

Significant increase of IGF-I concentration and of IGF-I/IGFBP-3 molar ratio in generation test predicts the good response to growth hormone (GH) therapy in children with short stature and normal results of GH stimulating tests

Joanna SMYCZYŃSKA^{1,2}, Maciej HILCZER^{1,2}, Renata STAWERSKA^{1,2}, Andrzej LEWIŃSKI^{2,3}

¹ Department of Pediatric Endocrinology, Medical University of Lodz, Poland

² Department of Endocrinology and Metabolic Diseases, Polish Mother's Memorial Hospital – Research Institute in Lodz, Poland

³ Department of Endocrinology and Metabolic Diseases, Medical University of Lodz, Poland

Correspondence to: Prof. Andrzej Lewiński, MD., PhD.
Department of Endocrinology and Metabolic Diseases
Polish Mother's Memorial Hospital – Research Institute
Rzgowska Street 281/289; 93-338 Lodz, Poland.
TEL: +48 42 271 17 15; FAX: +48 42 271 13 43; E-MAIL: alewin@csk.umed.lodz.pl

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Abstract

BACKGROUND: Insulin-like growth factor-I (IGF-I) generation test has been introduced for the assessment of growth hormone (GH) sensitivity, however, its significance in predicting growth response to GH therapy has also been brought up. The molar ratio of IGF-I to its binding protein-3 (IGFBP-3) determines IGF-I bioavailability.

AIMS: Evaluation of usefulness of IGF-I and IGFBP-3 generation test in predicting the effectiveness of rhGH therapy in children with short stature.

PATIENTS AND METHODS: The analysis comprised 60 children with short stature, normal results of GH stimulating tests but decreased IGF-I secretion. In all the patients, GH insensitivity was excluded on the basis of IGF-I and IGFBP-3 generation test. Next, GH therapy was administered and height velocity (HV), together with IGF-I and IGFBP-3 secretion, was assessed every year, during 3 years. The comparative group consisted of 30 children with partial GH deficiency (pGHD).

RESULTS: Both IGF-I secretion and IGF-I/IGFBP-3 molar ratio increased significantly during generation test ($p < 0.05$) and – further – during GH therapy (however insignificantly), together with at least doubling of pretreatment HV. There was no significant difference between the studied group of patients and children with pGHD.

CONCLUSIONS: Significant increase of IGF-I in generation test speaks for GH therapy effectiveness in short children, despite normal results of GH stimulating tests.

INTRODUCTION

Growth hormone (GH) is necessary for normal linear growing. Standard procedures in diagnosing GH deficiency (GHD) are GH stimulating tests (GHST). However, disorders of spontaneous GH secretion (neurosecretory dysfunction – NSD) or a decreased GH bioactivity may occur in the patients with normal results of GHST in whom the therapy with recombinant human GH (rhGH) is potentially effective.

Insulin-like growth factor-I (IGF-I) is the main peripheral mediator of GH activity. In GH-sensitive subjects, IGF-I plasma concentration reflects GH secretion, while in case of GH insensitivity (GHI), IGF-I level remains low despite normal or even elevated GH secretion. Both IGF-I bioavailability and stability of its concentration is determined by binding to specific proteins, especially – to insulin-like growth factors binding protein-3 (IGFBP-3). The molar ratio of IGF-I to IGFBP-3 has been regarded as an important index of IGF-I bioavailability (Juul *et al.* 1995; Tillmann *et al.* 2000).

Assessment of IGF-I and IGFBP-3 secretion was proposed to be a screening procedure in children with short stature, suspected for GHD by Rosenfeld (1996). Recently, IGF-I deficiency (IGFD) has been defined as a separate diagnosis which encompasses the abnormalities either in GH secretion (secondary IGFD, potentially responding to rhGH therapy) or in GH responsiveness (primary IGFD) (Cohen 2006; Wit *et al.* 2007).

