

Regulation of Circulating Soluble Leptin Receptor Levels By Gender, Adiposity, Sex Steroids, and Leptin

Observational and Interventional Studies in Humans

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Leptin is an adipocyte-secreted hormone important in energy homeostasis and diverse physiological processes. A circulating soluble form of the leptin receptor [soluble leptin receptor (sOB-R)] is the main leptin-binding protein and determinant of free leptin index (FLI), the presumed biologically active form of leptin. We performed observational and interventional studies to elucidate the regulation of sOB-R and FLI in humans. In a cross-sectional study ($n = 118$), leptin, gender, and adiposity were significant determinants of sOB-R. By multivariate analysis, estradiol (E2) and testosterone predict sOB-R, whereas insulin predicts leptin and FLI. In a frequent-sampling study ($n = 6$), sOB-R followed a significant circadian rhythm inverse to that of leptin, suggesting that leptin's biological activity may have an even more pronounced diurnal variation than originally thought. A 72-h fast in eight men decreased leptin levels by 80% and increased lymphocyte expression of leptin receptor mRNA and serum sOB-R levels by 100%. Physiological and pharmacological doses of recombinant-methionyl human leptin (rhLeptin) administered to fasted men prevented the fasting-induced increase of sOB-R levels, and pharmacological doses resulted in a decrease in sOB-R levels. These studies provide evidence that sOB-R is regulated by gender, adiposity, hormones, and rhLeptin administration. This may have important implications for the biological activity of leptin in disease states associated with abnormal leptin levels (e.g., obesity and anorexia nervosa). *Diabetes* 51: 2105–2112, 2002

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%FM, percent fat mass; BIDMC, Beth Israel Deaconess Medical Center; CSF, cerebrospinal fluid; E2, estradiol; ELISA, enzyme-linked immunosorbent assay; FFM, fat-free mass; FLI, free leptin index; FT, free testosterone; GCRC, General Clinical Research Center; LIFA, ligand-immunofunctional assay; rhLeptin, recombinant-methionyl human leptin; RIA, radioimmunoassay; sOB-R, soluble leptin receptor.

Leptin, the protein product of the *ob* gene, is produced by adipose tissue and is secreted into circulation. It acts mainly in the hypothalamus but also in other tissues by binding to specific leptin receptors, which belong to the cytokine receptor family (1,2). Multiple isoforms of the leptin receptor are generated through alternative splicing of the leptin receptor gene, including a long isoform expressed primarily in the hypothalamus and several short isoforms with a much wider tissue distribution (3). A soluble form of the leptin receptor [soluble leptin receptor (sOB-R)], consisting of only the extracellular region (4), binds leptin with an affinity similar to that of membrane-bound receptors (5) and represents the main leptin binding activity in serum (6).

Recent work in rodent models has demonstrated that the sOB-R modulates serum leptin levels by delaying its clearance and determines the amount of free versus bound leptin in serum (7). It has also been proposed that free leptin, the form present in cerebrospinal fluid (CSF), is the biologically active form of leptin (8). These data suggest that sOB-R plays an important role in the pathophysiology of energy homeostasis in rodents (7) and humans (9). Thus, understanding the regulation of circulating sOB-R and free leptin in humans may have important physiological and therapeutic implications for human obesity and eating disorders.

In obese subjects, a significantly higher percentage of leptin circulates in the free form compared with lean subjects (10,11), and the amount of free leptin increases with BMI, suggesting that leptin-binding proteins are saturated in states of increased adiposity (12). Short-term fasting results in an acute and marked decline in total leptin levels (13) as well as free leptin (9). Adiposity also affects the response to short-term fasting (24 h or less), with a blunted decline of free (10), bound, and total leptin levels in obese women compared with lean women (9). However, previous studies have either not measured sOB-R directly (10,12) or have evaluated only the effect of sex, adiposity, and short-term fasting on sOB-R levels in a limited number of subjects (9,11). In addition, the role of hormonal factors in regulating sOB-R and free leptin and whether sOB-R follows a circadian rhythm remains largely unknown. Finally, the role of endogenous or exogenously administered leptin in regulating sOB-R has not yet been

studied directly. Because most hormone systems are based on a feedback loop to permit precise regulation of active hormone levels, the recently reported regulation of serum leptin levels by sOB-R in rodents (7) raises the question of whether leptin may in turn regulate sOB-R.

To further elucidate the regulation of leptin, sOB-R, and free leptin index (FLI), we performed observational and interventional studies, including a prolonged 72-h fasting study on sOB-R regulation in eight lean men and a leptin administration study in five lean men who were given low (physiological) and high (pharmacological) doses of recombinant human leptin after fasting for 24 and 48 h, respectively.

RESEARCH DESIGN AND METHODS

Study designs

A. Cross-sectional study. A total of 120 consecutively enrolled Greek students participated in this study. Blood samples were obtained after an overnight fast in all but 2 subjects, leaving 118 subjects (62 women and 56 men, mean age 17.6 ± 0.16 years, mean BMI 22.3 ± 0.33 kg/m²) for subsequent analysis. Waist, hip, and arm circumferences were measured to a precision of 0.1 cm, and the waist-to-hip ratio was calculated. Triceps, biceps, subscapular, and suprailiac skinfolds were measured twice by one observer on the right side of the body to a precision of 0.2 mm, and the average of the two measurements was used. Body composition was assessed by bioelectrical impedance analysis, and fat-free mass (FFM) and percent fat mass (%FM) were calculated as previously described (14). Subjects completed a self-administered questionnaire on demographic and anthropometric characteristics, general health status, daily exercise, duration and intensity of smoking, and alcohol drinking habits.

