Pediatric Endocrinology Diabetes and Metabolism
Endokrynologia, Diabetologia i Choroby Przemiany Materii Wieku Rozwojowego

Original Paper | Praca oryginalna
Pediatr Endocrinol Diabetes Metab 2015;21,3:102-110
DOI: 10.18544/PEDM-21.03.0031

redakcja@pediatricendocrinology.pl
www.pediatricendocrinology.pl
www.pteidd.pl

Assessment of ghrelin, leptin, orexin A and alpha-MSH serum concentrations and the levels of the autoantibodies against the aforementioned peptides in relation to *Helicobacter pylori* infections and *Candida albicans* colonization in children with short stature

Ocena stężenia greliny, leptyny, oreksyny A i alfa-MSH w surowicy oraz poziomu przeciwciał skierowanych przeciwko wymienionym peptydom w odniesieniu do zakażenia *Helicobacter pylori* i zasiedlenia *Candida albicans* u dzieci z niedoborem wzrostu

[1]Renata Stawerska, [2]Elżbieta Czkwianianc, [3]Agnieszka Matusiak, [1]Joanna Smyczyńska, [1,4]Maciej Hilczer, [3]Magdalena Chmiela, [1,5]Andrzej Lewiński

[1]Department of Endocrinology and Metabolic Diseases, Polish Mother's Memorial Hospital – Research Institute, Lodz, Poland
[2]Department of Gastroenterology, Allergology and Pediatrics, Polish Mother's Memorial Hospital – Research Institute, Lodz, Poland
[3]Department of Immunology and Infectious Biology, Faculty of Biology and Environmental Protection, University of Lodz, Lodz, Poland
[4]Department of Pediatric Endocrinology, Medical University of Lodz, Lodz, Poland
[5]Department of Endocrinology and Metabolic Diseases, Medical University of Lodz, Lodz, Poland

Abstract

Introduction. Some of the gut-brain-adipose tissue peptides play an important role in growth hormone (GH) secretion and in the regulation of food intake. Based on the molecular mimicry hypothesis, intestinal microbe-derived antigens may trigger the production of autoantibodies cross-reacting with many regulatory peptides and modify their actions. **The aim of the study** was to assess ghrelin, leptin, orexinA and alpha-melanocyte-stimulating-hormone (αMSH) serum concentrations and autoantibodies against the aforementioned peptides levels in children with idiopathic short stature (ISS) and with GH deficiency (GHD) in relation to *Helicobacter pylori* (*H.pylori)* and *Candida albicans (C.albicans)* infections. **Material and methods.** The study group comprised 89 short children (aged 10.24±3.52 years): 64 with ISS and 25 with GHD and 36 normal height children (Controls) (aged 11.41±2.72 years). In each child, the concentration of ghrelin, leptin, orexinA and αMSH, the level of IgG autoantibodies against mentioned peptides as well as against *H.pylori* were assessed in serum, while presence of *C.albicans* – in stool samples. The control group was selected in such a manner that the prevalence of *H.pylori* and *C.albicans* was similar to the study group. **Results.** The levels of IgG antibodies against ghrelin and leptin were significantly higher in ISS than in Controls. In GHD children, the ghrelin concentrations were significantly higher than in Controls. In ISS children the leptin concentrations (as well as body mass index) were significantly lower than in GHD and in Controls. We did not find any differences among groups as regards orexinA and αMSH concentrations. **Conclusions.** The higher levels of autoantibodies against ghrelin and leptin in children with ISS may be connected with worse growth and lower body mass in these children through the modification of ghrelin and leptin activity. It is possible that these autoantibodies are formed in molecular mimicry mechanism between *H.pylori* and *C.albicans* and the aforementioned neuropeptides. However, further studies are necessary.

Key words

autoantibodies, ghrelin, leptin, orexin A, alpha-MSH, short stature

The study was financially supported by funds from the Ministry of Scientific Research and Information Technology, the present name – the Ministry of Science and Higher Education of Poland, project No. N 407 570038.

The authors declare no conflict of interest

Prof. Andrzej Lewiński, MD, PhD
Department of Endocrinology and Metabolic Diseases,
Polish Mother's Memorial Hospital – Research Institute;
Rzgowska St. No. 281/289, 93-338 Lodz, Poland
phone: +48 42 271 11 41, fax:+48 42 271 11 40
e-mail: alewin@csk.umed.lodz.pl

Received: 2015-12-01.
Accepted: 2015-12-31.
Conflict of interests: none declared.

