Regulation of insulin-like growth factor-binding protein-3 ternary complex formation in pregnancy

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Abstract

The IGFs are believed to be important in pregnancy and are implicated in the pathophysiology of pre-eclampsia. In adults the IGFs circulate primarily with IGF-binding protein-3 (IGFBP-3) and an acid-labile glycoprotein (ALS) in a 140 kDa complex which limits IGF bioavailability. Less than 10% of IGFBP-3 is in lower molecular weight forms. We have investigated the developmental regulation of the IGF/IGFBP system in normal and pre-eclamptic pregnancies with particular emphasis on the IGFBP-3 ternary complex. Circulating levels of IGF-I, IGFBP-3 and ALS, and their degree of association in the ternary complex in the fetus increased with gestational age. In neonatal serum from deliveries <35 weeks' gestation IGFBP-3 was predominantly in 30–50 kDa form(s)

and ALS was a limiting factor for ternary complex formation. In serum from deliveries >35 weeks both ALS and IGFs were limiting but approximately 25% of IGFBP-3 was unable to form the ternary complex even in the presence of excess ALS and IGF-I. Serum IGFBP-1, -2 and -6 concentrations tended to decrease with increasing gestational age. In pre-eclamptic pregnancies, amniotic fluid IGFBP-2, -3 and -6 levels decreased with gestational age while IGFBP-1 levels did not show the normal decline. We speculate that the endocrine IGF system develops in the fetus during the third trimester of pregnancy when ALS levels increase.

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Introduction

The insulin-like growth factor (IGF) system is believed to be important in fetal growth (Daughaday & Rotwein 1989, Wang & Chard 1992). IGF-I and IGF-II and their receptors are widely expressed in the human fetus, suggesting that they might play important paracrine or autocrine roles (Daughaday & Rotwein 1989). The bioavailability and activity of the IGFs is determined by their association with a family of IGF-binding proteins, the IGFBPs, which are also expressed and secreted by a wide range of human fetal tissues (Hill & Clemmons 1992, Delhanty *et al.* 1993, Pannier *et al.* 1994, Bralke *et al.* 1996). Protease activity may be an important determinant of the paracrine function of the IGFBPs in the fetus (Bralke *et al.* 1996). The IGFs appear to have an important endocrine function in the adult. They circulate predominantly in a 140 kDa complex with IGFBP-3 and an acid-labile glycoprotein (ALS) and also in association with IGFBP-3 and the other IGFBPs in binary complexed forms (Baxter 1994). IGF-I and IGFBP-3 concentrations in the adult circulation tend to vary in parallel, supporting the observation that association of an IGF with IGFBP-3 is

required before a ternary complex with ALS can form *in vitro* (Baxter & Martin 1989) or *in vivo* (Lewitt *et al.* 1993). Furthermore ALS is crucial to the stability of the IGFs and of IGFBP-3 in the circulation (Lewitt *et al.* 1993) and determines their circulating half lives of many hours (Guler *et al.* 1989). A proportion of IGFBP-3 also exists in forms of lower molecular weight in the circulation and these forms have half lives of approximately 10 min (Lewitt *et al.* 1993). It is not yet clear whether the IGFs have a significant endocrine role in the human fetus. Certainly IGF-I and IGFBP-3 also appear to be regulated in a coordinated manner, increasing with gestational age with a significant positive relationship to parameters of fetal growth (Fant *et al.* 1993). In contrast, circulating IGFBP-1 and IGFBP-2 levels are higher than in the adult circulation (Wang & Chard 1992, Blum *et al.* 1993, Schwander & Mary 1993, Bang *et al.* 1994b) and correlate inversely with IGF-I levels (Fant *et al.* 1993) and with birthweight (Verhaeghe *et al.* 1993, Giudice *et al.* 1995).

