The Orexigenic Peptides in Children with Celiac Disease

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ABSTRACT

Celiac disease is defined as a permanent gluten intolerance, which if untreated leads to malnutrition. The effect of that disease on the gut-brain axis remains unknown. This paper reports the level of plasma ghrelin, orexin A and B in a group of fifteen 8.6 ± 3.4-year-old children with untreated celiac disease and experimentally maintained on a gluten-free diet. A group of 15 age-matched healthy children served as control. Ghrelin and orexins were determined by EIA. The initial mean plasma concentration of ghrelin in the group with acute celiac disease (748.3 ± 41.1 pg/ml) was lower than that after 6 months of a gluten-free diet (812.4 ± 23.6 pg/ml; p<0. 05), but the values did not differ statistically from the values found in the control group (798.0 ± 102.5 pg/ml). The mean plasma levels of orexin A and B in children with untreated celiac disease (A: 0.99 ± 0.12 ng/ml, B: 0.59 ± 0.09 ng/ml) resembled those of healthy children (A: 0.89 ± 0.16 ng/ml; B: 0.58 ± 0.11 ng/ml). Within few months of treatment with a gluten-free diet, plasma levels of both orexins significantly increased over the levels before treatment (A: 1.19 ± 0.12 ng/ml, p < 0.001; B: 0.71 ± 0.06 ng/ml, p<0.002). Thus, a short period of gluten-free diet normalized synthesis of ghrelin in the gut. Plasma levels of orexins in the celiac children depended on the degree of malnutrition and condition of the gut mucosa.

INTRODUCTION

Ghrelin is a 28-amino acid peptide produced mainly in the stomach and small intestine. [1] Initially, ghrelin was considered responsible for release of growth hormone. [2] Furthermore, its roles in the formation of eating behavior and weight regulation were also recognized. Ghrelin is up-regulated in fasting, and it has been proposed that ghrelin provides a signal for initiation of eating. [3] Intracerebroventricular administration of ghrelin stimulates both growth hormone secretion and food intake. [4,5] Ghrelin is one of the peptides of the gut-brain axis. This peptide stimulates feeding through neuropeptide Y (NPY), agouti-related protein (AgRP), and orexigenic peptides co-localized in neurons of the hypothalamic arcuate nucleus. [6] NPY fibers directly project to orexin neurons. [7] Intracerebroventricular injection

***Table I**. Characteristics of studied groups*

	Control group	Celiac disease group	
		before treatment	during the treatment
Sex	8 boys, 7 girls	6 boys, 9 girls	
Age ± SD [years]	10 ± 4,5	8,5 ± 3	9 ± 3
% of optimal body weight	98,3 ± 15,3	93,7 ± 30,6	95,1 ± 31,2

of anti-orexin antiserum prior to the NPY injection significantly attenuated NPY-induced feeding, indicating that NPY interacts with orexins anatomically and functionally. [8] Orexins are neuropeptides produced by neurons located in the hypothalamus and neurons in the spinal cord. [9] However, neurons and endocrine cells in the gut and pancreas exhibit orexin-like immunoreactivity. [10] Both orexins A and B, the 33 and 28 amino acid peptides, respectively, are derived from the 130 amino acid preproorexin.[1,2] In humans, orexins are involved in some basic functions, mainly in behavior patterns controlled by the hypothalamic region, but the ghrelin-orexin system is postulated to integrate and regulate the homeostasis of energy balance and feeding. [11] Data on the concentration of ghrelin in conjunction with orexins in humans with chronic diseases of the gut resulting in malnutrition are lacking. Celiac disease (CD) is an example of one such disorder. CD is a permanent intolerance of gluten and other prolamins derived from wheat, barley and rye. [12] The typical presentation of untreated CD is associated with chronic diarrhea and failure to thrive. In older children abdominal pain, anorexia, growth retardation, osteoporosis and anemia are the most typical symptoms. Clinical symptoms are accompanied by characteristic intestinal lesions with villous atrophy, crypt hypertrophy and lymphocytic infiltrations. [13] After gluten withdrawal, rapid clinical improvement is observed, followed by small bowel mucosa regeneration. [14] In this study, the plasma concentrations of ghrelin and orexins (A and B) in children with celiac disease were estimated.

MATERIAL AND METHODS

A group of 15 children with celiac disease and 15 age matched healthy children (a control group) were examined. The Local Bioethics Committee approved the protocol of the study. Written consent to participate in the study was obtained from all children and their parents.

All children were diagnosed and followed-up by the Department of Pediatrics, Gastroentrology and Nutrition of the University Children's Hospital of Krakow. In all CD children, the diagnosis was established according to the revised ESPGAN criteria (1990). All children had positive IgA endomysial antibodies and characteristic small bowel atrophy of type III or IV. All control group patients were admitted for clinical observation that ruled out any pathology, or for follow-up to confirm that they were healthy. The children were subjected to anthropometric measurements. Their weight and height were determined with an electronic balance and a stadiometer. The percentage of optimal body weight was calculated according to the growth curves of weight for boys and girls in Polish populations as established by M. Krawczyński et al. in 2000. [15] The clinical characteristics of the studied groups are presented in table I.

