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Diurnal and Ultradian Dynamics of Serum Adiponectin in Healthy Men: Comparison with Leptin, Circulating Soluble Leptin Receptor, and Cortisol Patterns

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Adiponectin is an abundant serum adipokine secreted exclusively from differentiated adipocytes, which plays an important role in regulating insulin sensitivity. The dynamics of circulating adiponectin concentrations have yet to be systematically investigated. We sought to determine whether serum adiponectin levels exhibit diurnal or ultradian rhythms in healthy normal-weight men and to compare the 24-h profile of adiponectin fluctuations with those of leptin, leptin-binding protein (sOB-R), and cortisol.

We collected blood samples at 15-min intervals over 24 h from six subjects receiving an isocaloric diet, and we measured adiponectin, leptin, sOB-R, and cortisol levels. Fourier and cross-correlation analyses were performed on these time series to study diurnal variations, and the Cluster7 program was used for pulsatility analysis.

Circulating adiponectin and sOB-R levels exhibited ultradian pulsatility as well as a diurnal variation with a significant decline at night, reaching a nadir in the early morning. The 24-h variations of serum adiponectin and sOB-R were nearly identical and followed those of cortisol after a few hours, but were out-of-phase with leptin diurnal rhythms. These data suggest that adiponectin and sOB-R levels might be influenced by common regulatory factors and challenge the notion that cortisol may have a direct inhibitory effect on adiponectin in humans. (*J Clin Endocrinol Metab* 88: 2838–2843, 2003)

A VARIETY OF endocrine systems exhibit circadian (~24 h) and ultradian (<24 h) variations. Although these features were first demonstrated for hormones of the hypothalamic-pituitary axis, similar patterns apply to other hormones as well (1). Peripheral insulin action, for example, shows diurnal variations in part mediated by serum cortisol, which is known to have circadian and ultradian rhythmicity under hypothalamic-pituitary control (1, 2).

Adipose tissue also has the capacity to regulate insulin sensitivity through several proteins (adipokines) that are secreted into the circulation (3). Accumulating evidence shows that adiponectin, an abundant serum adipokine expressed and secreted exclusively from differentiated adipocytes (4, 5), plays an important role in insulin sensitivity. Adiponectin knockout mice develop insulin resistance (6), whereas adiponectin administration improves insulin resistance in obese mice with low circulating adiponectin levels (7). In humans, serum adiponectin is decreased in obesity and diabetes (8, 9), and low levels precede and correlate with the decline of insulin sensitivity (10, 11). Leptin, another intensively studied adipokine, has an important role in regulating energy homeostasis (12) as well as insulin sensitivity in rodents and leptin-deficient humans with congenital lipodystrophy (7, 13, 14). The biological activity of leptin is modulated by the soluble cleaved extracellular part of the leptin receptor (sOB-R), which is its main binding protein in the serum (15). Although the tissue source(s) and the exact molecular basis of its production remain to be fully elucidated, recent evidence suggests that sOB-R is secreted by adipose tissue alone or in combination with leptin (16–19). Serum leptin levels have been reported to have ultradian and circadian rhythmicity (20–22), whereas sOB-R shows a diurnal variation opposite to that of leptin (23).

The dynamics of circulating adiponectin concentrations have yet to be systematically investigated. In this study, we sought to determine whether serum adiponectin levels exhibit a diurnal rhythm or pulsatile patterns of secretion in healthy normal-weight men. Because leptin and cortisol influence insulin sensitivity and have well established circadian and ultradian rhythmicity, we also compared the diurnal adiponectin profile with those of leptin, sOB-R, and cortisol.

Subjects and Methods

Subjects

Six normal-weight healthy Caucasian men (mean age, 20.3 ± 0.6 yr; mean body mass index, 22.8 ± 0.9 kg/m^2) gave informed consent to participate in this study, which was approved by the Institutional Review Board at the Beth Israel Deaconess Medical Center. Subjects were screened for any significant medical history based on physical examination and routine laboratory tests. None of the subjects had abnormal glucose tolerance based on screening serum glucose measurements (mean random serum glucose, 75.7 ± 13.4 mg/dl).