Concentrations of IGFs and IGFBPs are also altered in the maternal circulation compared with the non-pregnant state. There is an increase in IGFBP-3 levels measurable by RIA (Baxter & Martin 1986). However, when samples

are subjected to SDS-PAGE and detected by blotting with radioiodinated IGF there is an apparent reduction in IGFBP-3 (Giudice *et al.* 1990, Hossenlopp *et al.* 1990). This discrepancy is explained by the observations that, although IGFBP-3 in pregnancy serum is able to bind endogenous IGF-I and radiolabelled ALS (Suikkari & Baxter 1992), it has a lower affinity and accelerated dissociation kinetics for iodinated IGF-I (Suikkari & Baxter 1991, Lassarre & Binoux 1994) due to a cation-dependent protease (Liu *et al.* 1992, Lassarre & Binoux 1994). The size distribution of circulating IGFs is not altered in maternal serum (Davies *et al.* 1991, Gargosky *et al.* 1991, Liu *et al.* 1992). Although the potential exists for altered bioavailability of IGFs the actual effect of this protease on IGF delivery to the tissues during pregnancy is not known. Compared with non-pregnant values, when measured by RIA, maternal IGFBP-1 levels increase rapidly, peak in the first trimester (Rutanen *et al.* 1982, Wang *et al.* 1991b) and have been shown to correlate inversely with birthweight (Howell *et al.* 1985, Hall *et al.* 1986). Maternal IGFBP-2 concentrations appear to decrease on ligand blotting (Giudice *et al.* 1990); however, the levels measured by RIA are variably reported to be low-normal (Schwander & Mary 1993) or to increase (Chard *et al.* 1994).

The pre-eclampsia syndrome is a major cause of perinatal morbidity. It has been suggested that the IGF system might play a role in its pathogenesis, since there is increased mitogenic activity of unknown origin in the maternal plasma (Musci *et al.* 1988). Increased circulating levels of maternal IGFBP-1 have been observed in pre-eclampsia, although there is substantial overlap of values with control subjects at similar stages of pregnancy (Than *et al.* 1984, Iino *et al.* 1986, Howell *et al.* 1989). Maternal IGFBP-3 levels have variably been reported to be normal (Varma *et al.* 1993) or low (Wang *et al.* 1996).

Since the IGFBP-3 ternary complex has a fundamental role in determining IGF bioavailability, the primary aim of this study was to examine its formation in the circulation of neonates of various gestational ages. In addition, because of the uncertain role of IGFs in pre-eclampsia, we obtained samples from pre-eclamptic pregnancies. We have measured the components of the complex (IGF-I, IGF-II, IGFBP-3 and ALS) and examined the factors determining its formation. In addition we describe the levels of IGFBP-1, -2 and -6 in these neonates. The above parameters were also determined in maternal serum, neonatal arterial serum and amniotic fluid from the same pregnancies.

Materials and Methods

Samples

This study was conducted in accordance with the guidelines of the Declaration of Helsinki and with the approval

of the Human Ethics Committee, Royal Prince Alfred Hospital. Samples were collected at the time of caesarean section from ten singleton pregnancies of 26–39 weeks' gestation complicated by pre-eclampsia, as defined by the Australasian Society for the Study of Hypertension in Pregnancy (Australasian Society for the Study of Hypertension in Pregnancy 1993). The degree of pre-eclampsia ranged from mild to severe. There were two control groups, one not in labour and undergoing caesarean section at 38–39 weeks' gestation ($n=11$), and a second having spontaneous vaginal deliveries at 22–40 weeks ($n=15$). Glucose tolerance was normal in all of the pregnancies and there were no serious maternal illnesses or evidence of fetal distress in either control group. Maternal serum, umbilical artery and vein sera, and amniotic fluid samples were obtained at the time of the caesarean section deliveries. Umbilical vein serum was obtained from the vaginal delivery group. All samples were stored at -80°C until analysis.

