Nocturnal Rise of Leptin in Lean, Obese, and Non–Insulin-dependent Diabetes Mellitus Subjects

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Abstract

We studied 24-h profiles of circulating leptin levels using a sensitive and specific RIA in lean controls and obese subjects with or without non–insulin-dependent diabetes mellitus (NIDDM) during normal routine activity. Serum leptin levels were significantly higher in obese (41.7±9.0 ng/ml; $n = 11$) and obese NIDDM (30.8±6.7; $n = 9$) subjects compared with those in lean controls (12.0±4.4, $n = 6$). In all the three groups, serum leptin levels were highest between midnight and early morning hours and lowest around noon to midafternoon. The nocturnal rise in leptin levels was significant when data were analyzed by ANOVA (lean: $F = 3.17$, $P < 0.0001$, $n = 4$; obese: $F = 2.02$, $P < 0.005$, $n = 11$; and obese NIDDM: $F = 4.9$, $P < 0.0001$, $n = 5$). The average circadian amplitude between acrophase and nadir was 75.6% in lean, 51.7%, in obese and 60.7% in obese NIDDM groups, respectively. No significant correlations ($P > 0.05$) were observed between circulating levels of leptin and either insulin or glucose levels in any of the 20 subjects studied for 24-h profiles. The nocturnal rise in leptin observed in the present study resembles those reported for prolactin, thyroid-stimulating hormone, and free fatty acids. We speculate that the nocturnal rise in leptin could have an effect in suppressing appetite during the night while sleeping. (*J. Clin. Invest.* 1996. 97:1344–1347.) Key words: leptin • obesity • NIDDM • circadian rhythms • nocturnal changes

Introduction

Successful isolation of the mouse (ob)[1] gene by positional cloning and the demonstration that obesity in ob/ob mouse is due to mutations in the gene (1) has considerably renewed interest in the etiology and pathophysiology of human obesity. Leptin (the protein product of ob gene) administration in ob/ob mouse results in weight loss by reduction in food intake and increased expenditure (2–5). In contrast, ob gene expression is increased in human obesity (6–9) and in various animal models of obesity (10, 11). Recently, we developed a specific and sensitive RIA of human leptin and demonstrated increased circulating levels of leptin in obese subjects which can be modulated by weight loss (12). It has been postulated that leptin acts as a satiety factor in regulating food intake, possibly through specific receptors in the brain (13, 14). It is feasible that leptin production by adipose tissue is under neuroendocrine control and shows similar secretory characteristics as those of other hormones (15). Therefore, we studied 24-h secretory patterns of leptin in lean, obese, and obese non–insulin-dependent diabetes mellitus (NIDDM) subjects. It appears that leptin is secreted in circadian rhythms with a nocturnal rise over daytime secretion which could be helpful in suppressing appetite while sleeping.

Methods

Human subjects and experimental protocol. A total of 26 human subjects whose clinical and biochemical characteristics are shown in Table I were studied. Obesity was defined as a body-mass index (BMI) > 27.3 for males and 27.8 for females according to the National Institutes of Health Consensus Development Panel (16). Every subject had an oral glucose tolerance test according to the National Diabetes Data Group (17). All the subjects had a normal physical examination and routine laboratory screening (SMA-12, HAA, CBC, HIV, urinalysis, urine drug screen, and HbA1C). The subjects had no other diseases except for obesity, diabetes, or mild hypertension. None of the patients were in an active weight loss program or taking any drug for the treatment of obesity. None of the subjects received thiazides, beta-blockers, psychotropic drugs, or any drug known to alter carbohydrate metabolism.

