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Plasma Levels of Neuropeptide Y and Peptide YY in Patients Diagnosed with Anorexia Nervosa and Type 2 Diabetes Morbid Obese Subjects

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Abstract

Anorexia Nervosa (AN) and Obesity are prevalent in modern societies.

This study evaluate circulating levels of Neuropeptide Y (NPY) and of Peptide Tyrosine-Tyrosine (PYY) in 60 women: 20 affected by AN (Body Mass Index = BMI 15.74 ± 2.09 kg/m^2, age 30.19 ± 10.52 yr), 10 restrictor (BMI 14.89 ± 1.64 kg/m^2) and 10 binge-purge subtype (BMI 18.27 ± 0.81 kg/m^2); 20 affected by severe Obesity (BMI>40 kg/m^2; age 33.56 ± 5.2 yr) with type II diabetes with no therapy, and 20 healthy controls (BMI 22.06 ± 0.93 kg/m^2, age 32.44 ± 4.35 yr).

NPY is higher in AN than obese and controls (70.17 ± 20.84 vs 25.12 ± 7.26 and 52.20 ± 10.88 pmol/L; p<0.001) and lower in obese than controls (p<0.001). PYY is higher in AN than obese and controls (219.77 ± 83.51 vs 116.42 ± 41.42 and 94.97 ± 12.74 pg/ml; p<0.001), with no differences between obese and controls.

In AN, NPY and PYY are quite higher (p=0.059; p=0.06) in restrictor (75.77 ± 20.86 pmol/L; 241.78 ± 84.6 pg/ml) than in binge-purge subtype (53.39 ± 8.72 pmol/L; 153.73 ± 29.45 pg/ml).

Increase of NPY despite simultaneous PYY increase in AN might be related to reduced sensitivity to PYY inhibitory effect on NPY production or increased production of NPY from sympathetic peripheral nervous system, a finding evident mainly in restrictor AN.

In obese PYY is close to controls suggesting a reduced intestinal production of this peptide because of the stimulus of continuous overfeeding, whereas the reduced NPY production could be explained by increased levels of insulin and leptin. Reduced NPY levels suggest that in these obese the overfeeding is not dependent on increased hungry signal, but on inadequate satiety signal.

Keywords: Neuropeptide Y; Peptide YY; Food intake; Restrictor and binge-purge subtype of Anorexia Nervosa

Introduction

Anorexia Nervosa (AN) and obesity are prevalent in modern societies. AN is a multifaceted disease characterized by disorganized feeding behavior, food adversion and strong attention to body shape and weight [1], accompanied by psychiatric symptoms such as depression and/or obsessive-compulsive disorder [2,3]. AN is the most common form of eating disorders in western society having a prevalence of 0.1-1% in general population, 0.3% in women and 0.1% in men. This condition largely affects young adolescent women, with between 15 and 25 years old making up 40% of all cases and the risk of mortality is 5–20% [4-7]. Obesity is a chronic condition characterized by an accumulation of body fat. The prevalence of the disease is progressively increasing in industrialized nations as a consequence of adoption of those life style changes typical of the western culture, especially regarding diet and physical inactivity [1,5].

