ORIGINAL ARTICLE

Ghrelin concentration is correlated with IGF-I/IGFBP-3 molar ratio but not with GH secretion in children with short stature

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Abstract

OBJECTIVES: In children with growth hormone deficiency (GHD) and neurosecretory dysfunction (NSD) ghrelin concentrations are significantly higher than in children with idiopathic short stature (ISS), however the correlation between serum ghrelin and growth hormone (GH) is not observed. The aim of the study was to compare ghrelin concentrations with IGF-I/IGFBP3 molar ratio in children with short stature due to different etiology.

MATERIAL: Analysis comprised 136 children (58 girls and 78 boys), aged 3.86–16.82 years with short stature (below –2.0 SD); in 21 of them GHD was diagnosed, in 23 – NSD and 92 – ISS. In each child, fasting ghrelin, insulin-like growth factor type I (IGF-I) and its binding protein type 3 (IGFBP-3) concentrations were measured. The results were analysed separately in younger and in older children. Depending on IGF-I/IGFBP-3 molar ratio, children were divided into two (2) groups: with lower IGF-I/IGFBP-3 and with higher IGF-I/IGFBP-3 ratio value.

RESULTS: Both in younger and in the older age groups, ghrelin concentration was significantly higher in children with lower IGF-I/IGFBP-3 ratio than in children with higher IGF-I/IGFBP-3 value (1937.3±1232.4 vs 1365.3±632.1 pg/ml in younger children and 1205.4±548.8 vs 867.4±282.9 pg/ml in older children). The negative correlation between ghrelin and IGF-I/IGFBP-3 ratio was observed in both age groups. Not only children with GHD and NSD, but also as much as 39% out of all children with ISS were qualified into the subgroups with lower IGF-I/IGFBP-3 ratio.

CONCLUSIONS: Ghrelin secretion is elevated in children with lower IGF-I/IGFBP-3 ratio. It seems that lower bioactivity of IGF-I is stimulating factor for ghrelin synthesis.

INTRODUCTION

The role of endogenous ghrelin in growing processes of children is unclear. Ghrelin is the natural ligand of the type 1a growth hormone (GH) secretagogue receptor and it is considered as a natural GH secretagogue (Bednarek *et al.* 2000, Cunha & Mayo 2002). Several studies have shown that, on a molar basis, ghrelin is significantly more potent at inducing GH secretion than GH releasing hormone (GHRH). Moreover, ghrelin and GHRH act synergistically, inducing a substantial GH response when given in combination (Alvarez-Castro *et al.* 2004, Veldhuis & Bowers 2010). However, in clinical studies correlations between ghrelin and GH concentrations are not observed, although in patients with growth hormone deficiency (GHD) and neurosecretory dysfunction (NSD), the ghrelin concentration is significantly higher than in those with idiopathic short stature (ISS) and in controls (Ghizzoni *et al.* 2004, Iñiguez *et al.* 2010, Stawerska *et al.* 2012). Insulin-like growth factor-I (IGF-I) is the main peripheral mediator of GH activity. Both IGF-I bioavailability and stability of its concentration is determined by binding to specific proteins, especially – insulin-like growth factors binding protein-3 (IGFBP-3). The molar ratio of IGF-I to IGFBP-3 is considered to be an index of IGF-I bioavailability (Juul *et al.* 1995). Assessment of IGF-I and IGFBP-3 secretions were proposed to be a screening procedure in children with short stature, suspected for GHD (Rosenfeld 1996), as their better reflect GH activity. Thus, the aim of the present study was to compare ghrelin concentrations with IGF-I/IGFBP-3 molar ratio in children with short stature due to different etiology.

MATERIAL AND METHODS

An approval for the study was obtained from the Bioethical Committee in the Polish Mother's Memorial Hospital – Research Institute (PMMH-RI) in Lodz. Analysis comprised 136 children (58 girls and 78 boys), aged 3.86–16.82 years (mean±SD: 10.38±3.42 years) with short stature, defined as body height below –2.0 SD from the mean value for child's age and sex, determined on the basis of actual population standards.

