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ORIGINAL ARTICLE

Prospective evaluation of leptin and neuropeptide Y (NPY) serum levels in girls with anorexia nervosa

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Abstract

OBJECTIVES: The pathogenesis of anorexia nervosa (AN) remains still unclear. It has been reported that neuropeptides may play a role in the control of appetite and hormone release contributing to hormonal disturbances in AN. However the question if neuropeptide alterations are consequence or cause of malnutritrion is still unresolved.

METHODS: Serum leptin, neuropeptide Y (NPY) concentrations as well as hormones (FSH, LH, estradiol, cortisol and fT4) serum levels were prospectively estimated in 19 girls aged 11,7–17,7 years (mean 15,5 years) with anorexia nervosa (AN) at the admission to the hospital (baseline) and at follow–up after 7,21±2,32 months of treatment. The treatment consisted of hypercaloric diet, psychotherapy and vitamins supplementation.

RESULTS: Mean leptin concentration significantly increased from 7,99±2,6 to 9,98 ± 2,48 μg/ml ($p < 0,01$), whereas mean NPY concentration significantly decreased from 34,10 ± 9,81 to 29,6 ± 8,04 pmol/l ($p < 0,01$). leptin/BMI ratio was constant, while NPY/BMI ratio decreased. There were no significant differences between leptin and NPY serum concentrations at baseline and follow-up in eumenorrheic vs. amenorrheic patients. Simple linear correlation analysis showed negative correlation between leptin and NPY concentrations at baseline (r=–0,67; $p < 0,05$) and at follow-up (r=–0,76; $p < 0,05$) only in eumenorrheic subgroup. There were no significant correlations between leptin, NPY and BMI and body weight values.

CONCLUSIONS: (1) Serum concentration of leptin increases and serum concentration of NPY decreases significantly during the treatment of anorectic girls. (2) These changes do not correspond with increasing body weight and BMI suggesting disregulation of appetite and body weight control mechanisms in AN. (3) Altered neuroregulation of the neuropeptides (leptin and NPY) secretion may contribute persistent amenorrhea after weight gain in anorectic patients with low initial BMI.

Abbreviations and units

AN	anorexia nervosa
NPY	neuropeptide Y
LH	luteinizing hormone
FSH	follicle stimulating hormone
fT4	free thyroxine
BMI	body mass index
IU	international units

Introduction

The pathogenesis of anorexia nervosa remains still unclear. It is postulated that some neuropeptides playing an important role in the control of appetite and hormonal secretion may be involved in development of this disease [1,4,6,10,11,16–18,23].

NPY is 36-amino-acid peptide that is a potent activator of feeding behaviour regulating appetite via central and peripheral mechanisms. [11,16]. Leptin is a hormone product of ob gene secreted predominantly by adipose tissue cells. It has been regarded as a hormonal signal linking adipose tissue status with a number of key central nervous system circuits [1,4,6,17,23]. Leptin may decrease food intake and reduce body weight by decreasing NPY synthesis or by inhibiting NPY's action as an appetite stimulant [19,20]. Previous studies demonstrated reduced leptin concentrations in patients with AN that increase during refeeding and weight gain [8,13,14]. Underweight AN patients have significantly elevated CSF NPY levels normalising after long-time recovery, whereas plasma NPY concentration remained unchanged [15].

Alterations of neuropeptide and neurotransmitter secretion affect the hormonal secretion in AN [6,22]. However it is uncertain whether these disturbances are a cause or consequence of symptoms. Some patients with AN cannot easily "reverse" their illness and even after weight gain and normalized eating patterns, many individuals who have recovered from AN or BN have physiological, behavioral and psychological symptoms that persist for extended periods of time [2].

Therefore the aim of this study was the longitudinal prospective evaluation of relationship between leptin and NPY and hormones (FSH, LH, estradiol, cortisol and fT4) during the treatment in anorexia nervosa.

Subjects and methods

The subjects were 19 adolescent girls aged 11,7–17,7 years (mean 15,5 years) meeting the diagnostic DSM-IV criteria for anorexia nervosa hospitalized due to acute phase of the disease. All girls were amenorrhoeic at the admission. The duration of clinical symptoms of AN was 4–44 months. After 4–8 weeks of hospitalization the patients were discharged and treated as outpatients. The treatment included hypercaloric diets, psychoterapy and oral administration of multivitamin tablets (Multivitamin, 3 tablets a day) containing 500 IU of vitamin D3, 1250 IU of vitamin A, 2.5 mg of vitamin E, 5 mg of vitamin B1, 2.5 mg of vitamin B2, 1.5 mg of vitamin B6, 25 mg of vitamin C, 60 mg of nicotinamide and 10 mg of calcium pantothenate. All patients gave their informed consent for the study. Antropometric measurements (body weight, height, BMI) as well as leptin, NPY, hormones (FSH, LH, estradiol, cortisol and fT4) serum concentrations evaluation were performed at the admission to the hospital (baseline) and at follow–up after 7,2 ± 2,3 months of treatment. Clinical characteristic of the group is given in *Table 1*.