Pediatr Endocrinol Diabetes Metab 2015;21,3:102-110

Streszczenie

Wstęp. Peptydy produkowane w przewodzie pokarmowym, tkance tłuszczowej i w mózgu odgrywają ważną rolę w wydzielaniu hormonu wzrostu (GH) oraz regulacji przyjmowania posiłków. Zgodnie z hipotezą molekularnego podobieństwa antygeny mikroorganizmów bytujących w przewodzie pokarmowym mogą stać się mechanizmem spustowym dla produkcji przeciwciał, które reagują krzyżowo z peptydami regulatorowymi i modyfikują ich działanie. **Celem pracy** była ocena stężenia greliny, leptyny, oreksynyA i αMSH oraz poziomu przeciwciał skierowanych przeciwko wymienionym peptydom u dzieci z idiopatycznym niedoborem wzrostu (ISS) i niedoborem GH (GHD) w odniesieniu do infekcji *Helicobacter pylori* (*H.pylori)* i zasiedlenia *Candida albicans* (*C.albicans)*. **Materiał i metody.** Analiza obejmowała 89 dzieci z niedoborem wzrostu (w wieku 10,24±3,52 lat): 64 z ISS i 25 z GHD oraz 36 dzieci prawidłowego wzrostu (grupa kontrolna) (w wieku 11,41±2,72 lat). U każdego dziecka oceniono w surowicy stężenie greliny, leptyny, oreksynyA i αMSH (*alpha-melanocyte-stimulating hormone*), poziom przeciwciał IgG skierowanych przeciwko wymienionym peptydom oraz przeciwko *H.pylori*, zaś obecność *C.albicans* na podstawie badania próbki kału. Grupa kontrolna została dobrana tak, aby częstość występowania infekcji *H.pylori* i zasiedlenia *C.albicans* była podobna do grupy badanej. **Wyniki.** Poziom przeciwciał IgG przeciwko grelinie i leptynie był znamiennie wyższy w grupie ISS niż w grupie kontrolnej. Stężenie greliny było istotnie wyższe u dzieci z GHD niż w grupie kontrolnej, zaś stężenie leptyny (jak również wskaźnik masy ciała) istotnie niższe w grupie ISS niż w grupach GHD i kontrolnej. Nie wykazano różnic pomiędzy grupami w odniesieniu do stężenia oreksynyA i αMSH ani przeciwciał skierowanych przeciwko nim. **Wnioski.** Podwyższony poziom przeciwciał skierowanych przeciwko grelinie i leptynie u dzieci z ISS jest związany z upośledzeniem wzrastania i gorszymi przyrostami masy ciała, prawdopodobnie poprzez modyfikację aktywności greliny i leptyny. Możliwe, że te przeciwciała reagują krzyżowo z peptydami na skutek molekularnego podobieństwa między wymienionymi peptydami a *H.pylori* i *C.albicans*, jednak potrzebne są dalsze badania wyjaśniające tę kwestię.

Słowa kluczowe

przeciwciała, grelina, leptyna, oreksyna A, alfa-MSH, niedobór wzrostu

Introduction

The well known causes of short stature in children are hormonal disorders, i.e. growth hormone (GH) deficiency (GHD) resulting in secondary IGF-I deficiency, primary IGF-I deficiency, hypothyroidism and gastrointestinal tract (GI) diseases resulting – among others – in malnutrition (and secondary IGF-I deficiency). If the height of the individual is more than 2 standard deviations (SD) below the mean for an age and sex and there is no evidence of systemic, endocrine, nutritional or chromosomal abnormalities, idiopathic short stature (ISS) is recognized [1,2]. In some of the ISS children familial short stature or constitutional delay of growth and puberty are diagnosed, however, in many the causes of short stature remain unknown [1,2]. This paper is an attempt to search for other factors and mechanisms that affect growth in children with ISS.

In short children, a higher incidence of *Candida albicans* (*C. albicans*) colonisation and *Helicobacter pylori* (*H. pylori*) infection than in normal height children was proved [3–5]. The GI microflora is an antigenic source. Based on the molecular mimicry hypothesis, intestinal microbe-derived antigens may trigger the production of autoantibodies cross-reacting with many regulatory peptides. Recently, Fetissov et al. [6] have described autoantibodies directed against 14 key appetite--regulating peptides in serum of healthy women. Numerous cases of sequence homology with these peptides were identified among commensal and pathogenic microorganisms, including *C. albicans* and *H. pylori* [6]. Among others, the authors demonstrated the phenomenon of molecular mimicry between *H. pylori* and leptin and alpha-melanocyte-stimulating hormone (MSH) and between *C. albicans* and ghrelin, leptin, orexin, and MSH [6].

These gut-brain-adipose tissue peptides play an important role in GH secretion and in the regulation of food intake. Among them, there are peptides of orexigenic axis, which stimulate food intake (i.e. ghrelin, orexin A) and of anorexigenic axis, which are responsible for suppressing appetite (i.e. leptin and αMSH) [7,8]. Ghrelin is also a strong stimulator of GH secretion [9], however its acting is mediated by other peptides, such as neuropeptide Y and leptin [10,11].

The autoantibodies present in the serum modify the action of these peptides blocking or intensifying effects exerted by them [12–15].

In our earlier work [16], we found a high prevalence of cases with elevated level of autoantibodies against ghrelin, leptin, orexin A or αMSH in ISS children with *C. albicans* and/or *H. pylori*. It is probable that it was connected with molecular mimicry between antigens of these microbiota and the mentioned peptides.

Thus, **the aim of the present study** was to assess the ghrelin, leptin, orexin A and αMSH serum concentrations as well as the autoantibodies against mentioned peptides levels in ISS, GHD and normal height children in relation to *Helicobacter pylori* (*H.pylori)* and *Candida albicans (C.albicans)* infections.

Material and methods

An approval for the study was obtained from the Bioethical Committee at the Polish Mother's Memorial Hospital – Research Institute (PMMH-RI) in Lodz.

We analyzed 89 children with short stature. In all patients, the body height was measured using a stadiometer and the height standard deviation score (HSDS) was calculated accor-

ding to current population standards [17]. Only children with HSDS below -2.0 were qualified into the study group. Next, based on the child's current percentile position, the height age (HA) was calculated (as the age ascribed to the 50th percentile for a given child's height). The body mass was assessed in all the qualified patients and that was followed by the calculation of the body mass index (BMI) standard deviation score for chronological age (BMI SDS for CA) and for height age (BMI SDS for HA). BMI SDS for HA is a better indicator for the assessment of the nutrition status in short children than BMI SDS for CA and it is used for comparing the body mass in normal height and short stature children. Children with thyroid dysfunction, autoimmune diseases, eating disorders, suffering from chronic cardiovascular, respiratory or urinary system diseases as well as girls with Turner's syndrome (diagnosed with the use of chromatin X and/or karyotype tests) were excluded from the study group. None of the children reported symptoms from the GI tract or previously had been diagnosed with or treated for GI diseases.

