Developmental pattern of fetal growth hormone, insulin-like growth factor I, growth hormone binding protein and insulin-like growth factor binding protein-3

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Abstract
Aims—To evaluate the developmental pattern of fetal growth hormone (GH), insulin-like growth factor I (IGF-I), GH binding protein (GHBP) and IGF binding protein-3 (IGF-3); to determine the implications for fetal growth.
Methods—Serum GH, IGF-I, GHBP and IGFBP-3 were measured in 53 fetuses, 41 aged 20–26 weeks (group A) and 12 aged 31–38 weeks (group B). Fetal blood samples were obtained by direct puncture of the umbilical vein in utero. Fetal blood samples were taken to rule out β thalassaemia, chromosome alterations, mother to fetus transmissible infections, and for maternal rhesus factor. GHBP was determined by gel filtration chromatography of serum incubated overnight with ^{125}I-GH. GH, IGF-I and IGFBP-3 were determined by radioimmunoassay.
Results—Fetal serum GH concentrations in group A (median 29 µg/l, range 11–92) were significantly higher (P<0.01) than those of group B (median 16.7 µg/l, range 4.5–29). IGF-I in group A (median 20 µg/l, range 4.1–53.3) was significantly lower (P<0.01) than in group B (median 75.2 µg/l, range 27.8–122.3). Similarly, IGFBP-3 concentrations in group A (median 950 µg/l, range 580–1260) were significantly lower than those of group B (median 1920 µg/l, range 1070–1770). There was no significant difference between GHBP values in group A (median 8.6%, range 6.6–12.6) and group B (median 8.3%, range 6–14.3). Gestational age correlated positively with IGF-I concentrations (P<0.0001) and IGFBP-3 (P<0.0001) and negatively with GH (P<0.0001). GHBP values did not correlate with gestational age. Multiple regression analysis showed a negative correlation between GH:IGF-I ratio and fetal growth indices
Conclusions—The simultaneous evaluation of fetal GH, IGF-I, IGFBP-3 and GHBP suggests that the GH-IGF-I axis might already be functional in utero. The progressive improvement in the efficiency of this axis in the last part of gestation does not seem to be due to an increase in GH receptors.
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Growth hormone (GH) and insulin-like growth factor I (IGF-I) are the major promoters of postnatal growth, but their role in human fetal growth has yet to be defined. It is widely accepted that GH is not critical for fetal growth, in spite of very high circulating concentrations.[1–5] However, clinical and laboratory findings suggest that GH has an important role in the control of fetal growth, at least in the last part of intrauterine life.[6 7] A correlation between fetal growth and IGF-I has been found in various studies,[8–10] and the recent report of a patient with intrauterine growth retardation and deletion of the IGF-I gene[11] indicates that IGF-I is essential for prenatal growth.

The bioavailability of IGFs and GH at the cellular level is modulated by specific binding proteins. Six IGF binding proteins (IGFBP) have been identified and IGF-I is bound predominantly to IGFBP-3, the major form present.[12] To date, only a few studies on fetal serum IGFBP-3 concentration have been carried out.[13–15] The high affinity, low capacity GH binding protein (GHPB), identical with the extracellular binding domain of the GH liver receptor, is considered to be a good indicator of tissue GH receptor numbers.[16] The finding in fetal human serum of low concentrations of GHBP has confirmed that there are relatively few GH receptors in fetal tissues,[17] although it has also been reported that intrauterine growth is positively correlated with GHBP.[18]

The associations between GH, IGF-I, GHBP-3, GHBP and postnatal growth have been widely studied, but few investigations have reported on these growth factors in fetal blood samples taken in utero.[8–10 19 20] As far as we are aware, no studies have simultaneously evaluated GH, IGF-I, and their binding proteins. Therefore, having had the opportunity to examine blood samples from fetuses ranging from 20 to 38 weeks of gestation, we thought it would be useful to evaluate GH, IGF-I, GHBP and IGFBP-3 values during fetal life.