Table. 1 Baseline and follow-up body weight, BMI, plasma leptin, NPY and hormones concentrations in girls with anorexia nervosa

	Baseline	Follow-up	
Body weight [kg]	41,51±6,85	48,98±9,08	$p<0.0001$
BMI [kg/m2]	15,79±2,01	18,50±2,51	$p<0.0001$
Leptin [µg/l]	7,99±2,60	9,98±2,48	$p<0.01$
Leptin/BMI ratio	0,51±0,19	0,55±0,16	ns
Neuropeptide Y [pmol/l]	34,10±9,81	29,63±8,04	$p<0.01$
Neuropeptide Y/BMI ratio	2,16±0,60	1,60±0,39	ns
LH [mIU/ml]	2,02±2,60	3,68±3,20	$p<0.05$
FSH [mIU/ml]	3,35±3,36	4,00±2,52	ns
Estradiol [pg/ml]	38,27±32,07	61,81±55,02	ns
Cortisol [µg/dl]	16,21±5,56	18,38±10,78	ns
fT4 [ng/dl]	1,16±0,25	1,19±0,29	ns

Follow-up data were analysed in whole group and also in amenorrheic (n=9) and eumenorrheic (n=10) subgroups. Patient was considered as eumenorrheic if menses occurred for 3 consecutive months. Weight and BMI initial values were significantly higher in eumenorrheic vs. amenorrheic subgroup (44,5 ± 5,3 kg vs. 38,2 ± 6,9 kg; $p < 0,05$ and 16,7 ± 1,7 kg/m2 vs. 14,8 ± 1,9 kg/m2 respectively) (data not shown).

All blood samples were collected in fasting state between 8.00 and 9.00 a.m. Serum NPY and leptin concentrations were measured by radioimmunoassay commercial kits (Euria NPY, Netherlands and Human Leptin Linco Research, USA) Sensitivity of NPY assay was 6 pmol/l and inter assay and intraassay coefficients of variance were 3,9 % and 12,7% respectively. Sensitivity of leptin assay was 0,5 ng/ml and inter assay and intraassay coefficients of variance were 8,3 % and 6,2 % respectively. Hormones concentrations were performed at the hospital laboratory using Elecsys 2010 analyser (Roche Diagnostics).

The data are presented as means ± SD. Statistical analysis was performed using the program Statistica 6.0. T-test for dependent and independent samples and Wilcoxon test or U-Mann Whitney test (for distribution different than normal) were used. Correlations were analyzed by Pearson linear correlation or Spearman test.

Results

Mean leptin concentration significantly increased, but leptin/BMI ratio remained unchanged. Mean NPY concentration significantly lowered during the observation period and NPY/BMI ratio decreased non-sig-

nificantly. We observed significant increase in serum LH concentration, FSH and estradiol concentrations also tended to increase (Tab.1). There were no significant differences between leptin, NPY serum concentrations and leptin/BMI, NPY/BMI ratios at baseline and follow-up in eumenorrheic vs. amenorrheic patients. Eumenorrheic patients had non-significantly greater serum LH, FSH and estradiol concentrations and significantly lower concentration of cortisol (Tab.2). Simple linear correlation analysis showed negative correlation between leptin and NPY concentrations at baseline (r=–0,67; $p < 0,05$) and at follow-up (r=–0,76; $p < 0,05$) only in eumenorrheic subgroup. There were no significant correlations between leptin, NPY and body weight, BMI values and hormonal parameters.

Table 2: Comparison of antropometric and hormonal follow-up data between amenorrheic and eumenorrheic patients

	Amenorrheic	Eumenorrheic	
Body weight [kg]	44,86±7,18	52,69±9,32	ns
BMI [kg/m2]	17,34±2,39	19,54±2,23	ns
Leptin [µg/l]	10,27±1,8	9,72±3,03	ns
Leptin/BMI ratio	0,60±0,11	0,51±0,19	ns
Neuropeptide Y [pmol/l]	26,77±7,85	32,2±7,68	ns
Neuropeptide Y/BMI ratio	1,54±0,41	1,65±0,38	ns
LH [mIU/ml]	2,82±3,19	4,46±3,16	ns
FSH [mIU/ml]	3,06±2,37	4,94±2,12	
nsEstradiol [pg/ml]	64,81±79,42	59,72±34,13	ns
Cortisol [µg/dl]	18,26±10,20	11,73±6,66	p<0,005
fT4 [ng/dl]	1,09±0,32	1,28±0,25	ns

Discussion

We observed a significant increase in serum leptin concentration during weight restoration in anorectic patients. These results are consistent with our [24] and other authors' previous findings [1, 8,12,13].

