

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

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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 (≤ 10 th 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 (R^2 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.

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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/and 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 hypoxemic 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 (Brønshøj, Denmark); values for pH, PCO_2 , PO_2 , HCO_3^- and base deficit were recorded. All blood samples were centrifuged within a short interval, and serum was stored at $-20^\circ C$. 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 ($\leq P_{10}$, P_{11-50} , P_{51-90} , and $>P_{90}$) 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 \pm s.d.); $P=0.067$). Mean \pm s.d. (range) values for IGFBP-1 were 112 ± 128 (12–1090) $\mu g/l$ (3.50 ± 3.99 nmol/l) in UA ($n=97$) and 113 ± 115 (9–878) $\mu g/l$ (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 \log_{10} -transformed data was compatible with a Gaussian distribution (data not shown). \log_{10} IGFBP-1 concentrations in both UA and UV were negatively correlated with \log_{10} C-peptide concentrations ($r=-0.40$ for both UA and UV; $n=96$ and 97 respectively; $P<0.001$).

Blood gas analyses showed lower PO_2 (-8.4 ± 6.3 mm Hg, mean \pm s.d.; $n=94$) and pH (-0.045 ± 0.064 , $n=98$) values in UA than in UV (both $P<0.001$), but higher PCO_2 (7.6 ± 5.6 mm Hg, $n=95$) and HCO_3^- (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 parameters (biometric parameters at birth, blood gas values, and IGFBP-1 and C-peptide measurements) were available, showed that UA \log_{10} IGFBP-1 was negatively correlated with weight at birth ($r=-0.20$; $P=0.06$), ponderal index ($r=-0.39$; $P<0.001$), UA PO_2 ($r=-0.39$; $P<0.001$) and UA \log_{10} C-peptide ($r=-0.46$; $P<0.001$). In the UV, \log_{10} IGFBP-1 was negatively correlated with birth weight ($r=-0.19$; $P=0.07$), ponderal index ($r=-0.39$; $P<0.001$), UV PO_2 ($r=-0.34$; $P<0.001$), UV pH ($r=-0.20$; $P=0.06$) and UV \log_{10} C-peptide ($r=-0.44$; $P<0.001$). \log_{10} C-peptide concentrations in UA and UV were positively correlated ($P<0.10$) with weight and length at birth as well as PO_2 and pH values but negatively correlated with pCO_2 (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 ($\leq P_{10}$) ($n=15$), average-for-gestational age (AGA) between P_{11} and P_{50} ($n=39$), AGA between P_{51} and P_{90} ($n=34$), and large-for-gestational age ($>P_{90}$) ($n=10$). There was a significant overall difference in UA and UV \log_{10} IGFBP-1 levels (ANOVA for both: $P=0.007$); UA and UV \log_{10} 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 ($n=72$), \log_{10} 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 \log_{10} IGFBP-1 and \log_{10} 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): \log_{10} 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).

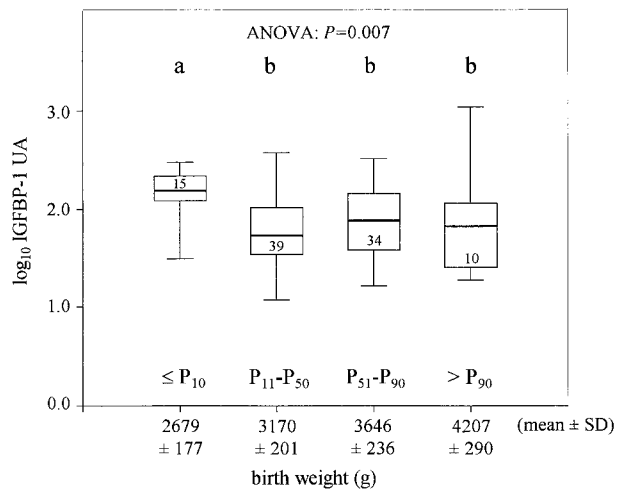


Figure 1 Box-plots of serum concentrations of IGFBP-1 in the umbilical artery in relation to birth weight percentiles: ≤ 10 th percentile ($\leq P_{10}$), P_{11-50} , P_{51-90} and $>P_{90}$. The number of samples in each group is given in the box. Groups that are significantly different ($P<0.05$) from one another are denoted by a different letter.

