Leptin and body weight regulation in patients with anorexia nervosa before and during weight recovery[1,2]

Verena Haas, Simone Onur, Thomas Paul, Detlev O Nutzinger, Anja Bosy-Westphal, Maren Hauer, Georg Brabant, Harald Klein, and Manfred J Müller

ABSTRACT

Background: Leptin has been considered a starvation hormone, but its role in malnourished patients is unknown.

Objective: We aimed to characterize the role of leptin in metabolic adaptation in women with anorexia nervosa (AN).

Design: In a cross-sectional study, 57 women with AN [mean (±SD) body mass index (kg/m^2) on admission: 15.2 ± 1.5] were compared with 49 healthy, normal-weight women (mean body mass index: 22.3 ± 2.3). Nineteen patients were reinvestigated during weight gain 43 and 84 d after baseline. We measured serum concentrations of leptin, soluble leptin receptor, insulin, ghrelin, and thyroid hormones [thyrotropin, triiodothyronine (T_3), and thyroxine]; fat mass (FM) and fat-free mass (FFM); resting energy expenditure (REE); energy intake; and eating behavior.

Results: Compared with values in the control women, leptin, T_3, REE, FM, and FFM were lower in the women with AN, but the leptin secretion rate was not significantly different. Leptin correlated with FM ($r = 0.83$, $P < 0.001$), T_3 ($r = 0.68$, $P < 0.001$), respiratory quotient ($r = -0.47$, $P < 0.001$), and REE ($r = 0.58$, $P < 0.001$). The association with REE weakened after adjustment for FFM and disappeared after further adjustment for T_3. Hunger and appetite had positive, whereas satiety and restraint had negative, associations with leptin. During weight gain (9.0 ± 3.3 kg in 84 d), serum leptin and the leptin secretion rate increased. Changes in leptin secretion were associated with energy intake and REE. The initial changes in the leptin secretion rate (ie, the difference between baseline and 43 d) were negatively associated with changes in body weight from 43 to 84 d.

Conclusions: Leptin contributes to metabolic adaptation in women with AN. The leptin response is associated with weight gain. *Am J Clin Nutr* 2005;81:889–96.

KEY WORDS Leptin, anorexia nervosa, body weight regulation, resting energy expenditure, thyroid hormones, energy intake, restraint, appetite, hunger

INTRODUCTION

When the hormone leptin (also termed Ob protein) was discovered about a decade ago (1), it was acclaimed the missing link in a complex system regulating energy homeostasis. Leptin acts as a signal to inform the brain about fat storage. Earlier research focused mainly on the role of leptin in obesity. Animal experiments showed that administration of leptin decreases food intake (2, 3) and increases resting energy expenditure (REE; 3–5). In humans, the data on the role of leptin in the regulation of energy balance are controversial. The plasma leptin concentration is proportional to body fat mass (FM) during energy balance (6) and declines sharply in periods of energy deficit (7–9). The fall in leptin exceeds the rate at which FM is lost (5), which suggests that leptin secretion per unit FM is reduced during acute negative energy balance. This may preserve body weight by contributing to a reduction in energy expenditure brought about by decreasing plasma triiodothyronine (T_3) and thus thyroid-hormone-induced thermogenesis (5, 10–13). These findings are in line with the idea that leptin is a "starvation hormone" that signals and counteracts an energy deficit (13, 14).

Anorexia nervosa (AN) is an eating disorder characterized by weight loss due to voluntarily restricted caloric intake and resistance toward efforts to increase body weight. The great variety of metabolic abnormalities in AN patients include those of severe malnutrition, such as decreased FM and body temperature, hypotonia, low REE, and bradycardia (15). Endocrine signs of undernutrition include the lowering of T_3 (13, 16) and leptin (17–23) and also the leptin secretion rate (18). Longitudinal studies of leptin secretion during nutritional rehabilitation in AN patients show contradictory results. Some authors suggest an increased leptin secretion rate during weight gain, but others did not confirm these results and related leptin to changes in BMI and not to FM (18). Additionally, leptin concentrations were found not to respond to short-term refeeding in AN patients (22). Adequate weight restoration during inpatient treatment confers positive long-term outcome (24) and represents a crucial but most difficult element in the treatment of AN.

The current study was designed to investigate the role of leptin and body weight regulation in women with AN. First, we hypothesized that in women with AN before treatment, a low serum leptin concentration or secretion rate would be associated with

[1] From the Institut für Humanernährung und Lebensmittelkunde der Christian-Albrechts-Universität zu Kiel (VH, SO, AB-W, MH, and MJM); Medizinisch Psychosomatische Klinik Bad Bramstedt (TP and DON); Universitätsklinikum Schleswig Holstein, Universität Lübeck (DON and HK); and Abteilung Gastroenterologie und Endokrinologie, Medizinische Hochschule Hannover (GB), Germany.

[2] Address reprint requests to MJ Müller, Institut für Humanernährung und Lebensmittelkunde, Agrar- und Ernährungswissenschaftliche Fakultät, Christian-Albrechts-Universität zu Kiel, Düsternbrooker Weg 17-19, D-24105 Kiel, Germany. E-mail: mmueller@nutrfoodsc.uni-kiel.de.

Received April 19, 2004.

Accepted for publication November 24, 2004.

the adaptation to starvation (ie, lowered T_3 and REE) and with psychological variables of energy intake (hunger, appetite, satiety, and restraint). Then, we investigated whether a disproportionate increase (ie, surpassing the increase expected by the gain in FM) in leptin during weight gain inhibits energy intake and appetite, raises satiety and restraint, and stimulates T_3-induced energy expenditure, thus limiting further weight gain.