Twenty-four-hour blood sampling protocol

Subjects were acclimatized to a research bed in the General Clinical Research Center for 48 h before the study and placed on an isocaloric

Abbreviations: CV, Coefficient of variation; sOB-R, soluble leptin receptor.

weight-maintaining diet with breakfast at 0800 h (20% of calories), lunch at 1300 h (35% of calories), dinner at 1800 h (35% of calories), and snack at 2200 h (10% of calories), which was followed for the entire duration of the study. Subjects were exposed to light from 0700 h to 2300 h and were in the dark from 2300 h to 0700 h. They were not allowed to do strenuous activity during the day, but otherwise activity was not limited. On the third day, starting at 0830 h, blood samples were collected every 15 min for 24 h (a total of 97 samples per subject) through an iv catheter inserted in an antecubital vein. During the night, blood samples were drawn outside the subjects' rooms to avoid disturbing their sleep. Samples were centrifuged after collection and stored frozen at −80 C until assayed for adiponectin, leptin, sOB-R, and cortisol.

Hormone measurements

Serum adiponectin levels were measured using a RIA (Linco Research, Inc., St. Charles, MO) with a sensitivity of 1 ng/ml and an intra-assay coefficient of variation (CV) of 6.6%. Serum leptin levels were measured using a RIA (Linco Research, Inc.) with a sensitivity of 0.5 ng/ml and intra-assay CV of 8.3%. Serum sOB-R levels were measured using a commercially available ELISA (BioVendor Laboratory Medicine, Brno, Czech Republic), with a sensitivity of 0.8 ng/ml and intra-assay CV between 5.5% and 6.6%. Serum cortisol levels were measured using a RIA (Diagnostic Systems Laboratories, Inc., Webster, TX) with a sensitivity of 0.5 μg/dl and intra-assay CV between 5.3% and 8.4%. To minimize assay variability, all samples for each subject were assayed at the same time.

Diurnal variability analysis

Twenty-four-hour time series analysis. Fourier analysis (24) was applied to the 24-h analyte time series to study fluctuations on selected time scales. Serum levels of adiponectin, leptin, sOB-R, and cortisol for each subject were first low-pass filtered over a frequency range of 0–0.1 cycles/h to extract the low-frequency components. These low-pass filtered time series were used to study the long-term (~24 h) variations. Each filtered dataset was rescaled so that the 24-h average was set equal to 100%, and data at each time point were defined as a percentage of the 24-h average. Group averages over all six subjects were then calculated for each of the four analytes–adiponectin, leptin, sOB-R, and cortisol. The acrophase and nadir of the diurnal variations were defined as the times of occurrence of the maximum and minimum values of these group averages, respectively.

Cross-correlation analysis. To assess the temporal relationships of diurnal variations among the four analytes, we applied standard cross-correlation measurements (24) on the following pairs of low-pass filtered time series from each subject: adiponectin *vs.* leptin, adiponectin *vs.* sOB-R, adiponectin *vs.* cortisol, leptin *vs.* cortisol, sOB-R *vs.* cortisol, and leptin *vs.* sOB-R. The cross-correlation functions were then averaged over all six subjects, and the lag relationships among all four analytes were calculated. We also calculated Spearman's correlation coefficients between simultaneous values of the four analytes using averaged 24-h profiles over all six subjects.

Ultradian variability (pulsatility) analysis

Cluster7, a computerized algorithm (25), was used to identify putative pulses in the 24-h adiponectin, leptin, sOB-R, and cortisol time series. Pulses are defined by this program as significant increases followed by significant decreases when analyzed using pooled *t* testing. To diminish the false-positive rate, several test parameters are operator-selected, including the intra-assay CV, the number of degrees of freedom, the width of the nadir and peak clusters, as well as the level of significance of the *t* statistic score. For the 15-min sampling procedure used in the study, we considered peaks being represented by one time point, and nadirs by two consecutive time points. We used a conservative intra-assay CV of 10%, chosen to be higher than those of each of the four assays. We analyzed the 24-h pulsatility patterns of the four analytes using different *t* statistic scores and report herein data obtained with a *t* statistic value of 2, which resulted in cortisol and leptin pulsatility close to previously reported patterns (1, 21, 22). To further validate that the pulsatile peaks detected by the Cluster algorithm are not simply due to random noise with a circadian variation, we tested the algorithm on surrogate data obtained by superimposing random Gaussian noise with 8% CV in the low-pass filtered adiponectin time series.

