Increased Serum IGF-I During Pregnancy Is Associated With Progression of Diabetic Retinopathy

Finn F. Lauszus,[1] Joachim G. Klebe,[1] Toke Bek,[2] and Allan Flyvbjerg[3]

The IGF system has been associated with development and progression of diabetic retinopathy. We examined whether a simple measurement of the IGF system (serum total IGF-I) correlated with progression of diabetic retinopathy in pregnancy in type 1 diabetes. A prospective observational study was performed in 103 pregnant women with type 1 diabetes. Serum IGF-I was measured in maternal serum from week 14, every fourth week until week 30, and every second week until delivery. Twenty-four–hour blood pressure was measured with a portable oscillometry monitor. The women had visual acuity testing and fundus photography before pregnancy, once in each trimester, and 4 months after birth. Each eye was assigned an overall retinopathy grade on a scale from 1 to 6 independently by two experienced graders. During pregnancy, serum IGF-I increased with increasing gestational age until a plateau was reached in week 32. Progression of retinopathy was significantly associated with a higher level of IGF-I ($P < 0.01$). Serum IGF-I increased with increasing progression of retinopathy. Change of retinopathy was significantly associated with level of IGF-I ($P < 0.01$). During pregnancy, serum IGF-I increased with increasing birth weight until a plateau was reached in week 32. Birth weight was significantly associated with a higher level of serum IGF-I ($P < 0.01$). *Diabetes* 52:852–856, 2003

Pregnancy is a prominent risk factor for the development and progression of retinopathy in women with type 1 diabetes. Progression of retinopathy has been reported to be associated with suboptimal regulation of blood glucose and blood pressure, albuminuria, and adverse perinatal outcomes. However, after it was shown that pituitary ablation could halt the progression of proliferative diabetic retinopathy, increased focus has been put on the hormonal changes with growth-stimulating potential that accompanies pregnancy (1). Growth hormone (GH) exerts many of its effects via the synthesis and release of IGF-I. IGF-I has been variably associated with diabetic microvascular disease, and a transient rise in IGF-I has been reported in the early phase of neovascularization of the retina (2).

IGFs constitute a system of peptides that promote mitosis and growth of various organs including the fetus during pregnancy. Although some regression of nonproliferative retinopathy occurs after delivery, new cases with progression of retinopathy during pregnancy have been reported with a prevalence ranging between 20 and 85% (3–5).

The aim of the present study was to examine the correlation between serum IGF-I and progression of retinopathy in diabetic pregnancy in 103 pregnant women with type 1 diabetes. Changes in serum IGF-I and progression of retinopathy, as well as their time course during and after pregnancy, were studied in a cross-sectional manner.

RESEARCH DESIGN AND METHODS

Subjects

Among 142 consecutive pregnancies of which 18 were repeated pregnancies, 124 pregnancies in women with type 1 diabetes were included from the outpatient clinic at Skejby Hospital, Aarhus, Denmark. Data were sufficient for evaluation in 103 of the 124 women. Participants were referred to the Department of Ophthalmology, Aarhus University Hospital, between 1993 and 1997. The women were scheduled for ophthalmologic examination, including visual acuity testing and fundus photography, before pregnancy, once in each trimester, and 4 months after birth, according to the principles defined in the Early Treatment for Diabetic Retinopathy Study (6). Ophthalmologic examination was done after induction of cycloplegia and mydriasis by phenylephrine 10% and tropicamide 1% eye drops. Fundus photography was performed with a Canon 60UV fundus camera on Kodak ectachrome 64 color diapositive film. In each eye a standard photograph of 60° was taken centered on the foveal region, thus covering an area corresponding to approximately fields 1–5 of the standard fields used in the Early Treatment for Diabetic Retinopathy Study (6). For the grading, each photograph was projected to a size of 1×1 m on a wallboard. The number of each type of pathological lesion—hemorrhages and/or microaneurysms, hard exudates, or cotton wool spots—was counted (truncated at 99), and the presence of laser scars or vascular abnormalities, such as intraretinal microvascular abnormalities (IRMA vessels), venous beading, or neovascularizations, was noted. Furthermore, the presence of diabetic maculopathy was noted, defined as hard exudates within one disk diameter of the fovea. Two experienced graders evaluated each photograph independently. When the two evaluations of a photograph were discrepant, the two graders reassessed it together. If there was still discrepancy, the opinion of the most senior grader was used. On the photograph, each eye was assigned an overall retinopathy grade on a scale from 1–6 according to the principles used in the Wisconsin Epidemiological Study of Diabetic Retinopathy (7,8). Consequently, the assignment of retinopathy grade was made according to the following guidelines (7): 1, no retinopathy; 2, <20 hemorrhages and/or microaneurysms or cotton wool spots alone; 3, ≥20 hemorrhages and/or microaneurysms, hard exudates combined with any number of hemorrhages and/or microaneurysms, or less than five cotton wool spots combined with hemorrhages and/or microaneurysms or hard exudates; 4, five or more cotton wool spots or IRMA vessels combined with hemorrhages and/or microaneurysms with or without hard exudates; 5, venous beading combined with hemorrhages and/or microaneurysms with or without hard exudates, IRMA vessels, or cotton wool spots; and 6, proliferative diabetic retinopathy or scars of photocoagulation known to have been directed at new vessels.

