The Relationship of Growth Rate, Plasma Growth Hormone (GH) Concentration, and GH-Binding Protein

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Growth hormone (GH)-binding protein (GHBP) and GH secretion are potential mediators of linear growth in children. To study the relationship between these variables, we measured GHBP activity, peak stimulated GH (PKGH), and 24-hour integrated GH concentration (ICGH) in 76 children referred for evaluation of growth. Linear growth was expressed as an age- and sex-specific growth rate standard deviation score (GRSD), which was calculated from sequential height measurements in the 6-month period immediately before GH testing. Using multiple regression models, we found that the relationship between GHBP and growth (GRSD) depended on height (height standard deviation [HGTSD] expressed as an age- and sex-specific *z* score) controlling for ICGH or PKGH. In further analysis of this relationship, we divided the subjects by HGTSD in subsequent analyses. In 19 children of normal stature (HGTSD > −2), GRSD increased with GH concentration (measured both as PKGH and ICGH: $P < .013$, $R^2 = .56$) but decreased with higher levels of GHBP ($P < .005$, $R^2 = .62$). In contrast, for 57 subjects with severe short stature (HGTSD ≤ −2), GRSD could not be predicted from GHBP, GH secretion, HGTSD, or interactions involving these variables. These data suggest the hypothesis that under normal conditions, GHBP and GH level may be important predictors of growth rate in children.

HUMAN GROWTH HORMONE (GH) has been found to circulate bound in part to a plasma protein that has been termed GH-binding protein (GHBP).[1] GHBP is identical in structure to the extracellular domain of the tissue GH receptor.[2-4] Plasma GHBP activity may thus reflect cellular GH receptor density and function.[4] For example, patients with Laron-type dwarfism have adequate levels of plasma GH, but a complete absence or deficiency of plasma GHBP activity corresponding to a decreased number of cellular GH receptors.[5]

Several clinical studies have evaluated factors that influence plasma GHBP activity. GHBP increases with age[6,7] and body mass index (BMI).[8] Circulating GH levels are inversely related to plasma GHBP activity[9]: GH-deficient patients have increased levels of GHBP, and acromegalics have reduced levels of GHBP. In response to GH therapy, some GH-deficient children may have an increase in GHBP activity.[10-12] GHBP activity is altered by diabetes[13,14] and renal and thyroid disorders.[15] Thus, multiple factors may influence plasma GHBP activity.

The impact of GHBP on linear growth is not clear. The fraction of circulating GH bound to GHBP may have a diminished access to tissue GH receptors.[16] And conditions that increase plasma GHBP may reduce the amount of unbound GH available to interact with tissue receptors. Alternately, conditions leading to increases of GH relative to GHBP would increase the amount of free GH able to stimulate cell-surface receptors. Possible clinical data supporting this hypothesis were recently reported in a cohort of normal-height boys, whose pubertal growth spurt was associated with the time of the highest ratio of GH relative to GHBP.[17]

However, there is other evidence suggesting that GHBP may act to prolong the presence of GH in the circulation.[18] The decreased clearance of GH may enhance the growth-promoting effect.[19]

A limited number of clinical studies have been conducted to evaluate changes in GHBP during childhood, but no pattern has emerged regarding the relationship of GH and GHBP with regard to linear growth in healthy children of normal stature and patients with growth failure. The purpose of this study was to examine the relationship of GHBP and GH with regard to growth velocity in a large cross-sectional sample of children who had undergone sophisticated quantitative assessment of GH secretion. From these data, we were able to derive statistical models that predicted growth rate based on level of GHBP activity, GH concentration, and height z-score.

SUBJECTS AND METHODS

Patient Selection

GHBP level was measured in patients undergoing endocrine diagnostic evaluation. These subjects had been referred for evaluation to the Pediatric Endocrine Clinic at the University of Maryland. Before endocrine diagnostic studies, all subjects had an intake history and underwent physical examination. Laboratory studies (complete blood cell count, sedimentation rate, electrolytes, liver function, thyroxine, thyrotropin, calcium, phosphorus, and urinalysis) were also performed. A karyotype was performed in all female subjects. Subjects were not included in the analysis if they were hypothyroid or had clinical or laboratory evidence of nonendocrine systemic illness or syndromes causing growth failure. Growth rates were calculated from sequential height data measured by stadiometer for periods of observation between 4 and 8 months immediately before GH diagnostic studies. Pubertal status for each subject was recorded during physical examination at the time of GH testing. BMI was calculated at the time of testing by dividing body weight (in kilograms) by height (in meters) squared. For comparison of growth velocity and height in different age and sex groups, both growth rate and height were converted to a standard deviation *z* score (GRSD and HGTSD, respectively).