Diagnostic algorithm in children with short stature but with excluded classic form of GHD leads to carrying out IGF-I generation test. However, several variants of the test were proposed, differing in the test duration, daily doses of rhGH, or in the rules of test interpretation (Buckway *et al.* 2001; Blum & Schweitzer 2003; Blair *et al.* 2004; Darendeliler *et al.* 2005, Tetlow & Clayton 2005). As a matter of fact, IGF-I generation test has become a very important diagnostic procedure for the patients suspected for GHI (Buckway *et al.* 2001). The lack of increase of IGF-I secretion during short-term rhGH administration speaks for GHI. On the other hand, significant IGF-I increase may support the indications to rhGH therapy. Unfortunately, data concerning that issue seem to be rather scarce and inconsistent. For instance, the correlation between an increase of IGF-I secretion during rhGH administration and growth response to the therapy has been confirmed in several studies (Schwarze *et al.* 1999; Kamp *et al.* 2002; Spiliotis *et al.* 2009; Pagani *et al.* 2011), while it has been denied by others authors (Jørgensen *et al.* 2001; Savage *et al.* 2010). The observations concerning the relationships between IGFBP-3 levels (and IGF-I/IGFBP-3 molar ratio) and height velocity (HV) during rhGH therapy are also inconsistent, either confirming (Kiström *et al.* 1997; Scirè *et al.* 2008) or denying (Tillmann *et al.* 2000; Lanes & Jakubowicz 2002) the existence of any correlation between IGF-I and IGFBP-3 secretion during rhGH therapy and HV.

The indications to rhGH therapy in short children with GHD have been established quite clearly (GH Research Society 2000). However, in the recent years some reports have been published, confirming the good effectiveness of rhGH therapy in children with idiopathic short stature (ISS) (Hintz 2005; Kemp *et al.* 2005; Ranke *et al.* 2005). On the other hand, the problem of varied growth responses among patients and a large drop-out of poor responders during the therapy have also been raised (Richmond & Rogol 2010). Thus, the criteria of qualification of children with short stature to rhGH therapy have still been the matter of discussion (Badaru & Wilson 2004; Rosenfeld *et al.* 2004; Richmond & Rogol 2010).

The aim of the study was to evaluate the significance of the results IGF-I and IGFBP-3 generation test in predicting the effectiveness of rhGH therapy in children with short stature.

PATIENTS AND METHODS

The analysis comprised the results of IGF-I and IGFBP-3 generation test, performed in 60 children (48 boys, 12 girls), age 12.5 ± 2.3 years (mean \pm SD), with short stature (below 3rd centile for age and sex), slow HV (below 4 cm/year) and with decreased IGF-I serum concentration despite normal GH secretion in 2 standard GHST (*i.e.* GH peak – in at least one test – above 10.0 ng/ml), with clonidine (0.15 mg/m², orally) and with glucagon (30 µg/kg *i.m.*, not exceeding 1 mg). Decreased IGF-I concentration was defined as IGF-I SDS for age and sex below –1.0, according to the criteria proposed by Cianfarani *et al.* (2005). Chronic diseases that might disturb IGF-I synthesis, including malabsorption syndromes, malnutrition, liver diseases, were excluded in every case; all the girls had normal female karyotype (46,XX). In all the patients thyroid function was normal during the study period, none of them required other hormonal substitution.

Blood samples for IGF-I and IGFBP-3 concentration measurements were collected in morning hours, before the 1st rhGH injection and after 7 daily doses of 0.033 mg/kg (0.1 IU/kg), administered at 8 p.m. for 7 consecutive days. In all the patients, an increase of IGF-I secretion during generation test was observed, leading to the normalisation of previously decreased IGF-I concentration and/or to doubling the initial IGF-I level. Moreover, IGFBP-3 secretion was normal in every case. In all the children, GHI was excluded, according to the criteria proposed by Blum *et al.* (1994). Moreover, in all of them, IGF-I concentration was still decreased during the 2nd evaluation. So, they all seemed to be the candidates to rhGH therapy.

Before treatment, the patients were qualified to the following groups:

1. Neurosecretory dysfunction of GH secretion (NSD) – 24 patients with decreased spontaneous nocturnal GH secretion, assessed during 6

hours, every 30 minutes (the cut-off level of GH peak was 10.0 ng/mL).

2. IGFD – 36 patients with decreased basal IGF-I levels, well responding to short-term rhGH administration, despite normal (or even high) GH peak both in nocturnal profile and in GHST.

In all the patients, rhGH therapy in a dose of 0.20 ± 0.02 mg/kg/week (0.60 ± 0.06 IU/kg/week) was administered. Patient's height SDS (HSDS) was calculated before treatment (H_0 SDS) and after 1, 2 and 3 years of rhGH therapy (H_1 SDS, H_2 SDS and H_3 SDS, respectively), HV was assessed before treatment (HV_0) and after 1, 2 and 3 years (HV_1 , HV_2 and HV_3 , respectively). Blood samples for assessment of IGF-I and IGFBP-3 concentration were additionally collected once a year during the therapy.

