Insulin-like growth factor-binding protein-1 in umbilical artery and vein of term fetuses with signs suggestive of distress during labor

J Verhaeghe, J Billen and L C Giudice

Department of Obstetrics and Gynaecology, and Laboratorium voor Experimentele Geneeskunde en Endocrinologie, Katholieke Universiteit Leuven, Leuven, Belgium

Abstract

Insulin-like growth factor-binding protein-1 (IGFBP-1) is believed to be an inhibitory factor for fetal growth. The regulation of IGFBP-1 secretion in the fetus is uncertain, although insulin and oxygen tension (PO2) and saturation are thought to play a role. We studied IGFBP-1 levels in umbilical cord artery (UA) and vein (UV) of 98 singleton fetuses at term with clinical signs of distress during labor, i.e. meconium-stained liquor or/and an abnormal fetal heart rate tracing. Blood gas values and serum C-peptide and IGFBP-1 concentrations were measured in both UA and UV. Twenty-five fetuses had an UA pH<7.20. The concentrations of IGFBP-1 were similar in UA and UV and were highly correlated (r=0.98). IGFBP-1 levels were inversely correlated with birth weight, with increased concentrations in small-for-gestational age fetuses (≤10th weight percentile). IGFBP-1 levels were negatively correlated with C-peptide concentrations, and remained so after correction for birth weight (r=−0.37 for both UA and UV; P<0.001); more specifically, IGFBP-1 levels were increased in the lowest C-peptide quartile (<0.23 nmol/l) compared with the other quartiles. In addition, IGFBP-1 levels were inversely correlated with PO2 values (r=−0.39 in UA and r=−0.34 in UV; P<0.001); quartiles of UA and UV PO2 showed a gradual increase in IGFBP-1 concentrations with lower PO2 values. A regression model with C-peptide and PO2 values as independent variables predicted IGFBP-1 concentrations (R² of model was 0.25 and 0.22 for UA and UV respectively; P<0.001). Other blood gas values (pH, PCO2, HCO3− and base deficit) did not correlate with IGFBP-1 levels. The data of this study indicate that serum IGFBP-1 levels in term fetuses are determined by both insulin and PO2 levels, and suggest that acute hypoxemia stimulates IGFBP-1 secretion in the fetus.

Introduction

There is suggestive evidence that insulin-like growth factor (IGF)-binding protein-1 (IGFBP-1), one of the six IGF-binding proteins that bind the IGF ligands (IGF-I and IGF-II), is an inhibitory factor for fetal growth. In the fetus, the IGFBP-1 gene is mainly expressed in the liver (Han et al. 1996). IGFBP-1 levels have been documented to be increased in mixed umbilical cord (UC) serum or in umbilical artery (UA) and vein (UV) serum of small-for-gestational age (SGA, <10th birth weight percentile) fetuses or fetuses with clinically recognized intra-uterine growth retardation (IUGR) (Wang et al. 1991, Verhaeghe et al. 1993, Giudice et al. 1995, Osorio et al. 1996, Cianfarani et al. 1998b). Similar results were obtained with fetal serum sampled in utero by cordocentesis (Langford et al. 1994, Östlund et al. 1997). In a small group of twins discordant for birth weight, UC serum IGFBP-1 levels were markedly higher in the growth-retarded members (Verhaeghe et al. 1996). Serum IGFBP-1 concentrations were found to be negatively correlated with total and free IGF-I concentrations in normal fetuses at birth (Klauwer et al. 1997) and in IUGR fetuses in utero (Östlund et al. 1997) and at birth (Osorio et al. 1996, Cianfarani et al. 1998b). IUGR in animal models (rats and sheep) – induced by uterine artery ligation/clamping or maternal fasting – is consistently associated with increased liver IGFBP-1 mRNA abundance (Unterman et al. 1990, Straus et al. 1991, McLellan et al. 1992, Osborn et al. 1992, Price et al. 1992a). Conversely, birth weight was found to be reduced in some strains of transgenic mice that overexpress the IGFBP-1 gene (Rajkumar et al. 1995). Finally, the in vivo administration of IGFBP-1 abrogated the stimulatory effect of IGF-I on body growth in postnatal pituitary-ablated rats (Cox et al. 1994).
The regulation of IGFBP-1 production in the fetus is uncertain. The stimulatory effect of a drop in utero placental blood flow on fetal hepatic IGFBP-1 gene expression may be mediated, at least in part, by hypoinsulinemia or hypoxemia in these fetuses. Indeed, insulin is the major determinant of circulating IGFBP-1 levels after birth in humans and experimental animals, with increased levels in type 1 diabetes but suppressed levels under hyperinsulinemic conditions (reviewed by Lee et al. 1997). In some studies, IUGR in human fetuses (Cianfarani et al. 1998b) and uterine artery ligation in the rat (Unterman et al. 1990) was associated with lower insulin levels. However, we found no difference in UC serum IGFBP-1 levels among two groups of 18 gestational age-matched fetuses with widely different C-peptide levels (Verhaeghe et al. 1993). Regarding fetal oxygenation, UV IGFBP-1 levels were found to be higher in 22 fetuses with an abnormal cardiotocograph tracing during labor than in 19 control fetuses, and there was a negative correlation between the UV pH value and IGFBP-1 levels in the distressed fetuses (Crawford et al. 1995). A chronic reduction in oxygen saturation without uterine artery manipulation in pregnant rats resulted in IUGR and a robust increase in IGFBP-1 mRNA abundance in the fetal liver (Tapanainen et al. 1994). Experiments in sheep showed that the infusion of nitrogen into the maternal trachea acutely raises fetal plasma IGFBP-1 levels, as quantified by ligand and immunoblot analysis (Iwamoto et al. 1992); however, intermittent cord occlusion had no effect on fetal liver IGFBP-1 mRNA abundance (Green et al. 2000).