The food intake is regulated by long- and short-term acting mediators [8-12] and involves complicated associations between neuropeptides and other neurotransmitters in the central nervous system (CNS) [13-17]. In people with eating disorders the disturbances of these neuroendocrine pathways could be responsible of frequent symptoms as refusal to eat, denial of hunger, irritability, feeling of physical efficiency up to excessive exercise [2]. Long-term acting mediators are hormonal signals produced by the pancreas such as Insulin, as well by the adipose tissue like Leptin and Adiponectin, all communicating with the brain about body fat and energy storage. These hormones do participate in the long-term regulation of energy homeostasis and body weight maintenance. Short-term acting mediators are peptides produced by enteroendocrine cells interspersed among the gastrointestinal tract; they act through the bloodstream or the vagus nerve on the CNS. These satiety signals [18,19] are meal-related and are effective in maintaining adequate meal size according to the energy expenditure and long-term maintenance of body weight [20,21]. Peptide Tyrosine-Tyrosine (PYY) is the most important anorexigenic substance [22] belonging to the pancreatic polypeptide family [23-25]. Anorexigenic effect is likely the consequence of PYY binding to the Y2 receptor, resulting in presynaptic inhibition of neuropeptide Y (NPY) neurons in the accurate nucleus (ARC) [25-31]. The hypothalamic ARC seems to play a crucial role in receiving and integrating these signals, it is incompletely isolated from the general circulation by the blood–brain barrier, allowing direct access of circulating factors to ARC neurons [32]. NPY is a 36-aminoacid neurotransmitter also belonging to the pancreatic polypeptide family; it is one of the most important orexigenic agents [33]. Animal experimental evidences suggest that NPY is coreleased with norepinephrine (NE) from sympathetic nerve endings and is involved in noradrenergic neurogenic vascular control of skeletal muscle [34]. However, most of NPY neurons are in the ARC: they are inhibited by insulin and/or leptin [35,36] and by PYY [25,37], while they are stimulated by restriction of food, starvation and ghrelin

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[38]. NPY interacts with the orexigenic Y_1 and Y_5 receptors in different brain areas [39].

The food intake disorders could be associated to the variation of some neuroendocrine signals that would then be involved in the etiopathogenesis of AN and morbid obesity.

The aim of this study is to measure circulating levels of NPY, a powerful feeding stimulator, and of PYY, inhibitor of NPY neurons as well an anorexigenic peptide, in AN and morbid obesity to evaluate whether peptide disturbances are cause or consequence of eating disorders.

Material and Methods

Subjects

The study group involved forty consecutive women attending to the Diet Health Education Clinic of Internal Medicine Department from May 2010 to May 2011: 20 diagnosed with AN according to the criteria of Manual Statistic Diagnostic IV edition (DSM-IV) and 20 diagnosed with no treated type 2 diabetes morbid obese subjects.

Ten of 20 (50%) AN subjects were restrictors, 10 were *binge-purge* subtype.

Twenty healthy, normal weight ages matched women recruited from the Department staff were enrolled as control group (Table 1).

All subjects gave informed consent and the ethical committee of the hospital approved the study proposal.

Data collection

Age was defined as age in years at the time of the medical visit. Weight and height were performed in the morning before 8 a.m. after an overnight fast (12 h). The body mass index (BMI) was calculated as body weight/height^2 (in kg/m^2). The depression and the obsessive-compulsive disorder were employed by Beck Depression Inventory (BDI). Fasting venous blood samples were collected into EDTA (ethylenediaminetetraacetic acid) to 6% (100 µl for 5 ml of blood)-treated tubes and immediately centrifuged at 4°C for 15 min. Plasma was divided into aliquots and stored at -70°C until assay.

Laboratory analysis

NPY in plasma samples was assayed by a competitive radioimmunoassay (RIA) (Euro-Diagnostic Sweden) using an antiserum raised against synthetic NPY conjugated to bovine thyreoglobulin. NPY in standards and samples competes with ^{125}I-labelled NPY in binding to the antibodies. ^{125}I–NPY binds in a reverse proportion to the concentration of NPY in standards and samples. Antibody-bound ^{125}I–NPY is separated from the unbound fraction by using a double antibody coupled to solid phase. The radioactivity of the antibody-bound ^{125}I–NPY is measured. The antiserum used in this method cross-reacts less than 2.0% with human PYY. The intra- and inter-assay variability for plasma NPY was 5.0% and 8.4%, respectively, and the lower limit of sensitivity was 3 pmol/l.

Plasma PYY concentrations were measured using a human PYY (3-36) RIA (DRG Germany), with 100% cross-reactivity with the two biologically active forms of PYY (1-36 human PYY and 3-36 human PYY) and no cross-reactivity with NPY, pancreatic polypeptide, insulin, glucagon, amylin amide, or substance P. Intra and inter-assay coefficient of variation of the assay were 5.6% and 6.7% respectively, and the lower limit of detection was 2.8 pg/ml.

