J. Endocrinol. Invest. 31: 153-158, 2008

Growth hormone therapy does not alter the insulin-like growth factor-I/insulin-like growth factor binding protein-3 molar ratio in growth hormone-deficient children

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ABSTRACT. ***Background:*** **Recent studies have linked raised levels of IGF-I and/or reduced levels of its main binding protein, IGF binding protein (IGFBP)-3, with the risk of developing cancer. A GH dose-dependent increase in IGF-I/IGFBP-3 molar ratio has been reported in subjects treated with GH, raising concern about the long-term safety.** ***Objective:*** **The aim of this study was to evaluate changes in serum IGF-I, IGFBP-3, and IGF-I/IGFBP-3 molar ratio over the first 12 months of replacement GH therapy in GH deficient (GHD) children.** ***Methods:*** **The study included 20 GHD children who had not previously received GH treatment, and 40 untreated non-GHD short children closely matched for age, gender, pubertal stage, and body mass index (BMI), as controls. Serum IGF-I, IGFBP-3 levels were measured before and after 12 months of GH treatment. Based on the molecular weight of IGF-I (7500) and IGFBP-3 (40,000, mean of glycosylated variants), we calculated the molar ratio of IGF-I/IGFBP-3.** ***Results:*** **IGF-I/IGFBP-3 molar ratio significantly increased during GH therapy (p=0.01). No significant difference in IGF-I/IGFBP-3 ratio was found between GHD children and controls at the different time points. In the multiple regression analysis, BMI (β=0.33) and age (β=0.33) proved to be the major predictors of the IGF-I/IGFBP-3 molar ratio (adjusted r^2=0.53, p<0.0001).** ***Conclusions:*** **Our results suggest that at a conventional replacement dose GH does not alter the IGF-I/IGFBP-3 molar ratio. Potential fears related to long-term cancer risk are likely to be greatest in patients exposed to high-dose GH therapy and with genetic predisposition to high IGF-I and/or low IGFBP-3 concentrations.**

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INTRODUCTION

The role of GH in carcinogenesis is unclear. Results from *in vitro* and animal studies suggest that GH might increase the risk of hyperplasia and malignancy, raising serum concentrations and peripheral tissue production of IGF-I, which is mitogenic and anti-apoptotic (1, 2). The IGF system plays a pivotal role in the development and/or maintenance of cancer (3-5). The IGF, their binding proteins (IGFBP) and receptors are important regulators of cell growth. IGF-I has significant effects on cell proliferation and differentiation; it is a potent mitogen and powerful inhibitor of programmed cell death or apoptosis. Most of the physiological effects of IGF-I are mediated through the type I IGF receptor (IGF-IR), which is overexpressed in many different types of cancers (6). By contrast, the IGFBP, in particular IGFBP-3, can inhibit IGF mitogenic effects as well as promote apoptosis of cancer cells directly and independently of IGF-I (7). Large-scale epidemiological studies have demonstrated a link between high concentrations of IGF-I and many of the common cancers of adulthood, such as carcinomas of prostate, lung, breast, and colon (3, 4, 7). Overall, available data suggest that high levels of IGF-I and low levels of IGFBP-3 are predictive of an increased cancer risk. There is increasing evidence that IGFBP-3 acts as a modulator of IGF action on normal somatic growth but participates in the regulation of tumor cell survival as an IGF-independent entity. Indeed, IGFBP-3 has demonstrated an expanding significance in can-

Key-words: GH deficiency, GH therapy, IGF-I, IGFBP-3.

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Accepted April 23, 2007.

cer. Epidemiological studies have shown IGFBP-3 to be protective against prostate cancer and other malignancies (8-11). GH causes raised serum IGF-I and to a lesser extent IGF binding protein-3 (IGFBP-3), and consequently increases the ratio of IGF-I to IGFBP-3 (12-14), thus raising concern on safety of the long-term GH therapy.

The aim of this study was to evaluate changes in serum IGF-I, IGFBP-3, and IGF-I/IGFBP-3 molar ratio, during the first year of GH treatment in children with GH deficiency.

