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Evaluation of ghrelin and leptin levels in obese, lean and undernourished children

Ispitivanje nivoa leptina i grelina u serumu gojazne, normalno uhranjene i mršave dece

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Abstract

Background/Aim. Energy homeostasis is a balance between energy intake and energy expenditure. Leptin and ghrelin are two orexitropic hormones with opposite effect on energy homeostasis. We investigated fasting ghrelin and leptin serum levels in children with different nutritional conditions. **Methods**. In 30 obese children of both sexes, aged from 6 to 17.67 years (mean 13.04 ± 2.95 years), fasting ghrelin and leptin levels were determined in the serum, along with auxological assessment and pubertal staging. Obtained values were analyzed and compared with those of the same parameters in 33 lean and 25 undernourished (UN) children. **Results**. Mean ghrelin/body mass (BM) ratio was the lowest in obese children (21.75 ± 12.60 pg/mL/kg), which was significantly different in comparison with that in lean and UN subjects. Mean leptin/BM ratio of 0.62 ± 0.86 pg/mL/kg in obese children was significantly higher than that in lean and UN children ($p < 0.01$ and $p < 0.001$, respectively). Ghrelin and leptin levels showed different profiles in obese, lean and UN children. An inverse relationship was discovered among study groups in ghrelin/leptin and leptin/ghrelin ratios. **Conclusion**. Obese children, compared to other children, have significantly higher values of leptin, and UN children have significantly higher values of ghrelin per kilogram of body mass. The results also illustrate the inverse ratio of ghrelin and leptin, which has been demonstrated as a clinically reliable marker of the status of obesity or undernutrition in children, with significant implications concerning rather large variations in the concentration of these hormones in relation not only to the body mass but also to the children's age.

Key words:
nutritional status; child; leptin; ghrelin; obesity.

Apstrakt

Uvod/Cilj. Homeostaza energije je balans između energetskog unosa i potrošnje energije. Leptin i grelin su dva oreksitropna hormona sa suprotnim efektom na homeostazu energije. Ispitivane su vrednosti leptina i grelina u serumu dece različititog tipa uhranjenosti. **Metode**. Kod 30 gojazne dece oba pola, uzrasta od 6 do 17,67 godina (srednja vrednost 13,04 ± 2,95 godine), određivane su vrednosti leptina i grelina u serumu, uz određivanje auksoloških parametara i pubertetskog statusa. Dobijeni rezultati su analizirani i upoređivani sa vrednostima istih parametara kod 33 normalno uhranjene i 25 mršave dece. **Rezultati**. Vrednosti odnosa grelin/telesna masa (TM) bile su najniže kod gojazne dece (21,75 ± 12,60 pg/mL/kg) što je statistički značajno različito u odnosu na vrednosti kod mršave i normalno ishranjene dece. Vrednost odnosa leptin/TM bila je statistički značajno veća kod gojazne dece (0.62 ± 0.86) u odnosu na normalno uhranjene ($p < 0,01$) i mršave ($p < 0,001$). Vrednosti leptina i grelina pokazale su različit profil kod gojazne, normalno uhranjene i mršave dece. U ispitivanim grupama dokazane su inverzne vrednosti odnosa grelin/leptin i leptin/grelin. **Zaključak**. Gojazna deca imaju značajno više vrednosti leptina, a mršava deca značajno više vrednosti grelina po kilogramu telesne mase u odnosu na ostalu decu. Rezultati ukazuju i na inverzni odnos grelina i leptina koji se pokazao kao klinički pouzdan pokazatelj prisustva gojaznosti ili neuhranjenosti kod dece, što je od velikog značaja, uzimajući u obzir velike varijacije u koncentraciji ovih hormona, ne samo prema telesnoj masi, već i prema uzrastu dece.

Ključne reči:
uhranjenost; deca; leptin; grelin; gojaznost.

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Introduction

The ghrelin-ghrelin receptor system is one of the most important mechanisms regulating energy balance and metabolism. Ghrelin, a 28-amino acid peptide, is mainly produced in the stomach from a distinct group of endocrine cells. It is discovered as potent growth hormone secretagogue and appetite stimulator by Kojima et al. [1] in 1999. Identified as a natural ligand for growth hormone secretagogue receptor (GHsR) the small peptide provoked a burst of new enthusiasm among scientists and clinicians. Many types of research were hunting for this hormone for years, but Kojima et al. [1] made great discovery by switching the search from the brain to the stomach [1–3].