Assays

Using methods previously described, IGFBP-1 (Baxter *et al.* 1987), IGFBP-2 (Baxter *et al.* 1995), IGFBP-3 (Baxter & Martin 1986), IGFBP-6 (Baxter & Saunders 1992) and ALS (Baxter 1990) levels were determined by RIA. Preliminary data were reported in the proceedings of an international workshop on IGFBPs (Lewitt *et al.* 1995). IGF-I levels were measured by RIA after acid-ethanol extraction as previously described (Baxter *et al.* 1982), except that des(1–3)IGF-I (GroPep, Adelaide, Australia), iodinated using the chloramine-T method to a specific activity of approximately $100\text{ }\mu\text{Ci}/\mu\text{g}$, was used as tracer. The effective assay range was $0.01\text{--}5.0\text{ ng/assay tube}$. The within-assay imprecision for $20\text{ }\mu\text{l}$ samples was 6.9% measured at $0.25\text{ ng/assay tube}$. IGF-II levels were measured after acid-ethanol extraction by RIA using radioiodinated des(1–6)IGF-II (GroPep) as previously described (Baxter *et al.* 1995). Radioiodinated des(1–3)IGF-I and des(1–6)IGF-II have reduced affinity for IGFBPs (Yamamoto *et al.* 1996).

Superose-12 chromatography

The molecular weight distribution of IGFBP-3 in serum was determined by Superose-12 gel permeation chromatography (Pharmacia, Uppsala, Sweden) in 50 mmol/l sodium phosphate, 100 mmol/l sodium chloride and 0.02% sodium azide, pH 6.5. The column calibration was performed as previously described (Baxter 1990). Serum ($30\text{ }\mu\text{l}$) was diluted to $120\text{ }\mu\text{l}$ in column buffer and $100\text{ }\mu\text{l}$ were injected via a V-7 valve (Pharmacia). The column was run at 1 ml/min and 0.5 ml fractions collected. Aliquots of $200\text{ }\mu\text{l}$ (umbilical cord samples) and $25\text{ }\mu\text{l}$ (maternal samples) were assayed in duplicate for IGFBP-3. Fractions 22–24 represent approximately 140 kDa and

Table 1 Maternal levels of IGFs and IGFBPs (ng/ml). IGF and IGFBP concentrations were measured by RIA in serum samples from 11 women not in labour undergoing caesarean section at 38–39 weeks' gestation and from 10 women undergoing caesarean section for pre-eclampsia. The pre-eclamptic group is divided into <35 weeks' gestation ($n=5$) and >35 weeks' gestation ($n=5$). Results are means $\pm$ S.E.M.

Peptide	Term controls (38–39 weeks)	Pre-eclamptic (<35 weeks)	Pre-eclamptic (>35 weeks)
IGF-I	457 $\pm$ 75	235 $\pm$ 55*	501 $\pm$ 84
IGF-II	432 $\pm$ 44	396 $\pm$ 98	548 $\pm$ 118
IGFBP-1	317 $\pm$ 37	424 $\pm$ 101	383 $\pm$ 134
IGFBP-3	4670 $\pm$ 610	5120 $\pm$ 1600	7040 $\pm$ 1100
ALS	24 260 $\pm$ 1800	25 970 $\pm$ 2020	35 220 $\pm$ 2980

* $P<0.05$, less than term control and less than pre-eclampsia >35 weeks' gestation.

fractions 25–27 represent 30–50 kDa forms of IGFBP-3. In experiments designed to determine the factors limiting formation of the IGFBP-3 ternary complex, serum samples of 30 μ l were incubated *in vitro* with and without 1 μ g recombinant human IGF-I (Kabi Pharmacia Peptide Hormones, Stockholm, Sweden) plus or minus 0.5 μ g human ALS purified from human serum (Baxter 1990) in a final volume of 120 μ l in column buffer. After a 30 min incubation at 22 °C, 100 μ l were size fractionated by Superose-12 chromatography and 200 μ l aliquots assayed for IGFBP-3 by RIA.

Statistics

Results are expressed as the mean $\pm$ S.E. Comparisons between groups were made using ANOVA and Fisher's protected least significant difference post hoc test and correlations were analysed using StatView (Abacus Concepts, Berkeley, CA, USA). The groups with samples from a range of gestational ages were also divided into

groups of <35 weeks and >35 weeks. The latter group was comparable with the term caesarean controls.