The children were recruited from the patients of the Outpatient Endocrinology Clinic and Outpatient Gastroenterology Clinic, where they referred due to short stature. Children with chronic diseases, as well as with complaints of gastrointestinal tract, were not qualified into the study group. Body height was assessed by using a stadiometer and height standard deviation score (height SDS) was calculated. Next, based on the actual child's position on percentile charts, the height age (HA) was calculated (as the age, given for 50th percentile for child's height). Body mass was assessed in all the qualified patients, followed by calculation of body mass index standard deviation score for chronological age (BMI SDS for CA) and for height age (BMI SDS for HA). The stage of puberty was assessed by the Tanner's scale and bone age (BA) was determined based on the roentgenograms of the non-dominant hand and wrist according to Greulich-Pyle's standards.

Children with thyroid dysfunction (based on TSH and FT4 serum concentrations) and with Turner's syndrome (diagnosed with use of chromatin X and/or karyotype tests) were excluded from the study. Next, children were referred to the Department of Endocrinology and Metabolic Diseases, PMMH-RI.

In each child a 3-hour nocturnal profile of GH secretion was obtained during sleep, with half-hour intervals, starting from the first hour after falling asleep. Then, two GH-stimulating tests were performed on subsequent days of hospitalisation: the oral clonidine test, with dose of 0.15 mg/m^2 and with GH measurements at 0, 30th, 60th, 90th and 120th minute of the test, and the test with intramuscular glucagon administration in dose of 30 μg/kg (not exceeding 1 mg), during which GH was measured at 0, 90th, 120th 150th and 180th minute. Peak GH concentration (GH_{max}) was determined in both tests and after falling asleep.

The following diagnoses were confirmed:

1. growth hormone deficiency – GHD, n=21 (GH_{max} was decreased in both stimulating tests and during sleep),
2. neurosecretory dysfunction – NSD, n=23 (GH_{max} was decreased in sleep but in normal range in – at least – one stimulating test, low IGF-I concentration) or
3. idiopathic short stature – ISS, n=92 (GH_{max} was in normal range during sleep and in – at least – one stimulating test).

On the 2nd day of hospitalisation (before performing any stimulating tests), fasting serum concentrations of total ghrelin, IGF-I and IGFBP-3 were measured. For calculation of IGF-I/IGFBP-3 molar ratio, the following molecular masses were used: 7.5 kDa for IGF-I and 42.0 kDa for IGFBP-3. IGF-I concentrations were also expressed by IGF-I SDS for sex and chronological age (IGF-I SDS for CA) and for HA and BA (IGF-I SDS for HA, IGF-I SDS for BA), according to reference data.

In whole group of children we have observed the negative correlation between age and ghrelin, age and IGF-I, as well as age and IGF-I/IGFBP-3 molar ratio. In order to eliminate influence of age on results of statistical analysis we have divided the children into two subgroups, taking into consideration CA, BA and puberty stage:

- younger children – CA below 11 years, BA below 9 years in boys and below 8 years in girls, lack of pubertal signs,
- older children – CA more than 11 years and/or with pubertal signs and/or BA more than 9 years in boys and more than 8 years in girls.

In both groups we have assessed ghrelin concentrations in children with lower and with higher IGF-I/IGFBP-3 molar ratio (cut off point was 0.16 – for younger children and 0.28 – for older children (the median values for the subgroups).

Growth hormone levels were measured by the immunometric method. The measurements were performed by IMMULITE, DPC assay sets, calibrated vs. the WHO IRP 80/505 standard set, with the following sensitivity level: 0.01 ng/ml, range: up to 40 ng/ml, the conversion index: ng/ml × 2.6 = mIU/l, the intra-assay CV: 5.3–6.5% and inter-assay CV: 5.5–6.2%.

Total ghrelin concentration was measured by radioimmunometric assay by Millipore assay sets, with the following sensitivity level: 100–10.000 pg/ml, the intra-assay CV: 3.3–10.0% and inter-assay CV: 14.7–17.8%.

Both IGF-I and IGFBP-3 concentrations were assessed by IMMULITE, DPC assays. For IGF-I, WHO NIBSC 1st IRR 87/518 standard was applied, with analytical sensitivity of the assay 20 ng/ml, the calibration range up to 1600 ng/ml, the intra-assay CV – 3.1–4.3% and the inter-assay CV – 5.8–8.4%. The assay for IGFBP-3 assessment 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%.

