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Investigation of the role of peptides that regulate food intake and nutritional status in children and adolescents

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Abstract of doctoral thesis

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ABBREVIATIONS

ACTH – adrenocorticotropic hormone
ANOVA – analysis of variance
BH – body height
BMI – body mass index
BW – body weight
CG – control group
CN – Crohn´s disease
CNS – central nervous system
CS – celiac disease
DEXA – dual-energy X-ray absorptiometry
DM – diabetes mellitus
EDTA – ethylenediaminetetraacetic acid
ELISA – enzyme-linked immunosorbent assay
FW – formular weight
GH – growth hormone
GHS – growth hormone secretagogues
GIT – gastrointestinal tract
IGF-1 – Insulin-like growth factor-1
IGFBP-3 – IGF-1 binding protein-3
IRMA – immunoradiometric assay
mRNA – messenger ribonucleic acid
MSH – melanocortin
MUP – 4-Methylumbelliferyl Phosphate
OP – osteoporosis
RIA – radioimmunoassay
SD – standard deviation
SDS – standard deviation score
SEM – standard error of mean
TFA – trifluoroacetic acid

1. INTRODUCTION

1.1. REGULATION OF FOOD INTAKE

Food intake and nutritional status of the organism are regulated mainly by interactions of the central nervous system (CNS) - especially the hypothalamus with the gastrointestinal tract (GIT) and the adipose tissue.

In the hypothalamus, there are mainly two pathways involved in the regulatory mechanisms. Activation of the orexigenic pathway leads to increase in food intake and in long term period it may cause weight gain. The orexigenic pathway involves several regulatory peptides, e.g. neuropeptide Y, agouti-related protein and orexin A. On the other hand, activation of the anorexigenic pathway (proopiomelanocortin, cocaine and amphetamine-regulated transcript) leads to decrease in food intake and loss of body weight.

In the GIT there are many regulatory peptides produced, such as ghrelin (orexigenic) or cholecvystokinin, glucagon-like peptide, amylin (anorexigenic) and other. These peptides are secreted as reaction to signals coming from the CNS or generated as reaction to distension of the GIT or to cleaved parts of the digested food.

The adipose tissue is considered to be an active endocrine organ producing wide spectrum of regulatory hormones, including peptides that influence nutritional status of the organism. Leptin is an important anorexigenic hormone which influences hypothalamic areas through leptin receptors. Adiponectin is a potent stimulator of insulin sensitivity with reverse relationship to the amount of body fat in the organism.

The CNS, GIT and adipose tissue are interconnected by system of negative feedback loops and influence each other by hormonal and neuronal pathways. This interplay of signals contributes to complex regulation of food intake and nutritional status of the organism and its disturbance may lead to disorders such as obesity or malnutrition. Moreover, secondary changes of above-mentioned regulatory mechanisms may occur in chronic diseases associated with impaired nutritional status, such as Crohn´s disease and celiac disease.

1.2. OREXIN A

Orexin A (hypocretin 1) is a hypothalamic neuropeptide composed of 33 aminoacid residues (FW=3562) that is involved in regulation of food intake, energy homeostasis, sleep/wake cycle and neuroendocrine functions (1). It was discovered in 1998 (2). Two types of orexin receptors are known

- OXR1/Hcrtr1 and OXR2/Hcrtr2. Cells producing these neuropeptides are localized in a small population within lateral and perifornical hypothalamic area. Fibers of these cells are widespread through many areas of the central nervous system and have mainly excitatory effect.

Previous studies show that intracerebroventricular administration of orexin A increases food intake in rodents and intraperitoneal injection of the selective antagonist against type 1 orexin receptor or central injection of anti-orexin antibody significantly reduces food consumption in rats. Fasting increases preproorexin mRNA content in lateral hypothalamus (3,4).

Orexin A was detected in human plasma in 2001 (5) and decreased plasma levels of orexin A were described in adult obese subjects compared to healthy controls. Changes of orexin A plasma levels were also described in adult fasting non-obese subjects (6,7).

1.3. GHRELIN

Ghrelin is a peptidic hormone composed of 28 aminoacid residues. It is produced by enteroendocrine cells of stomach and intestine. It is also produced in pancreas, kidney, placental tissue, thyroid gland, hypothalamus, and hypophysis. Gastrectomy leads to 65 – 80 % decrease of plasma levels of ghrelin. In human organism, ghrelin stimulates secretion of growth hormone, prolactin, and ACTH. Ghrelin also has orexigenic activity (increases food intake), influences the sleep/wake cycle, gastric motility and secretion, cardiovascular functions, regulates endocrine function of pancreas and metabolism of glucose and shows an antiproliferative effect (8).

Ghrelin is an important regulatory part of the homeostasis of the organism, and iterconnects neuroendocrine and metabolic response of the organism to starvation, and it is considered as a counterpart to leptin. Ghrelin was discovered by japanese scientists in 1999 as a natural ligand of an ”orphan” receptor GHS1a, which is specific for a group of synthetic peptides (growth hormone secretagogues - GHS) stimulating secretion of growth hormone (9). Plasma levels of ghrelin reflect short-time changes of food intake, as well as long-time changes of the nutritional state of the organism. Plasma levels of ghrelin are decreased after food intake and in obese humans, and they are increased during starvation and in patients with anorexia nervosa. Plasma levels of ghrelin in humans correlate negatively with body mass index, amount of body fat, size of adipocytes, and plasma levels of insulin, glucose, and leptin. Thus, ghrelin probably plays a role as a metabolic signal of hunger (10).

1.4. ADIPONECTIN

Adiponectin is a recently discovered product of adipocytes. Its plasma concentrations are decreased in obese adults and in patients with type 2 diabetes mellitus. Adiponectin stimulates oxidation of fatty acids, decreases plasma levels of triglycerides and increases insulin sensitivity. It has probably also antiinflammatory effect through inhibition of migration of monocytes and macrophagues and their conversion to foam cells. By this mechanism, it also acts against development of atherosclerotic processes. Plasma levels of adiponectin are decreased in patients with ischemic coronary disease. Hypoadiponectinemia may contribute to development of insulin resistance, atherosclerosis and obesity (11).

Insulin resistance is a common primary factor of all parts of the metabolic syndrome. The research of the base of this syndrome is recently more focused on molecular level and in this context, the function of adipose tissue as an endocrine organ is stressed. It is an origin of many various regulatory molecules, such as tumor-necrotising factor alpha, leptin, adiponectin and resistin. These molecules are structurally similar to cytokines and therefore they are named in the literature as adipokines. On the base of the scientific research, the gene responsible for development of insulin resistance was identified at locus 3q21. This locus contains also gene for adiponectin (12).

1.5. AMYLIN

Amylin is a polypeptide hormone composed of 37 aminoacids, that is produced in pancreatic beta-cells, and that was discovered in 1987. Releasing amylin into the circulation is increased postprandially, proportionally to the amount of digested food. Receptors for amylin are highly concentrated especially in the CNS – area postrema and nucleus accumbens. There is a 20% sequence homology between amylin, calcitonin and adrenomedullin and 44% homology with calcitonin gene-related peptide (13).

Amylin contributes to the regulation of postprandial glycaemia by suppression of glucagon release and regulation of gastric emptying. Deficit of amylin is typical for type I DM or late stage of type II DM. Amylin decreases food intake and participates in the regulation of body weight (14). Some biochemical forms of amylin cause proliferation of osteoblasts and inhibit bone resorption. Amylin modulates insulin sensitivity of skeletal

muscle, contributes to the regulation of blood pressure and causes vasodilatation (15).

2. OBJECTIVE

The aim of this study was to evaluate the role of regulatory peptides of the CNS, GIT and the adipose tissue in the pathogenesis of changes of body weight and nutritional status in children and adolescents and to evaluate the role of amylin in adult patients with osteoporosis.

We used radioimmunoassay to measure plasma levels of orexin A, total ghrelin and serum levels of leptin and enzyme-linked immunoassay to measure serum levels of adiponectin in obese children, patients with anorexia nervosa, Crohn´s disease, celiac disease and in healthy subjects. Plasma levels of amylin were measured by enzyme-linked immunoassay in adult patients with osteoporosis, type II DM and in healthy controls.

Specific aims of the project:

1. *To measure plasma levels of orexin A, total ghrelin and serum levels of adiponectin in obese children and to evaluate their relationship to serum levels of leptin, IGF-1 and IGFBP-3. To evaluate the influence of weight reduction on these levels.*

2. *To measure plasma levels of orexin A, total ghrelin and serum levels of adiponectin in patients with anorexia nervosa and to evaluate their relationship to serum levels of leptin, IGF-1 and IGFBP-3. To evaluate the influence of realimentation on these levels.*

3. *To measure plasma levels of orexin A in healthy children, and in children with Crohn´s disease and celiac disease.*

4. *To measure plasma levels of amylin in adult patients with osteoporosis, type II DM and in the control group.*

3. SUBJECTS AND METHODS

3.1. SUBJECTS

Obese subjects:

We recruited 58 obese subjects (33 girls and 25 boys) undergoing weight-reduction therapy in health resort. We included in the study all the

patients with body weight / body height ratio above 97th percentile according to standards for Czech children population based on 5th nation-wide anthropological survey performed by National Institute of Public Health, Prague (16). Mean age of our subjects was 13.6 ± 0.5 years (range 7.8 – 18.1) in girls and 12.4 ± 0.6 years (7.0 – 18.5) in boys. Mean body height was 160.0 ± 2.0 cm (134.6 – 177.6) in girls and 157.2 ± 3.1 cm (126.8 – 178.7) in boys, respectively. Body weight and body mass index (BMI) before and after weight-reduction therapy are shown in table 1.