Ghrelin quickly demonstrated its pleiotropic nature. Opposite to leptin, it stimulates food intake and rises body mass index (BMI) in rodents and humans. In fact, this hormone is one of the most important factors known for regulating appetite and energy expenditure. Ghrelin is also known as "starvation hormone", potent orexigenic signal acting *via* neuropeptide Y (NPY)/Agouti Related Peptide (AGRP) and orexin neurons stimulation in *nucleus arcuatus.* Both peripheral and central administration of ghrelin potently promotes body weight gain and adiposity through the stimulation of food intake while decreasing energy expenditure and body fat [4–8].

Ghrelin which operates as a signal of energy insufficiency and functional antagonist of leptin may play a physiological, and eventual pathophysiological role in the regulation of puberty onset and gonadal function [9–11].

Besides a role in energy homeostasis, growth and puberty, the influence of ghrelin on sepsis, atherogenesis, apoptosis, angiogenesis and addictional habits is among its most prominent effects with the potential of clinical application [8–12].

Since the discovery of leptin in 1994, it has been assumed that adipose tissue is not just fat storage organ, but plays a role in many physiological and pathological processes, including appetite regulation, glucose homeostasis, immune response, growth and differentiation, angiogenesis, hypertension, atherosclerosis and cancer. Finding that leptin signals to the brain that the stomach is full with consequent suppression of NPY production, a stimulator of food intake was among the first actions discovered in rats and confirmed in humans [13–18]. It controls the start of puberty [19], stimulates sympathetic nervous system activity and energy expenditure and influences thyroid, growth, and sex hormone axes [20, 21].

Taking into account the influence of both hormones on appetite regulation and energy expenditure, we found that it would be of importance to determine and compare levels of ghrelin and leptin in children and adolescents with different nutritional status. Also, it was intriguing to investigate ghrelin/leptin and leptin/ghrelin ratios in these groups and to explore correlations of two hormones with auxological data in children with different nutritional conditions.

Methods

The study was designed as cross-sectional and conducted in Pediatric Clinic of University Clinical Centre Niš, in the south-east region of Serbia.

Examinees

Our sample included 88 children and adolescents aged 6 to 17.67 years, stratified as obese (30 subjects, 11 females), undernourished – UN (25 subjects, 19 females) and lean (33 subjects, 24 females). Candidates for the study were selected from patients who were referred to the endocrinology examination due to obesity or consulted endocrinologist because of difficulties in gaining weight. Healthy and normal weight children, assigned as lean, with ideal weight for height, 33 of them, acted as controls. All these children underwent a complete physical examination by a pediatric endocrinologist in order to rule out organic disease or abnormalities in growth and development.

In all participants height and weight measurements were performed and calculation of height percentile (P), height standard deviation score (Height SDS) for chronological age (CA) and gender, body mass index (BMI kg/m²), percentiles of BMI and SDS of BMI for CA and gender, were established. Obesity was defined as BMI greater than P95 (+3SD) for their CA and gender. Undernutrition was defined as BMI-P < 3 (-2SD) for CA.

The informed consent was obtained from all participants and their legal representatives. The study was approved by the Ethical Committee of the Faculty of Medicine, University of Niš.

Hormone assays

Total ghrelin levels in the serum were measured using a commercial Human Ghrelin Elisa Kit Cusabio Biotech Co.,LTD. The assay sensitivity was 0.156 pg/mL, and the intra-assay coefficient of variation (CV) was < 8% and the inter-assay CV < 10%.

Serum leptin was measured using Quantikine Elisa Human Leptin Immunoassay USA&Canada. The assay sensitivity was 7.8 pg/ml with intra-assay CV of 3.3% and inter-assay CV of 5.4%.

Children fasted for at least 8 hours before specimen collection.