In the current study, we measured C-peptide and IGFBP-1 levels in the UA and UV of human fetuses with clinical suspicion of hypoxemia during labor. Complete blood gas analysis was carried out in both UA and UV.

Materials and Methods

We sampled UA and UV blood from 98 term pregnancies (37–42 weeks) at delivery. Multiple pregnancies, and pregnancies with congenital malformations, diabetes mellitus or other medical conditions were excluded. All fetuses were clinically suspected to be hypoxicemic in the course of labor either by the presence of meconium-stained liquor (n=9) or the presence of an abnormal electronic fetal heart rate tracing (n=61), or both (n=28). Meconium-stained liquor included any degree of meconium-staining of amniotic fluid (brown or green), detected at spontaneous or artificial rupture of membranes or later in the course of labor. Abnormalities in the fetal heart rate were defined as two or more periods of bradycardia, or repeated late (type 2) or variable decelerations, detected at any time during labor before the delivery process started. Some patients underwent one or more fetal scalp blood gas measurements during labor to guide clinical management. Because of clinical suspicion of fetal distress, delivery was expedited; 25 patients delivered spontaneously, 33 had an instrumental delivery (forceps or vacuum-extraction) and 40 underwent an (semi-) urgent cesarean section. All patients consented orally that blood be sampled from the cord, or consented orally that sampled cord blood could be used for scientific purposes. Blood samples were taken after cleaning the cord with a sterile swab before the placenta was delivered. For blood gas analysis, a heparin-containing syringe was used; for the other measurements, the blood sample was collected into a dry tube. Blood gas analysis was performed within a few minutes on an ABL 700 Analyzer (Bronsnoj, Denmark); values for pH, PCO2, PO2, HCO3− and base deficit were recorded. All blood samples were centrifuged within a short interval, and serum was stored at −20 °C. The series included 42 girls and 56 boys; their birth weight, length and head circumference, as well as gestational age at birth were recorded. The ponderal index (the fetal equivalent of the body mass index) was calculated as birth weight/length3. The percentile (P) of birth weight was classified into categories (≤P10, P11–50, P51–90, and >P90) according to recently updated birth weight charts derived from about 429,000 births in Flanders, Belgium (Devlieger et al. 2000).

IGFBP-1 was measured with an in-house radioimmunoassay (RIA), using as standard purified IGFBP-1 from amniotic fluid, which was standardized with an enzyme-linked immunoassay from Medix Biochemica (Kauniainen, Finland) that measures total IGFBP-1. The detection limit is 25 pg IGFBP-1 per tube. The within- and between-assay coefficients of variation are <10% (Verhaeghe et al. 1999). C-peptide was measured by RIA, as described previously (Verhaeghe et al. 1993).