Reduction of body weight was achieved during 5 weeks in health resort using both decrease of food intake and enhanced energy expenditure. No medication was used to decrease body weight in the subjects.

Patients with anorexia nervosa:

In the study, we included 36 girls with anorexia nervosa (10 premenarcheal and 26 postmenarcheal) treated by realimentation in department of pediatric psychiatry. We included in the study girls with BMI below 18.5. Mean age was 13.9 ± 0.4 years (range 12.6 – 16.6) in premenarcheal girls and 15.4 ± 0.3 years (13.5 – 18.8) in postmenarcheal. Mean body height was 158.2 ± 2.7 cm (148.5 – 176) in premenarcheal girls and 166.0 ± 1.3 cm (156.5 – 182.2) in postmenarcheal. Body weight and body mass index before and after realimentation are shown in table 1.

Increase of body weight was achieved during 8 weeks using increase of food intake, supervision during meals and psychotherapeutical approach. No medication was used to increase appetite in the subjects.

mean ± SEM	BW before (kg)	BW after (kg)	BMI before (kg/m2)	BMI after (kg/m2)
Obesity	74.2 ± 2.8	67.4 ± 2.6	28.8 ± 0.6	26.1 ± 0.6
Anorexia nervosa	42.4 ± 1.0	48.6 ± 1.0	15.8 ± 0.3	18.0 ± 0.2

Table 1. Body weight (BW) and BMI before and after therapeutical intervention in patients with obesity and anorexia nervosa

Patients with Crohn´s disease, celiac disease and healthy controls:

Patients with Crohn´s disease and with celiac disease were recruited on the base of common diagnosti criteria - clinical (typical gastrointestinal symptoms), laboratory (inflammatory markers, antibodies) and histological (intestinal biopsy). Patients were not previously treated with any drugs influencing food intake or nutritional status. In the study, we included 23 patients with Crohn´s disease (15 boys, 8 girls) and 18 patients with celiac disease (9 boys, 9 girls).

As control group, we recruited patients from outpatient department of ear-nose-throat clinic undergoing basic pre-operation investigation before small surgical interventions in the head and neck. In the study, we included 28 patients (14 boys and 14 girls), who did not exert any signs of impaired food intake or nutritional status.

Basic characteristics of the patients are summarized in table 2.

mean ± SEM	Age (yrs)	BH (m)	BW (kg)	BMI (kg/m2)
Crohn´s disease	14.8 ± 0.5	1.56 ± 0.03	47.0 ± 3.1	18.0 ± 0.7
Celiac disease	10.6 ± 1.0	1.36 ± 0.06	33.6 ± 4.1	16.8 ± 0.7
Control group	12.8 ± 0.6	1.55 ± 0.03	46.1 ± 2.4	18.7 ± 0.5

Table 2. Basic characteristics of patients with Crohn´s disease, celiac disease and the control group; BH = body height; BW = body weight

Patients with osteoporosis, type II diabetes mellitus and control group:

28 patients (3 men and 25 postmenopausal women with mean age of 65 years; range 42 – 82 yrs) with osteoporosis (OP) were selected for the study. Subjects were included on the base of clinical criteria (pain in axial skelet and/or hip joints) and/or previously radiographically diagnosed fractures. We performed a dual-energy X-ray absorptiometry (DEXA) in all the subjects and bone mineral density under 2.5 standard deviations (T-score < -2.5) was considered to confirm the diagnosis.

The second group of subjects was composed of patients with late stage type 2 DM (n = 10; 5 men, 5 women; mean 64 yrs; range 53 – 79 yrs) and served as negative controls with expected low amylin levels due to impaired beta-cell function. This was indicated by fasting serum C-peptide levels reaching lower level of reference range (300 pmol/l).

24 patients (11 men, 13 women; mean 53 yrs; range 35 – 73 yrs) followed in our outpatient department for mild compensated dyslipidaemia were chosen as the control group (CG). All these patients undergo only dietary treatment and physical examination to keep serum levels of cholesterole and triglycerides in reference range.

All subjects were informed of the nature of the study. Written consent was obtained from each participant in the presence of a witness.

3.2. LABORATORY METHODS

Blood withdrawal:

Ten ml of fasting venous blood were taken to 2 tubes (5 ml in plasma tubes containing EDTA and Aprotinin (antilysin, Spofa) and 5 ml in standard serum tubes (S-Monovette, Sarstedt)) from cubital vein between 7-9 a.m. In patients with obesity and anorexia nervosa, samples were taken at the beginning of the investigation and after 5 (respectively 8) weeks of the therapeutical intervention. Samples were centrifuged by 2,000 x g for 15 min. at 4° C. Separated plasma and serum were immediately frozen and stored at –80° C until the analysis. In children, there was anthropometrical investigation performed paralelly with the blood withdrawal.

Measurement of orexin A:

Orexin A concentrations were measured in duplicate after extraction procedure. Acidified plasma using 1% trifluoroacetic acid (TFA) was centrifuged and supernatant was loaded on a SEP-COLUMN containing 200 mg of C18 (Code RK-SEPCOL-1, Diagnostics inc., Lake Forest, CA, USA) washed by 1% TFA and eluted by 60% acetonitrile in 1% TFA. The eluants were evaporated to dryness in a speed vacuum centrifuge from Heto (Melsungen, Germany). The residues were dissolved in buffer for radioimmunoassay (RIA). We used commercially available RIA kit (Phoenix Pharmaceuticals, Belmont, CA, USA). The RIA is based on competitive-binding principle and makes use of polyclonal rabbit antibodies against human orexin A and goat anti-rabbit antibody to separate bound and free fraction. Human orexin A was used as standard. The intra-assay and inter-assay variations were 5.2 % and 14.2 %, respectively, and the lowest detectable concentration was 1 pg/ml. There is no crossreactivity with the fragment 16-33 of human orexin A, with orexin B, agouti-related protein amide (83-132), neuropeptide Y, alpha-MSH and human leptin.

Measurement of total ghrelin:

Total ghrelin plasma levels were measured in duplicate using competitive RIA (Phoenix Pharmaceuticals) using polyclonal rabbit antibodies against total human ghrelin and goat antibodies against rabbit immunoglobulins to separate bound and free fraction. Human ghrelin is used as standard. The amount of radioactivity in each sample is proportional to concentration of the peptide. The intra-assay and inter-assay variations were 7.4 % and 13.5 %, respectively, and the lowest detectable concentration was 10 pg/ml. There is no crossreactivity with the fragment 1-

14 of human ghrelin, with secretin, vasoactive intestinal peptide, galanin, neuropeptide Y and orexin A or B.

Measurement of adiponectin:

Adiponectin serum levels were measured in duplicate using sandwich enzyme-linked immunosorbent assay (ELISA; Linco Research, Inc., St.Charles, Missouri, USA) using primary antibodies against human adiponectin and secondary biotin-labeled mice antibodies. Horseradish peroxidase conjugated with streptavidin is bound to biotin and the reaction leads to increase of absorbance which is proportional to concentration of the peptide. Recombinant human adiponectin is used as a standard. The intra-assay and inter-assay variations were 3.5 % and 6.5 %, respectively, and the lowest detectable concentration was 0.5 ug/ml of serum. There is no crossreactivity with human amylin, C-peptide, insulin, leptin or resistin.

Measurement of amylin:

For the quantification of human amylin in plasma, we used the monoclonal antibody-based sandwich immunoassay (ELISA kit with immunofluorescent detection; Linco Research) derived from the assay principles described by Percy et al. in 1996 (17). The assay was run in duplicate using 50 µl of sample in each well.

In the total amylin assay, the capture antibody binds to an epitope near the midpoint of the peptide and recognizes reduced human amylin and human amylin acid (deamidated amylin) but not the 1-20 fragment of amylin. The detection antibody in this assay binds to reduced or unreduced human amylin but not amylin acid. Using this assay we are able to detect both reduced and unreduced amylin composed of all 37 aminoacids with amidated carboxy-terminus of the peptide. Moreover, as described by Percy et al., this assay is also able to detect „amylin-like peptides" (17). The intra-assay variation is 2.5% and the inter-assay variation is 10%.

In the unreduced amylin assay the capture antibody requires an intact disulphide bond between positions 2 and 7 of the peptide and recognizes human amylin, amylin acid (deamidated amylin), the 1-20 fragment of amylin, but not reduced amylin. The detection antibody in this assay binds to reduced or unreduced human amylin but not amylin acid. Using this assay we are able to detect only unreduced amylin composed of all 37 aminoacids with amidated carboxy-terminus. The intra-assay variation is 2.2% and the inter-assay variation is 5.9%.

