Clinical Significance of Growth Hormone-Binding Protein Measurements in Children (43768)

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Abstract. **To investigate the role of growth hormone (GH) and its downstream axis in normal growth and growth disorders, we measured serum GH-binding protein (GHBP) levels in children by ligand-mediated immunofunctional assay (LIFA). Samples were taken from 512 healthy children of normal stature, 146 healthy neonates, 153 short but otherwise normal children, and 27 patients with GH deficiency. Serum GHBP showed no significant diurnal variation. Serum GHBP levels were low in neonates, acutely rose within 6 months, and gradually increased toward midpuberty in normal children (r = 0.2, $P < 0.01$, assessed among subjects over 1 year old). Neither significant sexual dimorphism nor rapid pubertal changes were observed. In normal short children and patients with GH deficiency, GHBP was lower than normal, but not significant. These data suggest that one of the etiologies for growth disorder in children could be resistance to, or low efficacy for, utilization of GH.** [P.S.E.B.M. 1994, Vol 206]

A growth hormone-binding protein (GHBP) is identical to the extracellular binding domain of the membrane growth hormone receptor (GHR) and circulates in plasma (1). In humans, GHBP is thought to be derived from membrane receptors by proteolytic cleavage (2); thus, the measurement of GHBP may provide information on GHRs in each individual. The role of GHBP, however, is not well known. Recent data show both inhibitory and stimulatory regulating effects of GHBP in GH action (3, 4). These data suggest that the measurement of GHBP, which offers the evaluation of GH and its "downstream" axis, may be necessary for investigating the pathophysiology of growth disorders in children. In this study, we measured serum GHBP levels using ligand-mediated immunofunctional assay (LIFA) (5) in short but otherwise normal children and children with GH deficiency, and compared them with normal controls.

Subjects and Methods

Subjects. Serum samples were obtained from 514 normal controls (male/female: 264/250, ages 0–16 years old), 153 normal short children (103/50; 3–16 years old), 27 patients with GH deficiency (17/10, 2–22 years old), and 146 5-day-old normal neonates. Normal controls were defined as children with stature within ±2 standard deviation (SD) of the mean of the same age group in Japan, and whose percent overweight was within ±20%. Normal short children were subjects with normal GH secretion but with stature under −2 SD of the mean. To determine diurnal variation of GHBP, serum samples were obtained every 20 min during 4-hr nocturnal sleep from seven normal short children. We also assessed the short-term effect of GH administration on serum GHBP levels in eight GH deficient children. Recombinant human GH (rhGH), 0.1 U/kg, was administered subcutaneously daily for 10 days and plasma insulin-like growth factor-I (IGF-I) and GHBP levels were measured.

Methods. Growth hormone-binding protein levels in serum were measured in duplicate using LIFA, as previously described by Carlsson *et al.* (5), with a

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modification. Briefly, the 96-well immunoplates were coated with a monoclonal antibody to GHR (Mab263; Agen, Australia). After overnight incubation at 4°C, and washing, nonspecific binding sites on the plate were blocked. Standards or samples were dispensed into the coated wells with rhGH at a concentration of 200 ng/ml in assay buffer (PBS containing 3% BSA and 5 m*M* EDTA). Recombinant human GHBP (rhGHBP) (Novo Nordisk A/S, Denmark), with an amino acid sequence totally identical to the extracellular domain of GHR, was used as standard. Plates were incubated overnight at 4°C. After washing, a biotinylated mouse monoclonal antibody against GH (Sumitomo Pharmaceuticals, Japan) was added. After 30-min incubation at room temperature, and 10 washes, horseradish peroxidase avidin D (Vector Laboratories, CA, USA) was added to each well; plates were further incubated for 30 min at room temperature, and washed again before adding a freshly prepared substrate solution with Azino-bis(3-ethylbenztiazoline-t-sulfonic acid) (Serva, Germany). After 15-min incubation at 37°C, absorbance at 405 nm was determined.

Serum GH and IGF-I (after acid-ethanol extraction) were measured by a commercially available RIA kit (Eiken I. C. L. and Chiba Corning, Japan, respectively).

