Leptin, Neuropeptide Y, and Peptide YY in Long-Term Recovered Eating Disorder Patients

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Background: *Disturbances of leptin, neuropeptide Y (NPY), and peptide YY (PYY) have been found in women who are ill with anorexia or bulimia nervosa. It is not certain whether peptide disturbances are cause or consequence of eating disorders.*

Methods: *Plasma leptin and cerebrospinal fluid leptin, NPY, and PYY concentrations were measured in women who were recovered from anorexia or bulimia nervosa to determine whether alterations persisted after recovery.*

Results: *NPY, PYY, and leptin concentrations were similar across all diagnostic groups.*

Conclusions: *Alterations in NPY, PYY, and serum leptin concentrations are probably secondary to pathological eating behaviors. Alterations of these peptides are unlikely to be trait-related disturbances that contribute to the etiology of eating disorders.* Biol Psychiatry 1999;46: 292–299

Key Words: Leptin, neuropeptide Y, peptide YY, recovered eating disorders

Introduction

Anorexia nervosa (AN) and bulimia nervosa (BN) are disorders of unknown etiology that are characterized by aberrant patterns of feeding behavior and weight regulation. In addition, there are disturbances in attitudes toward weight and shape and the perception of body shape. In AN there is an inexplicable fear of weight gain and unrelenting obsession with fatness even in the face of increasing cachexia. BN usually emerges after a period of dieting, which may or may not have been associated with weight loss. In BN, binge eating is usually followed by either self-induced vomiting, or by some other means of compensation for the excess of food ingested. The majority of people with BN have irregular feeding patterns, and satiety may be impaired. Although abnormally low body weight is an exclusion for the diagnosis of BN, some 25–30% of bulimics presenting to treatment centers have a prior history of AN; however, all bulimics have pathological concern with weight and shape. Common to both individuals with AN and BN are low self-esteem, depression, and anxiety. In the past decade, a number of neuropeptides have been discovered that have profound effects upon appetite and weight regulation. These include neuropeptide Y (NPY), peptide YY (PYY), and leptin. It is theoretically possible that alternations in the activity of these brain peptides could contribute to the pathophysiology of AN or BN.

Leptin is the hormone product of the mouse ob gene and human homologue gene, LEP (Zhang et al 1994). It is secreted predominantly by adipose tissue cells, and it is thought to contribute to the regulation of body fat (Considine et al 1996). Leptin may decrease food intake and reduce body weight by decreasing NPY synthesis or by inhibiting NPY's action as an appetite stimulant (Stephens et al 1995), increasing metabolic rate by activation of β-adrenergic receptors, and possibly by having its own, or other peptide-mediated anorexigenic properties (Erikson et al 1996). In addition to its role in body weight regulation, it has become apparent that leptin may also be a metabolic signal that mediates impaired reproductive ability in conditions of extreme over- and underweight (Chehab 1996; Chehab et al 1996; Kopp et al 1997).

People with AN who are underweight and malnourished have consistently been found to have significantly reduced plasma and cerebrospinal fluid (CSF) leptin concentrations compared to normal weight control subjects (Hebebrand et al 1995; Grinspoon et al 1996; Baranowska et al 1997; Ferron et al 1997; Mantzoros et al 1997a). Reduced leptin concentrations are probably a normal physiological response to starvation in anorexia nervosa. Interestingly, people with AN have an increase in plasma leptin levels during refeeding and weight gain. Moreover, leptin concentrations may reach normal values before full weight restoration (Hebebrand et al 1997; Mantzoros et al 1997a),

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suggesting that premature normalization of leptin concentrations might contribute to difficulty in achieving and sustaining a normal weight in anorexia nervosa. Less work has examined the leptin status of individuals with bulimia nervosa; however, it appears that serum leptin concentrations in ill bulimics are similar to those of normal control women and are correlated with body mass (Argente et al 1997; Ferron et al 1997; Kopp et al 1997; Zipfel et al 1998).

NPY and PYY are related 36-amino-acid peptides that are potent activators of feeding behavior. Our group (Kaye et al 1990) found that underweight anorexia nervosa patients had significantly elevated concentrations of CSF NPY compared to healthy volunteers; however, CSF NPY concentrations normalized after long-term recovery. In contrast patients with anorexia nervosa, whether underweight or recovered, had normal CSF PYY concentrations.

The powerful permissive effect (without the development of tolerance) that central PYY administration has on food ingestion has led to speculation that increased activity of PYY may contribute to bulimia nervosa (Morley et al 1985; Morley and Blundell 1988). CSF PYY values for normal weight bulimic women studied when bingeing and vomiting are similar to control subjects (Kaye et al 1990). In contrast, CSF PYY concentrations were significantly elevated in bulimic women studied after a month of abstinence from bingeing and vomiting compared to healthy volunteer women and patients with anorexia nervosa. This CSF PYY finding has been confirmed in a new and larger group of normal weight bulimic women (M. Lesem, personal communication). In contrast to women with anorexia nervosa, patients with bulimia nervosa were found to have normal CSF NPY levels.

