Hemoglobin Level Is Linked to Growth Hormone-Dependent Proteins in Short Children

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Erythropoiesis was investigated in 32 children with short stature and in eight children with skeletal dysplasia by studying blood hemoglobin in relation to growth and to serum concentrations of insulin-like growth factor I (IGF-I), IGF binding protein-3 (IGFBP-3), and erythropoietin (EPO) before, during, and after 12 months of recombinant human growth hormone (GH) treatment. Blood hemoglobin concentration was positively correlated with relative body height and with serum IGF-I and IGFBP-3 levels \(P = .001 \text{ to } .02\), but not with the concentrations of EPO. The normal age-dependency of hemoglobin was lacking. Hemoglobin levels and their responses to GH treatment were similar in the patients with GH deficiency and those with normal GH secretion. Treatment with GH accelerated growth and elevated the concentrations of hemoglobin, IGF-I, and IGFBP-3. In the eight patients with skeletal dysplasia, body mass increased similarly, but gain in height was less than in the other patients, and the increase in hemoglobin was markedly pronounced. In this group, the correlations between hemoglobin, IGF-I, and IGFBP-3 were extremely close \(r = 0.90 \text{ to } 0.85, P = .031 \text{ to } .001\). These findings are in accord with earlier observations from in vitro and animal studies, and suggest that the GH-IGF axis is involved in the physiologic elevation of hemoglobin levels during childhood.

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GROWTH HORMONE (GH) and its principal regulator, insulin-like growth factor I (IGF-I), have been demonstrated to stimulate erythropoiesis under various conditions \(^1\text{ to } 10\) and in animal studies. \(^11\text{ to } 14\) In man, the view that GH plays a role in hematopoiesis is complicated by observations that individuals with GH deficiency are generally not anemic. With respect to erythropoiesis, the effects of treatment with recombinant human GH have not been thoroughly studied.

The mechanism linking increasing erythrocyte mass with body growth is poorly understood. Erythropoietin (EPO), the primary substance regulating erythropoiesis, was formerly believed to increase during growth as a consequence of renal hypoxia related to kidney growth. \(^1\text{ to } 12\) However, experiments on neonatal mice indicated that prevention of hypoxia by hypertransfusion does not totally suppress erythropoiesis during growth, \(^11\) although it does so in adult mice. \(^13\) Moreover, in rats, serum EPO decreased during growth, while the increasing concentrations of serum IGF-I were linearly correlated with total incorporation of iron into erythrocytes. \(^13\)

These findings suggest that factors related to growth itself are involved. \(^13\text{ to } 16\)

In vitro experiments have shown that physiologic concentrations of IGF-I stimulate human and murine erythroid precursors, whether EPO is present or not, \(^1\text{ to } 10\) whereas large doses of GH are required for the same effect. \(^1\text{ to } 4\) In an experiment with murine erythroid colony-forming units, \(^1\) the relative effect of IGF-I was strongest in the absence of EPO and, with increasing concentrations of EPO, the effect became weaker. Furthermore, administration of IGF-I to neonatal rats led to accelerated red blood cell production. \(^14\) In a study on hypophysectomized rats, both human GH and recombinant human IGF-I stimulated erythropoiesis and increased the serum EPO concentration. \(^12\) However, the stimulatory effect on erythropoiesis occurred before the serum EPO levels had increased. In uremic patients with anemia, the serum EPO concentration was within the normal range, but the serum IGF-I level was elevated. \(^17\) In these patients, IGF-I was positively correlated with hematocrit values, but serum EPO was not.

Treatment of short children with GH offered an opportunity to study the effects of GH on erythropoiesis in humans. GH stimulates the circulating levels of IGF-I and one of its binding proteins, IGF binding protein-3 (IGFBP-3). \(^18\) While GH has a short half-life and its secretion is pulsatile, \(^19\) IGF-I and IGFBP-3 show little, if any, diurnal variation. \(^20\text{ to } 21\) Of the six currently known IGF-binding proteins, the most abundant is IGFBP-3, \(^22\) regulated by GH \(^20\) and IGF-I. \(^18\text{ to } 24\)

The present study was designed to determine the relations between GH, growth, and erythropoiesis in humans. For this purpose we investigated the associations between hemoglobin, EPO, IGF-I, IGFBP-3, and body growth during GH treatment of 40 short children.