Results

Maternal values

Maternal serum samples were available from the two caesarean section groups. In the pre-eclamptic women IGF-I levels were seen to increase during gestation ($r=0.583$, $P<0.05$). Table 1 shows the IGF-I, IGF-II, IGFBP-1, IGFBP-3 and ALS values with the samples in the pre-eclampsia group divided into those <35 weeks' and those >35 weeks' gestation. There was no difference between the IGF-I levels in the five pre-eclamptic women >35 weeks' gestation and in the term caesarean controls. IGF-II, IGFBP-1, IGFBP-3 and ALS levels did not change with gestation and in the pre-eclamptic group were not significantly different from those in caesarean controls. IGFBP-2 levels varied over a wide range in the pre-eclampsia group. There were two very high values of 1103 and 1372 ng/ml in pregnancies of 27 and 31 weeks' gestation and in the other eight pre-eclamptic pregnancies the values were 278 ± 34 ng/ml, not significantly different from those in term controls (220 ± 59). No clinical parameters separated the women with the high IGFBP-2 levels from the rest of the group. IGFBP-6 levels decreased with gestation ($r = -0.805$, $P<0.001$).

Amniotic fluid values

Samples of amniotic fluid were available from the two caesarean section groups. Table 2 shows the IGF-I, IGF-II, IGFBP-1 and IGFBP-3 values in amniotic fluid with the pre-eclampsia group divided into <35 weeks' and >35 weeks' gestation. Low concentrations of IGF-I and -II were detected in amniotic fluid. They did not vary with gestational age and the pre-eclamptic values were similar to those in controls. IGFBP-1 levels were higher in the

Table 2 Amniotic fluid levels of IGFs and IGFBPs (ng/ml). IGF and IGFBP concentrations were measured by RIA in amniotic fluid samples from 11 women not in labour undergoing caesarean section at 38–39 weeks' gestation and from 10 women undergoing caesarean section for pre-eclampsia. The pre-eclamptic group is divided into <35 weeks' gestation ($n=5$) and >35 weeks' gestation ($n=5$). Results are means $\pm$ S.E.M.

Peptide	Term controls (38–39 weeks)	Pre-eclamptic (<35 weeks)	Pre-eclamptic (>35 weeks)
IGF-I	11.1 $\pm$ 1.0	8.9 $\pm$ 1.2	10.5 $\pm$ 1.3
IGF-II	59 $\pm$ 4	57 $\pm$ 8	51 $\pm$ 6
IGFBP-1	20 400 $\pm$ 5400	55 500 $\pm$ 12 200*	106 400 $\pm$ 89 300*
IGFBP-3	2860 $\pm$ 320	7210 $\pm$ 730 [†]	3770 $\pm$ 720

* $P<0.05$, greater than term controls.

[†] $P<0.05$, greater than pre-eclampsia >35 weeks.

Figure 1 Relationship between gestational age and IGFBP-2 levels measured by RIA in samples from (A) amniotic fluid and (B) umbilical cord vein serum. □ Caesarean section controls ($n=11$); ○ vaginal deliveries ($n=15$); ■ pre-eclamptic pregnancies ($n=10$).

pre-eclamptic group compared with the caesarean controls ($P<0.05$) and, unlike our previous observations in normal pregnancies (Baxter *et al.* 1987), did not vary with gestational age ($r = -0.090$). IGFBP-3 levels diminished with gestational age in pre-eclamptic pregnancies ($r = -0.890$, $P<0.001$), in a similar manner to that previously reported for normal deliveries (Baxter *et al.* 1987). ALS was not detected in amniotic fluid. Figure 1A shows that the IGFBP-2 levels in amniotic fluid diminished with gestational age in the pre-eclamptic group ($r = -0.695$, $P<0.005$). In the pregnancies >35 weeks the amniotic fluid IGFBP-2 concentrations did not differ significantly from those in caesarean controls. IGFBP-6 levels diminished with gestational age ($r = -0.854$, $P<0.001$). Thus, total amniotic fluid IGFBP levels greatly exceeded IGF levels and diminished during gestation.