Growth hormone secretion assessment

In each individual, laboratory tests were performed as a part of the diagnostics of short stature conducted during hospitalisation at the Department of Endocrinology and Metabolic Diseases, PMMH-RI in Lodz.

In order to assess GH secretion, in each child, a 3-hour, nocturnal profile of GH secretion was recorded every half-hour, starting from the first hour after falling asleep. Next, two stimulation tests were performed: one after oral administration of clonidine (with the dose of 0.15 mg/m^2 of body surface and GH measurements at time 0 and at the 30th, 60th, 90th and 120th minute of the test), and the other one after intramuscular administration of glucagon (with the dose of 30 μg/kg body mass, with GH measurements at time 0 and at the 90th, 120th, 150th and 180th minute of the test). Peak GH concentration (GH_{max}) was determined in both tests and after falling asleep. The children with GH_{max} values $<$ 10 ng/ml were qualified as those with GHD, while in the patients with GH_{max} value $\geq$ 10 ng/ml, ISS was diagnosed.

Finally, the study group consisted of 64 children with ISS (27 girls and 37 boys) and 25 children with GHD (7 girls and 18 boys).

In each child, IGF-I, IGFBP-3, IgG antibodies against ghrelin (anti-ghrelin), leptin (anti-leptin), orexin A (anti-orexin A) and αMSH (anti-αMSH) and concentrations of these hormones were assessed, serologic tests for *H. pylori* were performed and stool samples for *Candida sp*. were taken.

Hormonal assessment

The growth hormone levels were measured using the immunometric method. The measurements were performed with Immulite, DPC assay kits, calibrated to the WHO IRP 98/574 standard set, with the sensitivity level: 0.01 ng/ml, range: up to 40 ng/ml, the conversion index: ng/ml x 2.6 = mIU/l, the intra-assay CV: 5.3–6.5% and inter-assay CV: 5.5–6.2%.

IGF-I was assessed by Immulite, DPC assays; WHO NIBSC 1st IRR 87/518 standard was applied, with the analytical sensitivity of 20 ng/ml, calibration range up to 1600 ng/ml, intra-assay CV – 3.1–4.3% and inter-assay CV – 5.8–8.4%. For comparison of children with different age and sex, IGF-I concentrations were expressed as IGF-I SDS, according to reference data.

The assay for IGFBP-3 assessment was also assessed by Immulite, DPC; was calibrated to WHO NIBSC Reagent 93/560 standard, with analytical sensitivity 0.02 μg/ml, the calibration range up to 426 μg/ml, the intra-assay CV: 3.5–5.6% and the total CV: 7.5-9.9%.

The total ghrelin concentration was measured using the Millipore RIA kit (Linco Research) with sensitivity level: 100–10.000 pg/ml, the intra-assay CV: 3.3–10.0% and inter-assay CV: 14.7–17.8%.

The leptin concentrations were measured using the Millipore ELISA kit (Linco Research). Sensitivity level, the intra-assay CV and inter-assay CV were: 0.5–100 ng/ml, 1.4–4.9% and 1.3–8.6%.

Orexin A and αMSH levels in the plasma samples were analysed by an ELISA method, an immunoassay that determines antigen-antibody interactions, using ELISA kits purchased from Phoenix Pharmaceuticals, Inc. According to the manufacturer's instructions, the minimum detection limits of orexin A and αMSH were 0.2 and 0.16, respectively, range for both: 0–100 ng/ml.

Determination of IgG antibodies against ghrelin, leptin, orexin A and αMSH

Laboratory ELISA assays were used for the assessment of the IgG antibodies towards appetite regulating peptide hormones and neuropeptides. Concentrations of standard antigens and labeled antibodies were determined in preliminary studies. The standard peptide hormones and neuropeptides: ghrelin (Abbiotec, San Diego, USA), leptin (Bio Vendor, Brno, Czech Republic), orexin A and αMSH (Phoenix Pharmaceuticals, Inc., Burlingame, USA), were diluted in carbonate buffer pH 9.6 to the concentration of 2 μg/ml and were distributed into the wells of 96 well plate of MaxiSorp type (Nunc, Kastrup, Denmark),100 μl/well. The plates were incubated for 18 h, at 4°C, then washed three times with phosphate buffered saline (PBS) supplemented with 0.5% Tween 20 (PBS/Tween), 250 μl/well. Excess binding sites were blocked for 2 h with 1% bovine serum albumin (BSA, fraction V, Sigma, St. Louis, Mo., USA) in PBS/Tween (300 μl/well). After five washings, the wells were supplemented with the serum samples diluted 1:100 in BSA/PBS/Tween (100 μl/well), and incubated for 1 h, in 37°C. Peroxidase conjugated rabbit antibodies against human IgG (Dako) were diluted 1:6000 in PBS/BSA/Tween and added into the wells for 1 h, 37°C. The colour reaction was developed as previously described. The optical density (OD) values were read at 450 nm wave length. In order to exclude the non-specific interactions, control wells were included for each plate: wells coated with the hormone and incubated with serum sample without secondary antibody, wells coated with antigen and incubated with peroxidise conjugated antibody, wells coated with antigen followed by incubation with the substrate, and non-co-

ated wells blocked with BSA/PBS/Tween followed by the serum dilution and secondary antibody.