One-way ANOVA was applied for statistical analysis with post-hoc tests to account for median differences (because of different patient numbers in group, Tukey's HSD [Honestly Significant Difference] test was used for assays). The typical regression and correlation analysis were used to assess the relationships among parametric date. The level of significance at $p < 0.05$ was accepted for all the performed tests and comparisons.

RESULTS

In whole analysed group of children, the significant correlations between age and ghrelin concentration (r=–0.46, $p<0.05$), between age and IGF-I concentration (r=0.62, $p<0.05$) and between age and IGF-I/IGFBP-3 molar ratio (r=0.52, $p<0.05$) were observed. Thus, in order to eliminate influence of age on results of analysis, we have decided to assess the relationships between ghrelin and IGF-I/IGFBP-3 molar ratio, separately – in younger group and in older group of short children (see the inclusion criteria in Material and Methods). In both groups (younger and older), the children were divided into two subgroups – with lower and with higher IGF-I/IGFBP-3 molar ratio.

In each subgroup, there were children with different diagnoses: among the children with lower IGF-I/IGFBP-3 molar ratio (100%) there were 23.5% with GHD, 23.5% children with NSD and as much as 53% children with ISS (Table 1). On the other hand, 5 out of 21 children (23.8%) with GHD and 7 out of 23 children (30.4%) with NSD presented higher IGF-I/IGFBP-3 molar ratio. In turn, in 36 out of 92 children (39%) with ISS the lower IGF-I/IGFBP-3 molar ratio was observed (Figure 1).

The auxological data and hormonal results in children with lower and with higher IGF-I/IGFBP-3 molar ratio in

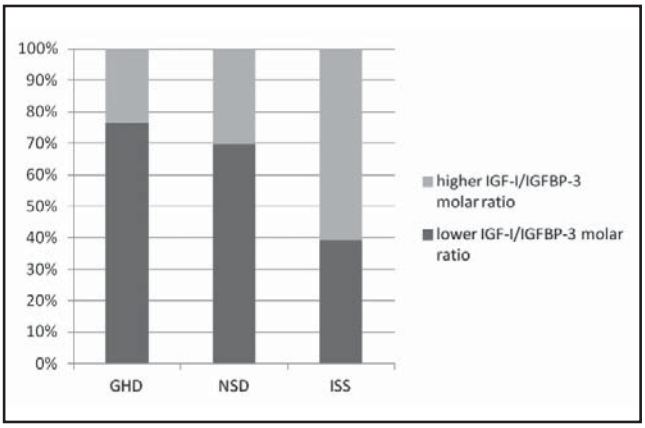

Fig. 1. Percentage of patients with lower and higher IGF-I/IGFBP-3 molar ratio value in particular diagnostic groups.

Tab. 1. Number of children with different diagnoses (GHD, NSD or ISS) which were qualified into subgroup with lower and with higher IGF-I/IGFBP-3 molar ratio.

		GHD n=21	NSD n=23	ISS n=92
lower IGF-I/IGFBP-3 molar ratio	younger children n=34	6	8	20
	older children n=34	10	8	16
	together n=68 (100%)	16 (23.5%)	16 (23.5%)	36 (53%)
higher IGF-I/IGFBP-3 molar ratio	younger children n=34	3	2	29
	older children n=34	2	5	27
	together n=68 (100%)	5 (7.4%)	7 (10.3%)	56 (82.3%)

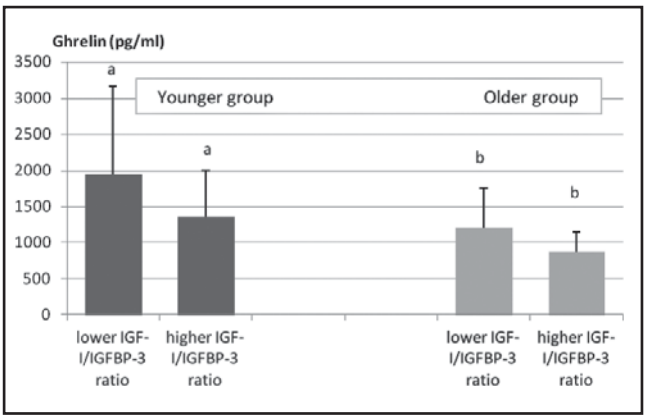

Fig. 2. Comparison of ghrelin concentration in children with lower and higher IGF-I/IGFBP-3 molar ratio value, depending on the age group (younger vs. older) ; a,b - $p=0.0001$.