Investigating the etiological role of neuropeptides in women who are ill with eating disorders is problematic, since the compromised nutritional status of these individuals may independently affect neuropeptides levels. Identification and assessment of individuals before the onset of the eating disorder would overcome the influences of impaired nutrition status, but such studies are prohibitive given the low prevalence rates and our current inability to accurately predict future sufferers of eating disorders. One way of determining whether a neuropeptide contributes to etiology of an eating disorder is to determine its status after long-term recovery. Abnormalities in a neurochemical after long-term recovery may indicate a trait-related, rather than state-related, disturbance. To determine the possibility of trait-related neuropeptide disturbances in women with eating disorders, the present study measured plasma leptin and CSF leptin, NPY, and PYY in individuals who were long-term recovered from an eating disorder in comparison to normal control women.

Methods and Materials

Participants

Women who had previously, in their lifetime, met DSM-III-R criteria for bulimia nervosa (RNWB), anorexia nervosa with bulimia (RBAN), and restricting anorexia nervosa (RRAN) were recruited for this study. Subjects had been previously treated in the eating disorders treatment program at Western Psychiatric Institute and Clinic, Pittsburgh, Pennsylvania or were recruited through advertisements. DSM-III-R eating disorder diagnoses were arrived at by clinical interview with a psychiatrist (WHK). To be considered "recovered," subjects had to have at least 1 year or more of 1) stable (defined by weight change of $< \pm 3$ kg) and normal weight (based on Metropolitan height and weight charts (Metropolitan Life Insurance Company 1959)) assessed by self-report; 2) regular menstrual cycles; and 3) having not binged, purged, or engaged in restrictive eating patterns prior to this study. In addition, subjects could not have used psychoactive medication such as antidepressants for the year prior to participating in this study.

Thirty-one healthy women served as a comparison group. These control women (NC) had no history of an eating disorder or any psychiatric, medical, or neurologic illness. They had normal menstrual cycles, and had been within a normal weight range since menarche. They also had no first-degree relatives with an eating disorder. These subjects were recruited through local advertisements.

Both the recovered eating disorder and control groups were medication-free, except for use of oral contraceptives, at the time of study. Past high and low weights of both groups were assessed retrospectively by self-report. Percent ideal weight was calculated by current weight/Metropolitan weight for height (Metropolitan Life Insurance Company 1959) $\times$ 100.

Protocol

Prior to entering the study, subjects gave signed informed consent. They completed a 7-day diary at home in which they listed individual food items consumed, portion sizes, and exercise frequency. Despite methodological limitations inherent to food diaries (Thompson and Byers 1994), a comparison of the diaries completed by the control and recovered eating disorder women confirmed the presence of relatively normal dietary intake in the recovered eating disorder group. These data will be presented elsewhere.

Blood and CSF sampling were undertaken as part of a series of studies investigating the concentrations of various neuropeptides in recovered eating disordered women in 1994. Biological studies were only done during the first 10 days of the follicular phase of the menstrual cycle for all subjects. Subjects were admitted to a research laboratory on the eating disorders unit of Western Psychiatric Institute and Clinic on the evening prior to blood sampling and lumbar puncture (LP) for adaptation to the laboratory and for psychological assessments. Fasting blood sampling and LP were undertaken the next morning. Subjects were served the same standardized, monoamine-controlled diets during their stay in the laboratory.

Current Psychiatric Symptomatology

Current psychopathology was assessed with a battery of standardized instruments and by self-report. All interviews were conducted by trained master's and doctoral level clinical interviewers. Depression was assessed with the Beck Depression Inventory (Beck et al 1961). Obsessions and compulsions were assessed with the Cornell Yale–Brown Obsessive Compulsive Scale (CYBOCS) (Goodman et al 1989a, 1989b). Obsessive concerns with body image, weight, or caloric intake, or compulsive behaviors such as exercise and weighing oneself were excluded from the CYBOCS. Eating disorder symptoms were assessed by the Eating Disorder Inventory (EDI) (Garner et al 1983).

Lumbar Puncture and Blood Sampling

Subjects participated in the LP and blood sampling protocol after completing psychological assessments. Seventeen of the NC, 9 of the RBAN, 10 of the RRAN, and 24 of the RNWB women agreed to have a LP. Subjects fasted from midnight until the procedure was completed (between 8 AM and 10 AM) on the next day. The LP was performed at the L 3–4 interspace with subjects in a left lateral position. The first 12 cc of spinal fluid was placed into a single aliquot that was stored on wet ice during the LP procedure. Immediately after the completion of the LP procedure, this 12-cc aliquot was divided by pipet into 1-cc and ½-cc aliquots, which were immediately frozen on dry ice and stored at −70°C. The aliquots for neuropeptide assay were preserved in ascorbic acid. Leptin and other CSF neuropeptide activities may be influenced by other factors, such as dieting and weight loss (Agren et al 1986; Brady et al 1990; Boden et al 1996; Considine et al 1996; Kolaczynski et al 1996), therefore plasma β-hydroxybutyrate (β-HBA), a ketone body, was assessed as an index of starvation.