Data analysis was performed with a software program (NCSS, Kaysville, UT, USA).

Results

UA and UV concentrations of IGFBP-1 and C-peptide were tightly correlated (r=0·98 for both parameters; P<0·001). There was no difference between IGFBP-1 levels in UA and UV (n=97; P=0·76), but there was a trend towards lower C-peptide concentrations in UA than in UV in the 96 available UA-UV pairs (Δ: 0·019 ± 0·101 nmol/l (mean ± s.d.); P=0·067). Mean ± s.d. (range) values for IGFBP-1 were 112 ± 128 (12–1090) µg/l (3·50 ± 3·99 nmol/l) in UA (n=97) and 113 ± 115 (9–878) µg/l (3·54 ± 3·58 nmol/l) in UV (n=98). Because IGFBP-1 and C-peptide data were unequally distributed, the data were log-transformed for further analyses. Each of the histograms of the log10-transformed data was compatible with a Gaussian distribution (data not shown). Log10 IGFBP-1 concentrations in both UA and UV were negatively correlated with log10 C-peptide concentrations (r=−0·40 for both UA and UV; n=96 and 97 respectively; P<0·001).
Blood gas analyses showed lower PO₂ (−8.4 ± 6.3 mm Hg, mean ± s.d.; n=94) and pH (−0.045 ± 0.064, n=98) values in UA than in UV (both P<0.001), but higher PCO₂ (7.6 ± 5.6 mm Hg, n=95) and HCO₃⁻ (2.9 ± 18.8 mEq/l, n=95) values (both P<0.001). There was a trend for higher base deficit values in UA than in UV (0.16 ± 0.95 mEq/l, n=94; P=0.076). Twenty-five fetuses (26%) had an UA pH<7.20, 11 fetuses had an UA pH<7.10, and 3 fetuses had an UA pH<7.00.

Pearson correlation analysis (row-wise deletion) in 91 UA and 92 UV samples in which all biometric parameters at birth, blood gas values, and IGFBP-1 and C-peptide measurements were available, showed that UA log₁₀ IGFBP-1 was negatively correlated with weight at birth (r=−0.20; P=0.06), ponderal index (r=−0.39; P<0.001), UA PO₂ (r=−0.39; P<0.001) and UA log₁₀ C-peptide (r=−0.46; P<0.001). In the UV, UV log₁₀ IGFBP-1 was negatively correlated with birth weight (r=−0.19; P=0.07), ponderal index (r=−0.39; P<0.001), UV PO₂ (r=−0.34; P<0.001), UV pH (r=−0.20; P=0.06) and UV log₁₀ C-peptide (r=−0.44; P<0.001). Log₁₀ C-peptide concentrations in UA and UV were positively correlated (P<0.10) with weight and length at birth as well as PO₂ and pH values but negatively correlated with pCO₂ (data not shown).

The negative correlation between UA and UV IGFBP-1 concentrations and birth weight was further explored by comparing IGFBP-1 levels in four groups of fetuses: SGA (≤ P₅₀) (n=15), average-for-gestational age (AGA) between P₁₁ and P₉₀ (n=39), AGA between P₅₁ and P₉₀ (n=34), and large-for-gestational age (>P₉₀) (n=10). There was a significant overall difference in UA and UV log₁₀ IGFBP-1 levels (ANOVA for both: P=0.007); UA and UV log₁₀ IGFBP-1 levels were increased in the SGA group compared with the other groups (Fig. 1 shows the UA data, UV data not shown). In the subgroup of fetuses with an UA pH ≥ 7.20 (n=72), log₁₀ IGFBP-1 levels in UA and UV were also higher in SGA fetuses (n=12) than in non-SGA fetuses (P=0.01).

Because both IGFBP-1 and C-peptide concentrations in UA and UV were correlated with birth weight, the negative correlation between IGFBP-1 and C-peptide was corrected for weight at birth: after correction, the negative correlation between log₁₀ IGFBP-1 and log₁₀ C-peptide remained highly significant (r=−0.37 for both UA and UV; P<0.001).

The negative correlation between UA and UV IGFBP-1 and C-peptide was further explored by comparing IGFBP-1 levels in quartiles of C-peptide levels. There was a significant overall difference (ANOVA: P<0.001 for both UA and UV). The analysis for UA is depicted in Fig. 2 (left panel): log₁₀ IGFBP-1 was significantly higher in the lowest quartile of C-peptide levels compared with the other three quartiles. The results were comparable for UV C-peptide (data not shown).

