Maternal and fetal insulin-like growth factors and their binding proteins in the second and third trimesters of human pregnancy

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The insulin-like growth factors (IGFs) are mitogenic polypeptides which circulate bound to a series of at least six binding proteins (IGFBPs). An increasing body of evidence supports a major role for the IGF in the control of human fetal growth although normal values in the human fetal circulation have not been established. In order to provide an accurate reflection of fetal IGFs and IGFBPs in utero, we have sampled fetal blood direct from the umbilical cord at 18–38 weeks of gestation using the technique of cordocentesis. We have measured IGF-I, IGF-II and IGFBP 1–3 in 91 fetuses in order to establish concentrations for these parameters in the second and third trimesters of human pregnancy.

Key words: human/IGFBP/IGF/pregnancy

Introduction

The insulin-like growth factors (IGFs) have been considered to be important in fetal growth and development since the observation of high concentrations of IGFs in the fetal rat (Moses et al., 1980). The IGFs and their binding proteins appear to have a role at all stages of pregnancy and are known to be present in embryological fluids in the first trimester (Miell et al., 1997). An increasing body of evidence has demonstrated an association between IGF concentrations and growth in human pregnancy (Foley et al., 1980; Bennet et al., 1983; Gluckman et al., 1983; Ashton et al., 1985; D’Ercole et al., 1986; Lassarre et al., 1991; Wang et al., 1991b; Kubota et al., 1992; Baldwin et al., 1993; Fant et al., 1993; Verhaeghe et al., 1993; Langford et al., 1994; Reece et al., 1994; Spencer et al., 1995; Giudice et al., 1995; Spencer et al., 1995; Nieto-Diaz et al., 1996). Such studies may not provide a true reflection of the fetus in utero as changes in the IGFs and insulin-like growth factor binding proteins (IGFBPs) may occur during fetal demise or during the process of delivery which makes profound demands on fetal reserves. Indeed changes in IGFBP-1 thought to be due to the stress of delivery have been recorded (Hills et al., 1994; Wang et al., 1995). Even when the delivery has been by elective Caesarean section there may be changes reflecting maternal fasting, stress, anaesthesia or transient hypotension.

A more accurate reflection of the fetal IGF/IGFBP axis may be found in serum obtained from the fetus in utero. This may be achieved by cordocentesis in which a needle is passed across the maternal abdomen, into the uterus and into an umbilical cord vessel under ultrasound guidance without maternal fasting or sedation from 18 weeks gestation onwards (Nicolaides et al., 1986). Studies using fetal serum obtained at cordocentesis found that IGF-I concentrations, but generally not IGF-II concentrations, increased with gestational age and correlated with fetal size (Lassarre et al., 1991; Bang et al., 1994; Langford et al., 1994; Reece et al., 1994; Arosio et al., 1995; Ostlund et al., 1997). IGFBP-3 has also been demonstrated to increase with gestation (Bang et al., 1994; Reece et al., 1994) and the overall fetal binding protein profile was found to be similar to that seen in hypopituitary adults (Lassarre et al., 1991).

Work in this field is currently hampered by the lack of reference ranges for IGFs and IGFBPs in fetal serum from normally grown fetuses. We therefore measured IGF-I, IGF-II, IGFBP-1, IGFBP-2 and IGFBP-3 in fetal blood obtained from 91 normally grown fetuses and their mothers in the second and third trimesters of human pregnancy. The values obtained were used to calculate reference ranges for fetal IGF-I, IGF-II and IGFBP 1–3 from 18–40 weeks gestation.

Materials and methods

IGF-I, IGF-II, IGFBP-1, IGFBP-2 and IGFBP-3 were measured in fetal blood from 91 pregnancies at 18–38 weeks gestation undergoing diagnostic cordocentesis. Samples were obtained by cordocentesis. In 57 cases maternal blood was obtained from the antecubital vein immediately before fetal blood sampling. The indications for sampling were: prenatal diagnosis of genetic disorder (n = 6); fetal karyotyping for advanced maternal age, failed amniocyte culture or abnormal maternal serum biochemistry (n = 14); investigation of maternal primary toxoplasmosis (n = 1); fetal blood grouping in isoimmunized pregnancies (n = 15); karyotyping for structural abnormality e.g. mild bilateral hydronephrosis, talipes (n = 55).