SUBJECTS AND METHODS

Study population and design

In this controlled prospective study, 57 women meeting the criteria for AN on the basis of the *Diagnostic and Statistical Manual of Mental Disorders IV* (ie, a body weight ≥15% lower than that expected for age and height, anxiety about and fear of obesity, disturbed attitudes toward body weight and shape, and hypothalamic amenorrhea in postpubertal females; 25) were recruited consecutively at the start of an inpatient treatment program in the Psychosomatic Clinic Bad Bramstedt, Germany. None of the patients had a medical condition (other than AN) or received medication known to affect body composition, energy intake, or energy expenditure. There was no age exclusion criterion for participation in the study. During the study period, the patients were enrolled in a psychotherapy and nutritional rehabilitation program and were encouraged to gain an average of 700 g body weight weekly. An age- and sex-matched control group consisted of 49 normal-weight, healthy women examined at the Institute of Human Nutrition, University of Kiel, Germany. Control subjects were recruited by advertisement among students of nutritional sciences.

The study protocol was approved by the ethical committee (Medical Faculty of the Christian-Albrechts University of Kiel, Germany). After being informed, patients, parents of underage patients, and control participants gave written consent.

Body composition, energy intake, serum hormone concentrations, and psychological indexes of eating behavior were assessed after referral (AN women at baseline). Nineteen nonrandomly-selected patients of the initial 57 took part in 2 follow-up measurements: at 43 and 84 d after baseline. The examination period and time for the follow-up examinations were chosen according to the results of a pilot study in which serum hormone concentrations were analyzed at weekly intervals in 6 AN patients (data not shown). These data suggested that normal leptin concentrations (ie, 8.5 ± 4.1 μg/L) could be reached within 12 wk of the psychotherapy and nutritional rehabilitation program. The high dropout rate of 67% occurred because patients were either discharged or discharged themselves before their 12th week of treatment and were not followed up. The exact times of the measurements were 9 ± 3 d (baseline), 43 ± 5 d after admission, and 84 ± 11 d after admission. The delay at baseline was due to administrative reasons (eg, combining the blood sampling with routine clinical investigations). The women with AN were compared with the control group at baseline. In addition, intraindividual data of AN patients at baseline, 43 d, and 84 d were used for longitudinal analysis of the effect of leptin on weight gain.

Body composition

Standard procedures were used to measure body height and weight (SECA, model 2200; Vogel & Halke, Hamburg, Germany). Skinfold thickness was measured by using a skinfold caliper (model 01127; Lafayette Instrument Company, Lafayette, IN) at 4 sites (biceps, triceps, subscapula, and suprailium). Body fat was calculated by using the equation of Durnin and Womersly (26). Bioelectrical impedance analysis was carried out (body composition analyzer model TVI-10; Danninger Medical Technology Inc, Detroit, MI) and FM and fat-free mass (FFM) were calculated with the appropriate software package (BODYCOMP, Danninger Medical) as described previously (27). FM as assessed by bioelectrical impedance analysis and skinfold-thickness measurements showed a strong correlation ($r = 0.89$ for all AN patients and control subjects and $r = 0.76$ for AN patients alone).

Energy expenditure and energy intake

REE was measured by indirect calorimetry (Deltatrac TMI; Datex Instrumentarium Corp, Helsinki, Finland) as described previously (27) and was calculated by using Weir's equation (28). REE was also predicted according to Harris and Benedict (29). Energy and macronutrient intakes were assessed by the use of a 3-d diet record and a software program for data analysis (PRODI 4.5 LE 2001 EXPERT; Wissenschaftliche Verlagsgesellschaft mbH, Stuttgart, Germany). All subjects received detailed instructions for completing the 3-d food records and the psychological questionnaires. Dietary intake data were obtained from 95.9% (47/49) of the control subjects and 82.5% (47/57) of the patients. Food records were tested for plausibility by using REE × 1.25 as a cutoff for energy intake: 4.3% of the control subjects and 13.8% of the AN patients were below the cutoff. These records were excluded from further analyses. There were no significant differences in mean BMI between patients with energy intakes above and below the cutoff. With the use of >2 SDs as an alternative cutoff criterion, only 2 protocols were excluded. Mean energy intake was 1776 ± 629 kcal/d in the AN patients and 1976 ± 307 kcal/d in the control subjects (NS).

Psychological variables of eating behavior

The Three Factor Eating Questionnaire (30, 31) scales for cognitive control (scale 1), disinhibition and cognitive control (scale 2), and hunger (scale 3) were completed by the patients at the beginning and at the end of inpatient treatment and by the control subjects. High values on scale 1 characterize participants with distinct, restrained eating behavior and a predominantly high cognitive control of eating behavior. Low values characterize participants with spontaneous and unrestrained eating behavior that is regulated mostly via internal signals of autonomous appetite and satiety regulation. Scales 2 and 3 were assessed but are not presented here because scale 2 does not produce comparable results for restrained and uninhibited eaters and scale 3 depends on scale 2. In addition, average hunger, appetite, and satiety scores were assessed daily after dinner on visual scales ranging from 0 (low) to 10 (high).