Methods

Fetal blood samples were obtained from 53 fetuses, 41 aged 20–26 weeks (group A) and 12 aged 31–38 weeks (group B). Fetal blood samples were taken in group A to rule out β-thalassaemia, chromosome alterations, and mother to fetus infections. In group B fetal blood was sampled for rapid fetal karyotyping and for maternal rhesus factor (the obstetric history included perinatal mortality). There were no cases of maternal glucose intolerance, hypertension, and other complications of pregnancy. The fetal blood sample tests in the cases selected for this study were normal and pregnancy continued to term.

Fetal development was assessed from ultrasonographic assessment of cranial biparietal diameter, femoral length, and abdominal circumference. Fetal weight was estimated according to the method of Hadlock *et al.*[21] The estimated weights for each fetus were appropriate for gestational age.

Fetal blood samples were obtained by direct puncture of the umbilical vein in utero, as described before.[22] The size of the red cells was determined immediately before removing the needle from the umbilical vein using a cell analyser (Coulter Counter; Coulter Electronics Ltd, Harpenden Herts, UK) to ensure that no maternal blood was mixed. Plasma was separated and stored at −20°C.

GHBP was determined by gel filtration chromatography (GFC). Serum (200 μl) was incubated overnight at room temperature with ^{125}I-GH (previously purified by GFC) in the presence or in absence of 5 μg of unlabelled GH. The incubation mixture was filtered on Millex HV filters (0.45 μ, 13 mm, Millipore; Bedford, USA) and injected in the chromatograph equipped with a Superdex 200-HR 10/30 column (Pharmacia, Uppsala, Sweden). Elutions were carried out with PBS buffer (0.1 M, pH 7.4) at 0.5 ml/min. Fractions of 1 min were collected and counted for radioactivity in a gamma counter. This method identifies three separate peaks: the first peak represents a low affinity GH binding (GHBP-1), the second a high affinity and low capacity GH binding (GHBP-II), and the third free ^{125}I GH.

Total binding of ^{125}I-GH to high affinity GHBP was expressed by dividing the radioactivity in the second peak by the sum of the radioactivities in the first, second, and third peaks, multiplied by 100. Non-specific binding was calculated in the same way using the radioactive elution profile of serum incubated with ^{125}I-GH in the presence of unlabelled GH. The GHBP is given as the percentage of specific binding calculated as the difference between total and non-specific binding. These results were corrected for occupancy of endogenous GH on the basis of a displacement curve obtained by adding increasing concentrations of unlabelled human GH to the reference serum. The intra-assay coefficient, at 34.1% of binding, was 3.6% (n=5) and the interassay coefficient, at the levels of 29.8%, was 3.3% (n=5).

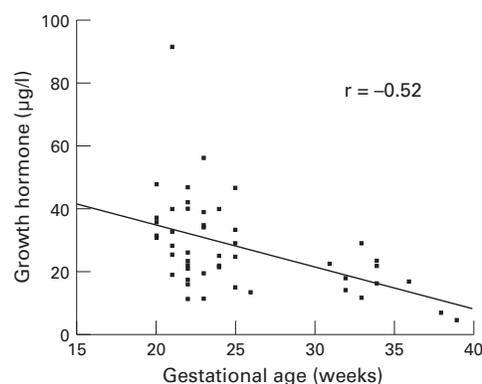Serum IGF-I values were determined using a commercial kit (Nichols Institute Diagnos-

Figure 1   Correlation between GH values and gestational age.

tics, San Juan Capistrano, CA) after an acid–ethanol extraction. The sensitivity of the method was 0.06 ng/ml and intra- and interassay coefficients of variation were 2.6 and 9.5 %, respectively, at the level of 1.1 ng/ml.

Serum IGFBP-3 concentrations were evaluated using an RIA kit (DSL Inc., Webster, Texas). The minimum detectable of IGFBP-3 that can be distinguished from 0 is 1.1 ng/ml at the 95% confidence limit. Intra- and interassay coefficients of variation were 5.2% and 6.9%, respectively, at the level of 2.5 ng/ml.