From the [1]Department of Obstetrics/Gynecology, Skejby Hospital, Aarhus University Hospital, Aarhus, Denmark; the [2]Ophthalmologic Department, Aarhus University Hospital, Aarhus, Denmark; and the [3]Medical Research Laboratories, Aarhus University Hospital, Aarhus, Denmark.

Address correspondence and reprint requests to Finn Lauszus, MD, PhD, Department of Gynecology and Obstetrics, Holstebro Hospital, Laegaardsvej 12, DK-7500 Holstebro, Denmark. E-mail: affl@ringamt.dk.

Received for publication 23 January 2002 and accepted in revised form 6 December 2002.

GH, growth hormone; IGFBP-1, IGF binding protein-1; IRMA, intraretinal microvascular abnormalities.

In each patient the retinopathy grade on the worst eye was used for the analysis. Significant change in retinopathy was defined as a change of at least two grading levels between two examinations or as a change of one grading level that was not reversed at the following examinations.

The women were followed at the Department of Gynecology and Obstetrics at Aarhus University Hospital, where routine visits were planned every other week during pregnancy for the measurement of HbA_{1c}. The aim was to reach normoglycemia in all patients (HbA_{1c} 4.3–6.5%). This was achieved by frequent home measurements using a glucometer and by increasing the frequency of insulin administration to 4–6 times daily. In addition to HbA_{1c}, 24-h urine was collected for the measurement of urinary albumin excretion, glucose, and creatinine clearance. Of the 103 women, 68 attended the prepregnancy clinic within 6 months before conception. In 36 women fundus photography was performed during the same period.

Blood pressure was measured using a portable oscillometry monitor (SpaceLab 90202 and 90207; Redmond, WA) in every trimester and after delivery. The equipment was programmed for cuff insufflation every 20 min from 0600 to 2300 and every hour during the night. Seven women had hypertension and received medical treatment with an ACE inhibitor, which was discontinued before conception. Two women had gestational hypertension, and 21 women developed preeclampsia. In all pregnant women hypertension was treated with either labetalol or methyldopa.

During pregnancy 81, 13, and 9 women had normo-, micro-, and macroalbuminuria, respectively. Normoalbuminuria was defined as albumin excretion rate <30 mg/24 h, microalbuminuria as 30–299 mg/24 h, and macroalbuminuria as excretion ≥300 mg/24 h. Nine women with normoalbuminuria during pregnancy had persistent microalbuminuria after pregnancy. One of these women showed prepregnant microalbuminuria. One of the women with microalbuminuria during pregnancy progressed to macroalbuminuria.

Three women reversed to normoalbuminuria after pregnancy. These three women had had prepregnant normoalbuminuria. Three of the women with macroalbuminuria during pregnancy had microalbuminuria after pregnancy while on treatment with ACE inhibitor, as they had before pregnancy.

Adverse perinatal outcome was defined as delivery before 36 completed weeks of gestation, birth weight ratio <0.9 and >1.3, preeclampsia, maternal or neonatal septicemia, neonatal hypoglycemia, respiratory distress syndrome, malformation, or demise of the neonate. Birth weight ratio was defined as observed birth weight divided by the expected birth weight for the same gestational age and sex, which corresponded to IUGR and macrosomia, respectively. The local ethical committee approved the study, and all women gave their informed consent. The mean age (±SD) of the 103 women was 28 ± 5 years (range 17–40), and the median age of onset of type 1 diabetes was 17 years (95% range 13–20). The mean parity was 0.6 ± 0.4 (range 0–3).

Serum total IGF-I and IGF binding protein-1 and -3

Serum total IGF-I was measured as previously described (9). The noncompetitive time-resolved immunofluorometic assay is based on monoclonal antibodies and performed in microtest wells. IGF-I antibodies were immobilized on the solid matrix. The detection limit was 0.0025 μg/l for the IGF-I assay. The operating range included upwards of 2.5 μg/l (IGF-I). All clinically relevant serum concentrations could be measured in one final dilution (1:1,066 for IGF-I) after ethanol extraction. The interassay variation was <10%.