H. pylori infection assessment

In each child, the serology test was performed to detect *H. pylori* infection. Specific antibodies to *H. pylori* antigens were detected with a laboratory enzyme-linked immunosorbent assay (ELISA), as previously described by Rechciński et al. [18]. The assay was conducted with a glycine acid extract (GE, 0.01 mg/ml) from the reference *H. pylori* strain CCUG 17874 (Culture Collection, University of Gothenborg, Sweden). The antigen was adjusted to the required concentration (10 μg/ml) in 0.05 M carbonate buffer (0.015 M Na_2CO_3, 0.035 M $NaHCO_3$), pH 9.6. The serum samples were diluted from 1:500 to 1:64 000 (to determine IgG), and from 1:100 to 1:6400 (to determine IgA), peroxidise conjugated rabbit antibodies (Dako, Glostrup, Denmark) to human IgG were diluted 1:6000 and to human IgA were diluted 1:1000. For colour reaction, the chromogen *o*-phenylenediamine dihydrochloride (Sigma, St. Louis, MO, USA) was used in the concentration of 1 mg/ml in 0.1 M citric phosphate buffer (0.1 M citric acid, H_2O, 0.067 M Na_2HPO_4, 12 H_2O), pH 5.0, with 0.005 ml of 30% H_2O_2 per 1 ml of the buffer. The OD values were read at 450 nm wave length (1420 Victor 2, Oy, Turku, Finland). The panel of negative control sera was used to establish the cut off value between positive and negative ELISA results. The border value was defined as two standard deviations above the mean of control negative sera from subjects uninfected with *H. pylori*.

C. albicans colonization assessment

In order to diagnose *C. albicans* colonization, stool samples from patients were cultured for *Candida sp*. However, only significant levels of *C. albicans* were taken into consideration as *candidiasis mucosae* of GI.

Control group

The same tests (IGF-I, IGFBP-3, IgG antibodies anti-ghrelin, anti-leptin, anti-orexin A and anti-αMSH and concentrations of these hormones, serologic tests for *H. pylori* and analysis of stool samples for *Candida sp*.) were performed in a group of 36 normal height children (23 girls and 13 boys), diagnosed at other departments of PMMH-RI for different reasons (however, not meeting the exclusion criteria). The control group was selected in such a manner that the prevalence of *H. pylori* and *C. albicans* was similar to the ISS and GHD group.

Statistics

Statistical analysis was performed with STATISTICA 5.0 PL program. A one-way ANOVA was applied for statistical analysis with the subsequent use of a *post-hoc* test, in order to statistically assess differences between the particular pairs of groups; Tukey's test was selected because of the unequal data number in particular groups. $P < 0.05$ was accepted as significant value.

Results

Auxological data and hormonal test results of the children from the analysed groups are presented in Table I.

The height (expressed as HSDS) of children from both groups of short stature (GHD and ISS) was similar, while BMI SDS (for CA and for HA) values of individuals with ISS were significantly lower than in ones with GHD and in Controls. The results of GH_{max} concentration reflect the definition of the groups. The IGF-I SDS in ISS group was significantly lower than in Controls but higher than in GHD group.

It was found that in children with GHD, the mean ghrelin concentration was significantly higher than in children from the Controls. Also, in ISS children the leptin concentration (similarly as BMI SDS for CA and for HA) were significantly lower than in GHD and in Controls. However, we did not find any differences among groups as regards orexin A and MSH concentrations (Table II).

We found that the levels of IgG antibodies against ghrelin and leptin were significantly higher in children with ISS than in children from the control group (Figure 1). We did not find any differences as regarded IgG autoantibodies against orexin A and αMSH among the studied groups (Figure 1).

In the total analyzed group of children, it was found a negative correlation between the ghrelin concentration and the level of IgG antibodies against ghrelin ($r=-0.25$, $p<0.05$), however we did not find any correlations between serum levels of the other individual peptides and the autoantibodies directed against them.

The prevalence of the *H. pylori* infection and *C. albicans* colonization in individual groups were similar – see material and methods and Table III. We did not find any differences as regards the serum level of IgG antibodies against *H. pylori* among groups (Table III).

Discussion

In the present study, we have confirmed that the serum levels of autoantibodies against ghrelin and against leptin are significantly higher in ISS children than in children from the control group. In GHD children, the levels of the abovementioned autoantibodies were similar to Controls. Since higher levels of autoantibodies against main peptide of orexigenic axis (ghrelin) and of anorexigenic axis (leptin) are observed in ISS group, it is very interesting to determine what kind of influence they exert on the concentration and action of ghrelin and leptin, as well as – indirectly – on other neuropeptides of orexi- and anorexigenic axis. Another interesting question is whether their presence is connected in any important way with worse growth velocity and worse food intake in ISS children.

In our study, we did not find any differences among groups as regards analyzed peptides (besides ghrelin, which concentration was significantly higher in GHD children; we observed and discussed the high ghrelin concentration in GHD children in our earlier study [19]) and leptin level, which was the lowest

Table I. Auxological data and hormonal test results (mean ± SD) of the children from the ISS, GHD and control group
Tabela I. *Dane auksologiczne i wyniki badań hormonalnych (średnia ± SD) u dzieci z grupy ISS, GHD i z grupy kontrolnej*