Pulses were characterized with the following parameters, reported as mean ± SEM: peak frequency (number of peaks per 24 h), mean peak duration (minutes), mean interval between peaks (minutes), mean peak height expressed as absolute value and percentage from the preceding nadir value, and mean value between peaks. Because one subject (no. 3) had multiple consecutively missing values, only five of the six 24-h profiles could be used to calculate mean pulse parameters.

Results

Diurnal variability analysis

Twenty-four-hour time series analysis. Figure 1 shows the 24-h serum adiponectin, leptin, sOB-R, and cortisol profiles of each subject plotted at 15-min intervals (not filtered). Visual inspection of these time series reveals a complex pattern of fluctuations. Figure 2 presents the low-pass filtered values averaged for all six subjects and suggests diurnal patterns. Figure 3 summarizes all four serum concentration profiles on the same graph. Table 1 gives the diurnal variation parameters of the four analytes.

Adiponectin. Serum adiponectin showed a clear diurnal variation characterized by a nocturnal decline starting in the late evening and continuing throughout the night to reach a nadir in the early morning (Table 1 and Fig. 2). Adiponectin levels were higher during the day, with a peak in the late morning. After peak levels were reached, there was minimal daytime variation, with adiponectin levels decreasing slightly during the early afternoon and then plateauing until late evening. The peak levels represented a 14.9 ± 3.7% increase from the 24-h mean, whereas the trough levels represented a 22.6 ± 3.1% decrease from the 24-h mean adiponectin concentration. The maximum diurnal variation calculated as the difference between the mean peak and trough values was 1.7 ± 0.1 μg/ml, representing 37.5 ± 6.8% of the mean 24-h adiponectin level.

Adiponectin vs. leptin and sOB-R. Serum adiponectin and leptin showed out-of-phase 24-h profiles (Table 1 and Fig. 3). A progressive increase of leptin occurred during the day, reaching peak levels in the evening or early night. Leptin levels then declined during the night to a nadir in the early morning. In contrast to the adiponectin-leptin relationship, adiponectin and sOB-R profiles showed a striking similarity and tracked each other closely over 24 h, reaching their minimum and maximum levels at about the same time. The peak sOB-R level was attained around noontime, whereas the nadir was observed in the early morning. SOB-R had slightly larger percentage variations compared with adiponectin levels at all times (Table 1 and Fig. 3).

Adiponectin vs. cortisol. These two hormones had similar profiles; adiponectin, however, showed smaller percentage amplitude changes over the entire 24-h period (Table 1 and Fig. 3). Cortisol levels progressively increased during the morning with peak levels in the late morning, followed by a continuous decline during the rest of the day with a nadir in the early night. Although adiponectin and cortisol reached

