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Serum Ghrelin, IGF-I and IGFBP-3 Levels In Children with Normal Variant Short Stature

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Abstract. This study is planned to investigate the role of ghrelin in normal variant short stature. Serum ghrelin, IGF-I and IGFBP-3 levels were measured in 17 children with constitutional delay of growth, 19 children with familial short stature and 11 age matched healthy children. Mean bone age of the constitutional delay of growth group was lower compared to other groups. Constitutional delay of growth group had lower mean weight compared to the controls. Serum IGF-I values were lower in the constitutional delay of growth group compared to the familial short stature and control groups. IGFBP-3 levels of the groups were similar. Ghrelin levels were higher in the short stature groups compared to the controls. In the multiple regression analyses, weight ($\beta = -.54$, $p<0.0001$) and height SDS ($\beta = -.33$, $p = 0.01$) were the independent determinants of ghrelin. The results of this study, the first one in which ghrelin levels are investigated in normal variant short stature, suggest that ghrelin does not play a role as a cause, but as a consequence in these patients because it is negatively correlated with weight and height standard deviation score. These negative correlations can be attributed to the compensatory response of ghrelin, which deserves further attention in future studies.

Key words: Ghrelin, IGF-I, IGFBP-3, Short Stature, Children

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SHORT stature is among the most common complaints that pediatricians face with. In most of the patients with short stature, no definitive cause can be documented and they are considered to be "idiopathic". The definition of idiopathic short stature (ISS) remains somewhat controversial. Some authors prefer to categorize all the children with unknown cause of short stature as ISS [1, 2], while some authors prefer to classify those with bone age appropriate for chronological age whose parents are in the range of short stature as familial short stature (FSS) and those who has bone age retardation (at least one year) with a family history of delayed pubertal maturation as constitutional delay of growth (and puberty) [CDG(P)], giving the name ISS to the rest [3–5]. Although FSS and CDGP are considered as "normal variants", studies reveal that final height is compromised as a result of the natural course in FSS and as a result of yet undefined reasons in CDGP [3]. The suggested causes of poor growth in children with ISS are most likely heterogenous, *i.e.* delay in physical maturity [5–9], suboptimal nutrition [4, 10, 11], genetic factors and growth hormone (GH) — insulin-like growth factor (IGF) axis abnormalities [4, 5, 9, 12–14].

Ghrelin is a natural GH secretagogue that increases serum GH levels and has other endocrine activities such as orexigenic and adipogenic effects and modulation of the endocrine response to variations in energy balance [15–18] which are probably mediated by its action on hypothalamus [19]. Because of its close relationship with GH secretion and nutritional status, one can speculate that altered ghrelin levels may be responsible for the mechanisms, at least in part, causing "normal variant" short stature. To the best of knowledge, there is no data in literature that compares serum ghrelin levels between FSS, CDGP and normal controls.

This study is planned to investigate ghrelin levels in patients with CDG, FSS, its relationship with serum IGF-I and IGFBP-3 and to compare the results with healthy controls.

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Materials and Methods

The study was conducted on 17 children with CDG (three girls, 14 boys) aged 6.5–15 (11.0 ± 2.8) years, 19 children with FSS (12 girls, 7 boys) aged 7.0–15.2 (11.5 ± 2.3) years and 11 healthy children (six girls, five boys) aged 6.8–14.5 (11.5 ± 2.4) years.

All interviews and clinical examinations were performed by the same physician. The children in the short stature groups had heights less than 3rd percentile or height SDS <–2 for age and sex with annual growth velocity in the normal ranges [20]. All the children were otherwise healthy in terms of chronic or systemic disorders. Their physical examinations, except for the height measurements, were completely normal and no biochemical abnormalities were noted on standard testing, which included complete blood cell counts, erythrocyte sedimentation rates, routine chemical analysis including liver and renal function tests, urinalysis and thyroid functions. Celiac was excluded with normal values of gliadin and endomysium antibodies. The results of pharmacological GH stimulation tests (L-Dopa and insulin tolerance tests) were also normal. Psychological analyses performed by the same examiner revealed no evidence of psychiatric disease or emotional disturbance. All had normal medical history including being term at birth with weights and heights appropriate of gestational age.

Those with bone age delay more than 2 SD for chronological age whose parents have normal height SDS were grouped as CDG, while those with bone ages appropriate for their chronological age whose parents are considered as having short stature were grouped as FSS. In order to have a strict classification, those patients who could not be differentiated as FSS or CDGP were not included in the study. Target heights of the subjects (which are calculated from heights of their parents) [21] were similar to their predicted heights (which are calculated by using bone ages according to the Greulich and Pyle atlas) [5, 22, 23].