Group	ISS	GHD	Controls Grupa kontrolna
n: (girls/boys) n: (dziewczynki/chłopcy)	64: (27/37)	25: (7/18)	36: (23/13)
chronological age (years) wiek kalendarzowy (lata)	10.26±3.44	10.23±3.93	11.41±2.72
height age (years) wiek wzrostowy (lata)	7.77±2.83 [a]	7.84±3.13 [b]	11.04±3.08 [a,b]
H SDS	-2.46±0.71 [a]	-2.47±0.68 [b]	-0.24±0.92 [a,b]
BMI SDS for CA BMI SDS dla CA	-0.75±0.98 [a,b]	0.39±1.69 [a]	0.35±1.35 [b]
BMI SDS for HA BMI SDS dla HA	-0.28±1.25 [a,b]	0.94±1.67 [a]	0.27±1.75 [b]
GHmax after clonidine (ng/ml) GHmax po klonidynie (ng/ml)	14.60±8.40 [a]	7.87±2.65 [a]	-
GHmax after glucagone (ng/ml) GHmax po glukagonie (ng/ml)	9.03±6.09 [a]	6.21±2.00 [a]	-
GHmax nocturnal profile (ng/ml) GHmax w nocnym profilu	12.95±7.01 [a]	5.75±3.19 [a]	-
IGF-I (ng/ml)	181.66±120.73	138.64±88.00 [a]	256.71±156.83 [a]
IGF-I SDS	-0.96±0.98 [a,b]	-1.63±1.07 [a]	-0.26±1.60 [b]
IGFBP-3 (μg/dl)	4.12±1.04	3.77±1.34	5.01±0.40

a,b – in the individual rows of the table, the variables designated with the same letters differ significantly from each other with $p<0.05$
a,b – zmienne oznaczone tymi samymi literami w poszczególnych rzędach tabeli różnią się między sobą znamiennie, $p<0.05$
ISS – idiopathic short stature; GHD – growth hormone deficiency; H SDS – height standard deviation score; BMI SDS – body mass index standard deviation score, CA – chronological age; HA – height age; GH – growth hormone, IGF-I – insulin-like growth factor I, IGFBP-3 – insulin-like growth factor binding protein 3.
ISS – idiopatyczny niedobór wzrostu; GHD – niedobór hormonu wzrostu; H SDS – wskaźnik odchylenia standardowego wzrostu; BMI SDS – wskaźnik odchylenia standardowego wskaźnika masy ciała, CA – wiek kalendarzowy; HA – wiek wzrostowy; GH – hormon wzrostu, IGF-I – insulinopodobny czynnik wzrostu typu I, IGFBP-3 – białko wiążące insulinopodobny czynnik wzrostu typu 3.

in ISS children (the leptin levels strongly positively correlated with BMI). It is well known that the presence of autoantibodies in healthy individuals is not responsible for lower production or secretion of peptides, but for changing their activities. It may be expressed both by an enhancement or inhibition of these functions [6,15,20,21].

Ghrelin is a natural ligand of type 1a GH secretagogue receptor and a strong GH stimulator. It is also an important orexigenic peptide that stimulates hunger and food intake mainly by stimulating the release of other orexigenic peptides and neurotransmitters. Starvation and/or malnutrition results in an increase of the ghrelin level, while in individuals with obesity ghrelin concentration is reduced [9].

In turn, leptin is the main peptide of the anorexigenic axis which suppresses appetite. Leptin is secreted mainly by adipocytes, but it has also been found in the stomach and the pituitary gland. Circulating leptin strongly correlates with BMI. It was known that hyperleptinemia may suppress ghrelin and stimulate somatostatin, both of which result in lower GH secretion [22,23]. In turn, it is also suggested that malnutrition-dependent reduction of leptin levels may play a role in hypersomatotropism in anorexia nervosa (AN) subjects [24]. Thus,

Table II. The serum concentration of ghrelin, leptin, orexin A and αMSH (mean ±SEM) of the children from the ISS, GHD and control group
Tabela II. *Stężenie ghreliny, leptyny, oreksyny A i αMSH (średnia±SEM) u dzieci z grupy ISS, GHD i grupy kontrolnej*

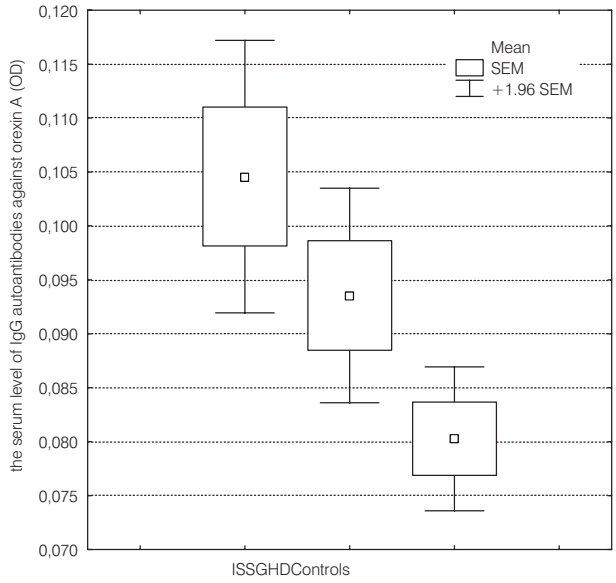
Group	ISS	GHD	Controls Grupa kontrolna
n: (girls/boys) n: (dziewczynki/chłopcy)	64: (27/37)	25: (7/18)	36: (23/13)
ghrelin (pg/ml) grelina (pg/ml)	1597.48±207.33	2221.58±496.19 [a]	1101.47±261.83 [a]
leptin (ng/ml) leptyna (ng/ml)	4.31±0.71 [a,b]	6.62±1.72 [a]	6.54±1.17 [b]
orexin A (ng/ml) oreksyna A (ng/ml)	0.84±0.04	0.78±0.06	0.94±0.05
αMSH (ng/ml) αMSH (ng/ml)	2.98±0.51	4.05±1.00	2.47±0.26

a,b – in the individual rows of the table, the variables designated with the same letters differ significantly from each other with $p<0.05$
a,b – zmienne oznaczone tymi samymi literami w poszczególnych rzędach tabeli różnią się między sobą znamiennie, $p<0.05$
ISS – idiopathic short stature; GHD – growth hormone deficiency;
αMSH – alpha-melanocyte-stimulating hormone