Pubertal development was assessed before treatment and after every year of treatment. Among the studied children, 16 boys and 5 girls were prepubertal during all the study period, while the remaining ones entered puberty either before or during rhGH therapy. The effectiveness of rhGH treatment in prepubertal ($n=21$) and pubertal ($n=39$) children was compared in order to estimate the "apparent" effectiveness of the therapy, connected with pubertal growth spurt, as puberty could be an independent factor leading to the increase of HV.

The results, obtained in the studied group, were compared with the age- and sex-matched group of 24 short children (18 boys, 6 girls, age 12.9 ± 2.6 years) with the isolated non-acquired partial GHD (pGHD; GH peak in 2 GHST ranging from 5 to 10 ng/ml). The latter analysis has been performed in order to compare the therapy effectiveness in the studied group and in children with unquestionable indications to rhGH administration.

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%. For comparison among children with different age and sex, IGF-I concentrations were expressed as IGF-I SDS. 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%. 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.

Growth hormone concentration was measured by hGH IMMULITE, DPC assay, calibrated to WHO IRP 80/505 standard, with the analytical sensitivity up to 0.01 ng/ml, the calibration range up to 40 ng/ml, the intra-assay CV – 5.3–6.5% and the inter-assay CV – 5.5–6.2%.

Statistical analysis included comparison of IGF-I concentrations in the particular time points, with use

of non-parametric statistical tests: Wilcoxon's test for dependent samples and Mann-Whitney's U test for independent samples.

The study was approved by the Local Committee of Ethics in the Polish Mother's Memorial Hospital – Research Institute.

RESULTS

During generation test, a significant ($p < 0.0001$) increase of IGF-I secretion (expressed as both IGF-I concentration and IGF-I SDS) was observed. Normal IGF-I concentration (*i.e.* IGF-I SDS over -1.0) was obtained in 53 children (88.3%). As a matter of fact, the remaining 7 patients did not achieve the normalisation of IGF-I concentration but the initial IGF-I levels were at least doubled in all of them. Interestingly, there was no correlation between GH peak in GHST and basal IGF-I concentration ($r = -0.12$, NS), while a negative correlation was observed between GH peak in GHST and IGF-I increase in generation test ($r = -0.31$, $p < 0.05$).

In both subgroups of the studied group (*i.e.*, NSD and IGFD) significant increase of both IGF-I SDS and IGF-I/IGFBP-3 molar ratio during generation test ($p < 0.05$), together with their further, however insignificant increase and more than twofold HV improvement in 1st year of rhGH therapy ($p < 0.05$), were observed, while all the differences in HV during consecutive years of treatment proved to be insignificant. Unfortunately, there was no correlation between any of the parameters of IGF axis, assessed in generation test (*i.e.*, an increase of both IGF-I and IGFBP-3 concentrations, and IGF-I/IGFBP-3 molar ratio) and an improvement of HV. The only significant correlation between the assessed parameters of IGF axis and the auxological indices of the therapy effectiveness was that between IGF-I/IGFBP-3 molar ratio after 1 year of rhGH therapy and the increase of HV during 1st year of treatment ($r = 0.35$, $p < 0.05$). It should be mentioned that generation test was not performed in pGHD comparative group.

Significant and of similar extent improvement of HV was observed in all the groups ($p > 0.05$), speaking for rhGH therapy effectiveness in those patients. Moreover, in particular time points (before the therapy and after 1, 2 and 3 years), all the differences among the subgroups of patients in HV or HSDS, or the increase of HSDS proved to be insignificant.

Similarly, in each time point all the differences in IGF-I secretion among the 3 subgroups of patients were insignificant. The improvement of HV during rhGH therapy in the studied subgroups (NSD and IGFD) was similar to that observed in the patients with pGHD. The differences in both IGF-I/IGFBP-3 molar ratio and HV between particular subgroups of patients, as well as differences between each of them and the children with pGHD, were also insignificant (see Table 1 and Figures 1–3).