Immunoreactive levels of IGF binding protein-1 (IGFBP-1) and -3 were determined in a subset of diabetic women (n = 45) by an enzyme-linked immunoassay (Medix Biomedica, Kainiainen, Finland) and IRMA (Diagnostic System Laboratories, Webster, TX). The intra- and interassay coefficients of variance were <5 and 10%, respectively.

Serum samples for measurement of IGF-I were drawn at weeks 14, 18, 22, 26, 30, 32, 34, 36, and 38 and in the women who came for check-up 3–6 months postpartum. Thus, 793 of 847 (94%) possible blood samples were obtained from weeks 14–38 (88 women delivered before week 38), and 49 of 103 possible samples were taken postpartum.

Statistical analysis

Differences between two means was tested with Student's t test if Gaussian distribution could be assured. Otherwise, Mann-Whitney's or Wilcoxon's test were used for unpaired and paired analysis, respectively. If non-Gaussian distribution was observed data were transformed logarithmically where appropriate. If any number was less than five, differences between two proportions were tested with Fisher's exact test, otherwise the χ^2 test was applied. Repeated measures (two-way) ANOVA was used for comparison between groups for measures taken over time from the same subject. Kruskal-Wallis test was applied with grade of retinopathy as the ordinal variable and HbA_{1c}, urinary albumin excretion rate, blood pressure, and onset of diabetes as continuous measurement variables. Spearman's ρ was calculated when appropriate. Values are given as mean ± SD, unless otherwise stated. A two-sided P value <0.05 was the level of significance. The statistical software was SOLO (BMPD, Los Angeles, CA). A birth weight ratio was computed by dividing the observed birth weight with the expected birth weight for the same gestational age and sex. The expected birth weights were calculated from a cohort of 4,742 singleton normal births during the same time period from the same clinic.

TABLE 1
Completeness of retinal grading dataset

	Before through during	During	During through after pregnancy
Retinopathy			
Progression	10	25	24
No change	21	62	49
Improvement	5	16	12
Total	36	103	85

Data are n.

RESULTS

Clinical parameters

HbA_{1c} was 8.1 ± 1.6% (range 4.8–12) before pregnancy; 7.5 ± 1.1 (5.3–10.2), 7.2 ± 1 (5–10.2), and 7.5 ± 1.1 (5.5–11.8) during the first, second, and third trimesters of pregnancy, respectively; and 8.7 ± 1.3 (6–13) after pregnancy. At all examinations during pregnancy, HbA_{1c} was significantly lower than before and after pregnancy ($P <$ 0.01). There was a positive correlation between retinopathy grade and HbA_{1c} before (Spearman's ρ = 0.57, $P <$ 0.01) and after pregnancy (0.42, $P < 0.01$). During pregnancy the correlation was gradually lost (0.29, $P < 0.01$; 0.29, $P < 0.05$; and 0.11, $P > 0.10$ in the first, second, and third trimesters, respectively). The women with no retinopathy differed significantly from the other groups in HbA_{1c} at week 14 (Table 1) and at onset of diabetes (Table 2).

In total, 25 women had progression of retinopathy, hereof 18 during pregnancy into the puerperium 4 months after delivery. Of the remaining seven women who progressed during pregnancy only, three women progressed from second to third trimester. None of the studied women showed progression of retinopathy requiring photocoagulation treatment. Significantly more women experienced progression of retinopathy after pregnancy than during pregnancy (Table 3), and this was accompanied by an increase, although nonsignificant, of the level of HbA_{1c}. No difference in glycemic regulation and insulin regimen was found throughout pregnancy in women with progression as compared with women without progression of retinopathy. Of the 103 women participating in the study, 27 women delivered before gestational week 36, 61 women at weeks 36–37, and the remaining 15 women at weeks 38–40.

TABLE 2
Change in retinal grading during and after pregnancy

	Total	During	During through after pregnancy
Retinopathy			
Progression	25	7	18
No change	62	—	—
Improvement	16	9	7
Total	103		

Data are n.

TABLE 3
Initial data of 103 pregnant women with type 1 diabetes

	n	Age (years)	Onset of diabetes (years)	Parity	Micro- and macro-albuminuria (*n*)	BMI (kg/m^2)
Retinopathy						
No	44	28	23 (21.25)*	0.7	3/0	28
Simple	47	28	13 (11.17)	0.5	9/0	27
Proliferative	12	29	12 (6.14)	0.5	1/9	28
All	103	28	17 (13.20)	0.6	13/9	28

*$P < 0.05$, no retinopathy vs. all other groups (ANOVA, Newman Keul's posthoc test). Onset of diabetes is presented as median (95% CI).

