Growth Hormone-Binding Proteins during Human Pregnancy: Maternal, Fetal and Neonatal Data (43767)

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Abstract. **Serum levels of growth hormone-binding protein (GHBP) were measured by high-performance liquid chromatography (HPLC) gel filtration in serum of 30 pregnant women and in cord serum of 69 preterm and term infants. In pregnant women, the mean ± SD serum level of GHBP was 32.4% ± 5.6%. Polynomial regression analysis showed a significant ($P = 0.01$) second-order relationship between the duration of pregnancy and serum GHBP levels, with increasing levels during the first half of pregnancy and decreasing levels thereafter. In cord serum, the mean ± SD serum level of GHBP was 3.1% ± 1.4% ($n = 51$) in preterm and 4.2% ± 1.3% ($n = 18$) in term neonates. Serum GHBP concentrations were related to gestational age ($r = 0.35$; $P < 0.005$) and to intrauterine nutritional state as evaluated by the ponderal index ($r = 0.30$; $P < 0.02$). In four term neonates, serum levels of GHBP were measured before and after an exchange transfusion (ET). Before the ET the serum GHBP level was 6.7% ± 2.4% and at the end it was 25.3% ± 2.6%. Thereafter, serum GHBP levels decreased slowly. Analysis of the elimination curve by exponential stripping showed a biexponential elimination pattern: GHBP (%) = $40 \cdot e^{(-5.1E-03 \times time)} + 64 \cdot e^{(-2.5E-04 \times time)}$. The serum half-life of the GHBP complex was estimated to be about 2 days.**

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Pregnancy influences the growth hormone (GH)-insulin-like growth factor-I (IGF-I) axis at different levels. During the second half of pregnancy, pituitary GH disappears from the circulation, while placental GH appears and serum levels of IGF-I increase (1). In contrast to animal studies showing a huge increase in GHBP levels during pregnancy (2), no differences in serum levels of GH-binding protein (GHBP) were reported between pregnant and nonpregnant women (3, 4). In spite of high levels of circulating GH, only low levels of GH receptors (5) and GHBP (4, 6) have been reported in the human fetus.

In the studies presented here, we measured serum levels of GHBP in pregnant women and in cord blood of preterm and term neonates in order to evaluate the effect of pregnancy on maternal GHBP levels and of fetal growth on fetal GHBP concentrations. The large differences in serum levels of GHBP between neonates and adults provided us with a tool to study the serum half-life of the high-affinity GHBP during and after an exchange transfusion (ET).

Subjects and Methods

Subjects. *Pregnant women.* Serum samples were obtained after informed consent in 30 women (mean ± SD age: 28.4 ± 3.7 years) at different stages of an uncomplicated pregnancy. In four of the women, samples were taken serially at 4- to 6-week intervals.

Nonpregnant women (control women). Twenty-four healthy adult women were studied: 14 (age: 34.8 ± 8.0 years) had spontaneous menstrual cycles, and 10 (age: 30.8 ± 7.5 years) were taking oral contraceptives.

Fetal samples. Cord blood samples were obtained at birth from 69 infants with a gestational age between 24 and 41 weeks. Fifty-one infants were born preterm (24–36 weeks) at 18 at term (37–41 weeks).

Neonatal samples. The effect of an ET on serum levels of GHBP was studied in four babies born at term and developing severe hyperbilirubinemia due to

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feto-maternal AB0-blood group incompatibility. Serum samples for the determination of GHBP were taken from the donor blood and from the patients before, at the end, and at various time intervals after termination of the ET.

Methods. The serum levels of GHBP were determined by high-performance liquid chromatography (HPLC) gel filtration as previously described (7). The binding affinity of the GHBP for pituitary or placental GH was calculated by Scatchard analysis. In nonpregnant subjects, basal levels of human GH (hGH) were measured by a commercially available immunoradiometric assay (GH-IRMA, Medgenix, Fleurus, Belgium). In pregnant women, pituitary GH (GH-N) was measured by a specific RIA based on the K24 monoclonal antibody. Pregnant women sera were also assayed for placental GH (GH-V) by a specific RIA which did not detect pituitary GH or human placental lactogen at doses up to 10 mg/l (8).

