abdominal region, and demonstrated that leptin was indeed produced in the adipose tissue in humans, and that plasma levels were controlled by production rate rather than clearance rate.

Because the diurnal rhythm of leptin is entrained to meal pattern, it is possible that insulin levels may alter leptin production. Previous studies using acute hyperinsulin clamps, however, have not caused acute increases in leptin levels, even when extended up to 6 h (22, 23). Extension of the hyperinsulin clamp up to 60 h, however, did result in elevated leptin and apparent ablation of the diurnal rhythm during the last 36 h, suggesting a chronic effect of insulin on leptin production (23). Still, the evidence linking insulin to leptin production remains weak. An alternative and speculative explanation of the diurnal rhythm of plasma leptin is that leptin production is coordinated with net triglyceride synthesis in the adipocyte, and hence is responsive to short-term energy balance.

In summary, phase shifts in plasma leptin levels are apparent within hours of changing the meal pattern, and are not correlated with cortisol, which is a robust marker of the circadian clock. The diurnal variation is also not altered by acute sleep deprivation, and thus is not likely to be regulated by growth hormone or other sleep-related hormones. This study provides direct evidence that diurnal variation in plasma leptin levels is entrained to meal timing in young healthy males.

Acknowledgments

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