Neonatal serum values

Neonatal serum was obtained from the two control groups and from the pregnancies complicated by pre-eclampsia. IGFBP-1 levels in neonatal serum fell with increasing gestational age ($r = -0.434$, $P<0.05$) but were not different between pre-eclamptic deliveries and the vaginal delivery control group. In the pregnancies <35 weeks neonatal IGFBP-1 levels were 520 ± 186 ng/ml and 981 ± 730 ng/ml in the pre-eclamptic and control group respectively (not significant (NS)). In the deliveries >35 weeks values were 161 ± 44 ng/ml and 278 ± 114 ng/ml respectively (NS). The relationship between IGFBP-2 levels and gestational age is shown in Fig. 1B. There was a trend for IGFBP-2 levels to fall during gestation in the pre-eclamptic group ($r = -0.608$, $P=0.06$). IGFBP-6 decreased with gestational age. This was statistically significant for the vaginal delivery control group ($r = -0.659$, $P<0.003$) and did not reach statistical significance for the group with pre-eclampsia ($r = -0.502$, $P=0.14$).

The ternary complex with IGFBP-3, ALS and IGF is of primary importance in limiting IGF availability from the circulation. IGF-I levels for the three groups are shown in Fig. 2A. The increase in IGF-I levels with gestational age was significant in the pre-eclamptic group ($r=0.706$, $P<0.005$). As previously published, IGF-II levels did not vary with gestational age and were not significantly different between the three groups. IGF-II levels in the vaginal delivery group were 183 ± 35 ng/ml, and the values in the pre-eclamptic group were 122 ± 42 ng/ml. Figure 2B and C show the IGFBP-3 and ALS levels respectively in umbilical cord vein samples. These peptides increased with gestational age in parallel with IGF-I and did not differ significantly between samples from pregnancies complicated by pre-eclampsia (IGFBP-3, $r=0.890$, $P<0.001$; ALS, $r=0.839$, $P<0.002$) and those from the vaginal delivery control group (IGFBP-3, $r=0.677$, $P<0.005$; ALS, $r=0.730$, $P<0.002$).

The above parameters were measured in umbilical cord arterial samples and there was no significant difference in the values obtained compared with those of umbilical cord vein samples (data not shown).

Studies of ternary complex formation

The size distribution of IGFBP-3 in cord vein serum was determined by subjecting the samples to Superose-12 chromatography. Figure 3A shows the size distribution of IGFBP-3 in the cord serum of neonates of the caesarean control group (38–39 weeks' gestation). Fractions 22–24 represent IGFBP-3 in the 140 kDa ternary complex and fractions 25–27, the ~ 50 kDa form(s) of IGFBP-3. In this control group most of the IGFBP-3 in the circulation was present in the ternary complex. Figure 3B shows the size distribution in cord serum taken at the time of caesarean section from pregnancies complicated by pre-eclampsia. In contrast to the pattern of distribution in the term controls,

Figure 2 Relationship between gestational age and umbilical cord vein levels of components of the IGFBP-3 ternary complex. (A) IGF-I, (B) IGFBP-3, (C) ALS levels. IGFBP-3 and ALS were measured by RIA. □ caesarean section controls ($n=11$); ○ vaginal deliveries ($n=15$); ■ pre-eclamptic pregnancies ($n=10$).

there was less IGFBP-3 in the ternary complex in the cord samples taken from pre-eclamptic pregnancies of <35 weeks' gestation so that the 140 kDa and 50 kDa peaks were of similar size. In the pre-eclamptic samples taken from >35 weeks' gestation the size distribution of IGFBP-3 was similar to that in the caesarean control group.