The detection antibody in both assays is complexed with streptavidin-alkaline phosphatase. The substrate, 4-Methylumbelliferyl Phosphate (MUP), is applied to the completed sandwich and the fluorescent signal,

monitored at 355 nm / 460 nm, is proportional to the amount of amylin present in the sample. The sensitivity of the assay is 1 pmol/l. The crossreactivity with human glucagon, insulin, pancreatic polypeptide, adrenomedullin, calcitonin and calcitonin gene-related peptide is less than 1% in both assays. There is no known interference with plasma protein, glucose or triglycerides.

Measurement of other analytes:

Leptin was measured in serum using commercially available human leptin RIA kit (Linco Research) based on competitive-binding principle. Insulin-like growth factor-1 (IGF-1) was measured in serum using immunoradiometric assay (IRMA; Beckman-Coulter, France) and IRMA method (Diagnostic Systems Laboratories, Inc., USA) was used to detect serum IGF binding protein-3 (IGF BP-3). Fasting serum C-peptide and insulin were measured by immuno-chemiluminiscence method (DPC Immulite 1). The above-mentioned methods are routinely performed in our biochemical laboratory.

3.3. ANTHROPOMETRY AND STATISTICAL ANALYSIS

Anthropometrical measurements were performed immediately after blood withdrawal. Body weight and body height were measured by standard techniques (calibrated weighing machine and stadiometer), in underwear and without shoes. BMI was counted as kg / m^2. Because the range of age of our subjects was wide and changes of anthropometrical parameters are not linear during childhood and puberty, we counted standard deviation scores (SDS) for body height, body weight, and BMI to use it in our correlation analyses. SDS were calculated from reference standards for Czech children population mentioned above (16).

Statistical analysis was performed using Prism 4.0 statistical software (Graph Pad Software, Inc.). Results are reported as mean $\pm$ SEM. In normally distributed values, the correlations were tested by Pearson´s correlation coefficient, the differences within groups were tested using paired t-test and differences between groups using unpaired t-test. Welch´s correction was used in cases of significantly different variances. One-way analysis of variance (ANOVA) was used for comparison of more than two groups. In case of non-normally distributed values, we used Spearman´s correlation coefficient, Wilcoxon test, Mann-Whitney test, and non-parametric ANOVA – Kruskal-Wallis test. A p value of less than 0.05 was considered statistically significant.

4. RESULTS

4.1. OBESE PATIENTS

Orexin A:

Fasting orexin A plasma levels before the weight-reduction were 33.3±2.0 pg/ml and after the therapy 51.7±3.1 pg/ml ($p<0.0001$). Levels of orexin A were not significantly different between girls and boys ($p=0.7842$), see fig. 1. We found negative correlation between orexin A and age ($r=-0.5395$; $p<0.0001$), body height ($r=-0.4751$; $p=0.0002$), body weight ($r=-0.4030$; $p=0.0017$) and BMI ($r=-0.2607$; $p=0.0481$). No correlation was found between orexin A and IGF-1, IGFBP-3 or leptin. Correlation data are summarized in table 3.

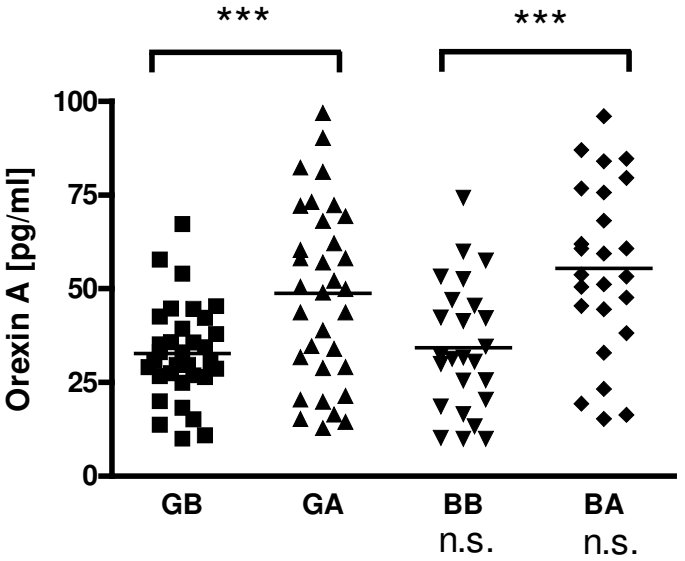

Figure 1. Plasma levels of orexin A in obese children before and after reduction of body weight
GB – girls before weight-reduction, GA – girls after, BB – boys before, BA – boys after; There was a statistically significant difference before vs after weight-reduction (*), but not between boys and girls (n.s.).

Ghrelin:

Fasting plasma levels of total ghrelin before the weight-reduction were 844.4±44.9 pg/ml and after the therapy 996.5±56.9 pg/ml ($p<0.0001$). In boys, ghrelin levels were significantly lower than in girls ($p=0.0149$). We did not find any correlation between ghrelin and age, but we found a negative correlation with body height ($r=-0.3196$; $p=0.0145$) and body weight ($r=-0.3173$; $p=0.0165$), see fig. 2. No correlation was found between ghrelin and IGF-1, IGFBP-3 or leptin, see table 3.

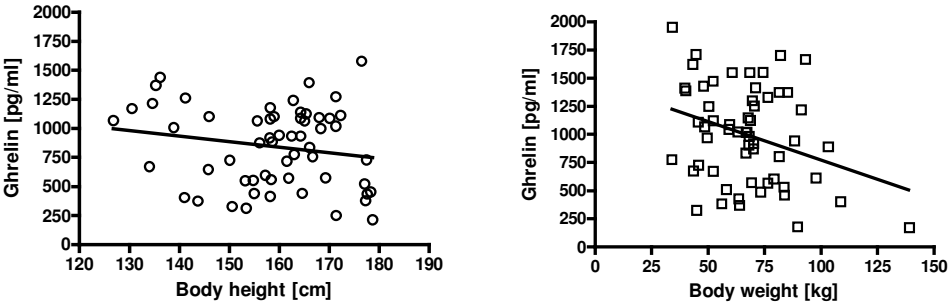

Figure 2. Correlation of ghrelin plasma levels with body height and body weight in obese children

Adiponectin:

Fasting adiponectin serum levels before the weight-reduction were 8.3±0.5 ug/ml and after the therapy 8.5±0.5 ug/ml ($p=0.2979$). There was a strong correlation between serum levels before and after the weight-reduction ($r=0.8986$, $p<0.0001$), see fig. 3. Levels of adiponectin were not significantly different between girls and boys ($p=0.5817$), We found a negative correlation between adiponectin levels and age ($r=-0.4625$; $p=0.0003$), body height ($r=-0.3998$; $p=0.0019$), body weight ($r=-0.3888$; $p=0.0026$), and BMI ($r=-0.3664$; $p=0.0047$). We found a negative correlation between adiponectin and IGF-1 ($r=-0.3219$; $p=0.0137$), insulin ($r=-0.4022$; $p=0.0017$) and C-peptide serum levels ($r=-0.4065$; $p=0.0015$). No correlation was found between adiponectin and leptin or IGFBP-3, see table 3.

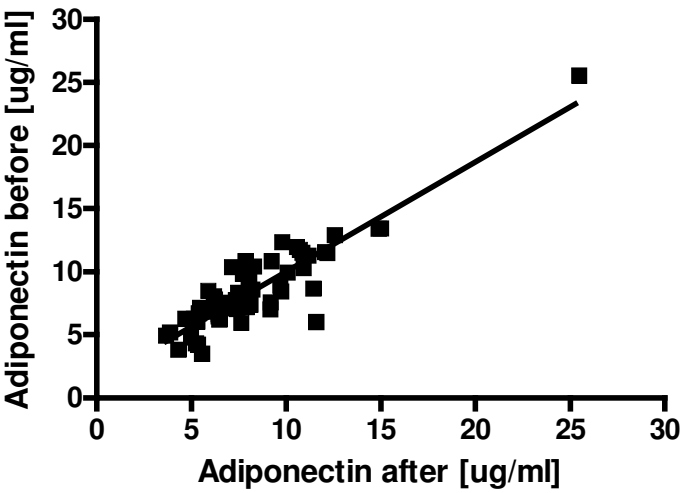

Figure 3. Correlation of adiponectin serum levels before and after weight-reduction in obese children.

4.2. ANOREXIA NERVOSA

Orexin A:

Fasting plasma orexin A levels before the realimentation were 56.2±2.4 pg/ml and after the therapy 47.5±1.4 pg/ml (p=0.0025). Levels of orexin A were not significantly different between premenarcheal and postmenarcheal girls, see fig. 4. We did not find any correlation between orexin A levels and age, body height, body weight or BMI. No correlation was found between orexin A and IGF-1, IGFBP-3 or leptin, see table 3.