Statistical analyses were performed with the Student's *t* test and Wilcoxon-Mann-Whitney test for difference of means and linear regression analysis for correlation.

Results

Assay Range, Specificity, and Accuracy. The LIFA standard curve was obtained by serial dilution of rhGHBP. The dilution curve for purified GHBP from human plasma was almost identical to that of rhGHBP. Serial dilution of human serum samples was parallel to the standard curve. The detection range for LIFA was 0.8–200 ng/ml. The intra- and interassay coefficients of variation were 6.9% and 9.7%, respectively. The mean recovery rate of five different concentrations of rhGHBP was 100.9% ± 10.4% (mean ± SD).

Diurnal Variation of GHBP. GHBP varied among different individuals; however, the changes in each individual were much smaller. All subjects showed pulsatile GH secretion during 4-hr nocturnal sleep.

Short-Term Effect of GH on GHBP. The plasma IGF-I was significantly elevated with GH administration in all subjects, but the GHBP levels were almost fixed and showed no significant changes. Thus, short-term changes for IGF-I stimulated by GH were not accompanied with changes in GHBP.

Serum GHBP Levels in Healthy Control Children with Normal Stature. The age-related changes of serum GHBP levels in healthy control children of stature within ±2 SD are shown in Fig. 1. The GHBP levels are low before 1 year of age. Five-day-old newborns showed quite low levels of GHBP, below 1 ng/ml. GHBP levels acutely elevated during the first 3 to 6 months, and gradually rose toward midpuberty. There was a weak but significant correlation between serum GHBP levels and chronological age (Fig. 1). Although significant sexual dimorphism was not observed, GHBP in females tended to be higher than that of males. Acute pubertal changes could not be observed. GHBP levels varied widely among individuals in each age group. When subjects were divided into age groups by 2-year intervals and compared with each other, the serum GHBP in subjects between 1 and 3 years of age were found to be significantly lower than that in subjects over 7 years old ($P < 0.05$). Children with a high percent of overweight had significantly high serum GHBP levels ($n = 178$, $r = 0.71$, $P < 0.01$).

Serum GHBP Levels in Normal Short Children. The GHBP levels in 127 short but otherwise normal children were plotted against chronological age (Fig. 2). In almost all of the subjects, serum GHBP levels were distributed within the normal range, but the distribution tended to be on the lower end of normal. In a small number of children, however, serum GHBP levels were definitely lower than that of normal subjects. Among children who showed low GHBP levels, neither extreme short stature nor remarkably high peak GH to provocation tests was found. However, endogenous GH secretion during 3-hr nocturnal sleep tended to be high, and IGF-I was lower than in normal subjects.

Serum GHBP in GH-Deficient Children. In 27 children with GH deficiency, the distribution of serum

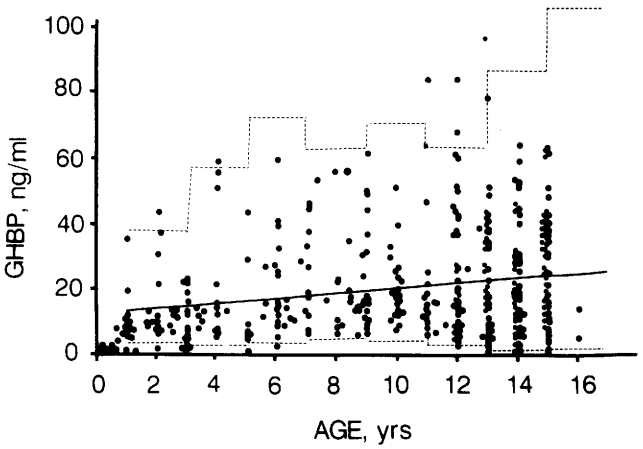

Figure 1. The age-related changes in serum GHBP levels in 574 normal children. The relationship between chronological age and serum GHBP was weak, but significant ($r = 0.18$, $P < 0.01$, $n = 428$; subjects under 1 year old were excluded). Subjects over 1 year old were divided into eight age groups by 2-year intervals. Each age group showed logarithmic normal distribution. Accordingly, a 95% confidence limit of normal distribution was calculated and is shown by the dotted line.