In all children, the blood samples were collected in the fasted state, between 8 and 9 a.m.. In the CD group, blood samples were taken twice: before the treatment and in the recovery state after gluten withdrawal from the diet (mean 6.2 ± 1.9 month of diet). In the control group, none of the children required any special diet. Blood samples for hormone measurements were collected into glass tubes containing 4 mg EDTA and 0.2 TIU (trypsin inhibitor unit) of aprotinin (Sigma, USA). Immediately after sampling, the tubes were transported to the laboratory in an icebox. Blood was centrifuged for 10

Fig. 1 *Mean value of the concentration of orexins in the studied groups*

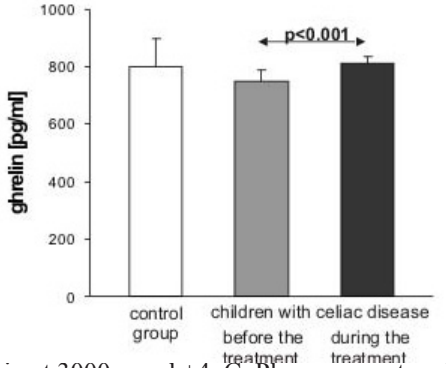
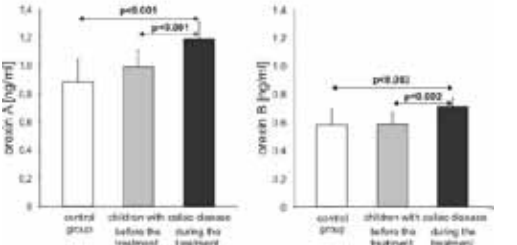


Fig. 2 *Mean value of the concentration of ghrelin in the studied groups*



min at 3000 g and +4oC. Plasma was stored at -20 oC until the measurements. Ghrelin and orexins were determined in plasma by EIA (Phoenix Pharmaceuticals Inc. USA). In the case of orexin, the measurements were performed after extraction of samples on C18 columns (Waters, USA). Plasma samples were acidified with 1% TFA (Sigma, USA) (1:1 v/v) followed by centrifugation at +4 oC and 7000 g for 20 min. The supernatant was applied to the column and washed with 3 x 3 ml of 1% TFA. The peptides were then eluted with 3 ml of 60% acetonitrile solution (BDH Chemicals Ltd, England) in 1% TFA. The eluates were evaporated in the stream of nitrogen. The dry residue was dissolved in a buffer immediately before the assays. The recovery from the control samples containing the known amount of the peptide was 81 ± 5% for orexin A and 84 ± 4% for orexin B. The intraassay coefficient of variation was below 5% in all methods. The orexin A assay exhibited 18% cross-reactivity with orexin B and no cross-reactivity with other substances, such as either NPY, melanocyte-stimulating hormone (MSH), leptin or corticotropin-releasing factor (CRF). The orexin B assay did not exhibit any cross-reactivity with orexin A, NPY, MSH, leptin and CRF. The sensitivity of the orexin assays was similar, that is 0.06 ng/ml. The ghrelin kit had no cross-reactivity with any other peptides. The minimum detectable concentration of ghrelin using this method was 0.08 ng/ml.

Data are expressed as mean+/-SD. Differences between the treatment groups were tested for statistical significance by one-way analysis of variance followed by Bonferroni multiple comparison procedure or, when appropriate, by Student's t-test. Correlations were based on Pearson's correlation coefficient. $P \leq 0.05$ was considered significant.

RESULTS

There were no statistically significant differences in weight and age between the control group and CD group before treatment and during the treatment. However, the mean percentage of optimal body weight in celiac children before the treatment was lower than in healthy controls (table I).

The mean plasma concentration of ghrelin in the group with acute CD (748.3 ± 41.1 pg/ml) was lower than that after the 6th month of the gluten-free diet (812.4 ± 23.6 pg/ml). The values observed in the control group (798.0 ± 102.5 pg/ml) did not differ statistically from values observed in acute CD and on gluten-free diet (fig. 1).

In all examined children, concentration of orexin A in plasma exceeded that of orexin B (fig.2). The mean plasma levels of orexin A and B found in the untreated CD group (orexin A – 0.99 ± 0.12 ng/ml; orexin B - 0.59 ± 0.09 ng/ml) were close to the mean levels of these peptides in the control group (orexin A – 0.89 ± 0.16 ng/ml; orexin B - 0.58 ± 0.11 ng/ml, respectively). Surprisingly, the mean plasma concentration of orexins in the CD group after a few months of the gluten-free diet (orexin A - 1.19 ± 0.12 ng/ml; orexin B - 0.71 ± 0.06 ng/ml) was higher than before the treatment ($p<0.001$ for orexin A and $p<0.002$ for orexin B). It was also higher than the mean

for the control group ($p<0.001$ for orexin A and $p<0.003$ for orexin B) (fig 2).