B. Pulsatility study. Six lean healthy men (mean age 20.3 ± 0.6 years and mean BMI 22.8 ± 0.9 kg/m²) were admitted to the General Clinical Research Center (GCRC) at Beth Israel Deaconess Medical Center (BIDMC) and placed on an isocaloric diet designed to keep weight stable. On the 3rd day starting at 8:30 A.M., blood samples for leptin and sOB-R levels were drawn every 15 min through an intravenous line for 24 h. Subjects were exposed to light from 7:00 A.M. to 11:00 P.M. and were in the dark from 11:00 P.M. to 7:00 A.M. During the night, blood samples were drawn outside the subject's room to avoid disturbing their sleep.

C. Interventional fasting study. Eight lean healthy men (mean age 23.3 ± 1.2 years and mean BMI 23.7 ± 0.6 kg/m²) were admitted to the GCRC and fasted for 3 days, drinking only calorie-free liquids and taking NaCl (500 mg), KCl (40 meq), and a standard multivitamin with minerals once per day. Blood samples for leptin and sOB-R were obtained on the 1st day after an overnight fast and on the 4th day after an additional 72 h of fasting. Body composition was assessed by dual energy X-ray absorptiometry using a Hologic 2000 scanner (Hologic, Waltham, MA) at the beginning and end of the fast.

D. Interventional leptin administration study. Five lean healthy men (mean age 22.2 ± 0.9 years and mean BMI 22.2 ± 0.4 kg/m²) were admitted to the GCRC under a similar fasting protocol and at 8:00 A.M. received recombinant-methionyl human leptin (rhLeptin) as a subcutaneous injection at a dose of 0.01 mg/kg (low dose). The same subjects were admitted again 2–3 weeks later under similar conditions except that they were given a higher pharmacological dose of leptin (0.1 mg/kg) at 8:00 A.M. for 2 consecutive days. Serum leptin and sOB-R levels were measured at time 0 before each dose of rhLeptin administered and at times +1, 2, 3, 4, 5, 6, 8, 10, 12, and 18 h after the dose. Leptin receptor mRNA expression in peripheral mononuclear cells was determined in blood samples drawn at 8:00 A.M., just before the rhLeptin dose on 3 consecutive days of fasting.

All study protocols were approved by the Institutional Review Board of the BIDMC, and the cross-sectional study was also approved by the Ethics Committee of Harokopio University. Clinical quality R-metHuLeptin was supplied by Amgen (Thousand Oaks, CA) and administered under an Investigational New Drug application submitted to the Food and Drug Administration. All subjects (and their parents for the cross-sectional study) gave informed consent to participate in these studies.

Hormone measurements. Serum leptin levels (ng/ml) were measured using radioimmunoassay (RIA) (Linco Research, St Louis, MO). Serum estradiol (E2) (pg/ml) and free testosterone (FT) (pg/ml) levels (DPC, Los Angeles, CA) and serum cortisol (μ g/dl) and insulin (μ IU/ml) levels (DSL, Webster, TX) were measured using RIA. Serum IGF-1 (ng/ml) and IGFBP-3 (ng/ml) levels were measured using immunoradiometric assay (DSL).

Serum sOB-R levels (U/ml) for all four studies were measured using a

commercially available enzyme-linked immunosorbent assay (ELISA) (BioVendor Laboratory Medicine, Brno, Czech Republic), with a sensitivity of 0.4 μ l (or 0.8 ng/ml) and an intra-assay CV of 3.9–5.6% for 16.9–69.5 units/ml. For the fasting and leptin administration studies, sOB-R levels were also determined by a newly developed ligand-immunofunctional assay (LIFA), which has been recently extensively described (6), and was calibrated using the rsOB-R preparation of Liu et al. (5). A standard curve of this rsOB-R demonstrated a paralleled course with native sOB-R. The lowest detectable sOB-R concentration in the assay was <2 ng/ml. Intra- and interassay CVs for two control samples were $<11.7\%$ ($n = 10$). Results of dilution and sOB-R spiking experiments showed a recovery of 97.2 ± 11.8 and $92 \pm 8.6\%$ (mean \pm SD; $n = 10$), respectively. The assay was insensitive to leptin interference.

Samples for the cross-sectional study were run in a single assay, as were samples for the interventional fasting study. For the pulsatility and interventional leptin studies, samples for each subject were run in a single assay on consecutive 96-well plates to minimize variability within subjects. Immunoreactivity of sOB-R measured by ELISA was free from leptin interference in the range of leptin values measured in the cross-sectional and pulsatility studies, and thus the data reported for these studies are those obtained using the ELISA method. In contrast, significant interference by leptin is possible in the range of leptin levels measured in the interventional studies. Thus, data using the LIFA method, which is free of leptin interference, are presented for these studies. Data obtained using the ELISA method resulted in similar sOB-R changes, but the absolute sOB-R levels are relatively lower (see below).