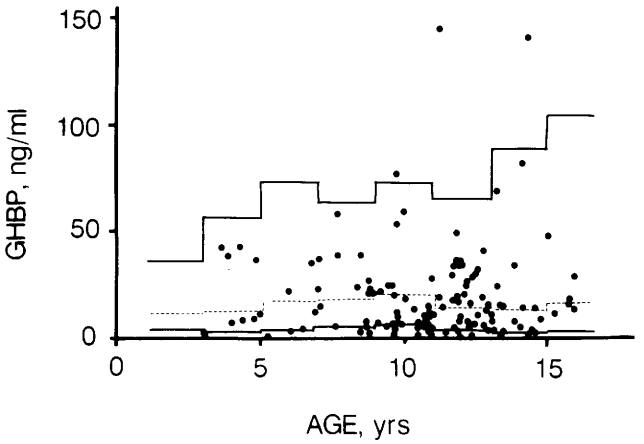

Figure 2. Serum GHBP levels in short but otherwise normal children (n = 127). Solid line, 95% confidence limit of normal distribution; dotted line, the mean value for each age group in normal controls.

GHBP considerably overlapped with the normal range. However, the mean value tended to be lower than that of normal controls (10.8 vs 12.4 ng/ml, the mean of normal logarithmic distribution), and slightly lower than that of normal short children (vs 11.3 ng/ml). These differences were not significant.

Discussion

In this study, we measured serum total GHBP levels by LIFA in healthy and short children. Serum total GHBP levels were almost stable, at least during nocturnal sleep, in each individual, with no relation to pulsatile GH secretion, as reported by Carlsson *et al.* (6). Thus, the rapid effect of GH on total GHBP, at least concerning the high-affinity GHBP, is quite small or negligible. Short-term GH loading for 10 days did not affect the GHBP levels. Long-term GH treatment induced increases of serum GHBP in seven out of 10 subjects, including both normal short children and patients with GH deficiency (data not shown), as previously reported (7–9). These results indicate that exogenous GH induces the GHR in an up-regulatory mechanism, and that a certain duration of time is necessary for this regulation.

In normal control children, serum GHBP levels were low in 5-day-old neonates, acutely increased by 6 months of age and gradually but significantly increased toward midpuberty. The rapid rise of GHBP in early infancy may indicate that the receptor-dependent actions of endogenous GH increase during the neonatal period (10, 11). Sexual dimorphism in GHBP levels was not apparent; however, levels in females were rather higher than those in males. Testosterone has been reported to act as a suppressor for serum GHBP levels in man (9). These data suggest sexual dimorphism of serum GHBP in humans. There are no apparent pubertal changes in GHBP in our study. Age-related changes in GHBP were reported by several authors (11–16), three of which showed an absence of acute pubertal changes (12–14). Martha *et al.* (14), using the LIFA GHBP assay, reported alterations of serum GHBP during normal puberty. They observed no dramatic changes in total GHBP levels during midpuberty in 11 normal boys. Although we did not investigate changes in GHBP longitudinally, our data also indicate that serum GHBP levels continuously rise before (mid)puberty, and reach and remain almost stable during midpuberty.

Recently, Baumann *et al.* (17) demonstrated that serum high-affinity GHBP levels were low in mountain Ok people of Papua New Guinea who were short in stature but had normal GH and IGF-I levels. Fontoura *et al.* (8) reported low levels of GHBP in idiopathic short stature subjects whose GH and IGF-I were also normal. We investigated whether there exists a conceptional group of short but otherwise normal children with low GHBP (low tissue GH receptors?), which contributed to their short stature. In 127 normal short children, GHBP levels were lower than that of normal subjects. We could not assert low GHBP levels as the etiology of short stature in these children; however, the low GHBP levels could suggest resistance to GH in peripheral tissue. GHBP levels were even lower in children with GH deficiency before treatment than the levels in normal short children in our study and those of others (8–10); this indicates deterioration of GH and its downstream axis, and that some amount of GH might be necessary to maintain the quantity of its binding proteins and receptors.

In conclusion, the measurement of GHBP in children with growth disorders will provide suggestive information on the action of GH and its axis.

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