After an overnight fast, patients reported to the Clinical Research Unit at 7:00 a.m. An indwelling IV catheter was placed. Fasting blood sample at 8:00 a.m. (time 0) was withdrawn, and, thereafter, either 30-min, (after meals), 60-min, or 120-min (during sleep) samples were withdrawn until 7:30 a.m. the next day. After 15-min clotting, serum was separated and stored frozen at −20°C until assayed for insulin and leptin. For glucose estimations, plasma was used. Each subject received breakfast at 8:00 a.m., lunch at 12:00 p.m., dinner at 5:00 p.m., and snack at 8:00 p.m. Each subject was given an isocaloric diet (30k cal/kg/d) comprised of 50% carbohydrates, 35% fat, and 15% protein. The carbohydrate portion of the diet included complex carbohydrates and simple sugars. Total calories calculated for whole-day consumption were distributed as 20% for breakfast, 30% for lunch, 40% for dinner, and 10% for a snack. The patients went to bed between

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1. *Abbreviations used in this paper:* NIDDM, non–insulin-dependent diabetes mellitus; ob, obese; rh, recombinant human.

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Table I. Clinical and Biochemical Characteristics of Patients

Groups	Lean	Obese	Obese NIDDM
n (M/F)	6 (3/3)	11 (3/8)	9 (4/5)
Age (yr)	40.7±1.6 (6)	43.0±2.3 (11)	49.3±2.1(9)
BMI (kg/m^2)	24.3±0.9 (6)	38.8±2.5 (11)	41.5±3.6 (9)
Glucose (mg/dl)	100.4±2.9 (6)	103.3±2.1 (11)	196.1±19.0(9)*
Hemoglobin A1C (%)	7.2±0.1 (6)	7.6±0.2 (11)	11.5±2.2(5)*
Insulin (μU/ml)	14.4±4.2 (6)	25.6±6.7 (11)	30.7±10.5 (9)
Leptin (ng/ml)	12.0±4.4 (6)	41.7±9.0 (11)*	30.8±6.7 (9)*
Cholesterol (mg/dl)	182±16.6 (6)	214.8±11.9 (11)	209±19.5 (5)
Triglyceride (mg/dl)	111.3±11.0 (6)	127.2±11.7 (11)	169.3±58 (5)
HDL-cholesterol (mg/dl)	41.5±2.6 (6)	43.4±4.4 (11)	42.0±7.0 (5)
LDL-cholesterol (mg/dl)	119.0±14.4 (6)	145.9±9.6 (11)	133.0±9.5 (5)

BMI, body-mass index. Data are mean±SE; *$P < 0.05$ compared to lean controls.

10:30 p.m., and midnight when television sets were turned off. All patients signed informed consent before participation in this study. The protocols were approved by the Institutional Review Board at Thomas Jefferson University.

Assays. Serum leptin levels were determined by RIA as previously described (12). Using recombinant human ob protein (rh leptin) synthesized in an *Escherichia coli* expression system and purified to homogeneity (5) as a source of standard, radiolabeled ligand and antibody production. Recombinant human leptin was labeled with ^{125}iodine by the Bolton-Hunter method followed by gel filtration on sephadex G-25 column. 100 μl of serum or recombinant human leptin (rh leptin) diluted in charcoal treated serum (standards 0–100 ng/ml) were incubated with anti-rh leptin rabbit serum (1:8,000 dil) in 50 mM PBS, pH 7.4, containing 0.1% Triton X-100, 0.1% BSA, and 0.01% sodium azide for 16 h at 4°C in a final vol of 300 μl. 100 μl ^{125}I-rh leptin (~ 30,000 cpm; ~ 30 μCi/μg sp act) was then added for an additional 24 h at 4°C, thereafter bound leptin was immunoprecipitated with 100 μl of sheep anti–rabbit IgG serum (1:2 dil; Antibodies Inc., Davis, CA), 100 μl normal rabbit serum (1:50 dil; Gibco BRL, Gaithersburg, MD), and 100 μl of 10% polyethylene glycol (PEG 8000; Fisher Scientific Co., Santa Clara, CA). After 20-min incubation at 22°C, the tubes were centrifuged for 20 min at 3,400 rpm, and the pellet was counted in Packard 5000 gamma counter. Serum leptin concentrations were calculated using unweighted four parametric logistic model. Our leptin RIA characteristics were as follows: (*a*) intrassay coefficient of variation (CV): 8.93 at 5.2 ng/ml (n = 12), 3.31 at 17.8 ng/ml (n = 13), 4.29 at 31.7 ng/ml (n = 13), 5.78 at 47.1 ng/ml (n = 24); (*b*) interassay CV: 15.9 at 3.31 ng/ml (n = 7), 7.4 at 12.7 ng/ml (n = 7), and 9.9 at 29.7 ng/ml (n = 7); (*c*) slope of the dose response curve: −1.62±0.05 (n = 8); (*d*) ED_{20} 2.84±0.18 ng/ml (n = 8), ED_{50} 8.02±0.41 (n = 8), and ED_{80} 23.75±2.85 (n = 8); and (*e*) detection limit 0.39 (n = 8). No cross reactivity was evident in leptin RIA for human insulin, glucagon, and IGF-1 up to 10 μg/ml concentrations. The dilution curves of two serum samples were parallel to leptin dose response curves (r = 1.0 and 0.99). Dose response curves showed linearity between 1.56–50 ng/ml concentrations. All serum samples from the same patient were run in triplicate in the same assay including same centrifugation step. Insulin levels were measured by specific RIA and plasma glucose levels by glucose oxidase method.