Assays

All assays were done by laboratory staff blind to the clinical data. Leptin was quantified using a commercial radioimmunoassay kit purchased from Linco Research, Inc. (cat# HL-81K). In brief, samples were incubated with 125I-leptin and leptin antibody overnight at 4°C. One milliliter of cold precipitating reagent was added, and the samples were incubated at 4°C for 20 min. The tubes were then centrifuged at 3000 *g* for 15 min at 4°C. The supernatant was decanted, and the pellets were counted. A complete set of blank standards (0.5–100 ng/mL) and quality controls were run with each assay. The lower detection limit of leptin is 0.5 ng/mL for plasma and 0.25 ng/mL for CSF. The intraassay coefficients of variation are 6.6 ± 0.8% and 5.5 ± 0.9%, respectively.

CSF NPY (Berrettini et al 1986) and PYY (Berrettini et al 1988) were assayed by radioimmunoassay. For measurement of plasma PYY levels, frozen CSF samples (1.8 mL) were thawed on ice and lyophilized using a speed vacuum concentrator (Savant, Farmingdale, NY). PYY samples were reconstituted in 250 μL of assay buffer, consisting of 0.063 mol/L sodium phosphate and 0.013 mol/L sodium edetic acid (pH 7.4), containing 0.02% sodium azide, 0.1% Triton-X-100, 250 kIU/mL aprotinin, and 1% normal rabbit serum and allowed to sit for 1 hour at 4°C. One hundred microliters of the sample was then pipetted in duplicate for the radioimmunoassay. PYY standard, human PYY antisera, and human PYY tracer were obtained from Penninsula Laboratories, Belmont, California. One hundred microliters of antiserum (1:80,000) was added to each sample and the PYY standard curve. The samples were then incubated for 48 hours at 4°C, after which 100 μL of I^{125} human PYY tracer (3500 counts per tube) was added to each tube, and the incubation continued for another 24 hours at 4°C. At this time, a second antiserum (goat antirabbit gamma globulin) was added, and the incubation continued for an additional 16 hours to precipitate the bound counts. The samples were centrifuged to separate bound from free PYY, and the pellets were counted to determine bound counts/min. The recovery of PYY added to hormone-free CSF was >90%. The detection limit of this assay was 0.9 pg/mL. All samples were measured in one assay run. The intraassay coefficient of variation at 1.5 pg/mL was 12%.

Samples for NPY assay were extracted prior to radioimmunoassay. Frozen CSF samples (1.8 mL for each) were thawed on ice and applied to Sep-Pak C18 cartridges (Waters, Milford, MA), which had been activated with 10 mL methanol followed by 10 mL 0.1% trifluoroacetic acid. The CSF samples were then applied to the cartridges. The cartridges were then washed with 10 mL of 0.1% trifluoroacetic acid. The absorbed peptides were then eluted with 4 mL of 60% CH_3CN and 40% 0.1% trifloroacetic acid. The elutant was collected and the solvent evaporated using a speed vacuum concentrator (Savant, Farmingdale, NY). The radioimmunoassay was performed as described above with the following differences. NPY standard and antibody were obtained from Penninsula Laboratories (Belmont, CA). I^{125}-labeled NPY was obtained from Amersham (Arlington Heights, IL). The recovery of NPY added to hormone-free CSF was >90%. The detection limit of the NPY assay was 25 pg/mL. All samples were measured in one assay run and the intraassay coefficient of variation was 5% at 300 pg/mL.

Radioimmunoassay was also used to measure estradiol (Diagnostic Product Corporation, Los Angeles, CA). β-HBA was measured by the enzymatic method of Williamson et al (1962).

Statistics

BMDP Statistical Software package (Dixon 1985) was used for data analysis. Between-group comparisons were made using one-way analysis of covariance. Post hoc comparisons between groups were made using two-tailed *t* tests. When the assumptions of homogeneity of variance were met according to Levine's test, pooled *t* tests are reported. When the assumption was not met, separate *t* tests are reported. Correlations were examined with linear-regression analysis. Values are expressed as mean ± SD. Bonferroni significance levels are reported to take into account the effects of multiple comparisons.

Results

Participant Characteristics

The characteristics of all participants and of the subset of participants who agreed to take part in the LP are presented in Table 1. Although the mean ages of all groups

