Cord serum insulin-like growth factor binding protein-1 and -3: effect of maternal diabetes and relationships to fetal growth

S Loukovaara1, RJ Kaaja2, RA Koistinen2

SUMMARY

Objectives: Insulin-like growth factor binding protein-1 and -3 (IGFBP-1 and -3) are the main insulin-like growth factor (IGF) carriers in fetal blood whose concentrations are regulated by hormonal factors such as insulin. IGFBPs may regulate fetal growth by altering the biological activity of IGF-I and IGF-II. We studied the effect of maternal diabetes on cord serum IGFBP-1 and IGFBP-3 levels, and the usability of IGFBP-1 and IGFBP-3 in the detection of birth weight variations.

Methods: Cord serum IGFBP-1 and IGFBP-3 concentrations were measured at birth by immunofluorometric assays in 67 pregnancies with type 1 diabetes and in 62 normal pregnancies.

Results: Concentrations of IGFBP-1 in cord serum were lower in diabetic pregnancies than in normal pregnancies (156 ± 28 µg/l vs 266 ± 29 µg/l, P = 0.007), whereas those of IGFBP-3 did not differ significantly (3327 ± 158 µg/l vs 2982 ± 105 µg/l, P = 0.076). IGFBP-1 correlated negatively and IGFBP-3 positively with birth weight z-score in diabetic pregnancies. The trend was similar in normal pregnancies. In multiple regression models, birth weight z-score was significantly associated with IGFBP-1 in diabetic and normal pregnancies, and with IGFBP-3 in diabetic pregnancies.

Conclusion: Maternal diabetes is associated with suppressed levels of IGFBP-1 in cord serum, whereas those of IGFBP-3 do not change markedly. In diabetic pregnancies, both cord serum IGFBP-1 and IGFBP-3 correlate with fetal growth.

Key-words: Insulin-like growth factor binding protein-1 · Insulin-like growth factor binding protein-3 · Macrosomia · Pregnancy · Type 1 diabetes.

RÉSUMÉ

Insulin-like growth factor binding protein-1 et -3 dans le sang du cordon : effet du diabète maternel et relations avec la croissance fœtale


Méthodes : Les concentrations d’IGFBP-1 et IGFBP-3 dans le sérum du cordon ont été mesurées à la naissance par immunofluorométrie au décours de 67 grossesses avec diabète de type 1 et 62 grossesses normales.

Résultats : Les concentrations d’IGFBP-1 dans le sérum du cordon étaient plus basses lors des grossesses diabétiques par rapport aux grossesses normales (156 ± 28 µg/l vs 266 ± 29 µg/l, P = 0.007), tandis que celles d’IGFBP-3 ne différaient pas significativement (3 327 ± 158 µg/l vs 2 982 ± 105 µg/l, P = 0.076). IGFBP-1 était corrélé négativement et IGFBP-3 corrélé positivement avec le z-score du poids de naissance lors des grossesses diabétiques. La tendance était similaire lors des grossesses normales. Dans des modèles de régression multiple, le z-score de poids de naissance était significativement associé à IGFBP-1 dans les grossesses normales et diabétiques, et à IGFBP-3 dans les grossesses diabétiques.

Conclusion : Le diabète maternel est associé à une réduction des concentrations d’IGFBP-1 dans le sérum du cordon, tandis que celles d’IGFBP-3 ne changent pas de façon marquée. Dans les grossesses diabétiques, IGFBP-1 et IGFBP-3 dans le sérum du cordon sont corrélés à la croissance fœtale.

Mots-clés : Insulin-like growth factor binding protein-1 · Insulin-like growth factor binding protein-3 · Macrosomie · Grossesse · Diabète de type 1.
there is extensive evidence that insulin-like growth factors (IGF-I and IGF-II) and their high affinity binding proteins (IGFBPs 1 through 6) play an important role in the regulation of fetal growth [1]. Concentration of cord blood IGF-I correlates positively with birth weight in term [2-5] and preterm [6] infants. Of the binding proteins, IGFBP-1 is the predominant IGF carrier during fetal life, with IGFBP-3 becoming the predominant form at term [7, 8]. Cord blood IGFBP-1 correlates negatively with fetal growth [4, 9, 10], which implies that IGFBP-1 acts as a growth inhibitor in the fetus. IGFBP-3 may also have an effect on fetal growth, as elevated levels were found in large-for-gestational age fetuses and suppressed levels in fetuses with intrauterine growth restriction [5, 10, 11].