Tab. 2. Comparison of auxological data and hormonal results in younger children with lower and with higher IGF-I/IGFBP-3 molar ratio value; p – level of significance.

	lower IGF-I/IGFBP-3 molar ratio	higher IGF-I/IGFBP-3 molar ratio	*p*-value
number of children	34	34	
CA (years)	7.03±1.84	8.16±1.91	NS
HA (years)	5.19±1.63	6.06±1.70	NS
height (cm)	111.16±10.88	117.08±10.87	NS
height SDS	–2.59±0.87	–2.25±0.70	NS
BMI (kg/m^2)	15.35±1.93	16.32±2.99	NS
BMI SDS for CA	–0.15±1.05	0.06±1.24	NS
BMI SDS for HA	–0.16±1.06	0.28±1.61	NS
BA (years)	5.1	6.3	NS
ghrelin (pg/ml)	1937.29±1232.43	1365.33±632.07	0.018
GH_{max} (ng/ml) after clonidine	17.89±9.60	17.76±9.08	NS
GH_{max} (ng/ml) after glucagon	9.68±7.69	9.86±6.29	NS
GH_{max} (ng/ml) in nocturnal profile	11.32±5.01	13.06±6.29	NS
IGF-I (ng/ml)	84.29±37.91	150.48±42.64	0.0001
IGFBP-3 (μg/ml)	4.07±1.73	4.12±0.90	NS
IGF-I/IGFBP-3 molar ratio	0.12±0.03	0.21±0.05	0.0001
IGF-I SDS for CA	–1.15±0.99	–0.44±0.69	0.001
IGF-I SDS for HA	–0.57±0.99	0.24±0.55	0.0002
IGF-I SDS for BA	–0.65±1.08	0.12±0.78	0.004

Tab. 3. Comparison of auxological data and hormonal results in older children subgroup with lower and with higher IGF-I/IGFBP-3 molar ratio; p – level of significance.

	lower IGF-I/IGFBP-3 molar ratio	higher IGF-I/IGFBP-3 molar ratio	*p*-value
number of children	34	34	
CA (years)	12.86±1.60	13.78±1.81	NS
HA (years)	9.53±1.23	10.62±1.71	NS
height (cm)	138.08±6.79	144.64±10.33	NS
height SDS	–2.80±0.80	–2.61±0.95	NS
BMI (kg/m^2)	16.97±2.16	18.97±3.04	0.003
BMI SDS for CA	–0.51±0.77	0.02±1.05	0.03
BMI SDS for HA	0.10±0.78	0.66±0.96	0.01
BA (years)	10.35±2.05	11.85±1.82	0.001
ghrelin (pg/ml)	1205.44±548.76	867.44±282.87	0.002
GH_{max} (ng/ml) after clonidine	15.03±8.99	14.92±8.74	NS
GH_{max} (ng/ml) after glucagon	10.24±8.00	13.50±6.74	NS
GH_{max} (ng/ml) in nocturnal profile	12.47±7.17	15.79±12.34	NS
IGF-I (ng/ml)	153.54±54.21	329.76±103.62	0.0001
IGFBP-3 (μg/ml)	4.59±1.00	4.97±1.16	NS
IGF-I/IGFBP-3 molar ratio	0.19±0.05	0.38±0.11	0.0001
IGF-I SDS for CA	–2.14±1.08	–0.49±0.79	0.0001
IGF-I SDS for HA	–0.65±1.08	0.12±0.78	0.004
IGF-I SDS for BA	–1.13±1.71	–0.23±0.81	0.01

younger and in older children are presented in Table 2 and in Table 3.

Both in younger and older children we have found that, despite on similar GH concentrations, in children with lower IGF-I/IGFBP-3 ratio, ghrelin concentration was significantly higher than in children with higher IGF-I/IGFBP-3 ratio (Figure 2). We did not observe any significant differences between children with lower and higher IGF-I/IGFBP-3 molar ratios, regarding age, height and body mass index (in younger children) and regarding age and height (in older children). In both age groups, we observed significantly lower IGF-I concentrations, as well as IGF-I SDS calculated for CA, HA and BA in children with lower IGF-I/IGFBP-3 molar ratio in comparison to children with higher IGF-I/IGFBP-3 ratio value, whereas IGFBP-3 concentrations did not differ between groups.