A similar developmental pattern of ternary complex formation was seen in the group of vaginal deliveries. Figure 4A demonstrates that in cord serum from these neonates delivered at <35 weeks' gestation the 140 kDa and 50 kDa peaks were also of similar size. In the vaginal delivery control group the IGFBP-3 molecular weight distribution pattern in neonatal samples from >35 weeks' gestation was similar to that in the caesarean control group

and to that of pre-eclamptic deliveries of >35 weeks' gestation. In Fig. 4B we have expressed the degree of ternary complex formation as a ratio of IGFBP-3 in Superose-12 column fractions 22–24 (140 kDa) to the IGFBP-3 in fractions 25–27 (50 kDa). There was a significant correlation between the 140 kDa/50 kDa ratio and gestational age in the cord serum from the control vaginal deliveries ($r=0.805$, $P<0.001$) and from the pre-eclamptic caesarean deliveries ($r=0.834$, $P<0.001$). These two groups did not differ significantly with respect to the degree of ternary complex formation, and at term approximately 30% of IGFBP-3 was in lower molecular weight form(s) in both groups. In contrast, when we subjected maternal serum from both the pre-eclamptic group and the caesarean controls to Superose-12 chromatography we

Figure 3 Molecular distribution of IGFBP-3 in umbilical cord serum taken at the time of caesarean section. Serum samples of 25 µl were size fractionated on a Superose-12 column and IGFBP-3 measured by RIA in each 500 µl fraction. (A) Samples from 11 controls of 38–39 weeks' gestation. (B) Samples from pre-eclamptic pregnancies of <35 weeks' gestation (□ $n=5$) and >35 weeks' gestation (■ $n=5$). Values are means $\pm$ S.E.M.

Figure 4 Molecular distribution of IGFBP-3 in umbilical cord serum from vaginal deliveries. Serum samples of 25 μ l were size fractionated on a Superose-12 column and IGFBP-3 measured by RIA in each 500 μ l fraction. (A) Samples from neonates of <35 weeks' gestation ($\circ$ $n=9$) and >35 weeks' gestation ($\bullet$ $n=6$). Values are means $\pm$ S.E.M. (B) Ternary complex formation is presented as a ratio of IGFBP-3 in the 140 kDa and 50 kDa forms. Ternary complex formation correlated with gestational age in cord samples from both the pre-eclamptic caesarean deliveries (dotted line, $r=0.834$, $P<0.001$) and the vaginal deliveries without pre-eclampsia (solid line, $r=0.805$, $P<0.001$). $\bullet$, Caesarean section controls ($n=11$); $\circ$, vaginal deliveries ($n=15$); $\blacksquare$, pre-eclamptic pregnancies ($n=10$).

found that less than 10% of circulating IGFBP-3 was not associated in the ternary complex (data not shown), confirming previous reports (Liu *et al.* 1992, Suikkari & Baxter 1992).

The size distribution of IGFBP-3 was measured in umbilical cord arterial samples and, compared with umbilical cord venous samples, there was no significant difference in the patterns observed (data not shown).

In order to determine whether IGFBP-3 in the ~ 50 kDa form(s) in neonatal serum was able to form a ternary complex with IGF-I and ALS we incubated serum

samples with excess IGF-I plus excess ALS and compared the IGFBP-3 molecular distribution with serum in the absence of additions. In Fig. 5A we show, using serum from premature vaginal deliveries in the presence of excess IGF-I and ALS, that a proportion of IGFBP-3 in the ~ 50 kDa peak was able to shift to the 140 kDa form. In term vaginal deliveries, the IGFBP-3 in the ~ 50 kDa form did not shift to 140 kDa in the presence of excess IGF-I and ALS but there appeared to be a significant increase in the recovery of IGFBP-3 in the 140 kDa form (Fig. 5B). These data are expressed as a 140 kDa/50 kDa

Figure 5 Molecular distribution of IGFBP-3 in umbilical cord serum in the presence and absence of excess IGF-I and ALS. Serum (25 μ l) was incubated at 22 $^{\circ}$ C for 2 h in the absence ($\blacksquare$) and presence ($\square$) of 1 μ g recombinant human IGF-I and 0.5 μ g human ALS, and then size fractionated on a Superose-12 column and IGFBP-3 measured by RIA in each 500 μ l fraction. (A) Serum from three vaginal deliveries of 20–31 weeks' gestation. (B) Serum from three vaginal deliveries of 37–38 weeks' gestation. Values are means $\pm$ S.E.M.