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Evaluation of GH Secretion

Testing was performed at the Endocrine Diagnostic Unit of the University of Maryland Hospital. GH secretory status was assessed by both pharmacological stimulation tests and 24-hour integrated concentration of GH (ICGH). The peak response of GH (PKGH) to stimulation was evaluated by sequential administration of insulin intravenously (0.075 U/kg [IV]), followed after 1 hour by 10% arginine infusion (5 mL/kg IV infused over 30 minutes).[20] Patients were not pretreated with gonadal steroids before testing of GH secretion.

The 24-hour ICGH profile was performed using a nonthrombogenic constant blood-withdrawal system,[21,22] and blood collection tubes were replaced every 30 minutes. Children were able to continue daily activities by carrying the portable pump in a small basket. Plasma samples were separated and stored at −20°C until assayed.

Signed informed consent for all diagnostic procedures was obtained from the parents and, when possible, the patient, in accordance with institutional policy at the University of Maryland.

Assays

GHBP in the pooled 24-hour sample was determined by a binding assay with dextran-coated charcoal separation, as previously described, and validated in detail against other methods.[23] In short, [^{125}I]hGH (1 ng per tube) was incubated with 0.05 mL human serum in the absence or presence of excess unlabeled hGH (1 μg) in a final volume of 0.27 mL at 4°C for 20 hours. Specific binding of [^{125}I]hGH was expressed as a percentage of the total counts per minute. With serum hGH levels of 7 μg/L or greater, specific binding to GHBP was corrected for displacement of the endogenous ligand, as previously described.[23] The assay does not allow discrimination between high- and low-affinity components of GHBP. However, low-affinity GHBP activity is estimated to account for only 3% of total GH-binding activity.[24] The assay sensitivity was 1% in 0.02 mL serum, and intraassay and interassay coefficients of variation were 5.8% and 7.1%, respectively. Normal-adult control levels by this methodology are 11.1% ± 5.6% (n = 23).[8]

Plasma GH levels were assayed by a double-antibody radioimmunoassay method[25] with a sensitivity of 0.78 ng/mL.

Statistical Analysis

HGTSD and GRSD scores were calculated for the subjects based on normative data previously reported.[26] The relationship between GHBP and several other variables was initially examined by simple univariate correlation analysis. The influences of age, pubertal status, sex, BMI, and GH secretory status on GHBP activity were evaluated by multiple regression techniques.[27-31] In these models, pubertal status was evaluated as a binomial variable, ie, either prepubertal or pubertal.

The relationship of GHBP, GH level (ICGH or peak GH), and HGTSD to GRSD was evaluated by least-squares multiple regression analysis. Each regression model was checked for fit and adherence to the assumptions of equality of variance and normality of residuals.[27-29] As part of the evaluation of each model's fit to the data, Cook's D statistic was used to search for data point outliers having undue influence on the regression model; such outliers were omitted from further evaluation.[29] Variance inflation statistics were used to detect multicolinearity.[27-29] Results are reported as the mean ± SD; statistical significance was indicated by P less than .05. The data set is available upon written request.

RESULTS

GHBP, GH Level, and Growth

Clinical data from the subjects are presented in Table 1. GH concentrations ranged from deficient to normal. Initially, GHBP, ICGH, and HGTSD data from all 76 subjects were analyzed in a multiple regression model as statistical predictors for GRSD. Diagnostic analysis in this model rejected two subjects with extreme values of GRSD having undue influence on the regression. When data from these two subjects were subsequently omitted, it was possible to construct a model that fit the data and fulfilled all underlying statistical assumptions necessary for multiple regression. This model is based on the 74 remaining subjects. GHBP, HGTSD, and the interaction of HGTSD with GHBP was predictive of GRSD ($P < .015$, $R^2 = .16$). Regression coefficients and significance levels for independent variables are presented in Table 2. In general, GRSD was inversely related to GHBP, and growth rate was faster in taller children. The significant interaction between HGTSD and GHBP indicated that the relationship between GHBP and GRSD changed with height.