However the correlation between serum leptin levels and BMI before and after refeeding was weak and non-significant. These data are in agreement with the results published by Eckert et al. [8], but not with those of Grinspoon and Haluzik [9,13]. However it should be noted that in Haluzik and Grinspoon's anorectic group there were no patients with BMI below 13 kg/m2. On the other hand, in our group as well in Eckert's group there were several patients with lower BMI (one of our patient had BMI 11,9 kg/m2). Thus may support hypothesis of Eckert et al. that under a critical threshold value of body fat leptin cannot further decrease physiologically [8]. Also Balligand et al. showed that the acute regulation of leptin by positive changes in energy balance is not preserved under critical threshold of body fat [3]. The dissociation between serum leptin levels and BMI after recovery is more difficult to explain. The similar results were obtained by Haluzik et al suggesting that the degree if correlation between serum leptin and the BMI depends on the initial value of BMI. It is known that the increase of the BMI in anorectics with lower initial BMI is predominantly the result of increased lean body mass. In our anorectic group initial BMI (15,8 kg/m2) was low and similar as in Haluzik's group (15,4 kg/m2).

We observed that the leptin/BMI index was constant before and after the treatment. Śmiarowska proved that leptin/body fat mass index do not differ between anorectic, obese and healthy subjects suggesting leptin secretion from adipose tissue is not related to the nutritional state [21].

We found that mean serum concentration of NPY decreased significantly after weight recovery. There is only a few data concerning prospective evaluation of NPY serum concentrations in anorectic patients. Nedvedkova et al. did not observe any difference between NPY serum concentrations in anorectic patients before and 1 month after partial recovery [17]. This period of observation is however very short in our opinion. Our patients were followed-up for over 6 months. On the other hand Escobar et al. demonstrated decreased NPY serum levels after weight recovery in anorectic patients who were nutritionally treated during 16 weeks [9]. There were no correlation between body fat percentage, BMI and serum NPY concentration in anorectic patients while serum NPY correlated significantly with total adiposity in healthy and obese subjects in this study. These findings are consistent with our observations suggesting that the same as for leptin a loss of physiological control between leptin, NPY and adipose tissue content occurs below treshold value of body fat percentage. The source of NPY in peripheral plasma and the probable mechanism of its effect on food intake are an open question. It can be speculated that NPY originates from pancreas and other sites of the gut or from the adrenal medulla rather than from the brain [5]. It is known that there are interactions between NPY and catecholamines as orexigenic signals stimulating carbohydrate intake [16]. Bartak et al. provided evidence of elevated baseline and exercise-induced sympathetic nervous activity in AN patients [7].

The most important finding derived by the study is lack of negative feedback between leptin and NPY serum concentrations in amenorrheic patients before and after the body weight normalisation. Mean serum concentrations of leptin and NPY were comparable in amenorrheic and eumenorrheic patients. However eumenorrheic subgroup had higher was initial BMI and weight value than amenorrheic group. Therefore we cannot ascertain if these changes are primary or secondary result of body weight loss. Taking into consideration that the dissociation between serum leptin and NPY concentrations in amenorrheic subgroup persists after normalisation of body weight it may be speculated that other factors than changes in body weight may be involved in it. It has been observed that weight recovery in patients with AN is not always associated with resumption of menses and frequently there is no difference between amenorrheic and eumenorrheic women with AN indicating that other endocrine axes should be normalised too [1]. Recently it has been proposed that

anorexiogenic effect of leptin may be mediated not only by the inhibition of NPY synthesis but also by stimulation of CRH synthesis in the hypothalamus [23]. Interestingly we observed significantly higher cortisol level in amenorrheic group (*Table 2*).

Taking these data together we speculate that altered neuroregulation of the neuropeptides (leptin and NPY) secretion in amenorrheic patients with AN is at least in part contributed by mechanisms independent on body weight and BMI. We cannot however ascertain if these mechanisms are primary or secondary to the lost of weight.

The authors are aware that this study has some limitations: lack of the control group and non-evaluated body composition.

We conclude that:

1) Serum concentration of leptin increases and serum concentration of NPY decreases significantly during the treatment of anorectic girls.
2) These changes do not correspond with increasing body weight and BMI suggesting disregulation of appetite and body weight control mechanisms in AN.
3) Altered neuroregulation of the neuropeptides (leptin and NPY) secretion may contribute peristent amenorrhea after weight gain in anorectic patients with low initial BMI.

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