Figure 6 Effect of excess IGF-I and ALS on IGFBP-3 molecular distribution in umbilical cord serum of <35 weeks' and >35 weeks' gestation. There were three serum samples in each group. Serum (25 µl) was incubated at 22 °C for 2 h in the presence of 1 µg recombinant human IGF-I (hatched bars), 11 µg IGF-I plus 0.5 µg human ALS (closed bars) or no additions (open bars), and then size fractionated on a Superose-12 column and IGFBP-3 measured by RIA in each 500 µl fraction. Ternary complex formation is presented as a ratio of IGFBP-3 in the 140 kDa and 50 kDa forms. Values are means $\pm$ S.E.M. Ternary complex formation was greater in cord serum from >35 weeks' gestation ($P<0.001$ in the presence of IGF-I, IGF-I plus ALS and no additions). * $P<0.05$, ** $P<0.01$, *** $P<0.001$ (compared with no additions). †† $P<0.01$, ††† $P<0.001$.

ratio in Fig. 6, where in addition we show the effect of an excess of IGF-I alone on ternary complex formation. In the premature sera, ALS and not IGF-I appeared to be a limiting factor for IGFBP-3 ternary complex formation. However, even in the presence of ALS, approximately one-third of IGFBP-3 was unable to shift to the 140 kDa form. In the term sera both ALS and IGF-I were limiting factors for ternary complex formation of IGFBP-3. The addition of excess IGF-I alone significantly increased the 140 kDa/50 kDa ratio ($P<0.05$), which increased further with the addition of excess ALS ($P<0.01$) so that less than 25% of IGFBP-3 was in the ~50 kDa form(s).

Discussion

In this paper we have further characterised the IGF/IGFBP system in normal pregnancy and in pregnancies complicated by pre-eclampsia. There were two comparison groups to control for the impact of caesarean delivery

and gestational age when examining the effect of pre-eclampsia. Our primary focus was to examine in neonates the developmental regulation of the IGFBP-3 ternary complex, since this complex is crucial to determining the bioavailability of endocrine IGFs. A clear developmental regulation of the ternary complex and its components was observed, with no difference between pre-eclamptic and control samples. IGFBP-3, ALS and IGF-I, and their association in the ~140 kDa complex, increased with gestational age during the second half of pregnancy. This increase in total IGF-I (Bennett *et al.* 1983, Ashton *et al.* 1985, Lassarre *et al.* 1991, Verhaeghe *et al.* 1993, Bang *et al.* 1994b) and the free form of IGF-I (Hasegawa *et al.* 1995) has previously been observed. IGFBP-3 is the primary circulating IGFBP in the second half of pregnancy, as it is in the adult non-pregnant circulation. The increase in IGFBP-3 with gestation has been documented previously (Bang *et al.* 1994b, Langford *et al.* 1994), although one study demonstrated no change with gestation (Giudice *et al.* 1995). In this study we have documented a concomitant increase in ALS levels. Bang and coworkers provided indirect evidence that ALS levels are low in fetal serum, by assessing the ability of endogenous ALS to shift exogenously added IGFBP-3 and IGF-II into the 140 kDa form (Bang *et al.* 1994a).