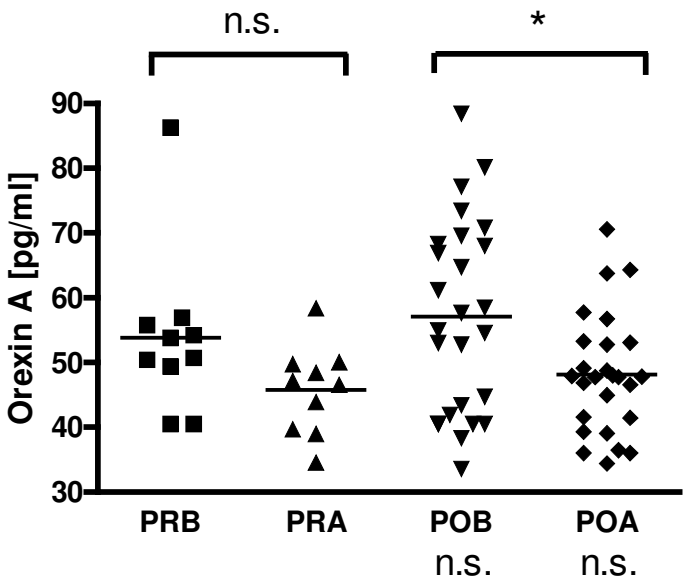

Figure 4. Plasma levels of orexin A in girls with anorexia nervosa
PRB – premenarcheal girls before realimentation, PRA – premenarcheal after, POB – postmenarcheal before, POA – postmenarcheal after; We foud a significant difference in postmenarcheal girls before realimentation vs after (*), but not in premenarcheal (n.s.); No difference was found between premenarcheal and postmenarcheal girls (n.s.).

Ghrelin:

Fasting plasma levels of total ghrelin before the realimentation were 3027±257 pg/ml and after the therapy 2148±213 pg/ml (p=0.0002). Levels of ghrelin were not significantly different between premenarcheal and postmenarcheal girls. We did not find any correlation between ghrelin levels and age, body height or body weight, but we found a negative correlation with BMI (r=-0.4392; p=0.0074), leptin serum levels (r=-0.6416; p<0.0001), see fig. 5, and IGFBP-3 serum levels (r=-0.3894; p=0.0189). No correlation was found between ghrelin and IGF-1 levels, see table 3.

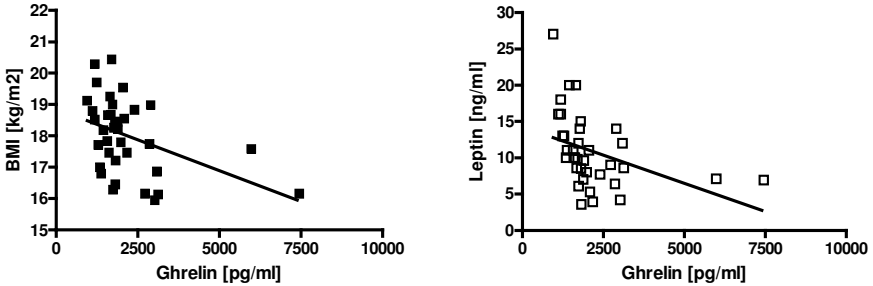

Figure 5. Correlation of plasma ghrelin levels with BMI and leptin in patients with anorexia nervosa

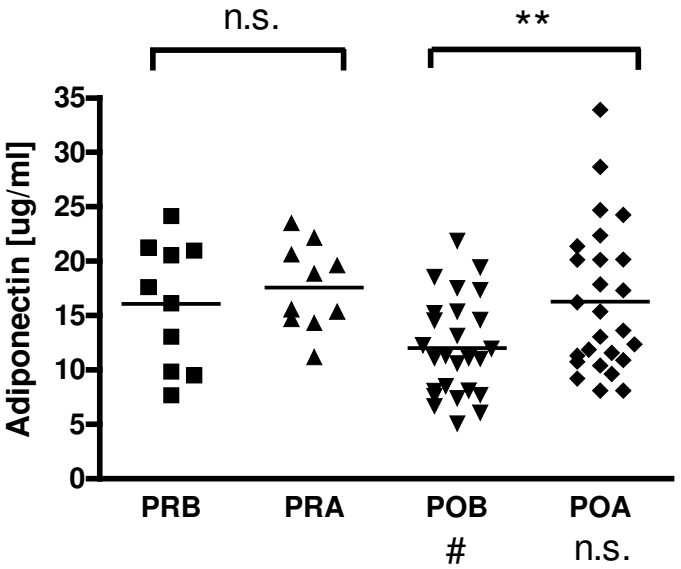

Figure 6. Serum levels of adiponectin in girls with anorexia nervosa
PRB – premenarcheal girls before realimentation, PRA – premenarcheal after, POB – postmenarcheal before, POA – postmenarcheal after; We foud a significant difference in postmenarcheal girls before realimentation vs after (*), but not in premenarcheal (n.s.); A significant difference was found between pre- and postmenarcheal girls before realimentation (#).

Adiponectin:

Serum adiponectin levels before the realimentation were 13.1±0.9 ug/ml and after the therapy 16.6±1.0 ug/ml (p=0.0024). Levels of adiponectin were significantly lower in postmenarcheal vs premenarcheal girls, but only before realimentation (p=0.0317), see fig.6. We did not find any correlation between adiponectin levels and age, body height and body weight, but we found a negative correlation with BMI (r=-0.4335; p=0.0083). No correlation was found between adiponectin and leptin, insulin or C-peptide levels, see table 3.

Obese:	Age	Gender	BH	BW	BMI	SDS BH	SDS BW	SDS BMI
Orexin A	-	0	-	-	-	0	0	0
Leptin	+	BL	+	+	+	+	+	+
Ghrelin	0	BL	-	-	0	-	0	0
Adiponectin	-	0	-	-	-	0	0	0

Obese:	Orexin A	Leptin	Ghrelin	Adiponectin	IGF-1	IGF BP-3	Insulin	C-peptide
Orexin A	X	0	+	0	0	0	NT	NT
Leptin	0	X	0	0	0	0	NT	NT
Ghrelin	+	0	X	0	0	0	NT	NT
Adiponectin	0	0	0	X	-	0	-	-

AN:	Age	Gender	BH	BW	BMI	SDS BH	SDS BW	SDS BMI
Orexin A	0	0	0	0	0	0	0	0
Leptin	0	0	0	0	+	0	+	+
Ghrelin	0	0	0	0	-	0	0	-
Adiponectin	0	POL	0	0	-	0	0	-

AN:	Orexin A	Leptin	Ghrelin	Adiponectin	IGF-1	IGF BP-3	Insulin	C-peptide
Orexin A	X	0	+	0	0	0	NT	NT
Leptin	0	X	-	0	+	0	+	+
Ghrelin	+	-	X	+	0	-	NT	NT
Adiponectin	0	0	+	X	0	0	0	0

Table 3. Summary of correlations with anthropometrical and biochemical parameters in patients with obesity and anorexia nervosa (AN)
(+) - positive correlation, (-) - negative correlation, (0) – no correlation, NT – not tested, BL – lower in boys, POL – lower in postmenarcheal girls; BH – body height, BW – body weight

4.3. CROHN´S DISEASE, CELIAC DISEASE AND CONTROL GROUP

Fasting plasma levels of orexin A in patients with Crohn´s disease (CN) were 57.6±3.7 pg/ml, in patients with celiac disease (CS) 55.2±3.3 pg/ml and in the control group (CG) 44.8±3.2 pg/ml. Levels in CN and CS were significantly higher than in CG (ANOVA, $p<0.05$), see fig.7. In CN, orexin A levels correlated positively with IGF-1 (r=0.6129, p=0.0089) and IGFBP-

3 (r=0.4674, p=0.0377). In CS, orexin A levels correlated with IGF-1 (r=0.6464, p=0.0316), but we did not find any correlation with leptin.

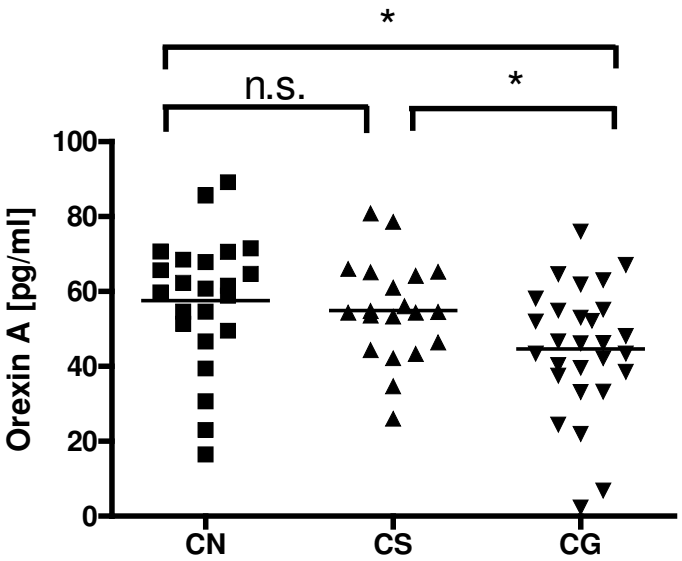

Figure 7. Plasma levels of orexin A in patients with Crohn´s disease (CN), celiac disease (CS) and in the control group (CG)

4.4. AMYLIN IN OSTEOPOROSIS, TYPE II DIABETES MELLITUS AND CONTROL GROUP

Fasting plasma levels of total amylin in patients with OP were 3.33 $\pm$ 0.46 pmol/l, in patients with DM 6.29 $\pm$ 1.47 pmol/l and in the CG 8.48 $\pm$ 3.12 pmol/l. Mean plasma levels were lower in patients with OP than in patients with DM and than in the CG, but the difference did not reach statistical significance (Kruskal-Wallis test, $p > 0.05$).