Serum GH was measured using a commercial liso-solid phase RIA assay (Technogenetics, Milan, Italy). The standards were calibrated against reference preparation WHO 80/205. The intra- and interassay coefficients of variation were, respectively, 5.8% and 9.7% at the level of 1.2 μg/l, and 5.3% and 8.9%, at the level of 12.5 μg/l. Cross reactivity was less than 1% for prolactin and human placental lactogen. Sensitivity of the assay was 0.1 μg/l, as determined by the mean + 2 SD of the zero standard.

In an attempt to express with one variable the efficiency of the GH-IGF-I axis, the ratio between these two factors was calculated in each blood sample.

For molar comparison between IGF-I and IGFBP-3, 7.5 and 30.5 kiloDaltons, respectively, were used.[23]

STATISTICAL ANALYSIS

The computer program Statistical Package for Social Science (SPSS Inc., Chicago, Ill) was
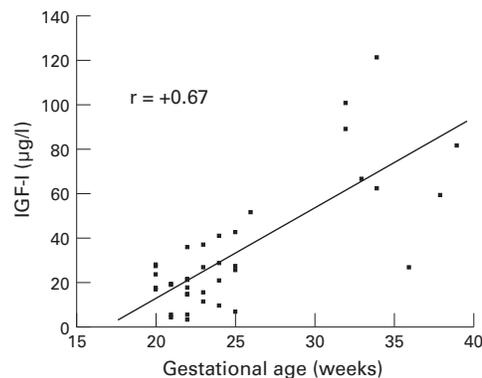

Figure 2   Correlation between IGF-I values and gestational age.

Table 1   Correlation between indices of growth and gestational age, GH:IGF-I ratio and IGF-I:IGFBP-3 ratio

Indices of growth	Fetal weight	Abdominal circumference	Femoral length
Correlation index	GA r = 0.93*	GA r = 0.94*	GA r = 0.90*
	GH:IGF-I r = -0.81*	GH:IGF-I r = -0.80*	GH:IGF-I r = -0.80*
	IGF-I:IGFBP-3 r = 0.53**	IGF-I:IGFBP-3 r = 0.53**	IGF-I:IGFBP-3 r = 0.53**
Multiple regression	GA R^2 = 0.92*, SE = 0.09,	GA R^2 = 0.92*, SE = 0.03,	GA R^2 = 0.84*, SE = 0.05,
	PRC = 0.89	PRC = 0.88	PRC = 0.78
	GH:IGF-I R^2 = 0.95*,	GH:IGF-I R^2 = 0.94*,	GH:IGF-I R^2 = 0.89*,
	SE = 0.07, PRC = −0.59	SE = 0.03, PRC = −0.51	SE = 0.04, PRC = −0.54

GA = Gestational Age; SE = Standard Error; PRC = Partial Correlation Coefficient.
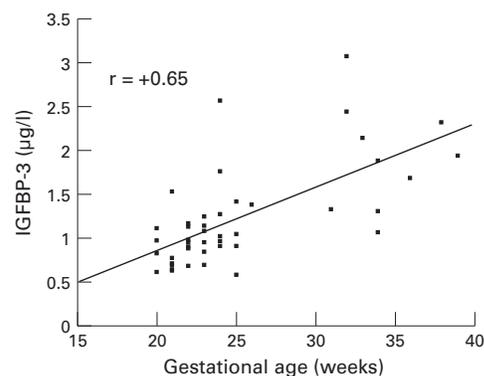* $P < 0.0001$, ** $P < 0.001$.