Serum IGFBP-1 levels are primarily regulated by insulin, which inhibits IGFBP-1 production in the liver [12, 13]. Serum IGFBP-3 levels fluctuate concomitantly with growth hormone and IGF-I levels, and it is possible that growth hormone increases IGFBP-3 production indirectly by increasing IGF-I production [14-16]. In addition, insulin may regulate IGFBP-3 levels in some situations, because insulin treatment raises IGFBP-3 levels in children with type 1 diabetes [17]. A regulatory role for insulin is also suggested by the observation that insulin stimulates IGFBP-3 production in vitro [18].

Although alterations in insulin secretion might be expected to be associated with altered production of both IGFBP-1 and IGFBP-3, data on their cord blood levels in diabetic pregnancies are inconsistent [19, 20]. Moreover, the relative contribution of IGFBP-1 and IGFBP-3 in producing birth weight variations has not been resolved. In the present study, we measured cord serum IGFBP-1 and IGFBP-3 concentrations in diabetic and normal pregnancies. We sought to answer the following questions: 1) What is the effect of maternal diabetes on fetal IGFBP-1 and IGFBP-3 levels? 2) What is the usability of IGFBP-1 and IGFBP-3 in the detection of birth weight variations in diabetic and normal pregnancies?

### Materials and methods

Sixty-seven women with type 1 diabetes were enrolled between June 2000 and May 2002 (Tab I). An attempt was made to enroll all patients with singleton pregnancies during this period. Sixty-two healthy pregnant women were enrolled prospectively during the same time period (Tab I). They were either without any risk factors for gestational diabetes (n = 23) or had a normal 2-hour oral glucose tolerance test (75 g) (n = 39). All study subjects gave their informed consent. The study was carried out with the approval of the local institutional review board, in accordance with the tenets of the Declaration of Helsinki.

Of the diabetic women, one had nephropathy, four had retinopathy, and three had both nephropathy and retinopathy. Nine women developed preeclampsia. Long-acting insulin was given 1-3 times daily and short-acting insulin at meals, mostly 3-4 times a day. Four women had an insulin subcutaneous infusion pump. Hemoglobin A1c (HbA1c) was assessed every 4-6 weeks by ion-exchange high-performance liquid chromatography (Diamat, Bio-Rad Laboratories, Hercules, California, USA). The last HbA1c value, obtained at a median of 15 days (range 0-41) before delivery, was used for statistical analyses.

Of the diabetic women, one had nephropathy, four had retinopathy, and three had both nephropathy and retinopathy. Nine women developed preeclampsia. Long-acting insulin was given 1-3 times daily and short-acting insulin at meals, mostly 3-4 times a day. Four women had an insulin subcutaneous infusion pump. Hemoglobin A1c (HbA1c) was assessed every 4-6 weeks by ion-exchange high-performance liquid chromatography (Diamat, Bio-Rad Laboratories, Hercules, California, USA). The last HbA1c value, obtained at a median of 15 days (range 0-41) before delivery, was used for statistical analyses.

Relative birth weight was expressed as standard deviation (SD) units (z-score) using a large Finnish standard population as a reference [21]. Newborn infants were considered large-for-gestational age when the relative birth weight was greater than 2 SDs.

After the delivery of the infant, the umbilical cord was double clamped and venous blood collected. Serum was separated by centrifugation, and samples were stored at -80°C until analyzed. IGFBP-1 concentration was measured by immunofluorometric assay with monoclonal antibodies F34-15C9 and F36-9G3, essentially as earlier described [22]. The sensitivity of the assay is 0.1 µg/l, intra-assay variation 3-11%, and interassay variation 4-10%. IGFBP-3 concentration was measured by immunofluorometric assay with monoclonal antibodies F42-1B6 and F41-5C11 [23].
The sensitivity of the assay is 0.3 µg/l, intra-assay variation 3.6-6.2%, and interassay variation 5.4-11%.