Blood samples

Blood samples were obtained between 0700 and 0800 after the subjects had fasted overnight. Serum was immediately frozen at −80 C° until analyzed. Serum total leptin concentrations were measured by radioimmunoassay with a commercial kit (human leptin RIA kit; Linco Research, St Charles, MO). Insulin was measured by using an enzyme-linked immunosorbent assay (DAKO Diagnostics, Cambridgeshire, United Kingdom). Serum

TABLE 1
Characteristics, nutritional status, serum hormone concentrations, resting energy expenditure (REE), energy intake, and eating behavior of women with anorexia nervosa (AN) studied on admission (baseline) and of healthy control subjects[1]

	AN patients (n = 57)	Control subjects (n = 49)	P[2]
Age (y)[3]	25 ± 7 (16–49)	25 ± 3 (20–39)	NS
Height (cm)	167 ± 6	168 ± 6	NS
Body weight (kg)	42.5 ± 4.5	63.1 ± 7.8	<0.001
BMI (kg/m^2)	15.2 ± 1.5	22.3 ± 2.3	<0.001
FM (%)	12.0 ± 6.8	32.6 ± 4.3	<0.001
FFM (kg)	37.3 ± 3.3	42.2 ± 3.4	<0.001
ΣSF (mm)	23.4 ± 9.4	51.9 ± 12.9	<0.001
Serum hormone concentrations			
Leptin (μg/L)	2.3 ± 1.6	8.5 ± 4.1	<0.001
Leptin adj (μg/L)	4.8 ± 1.4	5.1 ± 3.7	NS
SLR (nmol/L)	7.6 ± 3.7	13.3 ± 5.0	<0.001
FLI (nmol/L)	0.02 ± 0.02	0.05 ± 0.03	<0.001
Insulin (mU/L)	5.8 ± 3.9	5.7 ± 2.4	NS
Ghrelin (pmol/L)	374 ± 185	359 ± 115	NS
TSH (mU/L)	1.7 ± 1.0	1.4 ± 0.9	NS
fT_3 (pmol/L)	3.7 ± 0.9	5.4 ± 1.1	<0.001
fT_4 (pmol/L)	13.3 ± 1.7	16.7 ± 2.7	<0.001
REE (n = 29 and 49)			
Measured (MJ/d)	4.24 ± 0.56	5.48 ± 0.67	<0.001
Predicted (MJ/d)	5.28 ± 0.25	6.08 ± 0.35	<0.001
Measured − predicted (MJ/d)	−1.02 ± 0.43	−0.60 ± 0.57	<0.01
adj for FFM (MJ/d)	4.46 ± 0.42	5.27 ± 0.54	<0.001
adj for FFM and T_3 (MJ/d)	4.70 ± 0.41	5.05 ± 0.56	<0.01
RQ	0.96 ± 0.11	0.86 ± 0.04	<0.001
Energy intake and eating behavior variables (n = 47 and 47)			
Energy intake (kcal/d)	1779 ± 699	1977 ± 307	NS
Hunger	3.2 ± 2.3	5.0 ± 1.4	<0.001
Satiety	8.3 ± 1.6	6.0 ± 1.5	<0.001
Appetite	3.5 ± 2.3	5.2 ± 1.2	<0.001
Restraint	15.0 ± 4.5	5.7 ± 3.5	<0.001

[1] All values are $\bar{x}$ ± SD. FM, fat mass measured by bioelectrical impedance analysis; FFM, fat-free mass measured by bioelectrical impedance analysis; ΣSF, sum of 4 skinfold thicknesses; leptin adj, leptin concentration adjusted for FM; SLR, soluble leptin receptor; FLI, free leptin index; TSH, thyrotropin; fT_3, free triiodothyronine; fT_4, free thyroxine; REE predicted, REE predicted according to Harris and Benedict equations; RQ, respiratory quotient.
[2] Independent t test.
[3] Range in parentheses.

concentrations of T_3, thyroxine, and thyrotropin were analyzed by electrochemiluminescence immunoassay (Elecsys; Roche Diagnostics, Mannheim, Germany). Analytic differentiation of soluble leptin receptor (SLR) by specific radioimmunoassay systems was performed as previously described (32). A double-antibody radioimmunoassay procedure was used to measure serum ghrelin concentrations. Ghrelin antiserum was generated in rabbits by using a synthetic carboxyl-terminal fragment (aa 15–28 14Tyr; Biotrend, Köln, Germany) coupled to hemocyanin. A working titer of 1:1200 was used for subsequent studies. Tracer generated by the iodogen method was purified by HPLC on a C_{18} column. Full-length ghrelin (Bachem, Heidelberg, Germany) was used as a standard. The antibody-bound fraction and free peptide were separated by 2% dextran charcoal.

Statistics

Statistical analyses were performed with SPSS for WINDOWS (version 8.0; SPSS Inc, Chicago, IL). Data are presented as means ± SDs. Serum leptin concentrations were logarithmically transformed to normalize the distribution. Pearson correlations were used to evaluate relations among different variables. One-factor repeated-measures analysis of variance (ANOVA) with Bonferroni's post hoc test was used to analyze longitudinal changes in one variable within the group of patients during weight gain. P values < 0.05 were considered statistically significant.

Leptin concentrations were adjusted for FM as an index of leptin secretion by the use of a regression analysis. Accordingly, REE was adjusted for either FFM or FFM and T_3 by regression analyses. Free leptin index is calculated as leptin/SLR.

RESULTS

Role of leptin in the adaptation to underweight

Nutritional status, REE, and serum leptin

The nutritional status of the AN patients and the control subjects is presented in **Table 1**. On admission to the hospital, the AN patients were severely malnourished. Compared with the control subjects, the patients had a significantly lower mean body weight and BMI, which was predominantly explained by a reduced FM. Serum leptin concentrations were 73% lower in the

TABLE 2
Pearson correlation coefficients between the log of serum leptin concentrations and free triiodothyronine (T_3) concentrations versus resting energy expenditure (REE), respiratory quotient (RQ), fat mass (FM), and eating behavior variables in women with anorexia nervosa (AN) at baseline and in control subjects[1]

