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Serum Growth Hormone-Binding Proteins in the Human Fetus and Infant[1]

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ABSTRACT. Growth hormone-binding protein (GH-BP) levels were studied in cord serum of 69 human infants born after 24 to 41 wk of gestation and in serum of 14 infants aged 1 to 3 mo. GH-BP levels were measured by HPLC-gel filtration of serum incubated overnight with ^{125}I-hGH. The radioactive elution profile revealed two small ^{125}I-hGH peaks of high molecular weight and a large peak, corresponding to monomeric ^{125}I-hGH. The first peak of high molecular weight was variable, showed some of the characteristics (high molecular weight, displaceability by a large excess of unlabeled hGH) of the described low affinity, high capacity GH-BP, and did not correlate with gestational age or birth weight (peak I-BP). The second peak was identified as ^{125}I-hGH bound to the high affinity, low capacity GH-BP (peak II-BP). Mean ± SD specific binding of ^{125}I-hGH to this peak was significantly ($p < 0.0001$) different between preterm infants (3.1 ± 1.1%; $n = 51$), term infants at birth (4.2 ± 1.1%; $n = 18$), and 1- to 3-mo-old infants (8.5 ± 1.6%; $n = 14$). To evaluate the effect of intrauterine nutritional state, the ponderal index (weight/length^3) was calculated. Peak II-BP levels were lower ($p < 0.05$) in infants with the ponderal index < 2.35 (2.8 ± 1.0%; $n = 20$) than in those with the ponderal index between 2.35 and 2.65 (3.4 ± 1.2%; $n = 29$) or > 2.65 (3.8 ± 1.2%; $n = 20$). We conclude that GH-BP are present in human cord serum throughout the 3rd trimester of gestation. The levels of peak II-BP are influenced by fetal age and intrauterine growth and increase quickly after birth. (*Pediatr Res* 32: 69–72, 1992)

Abbreviations

BP, binding protein
GH, growth hormone
GH-BP, growth hormone-binding protein
hPL, human placental lactogen
hPRL, human prolactin
PI, ponderal index
NIDDK, National Institute of Diabetes and Digestive and Kidney Diseases

A specific GH-BP with high binding affinity and low binding capacity has recently been described in human and animal serum (1–4). Immunologic and biochemical studies have shown that this GH-BP is identical to the extracellular domain of the liver membrane GH receptor (5–8). Studies of the GH-BP in rats revealed a quantitative relationship between the binding of GH to the circulating GH-BP and to the hepatic somatogenic receptor (9, 10). These findings suggest that the serum levels of this circulating GH-BP may be an indicator of tissue GH receptor number. In addition to the GH-BP with high affinity and low capacity, another BP with low affinity and very high capacity for GH has been found in human plasma (11, 12).

In the human fetus, in spite of very high levels of circulating GH (13, 14), only low levels of tissue GH receptors (15, 16) and of serum GH-BP (17–19) have been reported. The physiologic significance of these findings remains unclear. We studied the presence of specific GH-BP in cord serum of human infants at term and preterm birth and investigated the relationship with gestational age and intrauterine growth.

MATERIALS AND METHODS

Blood samples. Mixed venous and arterial cord blood samples were obtained at birth from 69 infants with a gestational age between 24 and 41 wk. Fifty-one infants were born preterm (24–36 wk) and 18 infants were born at term (37–41 wk). Blood was collected into glass tubes; after clotting at 4°C, the blood was centrifuged and the serum kept frozen at −20°C until assayed. Twenty-eight infants were delivered by cesarean section. Thirty-two infants were born after multiplet pregnancies. In 25 cases, the mother was prenatally treated with corticosteroids and/or thyrotropin-releasing hormone to accelerate fetal lung maturation. To evaluate intrauterine nutritional state, the PI (weight/length^3) corrected for gestational age was calculated (20).

The results obtained in cord serum were compared with those obtained in 14 healthy infants aged 1 to 3 mo (spare serum obtained from blood sampling for other screening purposes). Adult male serum with low circulating GH levels (<1 μg/L) was used as reference serum.