Because of the interactive effect of GHBP and HGTSD, we further evaluated the effect of measured variables on growth rate in subgroups of subjects divided by height criteria. Of several possible cutoff points for dichotomizing the subject sample by height, we chose to further evaluate two examples. A HGTSD of −2, a cutoff point widely used in clinical practice, and a HGTSD of −2.34, where the slope of GRSD on GHBP in the overall regression model becomes zero, were used as cutoff points for subgroup analyses. Results of subgroup analyses at these cutoff points were similar. Therefore, only details from the groupings above and below a HGTSD of −2 are presented in detail. Clinical and laboratory data from the subject groups are presented in Table 3. In 19 subjects of normal stature, GRSD was correlated with both growth hormone secretion and GHBP. Figure 1A shows the linear relationship between GRSD and GHBP levels ($r = -.62$, $P < .005$). ICGH levels were also correlated with GRSD ($r = .56$, $P < .013$). The predictive value of both GHBP and GH secretion for GRSD was statistically evaluated in a multiple regression statistical model. In this model, GRSD was significantly related to both GHBP and GH secretion. The regression equation developed from this model using ICGH levels as the measure of GH secretion was GRSD = .36 (ICGH) − .47 (GHBP) + 2.1 ($P < .0007$, $R^2 = .60$ overall). The parameter estimates for ICGH ($P < .01$) and GHBP ($P < .004$) were both significant (units for the equation variables are GRSD in standard deviations, ICGH in micrograms per liter, and GHBP in percent). This statisti-

Table 1. Clinical and Laboratory Characteristics of Study Subjects (n = 74) Included in the Regression Model (mean ± 1 SD)

Age (yr)	Bone Age (yr)	ICGH (μg/L)	PKGH (μg/L)	HGTSD	GHBP (%)	BMI (kg/m^2)
11.6 ± 3.0	9.1 ± 2.9	3.1 ± 1.9	11.7 ± 7.9	−2.3 ± 0.6	8.1 ± 3.1	16.8 ± 2.5

Table 2. Multiple Regression Model (n = 74) for GRSD as the Dependent Variable With Independent Variables GHBP, ICGH, and HGTSD

Term	Coefficient	Standard Error	t	P
ICGH	.1795	.1268	1.415	.161
GHBP	−1.1784	.4340	−2.716	.008
HGTSD	4.5686	1.4144	3.230	.002
GHBP × HGTSD	−.5134	.1760	−2.917	.005
Constant	8.8249	3.5469	2.488	.015

NOTE. $R^2 = .16, P < .015$.

cal model is graphically represented in Fig 1B. Each line on the graph is derived from the above model by varying GHBP at different levels of ICGH. For any given level of ICGH, GRSD was inversely proportional to GHBP. GRSD also increases with increasing levels of ICGH.

A similar model was also derived using the PKGH as the measure of GH secretion: GRSD = .10 (PKGH) − .45 (GHBP) + 1.95 ($P < .001$, $R^2 = .58$ overall). The parameter estimates for PKGH ($P < .014$) and GHBP ($P < .008$) were both significant. There was no statistical interaction between GHBP and GH secretion on GHBP (units for the equation variables are GRSD in standard deviations, PKGH in micrograms per liter, and GHBP in percent). In a manner similar to the first model using ICGH, higher levels of PKGH were associated with higher GRSD; for any given level of PKGH, the growth rate was inversely related to GHBP activity. HGTSD was not a significant predictor in these models.

In contrast, in the model including 55 subjects whose HGTSD was not greater than −2 (two outliers omitted), GHBP and GH concentration were not predictive of GRSD (P = NS, $R^2 = .1$). Figure 2 illustrates the relationship of GRSD and GHBP for these subjects. The dispersion of GRSD and GHBP was much greater in patients with short stature than in the normal-stature group.