The elimination of GHBP from the neonatal serum was studied by JANA, a software program for exponential stripping of pharmacokinetic data (9). The serum half-life time of the GHBP was calculated as follows: $t_{1/2}$ = 0.693/slope of the elimination phase.

Results

Serum Levels of GHBP in Nonpregnant and Pregnant Women. Figure 1 shows the results for serum levels of GHBP in the women studied. The mean ± SD level of GHBP in the spontaneously menstruating women was 34.6% ± 6.7%. Women taking oral contraceptives had significantly ($P < 0.001$) higher serum concentrations of GHBP (47.0% ± 7.4%).

During pregnancy the mean ± SD serum levels of GHBP were not different from those found in control women. In these subjects, polynomial regression analysis revealed a significant second-order correlation between the duration of pregnancy and serum levels of GHBP ($R^2 = 0.19$; $F_{2,44} = 5.1$; $P = 0.01$). During the third trimester of pregnancy the binding affinity of the GHBP for placental GH was 4.8×10^8 l/mol, which was comparable to the binding affinity found in serum of control women (4.9×10^8 l/mol).

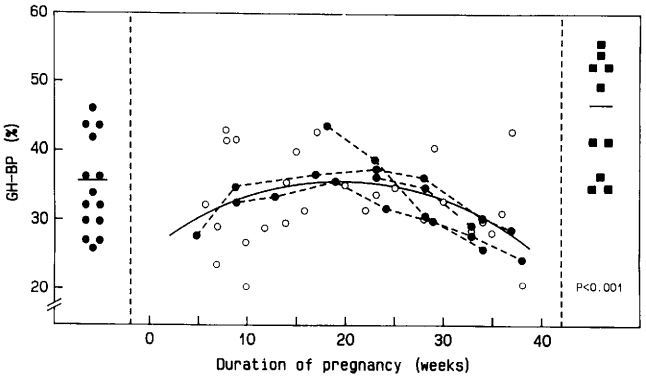

Figure 1. Serum levels of GHBP in spontaneously menstruating control women (left panel), in pregnant women in relation to the duration of pregnancy (middle panel; closed symbols represent serially obtained data), and in women taking oral contraceptives (right panel). Polynomial regression analysis showed a significant relationship between the duration of pregnancy and serum levels of GHBP (GHBP (%) = 25.5 + 1 × (weeks) − 0.03 × (weeks)^2; $R^2 = 0.19$, $F = 5.1$; $P = 0.01$). Results for GHBP are expressed in percentage of specific [^{125}I]hGH binding, corrected for circulating GH levels.

Serum Levels in the Human Fetus. The mean ± SD serum levels of GHBP was 3.1% ± 1.4% ($n = 51$) in preterm neonates and 4.2% ± 1.3% ($n = 18$) in term neonates. Scatchard analysis on a serum pool obtained from term neonates revealed a binding affinity of 8.2×10^8 l/mol and a binding capacity of 1.3×10^{-10} mol/l. Serum levels of GHBP were positively related to gestational age ($r = 0.35$; $P < 0.005$; Fig. 2) and to birth weight ($r = 0.45$; $P < 0.0005$). Birth length was also positively related to the serum GHBP concentrations ($r = 0.37$; $P < 0.005$). Serum levels of GHBP were positively related to the ponderal index (PI; $r = 0.30$; $P < 0.02$). The mean ± SD level of GHBP was 2.8% ± 0.2% ($n = 20$) in infants with PI < 2.35, 3.4% ± 0.2% ($n = 29$) in those with PI between 2.35 and 2.65, and 3.8% ± 0.3% ($n = 20$) in those with a PI > 2.65. The difference between the three groups was significant (ANOVA: $F = 3.36$; $P < 0.05$).