Orexin A significantly correlated with orexin B in CD children before and after the gluten free diet ($r= 0.44$ and; $r= 0.56$ respectively; $p<0.05$). For all children jointly, correlation between OXA and OXB: improved to $r=0.71$ ($p<0.01$). In the latter group, levels of ghrelin also correlated against the level of orexins (OXA $r= 0.49$ and OXB $r=0.49$; $p<0,05$). A statistically significant relationship between percentage of ideal body weight and studied peptides was found only in the case of orexin B ($r=-51$, $p<0.05$)

DISCUSSION

Differences in the secretion of ghrelin and orexin between subjects in the acute CD phase versus those on the gluten-free diet, and also compared with the healthy controls are incompletely understood. Several studies have revealed that in the acute stage of disease, celiac subjects have elevated fasting serum ghrelin levels compared to healthy subjects. [16, 17, 18] Although, other studies reported a lack of any difference between untreated celiac patients and normal adults.[19] Similar contradictions were observed in patients with a gluten-free diet: some papers described normalized ghrelin values and one study assessed the fasting plasma ghrelin levels lower than that in controls. [16,17,18,19] Chronic diseases resulting in malnutrition should be accompanied by elevated levels of orexigenic factors like ghrelin. Therefore, papers reporting subnormal ghrelin levels in patients with malnutrition produce confusing results. Nevertheless, our study also revealed diminished plasma ghrelin levels in untreated celiac children and normalized levels in children maintained for several months on the gluten-free diet.

In our opinion, there are several factors contributing to observed low ghrelin concentrations in children with acute celiac disease. Among them, limphocytic gastritis, common in persons with CD, is most likely. Over 60% of children with CD suffer from limphocytic gastritis, and their number continuously increases. [20] Gastritis may negatively affect the synthesis of ghrelin in the stomach. Reduced concentrations of plasma ghrelin were described in other chronic and acute types of cases including helicobacter pylori gastritis. [21, 22] Also, long-term activity of inflammation mediators could be taken into account. Celiac disease is an inflammatory disease of the gut. It is known that the same mediators of the inflammation (TNF alpha, IL 1 and IL 12) are also released in chronic arthritis, causing the down-regulation of ghrelin secretion. [23] Finally, impaired gastro-intestinal regulation could be a factor, as a damage of intestinal mucosa might result in either weaker hormonal or neural signals initiating the release of ghrelin.

Contradictions about the increase in the ghrelin plasma levels in untreated celiac patients might result from the persistence of disease, and some kind of adaptation, especially in adult patients.

Analysis of the secretion of central orexogenic factors - orexins A and B - could prove the observations and help in understanding the relationship between celiac disease and the gut-brain orexigenic axis. It is essential that orexins cooperate with ghrelin in the gut-brain axis involved in regulation of energy homeostasis.[5] Orexins are produced by the hypothalamic neurons that project to the autonomic centers and numerous brain sites important in neuroendocrine regulation. [24] However, the gut wall also exhibits orexin-like activity, constituting an integral part of the neurohumoral control of gastrointestinal function.[25, 26]

In this study, parallel to the increment of ghrelin, the increase in plasma orexin concentration was observed in children on the gluten-free diet compared to untreated celiac children. It is known that concentration of orexins negatively correlates with BMI. [27] In this work, the plasma orexin concentration in children with celiac disease,[28]and in healthy controls were found to be similar. Surprisingly, the gluten-free diet and improvement of the clinical state of the children resulted in an increase of both tested orexins. Nevertheless, orexin

B negatively correlated with percentage of the ideal body weight (more reliable index in children than BMI). Thus, lower levels of orexins than expected in untreated celiac children need explanation. One theory that might elucidate this phenomenon involves the fact that orexins are synthesized in the brain and in the gut. Mucosal atrophy and lack of appetite are the essential symptoms of celiac disease.[28] Recent experiments show that the mucosal content of some gastrointestinal hormones (like CCK) and their synthesis decreased in untreated celiac disease as opposed to the healthy subjects. In our opinion, lower than expected plasma levels of orexins observed in the untreated celiac children originate from the diminished synthesis of this peptide by the chronically damaged gut mucosa. The diminished orexin synthesis in the gut balanced the enhanced synthesis of orexins in the brain due to malnutrition. For this reason, children with celiac disease and healthy subjects exhibit no difference in the concentrations of these peptides. The gluten-free diet leads to the recovery of mucosa and correlates with clinically observed enhanced appetite in the celiac children. The recovery of mucosa should normalize peripheral synthesis of orexins. In our study, plasma concentrations of orexins after six months of the gluten-free diet were significantly higher than before introducing this diet. A six month period is sufficient for the mucosa to regenerate but, as it is presented in table I, it is insufficient to improve the body weight of children with celiac disease. Therefore, in malnourished children on gluten-free diets, orexin concentrations were higher than in healthy controls, as expected.

CONCLUSIONS

1. A short period of the gluten-free diet normalizes the synthesis of ghrelin in the gut.

2. Plasma levels of orexins in the celiac children depend on the degree of malnutrition and condition of the gut mucosa.

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