Statistical analysis

All statistical analyses were performed using SPSS version 12 software. The data are presented as mean ± SD and comparisons among groups were conducted by independent *t*-test, or Mann-Whitney test, depending on normality of variable distribution. The distributions of the continuous variables were assessed for normality by Shapiro-Wilk test. Pearson's correlation coefficients and Spearman's rho were calculated to evaluate the relationships between hormones values and auxological data $p < 0.05$ was considered to be statistically significant.

Results

The mean age of the obese group was 13.04 ± 2.95 (range 6–17.67) years and the mean puberty stage was 3.37.

Obese children had lipomastia, *genua valga*, and boys (with only two exceptions) pseudo-hypogenitalism.

The mean BMI was very high in obese children being 31.51 ± 4.78 kg/m², ranging from 23.95 kg/m² to 43.95 kg/m². The mean height percentile was P78.30 (1.17 ± 1.35 height SDS) and documented optimal growth in obese children. Mean height SDS of obese children was significantly higher than that of UN children. Children in the control group were also significantly taller than UN children. Overweight (body mass excess – BME), reached abnormal values: mean overweight being 27.45 (ranging from 10 to 52) kg (Table 1).

When absolute values of ghrelin levels in studied groups were compared we found statistical significance ($p < 0.05$). UN children (2,055.84 ± 579.37 pg/mL) and lean children (2,001.88 ± 598.75 pg/mL) had higher ghrelin levels than obese ones (1624.674.10 pg/mL). After adjusting ghrelin levels by calculation of ghrelin/kg BM the value of 72.25 pg/ mL/kg was significantly higher in the UN group in comparison with the control and the obese children ($p < 0.001$). Mean ghrelin/BM ratio was the lowest in the obese children, 21.75 (1.74–67.98) pg/mL/kg with high significance in comparison with the lean and UN subjects (Table 2).

The highest values of leptin were observed in the obese children (56.12 ± 96.94 pg/mL), which was statistically significantly higher compared to those in the controls ($p < 0.01$) and the UN children ($p < 0.001$). The values of leptin in normally nourished children (20.96 ± 12.74 pg/mL) were statistically significantly higher ($p < 0.001$) than those in the UN children (6.92 ± 8.10 pg/mL).

Table 1

Auxological data of investigated children

Parameters	Obese	UN	Control
Number	30	25	33
Sex (M/F)	19/11^{bc**}	6/19	9/24
Age (years), mean ± SD	13.04 ± 2.95	12.08 ± 3.70	13.23 ± 3.01
range	6.00 – 17.67	6.33 – 17.58	8.00 – 17.67
Height (cm), mean ± SD	160.66 ± 15.78^{b**}	144.16 ± 21.64	157.56 ± 13.92^{b*}
range	124.50 – 185.00	108.00 – 191.00	126.00 – 184.00
Height Percentile, mean ± SD	78.30 ± 22.22^{b***}	38.99 ± 33.23	66.98 ± 31.42^{b**}
range	4.60 – 99.90	0.70 – 99.90	0.80 – 99.90
Height SDS, mean ± SD	1.17 ± 1.35^{b***}	-0.35 ± 1.54	0.62 ± 1.23^{b**}
range	-1.70 – 6.50	-2.50 – 3.90	-1.28 – 3.00
BM (kg), mean ± SD	82.55 ± 21.72^{cb***}	31.69 ± 11.56	50.95 ± 16.43^{b***}
range	43.00 – 128.50	16.00 – 57.00	25.00 – 75.00
BME (kg), mean ± SD	27.45 ± 11.33^{bc***}	-11.71 ± 4.06	6.77 ± 9.61^{b***}
range	10.00 – 52.00	-2.00 – 18.00	-3.70 – 11.10
BMI (kg/m^2), mean ± SD	31.51 ± 4.78^{cb***}	14.65 ± 1.39	19.81 ± 3.37^{b***}
range	23.95 – 43.95	12.36 – 17.31	16.83 – 22.65
BMI z-score, mean ± SD	3.11 ± 0.18^{cb***}	-2.93 ± 0.49	0.9 ± 0.70^{b***}
range	3.00 – 4.70	-3.20 – 1.90	0.67 – 1.80
Puberty stage, mean ± SD	3.37 ± 1.35	2.88 ± 1.64	3.42 ± 1.46
range	1.00 – 5.00	1.00 – 5.00	1.00 – 5.00