	Log leptin			T_3		
	AN patients	Control subjects	All women	AN patients	Control subjects	All women
REE	0.11	0.04	0.58[2]	0.48[2]	0.04	0.56[2]
REE adj for FFM	0.02	−0.15	0.47[2]	−0.04	−0.30[3]	0.51[2]
REE adj for FFM and T_3	−0.04	−0.30[3]	0.19	—	—	—
RQ	0.11	−0.23	−0.47[2]	−0.02	−0.06	−0.44[2]
FM	0.57[2]	0.48[4]	0.83[2]	0.49[4]	0.12	0.67[2]
Hunger	0.05	−0.16	0.28[4]	−0.06	−0.06	0.2[3]
Satiety	−0.09	−0.13	−0.51[2]	0.16	0.11	−0.30[4]
Appetite	0.13	0.08	0.38[2]	−0.11	0.16	0.32[4]
Restraint	−0.23	−0.48[4]	−0.58[2]	−0.50	0.19	−0.56[4]
T_3	0.47[4]	0.21	0.68[2]	—	—	—

[1] adj, adjusted; FFM, fat-free mass.
[2] $P < 0.001$.
[3] $P < 0.05$.
[4] $P < 0.01$.

patients than in the control subjects, but the leptin secretion rate (as reflected by leptin concentrations adjusted for FM) did not differ significantly between groups (Table 1).

There were close associations between the log of serum leptin and FM in both AN patients and control subjects (**Table 2**), and the highest *r* value was obtained when the patients and control subjects were analyzed together (**Figure 1**, Table 2). SLR and the free leptin index were also lower in the patients than in the control subjects (Table 1). Both measured REE and REE adjusted for FFM were lower in the AN patients than in the control subjects (Table 1). When the patients and control subjects were analyzed together, significant correlations were seen between the log of serum leptin and REE, respiratory quotient, serum free T_3 concentrations, and variables of eating behavior (Table 2). By contrast, when the AN patients and control subjects were considered separately, there was no significant correlation between the log of serum leptin and REE, REE adjusted for FFM, or REE adjusted for FFM and T_3 (AN patients only). In the group as a whole, both the log of serum leptin and REE showed significant associations with T_3. There were no between-group differences in serum concentrations of insulin or ghrelin (Table 1). In both the patients and the control subjects, the log of serum leptin correlated with serum insulin concentrations ($r = 0.31$, $P < 0.05$, and $r = 0.32$, $P < 0.05$, respectively).

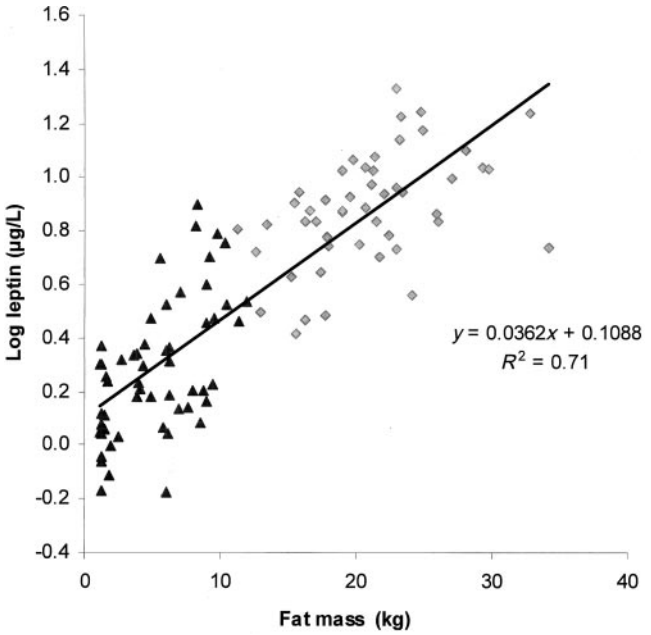

FIGURE 1. Correlation between fat mass measured by bioelectrical impedance analysis and serum leptin concentrations in 57 patients with anorexia nervosa before nutritional recovery (▲) and in 49 healthy control subjects (◆). Because the slopes of the group-specific regression lines were not significantly different, a combined regression line for patients and control subjects is shown ($P < 0.001$).

Psychological variables and leptin

Hunger and appetite were lower but satiety and restraint were higher in AN patients than in control subjects (Table 1). For all women combined, the log of serum leptin showed negative correlations with satiety and restraint, whereas positive correlations were seen between the log of serum leptin and hunger on the one hand and appetite on the other (Table 2). The correlations were not significant in control subjects or AN patients alone. In AN women, a positive correlation was seen between ghrelin concentrations and restraint ($r = 0.32$, $P < 0.05$).

Does the weight-induced increase in leptin limit further weight gain?

Nutritional status, REE, and serum leptin during nutritional recovery

Comparing the whole group of AN patients (Table 1) with those patients who could be followed for a mean period of 84 d after baseline (**Table 3**), no between-group differences in baseline nutritional status, REE, or endocrine variables were observed. However, the mean age in this subgroup of patients was lower: 21.6 ± 3.6 y compared with 25.0 ± 7.0 y in the whole group; $P < 0.05$). Weight gain over 84 d was 9.0 ± 3.3 kg. This increase resulted mainly from increases in FM (6.3 ± 3.5 kg), which were equivalent to 70.0% of the gained weight. After 84 d

TABLE 3
Nutritional status, serum hormone concentrations, resting energy expenditure (REE), energy intake, and eating behavior variables of women with anorexia nervosa (AN) studied on admission (baseline) and during nutritional recovery (43 and 84 d after admission)[1]