Influences on GHBP Activity

Among 76 subjects, GHBP activity was significantly correlated with age ($r = .25$, $P < .02$), BMI ($r = .56$, $P < .0001$), ICGH ($r = -.25$, $P < .02$), and PKGH ($r = -.31, P < .003$). A multiple regression analysis of the relationship of age, sex, BMI, pubertal status, and GH secretory status to GHBP activity was evaluated. Multiple regression models containing either ICGH ($P < .0001$, $R^2 = .45$) or PKGH ($P < .0001, R^2 = .43$) were both predictive of GHBP activity. Sex, BMI, and GH secretion were the only component variables that showed a statistically significant relationship with GHBP in this model when adjusted for the presence of the other variables. Males had lower GHBP levels (7.5% ± 2.6% *v* 9.3% ± 3.4% for females, $P < .003$ in the adjusted model). Pubertal status and HGTSD did not influence GHBP level.

DISCUSSION

A considerable amount of attention has been devoted to clinical conditions that may influence the circulating level of GHBP.[5-15,32] In contrast, clinical appraisal of the impact of GHBP on linear growth in children and its relationship to circulating GH levels has been limited. Difficulties arise in obtaining longitudinal measurements of growth and comprehensive evaluation of GH secretion in healthy normal-stature children. Merimee et al[7] studied GHBP and GH levels in a sample from 2,416 healthy children 3 to 16 years of age. The height and weight of participating subjects were not recorded. These investigators reported a correlation of mean GHBP levels for age in their subjects, with the 50th percentile of height for age derived from the literature.[7] In this population survey of a large number of healthy children, comprehensive GH testing was not performed, and GH secretion in the subjects was estimated from a single unstimulated specimen used to assay GHBP.

Martha et al[17] conducted a longitudinal study of GHBP levels and mean 24-hour GH concentrations in a cohort of 11 healthy boys (with height and weight within the normal range for age) every 4 months over a 4- to 5-year period. The level of GHBP was relatively stable over time in any one individual. In the sample cohort, the highest molar ratio of GH to GHBP was observed during the midpubertal years versus the years before or after. Since midpuberty corresponds to the time of the adolescent growth spurt, these investigators suggested that the increase in GH relative to GHBP during this critical period of growth may generate alterations in the amount of free versus bound GH. The association of GH and GHBP levels with temporal changes in linear growth velocity measured directly from the study subjects was not reported.

In the current study, we had the opportunity to evaluate the relationship of GHBP and comprehensive measures of GH concentration to 6-month growth velocity data in 76 children. Subjects ranged in height from normal to very short. We found that growth rate was related to GHBP and HGTSD, as well as to an interaction of HGTSD and GHBP. The interaction indicated that GHBP was more strongly associated with growth in taller children. Therefore, we further analyzed the data by subdividing subjects based on height.

We initially grouped subject data by the clinical convention of HGTSD above and below −2. In children who had heights in the normal range, we found that the combination

Table 3. Clinical and Laboratory Characteristics of Study Subjects Grouped According to Height *z* Score (mean ± 1 SD)

No. of Subjects	Age (yr)	Bone Age (yr)	ICGH (μg/L)	PKGH (μg/L)	HGTSD	GHBP (%)	BMI (kg/m²)
Normal-stature children (height > −2 SD)							
19	12.8 ± 2.3	10.6 ± 2.3	3.2 ± 2.2	10.7 ± 7.8	−1.6 ± 0.2	8.0 ± 1.9	17.1 ± 3.1
Short children (height ≤ −2 SD)							
55	11.3 ± 3.1	8.7 ± 2.9	3.1 ± 1.8	12.5 ± 8.3	−2.6 ± 0.5	8.0 ± 3.4	16.7 ± 2.2