SDS – standard deviation score; BM – body mass; BME – body mass excess; M – male; F – female; BMI – body mass index; BMI z-score – standard deviation of relative weight adjusted for child age and sex. UN – Undernourished group; a – vs Obese group; b – vs UN group; c – vs Control group; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Table 2

Orexitropic signaling proteins in investigated children

Parameters	Obese	UN	Control
Leptin (pg/mL), mean ± SD	56.12 ± 96.94^{c**b***}	6.92 ± 8.10	20.96 ± 12.74^{b***}
range	10.74 – 452.40	1.07 – 40.06	1.46 – 50.40
Leptin/BM (pg/mL/kg), mean ± SD	0.62 ± 0.86^{b***}	0.21 ± 0.17	0.37 ± 0.23^{b**}
range	0.15 – 3.93	0.04 – 0.80	0.02 – 0.90
Ghrelin (pg/mL), mean ± SD	1,624.93 ± 674.10	2,055.84 ± 579.37^{*a}	2,001.88 ± 598.75^{*a}
range	196.00 – 3195.00	838.00 – 3073.00	342.00 – 3124.00
Ghrelin/BM (pg/mL/kg), mean ± SD	21.75 ± 12.60	72.25 ± 32.82^{ac***}	38.68 ± 19.98^{a***}
range	1.74 – 67.98	37.36 – 180.59	5.23 – 96.21
Leptin/ghrelin ratio, mean ± SD	0.0582 ± 0.1536^{bc***}	0.0034 ± 0.0033	0.0116 ± 0.0079^{b***}
range	0.0042 – 0.8472	0.0004 – 0.0141	0.0008 – 0.0342
Ghrelin/leptin ratio, mean ± SD	59.69 ± 44.74	570.15 ± 507.42^{ac***}	194.36 ± 262.90^{a***}
range	1.18 – 238.43	70.74 – 2526.17	29.28 – 1297.95

BM – body mass; SD – standard deviation. UN – undernourished group; a – vs Obese group; b – vs UN group; c – vs Control group. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Leptin/BM ratio was highest in the group of obese children (0.62 ± 0.86 pg/mL/kg) – statistically significantly higher compared to that in the UN children ($p < 0.001$). Furthermore, the value of this ratio was statistically significantly higher in the group of normally nourished children compared to the UN children ($p < 0.01$) (Table 2).

Leptin/ghrelin ratio was highest in the group of obese children (0.0582 ± 0.1536), with a statistical significance compared to that in the UN children ($p < 0.001$) and the controls ($p < 0.001$). The value of leptin/ghrelin ratio was statistically significantly higher in the controls, compared to that in the UN children ($p < 0.001$). Ghrelin/leptin ratio was highest in the group of lean children (570.15 ± 507.42 pg/mL/kg), with a statistically significant difference when compared with the controls ($p < 0.001$) and the obese children ($p < 0.001$). The value of ghrelin/leptin ratio in the group of controls (194.36 ± 262.90 pg/mL/kg) was statistically significantly higher than that in the obese children ($p < 0.001$) (Table 2).

Leptin and ghrelin levels calculated per kilogram of BM showed different profiles in obese, lean and UN children (Figures 1a and 1b). An inverse relationship in leptin/ghrelin was found in the obese and UN children ghrelin/leptin ratios (Figures 2a and 2b).

Leptin levels significantly positively correlated with BM, BMI and high SDS ($p < 0.001$) (Figure 3), while ghrelin levels correlated significantly negatively with BM and BMI ($p < 0.01$), and nonsignificantly with high SDS (Figure 4).