In both age groups the negative correlations between ghrelin and IGF-I/IGFBP-3 ratio, as well as between ghrelin and IGF-I SDS calculated for CA, for HA and for BA were observed – see Figure 3 (a,b,c,d) and Figure 4 (a,b,c,d), however, the correlations between ghrelin and IGF-I/IGFBP-3 molar ratio were the strongest in both age groups.

DISCUSSION

As it was proved by Whatmore *et al.* (2003), ghrelin concentrations negatively correlated with the age of children, as well as with the body mass and IGF-I concentrations. Since, the two latter parameters increase with in child's age, it was necessary to create two separate age groups for children (younger short vs. older short children). Moreover, we have used objectified parameters, such as BMI SDS for HA and for BA and IGF-I SDS for CA, for HA and for BA, because in short children their biological development is usually markedly delayed. Application of our method, based on the above objectified parameters and separate examination of two age groups allowed us to provide an evidence that ghrelin concentration negatively correlated with IGF-I/IGFBP-3 molar ratio (as well as with IGF-I SDS

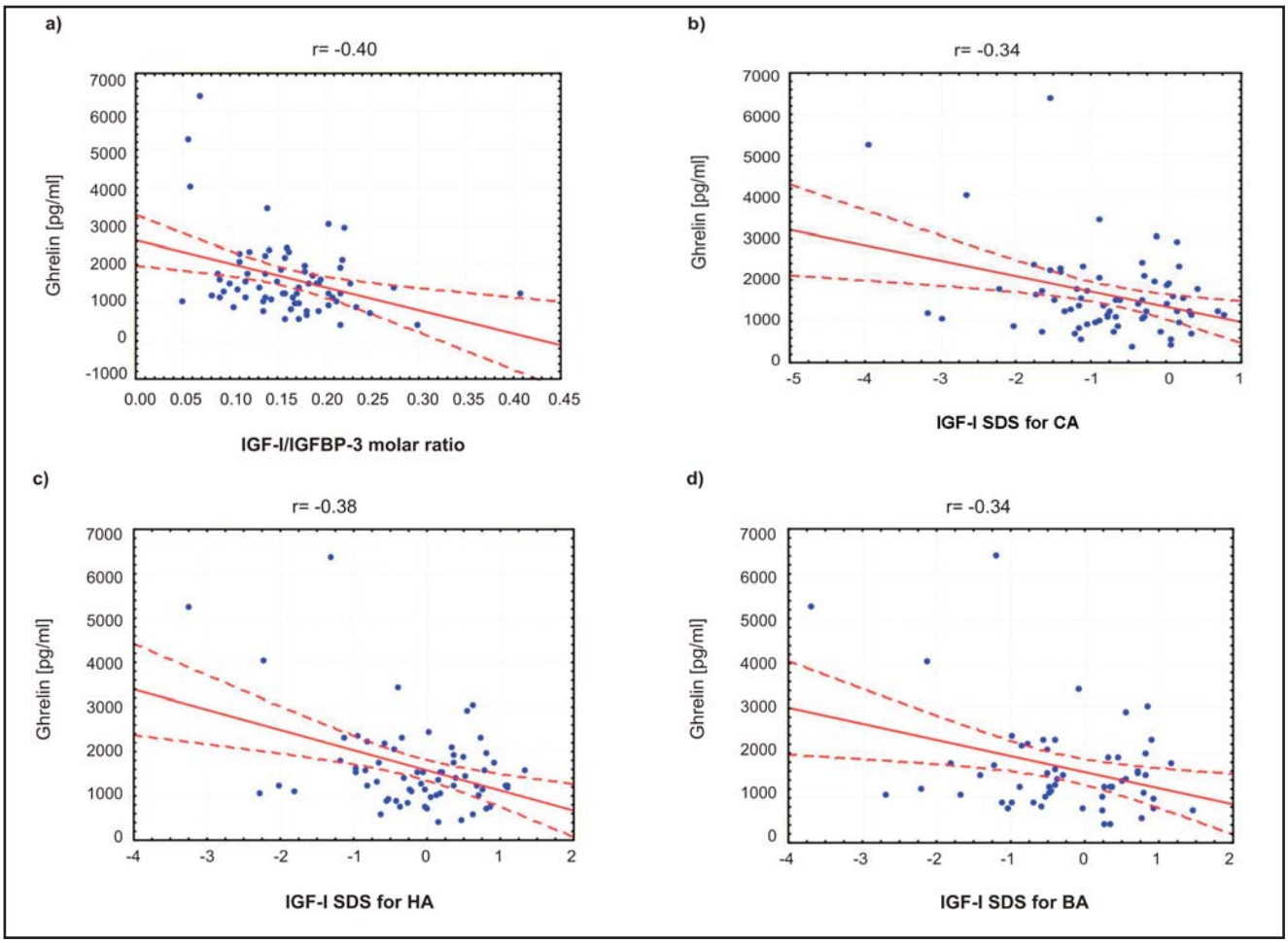

Fig. 3. Correlation between ghrelin concentration and IGF-I/IGFBP-3 molar ratio (**a**), ghrelin concentration and IGF-I SDS for CA (**b**), ghrelin concentration and IGF-I SDS for HA (**c**) and ghrelin concentration and IGF-I SDS for BA (**d**) in younger group of short children.

for CA, for HA and for BA), regardless of age and of body mass of children.