Methods. Serum levels of GH-BP were determined by the HPLC gel filtration method of Tar *et al.* (12), with the modifications described below. Biosynthetic hGH, labeled with ^{125}I-Na by the iodogen method (21) to sp act of 70–85 μCi/μg, was obtained from Novo-Nordisk, Gentofte, Denmark. ^{125}I-hGH was purified weekly on a 1.6- × 70-cm Sephadex G-100 column eluted with phosphate buffer (0.05 M, pH 7.5) with 0.1% BSA and 0.02% NaN_3. Serum (100 μL) was incubated overnight at 4°C with 40 000 cpm ^{125}I-hGH in potassium phosphate (0.1 M; pH 7.0) buffer with 0.1% BSA, in the absence or presence of 1 μg of unlabeled biosynthetic hGH (Novo-Nordisk) in a total volume of 200 μL. After filtration through a 0.2-μm Acrodisc LC polyvinylidine difluoride syringe filter (Gelman Sciences, Ann Arbor, MI), the incubation mixture was placed onto an HPLC Protein Pak 300sw column (Waters, Milford, MA) eluted with a 0.1 M Na_2SO_4 and 0.1 M potassium phosphate buffer (pH 7.0) at a flow rate of 0.5 mL/min to separate bound and free ^{125}I-hGH. The column was calibrated with blue dextran, β-amylase, BSA, ^{125}I-hGH, and free Na^{125}I. The eluted radioactivity was recorded on line using a Berthold HPLC radioactivity mon-

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itor LB 506 C-1 (Berthold, Wildbad, Germany) connected to an Olivetti M 290 S computer.

The binding of ^{125}I-hGH is expressed as the percentage of radioactivity. Total binding was calculated on the radioactive elution profile obtained when serum was incubated with ^{125}I-hGH in the absence of unlabeled hGH by dividing the radioactivity in the individual peak by the sum of the radioactivities in peaks I, II, and III, multiplied by 100. Nonspecific binding was calculated in the same way using the radioactive elution profile of serum incubated with ^{125}I-hGH in the presence of 1 μg unlabeled hGH. Because the first peak could not be displaced by 1 μg unlabeled hGH, only total binding to peak I-BP is presented. The binding to peak II-BP is given as the percentage of specific binding, calculated as the difference between total and nonspecific binding. Because high levels of circulating hGH, measured by an immunoradiometric assay (hGH-IRMA; Medgenix, Fleurus, Belgium), were present in most samples, the binding to peak II-BP was corrected for occupancy by endogenous GH. This was done on the base of a displacement curve obtained by adding increasing concentrations of unlabeled hGH to the reference serum; the percentage of ^{125}I-hGH bound to peak II-BP was divided by the fraction bound in the reference serum at the hGH concentration found in the unknown sample. No corrections were made for peak I-BP, which is not affected by GH levels in the range observed. The interassay coefficient of variation for peak II-BP was 8% at the level of 25.1% (n = 9) and 18% at the level of 3.6% (n = 10).

The specificity of peak II-BP was evaluated by incubating serum with ^{125}I-hGH in the presence of 0.5 μg hPRL (NIDDK hPRL RP-1) or 1 μg hPL (NIDDK hPL) obtained from Dr. S. Raiti through the NIDDK Pituitary Hormone Distribution Program. Binding affinity and capacity of the GH-BP were calculated by Scatchard analysis (22) of tracer binding-inhibition experiments with increasing concentrations of unlabeled hGH on three pools of cord serum from five different infants.

Statistics. Results are expressed as mean ± SD. Correlations between GH-BP levels and parameters studied were calculated by linear regression analysis and expressed as the Pearson correlation coefficient. Because of the interactions between gestational age and birth size, 1st order partial correlation coefficients are used to exclude the effect of gestational age on GH-BP levels. Comparisons between groups were made by one-way analysis of variance, followed by Newman-Keuls test for multiple comparisons.