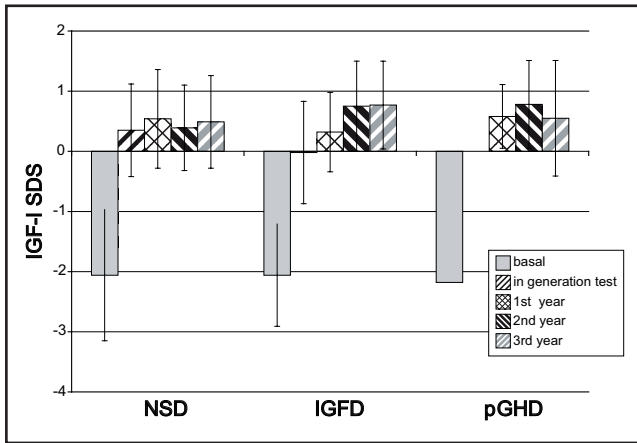


Fig. 1. IGF-I secretion in generation test and during rhGH therapy in particular groups of children. Significant differences were observed in all the groups between basal value and values in all other time points ($p < 0.05$). The differences among values in all remaining time points were insignificant.

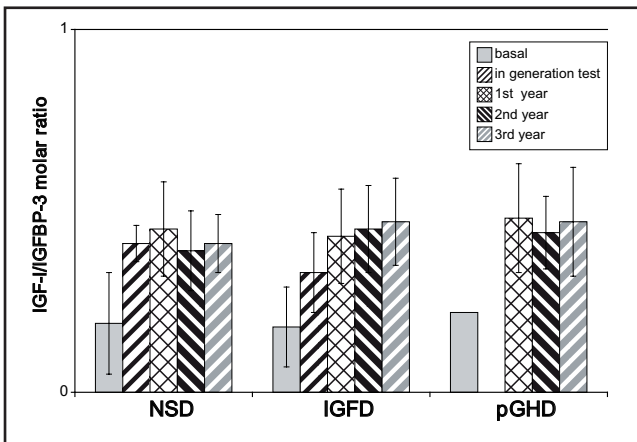


Fig. 2. Changes of IGF-I bioavailability during generation test and during rhGH therapy in particular groups of children. Significant differences were observed in all the groups between basal value and values in all other time points ($p < 0.05$). The differences among values in all remaining time points were insignificant.

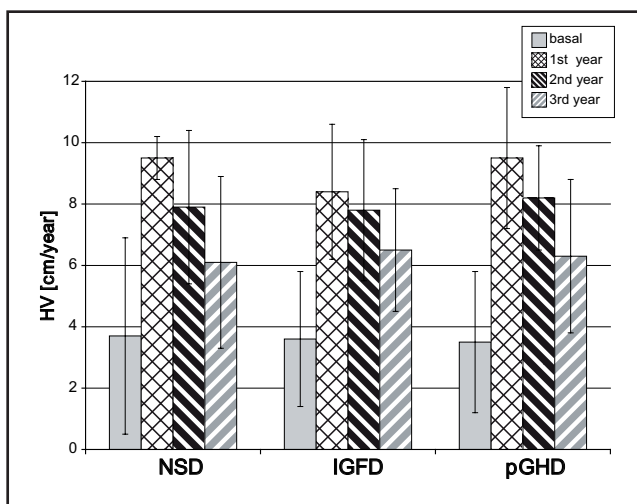


Fig. 3. Patients' HV before treatment and during rhGH therapy in particular groups of children. Significant differences were observed in all the groups between basal value and values in all other time points ($p < 0.05$). The differences among values in all remaining time points were insignificant.

Tab. 1. Selected data concerning generation test and rhGH therapy effectiveness in particular groups of children.

Group		NSD	IGFD	pGHD
IGF-I SDS	basal	-2.06 ± 1.21	-2.06 ± 0.77	-2.18 ± 0.58
	after generation test	0.35 ± 1.09	-0.02 ± 0.85	x
	1 year of rhGH therapy	0.54 ± 0.82	0.32 ± 0.66	0.58 ± 0.53
	2 years of rhGH therapy	$0.39 \pm 0.71^*$	0.75 ± 0.75	0.78 ± 0.76
	3 years of rhGH therapy	0.49 ± 0.77	0.77 ± 0.73	0.55 ± 0.96
IGFI/IGFBP-3 [molar ratio]	basal	0.19 ± 0.06	0.18 ± 0.05	0.22 ± 0.12
	after generation test	0.41 ± 0.14	0.33 ± 0.11	x
	1 year of rhGH therapy	0.45 ± 0.13	0.43 ± 0.13	0.48 ± 0.15
	2 years of rhGH therapy	0.39 ± 0.11	0.45 ± 0.12	0.44 ± 0.10
	3 years of rhGH therapy	0.41 ± 0.08	0.47 ± 0.12	0.47 ± 0.15
height SDS	H_0 SDS	-3.23 ± 0.89	-2.98 ± 0.85	-3.07 ± 0.79
	H_1 SDS	-2.60 ± 1.04	-2.82 ± 1.02	-2.68 ± 1.08
	H_2 SDS	-2.26 ± 1.04	-2.41 ± 0.92	-2.33 ± 0.95
	H_3 SDS	-1.57 ± 0.83	-1.93 ± 0.94	-1.74 ± 0.89
height velocity [cm/year]	HV_0	3.7 ± 1.1	3.6 ± 0.7	3.5 ± 1.1
	HV_1	9.5 ± 3.2	8.4 ± 2.2	9.6 ± 2.3
	HV_2	7.9 ± 2.5	7.8 ± 2.3	8.2 ± 1.7
	HV_3	6.1 ± 2.8	6.5 ± 2.0	6.3 ± 2.5