The negative correlation between UA and UV IGFBP-1 and PO_2 was further explored by comparing IGFBP-1 levels in quartiles of PO_2 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 \log_{10} IGFBP-1 levels with lower PO_2 values. The results were comparable for UV PO_2 (data not shown).

Because IGFBP-1 was negatively correlated with some blood gas values (PO_2 and pH) as well as C-peptide levels, we performed multiple regression analyses with PO_2 and \log_{10} C-peptide as independent variables for the UA, and PO_2 , 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 PO_2 ($P=0.05$) and \log_{10} C-peptide ($P<0.001$) were significant predictors (R^2 of model = 0.22; $P<0.001$). Consequently, the final regression model for both UA and UV contained PO_2 and \log_{10} C-peptide as independent variables, showing that both variables were significant predictors of \log_{10} 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.*

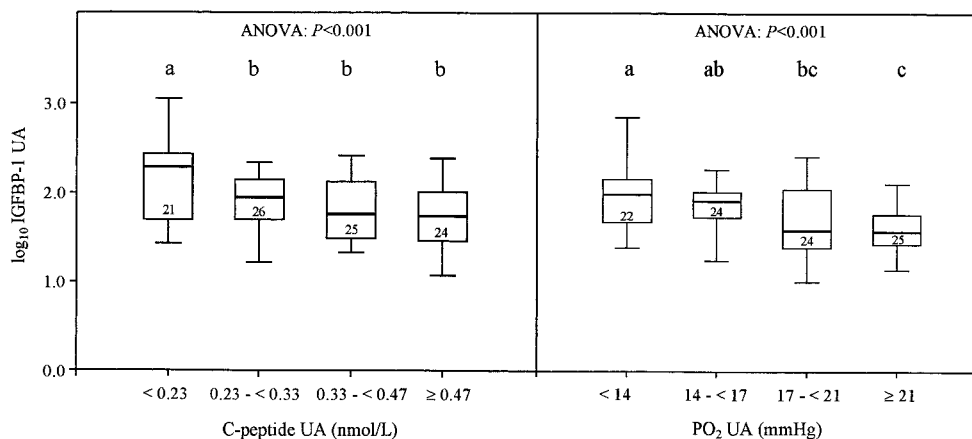


Figure 2 Box-plots of serum concentrations of IGFBP-1 in quartiles of serum concentrations of C-peptide (left panel) and PO_2 values (right panel) in the umbilical artery. The number of samples in each group is given in the box. Groups that are significantly different ($P < 0.05$) from one another are denoted by a different letter.

(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 PO_2 levels. The negative correlation with insulin concentrations in fetuses extends numerous studies in postnatal individuals (Lee *et al.* 1997). The negative correlation with PO_2 values is not surprising either, and suggests that acute changes in PO_2 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 PO_2 , 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 PO_2 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 PO_2 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 PO_2 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
PO_2	-2.6	0.01	-2.2	0.03
C-peptide	-3.4	0.001	-3.5	<0.001
Intercept	13.5	<0.001	13.8	<0.001
R^2 of model	0.25	<0.001	0.22	<0.001

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 \pm 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 (Thorp & 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 (HCO_3^- 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 very low – at least sixfold lower than IGF-I levels in adult sera (Verhaeghe *et al.* 1993) – 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 ($112 \pm 128 \mu\text{g/l}$ (mean \pm s.d.) in the UA of term fetuses in the current series, compared with $13.6 \pm 9.7 \mu\text{g/l}$ 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.

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