The mRNA-expression of the shortest membrane-bound leptin receptor splice variant (OBR_{sh}, huB219.3, or 6.4 short splice variant; GenBank Accession no. U66495) was quantified in circulating human mononuclear cells by RT-PCR as previously described (15). Blood samples (10 ml) were collected from five healthy men (mean BMI 22.2 ± 0.4 kg/m²) at 8:00 A.M. on 3 consecutive days of fasting. Isolation of total RNA and the RT-PCR reaction (using 1 μ g total RNA) were performed as previously described (15).

Statistical analysis. Descriptive characteristics of the group variables are expressed as mean values \pm SE. The OBR_{sh} mRNA expression data are indicated as the mean \pm SE (from arbitrary PhosphorImager units) normalized to the β -actin values to adjust for the potential variations in RNA input. Statistical analysis was performed using the Statview program (Abacus) and SPSS version 8 (Texas Instruments, Chicago, IL). Statistical significance was assessed by standard Student's *t* tests or paired two-tailed *t* test as well as ANOVA with post hoc tests. Values were considered to be significantly different at the $P \leq 0.05$ level (two-tailed).

Spearman's correlation coefficients were calculated to assess univariate associations between leptin, sOB-R, and the hormonal variables of the study. Simple and multiple regression analyses were performed to test for associations between dependent variables (leptin, sOB-R, and free leptin) and independent variables (including the hormones) after logarithmic transformation of non-normally distributed variables. In these analyses, leptin values were log transformed to normalize their distribution. This study had $>80\%$ power to demonstrate statistically significant associations at the conventional $\alpha = 0.05$ level if the underlying associations were strong or moderate, i.e., $r > 0.22$. To test for diurnal variation in serum concentrations of leptin and sOB-R, a Bayesian nonlinear hierarchical model (WinBUGS) was used, as previously described (16–18).

RESULTS

Table 1 shows the descriptive statistics for the men and women in the cross-sectional study.

Compared with men, women had significantly lower BMI ($P = 0.001$), higher sum of four skinfolds ($P = 0.002$), lower FFM values ($P < 0.001$), higher %FM ($P < 0.001$), higher leptin levels ($P < 0.001$), and lower sOB-R levels ($P < 0.01$). Thus, sex is a major predictor of sOB-R in healthy adults by bivariate analysis. We then explored hormonal and demographic predictors of sOB-R and FLI by examining univariate correlations between leptin, sOB-R, and the other hormones in this study population. Leptin was significantly and positively associated with %FM ($r = 0.78$, $P < 0.01$), whereas sOB-R was negatively associated ($r = -0.36$, $P < 0.01$), in bivariate analysis as well as when adjusted for sex ($r = -0.26$, $P = 0.02$). We found a statistically significant inverse and curvilinear correlation between leptin and sOB-R ($r = -0.34$, $P < 0.01$) as well as the expected significant correlations

TABLE 1
Cross-sectional study: descriptive characteristics of the study participants ($n = 118$)

Variable	Women ($n = 62$)			Men ($n = 56$)		
	Mean	SD	Range	Mean	SD	Range
Age (years)	17.5	1.6	14–26	17.8	1.8	15–25
BMI (kg/m^2)	21.3	3.1	16.2–30.1	23.4*	3.8	15.4–35.9
Sum of skinfolds (mm)	48.7	17.4	23.0–95.0	38.6†	18.4	17.0–106.0
FFM (kg)	39.5	3.8	28.4–47.9	59.6*	6.1	45.4–72.5
FM (kg)	15.5	4.8	7.9–29.1	13.3	7.3	3.0–35.0
FM (%)	27.6	5.0	17.6–38.4	17.3*	6.9	6.0–32.6
Leptin (ng/ml)	9.7	6.0	2.1–30.0	3.2†	2.6	1.2–12.8
sOB-R (ng/ml)	27.2	29	4.4–108.4	50.2*	39.8	4.4–164.2

* $P \leq 0.001$ vs. women by unpaired t test; † $P < 0.01$ vs. women by unpaired t test.

between leptin and insulin ($r = 0.36$, $P < 0.01$), cortisol ($r = -0.19$, $P < 0.05$), FT ($r = -0.49$, $P < 0.01$), and E2 ($r = 0.48$, $P < 0.01$) (Table 2). In contrast, only cortisol was significantly correlated with sOB-R ($r = 0.27$, $P < 0.01$).

Given that the study outcomes of interest are interrelated, we performed multivariate regression analysis adjusting for potential confounding variables. Table 3 presents a summary of the bivariate and multivariate regression analysis for leptin, sOB-R, and FLI in relation to the study hormones, adjusted for successively introduced covariates.

The only hormones significantly associated with sOB-R after adjusting for sex, age, alcohol, exercise, smoking, mutually for the study hormones, and fat mass in kilograms were FT ($r = -0.50$, $P < 0.05$) and E2 ($r = 0.23$, $P < 0.05$). Only insulin showed a significant and independent correlation with leptin and FLI ($r = 0.23$, $P < 0.001$ for both) after adjustment for the same variables. In summary, our observational data suggest that adiposity, gender, and sex steroids are significant determinants of sOB-R, whereas gender, adiposity, sOB-R, and insulin influence FLI.