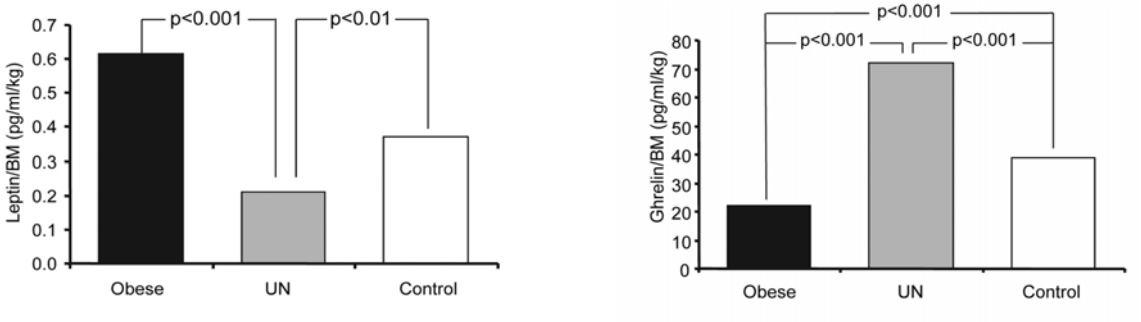

Fig. 1 – a) Leptin/BM and b) Ghrelin/BM ratios in children with different nutritional status. BM – body mass; UN – undernourished group.

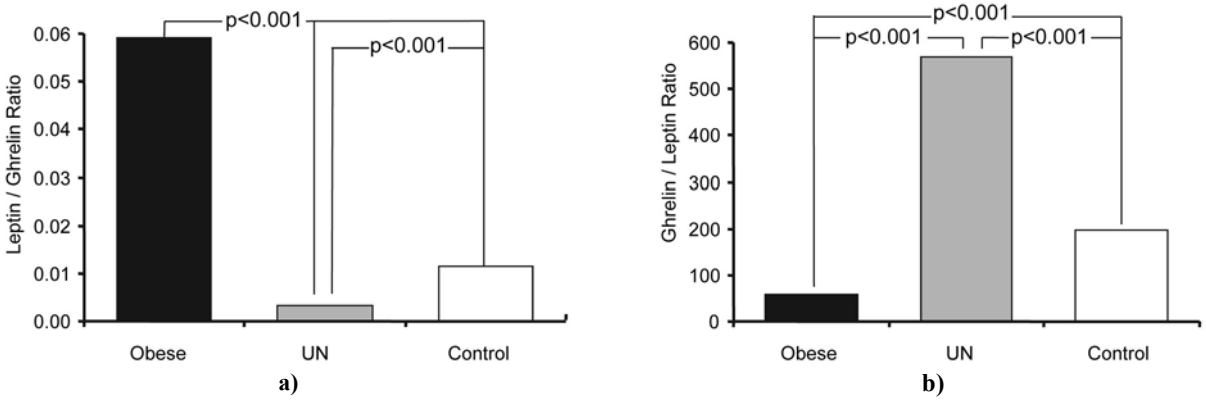

Fig. 2 – a) Leptin/ghrelin and b) Ghrelin/leptin ratios in children with different nutritional status. UN – undernourished group.

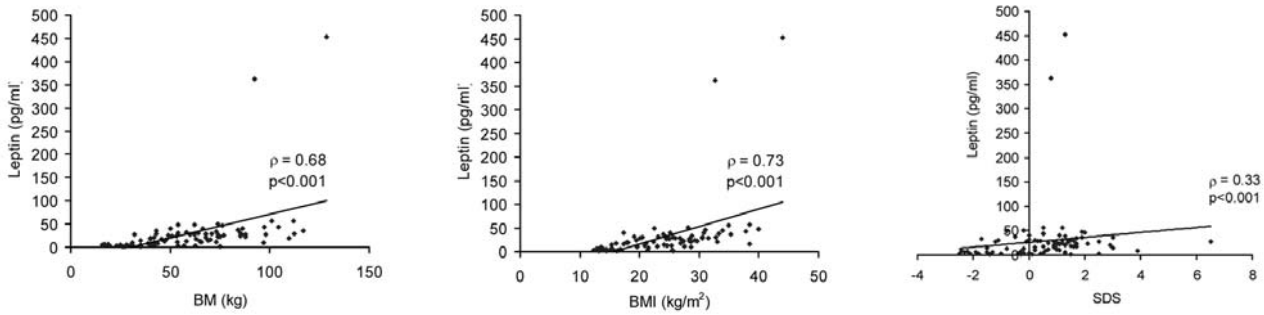

Fig. 3 – Leptin correlations with BM, BMI and SDS in children with different nutritional status. BM – body mass; BMI – body mass index; SDS – standard deviation score.