RESULTS

Presence and characteristics of GH-BP in human cord serum. Figure 1 shows the radioactive elution profile obtained under the described HPLC conditions of 100 μL of a term infant's serum, incubated overnight at 4°C with ^{125}I-hGH without (*panel A*) or with (*panel B*) an excess (1 μg) of unlabeled hGH. In the absence of unlabeled hGH, three peaks of radioactivity were present. The first peak eluted in the void volume, is not displaced by 1 μg hGH but could be abolished by a large excess of hGH (100 μg). These findings meet the identification criteria of ^{125}I-hGH bound to the low affinity, high capacity GH-BP (peak I-BP). The second peak eluted with an apparent molecular weight of about 80 kD; it is undetectable when the incubation is carried out in the presence of 1 μg hGH. This peak therefore seems to correspond to ^{125}I-hGH bound to the high affinity, low capacity GH-BP (peak II-BP). The third peak of radioactivity eluted at the same position as free ^{125}I-hGH in both test situations.

In the studied population of 69 cord serum samples and 14 samples of 1- to 3-mo-old infants, the percentage of ^{125}I-hGH bound to the peak I-BP was very low and variable, ranging from 0 to 5.4%. No differences were found between preterm newborns (2.4 ± 1.4%; n = 51), term newborns (2.1 ± 1.7%; n = 18), and the older infants (1.3 ± 1.5%; n = 14). In contrast, peak II-BP was present in all examined samples. The mean ± SD level of

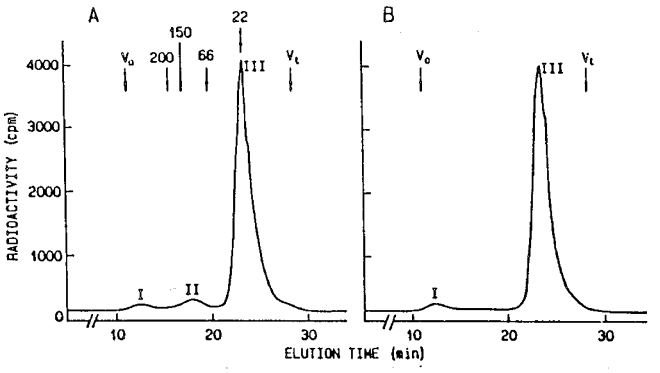

Fig. 1. Representative radioactive elution profiles of term human cord serum incubated with ^{125}I-hGH in the absence (*panel A*) or presence (*panel B*) of 1 μg unlabeled hGH. At the top of *panel A*, the void volume (V_o), the total volume (V_t), and the molecular weight (×10^3) of the markers used to calibrate the column are indicated. I = ^{125}I-hGH bound to peak I-BP; II = ^{125}I-hGH bound to peak II-BP; and III = free, monomeric ^{125}I-hGH.

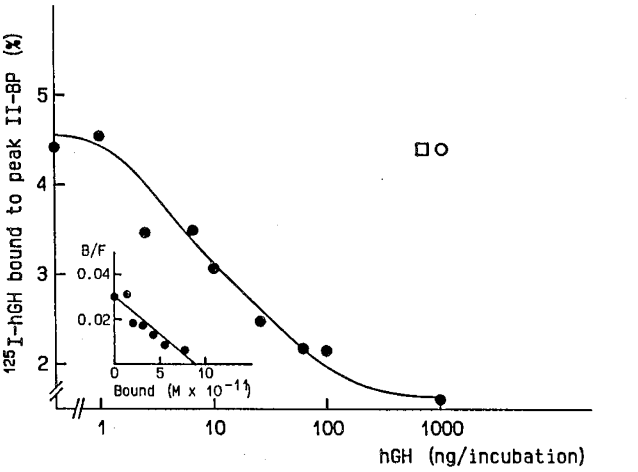

Fig. 2. Binding-displacement curve and Scatchard plot of ^{125}I-hGH binding to peak II-BP in 100 μL of a cord serum pool obtained from five term infants. The binding capacity is expressed per L of incubation volume (× 2 to obtain values expressed as nmol/L of serum). □, hPRL; ○, hPL.

peak II-BP was 3.1 ± 1.1% in preterm infants (n = 51), 4.2 ± 1.1% in term infants (n = 18), and 8.5 ± 1.6% in 1- to 3-mo-old infants (n = 14); the differences between these groups were highly significant ($F_{2,80}$ = 119.5; $p < 0.001$).