MATERIALS AND METHODS

Subjects. Fifty-one children with short stature were given GH medication between January 1992 and May 1993 at the Children’s Hospital, University of Helsinki, or at the Aurora Hospital, Helsinki, Finland. They were followed up during the first year of treatment. The children with chronic medication other than GH were excluded, leaving 20 boys and 20 girls for the study group. None of the patients had hematologic abnormalities nor had they any treatable diseases such as hypothyroidism or celiac disease. The patients’ age ranged from 1.6 to 13.3 years, with a mean of 7.0 years (Table 1). Seven patients had signs of early puberty during the study year. Two of them were boys. At the end of the follow-up period, they were at stage P2G2 according to the criteria of Tanner. \(^25\)

In eight children, the cause for their short stature was severe skeletal dysplasia. In the other 32 short children, endogenous GH secretion was evaluated by GH stimulation tests \(^26\) or overnight sampling analysis. Maximal plasma GH concentrations formed a continuum from very low serum values to normal values (Fig 1). The

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blood sample to be taken 1 week after the start of treatment. The tests were repeated if values were below 10 ng/mL. None of the blood tests on the day when treatment was started, and at 3, 6, and children were divided into four categories (Table 2). They grew 4.6 (range, 2.8 to 6.0) cm/year. For comparisons, the considered to be GH-deficient, the mean maximum GH response to stimulation tests was 5.8 (range, 1.0 to 9.9) ng/mL, and on average, they grew 4.6 (range, 2.8 to 6.0) cm/year. For comparisons, the patients were divided into four categories (Table 2).

The study was approved by the Ethics Committee of the Children’s Hospital, University of Helsinki. Informed consent was obtained from the children and their parents.

Protocol. The study protocol included physical examination and blood tests on the day when treatment was started, and at 3, 6, and 12 months of GH medication. In addition, 23 patients allowed a blood sample to be taken 1 week after the start of treatment. The investigations included body weight and height measurements with a Harpenden stadiometer, and the laboratory tests consisted of measurements of blood hemoglobin, hematocrit, reticulocyte count, and serum concentrations of ferritin, EPO, IGF-I, and IGFBP-3. The study protocol was followed strictly in 36 of the 40 patients. In four patients the protocol was followed for 6 months; three of these had skeletal dysplasia.

GH treatment. The indication for GH treatment was short stature. The recombinant human GH (Genotropin; Kabi Pharmacia, Stockholm, Sweden/Humatrope; Lilly, Indianapolis, IN/Nordropin; Novo Nordisk Gentofte, Denmark/Saizen; Serono Nordic, Geneva, Switzerland) was given as daily subcutaneous injections. The mean dose was 0.13 (range, 0.09 to 0.27) IU/kg/d or 3.2 (range, 1.8 to 6.5) IU/m2/d. Five patients (one girl with the Turner syndrome, two children with prenatal growth retardation, and two children who were likely GH deficient) were given iron medication (3 mg/kg/d) during the study because of signs of iron deficiency.

Biochemical analyses. Blood hemoglobin concentrations and hematocrit were measured with a Coulter Counter T 890 (Coulter, Miami, FL). Serum IGF-I concentration was determined by radioimmunoassay (RIA) (Incstar Co, Stillwater, MN) after acid extraction chromatography. The sensitivity of the assay was 2 nmol/L, the intraassay variation 7% to 8%, and the interassay variation 11% to 14%. Serum IGFBP-3 was measured by immunofluorometric assay. The serum samples were diluted 1:100 and the measurement range covered 0.6 to 650 ng/mL. The intraassay variation was 3.6% to 6.2% and the interassay variation 5.4% to 11%. EPO concentrations were determined by RIA (Incstar Co). At concentrations greater than the detection limit of 10 mU/mL, the intraassay variation of 7% to 8%, and the interassay variation 11% to 14%. Serum IGFBP-3 was measured by immunofluorometric assay. The serum ferritin was measured by RIA (Ferritin Ria Kit, Kodak Clinical Diagnostics, Amersham, UK). The total circulating erythrocyte volume was estimated in mL according to the following formula: hematocrit × body weight (kg) ÷ proportion of body weight assumed for blood volume (6.5 mL per kg).