MATERIALS AND METHODS

Study population

Twenty patients with isolated GH deficiency not previously treated with GH (12 boys and 8 girls, mean age 8.6±3.4 yr) and 40 untreated non-GH deficient (GHD) short children (24 males and 16 females) were evaluated. All subjects were recruited from the Outpatient Growth Clinic of the Rina Balducci Center of Pediatric Endocrinology, Tor Vergata University, Rome, Italy. Anthropometric measurements and pubertal stage evaluations were performed according to standard procedures (15), and data were compared with the standards of Tanner and Whitehouse (16). Eighteen GHD children were pre-pubertal and 2 at pubertal stage 2. Patients' heights, growth rates, and bone ages were expressed as z-scores for chronological age and sex. Bone age was estimated by the method of Greulich and Pyle (17). Body mass index (BMI) was calculated with the formula: BMI = weight (kg) / height (m^2) (18).

Informed consent was obtained from the patients or their parents or guardians.

GH deficiency diagnosis was based on fulfilment of all the following anthropometric, endocrine, and radiological criteria: stature less than –2 z-score, delayed bone age (at least 1 yr), growth rate less than 25th centile, peak GH response to 2 different provocative tests less than 10 µg/l (less than 19 µg/l for GHRH+arginine test), and catch-up growth during the first year of GH replacement therapy (growth rate ≥75th centile). Eight GHD children showed brain magnetic resonance imaging (MRI) positive for hypothalamus-pituitary abnormalities such as pituitary hypoplasia, stalk agenesis, ectopic posterior lobe, or empty sella (19).

Children referred for anthropometry resembling that of GH deficiency but with normal peak GH responses were diagnosed as non-GHD short children. Each GHD patient was coupled with a non-GHD short child closely matched for age (within 1 yr), gender, pubertal stage, and BMI (within 1.5 kg/m^2). Twenty non-GHD short children formed the pretreatment and 20 the 12-month control group.

In all subjects, IgA-endomysial and tissue transglutaminase antibody testing and free T_4 and thyroid-stimulating hormone assessment were performed to exclude celiac disease and hypothyroidism, respectively. Karyotype was normal in all girls, and no patient had dysmorphic features, malnutrition (percentage of ideal body weight was more than 85% in all patients), or chronic diseases.

Clonidine (100 µg/m^2 body surface area, orally), arginine (0.5 g/kg body weight up to a maximum of 30 g iv), and GHRH plus arginine (GHRH, 1 µg /kg iv) stimulation tests were used to assess GH secretion. All subjects were tested in fasting conditions. Pre-pubertal boys older than 10 yr were primed with 100 mg depot testosterone 3 d before testing. Pre-pubertal girls older than 9 yr were primed with 50 µg/d ethinyl estradiol for 3 consecutive days before testing.

Recombinant human GH was given sc, once daily, at bedtime, in a dose of ~ 5 mg/m^2 body surface area weekly (approximately 30 µg/kg daily). The GH dose was adjusted 6-monthly to body surface area.

Height, weight, BMI, growth rate, serum IGF-I, IGFBP-3, and IGF-I/IGFBP-3 molar ratio were measured prior to and after 1 yr GH treatment in GHD patients.

Hormone assays

Serum GH was measured by immunoradiometric assay (IRMA) (Diagnostic Systems Laboratories, Inc., Webster, TX). The intraassay coefficient of variation (CV) was 3.1-4.4%, the interassay CV was 5.9-11.5%, and the sensitivity limit was 0.01 µg/l.

Blood samples were collected without anticoagulant between 08:00 and 09:00 h from fasting subjects, allowed to coagulate, and centrifuged at room temperature. Serum samples were subdivided in 2 aliquots and stored at –20 C. Samples thawed just before the assays that were performed within 1 month from blood collection. Serum IGF-I was measured by IRMA (Nichols Institute Diagnostics). IGF-I was measured after extraction obtained by acidification followed by the addition of excess IGF-II to block the IGFBP binding sites. The intraassay CV was 3.3-4.6%, the interassay CV was 9.3-15.8%, and the sensitivity limit was 6 µg/l (0.8 nmol/l). Serum IGFBP-3 was measured by IRMA (Diagnostic Systems Laboratories, Inc.). The intraassay CV was 1.8-3.9%, the interassay CV was 0.5-1.9%, and the sensitivity limit was 0.5 mg/l (17.5 nmol/l). Both IGF-I and IGFBP-3 assays were validated in our laboratory by assessing the blood concentrations in 82 normal children and comparing the results with the normative data provided by the manufacturer's kit (19). Both IGF-I and IGFBP-3 values were expressed in z-scores according to our reference standards (20).