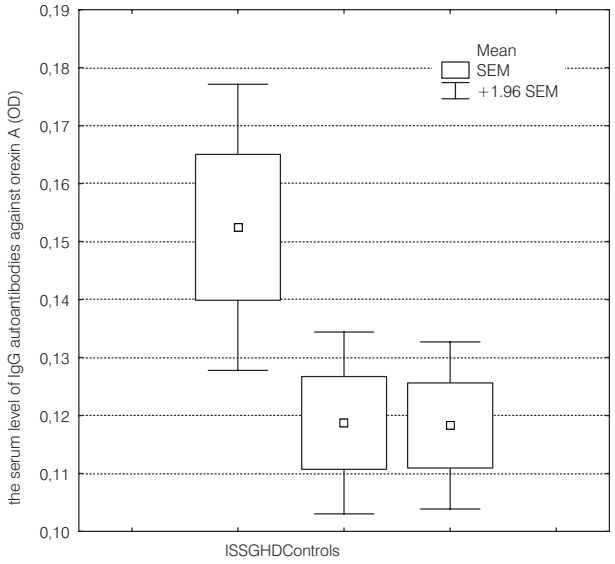
Table III. The level of IgG antibodies against *H. pylori* (mean ±SEM) and prevalence of the *H. pylori* infection and *C. albicans* colonization in ISS, GHD and control group
Tabela III. Poziom przeciwciał IgG przeciwko *H. pylori* (średnia ±SEM) oraz częstość występowania infekcji *H. pylori* i zasiedlenia *C. albicans* u dzieci z grupy ISS, GHD i z grupy kontrolnej

Group	ISS	GHD	Controls Grupa kontrolna	P=
N: (girls/boys) n: (dziewczynki/chłopcy)	64 (27/37)	25 (7/18)	36 (23/13)	
IgG against *H. pylori* (OD) IgG przeciwko *H. pylori* (OD)	0.28±0.40	0.45±0.56	0.32±0.38	NS
IgA against *H. pylori*	0.14±0.09	0.14±0.07	0.14±0.10	NS
Prevalence of positive results of *H. pylori* n(%) Częstość pozytywnych wyników dla *H. pylori* n(%)	13 (20.3%)	7 (28%)	11 (30.5%)	NS
Prevalence of positive results of *C. albicans* n(%) Częstość dodatnich wyników dla *C. albicans* n(%)	29 (45.3%)	10 (40%)	14 (38.9%)	NS

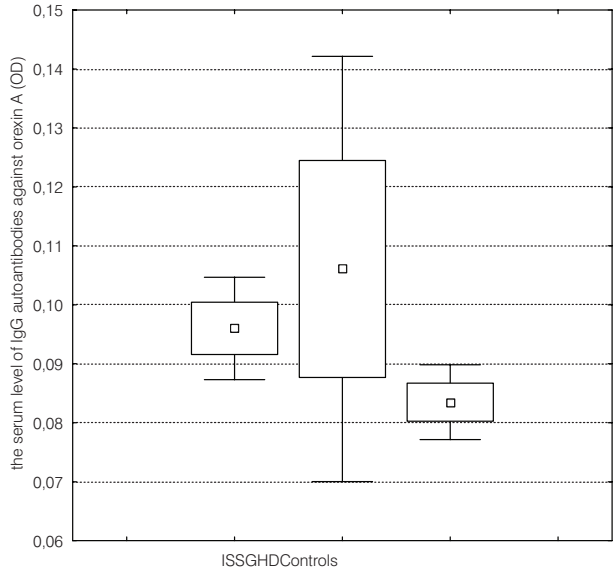
ISS – idiopathic short stature; GHD – growth hormone deficiency; OD – optic density, *C. albicans* – *Candida albicans*, *H. pylori* – *Helicobacter pylori*
ISS – idiopatyczny niedobór wzrostu; GHD – niedobór hormone wzrostu; OD – gęstość optyczna, *C. albicans* – *Candida albicans*, *H. pylori* – *Helicobacter pylori*



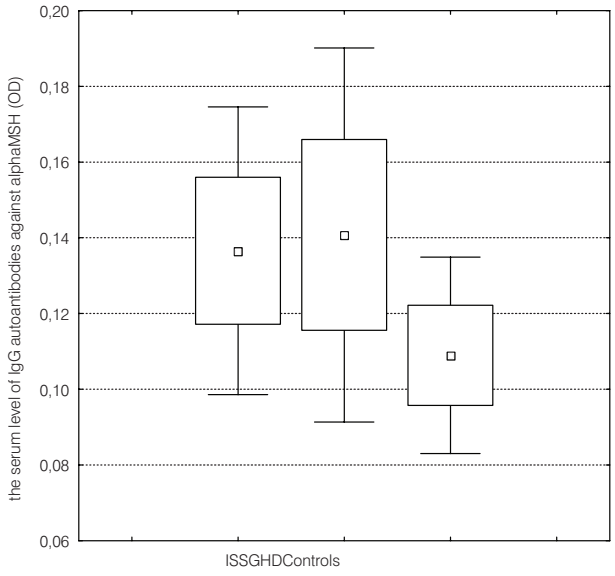
a – ISS vs Controls – $p<0.05$



b) ISS vs Controls , $p<0.05$



c)



d)

Figure 1. The serum levels of IgG autoantibodies against ghrelin (a), leptin (b), orexin A (c) and αMSH (d) in group ISS, GHD and Controls

Figure 1. *Poziom IgG autoprzeciwciał przeciw ghrelinie (a), leptynie (b), oreksynie A (c) i αMSH (d) w surowicy w grupie ISS, GHD i w grupie kontrolnej*

ghrelin and leptin generally have opposing effects, both on the secretory function of the somatotrophs, as well as on the activity of the orexigenic and anorexigenic axis.