	AN patients		
	Baseline (n = 19)	43 d (n = 19)	84 d (n = 19)
Body weight (kg)	41.9 ± 4.1[b]	47.8 ± 4.9[a]	50.9 ± 3.7[a]
BMI (kg/m^2)	14.6 ± 1.2[b]	16.8 ± 1.8[a]	17.7 ± 1.1[a]
FM (kg)	4.1 ± 2.7	8.6 ± 3.5	11.0 ± 2.5
FM (%)	9.5 ± 5.9	17.5 ± 6.1[a]	21.5 ± 3.9[a]
FFM (kg)	37.8 ± 3.5	39.5 ± 2.9	39.7 ± 2.4
ΣSF (mm)	22.2 ± 6.8[b]	40.4 ± 10.9[a]	48.1 ± 14.5[a]
Serum hormone concentrations			
Leptin (μg/L)	2.0 ± 1.1[b]	5.3 ± 3.2[a]	7.8 ± 5.26[a]
Leptin adj (μg/L)	5.0 ± 1.1[a]	6.5 ± 2.7[a,b]	7.2 ± 3.4[b]
SLR (nmol/L)	9.6 ± 4.3	8.2 ± 3.2	6.8 ± 2.3
FLI (nmol/L)	0.01 ± 0.01[a]	0.04 ± 0.02[a,b]	0.08 ± 0.05[b]
Insulin (mU/L)	5.0 ± 2.6	7.8 ± 5.1	7.1 ± 3.4
Ghrelin (pmol/L)	381 ± 183[a]	252 ± 83[b]	270 ± 119[a,b]
TSH (mU/L)	1.8 ± 1.2	1.8 ± 0.9	1.9 ± 1.1
fT_3 (pmol/L)	3.4 ± 0.9[b]	4.4 ± 0.9[a]	4.5 ± 0.8[a]
fT_4 (pmol/L)	12.5 ± 1.2	13.1 ± 1.8	13.4 ± 2.6
REE (n = 6)			
Measured (MJ/d)	4.14 ± 0.32[a]	4.79 ± 0.74[b]	4.70 ± 0.56[a,b]
Predicted (MJ/d)	5.28 ± 0.16[a]	5.54 ± 0.22[a,b]	5.61 ± 0.16[b]
Measured − predicted (MJ/d)	−1.15 ± 0.33	−0.75 ± 0.80	−0.91 ± 0.63
adj for FFM (MJ/d)	4.53 ± 0.36	5.27 ± 0.75	5.01 ± 0.56
adj for FFM and T_3 (MJ/d)	4.80 ± 0.29	4.93 ± 0.88	4.82 ± 0.51
RQ	0.98 ± 0.12	0.97 ± 0.07	0.96 ± 0.51
Energy intake and eating behavior variables			
Energy intake (kcal/d)	1802 ± 601	2141 ± 830	2015 ± 725
Hunger	3.0 ± 2.1	3.8 ± 2.7	3.4 ± 2.2
Satiety	8.4 ± 1.7	7.8 ± 2.2	7.9 ± 2.8
Appetite	3.8 ± 2.0	4.3 ± 2.5	3.9 ± 2.2
Restraint	15.0 ± 4.5	—	9.0 ± 4.1

[1] All values are $\bar{x}$ ± SD. FM, fat mass measured by bioelectrical impedance analysis; FFM, fat-free mass measured by bioelectrical impedance analysis; ΣSF, sum of 4 skinfold thicknesses; leptin adj, leptin concentration adjusted for fat mass; SLR, soluble leptin receptor; FLI, free leptin index; TSH, thyrotropin; fT_3, free triiodothyronine; fT_4, free thyroxine; REE predicted, REE predicted according to Harris and Benedict equations; RQ, respiratory quotient. Means in a row with different superscript letters are significantly different, $P < 0.05$ (ANOVA followed by Bonferroni-adjusted post hoc test).

of nutritional rehabilitation, BMI, body weight, and FM had increased significantly but were still lower than in the control subjects ($P < 0.001$; Tables 1 and 3). The rate of weight gain per week decreased significantly within the observation period (differences in mean weight gain between baseline and 43 d compared with between 43 and 84 d after baseline: 0.96 ± 0.39 compared with 0.52 ± 0.20 kg; $P < 0.05$).

Serum leptin concentrations increased significantly with weight gain (Table 3). Changes in FM were associated with changes in leptin ($r = 0.62$, $P < 0.01$). There were no significant differences in serum leptin between the AN patients after 84 d and the control subjects. The leptin secretion rate (leptin adjusted for FM) was significantly higher in the AN patients after 84 d than in the control subjects ($P < 0.01$; Tables 3 and 1). The free leptin index also increased and reached a value not significantly different from that in the control subjects (Tables 3 and 1). REE increased significantly with weight gain, but no significant baseline-to-43 d or 43-to-84 d differences in REE were seen after adjustment of REE for either FFM or FFM and T_3 (Table 3). After 84 d, REE was still lower in the AN patients than in the healthy control subjects ($P < 0.05$; Tables 3 and 1).

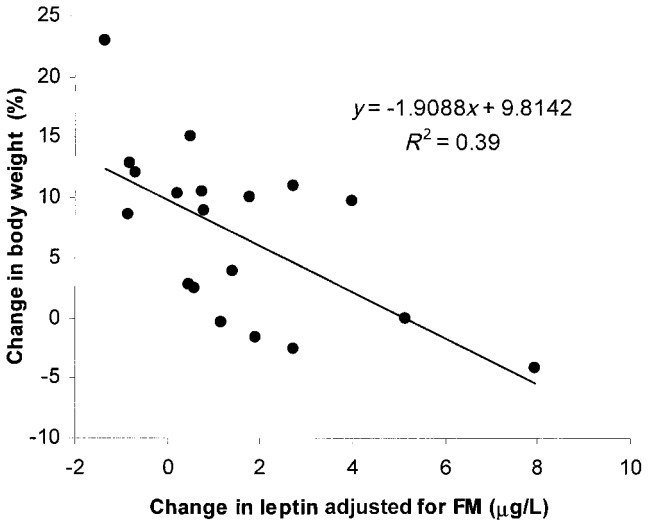

FIGURE 2. Association between the change in serum leptin secretion from baseline to 43 d (after 6 wk of recovery) and the change in body weight from 43 to 84 d in 19 women with anorexia nervosa. FM, fat mass.