Statistical analysis. All results are expressed as mean ±SEM. Statistical significant was determined by Student's *t* test using Macintosh Statview program. To demonstrate diurnal variations, a single factor ANOVA test was performed using Microsoft Excel program.

Results

Fig. 1 demonstrates fasting serum leptin levels in 6 lean controls, 11 obese nondiabetic, and 9 obese diabetic subjects. Serum leptin levels are significantly higher ($P < 0.05$) in obese subjects (41.7±9.0 ng/ml) and obese NIDDM (30.8±6.7) subjects compared to lean controls (12.0±4.4). Serum leptin levels between two obese groups, i.e., nondiabetics and diabetics, did not differ significantly ($P > 0.05$). Figs. 2, 3, and 4 demonstrate 24-h profiles of circulating leptin (*top*), insulin (*middle*), and glucose (*bottom*) levels in 6 lean, 11 obese, and 5 obese NIDDM patients. The 24-h profile of leptin in the lean group does not include data from two patients because of undetectable leptin levels throughout the 24-h period, whereas insulin and glucose profiles include all six lean patients. We observed time dependent changes (acrophase at 0230 h, i.e., 2:30 a.m. and nadir at 1300 h, i.e., 1:00 p.m.) in leptin levels (F = 3.098; $P < 0.0001$) when the data from all of the 20 patients of the three groups were pooled and analyzed by single factor ANOVA. In all of the three groups, i.e., lean (acrophase: 0230 h and nadir: 1430 h), obese (acrophase: 0030 h and nadir: 1300 h) and obese NIDDM (acrophase: 0430 h and nadir: 1430 h), we observed increased leptin levels when between midnight and early morning hours compared to lowest leptin levels observed in the afternoon. The peak night time leptin levels were 75.6, 51.7, and 60.7% higher compared to nadir afternoon leptin lev-

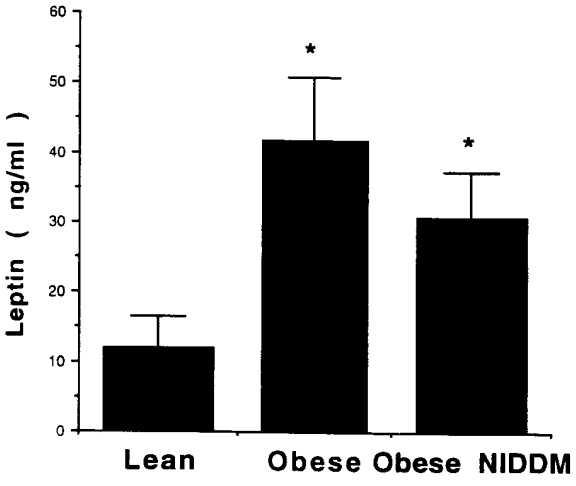

Figure 1. Fasting (0800 h) serum leptin levels (mean±SEM) in lean (n = 6), obese (n = 11), and obese NIDDM (n = 9) subjects. *Indicates levels of significance. ($P < 0.05$).