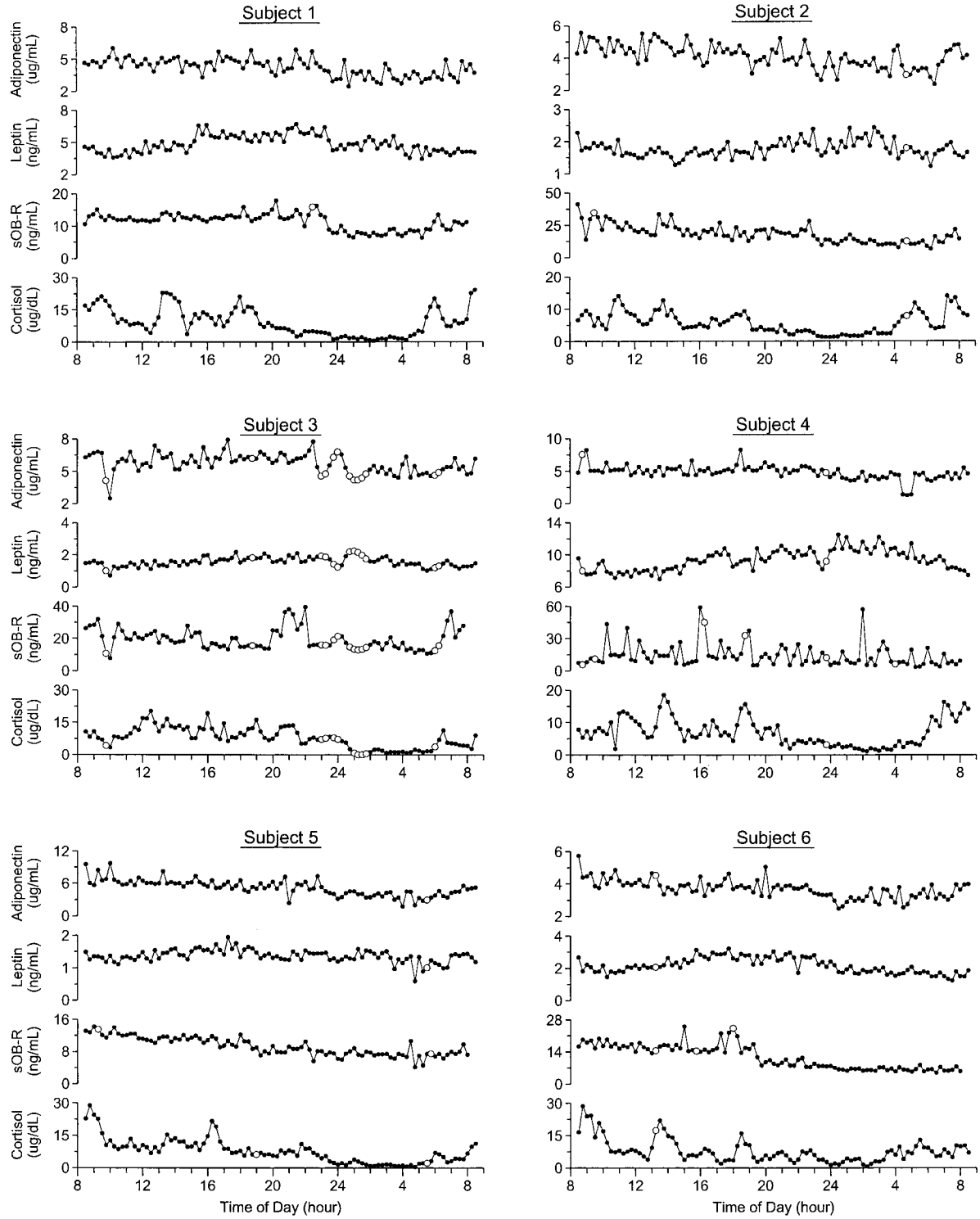

FIG. 1. Serum adiponectin, leptin, sOB-R, and cortisol levels for each of the six subjects sampled every 15 min over a 24-h period starting at 0830 h. The vertical axis scales differ between subjects due to individual variability in the ranges of these hormone levels. Missing data points were interpolated using a cubic spine interpolation and are indicated by *open circles*.

peak levels around the same time, adiponectin plateaued during the day, in contrast to cortisol, and reached its nadir 2 h after cortisol in the early morning hours.

Comparison of all four time series showed that serum adiponectin and sOB-R reached maximum levels in the late morning at approximately the same time with cortisol, and 4–5 h after the minimum leptin levels were observed. Adiponectin and sOB-R reached minimal levels early in the