Genital examination was performed according to the staging system of Tanner [24, 25]. Heights were measured to the nearest 0.1 cm with a Harpenden stadiometer. Body mass index (BMI), defined as the weight in kilograms divided by the square of the height in meters, was calculated. Neyzi's growth and development norms for Turkish children were used for the evaluation of heights and weights [20]. Blood samples were obtained in the fasting state at 8 a.m.

Serum IGF-I and IGFBP-3 levels were measured using IRMA (with DSL-5600 and DSL-6600 kits, respectively), and active n-octanoyl ghrelin levels were measured by using a commercial ELISA kit (Ghrelin human EIA kit; Phoenix Pharmaceuticals, Inc., Belmont, CA, USA) and samples for quality control were included in each assay. The intra-assay variation is <%5 and inter-assay variation is <%14 for the ghrelin kit. Blood samples, anticoagulated with EDTA and aprotinin were kept on ice, centrifuged at 1800 g for 15 min. at 4°C, plasma samples were separated and stored at –80°C until assayed.

All the values analyzed showed normal distribution according to the Kolmogorov-Smirnov test. Therefore, data are expressed as mean ± SD. ANOVA (*post hoc*; Bonferroni) and Pearson correlation analyses are used in order to compare values between groups and to investigate correlations between parameters respectively. Stepwise multiple regression analyses are used where available.

The study was approved by the local ethics committee and informed consent was obtained from all parents.

Results

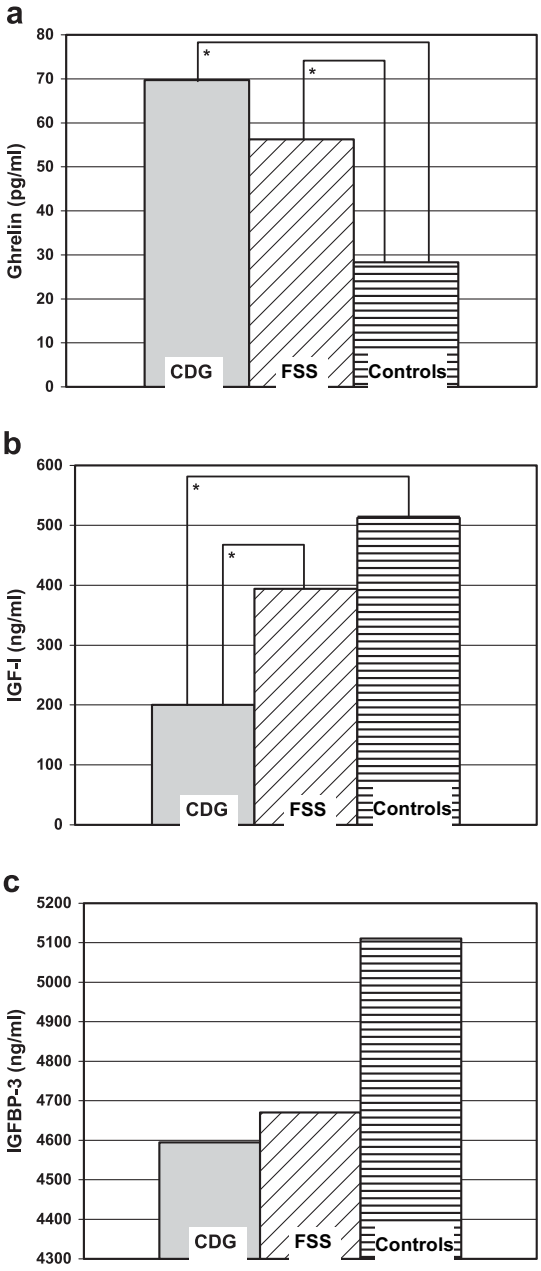
Age and anthropometric data of the patient and control groups are summarized in Table 1. Although chronological ages were similar between groups, mean bone age of the CDG group was lower than those of the other two groups as expected. Height and height SDS values were similar between CDG and FSS groups being significantly lower than controls. CDG group had lower mean weight compared to the control group and lower mean BMI compared to the FSS group. Mean ghrelin, IGF-I and IGFBP-3 levels are presented in Fig. 1 (a, b, and c, respectively). Serum ghrelin levels were higher in both groups with short stature compared to the control group (69.7 ± 33.1 pg/ml, 56.2 ± 29.0 pg/ml, 28.3 ± 23.3 pg/ml, respectively). Serum IGF-I values were lower in the CDG group compared to the FSS and control groups (200.3 ± 180.6 ng/ml, 394.0 ± 259.9 ng/ml, 512.1 ± 231.5 ng/ml, respectively). IGFBP-3 levels of the groups were similar (4594.8 ± 834.0 ng/ml, 4670.4 ± 751.9 ng/ml, 5110.4 ± 660.3 ng/ml, respectively).