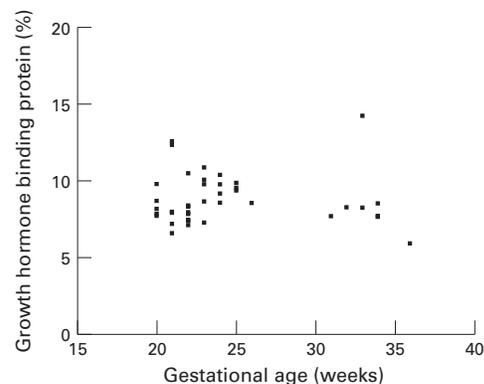*Figure 3   Correlation between IGFBP-3 values and gestational age.*

Figure 4   Correlation between GHBP values and gestational age.

used on an IBM computer. Data distribution was analysed with skewness and Kurtosis coefficients. Data are expressed as median and range. Where possible, a normal distribution was obtained after logarithmic transformation of data with a non-normal distribution. For normally distributed data, the significance was assessed using Student's t test, the Pearson correlation index and multiple regression analysis. For non-normally distributed data, the Pearson correlation index computed on the ranks and the Mann-Whitney test were used. All results nominally significant at $P < 0.05$ were indicated.

Results

The fetal GH, IGF-I, IGFBP-3, GHBP concentrations are reported in figs 1 to 4.

Fetal serum GH values in group A (median 29 µg/l, range 11–92) were significantly higher ($P < 0.01$) than those of group B (median 16.7 µg/l, range 4.5–29 µg/l). Overall, an inverse correlation between gestational age and GH was found (r = −0.52; $P < 0.0001$).

In group A, IGF-I concentrations (median 20 µg/l, range 4.1–53.3 µg/l) were significantly lower ($P < 0.01$) than those of group B (median 75.2 µg/l, range 27.8–122.3 µg/l). Gestational age correlated positively with IGF-I (r = 0.76; $P < 0.0001$), an inverse correlation also being found between GH and IGF-I (r= −0.67; $P < 0.0001$). However, this became non-significant by multiple regression analysis when gestational age was included in the regression.

The IGFBP-3 values (median 950 µg/l, range 580–1260 µg/l) were significantly lower in group A than in group B (median 1920 µg/l, range 1070–1770 µg/l). IGFBP-3 correlated positively with gestational age (r = 0.65; $P < 0.0001$), and with IGF-I (r = 0.72; $P < 0.0001$). This was confirmed by multiple regression analysis (gestational age $P < 0.0001$; IGF-I $P < 0.05$). The molar ratio IGF-I:IGFBP-3 in group A (median 0.08, range 0.06–0.12) was lower ($P < 0.01$) than that in group B (median 0.14, range 0.11–0.19). The molar ratio IGF-I:IGFBP-3 correlated positively with gestational age (r= 0.39; $P < 0.01$).

There was no significant difference between respective GHBP values in group A (median 8.6%, range 6.6–12.6%) and group B (median 8.3%, range 6.0–14.3%). GHBP values did not correlate with age.

The correlations between indices of growth and gestational age, GH:IGF-I ratio, and IGF:IGFBP-3 ratio are reported in table 1; multiple regression analysis confirmed the correlation between the indices of growth and the GH:IGF-I ratio, but not with the IGF-I:IGFBP-3 ratio.

Discussion

Our results, obtained in fetal serum samples at 20 to 38 weeks of gestational age, showed that as pregnancy progresses GH and IGF-I behave differently—that is, GH concentrations decrease as IGF-I increases.

The association between GH, IGF-I, and postnatal growth is well known. In contrast, a similar association between GH, IGF-I, and growth in the fetal period is more controversial. Clinical studies, have suggested that congenital GH deficiency is not associated with growth failure at birth, but the numbers of patients studied have been very small.[3] [5] However, this contradicts growth failure at birth associated with GH insensitivity[24] that occurs in many sibships with deletion of the GH gene.[25] Furthermore, a multicentre study of 52 patients[7] found that congenital GH deficiency

may cause significant prenatal growth failure. The central role of IGF-I as an endocrine promoter of fetal growth was initially suggested by the finding of a positive correlation between IGF-I and growth itself[8–10] and by the results obtained in animal models where growth delay was induced by hypoxia[26] or where quantitative[27 28]/qualitative[29] reduction of maternal food intake was associated with a fall in IGF-I concentrations. Moreover, Woods *et al*[11] have recently described a patient affected by severe intrauterine growth retardation and deletion of the IGF-I gene.