Using a nonlinear hierarchical model, we then explored whether, similar to leptin (19,20), sOB-R exhibits a circadian rhythm. Figure 1 shows the average leptin and sOB-R concentrations for six lean men over 24 h. Superimposed over these points, we traced out the posterior population mean predicted values at each time based on the model.

Table 4 shows the posterior mean and 95% credible intervals for the population amplitude, baseline, and phase parameters for leptin and sOB-R as well as estimates of the relative amplitude, a measure of oscillation that adjusts for varying baselines.

For both leptin and sOB-R, estimates of the population amplitudes are significantly nonzero, indicating that there is diurnal variation of the levels of both molecules. Leptin levels reach their maximal values in the late evening based on the posterior mean, whereas sOB-R levels reach their maximal values in the early afternoon. The phases between leptin and sOB-R are significantly different from one another. Further, the data may suggest an inverse relation between the two, with the peak sOB-R levels occurring shortly after the lowest leptin levels (between 2:47 and 7:22 h, 95% CI).

We then performed interventional studies to determine whether leptin directly regulates sOB-R by varying leptin levels. Two experiments were performed, including a prolonged fast causing a very low leptin state and leptin administration resulting in high leptin levels. Figure 2 shows the effect of a 72-h fast on leptin and sOB-R levels in eight lean men.

Leptin levels decreased to 20% of baseline (2.2 ± 0.64 to 0.31 ± 0.10 ng/ml, $P = 0.01$), whereas sOB-R levels increased significantly by >100% (21.7 ± 1.9 to 49.2 ± 2.8 ng/ml, $P < 0.001$) in response to a prolonged fast. Similarly, OBR_{sh}-mRNA expression in human peripheral mononuclear cells increased from day 1 to day 2 of fasting from 8.77 ± 3.99 to 22.88 ± 7.10 ADU (arbitrary density units) ($P = 0.12$). On day 3, OBR_{sh}-mRNA expression was upregulated to 29.38 ± 6.56 ADU compared with baseline levels ($P = 0.03$). Weight declined after the 72-h fast (73.4 ± 2.7 vs. 71.4 ± 2.7 , $P = 0.001$). FM also declined after the fast (12.3 ± 2.1 vs. 11.7 ± 2.2 kg, $P = 0.02$), but the magnitude of the change was small. Because FFM decreased as well after the fast (61.2 ± 2.6 vs. 59.7 ± 2.5 , $P = 0.003$), there was no significant difference in %FM (17.0 ± 2.4 vs. 16.8 ± 2.6).

TABLE 2
Cross-sectional study: Spearman's correlation coefficients between leptin soluble leptin-receptor sOB-R and the other measured hormones (FT, E2, IGF-1, and IGFBP-3)

	Leptin	sOB-R	Insulin	Cortisol	FT	E2	IGF-1
sOB-R	-0.34*						
Insulin	0.36*	-0.11					
Cortisol	-0.19†	0.27*	0.20†				
FT	-0.49*	0.08	0.13	0.30*			
E2	0.48*	-0.09	0.04	-0.14	0.50*		
IGF-1	-0.04	-0.06	0.16	0.23†	0.07	-0.14	
IGFBP-3	-0.04	-0.09	-0.04	0.03	-0.01	0.03	0.52*

*Correlation is significant at the 0.01 level (two-tailed); †correlation is significant at the 0.05 level (two-tailed).

TABLE 3
Univariate and multivariate regression analysis for leptin, sOB-R, and FLI in relation to study variables for the cross-sectional study

	r ₁	r ₂	r ₃	r ₄	r ₅
Leptin					
Insulin	0.41*	0.42*	0.41*	0.44*	0.23*
Cortisol	-0.20†	-0.02	0.02	-0.05	0.00
FT	-0.50*	0.11	0.10	0.02	-0.02
E2	0.30*	0.00	-0.00	-0.01	0.04
IGF-1	-0.08	0.02	0.03	-0.01	-0.08
IGFBP-3	-0.01	-0.03	-0.03	-0.02	-0.07
sOB-R					
Insulin	-0.10	-0.11	-0.11	-0.10	-0.02
Cortisol	0.29*	0.21†	0.16	0.12	0.10
FT	0.22†	-0.44†	-0.42†	-0.51†	-0.50†
E2	0.02	0.25†	0.26†	0.25†	0.23†
IGF-1	0.03	-0.02	0.03	0.07	0.10
IGFBP-3	-0.14	-0.13	-0.06	-0.10	-0.13
FLI					
Insulin	0.36*	0.39*	0.40*	0.42*	0.23*
Cortisol	-0.13	0.00	0.05	-0.03	0.01
FT	-0.46*	0.00	0.00	-0.08	-0.07
E2	0.32*	0.07	0.05	0.03	-0.06
IGF-1	-0.02	0.06	0.03	-0.01	-0.07
IGFBP-3	-0.04	-0.04	-0.03	-0.02	-0.06

r₁, Univariate standardized regression coefficient; r₂, multivariate standardized regression coefficient adjusted for sex only; r₃, multivariate standardized regression coefficient adjusted for sex, age, alcohol, exercise, and smoking; r₄, multivariate standardized regression coefficient adjusted for sex, age, alcohol, exercise, and smoking, and mutually for the study hormones; r₅, multivariate standardized regression coefficient adjusted for sex, age, alcohol, exercise, and smoking, mutually for the study hormones, and fat mass in kilograms. *0.001 < P < 0.01; †0.01 < P < 0.05.