Serum Levels in Neonates Before and After ET. Figure 3 shows the serum levels of GHBP in the four infants studied before, at the end, and at various time intervals after the ET. Before the ET, serum levels of GHBP were low (6.7% ± 1.2%). At the end of the ET, serum levels of GHBP were significantly higher (25.3% ± 2.6%; $P < 0.005$). These concentrations fell within the adult range, and represented about 75% of the levels found in the donor serum (34.4% ± 5.1%). After the ET serum levels of GHBP decreased slowly. In samples taken 84 to 96 hr after the ET, GHBP concentrations were still higher than before the ET.

Exponential stripping of the data by computer analysis revealed that the elimination curve was best

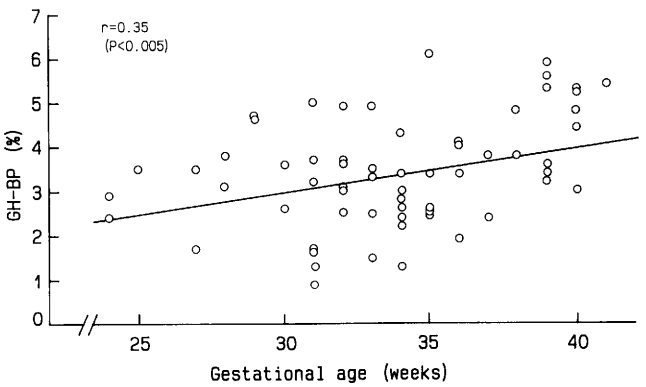

Figure 2. Relationship between gestational age and serum levels of GHBP. Results are expressed in percentage specific binding of [^{125}I]hGH, corrected for occupancy by endogenous GH.

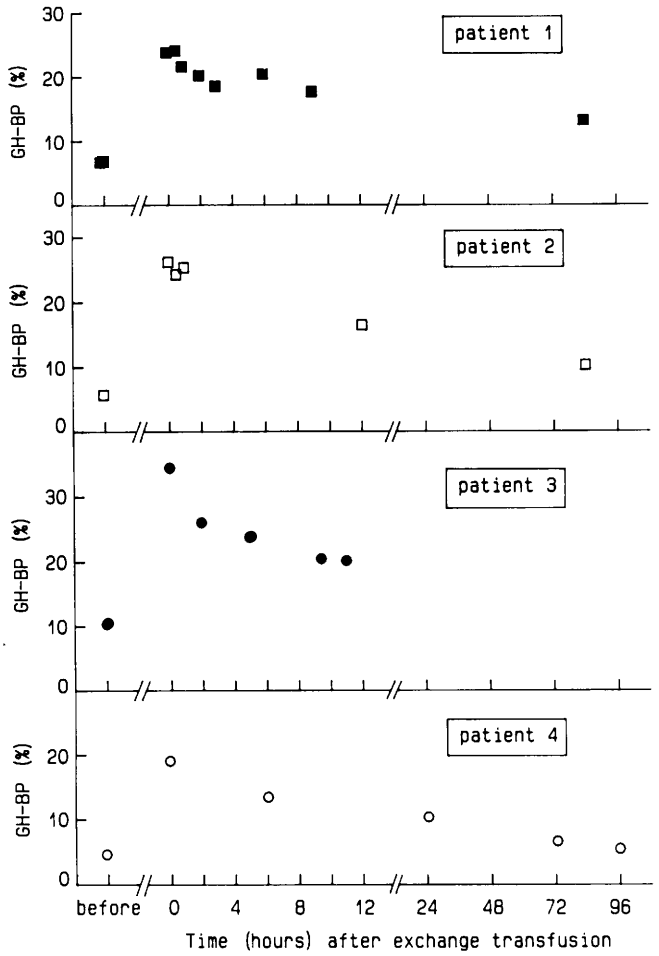

Figure 3. Serum levels of GHBP (%) in four neonates before, at the end (0), and at various time intervals after an ET for neonatal hyperbilirubinemia. Serum levels of GHBP were measured by HPLC gel filtration of serum incubated overnight at 4°C with [^{125}I]hGH. Results are expressed in percentage specific binding of [^{125}I]hGH, corrected for occupancy by endogenous GH.

described by a biexponential model: GHBP (%) = $40 \cdot e^{(-5.1E\text{-}03 \times \text{time})} + 64 \cdot e^{(-2.5E\text{-}04 \times \text{time})}$, with time expressed in minutes. The first part of this equation represents the distribution phase and the second part the elimination phase. Taking the slope of the elimination phase as the parameter to calculate the serum $t_{1/2}$, the $t_{1/2}$ of the GHBP complex was estimated at about 2 (=0.693/0.00025/1440) days.