The SPSS statistical package (SPSS Inc., Chicago, Illinois, USA) was used for data analysis. Continuous variables were compared by analysis of variance. Categorical data were compared by Fisher’s exact test. Spearman correlation coefficients were calculated to examine bivariate relationships. Multiple regression analyses were performed with the stepwise procedure with P ≤ 0.05 as the entry criterion. The results are presented as mean ± standard error of the mean (SEM) unless otherwise stated. P < 0.05 was considered statistically significant.

**Results**

Concentrations of IGFBP-1 in cord serum were lower in diabetic pregnancies than in normal pregnancies, whereas those of IGFBP-3 did not differ significantly (Tab II). A negative correlation existed between IGFBP-1 and IGFBP-3 in diabetic pregnancies (r = -0.373, P = 0.002) but neither of the binding proteins correlated with maternal serum HbA1c or duration of diabetes. In normal pregnancies, IGFBP-1 did not correlate significantly with IGFBP-3 (r = -0.223, P = 0.082). IGFBP-1 and IGFBP-3 did not correlate with maternal age, prepregnancy body mass index, or duration of pregnancy in diabetic or normal pregnancies.

Correlations of cord serum IGFBP-1 and IGFBP-3 with variables of intrauterine growth are shown in Table III. In diabetic pregnancies, IGFBP-1 correlated negatively and IGFBP-3 positively with birth weight, birth weight z-score, and placental weight. In normal pregnancies, the only significant correlation existed between IGFBP-1 and placental weight.

Multiple regression models with cord serum IGFBP-1 and IGFBP-3 as dependent variables are shown in Table IV. Birth weight z-score was significantly associated with IGFBP-1 in diabetic and normal pregnancies, and with IGFBP-3 in diabetic pregnancies. Vaginal delivery was significantly associated with IGFBP-1 in diabetic pregnancies. Other significant associations were not found.

**Discussion**

In the present study, we demonstrated that concentrations of IGFBP-1 in cord serum are decreased, whereas those of IGFBP-3 are not markedly altered in pregnancies complicated by type 1 diabetes near term. A trend toward higher than normal levels of cord serum IGFBP-3 was observed in diabetic pregnancies, but the difference to non-diabetic pregnancies was not statistically significant.

Earlier reports on fetal IGFBP levels in diabetic pregnancies are somewhat contradictory. Culler et al. found that cord serum IGFBP-1 concentrations decrease and IGFBP-3 concentrations increase in diabetic pregnancies [19]. In contrast, Liu et al. found raised cord serum levels of both of IGFBP-1 and IGFBP-3 [20]. Our finding of suppressed cord serum IGFBP-1 levels in maternal diabetes is in agreement with the report by Culler et al. [19]. This result is logically explained

---

**Table II**

<table>
<thead>
<tr>
<th>IGFBP-1</th>
<th>Diabetic pregnancies</th>
<th>Normal pregnancies</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>156 ± 28 µg/l</td>
<td>P = 0.007</td>
</tr>
<tr>
<td></td>
<td>266 ± 29 µg/l</td>
<td></td>
</tr>
<tr>
<td>IGFBP-3</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3327 ± 158 µg/l</td>
<td>P = 0.076</td>
</tr>
<tr>
<td></td>
<td>2982 ± 105 µg/l</td>
<td></td>
</tr>
</tbody>
</table>

**Table III**

<table>
<thead>
<tr>
<th>IGFBP-1</th>
<th>Diabetic pregnancies</th>
<th>Normal pregnancies</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>156 ± 28 µg/l</td>
<td>P = 0.007</td>
</tr>
<tr>
<td></td>
<td>266 ± 29 µg/l</td>
<td></td>
</tr>
<tr>
<td>IGFBP-3</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3327 ± 158 µg/l</td>
<td>P = 0.076</td>
</tr>
<tr>
<td></td>
<td>2982 ± 105 µg/l</td>
<td></td>
</tr>
</tbody>
</table>