Thus, fasting (a low-leptin state) results in a significant rise in sOB-R levels, suggesting that leptin per se may regulate serum levels of its own binding protein. To further address this notion, we then examined the effect of a high-leptin state on sOB-R levels by administering low physiological (0.01 mg/kg) and high pharmacological (0.1 mg/kg) doses of rhLeptin to five lean men. Figure 3 shows the effect of 0.01 mg/kg rhLeptin on serum leptin and sOB-R levels.

Serum leptin levels increased from 2.7 ± 0.2 ng/ml to a peak of 10.3 ± 1.0 ng/ml at 2 h in response to rhLeptin before gradually declining to baseline levels (2.4 ± 0.3 ng/ml) by 10 h. However, sOB-R levels did not increase significantly, as would be expected based on fasting only, but tended to decrease when leptin levels peaked (Fig. 3).

To evaluate the effect of high leptin levels on sOB-R, the same subjects were admitted a few weeks later and received rhLeptin at a 10-fold higher dose (0.1 mg/kg) at 8:00 A.M. for 2 consecutive days (Fig. 4).

On the first day, baseline leptin levels were similar to those in the low-dose study (3.4 ± 0.20 ng/ml) and peaked at 57.1 ± 6.96 ng/ml 2 h after the rhLeptin dose before declining back to baseline levels (2.1 ± 0.25 ng/ml) by 18 h. sOB-R levels, on the other hand, decreased significantly to a nadir at 3 h after the rhLeptin dose, i.e., shortly after the peak in leptin levels (Fig. 4). The same pharmacological rhLeptin dose was administered again at 8:00 A.M. on the next day with similar results. Baseline serum leptin levels were lower (1.2 ± 0.14 ng/ml) due to the effect of fasting

and reached a peak of 45.2 ± 6.14 ng/ml at 2 h. SOB-R again failed to increase in response to fasting, whereas leptin levels remained high, but SOB-R tended to drift to higher levels as leptin levels returned to baseline. Thus, with each dose of rhLeptin, sOB-R levels decline acutely and subsequently increase back to baseline in the fasted state, suggesting that leptin regulates circulating levels of its own binding protein. Similar changes in sOB-R levels (with an even more pronounced nadir) were observed when the ELISA method for measuring sOBR was used (data not shown).

DISCUSSION

The leptin receptor is a member of the cytokine receptor family encoded by the diabetes (*db*) gene (21). The product of this gene is alternatively spliced into at least five transcripts, including the long (OBRb), short (OBRa, c, and d), and soluble (OBRe) forms of the leptin receptor. All isoforms share the same extracellular domain, with the shorter isoforms having truncated cytoplasmic domains resulting in reduced signal transduction. OBRe (or sOB-R) circulates in human and rodent plasma and is capable of binding to leptin (22). In pregnant mice, leptin levels increase significantly due to a several-fold increase in leptin binding capacity from placental production of sOB-R, suggesting that this binding protein modulates leptin's availability and thus biological activity (23). Therefore, it has been proposed that sOB-R acts as a binding protein to elevate serum leptin by delaying its clearance and/or by directly competing with membrane receptors for the ligand (7). Leptin, with a molecular weight of only 16 kDa, would be more rapidly cleared or metabolized than leptin bound to a binding protein with a resultant complex of >300 kDa. Moreover, leptin in complex with sOB-R is unable to bind to or activate the leptin receptor because the soluble leptin receptor inhibits leptin binding to the cell membrane (5). This model of sOB-R serving as a binding protein for leptin is supported by recent data in rodent models. Adenovirus-mediated overexpression of the soluble leptin receptor (Ob-Re) without directly affecting leptin expression in Zucker lean rats results in higher total leptin levels caused by higher binding protein levels and has no major effect on food intake or body weight (7).

Although a significant body of work over the past 7 years has focused on leptin, the role and determinants of soluble leptin receptor in humans has remained largely unknown. We explored potential determinants of sOB-R and FLI and found that sex and adiposity are important determinants of sOB-R. Men have significantly higher sOB-R levels than women, and increasing adiposity further decreases sOB-R levels independently of sex. This is in agreement with previous studies showing that the proportion of bound leptin is higher in men versus women and in lean versus obese subjects (10,11,24–26). In addition, the significant negative correlation between leptin and sOB-R is consistent with the notion that lean subjects with lower leptin levels have a higher percentage of leptin present in the bound form (10,11,26). Women, who have higher total leptin levels than men, even after adjusting for BMI (21), have higher free leptin levels than men, suggesting that they may be more resistant to the effects of leptin.