Serum free T_3 concentrations increased significantly with weight gain but were still lower after 84 d than in the control subjects ($P < 0.05$; Tables 3 and 1). After 84 d but not after 43 d, serum free T_3 concentrations significantly correlated with leptin ($r = 0.82$, $P < 0.01$). There were significant and positive associations between changes in FM and changes in serum leptin during the first 43 and over the total 84-d period of treatment ($r = 0.51$, $P < 0.001$, and $r = 0.66$, $P < 0.001$, respectively). The initial change in the leptin secretion rate (ie, the difference between baseline and 43 d) was negatively associated with the change in body weight from 43 to 84 d (**Figure 2**; $P < 0.01$), energy intake ($r = -0.58$, $P < 0.05$), and REE ($r = -0.93$, $P < 0.01$) but not with REE adjusted for either FFM alone or FFM and T_3.

The patients were divided into 2 groups according to the median of the initial (between baseline and 43 d) leptin response (ie, 4 μg/L) to weight gain (ie, "high" versus "low" responders). High responders showed a lower weight gain between 43 and 84 d than did low responders (1.6 ± 2.9 compared with 4.7 ± 2.4 kg; $P = 0.02$).

Psychological variables and leptin

There were no significant correlations between changes in the leptin secretion rate and changes in psychological variables (hunger, appetite, satiety, or restraint). This was also true for ghrelin. By contrast, hunger and appetite showed positive associations with energy intake ($r = 0.47$ and 0.42, $P < 0.01$, respectively).

DISCUSSION

Leptin is widely viewed as the most important factor secreted by white adipose tissue (33, 34). Prentice et al (13) suggested that leptin is primarily a starvation signal that evolved to provide a readout of both dynamic (ie, during weight loss) and chronic (ie, at reduced body weight) states of energy stores. This idea is supported by our results. In addition, our data provide evidence that the leptin response to weight gain may affect the body weight regulatory system.

Role of leptin in the adaptation to underweight

Nutritional status and REE

Studies of AN patients have shown that serum leptin concentrations are low in the acute anorexic state and are still related to BMI (17, 19, 22). Few studies have focused on the leptin secretion rate. Hebebrand et al (18) described leptin secretion to be depressed in the malnourished state before treatment in adolescent anorexic girls. The results of the present study showed low leptin concentrations but a normal leptin secretion rate in severely malnourished women with AN. Our data reflected the adaptation to chronically low energy intake. Concomitantly, both REE and REE adjusted for FFM were low, which suggests that the low REE was not explained by reduced body mass alone. These results agree with previous findings (35) and suggest an active down-regulation of REE. Our results do not indicate a direct effect of leptin in this process. Although serum leptin concentrations showed a positive association with REE, this association became weaker after adjustment of REE for FFM and disappeared after further adjustment for serum T_3. It is tempting to speculate that a decrease in FM sensed by leptin could lead to a lowering of REE brought about by decreasing serum T_3. This is in line with the idea of Flier et al (12).

Psychological variables and leptin

Leptin secretion was positively related to appetite in the AN patients. When the AN patients and control subjects were considered together, hunger and appetite showed positive but satiety and restraint showed negative associations with leptin concentrations. These data do not support the hypothesis that leptin is a direct appetite suppressant. Our findings could be explained by at least 3 lines of evidence. First, serum concentrations may not reflect the biological activity of leptin. Second, the instruments used to assess quantitative and psychological variables of energy intake may not give accurate results. Third, the data may reflect a severe alteration of body weight regulation in AN patients.

Regarding the first idea, SLR is thought to be capable of modulating the action of leptin (36–38). Our data contrast with previous findings showing a normal or increased concentration of SLR in AN patients (36), which may function as a compensating mechanism during an energy deficit to suppress leptin action. A high SLR concentration may serve to delay the clearance of leptin by binding it. In the present study, such a compensation was not seen. By contrast, a low SLR concentration could serve to accelerate leptin clearance, thus reducing its anorexigenic effect and preventing further weight loss in AN patients. We confirmed a decrease in SLR with weight gain as documented earlier (36), which, together with marked increases in serum leptin, resulted in a disproportionate increase in the free leptin index.

We used established instruments to assess endocrine and psychological variables of energy intake and eating behavior. In AN, assessment of energy intake by use of standard methods produces relevant data (39–41). However, although we observed plausible associations between the various psychological variables, we assume that assessing eating behavior in patients with AN may lead to inaccurate results because of the patients' morbid fear of weight gain. The lack of correlation between humoral factors and appetite or energy intake may also be taken as evidence for the theory that the regulation of food intake is severely altered in AN patients.

Does the weight-induced increase in leptin limit further weight gain?

When energy balance is perturbed, the dynamic component of the leptin system greatly outweighs the static component (13). In the present study, the reduced serum leptin concentrations in the AN patients before treatment gave way to a disproportionately increased secretion rate during weight gain. In the former situation, leptin is a sensor of FM, whereas in the latter situation, leptin reflects both FM and energy balance. This finding confirms data from other authors (42). The positive association between changes in leptin secretion and changes in percentage body weight are most likely explained by increasing FM. However, leptin secretion per FM is also increased. Our data further suggest that the up-regulation of leptin secretion could exert negative effects on subsequent weight gain. When the changes in leptin secretion from baseline to 43 d were compared with the changes in either body weight or energy intake from 43 to 84 d, negative associations were found. These negative associations suggest an inhibitory effect of leptin on energy intake, which may add to long-term weight changes.