Fasting plasma levels of unreduced amylin in patients with OP ($n = 28$) were 2.51 $\pm$ 0.87 pmol/l, in patients with DM ($n = 10$) 4.15 $\pm$ 0.95 pmol/l and in the CG ($n = 5$) 13.50 $\pm$ 3.94 pmol/l. Plasma levels were significantly lower in patients with OP than in patients with DM and than in the CG (Kruskal-Wallis test, p value summary = 0.0003) - see fig. 8.

Mean body weight in OP, DM and CG group was 64.4, 71.1 and 69.3 kg, mean body height was 1.67, 1.64 and 1.64 m, and body mass index was

23.24 ± 0.68, 26.54 ± 1.06 and 25.82 ± 0.80 kg/m^2, respectively. No statistically significant difference was found between groups in body height. Mean body weight was lower in OP than in CG and BMI in OP group was significantly lower than in the CG (ANOVA, p = 0.0155). There was no correlation found in any group between amylin total or unreduced amylin levels and body weight, body height, or BMI. There was no correlation found in any group between amylin total or unreduced amylin levels and age. There was no significant difference in amylin levels between men (n=3) and women. Further statistical analysis was not performed due to low number of male subjects.

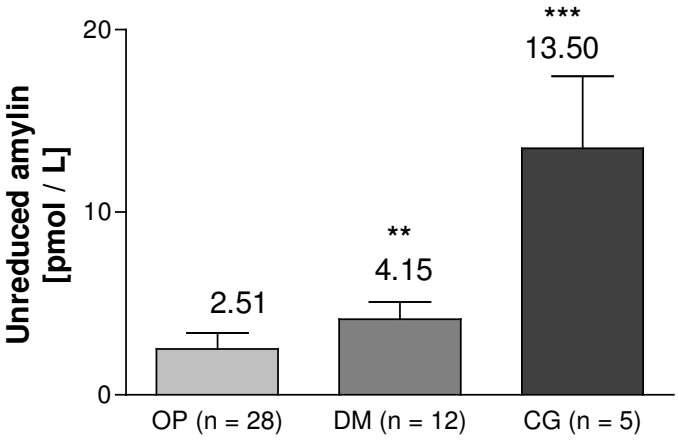

Figure 8. Unreduced amylin fasting plasma levels in patients with osteoporosis (OP), type 2 diabetes mellitus (DM), and in the control group (CG)

5. DISCUSSION

Orexin A:

In this study, we describe changes of plasma levels of the hypothalamic neuropeptide orexin A in obese children during reduction of body weight and in girls with anorexia nervosa during realimentation. Moreover, we show its relationship to other biochemical and anthropometrical parameters. Our results support the hypothesis that production of orexin A may be influenced by changes of body weight, but we did not prove that orexin A levels are directly related to circulating serum leptin levels. Although it was previously shown that neurons producing orexin A are in very close relationship to neurons expressing leptin receptors (18), there are probably more complex regulatory mechanisms involved in regulation of orexin A production (19,20). There may be other regulatory peptides (either peripheral – ghrelin, cholecystokinin or central – neuropeptide Y, agouti-related protein) which may possibly influence production of orexin A in the hypothalamus and furter studies are needed to identify such factors.

Moreover, detected plasma levels of orexin A may not precisely reflect hypothalamic expression of orexin A, although it crosses blood-brain barrier by simple diffusion (21). Expression of orexin A was also shown in other tissues distinct from central nervous system, such as testis (22-24). This fact may explain slightly higher orexin A levels in boys shown in our study, in spite of the fact that orexin A levels were not statistically different between girls and boys. These findings correspond to results obtained in studies performed in adult subjects (25,5).

In our study, we found a negative correlation between orexin A levels and age in obese children, but not in girls with anorexia nervosa. In 2002, Matsumura et al. described a positive correlation between orexin A levels and age in adult subjects (26). On the other hand, Kanbayashi in his paper did not find any differences in orexin A levels in cerebrospinal fluid in respect to age (27). In 2004, Tomasik et al. (28) published an analysis of orexin A and B plasma levels in children from newborns until adolescence. His paper shows the highest levels of orexins in neonates and in children during puberty with subsequent decrease. Our results in obese children aged 10 - 18 years are in agreement with his data, but there are probably other factors influencing orexin A levels in developing organism which need further investigation. Moreover, the negative correlation of orexin A levels and body height, body weight and BMI of the subjects disappears when the data are converted to SDS. This effect might be explained by age-related changes of orexin A in our subjects.

Although orexin A was shown to regulate growth hormone (GH) secretion both *in vitro* and *in vivo* (29,30), we did not find any relationship between orexin A levels and IGF-1 or its´ binding protein 3. We found a negative correlation between orexin A levels and body height, but after conversion of data to SDS the correlation was not signifficant. However, IGF-1 production is only partially dependent on GH and in obese children it is also regulated by nutritional factors. Thus, the effect of orexin A on GH secretion needn´t to be reflected in the relationship to IGF-1. To evaluate the orexin A-GH relationship in our subjects, it would be necessary to perform measurement of fasting GH under standard conditions with respect to its diurnal variation.

In anorexia nervosa, there are no data on orexin A levels available, thus it´s very difficult to compare data obtained in our study. Absence of any correlation with anthropometrical data in this group points to the previously published oppinion that anorexia nervosa is a disease with impaired both orexigenic and anorexigenic signalling (31).

Ghrelin:

In our study, we show significant changes of total ghrelin plasma levels during therapeutical intervention in children both with obesity and anorexia nervosa.

We did not show any relationship between ghrelin plasma levels and age in accordance with other authors who generally did not find any relationship between both variables (32-36). On the other hand, there are authors showing relationship between ghrelin levels and age, but only in healthy subjects with normal nutritional status (37,38). Thus, further studies are needed to evaluate ghrelin levels with respect to age in patients with impaired nutritional status.

In obese patients, we describe negative correlation of ghrelin with body height and its´ SDS. In spite of published data that ghrelin may enhance production of growth hormone, we did not find any relationship to IGF-1 levels. In anorexia nervosa, there was a negative correlation between ghrelin and IGFBP-3. Due to diurnal variation of GH secretion, we did not perform analysis of direct relationship of GH levels and ghrelin. There are several papers that discuss potential relationship of ghrelin plasma levels and growth (39-41), but none of them brings prove of clear relationship between ghrelin levels and production of GH.

Concerning ghrelin levels and other anthropometrical data, we have shown a negative correlation with body weight in obese children and negative correlation with BMI and SDS BMI in girls with anorexia nervosa. In spite of the fact that groups of subjects included in our study are

relatively small, we can conclude that ghrelin levels may be regulated by nutritional status of the organism both in excess and lack of energy supplies. There are many studies showing possible relationship between ghrelin levels and nutritional status of the organism (34,42-44). One of the factors that may play role in such regulation is leptin. This hypothesis is supported in our study by negative correlation of ghrelin and leptin levels in girls with anorexia nervosa.

One of the interesting issues of our study is finding that total ghrelin plasma levels correlate positively with orexin A plasma levels in patients both with obesity and anorexia nervosa. This correlation points to possible common regulatory mechanisms influencing both above-mentioned regulatory peptides. There are no data on orexin A and ghrelin correlation in the literature, thus the finding remains an important point for further investigation.

Adiponectin:

In our study, we didn´t show significant changes of adiponectin serum levels neither during reduction of body weight in obese children nor during realimentation in premenarcheal girls with anorexia nervosa.

The fact that there are no significant changes in adiponectin levels during 5 weeks of body weight-reduction shows that this time period is probably not sufficient for adequate regulation of adiponectin secretion. There are several papers supporting this hypothesis (45-47). Moreover, in our study adiponectin serum levels after weight-reduction correlate strongly with levels before weight-reduction, thus each of the patients keeps its adiponectin level during the whole period of therapeutical intervention. On the other hand, there are studies showing significant change during long term weight-reduction (48,49).

In postmenarcheal girls, there was a significant increase in adiponectin serum levels. This finding is surprising especially with respect to previously published data and generally accepted hypothesis that adiponectin under normal conditions is regulated negatively by the amount of body fat (11,50-52). The fact that adiponectin levels in postmenarcheal girls in our study change in unexpected manner may be caused by potential other factors that may influence production of this regulatory peptide, such as sex hormones that exceed under normal circumstances in perimenarcheal period, but may be negatively influenced by disturbed nutritional status in anorexia nervosa. Thus, it seems that the amount of body fat is not the only regulator of adiponectin production in perimenarcheal girls with anorexia nervosa (31). Moreover, in girls with anorexia nervosa we have shown positive correlation between adiponectin and ghrelin levels, which was not found in

obese patients. This fact supports the hypothesis that adiponectin serum levels may be regulated in different way under conditions of obesity and malnutrition.

In obese patients, but not in girls with anorexia nervosa,we have shown a negative correlation between adiponectin levels and body height, respectively IGF-1. In 2005 Nilsson described suppressive effect of GH on adiponectin expression *in vitro* (53). This may point to possible physiological negative relationship between GH production and adiponectin expression in adipose tissue.

Amylin:

In animal experiments, some biochemical forms of amylin seem to be important stimulators of osteoblastic proliferation and intact amylin also acts as an inhibitor of bone resorption, but it still remains unclear whether these functions are equivalent in humans.