Table 1. Participant Characteristics

Characteristic	NC	RBAN	RRAN	RNWB	ANOVA *F*	ANOVA *P*
n	31	13	10	29		
	(18)	(10)	(9)	(24)		
Age (years)	22.2 ± 3.8	25.9 ± 4.7	22.2 ± 3.1	26.1 ± 5.6[a]	4.9	.004
	(23.2 ± 4.3)	(25.9 ± 3.2)	(22.7 ± 3.2)	(26.6 ± 6.1)	(2.0)	(.07)
Body mass index	21.8 ± 1.4	21.3 ± 2.2	19.3 ± 1.2[b,c]	23.1 ± 2.2	10.0	<.00005
	(22.0 ± 1.1)	(20.8 ± 1.9)	(20.0 ± 0.9[c,d])	(23.0 ± 1.8)	(3.2)	(.02)
Current weight	103.5 ± 8.9	103.4 ± 10.3	94.7 ± 7.8[e]	111.5 ± 10.5[a]	7.3	.0002
(% of ideal)	(105.5 ± 7.6)	(100.9 ± 10.0[e])	(97.2 ± 8.9[e])	(111.4 ± 10.2)	(7.3)	(.0003)
Past high weight	108.2 ± 8.8	118.9 ± 16.6	106.4 ± 12.7[e]	124.2 ± 13.4[b]	10.5	<.00005
(% of ideal)	(110.2 ± 8.3)	(119.6 ± 18.7)	(102.6 ± 8.5[c])	(124.6 ± 14.3[d])	(8.4)	(<.00005)
Past low weight	94.6 ± 8.1	69.0 ± 7.3[b,c]	67.8 ± 8.5[b,c]	94.7 ± 8.3	58.1	<.00005
(% of ideal)	(96.3 ± 8.2)	(69.0 ± 7.9[b,c])	(66.3 ± 8.9[b,c])	(94.9 ± 8.3)	(37.5)	(<.00005)
Age of eating disorder onset	0 ± 0	14.6 ± 2.6	14.7 ± 2.1	16.1 ± 3.0	—	—
(years)	(0 ± 0)	(14.8 ± 2.4)	(14.7 ± 2.3)	(16.2 ± 3.2)	(—)	(—)
Duration of recovery	0 ± 0	38.0 ± 49.3	48.0 ± 35.2	36.9 ± 23.3	—	—
(months)	(0 ± 0)	(26.8 ± 19.0)	(51.8 ± 38.6)	(38.5 ± 24.3)	(—)	(—)
EDI—drive for thinness	1.1 ± 2.0	5.5 ± 5.5	4.1 ± 6.6	4.3 ± 5.1	2.7	.05
	(1.7 ± 2.5)	(5.4 ± 5.1)	(4.2 ± 6.3)	(4.4 ± 4.9)	(2.9)	(.04)
Bulimia	0.3 ± 0.8	1.0 ± 1.3	1.8 ± 2.5	1.0 ± 1.8	2.4	.08
	(0.1 ± 0.3)	(1.0 ± 1.3)	(1.8 ± 2.0[a])	(1.5 ± 1.5[a])	(2.5)	(.007)
Body dissatisfaction	3.9 ± 4.8	9.2 ± 8.7[d]	7.7 ± 8.7	12.6 ± 7.5[a]	5.1	.003
	(4.0 ± 6.2)	(9.1 ± 8.3)	(7.8 ± 8.2)	(13.3 ± 8.3[a])	(3.3)	(.02)
BECK	2.3 ± 3.4	7.5 ± 7.1	3.8 ± 4.2	6.8 ± 6.9	3.3	.03
	(3.4 ± 4.0)	(7.1 ± 7.1)	(3.8 ± 3.7)	(8.1 ± 7.9)	(1.6)	(.1)
CYBOCS	3.1 ± 4.1	8.9 ± 7.2	11.3 ± 8.7[a]	8.8 ± 7.5[a]	4.8	.002
	(2.8 ± 3.8)	(9.6 ± 7.6)	(11.0 ± 6.9)	(9.4 ± 7.4)	(2.7)	(.04)
Estradiol (pg/mL)	36.0 ± 35.0	43.8 ± 37.1	56.0 ± 51.0	33.0 ± 54.0	0.5	.6
	(36.2 ± 35.0)	(40.8 ± 25.1)	(56.2 ± 51.4)	(33.6 ± 56.0)	(0.5)	(.7)
β-HBA (μmol/L)	119.0 ± 89.0	133.0 ± 150.0	180.0 ± 21.0	127.0 ± 149.0	1.1	.4
	(118.8 ± 89.0)	(133.0 ± 150.0)	(108.4 ± 20.6)	(131.2 ± 151.6)	(1.1)	(.4)

Data shown are mean ± SD. Data in parentheses refer to characteristics of the subset of participants from whom CSF was collected. ANOVA, analysis of variance; NC, normal control subjects; RBAN, recovered bulimic anorexics; RRAN, recovered restricting anorexics; RNWB, recovered normal weight bulimics; EDI, Eating Disorder Inventory; BECK, Beck Depression Inventory; CYBOCS, Cornell Yale–Brown Obsessive Compulsive Scale; β-HBA, β-hydroxybutyric acid.

[a] Different from NC at 5% significance.
[b] Different from NC at 0.1% significance.
[c] Different from RNWB at 0.1% significance.
[d] Different from NC at 1% significance.
[e] Different from RNWB at 1% significance.

were in the 20s, the RNWB group was significantly older than the other groups (Table 1). The RRAN women had a significantly lower body mass index (kg/m^2) than the NC and RNWB groups. The percent ideal weight of the RRAN women was significantly lower, and the percent ideal current weight of the RNWB women was significantly higher than the NC women. The past highest weight of the RRAN women was significantly lower than that of the RNWB women, and the past highest weight of the RNWB women was significantly higher than the NC women. Not surprisingly, the past lowest weight of the recovered anorexic groups was significantly lower than the past lowest weight of the NC and the RNWB groups. The age of illness onset and the duration of recovery was similar across the recovered eating disorder groups. Despite meeting criteria for recovery, the previously eating disordered groups continued to exhibit elevated scores on the drive for thinness and body dissatisfaction subscales of the EDI, and were more depressed and obsessional than the control group. Plasma estradiol and β-HBA concentrations were similar across all diagnostic groups (Table 1).