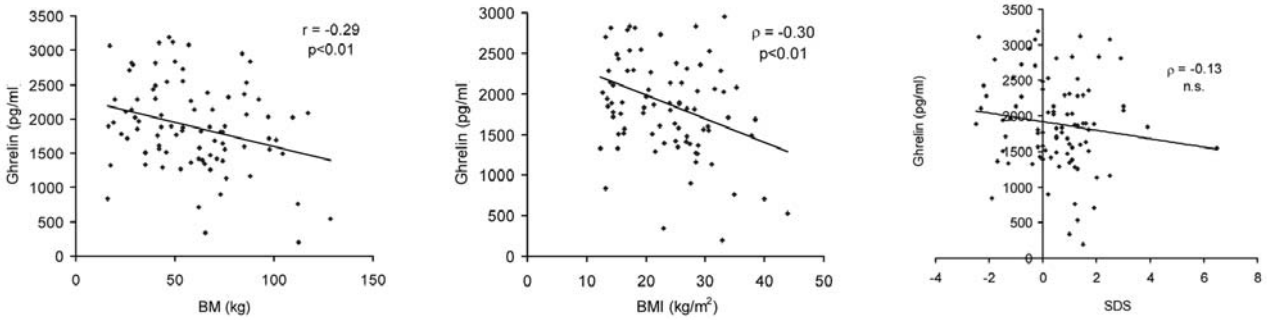

Fig. 4 – Ghrelin correlations with BM, BMI and SDS in children with different nutritional status. BM – body mass; BMI – body mass index; SDS – standard deviation score; n.s. – non significant.

Discussion

We intended to compare serum ghrelin and leptin levels in children with different nutritional status. These two hormones were chosen as potent appetite influencers in the opposite way. It was not surprising that we found the statistically significant difference among studied groups. In our study, the values of ghrelin were highest in the group of UN children. In the controls and in the UN children the values of ghrelin were statistically significantly higher compared to the group of obese children. The value of ghrelin/BM ratio was highest in the group of UN children, with a statistically significant difference compared to those of the obese children and controls, since ghrelin values were highest in the group of UN children with, at the same time, lowest BM, which suggested that in addition to the hunger/satiety status, the concentration of this hormone was also affected by BM, in agreement with the above-mentioned studies. The value of this ratio in the control group was statistically significantly higher compared to that in the obese children.

Mean ghrelin/BM level was more than tripled in the UN children in comparison with the same parameter in the obese ones. Modulatory effect of both forms of ghrelin, acylated and nonacylated on adipogenesis is well documented. It has been shown that both forms of ghrelin directly promote adipogenesis in rat bone marrow adipocytes [22, 23]. Although the orexigenic action of ghrelin itself predicts the impact on weight gain, ghrelin has also been shown to be able to directly act at the level of endocrine pancreas, liver and adipose tissue, thus modulating glucose and lipid metabolism [4, 5, 7]. In humans, ghrelin concentrations progressively decrease during childhood and adolescence, as well as with advancing puberty. In adolescents, similar to adults, ghrelin concentrations are inversely related to BMI and to circulating insulin [24]. One notable exception is the presence of elevated ghrelin concentrations in subjects with Prader-Willi syndrome, raising the possibility that ghrelin could be part of the etiology of excess food intake in this condition. The present study also shows that levels of ghrelin inversely correlated with BM and BMI.

In children with obesity, the decreased ghrelin and increased leptin levels suggest a possible adaptive process to positive energy balance. However, studies of ghrelin in children are scarce. Recently published study of Wali et al. [25] on ghrelin and obestatin level in obese children and children with failure to thrive showed significantly lower total ghrelin levels in children with obesity. In another study Shen et al. [26] investigated changes in ghrelin and obestatin levels before and after a meal in children with simple obesity (15 children) and anorexia (25 children). Their results confirmed the negative correlation between BMI and ghrelin, while the anorexia group had the highest values of obestatin and ghrelin. Recently Arrigo et al. [27] found that weight loss in prepubertal children was associated with a significant change in leptin, ghrelin and obestatin concentrations. They concluded that levels of these hormones are closely associated with obesity in childhood and might take part, as consequence but not as a cause, of weight changes.