The negative correlation between UA and UV IGFBP-1 and PO₂ was further explored by comparing IGFBP-1 levels in quartiles of PO₂ values. There was a significant overall difference (ANOVA: P<0.001 for UA, and P=0.005 for UV). The analysis for UA is depicted in Fig. 2 (right panel): there was gradual increase in log₁₀ IGFBP-1 levels with lower PO₂ values. The results were comparable for UV PO₂ (data not shown).

Because IGFBP-1 was negatively correlated with some blood gas values (PO₂ and pH) as well as C-peptide levels, we performed multiple regression analyses with PO₂ and log₁₀ C-peptide as independent variables for the UA, and PO₂, pH and C-peptide as independent variables for the UV. In the UV analysis, pH was not retained as a significant predictor (P=0.85), while PO₂ (P=0.05) and log₁₀ C-peptide (P<0.001) were significant predictors (R² of model=0.22; P<0.001). Consequently, the final regression model for both UA and UV contained PO₂ and log₁₀ C-peptide as independent variables, showing that both variables were significant predictors of log₁₀ IGFBP-1 concentrations (Table 1).

**Discussion**

Several observations were made in this study. First, IGFBP-1 concentrations were almost identical in the UA and UV, and all analyses were in essence similar for the UA and UV. It would therefore suffice in further studies to restrict measurements to the UV, which is easier to sample. Similar results were obtained by Wang et al.  

![Figure 1](https://www.endocrinology.org)
(1991), who found a correlation of 0.90 in 56 UA-UV pairs.

Secondly, IGFBP-1 levels in UA and UV were negatively correlated with both insulin (C-peptide) and PO2 levels. The negative correlation with insulin concentrations in fetuses extends numerous studies in postnatal individuals (Lee et al. 1997). The negative correlation with PO2 values is not surprising either, and suggests that acute changes in PO2 in the fetus, prior or during labor, stimulate IGFBP-1 secretion by the liver. In fetal sheep, there is suggestive evidence that acute hypoxemia provokes a rise in IGFBP-1 levels (Iwamoto et al. 1992). Moreover, chronic hypoxemia is a well known stimulatory factor of hepatic IGFBP-1 gene expression in fetal sheep (McLellan et al. 1992) and rats (Tapanainen et al. 1994). Tazuke et al. (1998) showed that IGFBP-1 gene expression is stimulated by hypoxia in HepG2 cells. Three consensus sequences for the hypoxia response elements (HREs) within intron 1 of the IGFBP-1 gene were identified, at least one of which is functionally responsive to hypoxia. The induction of IGFBP-1 gene expression was found to be mediated via hypoxia-inducible-factor-1 (HIF-1). The human IGFBP-1 promoter also contains other regulatory elements, including an insulin response element and glucocorticoid response elements (Powell et al. 1995). Regarding the effect of corticoids, maternal corticoid treatment in rats was reported to induce IUGR and stimulate fetal liver IGFBP-1 gene expression (Price et al. 1992b). Also, UC serum IGFBP-1 concentrations were found to be positively correlated with cortisol levels in 15 human term AGA fetuses (Cianfarani et al. 1998a).

Phosphorylation of IGFBP-1 increases its affinity for IGF-I binding. UV serum contains phosphorylated as well as less- and non-phosphorylated isoforms of IGFBP-1; SGA fetuses were found to have increased levels of phosphorylated, but not non-phosphorylated, IGFBP-1 (Iwashita et al. 1996). Future studies should examine the phosphorylation pattern of IGFBP-1 in the fetal circulation in relation to PO2, insulin and cortisol levels.