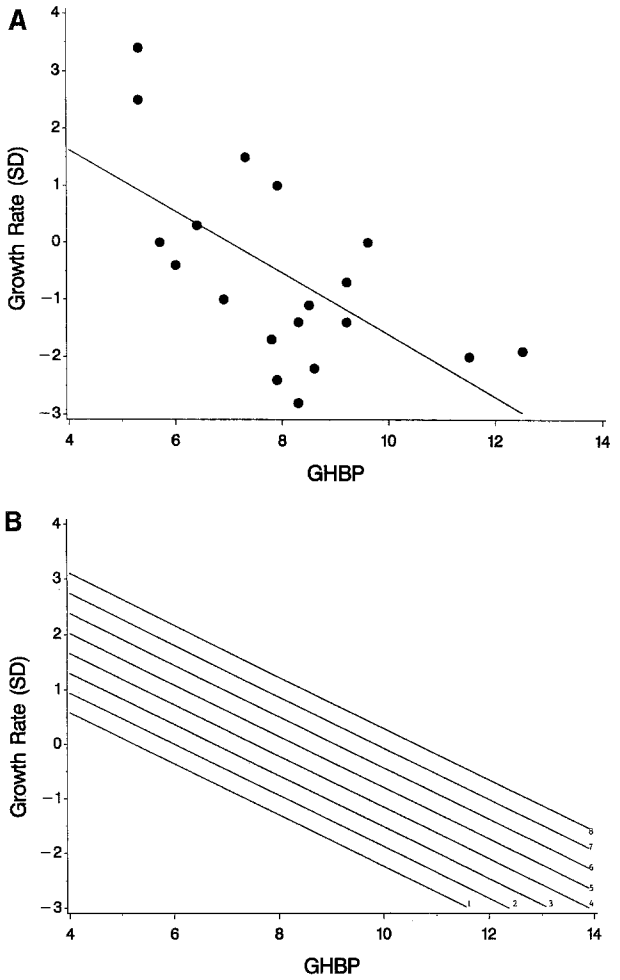

Fig 1. Influence of GHBP activity (%) and ICGH on GRSD in 19 children with height *z* score > −2. (A) Relationship between GRSD *v* GHBP for each subject (regression line for GRSD *v* GHBP: $r = -.62$, $P < .005$, GRSD = −.54 × GHBP + 3.8, n = 19). (B) GRSD is also significantly correlated with ICGH ($r = .56$, $P < .013$, n = 19). A multiple regression model was used to assess the influence of GHBP and ICGH together on GRSD. The regression equation generated for this model is GRSD = .36 × ICGH − .47 × GHBP + 2.1 ($P < .0007$, $r^2 = .60$, n = 19). To illustrate this relationship, regression lines for ICGHs of 1, 2, 3, 4, 5, 6, 7, and 8 μg/L were calculated from the regression model and drawn within the axes from the plot in A (ICGH regression lines start with 1 μg/L at lower left and progress to 8 μg/L at upper right). GRSD is increased for increasing levels of ICGH. For any given level of ICGH, GRSD is inversely related to GHBP.

of GHBP and GH concentration status were predictive of GRSD. In this statistical model, GHBP and GH concentration accounted for 60% of the variability in GRSD in this group of children. GRSD was increased at higher levels of GH secretion, and for any given GH level, GRSD was inversely related to GHBP activity. The model derived from these data suggests that GHBP and GH concentration may both have a role in mediating growth rate among children whose stature is in the normal range. Another grouping of the data based on the cutoff point of −2.34 HGTSD (from the overall regression model where the influence of GHBP on GRSD was minimal) yielded similar results.

Experiments performed in vitro suggest that GHBP may play a role in mediating GH activity. Lim et al[33] demonstrated that recombinant GHBP could inhibit GH binding of GH to GH receptors on IM-9 lymphocytes. In further studies by Mannor et al,[16] the high-affinity GHBP was shown to interfere with GH binding to tissue receptors for GH at physiologically relevant GHBP concentrations. Our present data and the data of Martha et al[17] cited above are consistent with the hypothesis that the concentration of circulating GH relative to the GHBP level plays an important role in mediating growth rate in normal-stature children. It is possible that GHBP decreases the amount of free GH able to bind with GH tissue receptors in vivo, with a consequent impact on linear growth. The data suggest that GHBP and GH level may both be important determinants of growth for normal healthy children.