Significant difference in IGF-I SDS after 2 years of rhGH therapy between NSD and other groups ($p < 0.05$) is marked by asterisk (*). Other differences between the groups in particular time points are insignificant.

Next, the effectiveness of rhGH administration in prepubertal and pubertal children was compared. There was no significant difference in IGF-I SDS between prepubertal and pubertal children in particular time points (before and after generation test, as well as during 3 years of rhGH therapy). Interestingly, IGF-I/IGFBP-3 molar ratio in the same time points presented significantly higher in pubertal children than in prepubertal ones. Despite better HV during rhGH therapy in pubertal children vs. prepubertal ones, all the differences in HSDS and/or HV before rhGH therapy and during 3 years of rhGH treatment turned out to be insignificant (see Table 2).

DISCUSSION

As it was mentioned before, IGF-I generation test was introduced for diagnosing GHI, with only slight increase of IGF-I secretion regarded as sufficient to exclude the resistance to GH. However, different criteria of normal response to rhGH administration in that test have been proposed by some authors. The increase of IGF-I concentration during 4-days generation test considered as sufficient to exclude GHI varied from 20 ng/ml (Tetlow & Clayton 2005) to 115 ng/ml (Darendeliler *et al.* 2005).

Tab. 2. Selected data concerning generation test and rhGH therapy effectiveness in particular groups of children according to the pubertal stage.

		Patients		
		all	prepubertal	pubertal
IGF-I SDS	basal	-2.12±0.95	-1.88±1.06	-2.17±0.86
	after generation test	0.11±0.96	0.12±1.14	0.11±0.83
	1 year of rhGH therapy	0.40±0.73	0.45±0.83	0.36±0.66
	2 years of rhGH therapy	0.57±0.80	0.54±0.78	0.48±0.82
	3 years of rhGH therapy	0.68±0.75	0.77±0.82	0.61±0.69
IGFI/IGFBP-3 [molar ratio]	basal	0.18±0.06	0.15±0.05	0.20±0.05
	after generation test	0.36±0.13	0.30±0.11	0.40±0.12
	1 year of rhGH therapy	0.42±0.13	0.37±0.13	0.44±0.12
	2 years of rhGH therapy	0.42±0.12	0.38±0.10	0.45±0.12
	3 years of rhGH therapy	0.45±0.13	0.45±0.12	0.45±0.11
height SDS	H ₀ SDS	-3.05±0.86	-3.06±0.57	-3.04±0.99
	H ₁ SDS	-2.75±1.03	-2.61±0.72	-2.82±1.16
	H ₂ SDS	-2.35±0.96	-2.17±0.88	-2.46±0.99
	H ₃ SDS	-1.83±0.81	-1.83±1.02	-1.82±0.85
height velocity [cm/year]	HV ₀	3.8±0.8	3.7±0.8	3.9±0.9
	HV ₁	8.8±2.6	8.3±2.0	9.1±2.9
	HV ₂	7.8±2.3	8.7±2.1	7.3±2.3
	HV ₃	6.4±2.7	7.8±2.0*	5.5±2.8*

Significant difference between HV of prepubertal and pubertal children in 3rd year of rhGH therapy is marked by asterisk (*). Other differences between the groups in particular time points are insignificant.

In turn, Blair *et al.* (2004) regarded IGF-I increase not exceeding the doubled value of CV of the assay as low. In the present study – according to the latter two papers – the cut-off value for IGF-I increase should be established on the level of approximately 17%, thus leading to exclusion of GHI in all the patients, qualified to the therapy.