The cut-off value for fetal hypoxemia during labor is uncertain. Thorp & Rushing (1999) reviewed data from 4 large studies (>1000 births) in which the UA PO2 was measured: mean values varied between 15·1 and 23·7 mm Hg, and −1 s.d. values were between 10·2 and 13·7 mm Hg. The mean UA PO2 value in this study was 17·7 mm Hg and the −1 s.d. value was 11·7 mm Hg. Although this study specifically sampled fetuses with signs suggestive of distress during labor, these clinical signs are known to have poor positive predictive value for fetal hypoxemia; in addition, action was taken to deliver the babies (semi-)urgently (74% of patients in this series were either instrumentally delivered or underwent an urgent cesarean section). The UA pH value is clinically the parameter

| Table 1 Multiple regression analysis of serum concentrations of IGFBP-1 (dependent variable) and PO2 and C-peptide values (independent variables) in the umbilical artery (UA) and vein (UV) of 98 fetuses with signs suggestive of distress during labor |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|
| **IGFBP-1 in UA**              | **IGFBP-1 in UV** |
| T-value | \(P\) level | T-value | \(P\) level |
| PO2   | \(-2.6\)        | \(0.001\) | \(-2.2\) | \(0.03\) |
| C-peptide | \(-3.4\) | \(0.001\) | \(-3.5\) | <0.001 |
| Intercept | \(13.5\) | <0.001 | \(13.8\) | <0.001 |
| R2 of model | \(0.25\) | <0.001 | \(0.22\) | <0.001 |

**Figure 2** Box-plots of serum concentrations of IGFBP-1 in quartiles of serum concentrations of C-peptide (left panel) and PO2 values (right panel) in the umbilical artery. The number of samples in each group is given in the box. Groups that are significantly different (\(P<0.05\)) from one another are denoted by a different letter.
most often used to assess in utero oxygenation. In the current study, 25/98 UA samples had a value below 7.20, commonly used in obstetrical practice as a cut-off value for cordocentesis or fetal scalp blood pH measurements. The [mean – 1 s.d.] UA pH value was reported to be between 7.11 and 7.23 in ten large population studies, with values of 7.16 and 7.19 in the largest two studies (Thorpe & Rushing 1999). However, the UA pH value is a measure of both the respiratory and metabolic acid-base status of the fetus. Tazuke et al. (1998) found elevated UA IGFBP-1 levels in fetuses with mixed metabolic/respiratory acidosis, but not in fetuses with respiratory acidosis. In this study, the pH value, as well as other measures of acid-base status (HCO3− and base deficit) were not significant predictors of UA or UV IGFBP-1 levels.

There is consensus that, on average, SGA fetuses have both lower IGF-I levels and higher IGFBP-1 levels in their serum than AGA fetuses. However, IGFBP-1 appears to have better discriminatory power to detect IUGR than IGF-I. Indeed, mean IGF-I levels in cord serum are at least sixfold lower than IGF-I levels in adult sera (Verhaeghe et al. 1999) – and there is a considerable overlap between IGF-I values in AGA and SGA babies. IGFBP-1 levels in cord serum, however, are severalfold higher than in adult sera (112 ± 128 µg/l (mean ± s.d.) in the UA of term fetuses in the current series, compared with 13.6 ± 9.7 µg/l in sera of adult women not taking oral contraceptives (Verhaeghe et al. 1999)); in twins with discordant birth weight, IGFBP-1 levels were markedly higher in the growth-retarded member (Verhaeghe et al. 1996). From these data, it would appear logical to evaluate the potential use of IGFBP-1 as a parameter to predict IUGR: to this end, IGFBP-1 could be measured in amniotic fluid or in maternal serum. We previously found that IGFBP-1 levels in second-trimester amniotic fluid at genetic amniocentesis, although present in high concentrations, cannot predict weight at birth (Verhaeghe et al. 1999). Maternal serum IGFBP-1 levels have been found to be increased at the time of diagnosis by ultrasound of severe IUGR at 22–26 weeks of pregnancy (Fowler et al. 1999). In addition, maternal serum levels of highly phosphorylated IGFBP-1 at 18 weeks of pregnancy were found to correlate negatively with birth weight in 44 women with type 1 diabetes (Gibson et al. 1999). Future studies on the predictive value of maternal serum IGFBP-1 are important and await further investigation.

Acknowledgements

We thank W Coopmans, M-J Leemput, R van Bree and E Van Herck for their advice and help, and the doctors and midwives of the Labor Ward for their help with the cord blood sampling.

This study was supported in part by National Institutes of Health Grant HD25220–09 to L C G.

References


McLellan KC, Cooper SB, Bocking AD, Delhanty PJD, Phillips ID, Hill DJ & Han VKM 1992 Prolonged hypoxia induced by the reduction of maternal uterine blood flow alters insulin-like growth


Received 7 November 2000
Accepted 30 May 2001