Neuropeptide Concentrations

The concentrations of CSF NPY, CSF PYY, CSF leptin, and serum leptin concentrations were similar for all groups (Table 2); however, the CSF/serum leptin ratio in the RNWB group was lower than in the other groups, with the difference being statistically significant between the RNWB and the RBAN groups.

Table 2. Neuropeptide Concentrations in Normal Control Women and Women Recovered from an Eating Disorder

					ANOVA	
Neuropeptide	NC	RBAN	RRAN	RNWB	*F*	*p*
CSF NPY (pg/mL)	185.0 ± 54.0	203.0 ± 36.0	171.0 ± 55.0	202.0 ± 63.0	0.8	.5
CSF PYY (pg/mL)	1.6 ± 0.5	1.4 ± 0.5	1.5 ± 0.3	1.4 ± 0.3	0.5	.7
Serum leptin (ng/mL)	11.0 ± 4.0	9.7 ± 6.0	12.7 ± 6.4	14.4 ± 6.4	1.6	.2
CSF leptin (ng/mL)	0.5 ± 0.1	0.5 ± 0.2	0.5 ± 0.2	0.6 ± 0.2	0.3	.8
CSF/serum leptin	0.05 ± 0.01	0.07 ± 0.04	0.05 ± 0.02	0.04 ± 0.01[a]	3.2	.03

Data shown are mean ± SD. ANOVA, analysis of variance; NC, normal control subjects; RBAN, recovered bulimic anorexics; RRAN, recovered restricting anorexics; RNWB, recovered normal weight bulimics; NPY, neuropeptide Y; PYY, peptide YY; CSF, cerebrospinal fluid.

[a]Different from RBAN at 5% significance.

Relationship of Body Mass Index to Neuropeptide Concentrations

Table 3 shows the correlations between current body mass index and neuropeptide concentrations in the separate diagnostic groups and in the recovered eating disordered women as a whole (RBAN + RRAN + RNWB). Body mass index was negatively correlated with NPY concentration only in the NC group. Body mass index was not significantly correlated with PYY in any of the diagnostic groups. In the RNWB women and in the recovered eating disorder women as a group, body mass index was positively correlated with both CSF and serum leptin concentrations. Body mass index was negatively correlated with the CSF/serum leptin ratio in the recovered eating disorder group as a whole.

Relationship of Serum Leptin Concentrations to Clinical Variables

Serum leptin was negatively associated with NPY concentrations only in the RRAN women (Table 4). In all diagnostic categories serum leptin levels were positively and strongly correlated to CSF leptin levels. Serum leptin concentrations were not associated with estradiol or β-HBA levels (data not shown).

Table 3. Correlations between Current Body Mass Index and Neuropeptide Concentrations in Women Recovered from Eating Disorders

Characteristic	NC	RBAN	RRAN	RNWB	All ED groups
CSF NPY	−.54[a]	.28	−.25	−.06	.16
CSF PYY	.35	.22	−.02	−.16	−.10
Serum leptin	.18	.67	.50	.55[b]	.57[a]
CSF leptin	.23	.53	.59	.56[a]	.56[a]
CSF/serum leptin	−.12	−.40	−.42	−.23	−.40[b]

NC, normal control subjects; RBAN, recovered bulimic anorexics; RRAN, recovered restricting anorexics; RNWB, recovered normal weight bulimics; ED, eating disordered; NPY, neuropeptide Y; PYY, peptide YY; CSF, cerebrospinal fluid.

[a]Significant at the 1% level.

[b]Significant at the 5% level.

Discussion

NPY, PYY, and leptin concentrations are normal in women who recover from AN and BN. These data suggest that alterations of these peptides found in ill AN and BN are likely to be secondary to malnutrition and are not traits that cause AN or BN. We replicated our initial finding of normal CSF NPY after long-term recovery from AN (Kaye et al 1990). Although PYY concentrations have been found to increase after short-term abstinence from bingeing and vomiting in BN (Kaye et al 1990), the present findings imply that this is a transient phenomena and reflects a reactive resetting of the modulation of CNS PYY, rather than a trait-related disturbance.