Statistical analysis

All data were expressed as mean ± standard deviation of the mean. Results were analyzed by a commercial software package (NCSS) using unpaired *t* test for single comparisons (all data were normally distributed). Correlations were sought by linear regression analysis. Statistical significance was defined as $P < 0.05$.

Results

By BDI, eleven of twenty (55%) AN presented mild depression, nine patient (45%) had obsessive-compulsive disorder. Morbid obese patients had no depressive symptomatology.

BMI was significantly lower ($p<0.001$) in AN patients than in no-treated type 2 diabetes morbid obese subjects and normal weight healthy subjects.

BMI of restrictor was significantly lower ($p < 0.001$) than *binge-purge* (14.89 ± 1.64 vs 18.27 ± 0.81 kg/m^2) subtype.

Higher NPY level was observed in AN than type 2 diabetes morbid obese and normal weight subjects (70.2 ± 20.8 vs 25.1 ± 7.3 and 52.2 ± 10.9 pmol/L, respectively; $p<0.001$) and lower in type 2 diabetes morbid obese and normal weight subjects (25.1 ± 7.3 vs 52.2 ± 10.9 pmol/L; $p<0.001$).

Higher PYY was also shown in AN than morbid obese and normal weight (219.8 ± 83.5 vs 116.4 ± 41.4 and 95 ± 12.7 pg/ml, respectively; $p<0.001$), while in type 2 morbid obese and normal weight was similar.

	Anorectics n=20	**AN restrictor** n=10	**AN binge-purge** n=10	**Morbid obese** n=20	**Controls** n=20
Age (years)	30.2±10.5	32.4±11.4	23.5±0.6	33.6±5.2	32.4±4.3
Body Weight (kg)	41.6±5.5	39.2±3.1	48.9±4.5	122.8±13.8	66.2±5.8
BMI (kg/m^2)	* 15.7±2.1	*** 14.9±1.6	18.3±0.8	** 52.8±3.9	22.1±0.9
NPY (pmol/L)	* 70.2±20.8	75.8±20.9	53.4±8.7	** 25.1±7.2	52.2±10.9
PYY (pg/ml)	* 219.8±83.5	241.8±84.6	153.7±29.4	116.4±41.4	95±12.7

BMI: * p<0.001 AN vs morbid obese/controls; ** p<0.001 morbid obese vs controls;
*** p<0.001 restrictor vs binge/purge AN subtypes
NPY: * p<0.001 AN vs morbid obese/controls; ** p<0.001 morbid obese vs controls
PYY: * p<0.001 AN vs morbid obese/controls

Table 1: Study subjects' characteristics and NPY and PYY plasma concentrations.

We observed tendency to higher NPY and PYY levels in restrictor than *binge-purge* AN subtype (75.8 ± 20.9 pmol/L vs 53.4 ± 8.7 pmol/L and 241.8 ± 84.6 pg/ml vs 153.7 ± 29.4 pg/ml, respectively) (Table 1).

There was an inverse correlation between BMI of patients and the NPY (r= -0.75; p <0.001) as well PYY (r= -0.40; p<0.001) concentrations (Figures 1 and 2). Direct correlation exists between NPY and PYY plasma levels (r= 0.3, p<0.05) (Figure 3).

Discussion

Studies on the role of the chemical mediators and their mechanisms in the regulation of food intake have been ongoing for several years but results are still conflicting [38,40,41].

In the present paper, we found in anorectic's women concomitant increase of both NPY and PYY concentrations. This unexpected result might be related to reduced sensitivity of Y2 receptors to PYY inhibitory effect on NPY production, or to increased production of NPY from sympathetic peripheral nervous system, a finding frequently reported mainly in restrictor AN.

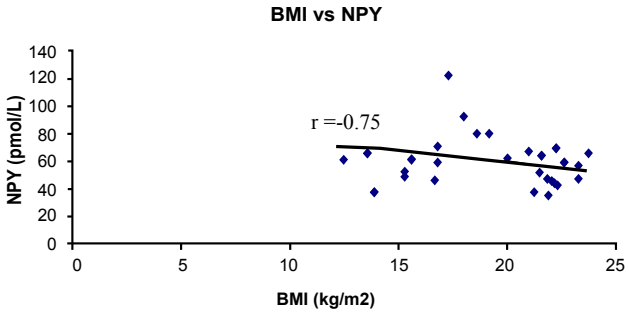

Figure 1: Correlation between BMI and NPY in all subjects.