Discussion

The presented studies describe serum GHBP levels in nonpregnant and pregnant women, and in preterm and term-born neonates. The serum binding of [^{125}I]hGH in women taking oral contraceptives containing EE2 was higher than in spontaneously menstruating women, suggesting a GHBP up-regulating effect of exogenous estrogens. This finding is in agreement with our report in Turner syndrome girls showing an increase in serum GHBP levels after oral administration of EE2 (10). Hence, GHBP seems to be a protein of which the production can be stimulated by oral estrogen administration.

In agreement with the data from the literature (3, 4), the mean ± SD GHBP level during pregnancy was not different from that found in nonpregnant spontaneously menstruating women. However, polynomial regression analysis revealed a biphasic effect of pregnancy on GHBP levels. During the first half of pregnancy, serum GHBP levels increase and during the second half they decrease. Blumenfeld *et al.* (11) also reported that in pregnancies resulting from ovulation induction cycles, a sharp increase in serum concentrations of GHBP during early gestation, followed by a gradual decrease beginning around the end of the first trimester, occurred.

During late gestation the binding affinity of the GHBP for placental GH was similar as in nonpregnant women. Moreover, the calculated binding affinity of 5×10^8 l/mol is comparable to the value we found in neonates and adults (7). This finding shows that the GHBP binds placental GH with the same affinity as pituitary GH. Moreover, our data suggest that pregnancy does not modify the binding affinity of the circulating GHBP.

In the human fetus and neonate, only low levels of tissue GH receptors (5) and GHBP (4, 6) have been reported. Our data confirm these findings and show moreover that the fetal GHBP has a binding affinity comparable to that found in adults. The GHBP concentrations increase with gestational age, and a positive relationship was found between serum levels of GHBP and birth weight. Interestingly, a similar positive relationship has been reported in the human fetus between the hGH-binding capacity of hepatic membranes and fetal body weight (12). Hence, it appears that the binding capacity of both the hepatic somatogenic receptor and the circulating GHBP increases with body weight, a finding compatible with a common regulatory mechanism.

A positive relationship was also found between the nutritional state, evaluated by PI, and the serum concentrations of GHBP, suggesting that the prenatal nutritional state influences the levels of circulating GHBP. These findings are in agreement with those of postnatal studies reporting positive relationships between nutritional state and GHBP levels (13, 14). Our data suggest that nutritional state is a modulator of the levels of circulating GHBP also during prenatal life.

In the literature, limited information is available about the metabolic clearance of the GHBP. Fairhall *et al.* (15) studied the metabolic clearance of intravenously injected recombinant hGHBP in guinea pigs and found that, when hGHBP and hGH were incubated together and then injected, the plasma disappearance was slower than when hGHBP or hGH were injected alone. These studies suggest that hGH and

GHBP alone can freely penetrate in the extravascular compartment, but that the GH-BP-GH complex is largely trapped in the vascular compartment and does not easily penetrate into the extracellular compartment. Thus, complex formation decreases the clearance rate of GH as well as the GHBP.

Although our data obtained after an ET in neonates are limited and have to be interpreted cautiously, they suggest that the GHBP disappears with a serum half-time time of about 2 days. It has to be stressed, however, that the observed elimination rate does not simply represent the metabolic clearance rate of the GHBP. Serum levels of hGH are high in the neonate, and it can be calculated that about 40% of the GHBP after the ET is saturated with GH. As suggested by the above-mentioned animal studies, the GH-BP-GH complex may be cleared more slowly from the circulation than the uncomplexed GHBP. Therefore, the serum half-life time of the uncomplexed GHBP is probably shorter than the estimated 2 days, calculated for a mixture of complexed and uncomplexed GHBP.

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