Data transformations. Because the age of the patients ranged from 2 to 14 years, we transformed all age-dependent values into standard deviation (SD) units. The transformation of body height was based on Finnish growth standards and, consequently, the transformed height is referred to as relative height. The mean relative height before treatment was -3.3 SD units (Table 1).

Because in the reference series hemoglobin, IGF-I, and IGFBP-
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3rd were age-dependent, these results were also transformed. The conversion of hemoglobin was based on a large body of Finnish-American data. The conversions of IGF-I and IGFBP-3 were based on the values we obtained from 139 healthy children between 0.2 and 15.9 years of age. The samples had been obtained when the children visited the general outpatient clinic because of allergy, a psychosomatic problem, minor surgery, or follow-up of these. The reference values were calculated for 2-year cohorts. In the transformation procedure, values were interpolated between the mean ages of the 2-year cohorts.

The EPO values were not converted into SD units, because EPO concentration remains stable after infancy. The distributions of EPO and ferritin concentrations were skewed, and these data were, therefore, subjected to logarithmic transformation.

Data presentation and statistical analyses. Data are presented in the text as means (95% confidence intervals) and, for the sake of clarity, in the figures as means ± standard error of mean (SEM). The total ranges of baseline values are given in Table 1. Both simple and multiple linear regression analyses were performed. We considered the various diagnostic categories, maximal serum value observed in GH stimulation tests, IGF-I, IGFBP-3, EPO, ferritin, age, sex, puberty, body mass index, relative height, and growth velocity as independent variables to explain the variation of hemoglobin level. Linear associations were tested with Pearson’s correlation coefficient. To compare the different diagnostic categories, analysis of variance (ANOVA) and the post hoc Student-Neuman-Keuls test were used. Values for the patients with skeletal dysplasia and the 32 other patients were compared by the two-tailed independent-samples t test. Paired samples t test was used to test changes in ferritin level. Differences were considered significant at P < .05.

RESULTS

Correlation of blood hemoglobin concentration with body height and with serum IGF-I and IGFBP-3. In the 32 patients with GH secretion ranging from normal to pathological, blood hemoglobin concentration (as g/dL) correlated positively with relative height (r = 0.37, P = .037), with IGF-I (r = 0.37, P = .004), and with IGFBP-3 (r = 0.50, P = .003), but was independent of EPO (P = .48). If the eight patients with skeletal dysplasia were included, the correlations were even closer (Fig 2). Multiple regression analysis was performed on 40 patients. The best three-variable model explained 56% of the variation in hemoglobin level. According to this model, hemoglobin was positively associated with IGFBP-3 (P = .002) and body height in cm (P < .001) and negatively associated with age (P < .001). The best four-variable model (R² = .61) also included serum ferritin (P = .060) in the list of variables that had positive associations with hemoglobin. Transformation of hemoglobin into SD units did not change the results.

Effect of GH on growth and hemoglobin, IGF-I, and IGFBP-3 concentrations. In the 40 patients, the average growth rate of 5.5 (4.8 to 6.1) cm per year before GH treatment increased to 9.8 (9.0 to 10.6) cm per year. Correspondingly, the relative height increased by a mean of 1.0 (0.8 to 1.1) SD units during the 1-year study period. Figure 3 illustrates the respective changes for the two subgroups.

Hemoglobin increased by 0.42 (0.16 to 0.68) g/dL during
The present study demonstrates that three parameters of growth, body height, IGF-I, and IGFBP-3, correlate closely with blood hemoglobin level. The results strongly suggest that the GH-IGF-I axis participates in the regulation of human erythropoiesis. In addition to corroborating the results of earlier studies conducted in vitro and in animals, the present results substantiate the previous anecdotal observations.

There is a fundamental difference in blood hemoglobin...
level between adults and growing children. In adulthood, aged erythrocytes are constantly replaced by equal quantities of new erythrocytes to maintain the hemoglobin level. During growth, in contrast, each new erythrocyte generation has to be more numerous than the previous, just to maintain a stable hemoglobin level. Yet, the mean blood hemoglobin concentration normally increases from about 11.0 g/dL at the age of 2 months to about 13.5 g/dL in adult women and to 15.5 g/dL in adult men. Our results suggest that the GH-IGF-I axis plays a role in growth of the continuously renewed erythrocyte mass. If GH promotes the increase in the erythrocyte mass during childhood, the system may be adjusted to overproduction of erythrocytes and a gradual increase in hemoglobin concentration. In light of the proposed possibility, it is not surprising that the children with growth failure did not demonstrate the normal age-dependency of hemoglobin, but they did demonstrate dependency on body height.