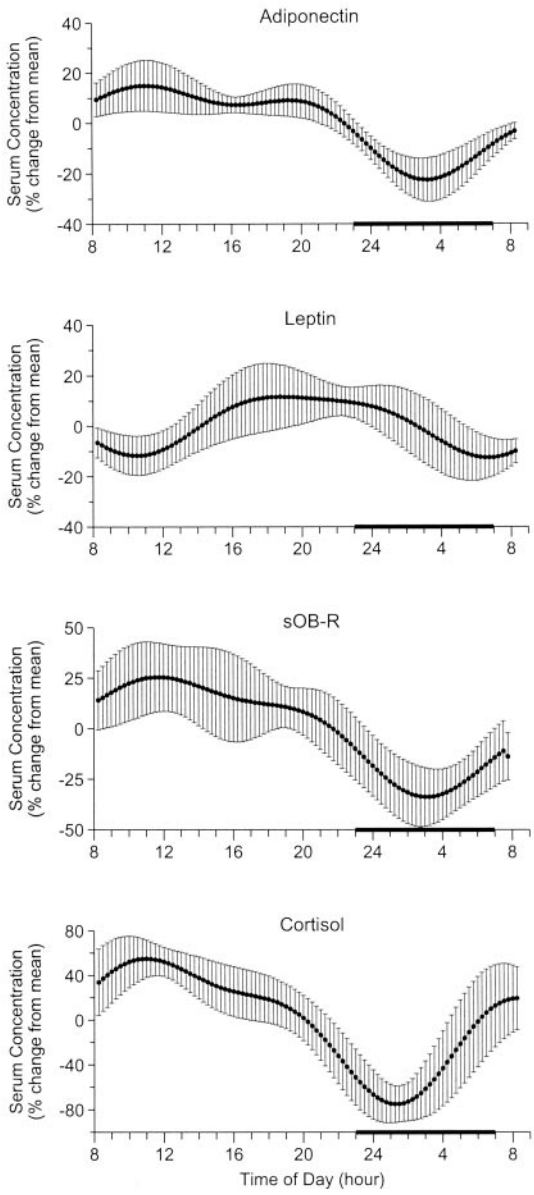

FIG. 2. Mean percentage changes of serum adiponectin, leptin, sOB-R, and cortisol levels over a 24-h period beginning at 0830 h. Hormone levels for each subject were first low-pass filtered over a frequency range of 0–0.1 cycles/h to extract the low-frequency components, expressed as the percentage change from their 24-h mean value, and averaged over all six subjects. *Error bars* represent the SD values of the average levels. The *solid horizontal line segment* indicates sleeping hours during the night (2300 h-0700 h).

morning, about 2 h after minimum cortisol levels and about 8 h after maximum leptin levels were observed.

Cross-correlation analysis. Table 2A presents results of the cross-correlation analysis. Maximum values of the mean cross-correlation coefficients were observed when the long-term sOB-R fluctuation led adiponectin by 30 min in average, when adiponectin led leptin by 3 h and 15 min, and when sOB-R led leptin by 2 h and 45 min. Maximum cross-correlation coefficients were also observed when cortisol led adiponectin by 1 h and when cortisol led sOB-R by 45 min.

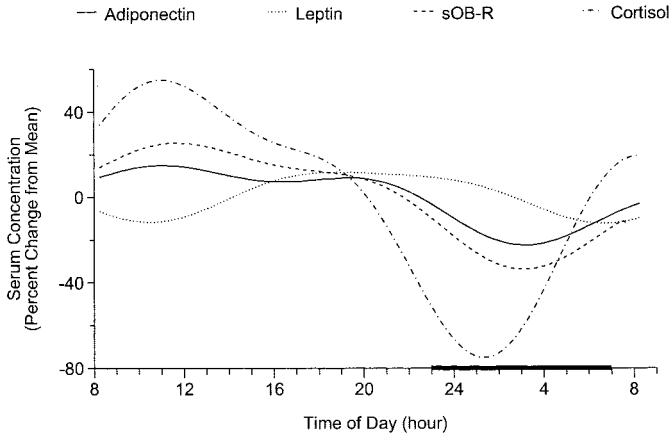

FIG. 3. Mean percentage changes of serum adiponectin, leptin, sOB-R, and cortisol levels shown in Fig. 2 are plotted together to facilitate comparison. The *solid horizontal line segment* indicates sleeping hours during the night (2300 h-0700 h).

Finally, the maximal cross-correlation coefficient between cortisol and leptin appeared when cortisol led leptin by 5 h and 30 min. sOB-R correlated most strongly with adiponectin (0.60), although the highest correlation was observed between adiponectin and cortisol (0.71). The lag periods calculated by cross-correlation analysis were consistent with the lag times of the trough levels during the night between different hormones (Fig. 3). Of note, however, the cross-correlation coefficient calculated the effect of all data points. Therefore, it was not completely determined by the trough level of the time series, although the trough level of the hormone had a significant contribution.