ALS is fundamental to formation of the IGFBP-3 ternary complex and our results would suggest that ALS levels are responsible for the coordinate regulation of IGF-I and IGFBP-3 in the fetal circulation. What determines the increase in ALS during the third trimester? ALS is a growth hormone (GH)-dependent protein in the adult human circulation (Baxter 1990). However, studies in isolated rat hepatocytes suggest that there may be additional regulators of its production although no other positive regulators have been identified (Dai *et al.* 1994). GH is present and is secreted by the fetal pituitary as early as 5 weeks of gestation. However, the high GH level until 24 weeks of gestation suggests the presence of GH insensitivity during the first and mid trimesters (Gluckman 1995). Falling GH concentrations in the third trimester and the ALS response observed during that time in our study would indicate the development of sensitivity to GH actions, as a result of changes in the pathways of GH signal transduction and/or ALS synthesis, in late gestation and support a role for GH in late fetal growth.

In contrast to the components of the ternary complex, IGFBP-1, -2 and -6 tended to fall with increasing gestational age. Although previously published data would suggest that circulating IGFBP-1 and IGFBP-2 are elevated in fetuses with evidence of uteroplacental insufficiency (Langford *et al.* 1994), in our study there was no difference between the pre-eclamptic and control groups. A confounding factor in comparing these two groups is the effect of labour. If IGFBP-1 levels were elevated in relation to the stress of labour (Hills *et al.* 1996) to a greater

degree than the stress of caesarean section, then any differences due to the effect of pre-eclampsia could have been masked. In addition our study group represented a range of severity of pre-eclampsia.

There was no difference in IGF and IGFBP levels between arterial and venous cord samples, supporting the concept of synthesis of endocrine IGFs (Wang *et al.* 1991a) and endocrine IGFbps within the fetus. Furthermore the degree of ternary complex formation was similar in arterial and venous samples, indicating that the placental unit does not contribute to ternary complex synthesis or degradation.

Total IGF levels are very low in amniotic fluid and are exceeded by IGFBP concentrations by three orders of magnitude. In the pre-eclamptic group amniotic fluid IGFBP-1 levels did not show the decline with gestational age (Baxter *et al.* 1987) and it is possible that the loss of this relationship related to fetal growth retardation as has been documented in early pregnancy (Hakala-Ala-Pietilä *et al.* 1993). The question of whether this was of specific importance would need to be addressed with a gestational age-matched control group. Levels of IGFBP-2, -3 and -6 in amniotic fluid decreased with weeks of gestation. Taken together with previous studies of IGFBP-1 (Rutanen *et al.* 1982, Baxter *et al.* 1987, Wathen *et al.* 1993, Nonoshita *et al.* 1994), IGFBP-2 (Chard *et al.* 1994) and IGFBP-3 (Nonoshita *et al.* 1994) it is likely that in amniotic fluid IGFbps increase to reach peak levels in the first half of pregnancy and then decline. The role of this huge excess of IGFbps in amniotic fluid is yet to be determined.

Previous observations have suggested that a paracrine IGF system is of primary importance in early fetal growth, with widespread tissue expression of both IGF-I and IGF-II in early-mid gestation and widespread expression of IGF receptors (Daughaday & Rotwein 1989, Wang & Chard 1992). The IGFbps also seem to have a paracrine role as they are ubiquitous in mid gestational tissues (Hill & Clemmons 1992, Delhanty *et al.* 1993, Pannier *et al.* 1994, Braulke *et al.* 1996). The endocrine role of IGF-I in postnatal growth is generally accepted (Wang & Chard 1992). Our observations would suggest that in the third trimester of pregnancy a circulating reservoir of IGFs is formed, supporting development of the endocrine IGF system in prenatal life. Further support for this concept is the observation that despite its relatively low concentrations, IGF-I is positively correlated with gestational age and birthweight. This endocrine system is not fully developed at birth and ALS deficiency appears to be the primary limiting factor in its formation, while in contrast in the adult ALS circulates in excess (Baxter 1990). In addition approximately one-third of IGFBP-3 in the term neonate is unable to form a ternary complex and the concentrations of the other IGFbps are higher than in the adult circulation. The immature endocrine IGF system is thus characterised by a predominance of IGFs in binary

complexes which could favour a high rate of IGF turnover and transport to the fetal tissues.

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