EPO, by reacting against any renal hypoxia, is unquestionably the principal factor preventing anemia. But although blood hemoglobin concentration remains constantly within the normal range, the GH-IGF-I system may elevate it further during growth periods. This increase would appear to guarantee better oxygen supply to growing tissues. In adulthood, the role of this latter mechanism in the regulation of erythropoiesis would be of minor importance. The notion that GH-IGF-I axis and EPO appear to have parallel functions is supported by observations from an in vitro study on mice in which, at normal EPO levels, about 40% of erythropoiesis depended on EPO alone, another 30% or less on the additive effect of IGF-I, and the remaining 30% or more on other unidentified serum factors.7

During GH treatment, erythropoiesis and rapid body growth increase iron needs, and serum ferritin concentration decreases.27 In the light of this observation, the slight increase in hemoglobin concentration observed seems even more remarkable. In early male puberty, the decrease in ferritin that precedes accelerated growth may berelated to early increase in GH secretion, and significant increase in hemoglobin concentration occurs later during pubertal maturation.35 It is not impossible that our observation of decreased ferritin levels in prepubertal children given GH is related to a similar mechanism as that seen in early puberty under physiological conditions. Further studies are needed to address this possibility.

The present study proved that the role of GH-IGF-I system in the control of hematopoiesis is obvious. However, the mechanism through which the GH-IGF-I system acts on erythroid progenitor cells is not clear. In a recent study, Ratajczak et al36 suggested that IGF-I does not have a direct effect on proliferation of erythroid progenitor cells, and other studies have suggested that, in hematopoietic cells, IGF-I acts principally by inhibiting apoptosis.37,38

In our patients, endogenous GH secretion varied from apparently normal to remarkably subnormal, although none had complete absence of GH secretion. In keeping with earlier observations,4,39 the baseline hemoglobin level was not significantly lower in the children with GH deficiency than in the other patients. In our study, these children were hardly distinguishable from the other patients in their 1-year growth response to GH treatment. This finding is also supported by several other studies.40-42 In fact, we observed no important differences between the subgroups classed according to GH secretion, but we cannot rule out the possibility that such differences may exist. As the diagnosis of GH deficiency is a matter of subjective agreement,43 it may be argued that very few, if any, of our patients had unequivocal GH deficiency. From the statistician’s point of view, the present study was not designed to detect similarities. For that purpose, the sample size would have had to be larger.

A remarkable hemoglobin response to GH treatment was observed in the children with skeletal dysplasia. Because of their chondrodystrophic bone disease, predominantly achondroplasia, they were not only short but their trunk/limb ratio was disproportionate for age. In achondroplasia, there are mutations in the gene encoding fibroblast growth factor receptor-3.44 Growth deformities in the spines and limbs of those affected reduce their growth potential, their final height usually being within the range of 112 to 145 cm.45 These rare conditions may help us understand the relationship between body growth and erythropoiesis. In our eight patients, GH treatment resulted in a slight body growth response only, whereas the other responses to GH, such as erythropoiesis, seemed unaffected, for their erythrocyte mass increased well. IGF-I and IGFBP-3 increased in a similar manner both in the patients with skeletal dysplasia and in the other patients, but the association between hemoglobin and these GH-related parameters was clearer in the former patients.

The role of EPO as an indicator of hypoxia and a regulator of erythropoiesis is well documented, and in anemic patients, serum EPO concentration is known to correlate negatively with hemoglobin level.46,47 However, a correlation between serum EPO and blood hemoglobin concentrations has not been reported in subjects with normal hemoglobin levels.16 In keeping with this, we found no such correlation. As expected from previous studies, EPO increased during the first week of GH treatment.12,48 The sodium-retaining action of GH causes fluid retention49,50 and, most likely, expansion of plasma volume. From these observations it is possible to speculate that initial hemodilution due to this phenomenon would explain the early temporary decline in hemoglobin concentration that coincided with the elevation in EPO concentration. The fact that the initial elevation of EPO correlated with the subsequent elevation of hemoglobin concentration confirms the involvement of EPO in the observed changes.

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Hemoglobin level is linked to growth hormone-dependent proteins in short children

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