This correlation between ghrelin concentration and IGF-I/IGFBP-3 molar ratio was observed in the whole group of short children, regardless of the diagnosis established on the basis of maximal GH levels during sleep and during stimulating tests. Moreover, the correlation between ghrelin and GH secretion was not observed. The explanation of this issue is not obvious. In 2004, Ghizzoni *et al.* have provided evidence that ghrelin levels in children with NSD are higher than in the children with ISS. In our previous work (Stawerska *et al.* 2012) we have also confirmed that both in children with NSD and GHD ghrelin concentrations are significantly higher than in healthy children. Moreover, in children with ISS, ghrelin concentrations were also higher than in controls. Similar results of studies in children populations are presented by other researchers. Camurdan *et al.* in 2006 confirmed that ghrelin concentration is also higher in short children with familial short stature and with constitutional delay of growth and puberty than in children from control group. Iñiguez *et al.* (2010) reported that ISS patients exhibited a higher level of ghrelin and similar IGF-I levels when compared to controls; moreover, in some of them ghrelin levels were even greater than +2 SD when compared to controls. In the latter cases, molecular analysis showed some polymorphisms of GHSR (Iñiguez *et al.* 2010).

Thus, the relationship between ghrelin, GH and IGF-I secretions are indisputable. However, it seems that higher ghrelin concentration does not play a role as a cause, but as a consequence and it takes place in compensatory response. It also seems that the most powerful stimulus for ghrelin secretion is low IGF-I bioavailability. In some GHD children, the IGF-I concentration is normal, while in some ISS children, the IGF-I concentration is lower. In GH-sensitive patients, IGF-I plasma concentration reflects GH secretion, but in case of GH insensitivity, IGF-I level remains low despite normal or