It is well known that children with ISS, despite normal GH secretion, are short. In many of them, the low body mass is also observed, but they do not have any GI disorders or malabsorption [25]. Many of them are poor-eaters [26]. In our study we also observed that children with ISS were slimmer than children from GHD group and from control group.

Thus, it seems that the higher levels of autoantibodies against ghrelin and leptin may be a reason of abnormal physical development of the child. So far, the modulatory effect of auto-

antibodies against ghrelin in eating disorders [20], stress and anxiety [12] and autoimmune diseases [27] have been analysed, but not in children with short stature. In our study, similarly as Terashi et al. [20] we observed a negative correlation between ghrelin concentration and autoantibodies against ghrelin. The authors of this study suggested that a decrease of bioavailable ghrelin autoantibodies might underline an increase of plasma ghrelin levels and the resulting phenomenon of ghrelin resistance in patients with AN with low IGF-I. In our group of ISS children, low concentration of IGF-I was observed as well. On the other hand, ghrelin-reactive IgG antibodies protect ghrelin form degradation and may enhance its orexigenic effect, depending on the affinity for ghrelin, which was increased in obese humans [13]. Thus, a lot of questions need to be elucidated.

It is worth mentioning that malnutrition also induces the nutritionally responsive proteins sirtuin 1 (SIRT1) and fibroblast growth factor 21 (FGF21). They inhibit GH signal transduction in the liver by blocking the JAK/STAT pathway, which restricts IGF-I production. It is probable that in short children with malnutrition similar mechanisms are observed [28].

In turn, anti-leptin autoantibodies may strengthen leptin action. Recent data suggest that leptin is a metabolic signal that regulates GH secretion, since the administration of leptin antiserum led to a decrease in spontaneous GH secretion [29]. Also in this area there are no data on the impact of such antibodies on GH. It could be speculated that the antibodies act as a stimulant, similarly to MSH autoantibodies. MSH autoantibodies appear as a physiologic modulator of feeding [30]. This modulating depends on affinity for MSH: low affinity of IgM and IgG (which dominate in rats) has no blocking properties for MSH but – instead – may increase its action probably playing the role of MSH carrier [30]. On the one hand, the enhanced action of leptin may inhibit appetite and on the other hand – inhibit GH production. These assumptions, however, require further research to confirm or deny the thesis.

Considering that the prevalence of *H. pylori* and *C. albicans* is higher in children with ISS than in normal height children [3], we tested the hypothesis that these microbiota may be a reason for a higher production of antibodies which cross-react with neuropeptides due to molecular mimicry between their antigens. It is especially important to the issues lately raised regarding the impact of the bacterial microflora in a number of aspects of our lives, including the area of eating disorders [31]. In our study, the control group was chosen in such a way that the percentage of children infected with *H. pylori* and *C. albicans* in the control group was similar to that in the study group. In our previous study [16], we showed that the number of patients with significantly elevated levels of antibodies against the aforementioned peptides, was significantly higher among short stature children with *H. pylori* and *C. albicans* than among short children without these pathogens, or among Controls [16]. Despite that, it was not possible to clearly determine the impact of *H. pylori* infection and *C. albicans* colonization on forming the antibodies. Although a causal relationship is likely, further studies are planned. We are going to compare the concentrations of neuropeptides and of autoantibodies directed against them in the group of children with *H. pylori* and/or *C. albicans* vs. the group of children without these microbiota. In the next studies, we intend to perform such comparisons among children with ISS, GHD and also in Controls (unfortunately, in the present study the numbers of children with GHD and Controls were too small for such analysis). We also intend to focus on these out of specific antigens of *H. pylori* and *C. albicans* that induce potentially autoreactive responses.

It should be taken into account that the molecular mimicry between the antigens of neuropeptides and intestinal microflora has been found for many microbiota, while in our study only two were analyzed.

Summing up, it should be emphasized that we found significantly higher levels of antibodies against ghrelin and leptin in children with ISS than in the control group. It indicates a possible effect of these antibodies on the growth deficit and lower body mass in these children. Further studies on the possible effects on the formation of these antibodies through the mechanism of molecular mimicry between *H. pylori* infection and *C. albicans* colonization and neuropeptides remain in the area of further consideration.

References

1. Wit JM, Clayton PE, Rogol AD et al. *Idiopathic short stature: definition. epidemiology. and diagnostic evaluation*. Growth Horm IGF Res. 2008;18:89-110.
2. Pedicelli S, Peschiaroli E, Violi E, Cianfarani S. *Controversies in the definition and treatment of idiopathic short stature (ISS).* J Clin Res Pediatr Endocrinol. 2009;1:105-115.
3. Stawerska R, Smyczyńska J, Hilczer M et al. *The high incidence of oligosymptomatic gastrointestinal tract diseases in children with short stature of different etiology*. Horm Res Ped. 2013;80(suppl.1):417.
4. Takahashi M, Kimura H, Watanabe K. *Helicobacter pylori infection in patients with idiopathic short stature*. Pediatr Int. 2002;44:277-280.
5. Fialho AM, Braga AB, Queiroz DM et al. *The association between Helicobacter pylori infection and height in children from an urban community in north-east Brazil.* Ann Trop Paediatr. 2007;27:55-61.
6. Fetissov SO, Hamze Sinno M, Coëffier M et al. *Autoantibodies against appetite-regulating peptide hormones and neuropeptides: putative modulation by gut microflora*. Nutrition. 2008;24:348-359.
7. Kalra SP, Dube MG, Pu S, et al. *Interacting appetite-regulating pathways in the hypothalamic regulation of body weight*. Endocr Rev. 1999;20:68-100.
8. Nakazato M, Murakami N, Date Y et al. *A role for ghrelin in the central regulation of feeding*. Nature. 2001;409:194-198.
9. Sato T, Nakamura Y, Shiimura Y et al. *Structure, regulation and function of ghrelin*. J Biochem. 2012;151:119-128.