The low levels of leptin in underweight anorexics and the elevated leptin levels found during refeeding in anorexia nervosa are likely to be secondary to substantial changes in dietary intake; however, the reduced CSF/serum leptin ratio in the RNWB compared to the RBAN women may be of potential interest. For circulating leptin to fulfil its role as signal of adiposity to the brain, it must cross the blood–brain or blood–CSF barriers. Recently, the relationship between plasma and CSF leptin has been best described as nonlinear, such that individuals with high plasma leptin levels have a relatively low proportional leptin concentration in CSF (Schwartz et al 1996). It has been suggested that a saturable, carrier-mediated system

Table 4. Correlations between Serum Leptin and Neuropeptide Concentrations in Women Recovered from Eating Disorders

Characteristics	NC	RBAN	RRAN	RNWB	All ED groups
CSF NPY	.10	.27	−.96[a]	−.10	−.20
CSF PYY	.35	.55	−.65	−.36	−.10
CSF leptin	.80[a]	.78[b]	.80[b]	.88[c]	.83[c]

NC, normal control subjects; RBAN, recovered bulimic anorexics; RRAN, recovered restricting anorexics; RNWB, recovered normal weight bulimics; ED, eating disordered; NPY, neuropeptide Y; PYY, peptide YY; CSF, cerebrospinal fluid.

[a]Significant at the 1% level.

[b]Significant at the 5% level.

[c]Significant at the 0.1% level.

operates to transport leptin into the brain and that, due to the saturation of available transporters, a reduced efficiency of leptin uptake into the brain exists in overweight individuals who tend to have higher leptin levels. It is possible that the reduced CSF/serum leptin ratio in the RNWB group may have been a consequence of saturation of available leptin transporters, since these women were significantly heavier and had slightly higher serum leptin concentrations than the other diagnostic groups; however, the serum leptin concentrations were only slightly, and not significantly, elevated in the RNWB group, and saturation of leptin transporters appears to only occur at leptin concentrations above the normal range (Schwartz et al 1996; Mantzoros et al 1997b). Thus it may be more likely that the reduced CSF/serum leptin ratio in RNWB is not a consequence of high serum leptin levels saturating available transporters, but may be due to some form of impairment in leptin transport to the brain. Such an impairment may erroneously signal a low body fat mass and contribute to the higher body mass index, the drive to overfeed, and/or to the susceptibility of relapse in RNWB relative to RBAN patients. It should be emphasized, however, that the CSF/serum leptin ratio in the RNWB group was not significantly different from the NC group. Thus, although it is possible that the CSF/serum leptin ratio may contribute to the differences in pathophysiology in RNWB and RBAN women, it does not appear to be a specific etiological factor for bulimia nervosa.

Consistent with numerous previous studies of individuals across all weight ranges (Grinspoon et al 1996; Hamann and Matthaei 1996; Sinha et al 1996; Ferron et al 1997; Haffner et al 1997; Mantzoros et al 1997b), body mass index was positively correlated with both serum and CSF leptin concentrations in the recovered eating disorder groups. Leptin messenger RNA expression and synthesis occurs almost exclusively in adipocytes, and it is the increased adipose tissue mass that accounts for the association between body weight and leptin concentrations (Houseknecht et al 1996). The absence of a correlation between body mass index and leptin concentrations in the control women may have been due to the relatively narrow weight range represented in this group. Conversely, the strongest association between body mass index and leptin levels was observed in the RNWB women, who had the widest range in body mass index.

Body mass index was negatively correlated with the CSF/serum leptin ratio in the recovered eating disorder group as a whole. This is consistent with the findings of Mantzoros et al (1997a), who suggested that increased CSF/serum leptin ratio in underweight anorexia nervosa patients reflected an increase in efficiency of leptin uptake under conditions of low plasma leptin and low body weight. Body mass index was also negatively related to NPY, but only in the NC women. This relationship suggests that reduced NPY expression in those with higher weights is a homeostatic mechanism to reduce appetite and body weight. It is not known why such a relationship was not observed among the recovered eating disorder groups, but prior starvation or bingeing and vomiting may uncouple this as a potential mechanism of homeostatic regulation of body weight.

It should be noted that percent body fat correlates with leptin levels to a higher degree than does body weight. A limitation of the present study is that we did not measure body fat, which may have differed between groups independently of body mass index; however, within the same gender and age range body mass index may represent average body fat estimates (Heitmann 1990).

Serum leptin concentrations were negatively correlated with NPY only in the RRAN group. The direction of this association is as would be expected given that, during starvation, leptin production decreases to inform the brain of reduced body fat and NPY levels increase to effect the stimulation of appetite. Conversely, during overfeeding leptin production increases to inform the brain of increased energy intake, whereas NPY levels decrease to effect inhibition of appetite (Sahu et al 1988). In addition, leptin itself has the ability to directly suppress hypothalamic NPY synthesis (Stephens et al 1995). Why, however, was a significant relationship between leptin and NPY only observed for the RRAN group? It is possible that prior starvation and the sustainment of a relatively low mean body weight even after recovery may sensitize these individuals into exceptionally tight coupling of leptin and NPY levels in an effort to counteract possible future extreme body weight states.

The lack of correlation between leptin concentrations and estradiol is similar to the findings if Grinspoon et al (1996). This group suggested that insulinlike growth factor-I (IGF-I), a hormone regulated by nutritional status that falls in response to energy deprivation, may be one factor controlling leptin secretion.