It has been suggested that the ratio of IGF-I to its principle binding protein IGFBP-3 provides a measure of biologically active IGF-I (21). Based on the molecular weight of IGF-I (7500) and IGFBP-3 (40,000, mean of glycosylated variants) we calculated the molar ratio of IGF-I/IGFBP-3 by multiplying the ratio by 5.33 (40,000/7500).

Statistical analysis

Results are reported as mean±SD. Differences between means were assessed using unpaired two-tailed Student's t-test. Differences between GHD and non-GHD short children in the case-control study, and between pre- and 12-month treatment were evaluated by the paired t-test. Multiple regression and forward stepwise regression analyses were used in the selection of predictors of IGF-I/IGFBP-3 molar ratio. Significance was assigned for $p<0.05$. A computer program was used for all statistical calculations (Statistica, StatSoft Inc., Tulsa, OK).

RESULTS

Anthropometric and endocrine features of GHD prior to the initiation of GH therapy and non-GHD short children are summarized in Table 1. GHD patients were significantly shorter (–2.17±0.84 *vs*

Table 1 - *Anthropometric and endocrine features of GH deficient (GHD) prior to the initiation of GH therapy and untreated closely matched [for age, gender, pubertal stage, and body mass index (BMI)] non-GHD short children (control group).*

	GHD children (pre-treatment) (no.=20) Mean±SD	Non-GHD short children (no.=20) Mean±SD	*p*
Age (yr)	8.66±3.47	9.5±3.0	ns
Height (cm)	120.22±17.24	125.1±17.00	ns
Height z-score	–2.17±0.84	–1.43±0.8	<0.005
BMI	15.72±2.00	16.7±1.6	ns
Puberty	1.15±0.36	1.16±0.38	ns
IGF-I (ng/ml)	127.67±73.33	216.9±154	<0.003
IGF-I (z-score)	–1.61±0.62	–1.0±0.8	0.02
IGFBP-3(mg/l)	3.44±1.06	3.9±0.9	ns
IGFBP-3 (z-score)	–0.05±1.46	0.65±0.86	ns
IGF-I/IGFBP-3	0.19±0.99	0.26±0.12	ns

–1.43±0.8 z-score, p=0.007). Pre-treatment serum IGF-I was lower (p=0.02) in the GHD compared with the non-GHD group. No significant differences were observed in IGFBP-3 levels and IGF-I/IGFBP-3 molar ratio.

As expected, after 12 months of GH therapy, GHD patients showed a significant increase in height, height velocity, IGF-I and IGFBP-3 levels (Table 2). In all GHD patients on GH therapy, serum IGF-I values did not exceed the upper limit of normality range for age and gender. The IGF-I/IGFBP-3 molar ratio significantly increased during GH therapy (0.19±0.99 pre-therapy up to 0.32±0.20 post-therapy, p=0.01), with wide interindividual variations (Fig. 1).

The strictly matched (for age, gender, pubertal stage, and BMI) group of non-GHD short children, enabled us to compare data of GHD patients after 12-month GH therapy with an appropriate control

Table 2 - *Anthropometric and endocrine features of GH-deficient (GHD) patients prior to and after 12 months of GH therapy.*

	GHD children (pre-treatment) (no.=20) Mean±SD	GHD children (1 yr therapy) (no.=20) Mean±SD	*p*
Age (yr)	8.66±3.47	10.15±2.98	0.01
Height (cm)	120.22±17.24	128.93±16.63	<0.0001
Height (z-score)	–2.17±0.84	–1.73±0.86	<0.005
Height velocity (cm/yr)	4.17±1.18	8.1±1.9	<0.0001
Height velocity (z-score)	–1.8±1.5	2.2±2.5	<0.0001
BMI	15.72±2.00	16.42±1.69	ns
Pubertal stage	1.15±0.36	1.40±0.75	0.02
IGF-I (ng/ml)	127.67±73.33	315.35±215.33	<0.001
IGF-I (z-score)	–1.61±0.62	–0.72±1.08	<0.001
IGFBP-3 (mg/l)	3.44±1.06	4.93±1.26	<0.0001
IGFBP-3 (z-score)	–0.05±1.46	0.84±1.16	<0.005
IGF-I/IGFBP-3	0.19±0.99	0.32±0.20	0.01

BMI: body mass index; IGFBP-3: IGF binding protein 3.