We also found that several hormones are independently

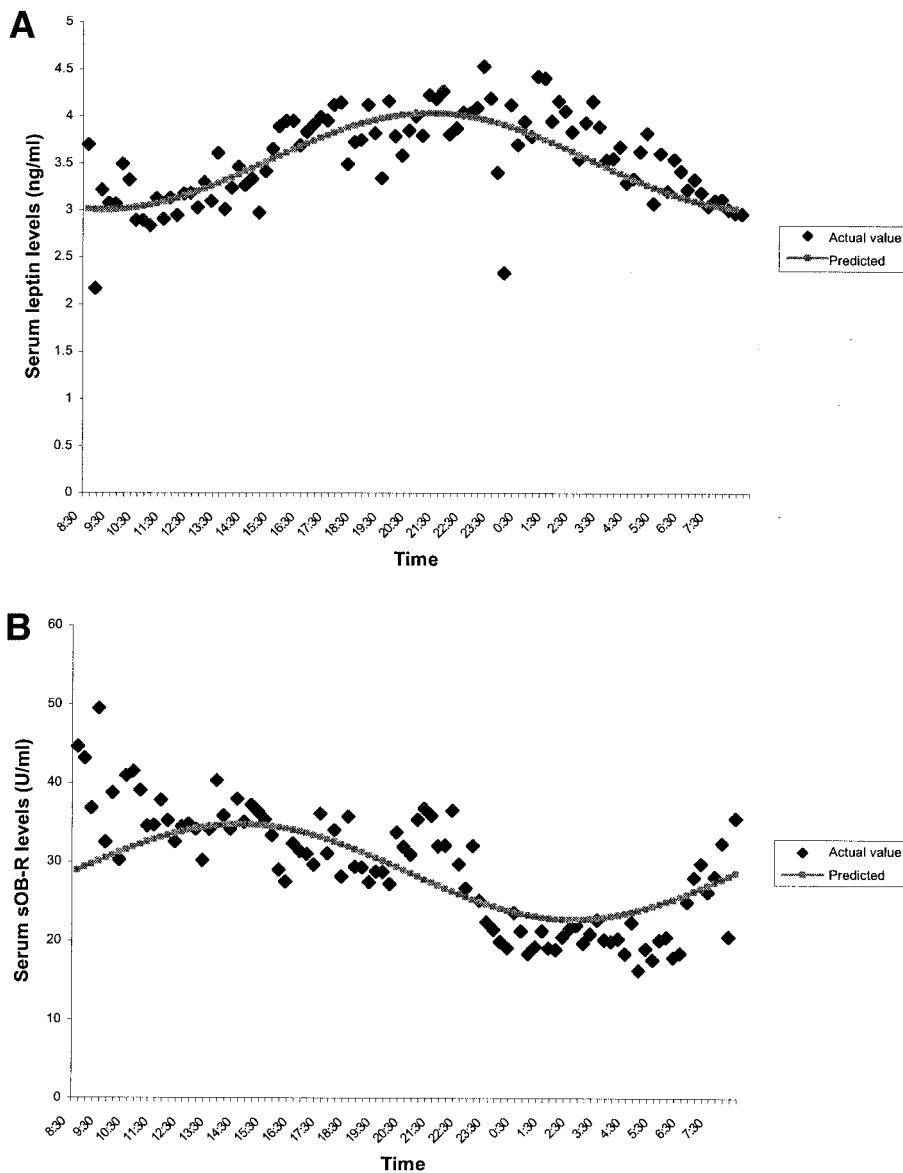


FIG. 1. Average serum leptin (A) and serum soluble leptin (B) receptor (sOB-R) levels over a 24-h period ($n = 6$ subjects). Superimposed lines trace out the posterior population mean model fit.

associated with sOB-R and FLI. Whereas insulin was significantly correlated with leptin and FLI after adjusting for potential cofounders in multivariate analysis models, E2 and testosterone were the main independent determinants of sOB-R. Our findings on the hormonal predictors of sOB-R are novel, and our data on FLI are consistent with a recent observational study that found a significant positive correlation between FLI and insulin in multivariate regression analysis (24). Further support of these observational data are provided by an interventional study using hyperinsulinemic-euglycemic clamps showing that

insulin increases free leptin in healthy subjects (27). Moreover, the approximate twofold increase of circulating leptin and sOB-R associated with pregnancy (28) is consistent with our data demonstrating a positive correlation with E2 and insulin, both of which are increased in pregnancy.

Another novel finding of this study is that, similar to total leptin levels, sOB-R follows a circadian rhythm with peak levels in the early to mid-afternoon. Interestingly, this pattern is the inverse of that seen in the circadian variation of leptin levels that peak in the late evening and nadir in

TABLE 4
Pulsatility study: posterior population estimates for testing diurnal variation in leptin and sOB-R levels

	Leptin (ng/ml)		Soluble leptin receptor (U/ml)	
	Mean	95% CI	Mean	95% CI
Amplitude (A)	0.56	0.05–1.08	6.26	1.98–10.14
Baseline (B)	3.54	0.28–6.83	27.0	17.74–36.3
Relative Amplitude (A/B)	0.24	0.01–0.74	0.21	0.08–0.44
Maximum	9:02 P.M.	5:50 P.M.–12:09 A.M.	2:24 P.M.	11:49 A.M.–4:24 P.M.
Variance (S^2)	0.12	0.03–0.33	30.48	8.5–79.1

