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Plasma levels of leptin and orexin A in the restrictive type of anorexia nervosa

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ABSTRACT

Objective: The aim of the study was to analyze the pattern of leptin and orexin A plasma levels in patients with the restrictive type of anorexia nervosa (AN-R), during the course of treatment. Thirty females with AN-R, aged 18.0 ± 1.6 years (mean ± SD), range of 15.5–21.0 years, were investigated before and after 2, 3, and 6 months of treatment, which included a normocaloric diet and cognitive–behavioral psychotherapy. The control group consisted of 20 age-matched, healthy control females.
Results: Before the therapy, both leptin and orexin A plasma levels were significantly lower than in the control group and were negatively correlated. During treatment, leptin levels increased and, after 6 months, showed a correlation with body mass index (BMI). Orexin A levels showed a further decrease during treatment, with no correlation with BMI.
Conclusions: The results corroborate those of other researchers showing a decrease of leptin levels in patients with AN-R and its increase with body mass increment. They may also suggest a possible relationship between leptin and orexin A plasma level patterns in such patients.

1. Introduction

Anorexia nervosa (AN) is a disorder characterized by abnormal eating behavior and by weight changes which are reflected in multiple endocrine and metabolic abnormalities. Current diagnostic criteria (Diagnostic and Statistical Manual — DSM-IV) distinguish between two subtypes of AN: restricting anorexics (AN-R) and binge-eating/purging anorexics (AN-BP) [1]. In both subtypes, dysregulation of anorexigenic and orexigenic signaling seem to play a prominent role. The main anorexigenic peptide is leptin, of which the primary source is adipose cells. Leptin regulates energy balance throughout the body by controlling processes involved in energy intake and utilization [2]. Underweight AN patients have consistently been found to have significantly lower plasma leptin concentrations compared to normal weight controls. In such patients, leptin levels showed an increase with concomitant weight gain and correlated with the body mass index (BMI) [3,4].

A number of orexigenic peptides have been identified, including ghrelin and orexins. Orexins are expressed in the lateral hypothalamic area, which has been described as the "feeding center", and these peptides were initially characterized as orexigenic (appetite-stimulating) factors [5]. However, subsequently, a number of other roles for orexins have been elucidated, including sleep, thermoregulation, energy homeostasis and reward systems [6–10]. An important functional difference between orexin A and orexin B is that only orexin A, but not orexin B can cross the brain–blood barrier [11]. Orexin A can be determined in human plasma and its level may reflect the peptide coming from the brain but also that produced in the gut [12,13]. The results of studies on orexin A plasma levels in patients with AN compared to control subjects are inconsistent [14,15].

It has been found that leptin and orexins may interact in a number of ways. Muroya et al. [16] demonstrated that orexins regulate the activity of rat hypothalamic neurons in a manner reciprocal to leptin. On the other hand, leptin receptor in the lateral hypothalamic area can exert a modulatory activity on orexin neurons [17].

In our previous study, we investigated plasma levels of another orexigenic peptide, ghrelin, in AN patients, in the course of treatment. Before therapy, ghrelin plasma levels were significantly higher compared with the control group, and after 6 months of treatment, the levels were significantly lower than in the controls and correlated with an increase of BMI [18].

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The aim of the current study was to analyze the pattern of leptin and orexin A plasma levels during 6-month treatment of patients with the restrictive type of anorexia nervosa (AN-R), as well as the relationship between these two peptides, and their association with BMI changes.

2. Participants

Thirty females suffering from anorexia nervosa of the restrictive type (AN-R), aged 18.0 ± 1.6 years (mean ± SD), range 15.5–21.0 years, who completed 6-month inpatient and outpatient treatment courses were included in the study. They were diagnosed as AN-R according to the DSM IV criteria [1]. Initially, they all had a ≥15% body weight loss and their body mass index (BMI) was below 17.5 kg/m^2. The mean duration of the disease before admission to the hospital, established by interviews with the patients and their families, was 11.5 ± 7 months. Patients with a concomitant diagnosis of depression or anxiety disorder (including obsessive–compulsive disorder) were not included into the study.

All the patients were hospitalized for up to 3 months in the Child and Adolescent Psychiatry and Psychotherapy Ward at the John Paul II Pediatric Center in Sosnowiec. During hospitalization the patients received treatment including a normocaloric diet (2300 kcal/day: 98.4 kcal/day of protein, 75 kcal/day of fat, and 376 kcal/day of carbohydrates), supervised meal consumption (the meals were provided 5 times a day) and cognitive–behavioral psychotherapy (CBT). CBT was performed by a team of psychiatrist, psychologist and a nurse. It was based on the constant number and time of meals. An individual system of positive and negative reinforcements was introduced. A positive reinforcement was given for an increase and a negative reinforcement for a decrease in body weight. Those patients who reached a BMI of 16.5 kg/m^2 were allowed to leave the hospital for weekends. Psychotherapy was performed for 45 min twice a week on an individual basis by one psychologist and 60 min twice a week in a group mode by two psychologists. When a BMI of 17 kg/m^2 was reached the patient was discharged from the hospital and ambulatory treatment was started. The patients were examined once a month and their weight was checked by a nurse and a psychiatrist. Pharmacological treatment was not performed except for occasional intake of anxiolytic drugs for immediate sedation or sleep promotion.