Figure 2 shows a binding-displacement curve of ^{125}I-hGH to peak II-BP as a function of the amount of unlabeled hGH added. Scatchard analysis of three experiments revealed a mean binding affinity of 8.2 × 10^8 L/mol and a binding capacity of 2.4 μg/L serum. In the reference adult serum, binding affinity was 6.2 × 10^8 L/mol and binding capacity was 26 μg/L serum. Figure 2 also indicates that the binding of ^{125}I-hGH to peak II-BP did not decrease when serum was incubated in the presence of 1 μg hPRL or hPL, supporting the binding specificity of this protein for GH.

Relation of cord serum levels of GH-BP to gestational age, birth size, and nutritional state. Table 1 summarizes the results of correlation analysis for GH-BP levels in cord serum. No relationship was found between the levels of peak I-BP and gestational age or birth weight. In contrast, peak II-BP levels were found to be positively related to gestational age (r = 0.35; $p < 0.005$) (Fig. 3) and birth weight (r = 0.45; $p < 0.0005$). To separate the effects of gestational age and birth size, the 1st order partial correlation coefficients between birth weight and peak II-BP levels were calculated to control for the effect of gestational

Table 1. *Correlation analysis for cord serum GH-BP levels**

	Peak I-BP		Peak II-BP	
	r	*p*	*r*	*p*
Gestational age	0.09	NS	0.35	<0.005
Birth weight				
Zero order *r*	0.02	NS	0.45	<0.0005
1st order *r*			0.29	0.01

* Pearson *r* and significance levels *p* are shown. Zero order values refer to initial analysis excluding any interaction between gestational age and birth size. First order value refers to partial correlation analysis where the effect of gestational age upon birth weight is controlled.

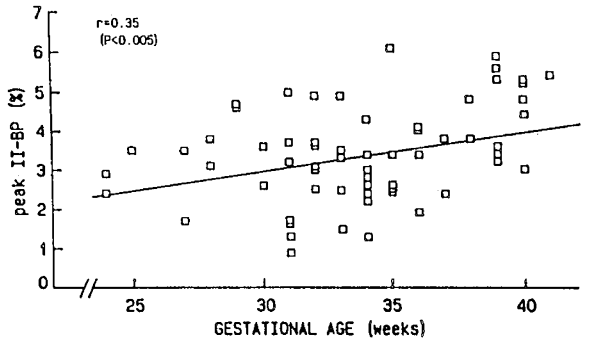

Fig. 3. Relationship between gestational age and serum levels of peak II-BP ($r = 0.35$; $p < 0.005$).

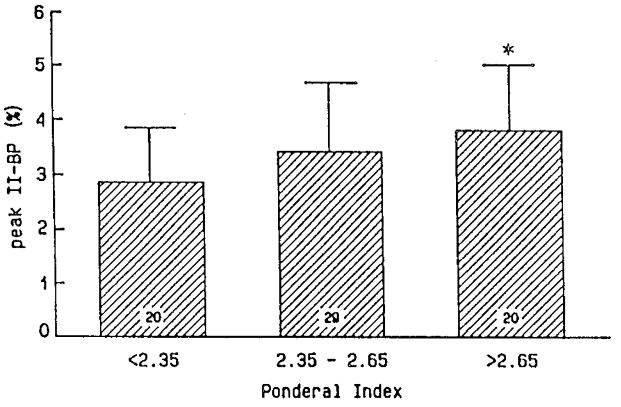

Fig. 4. Mean ± SD peak II-BP levels in infants with PI below 2.35, between 2.35 and 2.65, and above 2.65 (*, $p < 0.05$ compared to infants with PI < 2.35).

age. The association between cord serum levels of peak II-BP and birth weight remained significant ($r = 0.29$; $p = 0.01$).

To evaluate the effect of nutritional state, the PI corrected for gestational age was calculated. Mean ± SD level of peak II-BP was 2.8 ± 0.9% in infants with PI < 2.35 ($n = 20$), 3.4 ± 1.2% in those with PI between 2.35 and 2.65 ($n = 29$), and 3.8 ± 1.2% in those with a PI > 2.65 ($n = 20$) (Fig. 4). The differences between the three groups were significant ($F_{2,66} = 3.36$; $p < 0.05$). No influence of fetal sex or of prenatal treatment with corticoids or thyrotropin-releasing hormone was found on the levels of peak I-BP or II-BP.