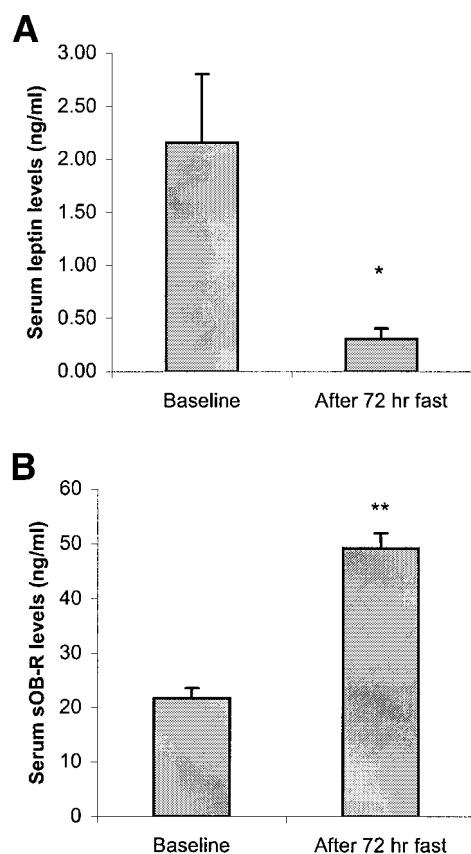


FIG. 2. Effect of 72-h fasting on serum leptin levels (A) and serum soluble leptin receptor (sOB-R) levels (B) ($n = 8$ subjects). Values are expressed as means \pm SE. ** $P < 0.001$, * $P < 0.01$ vs. baseline.

the late morning (19). Although these data suggest that leptin and sOB-R levels are reciprocally regulated, these observational data cannot prove causality. Alternatively, common factors (e.g., light and nutrition) may regulate both. Thus, the biological activity of leptin may have an even more pronounced diurnal variation than that suggested by studying leptin levels alone, with free leptin being highest in the late evening and early morning time periods. This peak in free leptin may play a physiological role by increasing satiety during the nighttime, when

feeding does not usually occur, but further interventional studies are warranted to test these hypotheses.

The above data showing a negative correlation between leptin and sOB-R as well as an out-of-phase (discordant) diurnal rhythm led us to further investigate leptin as a regulator of its own binding protein. With prolonged fasting, leptin levels decline to $\sim 20\%$ of baseline. This very low-leptin state was associated with a significant rise in sOB-R, suggesting that the binding protein may be upregulated in response to the relative lack of leptin. Because relatively short duration of fasting may have no statistically significant effect on sOB-R levels (9), the time course of sOB-R changes in response to a low-leptin state needs to be studied further.

Finally, a major finding of our interventional studies in humans is that leptin appears to reciprocally regulate its own binding protein acutely, because leptin administration in the fasted state prevents the expected significant increase in sOB-R and even results in a decrease in sOB-R, which inversely mirrors the changes in leptin levels. Relatively large changes in serum leptin levels are necessary to observe relatively less pronounced changes in sOB-R.

One can also speculate on the physiological significance of this finding. With fasting, an increase in sOB-R associated with a decrease in total leptin leads to a more pronounced decrease in free leptin. This may be an adaptive response to states of food deprivation because it would be advantageous to restrict the availability of free leptin (with its inhibitory effect on food intake) in states of nutritional deprivation, such as starvation or anorexia nervosa (29). At the other end of the energy homeostasis spectrum is the state of obesity in which higher serum leptin levels reflect increased adipose stores. In this situation, sOB-R may decrease as an adaptive response in order to increase the availability of active leptin, as suggested by the finding of higher FLI with increasing BMI. Previous data seem to support this possibility, as serum free leptin levels correlate well with CSF free leptin levels at the low end of the serum range, but higher serum free leptin levels correspond to only small increments in CSF free leptin levels (25). Thus, human obesity appears to be a state of resistance to free leptin. In addition to decreased