A limitation of the present study is that we did not measure IGF-I, insulin, or tumor necrosis factor-α, all of which are thought to up-regulate leptin production (Grinspoon et al 1996; Mantzoros et al 1997b). Nor did we measure adrenocorticotropic hormone, cortisol levels, or dietary restraint, which have been previously reported to be inversely related to serum leptin concentrations (Mantzoros et al 1996; Von Prittwitz et al 1997). A further limitation of the present study was that not all of the women studied participated in the LP procedure; however, CSF samples were available from the majority of women in each of the recovered eating disorder groups.

In summary, this study does not show evidence for the existence of trait-related alterations in absolute serum

leptin and CSF leptin, NPY, and PYY concentrations in women who have suffered from AN or BN. Such a conclusion assumes that long-term recovered women are in a similar physiological state as they were prior to the onset of their eating disorder, and only prospective studies can confirm this conclusion. We speculate that in RNWB, there may be an abnormality in the efficiency of brain uptake of leptin in bulimia nervosa compared to RBAN. Replication of this finding and studies that permit direct assessment and characterization of leptin uptake into the central nervous system will be required to further investigate this possibility. Although these neuropeptides are unlikely to be predisposing factors in eating disorders, continued exploration of their roles as precipitating and/or maintaining factors and their relationship to neuroendocrine and reproductive function during the acute stages and the recovery period of these illnesses remains warranted.

References

Agren H, Mefford IN, Rudorfer MV, Linnoila M, Potter WZ (1986): Interacting neurotransmitter systems. A non-experimental approach to the 5HIAA-HVA correlation in human CSF. *J Psychiatr Res* 20:175–193.

Argente J, Barrios V, Chowen J, Sinha MK, Considine RV (1997): Leptin plasma levels in healthy Spanish children and in adolescents, children with obesity, and adolescents with anorexia nervosa and bulimia nervosa. *J Pediatr* 131:833–883.

Baranowska B, Wasilawska-Dziubinska E, Radzikowska M, Plonowski A, Roguski K (1997): Neuropeptide Y, galanin and leptin release in obese women and in women with anorexia nervosa. *Metabolism* 46:1384–1489.

Beck, A, Ward C, Mendelson M, Mock J, Erlbaugh J (1961): An inventory for measuring depression. *Arch Gen Psychiatry* 4:561–571.

Berrettini WH, Nurnberger JI, Di Maggio DA (1986): Neuropeptide Y immunoreactivity in human cerebrospinal fluid. *Peptides* 7:455–458.

Berrettini WH, Kaye WH, Gwirtsman H, Allbright A (1988): Cerebrospinal fluid peptide YY immunoreactivity in eating disorders. *Neuropsychobiology* 19:121–124.

Boden G, Chen X, Mozzolli M, Ryan I (1996): Effect of fasting on serum leptin in normal human subjects. *J Clin Endocrinol Metab* 81:3419–3423.

Brady LS, Smith MA, Gold PW, Herkenham M (1990): Altered expression of hypothalamic neuropeptide mRNAs in food-restricted and food-deprived rats. *Neuroendocrinology* 52: 441–447.

Chehab FF (1996): A broader role for leptin. *Nat Med* 2:723–724.

Chehab FF, Lim ME, Lu R (1996): Correction of the sterility defect in homozygous obese female mice by treatment with the human recombinant leptin. *Nat Genet* 12:318–320.

Considine RV, Sinha MK, Heiman ML, Kriauciunas A, Stephens TW, Nyce M, et al (1996): Serum immunoreactive-leptin concentrations in normal-weight and obese humans. *N Engl J Med* 334:292–295.

Dixon J (1985): BMDP Statistical Software. Berkeley, CA, University of California Press.

Erikson JC, Clegg KE, Palmiter RD (1996): Sensitivity to leptin and susceptibility to seizures of mice lacking neuropeptide Y. *Nature* 381:415–421.

Ferron F, Considine RV, Peino R, Lado IG, Dieguez C, Casanueva FF (1997): Serum leptin concentrations in patients with anorexia nervosa, bulimia nervosa and non-specific eating disorders correlate with the body mass index but are independent of the respective diseases. *Clin Endocrinol* 46:289–293.

Garner DM, Olmstead MA, Polivy J (1983): The Eating Disorders Inventory: A measure of cognitive-behavioral dimensions of anorexia nervosa and bulimia. In: Darbey PL, Garfinkel PE, Garner DM, Coscina DV, editors. Anorexia Nervosa: Recent Developments in Research. New York, Alan R. Liss, pp 173–184.

Goodman WK, Price LH, Ramussen SA, Mazure C, Delgado P, Heninger GR, et al (1989a): The Yale-Brown Obsessive Compulsive Scale (Y-BOCS): II. Validity. *Arch Gen Psychiatry* 46:1012–1016.

Goodman WK, Price LH, Ramussen SA, Mazure C, Fleischmann CL, Heninger GR, et al (1989b): The Yale-Brown Obsessive Compulsive Scale (Y-BOCS): I. Development, use and reliability. *Arch Gen Psychiatry* 46:1006–1011.

Grinspoon S, Gulick T, Askari H, Landt M, Lee K, Anderson E, et al (1996): Serum leptin levels in women with anorexia nervosa. *J Clin Endocrinol Metab* 81:3861–3863.