In the first experiment, total amylin levels in patients with OP were not significantly different from those in the CG. Regardingless this fact, the mean value was the lowest in OP group. This may point to possible deficit of amylin in patients suffering from osteoporosis. Unreduced amylin fasting plasma levels were more significantly different in patients with OP than those of total amylin, in spite of lower number of patients in the investigated groups. On the base of this finding and the results of previous animal and *in vitro* experiments (54-56), the investigation of unreduced amylin seems to be more sensitive in the relationship to the effect on bone. Perhaps, the intact disulphide bond plays a key role in stimulation of osteoblasts. This effect may be negatively influenced by reduction processes in the human organism resulting in cleavage of the disulphide bond of the amylin molecule.

There were no anamnestic, clinical or biochemical data in patients with OP showing any dysfunction of pancreatic beta-cells that could result in decreased production or secretion of amylin into circulation. To reveal possible causes of amylin deficiency in these patients, more detailed study of bone metabolism is needed. Also biochemical analysis of possible modification of amylin in human plasma and its kinetics would be usefull to reveal the causes of our findings. There may be many factors influencing either amylin production, release into circulation, or its effect on receptors in bone tissue that may lead to primary or secondary changes in amylin plasma levels. There are very few studies available concerning this topic, especially regarding data in humans. In our study, amylin levels in DM patients were lower than in the CG, fulfilling the hypothesis that impairment of beta-cell function may result in deficit of amylin in peripheral blood.

In our study, the mean age of CG was significantly different from OP group. A possible role of this fact in differences of amylin levels was widely discussed. There are no published data showing relationship between fasting amylin plasma levels and age of subjects. The only available study in humans shows impairment of amylin release from pancreas after glucose infusion in elderly patients (57). Moreover, we did not find any correlation between age and amylin levels in any of the analysed groups. After adjustment to age, the differences in amylin levels did not change. We conclude that amylin fasting plasma concentrations in our subjects are not related to age.

For osteoporosis is more frequent in women, the gender ratio in OP group was not equal. Mean amylin levels did not differ in men comparing to women. When we compared only women from OP, DM and CG groups, the results did not differ from results obtained by analysis of complete groups, and pointed to deficiency of amylin in OP patients.

There is a well known relationship between body weight and bone density and recently there were some data published about molecular link between adipose tissue and bone (58). We investigated whether amylin levels are related to basic anthropometrical parameters. Mean body weight of subjects was the lowest in OP group and body mass index differed significantly from CG. However, we did not find any correlation between these parameters and amylin levels in any of the analysed groups and the differences in amylin levels did not change after adjustment to body weight. Thus, neither this anthropological parameter probably contributes to changes in amylin plasma concentrations.

The „unreduced-amylin assay" detects only whole-chain unreduced amylin. The „total-amylin assay" measures in addition reduced amylin and probably also some „amylin-like peptides", as described above. „Amylin-like peptides" have been shown by Western blotting to be larger than human amylin, and have been found in all samples from amylin-positive individuals tested by Percy et al. (17). These peptides may be e.g. amylin aggregates, amylin precursors such as proamylin, or posttranslationally modified amylin. This explains the differences in concentrations in OP and DM groups between both assays. The number of patients in our experiments is not high enough to postulate, whether amylin fasting plasma levels should be introduced as one of biochemical investigations in osteoporotic patients. In further experiments, the role of amylin according to the type and etiology of osteoporosis (postmenopausal, senile, secondary) should be investigated and higher number of patients should be included.

6. CONCLUSIONS

1. *Orexin A fasting plasma levels change significantly in patients both with obesity and anorexia nervosa during therapeutical intervention leading towards normalisation of nutritional status.*

2. *Orexin A fasting plasma levels differ significantly in patients with impaired nutritional status (Crohn´s disease, celiac disease) from healthy controls.*

3. *Total ghrelin fasting plasma levels change significantly both in obese patients during weight-reduction and in girls with anorexia nervosa during realimentation.*

4. *Adiponectin serum levels did not change significantly during five weeks of weight-reduction therapy. In girls with anorexia nervosa, there are probably other hormonal factors influencing adiponectin levels in perimenarcheal period.*

5. *Unreduced amylin fasting plasma levels are significantly decreased in patients with osteoporosis in comparison with type II diabetic patients and control subjects*

7. LITERATURE CITED

1. YANAGISAWA M, BEUCKMANN CT: Orexins: from neuropeptides to energy homeostasis and sleep/wake regulation. *J Mol Med* 80: 329-342, 2002.

2. SAKURAI T, AMEMIYA A, ISHII M, et al.: Orexins and orexin receptors: A family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. *Cell* 92: 573-585, 1998.

3. DUBE MG, KALRA SP, KALRA PS: Food intake elicited by central administration of orexins/hypocretins: identification of hypothalamic sites of action. *Brain Res* 842: 473-477, 1999.

4. HAYNES AC, JACKSON B, CHAPMAN H, et al.: A selective orexin-1 receptor antagonist reduces food consumption in male and female rats. *Regul Pept* 96: 45-51, 2000.

5. ARIHARA Z, TAKAHASHI K, MURAKAMI O, et al: Immunoreactive orexin-A in human plasma. *Peptides* 22: 139-142, 2001.

6. ADAM JA, MENHEERE PP, VAN DIELEN FM, et al.: Decreased plasma orexin-A levels in obese individuals. *Int J Obes Relat Metab Disord* 26: 274-276, 2002.

7. KOMAKI G, MATSUMOTO Y, NISHIKATA H, et al.: Orexin-A and leptin change inversely in fasting non-obese subjects. *Eur J Endocrinol* 144: 645-651, 2001.

8. CASSONI P, PAPOTTI M, GHÉ C, et al.: Identification, characterization, and biological activity of specific receptors for natural (ghrelin) and synthetic growth hormone secretagogues and analogs in human breast carcinomas and cell lines. *J Clin Endocrinol Metab* 86: 1738 – 1745, 2001.

9. KOJIMA M, HOSODA H, DATE Y, et al.: Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature* 402: 656 – 660, 1999.

10. TSCHÖP M, WAWARTA R, RIEPL RL, et al.: Post-prandial decrease of circulating human ghrelin levels. *J Endocrinol Invest* 24: RC19 – RC21, 2001.

11. NEDVÍDKOVÁ J, SMITKA K, KOPSKÝ V, HAINER V: Adiponectin, an adipocyte-derived protein. *Physiol Res* 54: 133-140, 2005.

12. FILIPPI E, SENTINELLI F, TRISCHITTA V, et al.: Association of the human adiponectin gene and insulin resistance. *Eur J Hum Genet* 12: 199-205, 2004.

13. WIMALAWANSA SJ: Amylin, calcitonin gene-related peptide, calcitonin, and adrenomedullin: A peptide superfamily. *Crit Rev Neurobiol* 11: 167 – 239, 1997.

14. WOOKEY PJ, CAO Z, VAN GEENEN RCI, et al.: Increased density of renal amylin binding sites in experimental hypertension. *Hypertension* 30: 455 – 460, 1997.

15. CHIN SY, HALL JM, BRAIN SD, MORTON IK: Vasodilator responses to calcitonin gene-related peptide (CGRP) and amylin in the rat isolated perfused kidney are mediated via CGRP1 receptors. *J Pharmacol Exp Ther* 269: 989–992, 1994.

16. LHOTSKÁ L, BLÁHA P, VIGNEROVÁ J, ROTH Z, PROKOPEC M. V. celostátní antropometrický výzkum dětí a mládeže 1991 (české země). SZÚ, Praha, 1993.

17. PERCY AJ, TRAINOR DA, RITTENHOUSE J, PHELPS J, KODA JE: Development of sensitive immunoassays to detect amylin and amylin-like peptides in unextracted plasma. *Clin Chem* 42: 576-585, 1996.

18. FUNAHASHI H, YAMADA S, KAGEYAMA H, TAKENOYA F, GUAN JL, SHIODA S: Co-existence of leptin- and orexin-receptors in feeding-regulating neurons in the hypothalamic arcuate nucleus – a triple labeling study. *Peptides* 24: 687-694, 2003.

19. ARCHER ZA, FINDLAY PA, RHIND SM, MERCER JG, ADAM CL: Orexin gene expression and regulation by photoperiod in the sheep hypothalamus. *Regul Pept* 104: 41-5, 2002.

20. KOK SW, MEINDERS AE, OVEREEM S, et al.: Reduction of plasma leptin levels and loss of its circadian rhythmicity in hypocretin (orexin)-deficient narcoleptic humans. *J Clin Endocrinol Metab* 87: 805-809, 2002.

21. KASTIN AJ, AKERSTROM V: Orexin A but not orexin B rapidly enters brain from blood by simple diffusion. *J Pharm Exp Ther* 298: 219-223, 1999.

22. KARTERIS E, CHEN J, RANDEVA HS: Expression of human prepro-orexin and signaling characteristics of orexin receptors in the male reproductive system. *J Clin Endocrinol Metab* 89: 1957-1962, 2004.

23. JÖHREN O, NEIDERT SJ, KUMMER M, DENDORFER A, DOMINIAK P: Prepro-orexin and orexin receptor mRNAs are differentially expressed in peripheral tissues of male and female rats. *Endocrinology* 142: 3324-3331, 2001.