**Table IV**

<table>
<thead>
<tr>
<th>IGFBP-1</th>
<th>Birth weight z-score:</th>
<th>Birth weight z-score:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β = -0.384, P = 0.001</td>
<td>β = -0.274, P = 0.031</td>
</tr>
<tr>
<td></td>
<td>β = 0.325, P = 0.004</td>
<td>R² for the model 0.266</td>
</tr>
<tr>
<td>IGFBP-3</td>
<td>Birth weight z-score:</td>
<td>Birth weight z-score:</td>
</tr>
<tr>
<td></td>
<td>β = 0.491, P &lt; 0.0001</td>
<td>β = -0.274, P = 0.031</td>
</tr>
<tr>
<td></td>
<td>R² for the model 0.241</td>
<td>R² for the model 0.266</td>
</tr>
</tbody>
</table>

Maternal age, prepregnancy body mass index, duration of diabetes, duration of pregnancy, HbA1c (in diabetic pregnancies), smoking, vascular complications (in diabetic pregnancies) and gender of the fetus showed no significant independent effect on the dependent variables in any of the models.
by hyperglycemia and raised fetal insulin levels, as insulin inhibits IGFBP-1 production in the liver [12, 13]. Although insulin raises IGFBP-3 levels in vivo [17] and stimulates IGFBP-3 secretion in vivo [18], we did not observe a significant change in cord serum IGFBP-3 levels in maternal diabetes. The result is in disagreement with earlier reports [19, 20], but may be explained by the relatively good glycemic control in our diabetic subjects, as evidenced by their low HbA1c levels. It could be hypothesized that insulin is a more potent regulator of IGFBP-1 levels than IGFBP-3 levels in the fetus. IGF-1 is a ubiquitous mitogen that has been suggested to enhance fetal growth [1-6]. Only the free fraction of the circulating IGF-1 is considered biologically active [8]. A decrease in cord serum IGFBP-1 concentration in diabetic pregnancies could be expected to increase the proportion of the bioavailable IGF-I. This may partly explain the increased occurrence of macrosomia in diabetic pregnancies [24]. The idea that IGFBP-1 acts as a growth inhibitor in the fetus is also supported by our finding of a negative correlation between cord serum IGFBP-1 and fetal growth in diabetic pregnancies. In this group, we also found a positive correlation between cord serum IGFBP-3 and fetal growth, which may be explained by the putative stimulatory effect of IGF-1 on IGFBP-3 production [15, 16].

Our data are in disagreement with earlier studies with diabetic women, in whom no correlation between cord blood IGFBP-1 and birth weight was observed [19, 20]. A positive correlation between cord blood IGFBP-3 and birth weight was observed in one [19] but not in another study [20]. Nevertheless, correlations between cord blood IGFBP-1 and -3 and birth weight in nondiabetic women [4, 5, 9-11] have been comparable to the present results. We observed stronger correlations between cord serum IGFBP-1 and -3 and variables of intrauterine growth in diabetic pregnancies than in normal pregnancies. Similarly, in multiple regression models, relative birth weight had a more significant independent effect on IGFBP-1 and IGFBP-3 in diabetic than in normal pregnancies. These differences may be related to the different growth patterns of fetuses in the two study groups, i.e. the higher relative birth weight and higher incidence of macrosomia in diabetic pregnancies. Vaginal delivery also had an independent increasing effect on IGFBP-1 in diabetic pregnancies. However, this may be due to the fact that vaginal delivery was avoided in pregnancies with macrosomia, in which IGFBP-1 levels tended to be lower than normal.

In conclusion, maternal diabetes is associated with suppressed levels of IGFBP-1 in cord serum, whereas those of IGFBP-3 do not change markedly. In diabetic pregnancies, both cord serum IGFBP-1 and IGFBP-3 correlate with fetal growth.

Acknowledgments – This study was supported by the Academy of Finland, Mary and Georg C. Ehrenroth’s Foundation, the Paulo Foundation, the University of Helsinki, and HUCH Clinical Research Grants (TKK4150 and TYH3218).

References
16. Ono T, Kanzaki S, Seino Y, Baylink DJ, Mohan S. Growth hormone (GH) treatment of GH-deficient children increases serum levels of insulin-like growth factors (IGFs), IGF-binding protein-3 and -5, and...