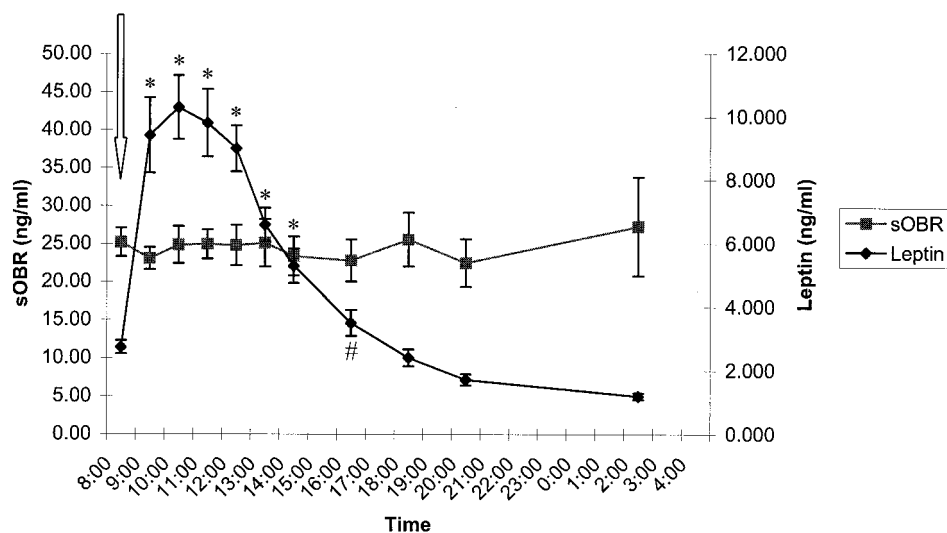


FIG. 3. Average percent change in serum leptin and soluble leptin receptor (sOB-R) levels in response to rhLeptin administration at a low dose (0.01 mg/kg) ($n = 5$ subjects). Arrow indicates rhLeptin administration. Values are expressed as means \pm SE. * $P < 0.01$, # $P < 0.05$ vs. baseline level before rhLeptin dose.

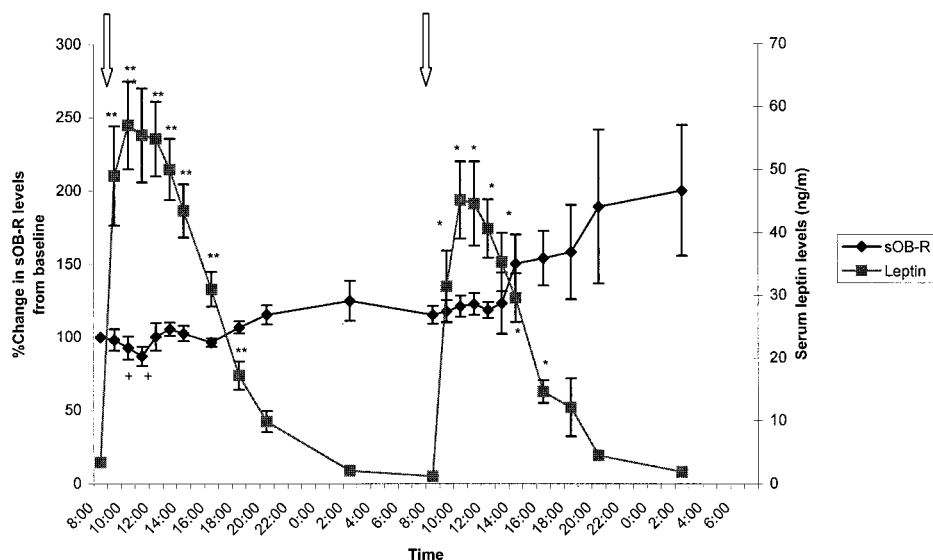


FIG. 4. Average change in serum leptin levels and percent change in soluble leptin receptor (sOB-R) levels in response to rhLeptin administration at a pharmacological dose (0.1 mg/kg) ($n = 5$ subjects). Arrows indicate rhLeptin administration. Values are expressed as means \pm SE. ** $P < 0.001$, * $P < 0.01$ vs. baseline level before rhLeptin dose. + $P < 0.05$ sOB-R levels measured at 1000 and 1100 on day 1 vs. sOB-R levels measured at all time points between 2000 on day 1 and 1200 on day 2, and $0.05 < P < 0.10$ vs. time points 1400, 1600, 0200 of day 2.

transport of leptin into CSF at the level of the blood-brain barrier or receptor/postreceptor defects (21), it is also possible that dysregulation of sOB-R secretion and function may represent another mechanism underlying the development of leptin resistance in a subset of obese subjects (7). Moreover, if levels of the soluble leptin receptor in serum are proportional to the density of cellular leptin receptor levels in the hypothalamus or to the expression of the leptin receptor isoform responsible for the transport of leptin through the blood-brain barrier, measuring sOB-R levels in the serum may help differentiate obese patients with relative leptin resistance (high leptin, low sOB-R levels) from those with relative leptin insufficiency (low leptin, high sOB-R levels), who would be expected to benefit most from exogenous leptin administration, but this remains to be shown by future studies.

Although the exact mechanism by which leptin and its binding protein are reciprocally regulated is currently the focus of ongoing research efforts, the time frame of the changes in sOB-R levels in relation to the changes in leptin levels (within a few hours) suggests that this may occur through posttranslational modifications or secretion of preformed sOB-R stored in secretory vesicles. However, changes in ObR gene expression, as indicated by the OBR_{sh} -mRNA expression data presented herein, could also contribute to the increase of serum sOB-R because sOB-R is the product of ObR cleavage, but the exact mechanism governing the production of sOB-R remains to be determined (7). Soluble leptin receptor is absent in *dbPas/dbPas* mice, which carry a duplication of exon 4 and 5 of leptin receptor DNA, leading to a premature stop codon at amino acid 281 (22). Although most of sOB-R may be produced by proteolytic cleavage of membrane-associated receptors (7), OBRe is also expressed in rodent placenta (23,30).

In many peptide hormone systems, the free form of the hormone is more biologically active than the bound form, and binding proteins function as a buffer to extend peptide hormone half-life (31). Alterations of the soluble leptin receptor in the serum may have significant implications in the pathophysiology and treatment of obesity and eating disorders either directly by regulating free leptin availabil-

ity and/or by serving as a marker of leptin receptor expression in the hypothalamus.

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