Haffner SM, Miettinen H, Mykkanen, L, Karhapaa P, Rainwater DL, Laakkso M (1997): Leptin concentrations and insulin sensitivity in normoglycemic men. *Int J Obes* 21:393–399.

Hamann A, Matthaei S (1996): Regulation of energy balance by leptin. *Exp Clin Endocrinol Diabetes* 104:293–300.

Hebebrand J, van der Heyden J, Devos R, Kopp W, Herpertz S, Remschmidt H, et al (1995): Plasma concentrations of obese protein in anorexia nervosa. *Lancet* 346:1624–1625.

Hebebrand J, Blum WF, Barth N, Coners H, Englaro P, Juul A, et al (1997): Leptin levels in patients with anorexia nervosa are reduced in the acute stage and elevated upon short-term weight restoration. *Mol Psychiatry* 2:330–334.

Heitmann BL (1990): Evaluation of body fat estimated from body mass index, skinfolds and impedance. A comparative study. *Eur J Clin Nutr* 44:831–837.

Houseknecht KL, Flier SN, Frevert EU, Frederich RC, Flier JS, Khan BB (1996): Leptin secretion by the adipocyte: Regulation with genetic and dietary obesity and search for an intracellular storage pool. *Exp Clin Endocrinol Diabetes* 104(suppl 2):8.

Kaye WH, Berrettini W, Gwirtsman H, George DT (1990): Altered cerebrospinal fluid neuropeptide Y and peptide YY immunoreactivity in anorexia and bulimia nervosa. *Arch Gen Psychiatry* 47:548–556.

Kolaczynski JW, Considine RV, Ohannesian J, Marco C, Openanova I, Nyce MR, et al (1996): Response of leptin to short-term fasting and refeeding in humans. *Diabetes* 45: 1511–1515.

Kopp W, Blum WF, Von Prittwitz S, Ziegler A, Lubbert H, Emons G, et al (1997): Low leptin levels predict amenorrhea in underweight and eating disordered females. *Mol Psychiatry* 2:335–340.

Mantzoros C, Qu D, Frederich R, Maratos-Flier E, Flier JS (1996): Activation of 3 adrenergic receptors suppresses leptin expression and mediates a leptin independent inhibition of food intake in mice. *Diabetes* 45:909–914.

Mantzoros C, Flier JS, Lesem M, Brewerton TD, Jimerson DC (1997a): Cerebrospinal fluid leptin in anorexia nervosa: Correlation with nutritional status and potential role in resistance to weight gain. *J Clin Endocrinol Metab* 82:1845–1851.

Mantzoros CS, Moschos S, Avramopoilos I, Kaklamani V, Liolios A, Doulgerakis D, et al (1997b): Leptin concentrations in relation to body mass index and the tumor necrosis factor-α system in humans. *J Clin Endocrinol Metab* 82: 3408–3413.

Metropolitan Life Insurance Company (1959): New weight standards for men and women. *Stat Bull Metro Metropolitan Insurance Company* 40:1–11.

Morley JE, Blundell JE (1988): The neurobiological basis of eating disorders: Some formulations. *Biol Psychiatry* 23:53–78.

Morley JE, Levine AS, Grace M, Kneip J (1985): Peptide YY (PYY), a potent orexigenic agent. *Brain Res* 341:200–203.

Sahu A, Kalra PS, Kalra SP (1988): Food deprivation and ingestion induce reciprocal changes in neuropeptide Y concentrations in the paraventricular nucleus. *Peptides* 9:83–86.

Schwartz MW, Peskind E, Rasking M, Boyko EJ, Porte D (1996): Cerebrospinal fluid leptin levels: Relationship to plasma levels and to adiposity in humans. *Nat Med* 2:589–593.

Sinha MK, Ohannesian JP, Helman ML, Kraiuciunas A, Stephens TW, Magosin S, et al (1996): Nocturnal rise in leptin in lean, obese and non-insulin-dependent diabetes mellitus subjects. *J Clin Invest* 97:1344–1347.

Stephens TW, Basinski M, Bristow PK, Bue-Valleskey JM, Burgett SG, Craft L, et al (1995): The role of neuropeptide Y in the antiobesity action of the obese gene product. *Nature* 377:530–532.

Thompson FE, Byers T (1994): Dietary assessment resource manual. *J Nutr* 124:2245S–2317S.

Von Prittwitz S, Blum WF, Ziegler A, Scharmann S, Remschmidt H, Hebebrand J (1997): Restrained eating is associated with low leptin levels in underweight females. *Mol Psychiatry* 2:420–422.

Williamson DH, Mellanby J, Krebs HA (1962): Enzymatic determination of D(-)β-hydroxybutyric acid and acetoacetic acid in blood. *Biochem J* 82:90–96.

Zhang Y, Proenca R, Maffeui M, Barone M, Leopold L, Friedman JM (1994): Positional cloning of the mouse obese gene and its human homologue. *Nature* 372:425–432.

Zipfel S, Specht T, Blum WF, Hebebrand J, Englaro P, Hartmann M, et al (1998): Leptin — a parameter for body fat measurement in patients with eating disorders. *Eur Eating Disord Rev* 6:38–47.