Although well known as GHs (growth hormone secretagogue), ghrelin level was low in our group of obese children exerting optimal growth. We can conclude indirectly that our obese children growth because of insulin and/or *via* leptin stimulated conversion of T4 to T3 what is already documented in obese children [28, 29].

Plasma leptin concentrations correlate with the amount of energy stored as fat, and obese individuals express higher levels of leptin than lean individuals [14, 17]. Our study confirmed this positive correlation between leptin concentrations and BM and BMI. Also, our results demonstrated that leptin values were highest in the group of obese examinees, compared to the UN children and the controls. Moreover, leptin/BM ratio was lowest in the UN children in whom individual values of leptin and BM were lowest, while it was highest in the group of obese children, in whom individual values of leptin and BM were highest. Leptin/BM ratio was statistically significantly higher in the obese and control children, compared to the UN ones. This suggested a direct dependence of leptin values on BM, as shown by previously mentioned studies as well.

The height of investigated obese children was optimal (mean percentile being P78.30). High caloric and protein intake in overfed children provide full energy stores, high leptin values, and stimulation of growth hormone secretion and T4 to T3 conversion. This could explain the excellent growth in our study group. In addition, leptin *per se* stimulates T4 to T3 conversion and leptin receptors are identified in the thyroid gland [30]. Leptin, GH, insulin and thyroid hormones, acting synergistically, may be responsible for stimulation of growth in children with exogenous obesity [14, 17, 31].

The study was limited by small sample sizes and wide age range of investigated children. We avoided further dividing of study groups according to pubertal development, due to relatively small number of pubertal participants. The majority of studied children were in mid-puberty. We also recognize that boys dominated in the obese group and girls in the UN one, but the data about gender difference in orexitropic signals are still biased [28, 29].

Suppressed levels of ghrelin in our group of obese children and more than tripled in the UN children are in favor of the significant influence of this hormone on adaptation in conditions of overfeeding and starvation. Leptin levels were inversely regulated among study groups when compared to ghrelin. It is important to establish a healthy balance between these two hormones. In this interplay, genetic and environmental influencers are certainly of great importance, but this must be further investigated.

Leptin/ghrelin ratio was highest in the group of obese children, with statistical significance compared to the UN children and the controls. The value of leptin/ghrelin ratio was statistically significantly higher in the control group compared to that in the UN children. Leptin values were highest in the group of obese children, with the simultaneous presence of lowest ghrelin values, which could be possibly interpreted as the process of adaptation to a positive energy balance. Children with higher BMI had more adipose tissue, i.e. more leptin per kg of BM, as shown in the study. At the same time, leptin/ghrelin ratio was able to identify distinctly the obese children, and it can thus be used as an indicator of the status of obesity, supplementing BM and BMI.

Ghrelin/leptin ratio was highest in the group of UN children, with a statistically significant difference compared to that in the control group and the obese children. The value of ghrelin/leptin in the controls was statistically significantly higher than that in the obese children. The values of ghrelin were highest in the group of UN children, with simultaneous lowest leptin values; this association demonstrated that there was a mechanism of adaptation in the UN children to a negative energy balance. Ghrelin/leptin ratio clearly identified the UN children, and this relationship could be used as a supplemental indicant of undernutrition, in addition to the already known indicants (BM, BMI).

Such data are scarce for the children aged up to 12 years [27, 32], as those enrolled in this study. Moreover, there have been no studies involving all three groups of children (UN, normal weight and obese). This shows that in children of different ages a characteristic profile of secretion of these hormones is kept, as well as its association with BM, BMI SDS, and BMI-P.

Conclusion

The obese children, compared to other children, demonstrate significantly higher values of leptin, and the UN children demonstrate significantly higher values of ghrelin per kilogram of body weight. The results also illustrate the inverse ratio of ghrelin and leptin, which has been demonstrated as a clinically reliable indicator of the status of obesity or undernutrition in children, with significant implications concerning rather large variations in the concentration of these hormones not only with body mass but also with children age. The results provide a better understanding of hormonal regulation in different nutritional conditions. The important difference in appetite targeting hormones and their ratios (ghrelin/leptin and leptin/ghrelin profile) between the obese and the UN children was found.

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