Table 2B summarizes Spearman's correlation coefficients between pairs of the four analytes. Adiponectin levels correlated strongly and positively with sOB-R ($r = 0.76$; $P < 0.001$) and cortisol ($r = 0.69$; $P < 0.001$), but not with leptin levels ($r = -0.18$; $P = 0.08$). Cortisol and sOB-R levels also showed significant correlation ($r = 0.63$; $P < 0.001$).

Ultradian variability analysis

Adiponectin and sOB-R profiles of all six subjects showed significant pulsatility for the 15-min sampling frequency. Cortisol and leptin profiles analyzed with the same Cluster7 parameters as those used for adiponectin and sOB-R showed pulses with a 24-h frequency in the low range of values reported previously (1, 21, 22). For the surrogate data, the average number of peaks selected by the Cluster algorithm was significantly lower than the number of peaks detected in the original adiponectin time series (2.8 ± 1.2 *vs.* 10.0 ± 2.0; $P < 0.001$). This result indicates that the majority of peaks detected in the actual hormone time series are most likely not caused by random noise. Table 3 summarizes mean pulse characteristics of the 24-h serum adiponectin, sOB-R, leptin, and cortisol profiles.

Discussion

We demonstrate for the first time that serum adiponectin exhibits a diurnal variation with a significant decline at night, reaching a nadir in the early morning. Diurnal profiles of

TABLE 1. Diurnal variation parameters of serum adiponectin, sOB-R, leptin, and cortisol levels

Parameter	Adiponectin (μg/ml)	sOB-R (ng/ml)	Leptin (ng/ml)	Cortisol (μg/dl)
Mean 24-h concentration	4.6 ± 0.3 (3.7–5.7)	14.0 ± 1.6 (9.3–19.3)	3.5 ± 1.3 (1.4–9.4)	7.4 ± 0.4 (5.7–8.9)
Mean peak increase (% 24-h mean)	14.9 ± 3.7	25.4 ± 6.4	11.6 ± 4.8	54.9 ± 5.9
Peak increase range (% 24-h mean)	12.3–30.1	21.1–47.0	13.4–29.9	49.2–79.3
Time at peak (h)	1100 (1000–2000)	1145 (1045–2100)	1845 (1600–0145)	1100 (0715–1315)
Mean nadir decrease (% 24-h mean)	22.6 ± 3.1	33.9 ± 5.4	12.5 ± 2.8	75.1 ± 5.7
Nadir decrease range (% 24-h mean)	14.9–35.1	25.9–52.3	10.3–25.1	60.4–98.0
Time at nadir (h)	0315 (0215–0330)	0300 (0215–0615)	0645 (0515–1530)	0115 (0015–0330)
Maximal amplitude (% 24-h mean)	37.5 ± 6.8	59.3 ± 11.7	24.1 ± 7.6	130 ± 11.5

Values are expressed as mean ± SEM, and the range is presented in *parentheses.*

TABLE 2A. Maximum cross-correlation coefficients (Coeff) and temporal relationships between pairs of serum adiponectin, sOB-R, leptin, and cortisol diurnal profiles

	Adiponectin (μg/ml)		sOB-R (ng/ml)		Leptin (ng/ml)	
	Coeff	Lag period	Coeff	Lag period	Coeff	Lag period
sOB-R (ng/ml)	0.60	30 min				
Leptin (ng/ml)	0.46	−195 min	0.47	−165 min		
Cortisol (μg/dl)	0.71	60 min	0.42	45 min	0.35	330 min

Positive lag period means that changes of the analyte to the left appear earlier in time. Negative lag period means that changes of the analyte to the left appear later in time. Calculations were performed after low-pass filtering of data points (see text).

TABLE 2B. Pearson correlation coefficients (Coeff) between pairs of serum adiponectin, sOB-R, leptin, and cortisol diurnal profiles

	Adiponectin (μg/ml)		sOB-R (ng/ml)		Leptin (ng/ml)	
	Coeff	*P*	Coeff	*P*	Coeff	*P*
sOB-R (ng/ml)	0.76	<0.001				
Leptin (ng/ml)	−0.18	0.08	−0.26	0.01		
Cortisol (μg/dl)	0.69	<0.001	0.63	<0.001	−0.46	<0.001

TABLE 3. Pulse parameters of 24-h serum adiponectin, sOB-R, leptin, and cortisol levels