The study had the approval of the King’s College Hospital ethical
committee and written, informed consent was obtained from the mother in all cases.

IGF-I was measured by radioimmunoassay after acid-ethanol extraction of the binding proteins and displacement of IGF from binding proteins by addition of excess unlabelled IGF-II. This assay was performed by W.Blum (Tubingen, Germany). The intra- and interassay coefficient of variation (CV) was 4.0 and 9.6% respectively (Blum and Breier, 1994).

IGF-II was measured by radioimmunoassay after acid-ethanol extraction of the binding proteins and displacement of IGF from binding proteins by addition of excess unlabelled IGF-I. The intra- and interassay CV at 0.6 µg/l was 3.6 and 12.2% respectively and the assay had 0.05% cross-reactivity with IGF-I and a sensitivity of 0.018 ng/tube (Blum et al., 1988).

IGFBP-1 was measured by specific radioimmunoassay. The minimum detection limit of the assay was 1.5 µg/l. The interassay CV at 55 µg/l was 6.2% and the intra-assay CV at 35 µg/l was 4% (Miell et al., 1993).

IGFBP-2 was measured by specific radioimmunoassay by W. Blum (Tubingen, Germany). The assay has a sensitivity of 30 µg/l. Intra- and interassay CV was 3.7 and 9.6% respectively (Blum et al., 1993).

IGFBP-3 was measured by specific radioimmunoassay. The sensitivity of the assay was 0.03 µg/l. The intra- and interassay CV at 3.5 mg/l was 4.2 and 11.4% respectively (Miell et al., 1993).

All assays were not performed on all samples due to insufficient sample size in a few cases.

**Statistical analysis**

Regression analysis was used to examine any relationship between measured variables and gestational age. Data or residuals from linear regression were tested for normality. For those measurements that were not normally distributed, the distribution was made Gaussian by logarithmic transformation. For those that changed significantly with gestation, the regression lines were used to calculate the adjusted means and residual standard deviations (SD). To produce the reference ranges with gestation in the original units the limits of the calculated reference range in logarithms were subjected to anti-logarithmic transformation. Reference ranges are shown as mean, fifth and 95th confidence intervals.

Where parameters did not vary with gestation the mean and standard error of the mean are given.

**Results**

Fetal IGF-I concentrations increased with gestational age ($P < 0.0001$, $r = 0.780$) (Figure 1). Fetal IGF-II concentrations increased with gestational age ($P = 0.0005$, $r = 0.377$) (Figure 2). Fetal IGFBP-2 concentrations increased with gestational age ($P < 0.0001$, $r = 0.445$) (Figure 3). Fetal IGFBP-3 concentrations increased with gestational age ($P < 0.0001$, $r = 0.642$) (Figure 4). Fetal IGFBP-1 did not vary significantly with gestation (mean 300.0 ± 25.1 µg/l).

Maternal IGF-I concentrations increased significantly with gestation ($P = 0.0013$, $r = 0.415$) (Figure 5). Other maternal parameters did not vary significantly with gestational age (mean IGF-II: 499.9 ± 24.07 µg/l; mean IGFBP-1: 104.39 ± 13.15 µg/l; mean IGFBP-2: 110.4 ± 7.19; mean IGFBP-3: 3.95 ± 0.09 mg/l).