10. Watanobe H, Tamura T. *Stimulatory and inhibitory effects of neuropeptide Y on growth hormone secretion in acromegaly in vivo*. Neuropeptides. 1997;31:29-34.
11. Dieguez C. Carro E. Seoane LM, et al. *Regulation of somatotroph cell function by the adipose tissue*. International Journal of Obesity. 2000;24:100-103.
12. François M, Schaefer JM, Bole-Feysot C et al. *Ghrelin-reactive immunoglobulins and anxiety. depression and stress-induced cortisol response in adolescents. The TRAILS study*. Prog Neuropsychopharmacol Biol Psychiatry. 2015;59:1-7.
13. Takagi K, Legrand R, Asakawa A et al. *Anti-ghrelin immunoglobulins modulate ghrelin stability and its orexigenic effect in obese mice and humans*. Nat Commun. 2013;4:2685.
14. Coquerel Q, Sinno MH, Boukhettala N et al. *Intestinal inflammation influences α-MSH reactive autoantibodies: relevance to food intake and body weight*. Psychoneuroendocrinology. 2012;37:94-106.
15. Fetissov SO. Hamze Sinno M. Coquerel Q et al. *Emerging role of autoantibodies against appetite-regulating neuropeptides in eating disorders.* Nutrition. 2008;24:854-859.
16. Stawerska R, Czkwianianc E, Matusiak A et al. *Prevalence of autoantibodies against some selected growth and appetite-regulating neuropeptides in serum of short children exposed to Candida albicans colonization and/or Helicobacter pylori infection: the molecular mimicry phenomenon.* Neuroendocrinol Lett. 2015;36:101-107.
17. Palczewska I, Niedźwiecka Z. *Indices of somatic development of Warsaw children and adolescents.* Medycyna Wieku Rozwojowego 2001;5(suppl.1/2):17-118.
18. Rechciński T, Chmiela M, Małecka-Panas E et al. *Serologic indicators of Helicobacter pylori infection in adult dyspeptic patients and health blood donors*. Microbiol Immunol. 1997;41:387-393.
19. Stawerska R, Smyczynska J, Czkwianianc E et al. *High concentration of ghrelin in children with growth hormone deficiency and neurosecretory dysfunction.* Neuroendocrinol Lett. 2012;33:331-339.
20. Terashi M, Asakawa A, Harada T et al. *Ghrelin reactive autoantibodies in restrictive anorexia nervosa*. Nutrition. 2011;27:407-413.
21. Smitka K, Papezova H, Vondra K, Hill M et al. *The role of "mixed" orexigenic and anorexigenic signals and autoantibodies reacting with appetite-regulating neuropeptides and peptides of the adipose tissue-gut-brain axis: relevance to food intake and nutritional status in patients with anorexia nervosa and bulimia nervosa*. Int J Endocrinol. 2013;2013:483145. doi: 10.1155/2013/483145.
22. Kalra SP, Ueno N, Kalra PS. *Stimulation of appetite by ghrelin is regulated by leptin restraint: peripheral and central sites of action*. Journal of Nutrition. 2005;135:1331–1335.
23. Carro E, Seoane LM, Senaris R et al. *Interaction between leptin and neuropeptide Y on in vivo growth hormone secretion.* Neuroendocrinology. 1998;68:187-191.
24. Scacchi M, Pincelli AI, Cavagnini F. *Nutritional status in the neuroendocrine control of growth hormone secretion: the model of anorexia nervosa*. Frontiers in Neuroendocrinology. 2003;24:200-224.
25. Thibault H . Souberbielle JC. Taieb C. Brauner R. *Idiopathic prepubertal short stature is associated with low body mass index*. Horm Res. 1993;40:136-140.
26. Wudy SA, Hagemann S, Dempfle A et al. *Children with idiopathic short stature are poor eaters and have decreased body mass index*. Pediatrics. 2005;116:52-57.
27. Prodam F, Cadario F, Bellone S et al. *Obestatin levels are associated with C-peptide and antiinsulin antibodies at the onset, whereas unacylated and acylated ghrelin levels are not predictive of long-term metabolic control in children with type 1 diabetes.* J Clin Endocrinol Metab. 2014;99:599-607.
28. Griffin IJ. Catch-Up Growth: Basic Mechanisms. Nestle Nutr Inst Workshop Ser 2015;81:87-97.
29. Carro E, Señaris R, Considine RV et al. *Regulation of in vivo growth hormone secretion by leptin*. Endocrinology. 1997;138:2203-2206.
30. Lucas N, Legrand R, Ouelaa W et al. *Effects of rabbit anti-α-melanocyte-stimulating hormone (α-MSH) immunoglobulins on α-MSH signaling related to food intake control*. Neuropeptides. 2014;48:21-27.
31. Alcock J. Maley CC. Aktipis CA. *Is eating behavior manipulated by the gastrointestinal microbiota? Evolutionary pressures and potential mechanisms.* Bioessays. 2014;36:940-949.