The control group consisted of 20 healthy schoolgirls from Silesian colleges, aged 18.6 ± 0.3 years (mean ± SD), ranging from 18.0 to 19.8 years, with a mean BMI of 21.4 ± 2.1 (range 17.6–25.7). None was suffering from any psychiatric disorders, including eating disorders, or receiving any drugs. Their daily intake of food over the past year had per day a mean of 2100–2300 kcal. This information was obtained from each girl during a standardized clinical interview followed by psychiatric, neurological and physical examinations.

The protocol of the study was approved by the Local Bioethics Committee and written informed consent to participate in the study was obtained from all those participants and their parents.

3. Laboratory methods

Blood was collected by the method described by Baranowska et al. [19]. Venous blood samples were collected in the fasting state (between 8 and 9 a.m.) into Vacutainer tubes containing EDTA (1 mg/ml of blood). The blood samples were gently shaken and then transferred to centrifuge tubes containing aprotinin (0.6 TIU/ml of blood). They were centrifuged at 1600 × g for 15 min at 4 °C. The plasma collected was immediately frozen and kept at −70 °C.

The total orexin A plasma level was measured by commercial radioimmunoassay kits (Phoenix Pharmaceuticals, Mountain View, California). Buffer A, a 1% water solution of trifluoroacetic acid and buffer B, a 60% solution of acetonitrol in a 1% solution of trifluoroacetic acid were used, as well as chromatography columns, for the extraction (SEP-PAK C-18. Waters Associates). The leptin plasma level was measured by commercial enzymoimmunological assay (Leptin Sandwich ELISA, DRG Instruments GmbH Deutschland) using an ELISA counter (DRG Elisa MAT 2000). Plasma orexin A levels were determined by means of LKB Wallac Clini Gamma 1272 gamma counter. The minimal detectable quantity was 0.001 ng/ml.

The AN-R patients' blood was sampled four times: at admission and after 2, 3 and 6 months of therapy. The control participants have blood drawn only once.

4. Statistical analysis

Calculations were performed using the Statistica version 7.1 statistical package. The Shapiro–Wilk test was used to determine the normality of data distribution. The level of statistical significance was determined at $p<0.05$.

As the BMI results showed normal distribution, the ANOVA test for repeated measurements was used. To compare BMI parameters between periods of time, the LSD (least significant differences)-post hoc test was used. The non-paired *t*-test was used for comparison between patients and control participants.

As the leptin and orexin A values did not show a normal distribution, the nonparametric Friedman test was performed in order to detect differences between multiple measurements. The mean range-post hoc test was used to compare leptin and orexin A parameters during the period of the study. The non-parametric Mann–Whitney test was used for comparison between patients and control participants.

The non-parametric Spearman's test was used to investigate any correlation between leptin or orexin A levels with BMI, or any reciprocal correlation between leptin and orexin A. When leptin levels were transformed into logarithmic values, as lg10, and the BMI was expressed as a BMI SDS (z-score), the Pearson test was used to assess the correlation between these parameters.

5. Results

5.1. Body mass index (BMI)

The values of BMI expressed as kg/m^2, as a z-score and as percentiles are shown in Table 1 and Fig. 1.

The increases in the BMI after 2, 3 and 6 months of therapy were statistically significant (ANOVA test, $F=29.51$; $p<0.001$). A significant increase was noted between baselines, and after 2 and 3 months of therapy, however, the increase of BMI between 3 and 6 months was not significant.

The increase in BMI was also reflected in changes of z-score, and in the distribution of participants in given percentiles, going in the direction of values found in healthy participants.

5.2. Leptin

Leptin concentrations during treatment are shown in Fig. 2.

Statistically significant increases in leptin plasma levels were observed at each investigated period ($p<0.001$, Friedman test) although even after 6 months of therapy, the leptin levels in the patients were little more than half the control participants' values.

There was no correlation between the BMI and leptin plasma level in the AN-R patients before the therapy and after 2 and 3 months of the therapy ($R=0.22$; $p=0.239$; $R=0.22$; $p=0.240$; $R=0.16$; $p=0.387$, respectively; the Spearman correlation). There was, however, a correlation between the BMI and leptin plasma level in the AN-R patients after 6 months of the treatment ($R=0.59$; $p<0.001$). No correlation was observed between the BMI and leptin plasma level in the control group ($R=0.20$, $p=0.402$).