When short children were analyzed as a separate subgroup, GHBP and GH concentrations were not found to have predictive value for growth rate. This finding was suggested by the initial data analysis from all subjects. In short children, there may be several factors disturbing the relationship of GHBP and GH with growth rate, accounting for the observed difference from taller children. In addition, such factors may not be readily identifiable by current clinical methods. Candidates may be the secretion of bioinactive GH variants,[34] GHBP and GH surface receptors with altered binding characteristics, altered postreceptor response to GH, and inability to generate and respond to intermediary growth factors, among others. Occult illnesses such as celiac disease may be more prevalent among children of short stature and may elude detection on basic physical examination and laboratory screenings.[35] Such underlying processes may not only influence growth rate, but levels of GHBP and GH as well. It has previously been suggested that children with idiopathic short stature and reduced levels of GHBP may have GH insensitivity at the GH receptor level.[36,37] A report of two subjects with short

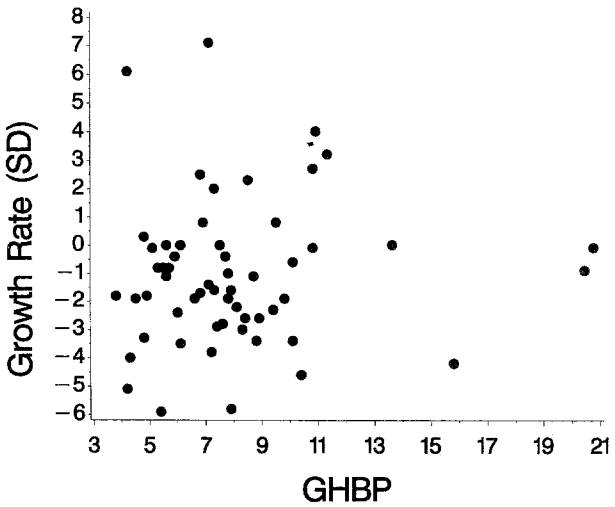

Fig 2. GRSD, GHBP activity (%), and ICGH secretory status in 57 children with short stature (height SD ≤ −2). In the statistical analysis, regression diagnostics excluded two subjects with GRSD > 5. There was no relationship between GRSD and GHBP or GH concentration (P = NS).

stature and very high GHBP and GH levels suggested another form of GH insensitivity.[38] Thus, in subjects with severe short stature, multiple pathologic factors may potentially obscure the relationship of GHBP and GH level with growth rate observed in children of normal stature.

Limitations in interpretation of the data should be pointed out. The results are based on two observations of height (to determine growth rate) and one measurement of GHBP and GH concentration. Growth rate and GH concentration vary over time.[17,21,26] Thus, we did not have the opportunity with the current data set to evaluate the consistency of relationships among study variables at multiple points in time. A lesser problem is the fact that these data are derived from a referred patient group and thus the sample subgroupings are not well-defined cohorts. Nevertheless, the study subjects represent a sample containing a wide range of HGTSD, growth rates, and GH concentrations. And the observed relationship of GHBP and GH with growth rate provides a basis for generating hypotheses concerning the physiology of linear growth in childhood.

Several previous investigations have reported univariate effects of age,[6,8] BMI,[8,9] and GH status[9,12] on GHBP in short- and normal-stature children. Using multiple regression analysis, we found that GH levels, BMI, and gender are strongly associated with GHBP activity when adjusted for the influence of age and pubertal status.

GHBP activity was higher in girls than in boys. The influence of sex on GHBP activity was independent of HGTSD, age, and GH secretion. In adults, it has been previously reported that GHBP activity is higher in women than in men.[39] In a recent study of 788 short-stature children, GHBP was also reported to be higher in girls than in boys.[36] Thus, our findings support these earlier studies in children with short stature. The mechanism responsible for sexual dimorphism of GHBP in short children is not clear. Testosterone levels may alter GHBP activity,[11] but the sex difference in GHBP levels remains when results are controlled for pubertal status. The lack of an effect of puberty on GHBP has previously been reported by several groups.[6,7,9,40] Other studies have not reported differences in GHBP between men and women[23,41] or normal-stature children.[7]

In conclusion, results of this cross-sectional study suggest several hypotheses concerning the relationship of GHBP and GH concentration with growth rate in childhood, as well as alterations that may occur with abnormal growth. Conclusive testing of these hypotheses will require a longitudinal clinical study of broader scope. Notwithstanding the difficulties in subject recruitment, such a study should include sufficient numbers of children who meet preestablished eligibility criteria and have a height range characterized as normal, borderline short, or severly short. The protocol should undertake frequent serial monitoring of pubertal status, height, weight, GHBP, and GH in the cohort of children over a period of several years.

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