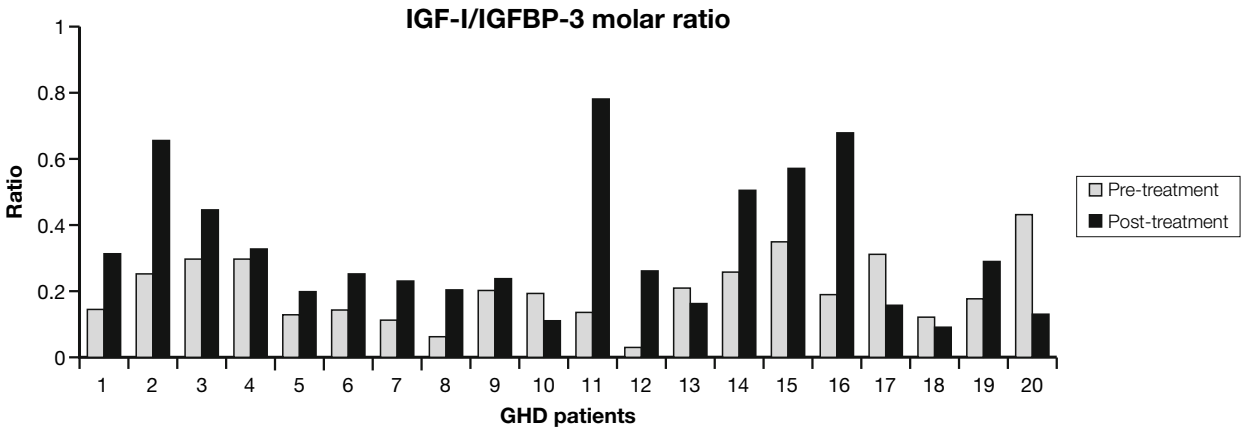

Fig. 1 - *Interindividual variability of IGF-I/IGF binding protein (IGFBP)-3 molar ratio in GH-deficient (GHD) children prior to and after 12 months of GH therapy.*

group. The comparison did not show any significant difference in IGF-I/IGFBP-3 molar ratio between GHD and control children at baseline and 12 months of GH treatment (Table 3).

However, when IGF-I levels were assessed relative to IGFBP-3 levels, controlling for age and gender, GHD subjects consistently had higher, though within the normal range, levels of IGF-I than control subjects at each level of IGFBP-3 (Fig. 2).

Stepwise regression analysis revealed that the major predictors of IGF-I/IGFBP-3 molar ratio in GHD patients were age (β=0.34) and BMI (β=0.33), with an overall adjusted r^2=0.53 (p<0.0001).

DISCUSSION

Previous reports from studies in adults or young adults showed that growth hormone raises serum IGF-I and to a lesser extent IGFBP-3, and consequently causes a raised ratio of IGF-I to IGFBP-3, with this ratio being greater as GH concentrations increase (12-14). More recently, an observational prospective longitudinal study with serial measurements during the first year of GH treatment in GHD and non-GHD short children (including patients with Turner and Noonan syndrome) has shown an increase in IGF-I/IGFBP-3 molar ratio with increasing dose of GH (22). The increase in IGF-I/IGFBP-3 mo-

Table 3 - *Anthropometric and endocrine features of GH-deficient (GHD) after 12 months GH therapy and untreated closely matched [for age, gender, pubertal stage and body mass index (BMI)] non-GHD short children.*

	GHD patients (1 yr therapy) (no.=20) Mean±SD	Non-GHD short children (no.=20) Mean±SD	*p*
Age (yr)	10.15±2.98	10.4±2.7	ns
Height (cm)	128.93±16.63	128.7±14.5	ns
Height (z-score)	–1.73±0.86	–1.5±0.8	ns
BMI	16.42±1.69	16.3±1.6	ns
Pubertal stage	1.40±0.75	1.3±0.6	ns
IGF-I (ng/ml)	315.35±215.33	216.4±140.1	ns
IGF-I (z-score)	–0.72±1.08	–1.2±0.8	ns
IGFBP-3 (mg/l)	4.93±1.26	4.03±0.9	0.02
IGFBP-3 (z-score)	0.84±1.16	0.5±1.2	ns
IGF-I/IGFBP-3	0.32±0.20	0.27±0.11	ns

BMI: body mass index; IGFBP-3: IGF binding protein 3.