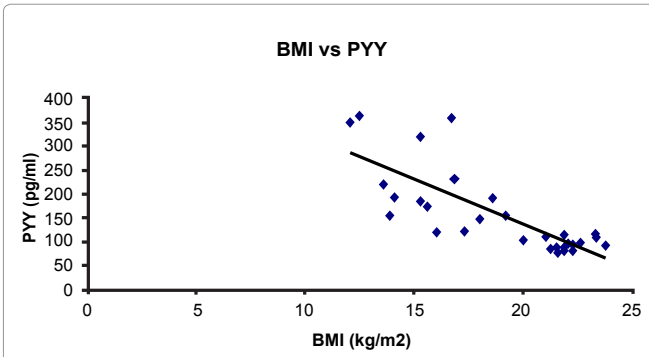

Figure 2: Correlation between BMI and PYY in all subjects.

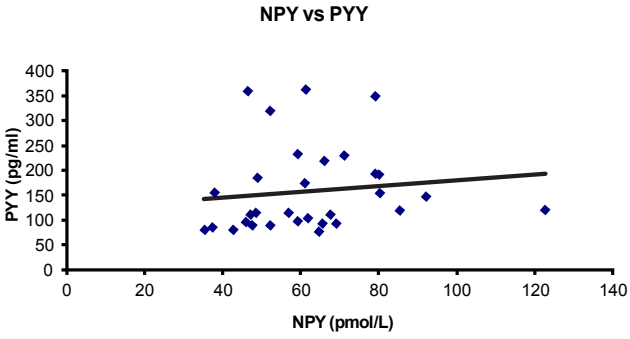

Figure 3: Correlation between NPY and PYY in all subjects.

In anorectic patients NPY, although elevated, could not carry on its orexigenic activity, as if a reduced responsivity of Y1 and Y5 receptors could be operative with a mechanism similar to the insulin resistance in DMT2 and obesity [42]. Alternatively NPY, although operative, could not succeed to stimulate eating because other signals interfere. At this regard, we did find that serotonin in AN is higher than in type 2 diabetes morbid obese patients and normal weight healthy subjects (unpublished data).

In *binge-purges* NPY was found lower than in restrictors. Since the food intake reduction in *binge-purges* alternates with binging [2-4,43], NPY production is irregular, thus the resistance of Y1-Y5 receptors unlikely may develop. Moreover, in *binge-purges* serotonin (unpublished data) is lower than in restrictors, this allowing rise of food intake.

These results, together considered, address the hypothesis that despite in AN there is an hyper-production both of NPY and PYY [44,45], neither seems working properly, because of the receptors desensitization. Since NPY and PYY bind to the receptors of the same family [25,39], they could be inactivated by the same pathways.

Finally, we did not find any correlations between mental symptoms and hormone concentrations in anorectic patients.

In morbid obese subjects, PYY is close to controls suggesting a reduced intestinal production because of continuous overfeeding, whereas the reduced NPY production could be explained by increased levels of insulin and leptin. Reduced PYY and NPY levels indicate that in these patients the overfeeding is not dependent on increased hungry signal, but on inadequate satiety signal. The PYY deficiency may contribute to the pathogenesis of obesity [46]. As a support, PYY administration leads to a decrease in food intake in rodent models [47] as well as in obese adults [48].

As suggested [45], changes in chemical mediator's activity, some of them still unknown, lead to food intake disorders which trigger a vicious circle; moreover the simultaneous hyper-production of orexigenic and anorexigenic signals sends confusing messages to hypotalamic areas [45].

Further, BMI is closely correlated with PYY concentrations, as well NPY, indicating a continuum between nutritional status and neuropeptides behavior.

In conclusion, for the first time this study shows significant differences between restrictor and *binge-purge* AN subtype as for as the evaluated parameters are concerned. The better nutritional status should positively influence the plasma levels of chemical mediators.

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