Parameter	Adiponectin	sOB-R	Leptin	Cortisol
Pulse frequency/24 h	10.0 ± 2.0	11.4 ± 5.4	5.6 ± 2.5	12.8 ± 1.9
Pulse duration (min)	88.1 ± 69.4	84.4 ± 70.7	152.1 ± 113.6	68.9 ± 41.9
Pulse height	5.4 ± 1.0 μg/ml	17.7 ± 6.6 ng/ml	4.6 ± 0.6 ng/ml	9.5 ± 5.7 μg/dl
Pulse height (% increase)	156.0 ± 57.9	215.3 ± 92.3	172.8 ± 98.6	264 ± 230.8
Interpeak interval (min)	136.5 ± 88.2	115.5 ± 72.1	242.6 ± 146.7	102.7 ± 42.5
Interpeak concentration	4.1 ± 1.0 μg/ml	10.3 ± 3.4 ng/ml	3.2 ± 0.9 ng/ml	5.2 ± 3.0 μg/dl

Values are expressed as mean ± SEM. Data analysis was done using the Cluster7 program (25).

leptin (20–22) and cortisol (1) were similar to those reported previously. Furthermore, the 24-h variations of serum adiponectin and sOB-R (23) were nearly identical and followed those of cortisol after a lag period, but were out-of-phase with leptin diurnal rhythms. We also report for the first time that circulating adiponectin and sOB-R show evidence for an ultradian pulsatility.

The similar 24-h profiles of adiponectin and sOB-R suggest that these two proteins might be under the influence of common regulatory factors and require further investigation. We also found similar but not overlapping diurnal variations of serum adiponectin and cortisol levels. Although the two hormones reached their maximal values around the same time in the morning, a lag period was observed later during the day and at night, with adiponectin reaching a nadir approximately 2 h after cortisol. Because adiponectin increases whereas cortisol decreases insulin sensitivity, one might hypothesize that the nocturnal cortisol decline indirectly determines compensatory adiponectin changes that would tend to keep the degree of insulin resistance stable. Our data do not provide evidence for an immediate direct effect of cortisol on adiponectin production and secretion *in vivo* in humans, however. Previous *in vitro* studies have shown that glucocorticoid exposure for 16–24 h inhibits adiponectin expression in 3T3-L1 and human mature adipocytes (26, 27).

Other regulatory factors could also potentially influence serum adiponectin levels. Adrenergic tone, which decreases early during the sleep period and then increases in the morning hours (28), has a direct inhibitory effect on adiponectin expression and secretion *in vitro* (29, 30). In addition, the fact that adiponectin starts to decrease before the sleep period in our study may indicate that the observed adiponectin fluctuations are probably not directly related to the sleep-wake cycle. Whether adiponectin variations are associated with sleep-wake cycles or adrenergic tone remains to be conclusively shown by further studies. We also found that, similar to a previous study (9), serum adiponectin concentrations are not noticeably influenced by meals, suggesting that, *in vivo*, insulin does not acutely regulate adiponectin levels in humans. Insulin exposure immediately alters basal adiponectin secretion from 3T3-L1 adipocytes *in vitro* (5, 31), and the lack

of insulin action in fat-specific insulin receptor knockout mice results in increased adiponectin expression and serum adiponectin levels *in vivo* (32). However, the effect of long-term insulin exposure on adiponectin mRNA expression remains controversial (26, 27). Future studies are needed to fully elucidate the potential effect of insulin on adiponectin levels *in vivo* in humans.

In conclusion, we report evidence that circulating adiponectin levels have a diurnal variation in healthy normal-weight male human subjects. Furthermore, serum adiponectin and sOB-R levels change in the same direction and at the same time and lag behind cortisol variations by a few hours, but are out-of-phase with leptin diurnal rhythms. Although the observed temporal correlations do not prove the existence of a causal relationship, they raise the possibility of significant regulation of one molecule by the other or by a yet unidentified common factor, and thus deserve further investigation. Further investigation is also needed to define whether the apparent ultradian patterns of serum adiponectin and sOB-R are related to each other and whether they are required for biological effects. Finally, the possible influence of gender and age, as well as effects of physiological changes and disease states on adiponectin and other hormones interacting with it warrant further study.

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