24. MITSUMA T, HIROOKA Y, KAYAMA M, et al.: Radioimmunoassay for orexin A. *Life Sci* 66: 897-904, 2000.

25. HIGUCHI S, USUI A, MURASAKI M, et al.: Plasma orexin-A is lower in patients with narcolepsy. *Neurosci Lett* 318: 61-64, 2002.

26. MATSUMURA T, NAKAYAMA M, NOMURA A, et al.: Age-related changes in plasma orexin-A concentrations. *Exp Gerontol* 37: 1127-1130, 2002.

27. KANBAYASHI T, YANO T, ISHIGURO H, et al.: Hypocretin-1 (orexin-A) levels in human lumbar CSF in different age groups: infants to elderly persons. *Sleep* 25: 337-339, 2002.

28. TOMASIK PJ, SPODARYK M, SZTEFKO K: Plasma concentrations of orexins in children. *Ann Nutr Metab* 48: 215-220, 2004.

29. CHEN C, XU R: The *in vitro* regulation of growth hormone secretion by orexins. *Endocrine* 22: 57-66, 2003.

30. SEOANE LM, TOVAR SA, PEREZ D, et al.: Orexin A suppresses *in vivo* GH secretion. *Eur J Endocrinol* 150: 731-736, 2004.

31. INUI A: Eating behavior in anorexia nervosa – an excess of both orexigenic and anorexigenic signalling? *Mol Psychiatry* 6: 620-624, 2001.

32. SHIYA T, NAKAZATO M, MIZUTA M, et al.: Plasma ghrelin levels in lean and obese humans and the effect of glucose on ghrelin secretion. *J Clin Endocrinol Metab* 87: 240-244, 2002.

33. GHIZZONI L, MASTORAKOS G, VOTTERO A, ZIVERI M, ILIAS I, BERNASCONI S: Spontaneous growth hormone (GH) secretion is not directly affected by ghrelin in either short normal prepubertal children or children with GH neurosecretory dysfunction. *J Clin Endocrinol Metab* 89: 5488-5495, 2005.

34. BELLONE S, CASTELLINO N, BROGLIO F, et al.: Ghrelin secretion in childhood is refractory to the inhibitory effect of feeding. *J Clin Endocrinol Metab* 89: 1662-1665, 2004.

35. BELLONE S, RAPA A, VIVENZA D, et al.: Circulating ghrelin levels in newborns are not associated to gender, body weight and hormonal parameters but depend on the type of delivery. *J Endocrinol Invest* 26: RC9-RC11, 2003.

36. BELLONE S, RAPA A, VIVENZA D, et al.: Circulating ghrelin levels as function of gender, pubertal status and adiposity in childhood. *J Endocrinol Invest* 25: RC13-RC15, 2002.

37. SORIANO-GUILLÉN L, BARRIOS V, CAMPOS-BARROS A, ARGENTE J: Ghrelin levels in obesity and anorexia nervosa: effect of weight reduction or recuperation. *J Pediatr* 144: 36-42, 2004.

38. SORIANO-GUILLÉN L, BARRIOS V, CHOWEN JA, et al.: Ghrelin levels from fetal life through early adulthood: relationship with endocrine and metbolic and anthropometric measures. *J Pediatr* 144: 30-35, 2004.

39. IKEZAKI A, HOSODA H, ITO K, et al.: Fasting plasma ghrelin levels are negatively correlated with insulin resistance and PAI-1, but not with leptin, in obese children and adolescents. *Diabetes* 51: 3408-3411, 2002.

40. MISRA M, MILLER KK, HERZOG DB, et al.: Growth hormone and ghrelin responses to an oral glucose load in adolescent girls with anorexia nervosa and controls. *J Clin Endocrinol Metab* 89: 1605-1612, 2004.

41. RIGAMONTI AE, PINCELLI AI, CORRA B, et al.: Plasma ghrelin concentrations in elderly subjects: comparison with anorexic and obese patients. *J Endocrinol* 175: R1-R5, 2002.

42. BUNT JC, SALBE AD, TSCHÖP MH, DELPARIGI A, DAYCHILD P, TATRANNI PA: Cros-sectional and prospective relationschips of fasting plasma ghrelin concentrations with anthropometric measures in Pima Indian children. *J Clin Endocrinol Metab* 88: 3756-3761, 2003.

43. BACHA F, ARSLANIAN SA: Ghrelin suppression on overweight children: a manifestation of insulin resistance? *J Clin Endocrinol Metab* 90: 2725-2730, 2005.

44. KANUMAKALA S, GREAVES R, PEDREIRA CC, et al.: Fasting ghrelin levels are not elevated in children with hypothalamic obesity. *J Clin Endocrinol Metab* 90: 2691-2695, 2005.

45. MANIGRASSO MR, FERRONI P, SANTILLI F, et al.: Association between circulating adiponectin and interleuin-10 levels in android obesity. Effects of weight loss. *J Clin Endocrinol Metab* 90: 5876-5879, 2005.

46. ANDERLOVÁ K, KŘEMEN J, DOLEŽALOVÁ R, et al.: The influence of very-low-calorie-diet on serum leptin, soluble leptin receptor, adiponectin and resistin levels in obese women. *Physiol Res*, 2005, in press.

47. IMBEAULT P, POMERLEAU M, HARPER ME, DOUCET E: Unchanged fasting and postprandial adiponectin levels following a 4-day caloric restriction in young healthy men. *Clin Endocrinol* 60: 429-433, 2004.

48. YANG W-S, LEE W-J, FANAHASHI T, et al.: Weight reduction increases plasma levels of an adipose-derived anti-inflammatory protein, adiponectin. *J Clin Endocrinol Metab* 86: 3815-3819, 2001.

49. REINEHR T, ROTH C, MENKE T, ANDLER W: Adiponectin before and after weight loss in obese children. *J Clin Endocrinol Metab* 89: 3790-3794, 2004.

50. MATSUBARA M, MARUOKA S, KATAYOSE S: Inverse relationship between plasma adiponectin and leptin concentrations in normal-weight and obese women. *Eur J Endocrinol* 147: 173-780, 2002.

51. DELPORTE ML, BRICHARD SM, HERMANS MP, BEGUIN C, LAMBERT M: Hyperadiponectinaemia in anorexia nervosa. *Clin Endocrinol (Oxf)* 58: 22-29, 2003.

52. IWAHASHI H, FUNAHASHI T, KUROKAWA N, et al.: Plasma adiponectin levels in women with anorexia nervosa. *Horm Metab Res* 35: 537-540, 2003.

53. NILSSON L, BINART N, BOHLOOLY-Y M, et al.: Prolactin and growth hormone regulate adiponectin secretion and receptor expression in adipose tissue. *Biochem Biophys Res Commun* 331: 1120-1126, 2005.

54. CORNISH J, CALLON KE, GASSER JA, et al.: Systemic administration of a novel octapeptide, amylin-(1-8), increases bone volume in male mice. *Am J Physiol: Endocrinology and Metabolism* 279: E730 – E735, 2000.

55. CORNISH J, CALLON KE, KING AR, et al.: Systemic administration of amylin increases bone mass, linear growth, and adiposity in adult male mice. *Am J Physiol: Endocrinology and Metabolism* 275: E694 – E699, 1998.

56. CORNISH J, CALLON KE, LIN CQ-X, et al.: Dissociation of the effects of amylin on osteoblast proliferation and bone resorption. *Am J Physiol: Endocrinology and Metabolism* 274: E827 – E833, 1998.

57. DECHENES CJ, VERCHERE CB, ANDRIKOPOULOS S, KAHN SE: Human aging is associated with parallel reductions in insulin and amylin release. *Am J Physiol: Endocrinology and Metabolism* 275: E785 – E791, 1998.

58. LIVSHITZ G, PANTSULAIA I, TROFIMOV S, KOBYLIANSKY E. Genetic variation of circulating leptin is involved in genetic variation of hand bone size and geometry. *Osteoporos Int* 14: 476–483, 2003.

8. AUTHOR´S PUBLICATIONS

8.1. ORIGINAL PAPERS AND REVIEWS IN IMPACTED BIOMEDICAL JOURNALS

BRONSKÝ J, NEDVÍDKOVÁ J, ZAMRAZILOVÁ H, PECHOVÁ M, CHADA M, KOTAŠKA K, NEVORAL J, PRŮŠA R: Dynamic changes of orexin A, leptin and anthropometrical data in obese children during reduction of body weight. *Physiol Res* 56: 2007, accepted. ISSN 0862-8408. IF = 1,140.

BRONSKÝ J, PRŮŠA R: Amylin fasting plasma levels are decreased in patients with osteoporosis. *Osteopor Int* 15: 243-247, 2004. ISSN: 0937-941X (printed version) 1433-2965 (online version). IF = 3,799.

BRONSKÝ J, CHADA M, KOTAŠKA K, PRŮŠA R: Hypothalamické neuropeptidy ovlivňující příjem potravy a regulaci energetické rovnováhy. *Čes a slov Neurol Neurochir* 66/99: 8-13, 2003. ISSN: 1210-7859. IF = 0,047.

CHADA M, PRŮŠA R, BRONSKÝ J, KOTAŠKA K, ŠÍDLOVÁ K, PECHOVÁ M, LISÁ L: Inhibin B, follicle stimulating hormone, luteinizing hormone and testosterone during childhood and puberty in males: changes in serum concentrations in relation to age and stage of puberty. *Physiol Res* 52: 45-51, 2003. ISSN 0862-8408. IF = 1,140.