**Discussion**

We have established normal values for gestation for fetal and maternal IGF-I, IGF-II and IGFBP-1 to -3 where these parameters vary with gestational age. Such data have previously been lacking in this field. This may be due to ethical issues raised by performing invasive procedures such as cordocentesis which carry a risk of miscarriage in order to obtain fetal blood for research.
In this study all women were undergoing cordocentesis in order to obtain fetal blood for prenatal diagnosis and the study involved collection of an additional small volume of blood at the time of sampling, thus minimizing any extra risk to the fetus. Fetuses requiring prenatal diagnosis may not be truly representative of the normal population; however, it would obviously be ethically unacceptable to expose entirely normal pregnancies to the risk of miscarriage for research purposes alone. The predominant indication for cordocentesis was karyotyping when an anatomical marker of chromosomal abnormality had been identified by ultrasound scanning. These markers included findings such as talipes, clinodactyly and mild hydronephrosis. Major differences in the IGF axis in such fetuses compared to entirely normal fetuses would not be expected on theoretical grounds.

Our data confirm that fetal IGF-I and IGF-II both increase with gestational age as has been previously reported in fetal blood obtained at cordocentesis (Lassarre et al., 1991; Bang et al., 1994; Reece et al., 1994; Ostlund et al., 1997). IGF-II concentrations were consistently higher than IGF-I concentrations. Fetal IGFBP-1 did not vary with gestational age. This supports the study of Bang et al. (1994) which also used samples obtained by cordocentesis. In serum obtained from the umbilical cord post-delivery, IGFBP-1 has been reported to decrease with gestation (Lewitt et al., 1995). However, since the process of delivery can have profound effects on fetal IGFBP-1 concentrations it is unclear whether this reflects IGFBP-1 concentrations in utero (Hills et al., 1994; Wang et al., 1995). IGFBP-2 in fetal serum showed a significant increase with gestation. Previously concentrations have been reported to show no change with gestation (Bang et al., 1994).

IGFBP-3 in fetal serum showed a significant rise with gestation. This would be consistent with previous reports that IGFBP-3 becomes the major IGF binding protein in fetal serum in the latter half of pregnancy (D’Ercole et al., 1980;
Baldwin, S., Chung, T., Rogers, M. et al. (1994). In maternal serum IGFBP-3 undergoes proteolysis which decreases its affinity for IGF-I and results in an increase in circulating free IGF-I (Hasegawa et al., 1995). IGFBP-3 proteolysis may result in spuriously high IGFBP-3 concentrations being reported by radioimmunoassay if intact IGFBP-3 and IGFBP-3 fragments are both recognized (Langford et al., 1995). However, significant IGFBP-3 proteolysis does not occur in human fetal serum (Bang et al., 1994; Langford et al., 1995) and it would therefore appear likely that the rise in fetal IGFBP-3 reported here is genuine. IGFBP-2 is also subject to proteolysis and it is possible that this may influence concentrations reported by radioimmunoassay. IGFBP-2 proteolysis is not known to occur in human fetal serum but this is an area where further work is needed.

In maternal serum only IGF-I was found to increase with gestation. This is consistent with previous reports (Wilson et al., 1982; Hall et al., 1984; Mirlesse et al., 1993; Hasegawa et al., 1995). None of the IGFBPs studied showed an increase in maternal serum with gestational age at the gestations studied here. IGFBP-1 is known to increase rapidly in early pregnancy before reaching a peak at 12–13 weeks then remaining raised until term (Wang et al., 1991a). Since our data apply to the latter half of pregnancy it is not surprising that no rise with gestation was observed and this has also been reported by Hills et al. (1996). IGFBP-2 concentrations assessed by Western ligand blotting have previously been reported to decrease throughout gestation, possibly due to proteolysis resulting in decreased ligand binding (Giudice et al., 1990). We did not find such a decrease but our method of assessment was radioimmunoassay which may still recognize proteolysed binding protein as is the situation with IGFBP-3. Maternal IGFBP-3 has been reported to rise in pregnancy when assessed by radioimmunoassay (Baxter and Martin, 1986) but we did not confirm such a rise despite a strong correlation with maternal IGF-I concentrations.

This paper presents data on IGF and IGFBP concentrations in pregnancies with normally grown fetuses. The pregnancies sampled may not be regarded as entirely representative of the normal population in that they required cordentesis for prenatal diagnosis. However, since ethical constraints do not allow collection from uncomplicated pregnancies we believe these data represent an important contribution to this field.

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References


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