DISCUSSION

The present study confirms on a large number of cord serum samples the presence of circulating GH-BP in the human fetus. After incubation of serum with ^{125}I-hGH, two small peaks of bound radioactivity were found. The first peak was very low and shared important characteristics of the described peak I-BP (high molecular weight, displaceability by a large excess of hGH) (11, 12). Its nature is presently unclear. No relationship was found between the levels of this first peak of bound ^{125}I-hGH and gestational age or birth size.

The second peak of bound ^{125}I-hGH was detectable in all samples and has characteristics identical to those of peak II-BP (2, 3, 11, 12). This BP has a molecular weight of about 60 kD and binds specifically GH and not hPRL or hPL. Moreover, its binding affinity is the same as in the adult reference serum and corresponds with the reported values (2, 12). The binding capacity, however, was about 10 times lower than in adult serum. The low binding of ^{125}I-hGH to peak II-BP in cord serum can therefore be explained by the lower concentration of circulating peak II-BP and not by a lower binding affinity.

The earlier evidence for GH-BP in the human fetus is limited. Daughaday *et al.* (17) described one peak of GH-BP that was virtually undetectable in 11 premature infants and very low in 17 term infants. Baumann *et al.* (18) examined cord blood from four neonates and found that about 4% of GH was bound to the low affinity peak I-BP and about 10% to the high affinity peak II-BP. Silbergeld *et al.* (19) examined arterial blood from six full-term infants and reported GH-BP activity at the detection level.

In agreement with the data of Daughaday *et al.* (17), our study shows that the levels of peak II-BP increase during the 3rd trimester of pregnancy. In infants aged 1 to 3 mo, peak II-BP levels are twice as high as in term infants at birth. This finding suggests that the amount of GH-BP—and possibly also of the tissue GH receptor—increases in the neonatal period, supporting the concept that the receptor-dependent actions of endogenous GH are limited in the fetus and increase in the neonatal period (23, 24).

A positive relationship was found between the levels of peak II-BP and birth weight. Interestingly, a significant positive relationship has been reported in the human fetus between the binding capacity of hepatic membranes for hGH and fetal body weight (15). Thus, it appears that the binding capacity of both the hepatic somatogenic receptor and the circulating GH-BP increases with body weight, suggesting a common regulatory mechanism.

In the present study, a relationship between the levels of peak II-BP and the PI, a parameter for intrauterine nutritional state, was also found, suggesting that the intrauterine nutrition influences the levels of circulating peak II-BP. We recently reported that in prepubertal and pubertal children nutritional state is positively related to peak II-BP levels (25). Moreover, it has been shown that prolonged fasting down-regulates the levels of the circulating GH-BP (26) and that the levels of serum GH-BP are decreased in celiac disease (27) and anorexia nervosa (27, 28). Studies in the rat also showed that fasting diminishes the levels of the hepatic GH receptor and the circulating GH-BP (29) and their respective hepatic mRNA levels (30). Hence, nutritional state seems to be an important regulatory factor for the level of GH-BP during both prenatal and postnatal life.

The concentration of total hGH is elevated in human fetal serum (13, 14), especially in conditions of suboptimal intrauterine growth (31). The fact that only low amounts of GH-BP are present in cord serum—particularly in infants with a low PI—suggests that large amounts of hGH in the fetus circulate as free monomeric hGH. Using the formula of Barsano and Baumann (32), it can be calculated that only about 2% of the circulating hGH in the human fetus is bound to the BP. This figure corresponds with the observation that virtually no high molecular weight forms of hGH are present in cord serum (33). Peak II-BP has been shown to decrease the metabolic clearance of GH (34). It is therefore possible that the exceptionally fast disappearance rate of hGH in the human infant (13) is in part due to the low levels of GH-BP.

In conclusion, low levels of GH-BP are present in the human fetus throughout the 3rd trimester of pregnancy. The levels increase with gestational age and are also influenced by the intrauterine nutritional state. Further studies are required to

strengthen the hypothesis that the levels of circulating GH-BP reflect the levels of the cellular GH receptor and to unravel the physiologic relevance of the high circulating hGH levels and low GH-BP and tissue GH receptor presence in the human fetus.

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