CHADA M, PRŮŠA R, BRONSKÝ J, PECHOVÁ M, LISÁ L: Inhibin B, follicle stimulating hormone, luteinizing hormone, and oestradiol and their relationship to the regulation of follicle development in females during childhood and puberty. *Physiol Res* 52: 341-346, 2003. ISSN 0862-8408. IF = 1,140.

BRONSKÝ J, PRŮŠA R, NEVORAL J: The role of amylin and related peptides in osteoporosis. *Clin Chim Acta*, accepted, 2006. ISSN 0009-8981IF = 1,961.

BRONSKÝ J, KARPÍŠEK M, BRONSKÁ E, PECHOVÁ M, JANČÍKOVÁ B, KOTOLOVÁ H, PRŮŠA R, NEVORAL J: Adiponectin, adipocyte fatty acid binding protein (AFABP), and epidermal fatty acid binding protein (EFABP) – proteins newly identified in human breast milk. *Clin Chem*, submitted.

8.2. OTHER ORIGINAL PAPERS AND REVIEWS

BRONSKÝ J, CHADA M, NEVORAL J, KRÁSNIČANOVÁ H, LISÁ L, PRŮŠA R: Orexiny - experimentální poznatky a perspektivy v pediatrické praxi. *Čes-slov Pediat* 57: 600 – 603, 2002. ISSN 0069-2328.

BRONSKÝ J, CHADA M, KOTAŠKA K, PRŮŠA R: Amylin – fyziologická úloha v lidském organismu. *Čs Fyziol* 51: 179-180, 2002. ISSN 1210-6313.

BRONSKÝ J: Orexiny – spát či jíst? *Lékařské listy* 1: 24, 2003. ISSN 0044-1996.

PRŮŠA R, BRONSKÝ J: Molekula roku 2002 – humanin. *Pelikán, Akademický bulletin UK 2.lékařské fakulty* 10: 15, 2002. ISSN 1214-2670.

PRŮŠA R, ZADINA J, BRONSKÝ J: Aktivovaný koagulační faktor XII u pacientů s hyperglykémií a dyslipoproteinémií. *Vnitř Lék* 50: 917-922, 2004. ISSN 0042-773X.

BRONSKÝ J, KOTAŠKA K, PRŮŠA R: Ghrelin – struktura, funkce a klinické využití. *Čs Fyziol* 53: 80-85, 2004. ISSN 1210-6313.

BRONSKÝ J, VESELÁ M, KRÁSNIČANOVÁ H, NEDVÍDKOVÁ J, SCHMIDTOVÁ J, HRDLIČKA M, KOUTEK J, PRŮŠA R, NEVORAL J: Celkový ghrelin v plazmě pacientek s mentální anorexií během osmitýdenní realimentace. *Čes a slov psychiatrie* 101: 45-48, 2005. ISSN 1212-0383.

BRONSKÝ J: Peptidové hormony v regulaci příjmu potravy a nutričního stavu organismu. *Výživa a potraviny* 5: 133-134, 2005. ISSN 1211-846X.

BRONSKÝ J, NEVORAL J, PRŮŠA R: Adiponektin - marker insulinové sensitivity. *Čs Fyziol* 54: 92-96, 2005. ISSN 1210-6313.

BRONSKÝ J.: Význam formulí ve výživě dítěte batolecího věku. *Čtvrtletní noviny Nutricia*, in press.

8.3. ABSTRACTS IN IMPACTED BIOMEDICAL JOURNALS

PRŮŠA R, LISÁ L, PECHOVÁ M, BRONSKÝ J, CHADA M: Inhibin B, follicle stimulating hormone, luteinizing hormone and testosterone and their relationships in males during childhood and puberty. *Clin Chem Lab Med, Spec Suppl* 40: 90, 2002. ISSN 1437-8523. IF = 1,685.

BRONSKÝ J, NEDVÍDKOVÁ J, ZAMRAZILOVÁ H, PECHOVÁ M, KOTAŠKA K, PRŮŠA R: Ghrelin, leptin, IGF1 and IGFBP-3 and their relationship in obese children during reduction of body weight. *Clin Chem Lab Med, Spec Suppl* 41: 347, 2003. ISSN 1437-8523. IF = 1,685.

BRONSKÝ J, ZADINA J, PECHOVÁ M, CHADA M, PRŮŠA R: Amylin plasma levels in patients with osteoporosis and type 2 diabetes mellitus.

Clin Chem Lab Med, Spec Suppl 41: 83, 2003. ISSN 1437-8523. IF = 1,685.

CHADA M, KOTAŠKA K, BRONSKÝ J, PECHOVÁ M, LISÁ L, PRŮŠA R: Dimeric inhibins, follicle stimulating hormone, luteinizing hormone and oestradiol and their relationship in girls during childhood and puberty. *Clin Chem Lab Med, Spec Suppl* 41: 94, 2003. ISSN 1437-8523. IF = 1,685.

BRONSKÝ J, PECHOVÁ M, NEDVÍDKOVÁ J, ZAMRAZILOVÁ H, PRŮŠA R: Orexin A fasting plasma levels increase after weight loss in obese children and adolescents. *J Pediatr Gastroenterol Nutr, Spec Suppl* 36: 520-583, 2003. ISSN 0277-2116 (printed version), 1536-4801 (online version). IF = 1,402.

BRONSKÝ J, PECHOVÁ M, NEDVÍDKOVÁ J, CHADA M, PRŮŠA R: Orexin A and leptin levels change inversely during reduction of body fat in obese children and adolescents. *Clin Chem, Suppl* 49: A142, 2003. ISSN 0009-9147. IF = 6,501.

PRŮŠA R, BRONSKÝ J, ČEPOVÁ J, PECHOVÁ M: A possible role of amylin in the pathogenesis of osteoporosis. *Clin Chem, Suppl* 49: A126, 2003. ISSN 0009-9147. IF = 6,501.

BRONSKÝ J, NEDVÍDKOVÁ J, SCHMIDTOVÁ J, HRDLIČKA M, PRŮŠA R, KRÁSNIČANOVÁ H, NEVORAL J: Ghrelin, leptin, IGF-1, IGF BP-3 and their relationship in girls with mental anorexia before and after realimentation. *J Pediatr Gastroenterol Nutr, Spec Suppl* 39: S74, 2004. ISSN 0277-2116 (printed version), 1536-4801 (online version). IF = 1,402.

BRONSKÝ J, NEDVÍDKOVÁ J, PRŮŠA R, VALTROVÁ V, NEVORAL J: Changes of ghrelin plasma levels during decrease of body weight in obese children. *Gut / Endoscopy, Spec Suppl* 53/36: A11, 2004. ISSN 0017-5749 / 0013-726X. IF = 6,601.

BRONSKÝ J, NEDVÍDKOVÁ J, ŠRÁMKOVÁ D, PECHOVÁ M, PRŮŠA R, NEVORAL J: Adiponectin, insulin and C-peptide serum levels in obese children before and after reduction of body weight. *J Pediatr Gastroenterol Nutr* 40: 688, 2005. ISSN 0277-2116 (printed version), 1536-4801 (online version). IF = 1,402.

PRŮŠA R, BRONSKÝ J, ŠRÁMKOVÁ D, PECHOVÁ M, NEVORAL J: Adiponectin serum levels in obese and non-obese children and its relationship to biomarkers of lipid metabolism. *Clin Chem, Suppl* 51: A232-233, 2005. ISSN 0009-9147. IF = 6,501.

9. SUMMARY

Introduction: Regulation of food intake and nutritional status is mediated by complex interactions of regulatory peptides of the central nervous system, gastrointestinal tract and adipose tissue. These systems are connected by feedback loops which inform the centre about amount of ingested food and energy reserves in the organism. Dysfunction of any of these regulatory areas may lead to changes in nutritional status of the organism.

Methods: We used radioimmunoassay to measure plasma levels of orexin A, total ghrelin and serum levels of leptin and enzyme immunoassay to measure serum levels of adiponectin in healthy subjects and in children with obesity, anorexia nervosa, Crohn´s disease and celiac disease and we evaluated the influence of nutritional therapy on these levels. Moreover, we evaluated relationship of these regulatory peptides to other biochemical and anthropometrical factors of nutritional status. We also measured plasma levels of total and unreduced amylin by enzyme immunoassay with immunofluorescence detection in adult patients with osteoporosis, type II diabetes mellitus and in the control group.

Results: During reduction of body weight in obese children and adolescents, there were statistically significant changes of plasma orexin A levels and total ghrelin levels, but we haven´t seen any changes in serum levels of adiponectin. In girls with anorexia nervosa during realimentation, statistically significant changes of all three analytes occured. Plasma levels of orexin A in patients with Crohn´s disease and celiac disease were significantly higher than in healthy controls. In adult patients with osteoporosis, there were significantly lower levels of unreduced amylin in comparison with type II diabetic patients and the control group.

Conclusions: We measured plasma levels of orexin A, ghrelin and amylin and serum levels of adiponectinu and leptin in patients with impaired nutritional status and evaluated their relationship to anthropometrical and biochemical parameters during nutritional therapy.