Taking the study group as a whole, serum ghrelin levels were significantly correlated with chronological

Table 1. Age and anthropometric data of children with short stature and controls*

	Constitutional delay of growth group (n = 17)	Familial short stature group (n = 29)	Control group (n = 11)
Chronological age (years)	11.0 ± 2.8	11.3 ± 2.2	11.5 ± 2.4
Bone age (years)	8.1 ± 2.7[a,b]	10.8 ± 2.1	11.5 ± 2.5
Height (cm)	128.4 ± 14.4[a]	132.2 ± 12.0[a]	146.1 ± 13.5
Height SDS	–2.4 ± 0.3[a]	–2.1 ± 0.2[a]	–0.1 ± 0.9
Weight (kg)	27.2 ± 7.3[a]	32.3 ± 7.1	35.9 ± 9.1
BMI (kg/m^2)	16.1 ± 1.9[b]	18.2 ± 2.2	16.6 ± 2.1

*: Values are expresses as Mean ± SD. [a]: Constitutional delay of growth group or Familial short stature group vs Controls, $p<0.05$. [b]: Constitutional delay of growth group vs Familial short stature group, $p<0.05$



age, bone age, pubertal stage, weight, height, height SDS, BMI and IGF-I, with weight and height SDS being the independent determinants on serum ghrelin levels in the stepwise multiple regression analyses (Table 2). Serum IGF-I values were significantly correlated with age, bone age, weight, height, height SDS and ghrelin. Although IGF-I levels were negatively correlated with ghrelin (Fig. 2), the multiple regression analyses revealed that bone age was the most important determinant of serum IGF-I levels (Table 2).

Because of the close relationship of ghrelin and IGF-I with other parameters, comparison between groups in terms of ghrelin and IGF-I were reperformed using weight and height SDS for ghrelin and bone age for IGF-I as the covariates. Using these adjustment models, ghrelin and IGF-I values were found to be similar between groups ($p>0.05$).

Discussion

The intimate pathogenic mechanism underlying the poor growth observed in patients with "idiopathic" short stature and/or "normal variant" short stature is not yet clearly documented. To date the data point out that the causes are most likely heterogenous, including delay in physical maturity [5–9], suboptimal nutrition [4, 10, 11], genetic factors and GH-IGF axis abnormalities [4, 5, 9, 12–14]. Therefore a careful investigation of the components related to the GH-IGF-I axis, nutrition and maturity should be carried out in patients with growth failure who are categorized as "idiopathic"

Fig. 1. Ghrelin (a), IGF-I (b) and IGFBP-3 (c) levels in the Constitutional delay of growth (CDG), Familial short stature (FSS) and control groups. *: $p<0.05$

Table 2. Simple and multiple regression analyses of ghrelin and IGF-I with other parameters in the study groups

	Ghrelin		IGF-I	
	Simple regression[a]	Multiple regression[b]	Simple regression[a]	Multiple regression[b]
Chronological age	–0.45 (0.001)	— (>0.05)	0.53 (0.000)	— (>0.05)
Bone age	–0.58 (0.000)	— (>0.05)	0.72 (0.000)	0.72 (0.000)
Pubertal stage	–0.39 (0.008)	— (>0.05)	— (>0.05)	— (>0.05)
Weight	–0.64 (0.000)	–0.47 (*0.001*)	0.64 (0.000)	— (>0.05)
Height	–0.61 (0.000)	— (>0.05)	0.64 (0.000)	— (>0.05)
Height SDS	–0.44 (0.002)	–0.33 (*0.01*)	0.32 (0.036)	— (>0.05)
Body mass index	–0.33 (0.025)	— (>0.05)	— (>0.05)	— (>0.05)
IGF-I	–0.49 (0.001)	— (>0.05)		
Ghrelin			0.49 (0.001)	— (>0.05)

[a]: Values are expressed as; r (p of r).
[b]: Values are expressed as; β (p of β).