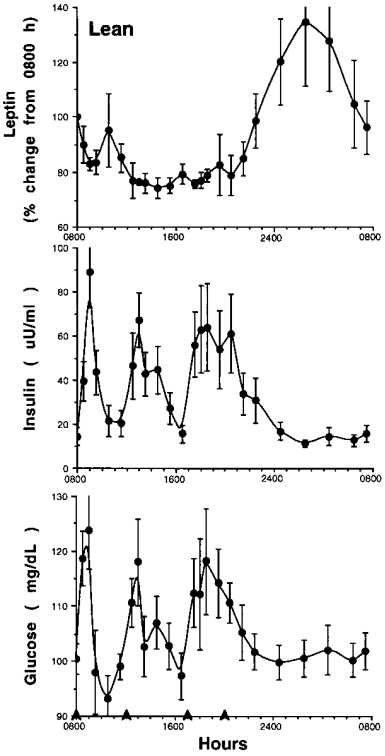

Figure 2. 24-h profiles of serum leptin levels expressed as percent change from fasting 0800 h values ($n = 4$), insulin ($n = 6$) and glucose ($n = 6$) in lean controls. Arrows indicate meal times. Each point represents mean±SEM.

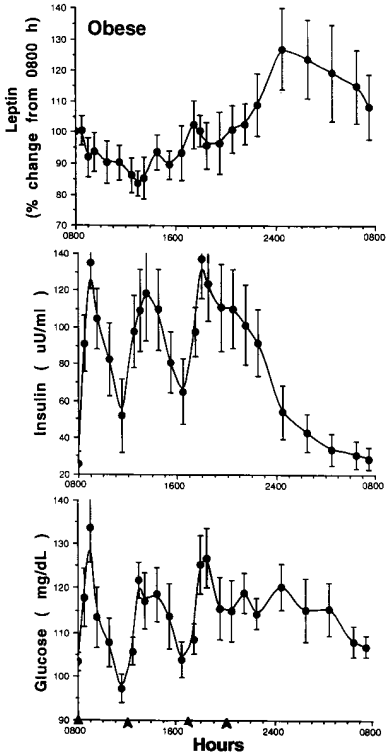

Figure 3. 24-h profiles of serum leptin, insulin, and glucose in 11 obese subjects.

els when represented as percent change from 0800 h fasting levels in lean (74.4±3.8 vs. 134.8±23.6), obese (83.8±4.2 vs. 127.0± 13.0), and obese NIDDM (74.8±9.3 vs. 120.3±7.6) groups, respectively. When leptin levels at different times of the 24-h period were analyzed by ANOVA in separate patient groups, significant differences were found in lean ($F = 3.17$; $P < 0.0001$), obese ($F = 2.02$, $P < 0.005$), and obese NIDDM ($F = 3.098$, $P < 0.0001$) groups.

To determine whether serum leptin levels were influenced by food ingestion, we analyzed pre and post ($^1/_2$, 1, and $1\,^1/_2$ h) meal leptin levels by ANOVA in all of the 20 subjects and three patient groups. After breakfast, a significant decrease in serum leptin levels was observed in lean ($F = 3.54$, $P < 0.05$), obese NIDDM ($F = 4.29$, $P < 0.05$), and all 20 patients ($F = 3.60$, $P < 0.05$) groups, but not in the obese group ($F = 0.75$, $P > 0.05$). However, no such decreases in leptin levels were noted in any of the groups after lunch, supper, or evening snack. It appears that the decrease in serum leptin levels after breakfast is the continuation of decline in night time acrophase and changes in leptin were not related to feeding. In contrast, both serum insulin (*middle* of Figs. 2, 3, and 4) and plasma glucose (*bottom* of Figs. 2, 3, and 4) levels increased in the expected manner after each meal in all the three groups. We observed no significant correlation ($P > 0.05$) between serum leptin changes and either insulin or glucose levels, in each of the 20 subjects studied for 24-h patterns of leptin secretion.