The negative association between changes in leptin secretion and changes in REE is in contrast with the positive association seen between leptin concentrations and REE at baseline. The different findings may reflect that the 2 leptin-sensitive systems (ie, the leptin–energy intake system and the leptin–energy expenditure system) operate differently in AN patients during weight gain. It is possible that the former system is activated, whereas the latter is still inactive, in response to an 84-d period of weight gain in AN patients. Accordingly, REE, T_3, and FFM are still low after both 43 and 84 d when compared with values in controls, which might give indirect evidence for a disturbed leptin-T_3 interaction in this situation.

The high leptin response from baseline to 43 d was associated with a lower weight gain from 43 to 84 d. Thus, patients who gained much weight during the first 6 wk may have voluntarily reduced their weight gain during the second period of observation. The finding that the responses from baseline to 84 d in high and low responders, respectively, in serum leptin (6.4 and 5.1 μg/L) and FM (7.0 and 6.8 kg) were not significantly different may argue in favor of the idea that leptin kinetics are a readout of energy stores. However, the negative association between leptin secretion and energy intake suggests a further regulatory role of leptin.

Treatment implications

Endocrine adaptation in AN patients at low body weights is a mechanism to prevent further weight loss. This adaptation may also explain why it is difficult for these patients to return to their

premorbid weights. Our data suggest that leptin presents one factor to prevent starvation and defend low body weight in women with AN. Accordingly, Holtkamp et al (42) suggested that leptin should be monitored to slow down weight gain in patients with high leptin concentrations. However, this method did not improve the outcome in another study (43). With the use of univariate variance analysis, 60% of the variance in nutritional status 1 y after discharge of AN patients was explained by leptin concentrations at discharge (44). Patients with poor outcome after 1 y had higher leptin concentrations at discharge than did healthy control subjects after adjustment for BMI and FM (44). These data also suggest that the leptin response to weight gain predicts long-term outcome. Further studies are necessary to assess the effects of leptin secretion on the clinical outcome of AN patients.

MJM was responsible for study concept and design; VH, SO, MH, TP, and DON contributed to data acquisition; VH, SO, MH, and AB-W investigated the subjects and performed the assessments of nutritional status, resting energy expenditure, and energy intake; GB and HK were responsible for laboratory analyses of hormones; TP and DON were responsible for the patients and for the assessment of the psychological variables; VH and SO had full access to all data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis, including statistical analyses; VH, AB-W, and MJM analyzed and interpreted the data and drafted the manuscript; all authors discussed the results and approved the final manuscript; and MJM and TP supervised the study. The authors had no conflicts of interest.