The most important problem in clinical practice is a discordance among the results of different studies, as regards the effects of short- and long-term rhGH administration on IGF-I and IGFBP-3 secretion (Schwarze *et al.* 1999; Buckway *et al.* 2001; Jørgensen *et al.* 2001; Kamp *et al.* 2002). In the present study, significant (and similar) increase of IGF-I secretion was observed in generation test and during rhGH therapy, speaking for the stability of the effect of rhGH administration on IGF-I secretion. This finding appears to be important, especially in the aspect of predicting the effectiveness of rhGH therapy in short children with uncertain diagnosis, especially in ones with IGFD and normal results of GHST who are not GH-insensitive.

The crucial issue in qualifying such patients to rhGH therapy – even more essential than an impact of this

treatment on IGF-I synthesis – seems to be its influence on growing rate. Thus, the studies on short-term tests, useful in prediction of the effectiveness of rhGH therapy, including IGF-I generation test – are particularly important. Unfortunately, the results of such studies led to divergent conclusions, from one side confirming the significance of generation test as a strong predictor of the growth response to rhGH therapy (Schwarze *et al.* 1999), on the other pointing at the lack of relationships between IGF-I secretion and the effects of rhGH therapy (Jørgensen *et al.* 2001). Recently, Spiliotis *et al.* (2009) have stated that IGF-I generation test might be an indicator of GH secretory status. Similar observations have been reported by Chatelain *et al.* (2010) who have proved that in GH-deficient children IGF-I increase in first month of rhGH administration correlates with growth response in 1st and 2nd year of treatment.

According to our results, a good IGF-I response to short-term rhGH administration seems to be an useful prognostic factor for long-term effectiveness of therapy, as the growth response proved to be good in all the children in whom the basal IGF-I level was low and, next, significantly increased during generation test. However, no correlation between IGF-I increase and HV has been found in our present study. The opinion that IGF-I response did not predict the growth response to rhGH therapy has also been stressed by Savage *et al.* (2010) who have not recommended that test, except for confirming severe GHI.

Recent reports of Rosenthal *et al.* (2007) and of Cohen *et al.* (2008) have suggested that focusing on the diagnosis of IGFD and searching out the patients who may benefit during rhGH therapy among all IGF-I deficient ones, may be more appropriate than distinguishing between GHD and ISS, based on the results of GHST.

In the recently published consensus on diagnosis and treatment of ISS, Cohen *et al.* (2008) have stated that the biochemical criteria for initiating rhGH therapy in children with ISS have not been established. With regard to IGF-I generation test, the above quoted authors have suggested that better normative data for that procedure should be drawn up. It seems that our present study may be a contribution to that issue.

In the aspect of predicting the effectiveness of rhGH therapy, the observations of changes in IGFBP-3 secretion during rhGH administration may be not less important than the assessment of IGF-I levels. Derandeler *et al.* (2005) proved that in the patients with subnormal results of GHST, an adequate IGFBP-3 response in generation test predicted the poor HV during rhGH therapy. In our present study, the increase of IGF-I secretion was much more pronounced than that of IGFBP-3, leading to doubling IGF-I/IGFBP-3 molar ratio. Our results are consistent with those presented by Tillmann *et al.* (2000) and Scirè *et al.* (2008). Moreover, an essential correlation between an increase of IGF-I/

IGFBP-3 molar ratio and improvement of HV has been found in our study.

The last observation that should be emphasized is the lack of significant differences in rhGH therapy effectiveness (including both IGF-I secretion and HV) not only among the subgroups of children with normal results of GHST but also between the examined group of 60 children and the patients with pGHD. Similar effectiveness of rhGH therapy in short children with either normal or subnormal results of GHST has previously been quite well documented (Hintz 2005; Kemp *et al.* 2005; Ranke *et al.* 2007). Thus, it may be impossible to clearly distinguish between good and poor responders to rhGH therapy, basing on the results of GHST. This observation confirms other data, suggesting the lack of evidence for diagnosing GHD and – consistently – qualifying children to rhGH therapy on the ground of the results of GHST only (Badaru & Wilson 2004).

Summing up, not only GH secretion but also GH sensitivity, as well as both IGF-I secretion and its bio-availability, should be taken into account, while qualifying (or disqualifying) children with short stature to rhGH therapy. At least twofold increase of previously low IGF-I concentration during generation test, leading to its normalisation, together with a similar increase of IGF-I/IGFBP-3 molar ratio, speak for the good effectiveness of rhGH therapy in short children, even despite normal results of GHST. Nevertheless, the limited value of GHST for exact prediction of the growth response in individual patients, should be considered. Further observations of such patients up to final height are needed to fully assess the significance of our finding.

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