Discussion

Hormones are characterized by a wide range of biological rhythms (15). Leptin is hypothesized to interact with specific receptors in the brain and control the appetite to regulate food intake and possibly energy metabolism (1–5, 13, 14). However, the precise mechanism(s) of appetite control by leptin and its own regulation in adipose tissue are not defined. In this study, we demonstrate the nocturnal rise of leptin in humans. The changes in leptin during a 24-h period do not appear to be influenced by meal ingestion and meal-related increases in circulating insulin and glucose levels. Leptin levels are low around noon and the early afternoon period and are high during the

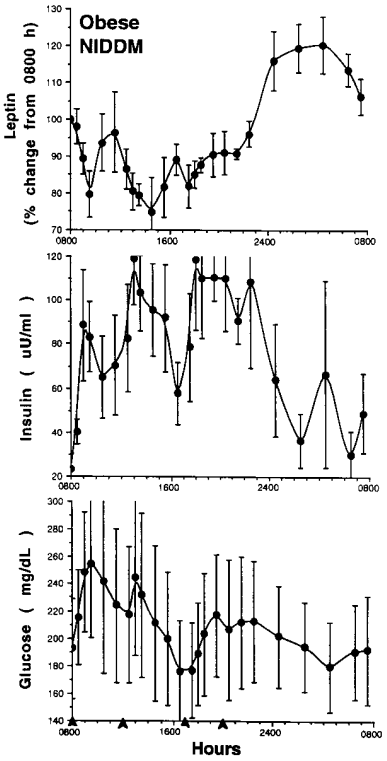

Figure 4. 24-h profiles of serum leptin ($n = 5$), insulin ($n = 4$), and glucose ($n = 4$) in five obese NIDDM subjects.

night. The nocturnal increase in leptin, when compared with 24-h secretory profiles of different hormones, closely resembles those of prolactin (18) and thyrotropin (19) and precedes the early morning rise of ACTH and cortisol (20, 21). During the night, growth hormone increase is associated with the onset of sleep and with slow wave sleep (22, 23). A progressive decrease occurs in the tolerance to oral or intravenous glucose beginning in midafternoon and reaches its minimum around midsleep in normal humans (24–26). Induction of ob gene expression by glucocorticoids (27) would be contrary to any association of low nocturnal cortisol levels and increased leptin levels, though acute effects could be different than chronic administration of pharmacological doses of glucocorticoids.

Due to the lack of in vitro and in vivo data on the hormonal regulation of leptin production and secretion by adipose tissue, it is difficult to ascertain which of these hormones, if any, are directly regulating the nocturnal rise of leptin in humans. The nocturnal rise of leptin also resembles the increase in nonesterified FFA levels during the night (28). In summary, we demonstrate a nocturnal rise of leptin in human subjects though its significance with regards to obesity is not evident. We speculate that the nocturnal increase of leptin in humans could be related to appetite suppression while sleeping.

After the completion of our study, Saladin et al. (29) demonstrated in rats that adipose tissue Ob mRNA levels were lowest during the light cycle, increasing soon after the rats started eating (2000 h), reaching a maximum around 0400 h. Thereafter, Ob mRNA steadily decreased, reaching a minimum in the afternoon. These investigators were able to demonstrate that the rhythmicity of adipose tissue Ob mRNA expression was entirely linked to changes in food intake. If the changes in adipose tissue Ob mRNA reported by Saladin et al. (29) can be equated to changes in circulating leptin levels, it seems that the diurnal rhythm of leptin production is different in humans and rodents. This would not be surprising since the physiology of adipocytes between these two species is somewhat different.

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