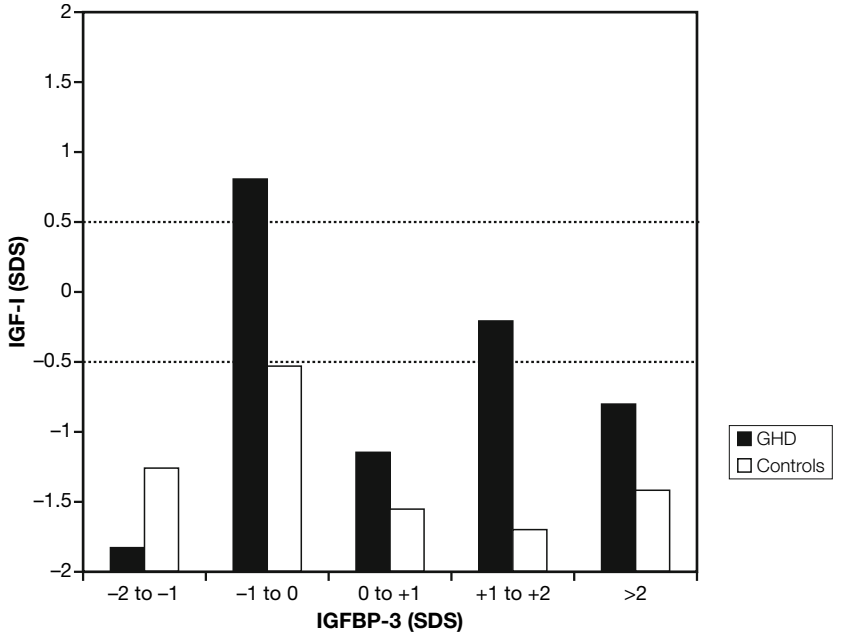

Fig. 2 - *Serum levels of IGF-I vs IGF-binding protein (IGFBP)-3 expressed in SD score (SDS). Horizontal lines represent –0.5 and +0.5 SDS.*

lar ratio with increasing dose of GH may reflect an increase in bioavailable IGF-I and, therefore, might be associated to a higher risk of cancer.

Our results confirm the significant increase in IGF-I/IGFBP-3 molar ratio in GHD patients on treatment with GH. However, the strictly matched control groups at baseline and at 12- month GH therapy enabled us to rebut evidence suggesting a promoting effect of GH therapy on overall IGF-I bioavailability.

GHD children at 12-month GH therapy, as a group, showed higher concentrations of IGFBP-3 than the control group, although with wide interindividual variations. Intrinsic factors may affect the reproducibility of IGF-I and IGFBP-3 assessment. The biological variability in IGF-I measurements is up to 32% in the same subject tested on different days (23). Furthermore, genetic determinants account for approximately 40% of the variability in serum IGF-I and 60% of the variability in serum IGFBP-3 concentrations (24). Direct sequencing of genomic DNA specimens from a multiethnic population has identified several single nucleotide polymorphisms in the promoter region of IGFBP-3. For the most common single nucleotide polymorphism, genotype was highly correlated to circulating levels of IGFBP-3 (25). Although classically serum IGFBP-3 levels are known to be closely GH dependent (26), other factors such as age, pubertal development, nutrition, and hepatic function influence the circulating IGFBP-3 levels (27). In addition, we have recently shown that GHD patients have increased IGFBP-3 proteolytic activity yielding additional low-molecular mass fragments that affect the results of IGFBP-3 measurements (20). Most of the available commercial kits, in fact, employ anti-IGFBP-3 antibodies that recognize all the IGFBP-3 circulating forms, including IGFBP-3 fragments (28-31).

The results of the present study suggest age and body mass index to be the major predictors of IGF-I/IGFBP-3 molar ratio. This finding is consistent with a recent report showing IGF-I/IGFBP-3 molar ratio to be closely related to age, BMI, and pubertal stage (32). In that study, the relationship with age was positive until mid-early puberty, then turned out to be negative in late puberty (32). As our study population was at pubertal stage 1 or 2, this accounts for the positive linear relationship observed in our subjects.

In conclusion, our results suggest that at least at a conventional replacement dose of GH, no abnormal elevation of IGF-I/IGFBP-3 molar ratio is observed. Potential fears related to long-term cancer risk are likely to be greatest in non-GHD patients exposed to high dose GH therapy and with genetic background leading to high IGF-I and low IGFBP-3 concentrations.

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