Only two studies have examined in vivo fetal GH concentrations.[19 20] The first showed that the increased GH values measured during gestation tended to fall after the 25th week, although this did not reach significance. The second described a significant and progressive decrease in GH values that was associated with a concomitant increase in IGF-I during the second half of gestation. Our investigation agrees with these results. Our finding of increased GH concentrations associated with very low IGF-I values seems to mirror that of Laron dwarfism[30] where receptor insensitivity blocks the influence of GH on IGF-I production. Nevertheless, similar to the data of Leger *et al*,[20] GH and IGF-I values do not remain stable, but change as fetal development progresses, eliciting particularly significant differences after the 30th week (figs 1 and 2). Animal studies have indicated the presence in utero of a GH-IGF-I axis[6 31] and Legers *et al*[20] recently found higher GH and lower IGF-I values in growth retarded human fetuses than in normally grown fetuses, supporting the hypothesis of an early functioning GH-IGF-I axis. In our study the inverse correlation between GH and IGF-I, after including gestational age in multiple regression analysis, became non-significant. However, we found an inverse relation between GH:IGF-I ratio and the indices of fetal growth when the correlation with gestational age at the time of cordocentesis was excluded. This would seem, therefore, to support the existence of a GH:IGF-I axis and also to underline the value of its efficiency.

In the circulation IGFBP-3 binds about 80% of IGFs[12] and its serum concentration is regulated in healthy children primarily by endogenous GH secretion.[32] This correlation between GH, IGF-I and IGFBP-3 is considered so close that evaluation of IGFBP-3 serum concentrations was suggested by Blum *et al*[33] as an excellent tool for screening GH deficiency. Our results confirm that fetal IGFBP-3 concentrations correlate with gestational age[10 34] and with IGF-I.[12] The IGF-I:IGFBP-3 molar ratio may reflect the free biologically active IGF-I, and the finding in the pubertal period of a marked increase in that ratio would seem to support its clinical importance.[23] Although our results seem to suggest that the gradual increase in IGF-I in fetuses is associated with a parallel increment in its bioavailability, we found no correlations between IGF-I:IGFBP-3 ratio and the indices of fetal growth when the influence of gestational age was included. This may be due, on the other hand, to the fact that many factors in the fetus can affect IGF-I bioavailability, such as IGFBP-3 protease activity. This is considered an important mechanism which may itself be related to the efficiency of the GH transduction pathway.[35]

Our study indicates that GHBP is already present in the fetus from the 20th week of gestation (fig 4). GHBP values in fetal blood seem to be much lower than after birth (Pirazzoli P, DallaCasa P, Salvioli GP, *et al*. Serum GH-BP II concentrations in the human fetus after blood collection in utero: comparison with infant. 34th Annual Meeting of the European Society for Paediatric Endocrinology 1995; abstract 151) also confirmed by the investigations using cord sera of premature and full term infants by Massa *et al*[18] and Barrios *et al*.[34] Unlike these studies, of premature neonates, our results did not show any correlation between GHBP, gestational age, and fetal growth. Our results seem to suggest the hypothesis that before birth the maturation of a GH-IGF-I axis is not the result of an increase in GHBP as this happens postnatally,[34] but rather a progressive maturation of the systems regulating IGF-I synthesis in which nutritional factors could have a determining role.[27] However, although GHBP values are widely assumed to reflect GH-receptor numbers, further studies are required to strengthen our hypothesis, because single GHBP measurements may not be sufficient to define the complexity of receptor status and turnover.

In conclusion, the simultaneous evaluation of fetal GH, IGF-I, IGFBP-3 and GHBP suggests that the GH-IGF-I axis could already be functional in utero. The progressive improvement in the efficiency of this axis in the last part of gestation does not seem to be due to an increase in the GH receptor numbers.

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