REFERENCES

1. Zhang F, Proenca M, Maffei M, Barone M, Leopold M, Friedman JM. Positional cloning of the obese gene and its human homologue. Nature 1994;372:425–32.
2. Eckel LA, Langhans W, Kahler A, Campfield LA, Smith FJ, Geary N. Chronic administration of OB protein decreases food intake by selectively reducing meal size in female rats. Am J Physiol 1998;275:R186–93.
3. Tang-Christensen M, Havel PJ, Jacobs RR, Larsen PJ, Cameron JL. Central administration of leptin inhibits food intake and activates the sympathetic nervous system in rhesus macaques. J Clin Endocrinol Metab 1999;84:711–7.
4. Scarpace PJ, Matheny M, Pollock BH, Tumer N. Leptin increases uncoupling protein expression and energy expenditure. Am J Physiol 1997; 273:E226–30.
5. Jequier E. Leptin signaling, adiposity, and energy balance. Ann N Y Acad Sci 2002;967:379–88.
6. Havel PJ, Kasim-Karakas S, Mueller W, Johnson PR, Gingerich RL, Stern JS. Relationship of plasma leptin to plasma insulin and adiposity in normal and overweight women. Effects of dietary fat content and sustained weight loss. J Clin Endocrinol Metab 1996;81:4406–13.
7. Kolaczynski JW, Considine RV, Ohannesian J, et al. Responses of leptin to short-term fasting and refeeding in humans: a link with ketogenesis but not ketones themselves. Diabetes 1996;45:1511–5.
8. Weigle DS, Duell PB, Connor WE, Steiner RA, Soules MR, Kuijper JL. Effect of fasting, refeeding, dietary fat restriction on plasma leptin levels. J Endocrinol Metab 1997;82:561–5.
9. Grinspoon SK, Askary H, Landt ML, et al. Effect of fasting and glucose infusion on basal and overnight leptin concentrations in normal weight women. Am J Clin Nutr 1997;66:1352–6.
10. Müller MJ, Seitz HJ. Thyroid hormone action on intermediary metabolism. Part 1: respiration, thermogenesis and carbohydrate metabolism. Klin Wochenschr 1984;62:11–8.
11. Müller MJ, Burger AG, Ferrannini E, Jequier E, Acheson K. Glucoregulatory function of thyroid hormones: role of pancreatic hormones. Am J Physiol 1989;256:E101–10.
12. Flier JS, Harris M, Hollenberg AN. Leptin, nutrition, and the thyroid: the why, the wherefore, and the wiring. J Clin Invest 2000;105:859–61.
13. Prentice AM, Moore SE, Collinson AC, O'Connell MAO. Leptin and undernutrition. Nutr Rev 2002;60:S56–67.
14. Havel PJ. Control of energy homeostasis and insulin action by adipocyte hormones: leptin, acylation stimulating protein, and adiponectin. Curr Opin Lipidol 2002;13:51–9.
15. Keys A, Brozek J, Henschel A, Mickelsen O, Taylor HL. The biology of human starvation. Minneapolis, MN: University of Minnesota Press, 1950.
16. Müller MJ. Thyroid hormones and energy and fat balance. Eur J Endocrinol 1997;136:267–8.
17. Grinspoon S, Gulick T, Askari H, et al. Serum leptin levels in women with anorexia nervosa. J Clin Endocrinol Metab 1996;81:3861–3.
18. Hebebrand J, Blum WF, Barth N, et al. Leptin levels in patients with anorexia nervosa are reduced in the acute stage and elevated upon short-term weight restoration. Mol Psychiatry 1997;2:330–4.
19. Casanueva FF, Dieguez C, Popovic V, Peino R, Considine RV, Caro JF. Serum immunoreactive leptin concentrations in patients with anorexia nervosa before and after partial weight recovery. Biochem Mol Med 1997;60:116–20.
20. Eckert E, Pomeroy C, Raymond N, Kohler P, Thuras P, Bowers CY. Leptin in anorexia nervosa. J Endocrinol Metab 1998;83:791–5.
21. Herpertz S, Albers N, Wagner R, et al. Longitudinal changes of circadian leptin, insulin and cortisol plasma levels and their correlation during refeeding in patients with anorexia nervosa. Eur J Endocrinol 2000;142: 373–9.
22. Pauly R, Lear SA, Hastings FC, Birmingham CL. Resting energy expenditure and plasma leptin levels in anorexia nervosa during acute refeeding. Int J Eat Disord 2000;28:231–4.
23. Lear SA, Pauly RP, Birmingham CL. Body fat, caloric intake, and plasma leptin levels in women with anorexia nervosa. Int J Eat Disord 1999;25:283–8.
24. Gross G, Russell JD, Beumont PJ, et al. Longitudinal study of patients with anorexia nervosa 6 to 10 years after treatment, Ann N Y Acad Sci 2000;904:614–6.
25. American Psychiatric Association. Diagnostic and statistical manual of mental disorders, 4th ed. Washington, DC: American Psychiatric Association, 1994.
26. Durnin J, Womersly J. Body fat assessed from body density and its estimation from skinfold thickness: measurements on 481 men and from 16 to 72 years. Br J Nutr 1974;32:77–97.
27. Müller MJ, von zur Mühlen A, Lautz HU, Schmidt FW, Daiber M, Hürter P. Energy expenditure in children with type 1 diabetes: evidence for increased thermogenesis. BMJ 1989;299:487–91.
28. Weir JB. New methods for calculating metabolic rate with special reference to protein metabolism. J Physiol (Lond) 1949;109:1–9.
29. Harris JA, Benedict FG. A biometric study of basal metabolism in man. Washington, DC: Carnegie Institute of Washington, 1919. (Carnegie Institute of Washington publication 279.)
30. Stunkard AJ, Messick S. The three factor eating questionnaire to measure dietary restraint, disinhibition and hunger. J Psychosom Res 1985; 29:71–83.
31. Pudel V, Westenhöfer J. Handanweisung, Fragebogen zum Essverhalten (FEV). [The Three Factor Eating Questionnaire–German version.] Göttingen, Germany: Hogrefe Verlag fuer Psychologie, 1989:7–10 (in German).
32. Lewandowski K, Horn R, O'Callaghan CJ, et al. Free leptin, bound leptin, and soluble leptin receptor in normal and diabetic pregnancies. J Clin Endocrinol Metab 1999;84:300–6.
33. Friedman JM, Halaas JL. Leptin and the regulation of body weight in mammals. Nature 1998;395:763–70.
34. Trayhurn P, Beattie JH. Physiologic role of adipose tissue: white adipose tissue as an endocrine and secretary organ. Proc Nutr Soc 2001;60:329–39.
35. Polito A, Fabbri A, Ferro-Luzzi A, et al. Basal metabolic rate in anorexia nervosa: relation to body composition and leptin concentrations. Am J Clin Nutr 2000;71:1495–502.
36. Kratzsch J, Lammert A, Bottner B, et al. Circulating soluble leptin receptor and free leptin index during childhood, puberty and adolescence. J Clin Endocrinol Metab 2002;87:4587–94.
37. Chan JL, Bluher S, Yiannakouris N, Suchard MA, Kratsch J, Mantzoros CS. Regulation of circulating soluble leptin receptor levels by gender, adiposity, sex steroids, and leptin. Diabetes 2002;51:2105–12.
38. Laimer M, Ebenbichler CF, Kaser S, et al. Weight loss increases soluble leptin receptor levels and the soluble receptor bound fraction of leptin. Obes Res 2002;10:597–601.
39. Affenito SG, Dohm FA, Crawford PB, Daniels SR, Striegel-Moore RH.

Macronutrient intake in anorexia nervosa: the National Heart, Lung, and Blood Institute Growth and Health Study. J Pediatr 2002;141:701–5.
40. Hadigan CM, Anderson EJ, Miller KK, et al. Assessment of macronutrient and micronutrient intake in women with anorexia nervosa. Int J Eat Disord 2000;28:284–92.
41. Nova E, Varela P, Lopez-Vidriero I, et al. A one-year follow-up study in anorexia nervosa. Dietary pattern and anthropometrical evolution. Eur J Clin Nutr 2001;55:547–54.
42. Holtkamp K, Hebebrand J, Mika C, et al. The effect of therapeutically induced weight gain on plasma leptin levels in patients with anorexia nervosa. J Psychiatr Res 2003;37:165–9.
43. Lob S, Pickel J, Bidlingmaier M, et al. Serum leptin monitoring in anorectic patients during refeeding therapy. Exp Clin Endocrinol Diabetes 2003;111:278–82.
44. Holtkamp K, Hebebrand J, Mika C, Heer M, Heussen N, Herpertz-Dahlmann B. High serum leptin levels subsequent to weight gain predict renewed weight loss in patients with anorexia nervosa. Psychoneuroendocrinology 2004;29:791–7.

The American Journal of Clinical Nutrition