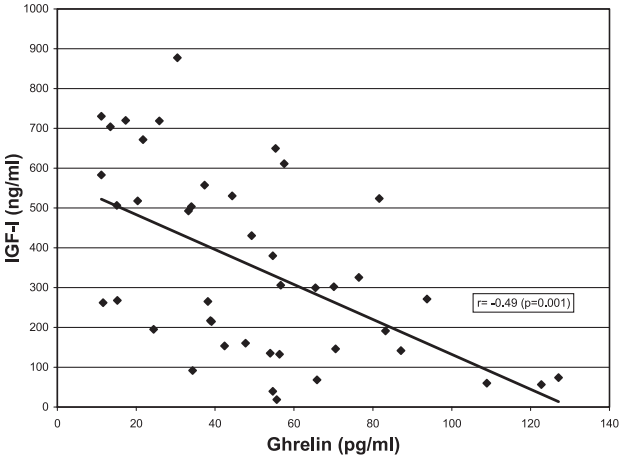


Fig. 2. Relationship of ghrelin with IGF-I in the study groups.

or "normal variant" short stature. One of these components is ghrelin, the novel peptide which is strongly related to growth with its orexigenic properties and being a potent GH secretagogue. There is not enough data in literature considering ghrelin levels and their relationship to other parameters of growth in children with CDG and FSS.

The results of our study suggest that ghrelin plays a role not as a cause, but as a consequence — probably in a compensatory manner — of the pathological events observed in patients with "normal variant" short stature. One would expect lower ghrelin levels in patients with short stature compared to controls if it was the underlying cause, but our results reveal that serum ghrelin levels tend to be higher in these patients.

Investigating the reason for this elevated ghrelin levels in our patients, multiple regression analyses revealed that weight and height SDS were the independent determinants of serum ghrelin levels, which in turn suggest that elevated ghrelin levels are the consequence of lowered weight (being statistically significant in the CDG group compared to controls) and height SDS observed in "normal variant" short stature patients. This result leads to a suggestion that ghrelin levels could be elevated in our patients in a compensatory manner because of its two major functions: orexigenic functioning as a response to lower body weight and a strong GH secretagogue functioning as a response to lowered height SDS. Previous studies support our finding that children with CDG and FSS tend to be lean [9, 26, 27]. Ghrelin, as is very well known, has profound orexigenic and adipogenic properties functioning as a signal for the hypothalamus when an increase in metabolic substrates is needed [15–18, 28]. Therefore, it seems to be logic that ghrelin levels might be elevated in our patients, especially in the CDG group as an adaptive mechanism to lowered body weight, as seen in patients with anorexia nervosa and in obese subjects who lose weight with diet [29, 30]. This suggestion cannot solely explain the elevated ghrelin levels in our patients because of two reasons; first, although our FSS group had lower body weight compared to controls, the difference did not reach statistical significance so that there should be another reason for the elevated ghrelin levels in our FSS group; second, in the multiple regression model, body weight was not the only predictor of serum ghrelin levels, with height SDS being the other independent determinant. To the best of our knowledge, our study is the first one in which an

independent negative correlation between height SDS and ghrelin could be demonstrated. The independency of height SDS is supported with the findings that besides being an independent predictor of ghrelin in the regression analyses, our FSS patients with similar body weights had higher ghrelin levels compared to controls before the adjustment model.

Although it seems to be logic for a strong GH secretagogue like ghrelin to be elevated in patients with low height SDS, the exact mechanism of the inverse relationship between serum ghrelin levels and height SDS cannot be strictly explained with our data. The role of lower IGF-I levels could be an explanation; one could assume that ghrelin levels were elevated in a compensatory manner because of the lower IGF-I values leading to lower height SDS. This assumption is supported by the findings that serum ghrelin and IGF-I levels are found to be negatively correlated in healthy children [31, 32]. We have also found this negative correlation in our study groups, but the multiple regression analyses revealed that it was not an independent one. Even if this assumption was true for the CDG group, it does not explain the higher ghrelin levels observed in the FSS group whose IGF-I values were similar to the control group. Besides, there is a considerable amount of data in literature that does not support the possible "negative feed-back loop" of GH-IGF-I axis with ghrelin [33, 34]. Only Matsuoka *et al.* [35] with their very recent data were able to demonstrate such a negative feed-back loop. Another possible explanation could be that the suboptimal nutritional state in patients with short stature that ends up with lower height SDS could be the reason for the compensatory elevation of ghrelin as an orexigenic factor. It is shown that suboptimal nutrition in childhood can contribute to impaired development of body height, and that this is the case in at least some of the patients with "idiopathic" short stature [4, 10, 11, 36]. Our data does not include appetite status of the patients to be in favor or against this possible explanation, but overall, we suggest that the finding of the height SDS being an independent determinant of serum ghrelin levels in children with "normal variant" short stature deserves further attention in future studies.

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