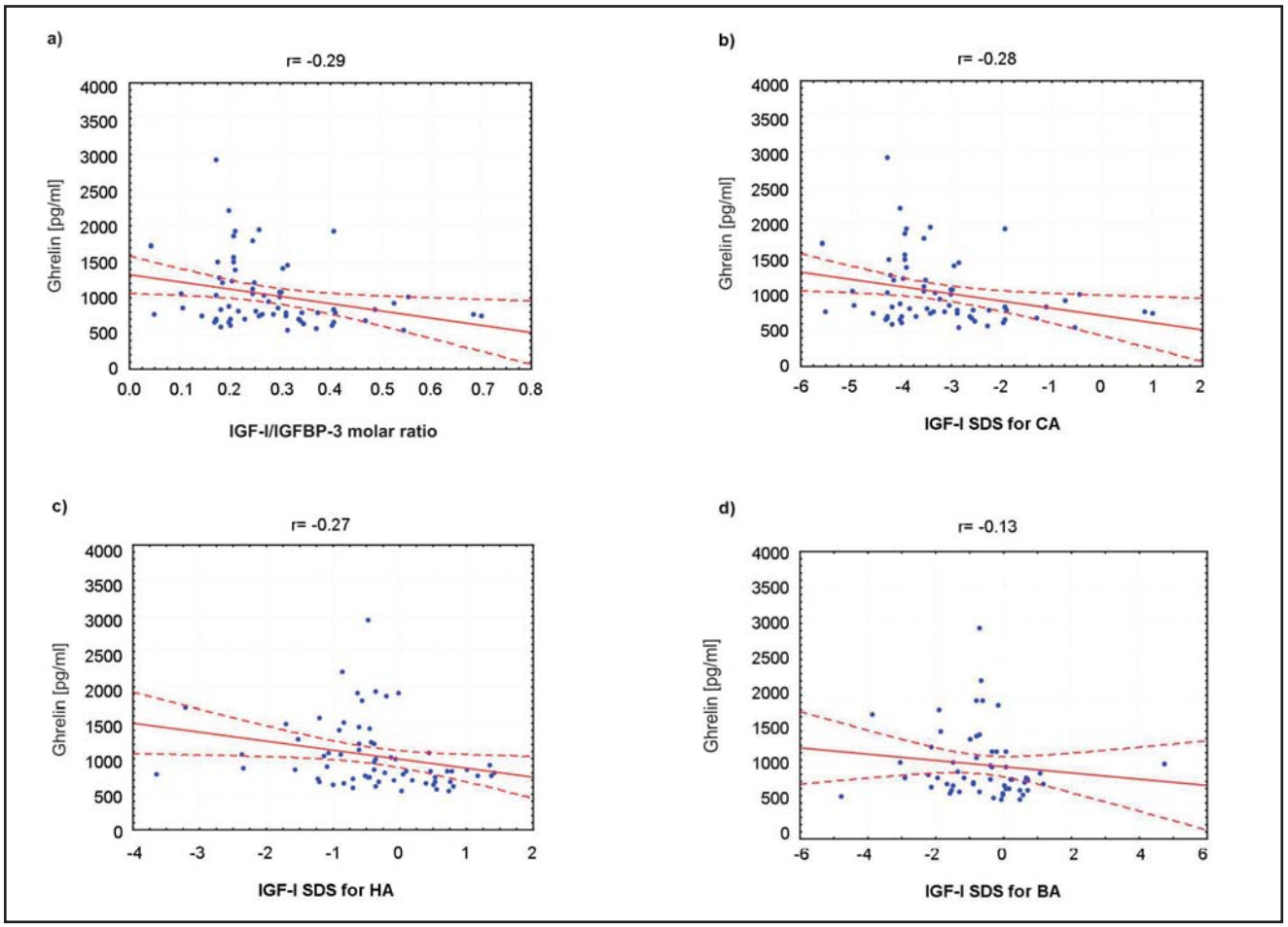

Fig. 4. Correlation between ghrelin concentration and IGF-I/IGFBP-3 molar ratio (a), ghrelin concentration and IGF-I SDS for CA (b), ghrelin concentration and IGF-I SDS for HA (c) and ghrelin concentration and IGF-I SDS for BA (d) in older group of short children.

even elevated GH secretion. It is well known that the poor agreement between IGF-I concentration and levels of GH in pharmacological test is observed (Hauffa *et al.* 2007). Moreover, it should be emphasized that low IGF-I concentration is observed not only in children with GHD and NSD but also in children with, i.e., celiac diseases (Selimoglu *et al.* 2006), Prader-Willi syndrome (Tauber *et al.* 2004) or children born small for gestational age (Darendeliler *et al.* 2008, Lebl *et al.* 2011). In these cases the higher ghrelin concentrations are observed, although short stature is not specific for these cases. Further studies are needed to clarify this issue.

Summing up, the decreased value of IGF-I/IGFBP-3 molar ratio appears to be a more sensitive index to detect the lack of GH action in short children than GH concentration in stimulating tests and/or nocturnal profile. It is possible that the low value of molar ratio in question may be a stimulating factor for ghrelin production, most probably as a compensatory mechanism.

In consequence, in some children diagnosed as ISS, with low IGF-I/IGFBP-3 molar ratio and high ghrelin concentration, verification of diagnosis should be considered.

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