Fundus photography was performed in 36 women before pregnancy and in 85 women after delivery (Table 4). In 62 women no change in retinopathy was seen. In 16 and 25 women, respectively, regression and progression was seen. No association was found with nephropathy or proliferative retinopathy. Progression was seen in 35, 22, and 11% of grades 1, 2, and 3 and 4 combined, respectively (Table 5). All women with progression increased one grade only.

Serum IGF-I and IGFBP-1 and -3

Serum IGF-I showed a steady and significant increase of ~82% (weeks 14–34, $P < 0.01$) during pregnancy, reaching a plateau in weeks 32–36 before a significant decline in week 38 (weeks 36–38, $P < 0.01$). Serum IGF-I increased significantly more during pregnancy in women with progression than in women with no progression (IGF-I weeks 14–34: 94 ± 21 to 209 ± 103 μg/l vs. 96 ± 37 to 161 ± 68 μg/l, $P < 0.01$). In gestational weeks 30 and 32, serum IGF-I was associated with progression of retinopathy adjusted for 24-h blood pressure, HbA_{1c}, and BMI ($P < 0.05$). Serum IGF-I increased with retinopathy grade. The change in retinopathy was significantly associated with the level of serum IGF-I ($P < 0.01$; Fig. 1).

In the period of weeks 14–38, serum IGF-I was significantly associated with birth weight group ($P < 0.01$, Fig. 2); the higher the birth weight ratio the higher the serum IGF-I. In gestational week 34, the level of serum IGF-I was 34% higher in women from the group with birth weight ratio >1.4 than in the group with a birth weight ratio <1.18 (Fig. 2). However, progression of retinopathy was not associated with higher birth weight. The IGFBP-1 and -3 levels showed no correlation with progression of retinopathy.

DISCUSSION

The major new finding of the present study is an apparent stepwise increase in serum total IGF-I associated with progression of diabetic retinopathy. This result presents further evidence and extends previous observations suggesting a possible role of elevated serum IGF-I for accelerating diabetic retinopathy during pregnancy (2,10–12). The maternal serum IGF-I levels were time-dependent with increasing levels during pregnancy until week 34, with a decrease from week 36 to 38 and full normalization after delivery. The dynamic changes in IGF-I may in part explain previous conflicting reports on correlations between retinopathy and IGF-I in type 1 diabetes (2,10–15).

Clinical evidence has suggested that IGF-I may be involved in the development of retinopathy, as an increase in serum IGF-I is observed in response to a reduction in hyperglycemia that precedes the progression of retinopathy (11,12). In the phase where new vessels have developed, the vitreous body shows higher IGF-I levels in diabetic than in nondiabetic patients (10). Paradoxical worsening of preexisting diabetic retinopathy after improved diabetes control has been noted repeatedly and has been named "normoglycemic re-entry phenomenon." Relative or absolute insulin deficiency is believed to result in decreased hepatic IGF-I production followed by elevated GH levels and a further deterioration of metabolic control. In contrast, adequate insulin substitution to improve glycemic control reverses the situation by upregulating circulating IGF-I and lowering GH. The present data show, however, that despite good glycemic control in pregnancy, an increase in circulating IGF-I takes place concomitantly with progression of diabetic retinopathy. A cause-effect

TABLE 4
Pregnancy data of 103 women with type 1 diabetes

	n	Trimester	HbA_{1c} (%)	Creatinine clearance (ml/min)	Systolic BP (mmHg) (*n* = 93)	Diastolic BP (mmHg) (*n* = 93)	Weight (kg)
Retinopathy							
No	44	First	7.0 ± 1*	130 ± 26	118 ± 8	72 ± 6	69 ± 13
		Third	7.3 ± 1.1	117 ± 31	121 ± 8	76 ± 6	78 ± 15
Simple	47	First	7.7 ± 0.9	127 ± 26	122 ± 7	73 ± 5	66 ± 7
		Third	7.7 ± 1.2	111 ± 33	129 ± 12	80 ± 8	74 ± 8
Proliferative	12	First	8.2 ± 1.1	96 ± 39†	136 ± 15†	84 ± 7†	70 ± 18
		Third	7.5 ± 0.9	86 ± 46†	140 ± 17	89 ± 10†	76 ± 13
All	103	First	7.5 ± 1	125 ± 29	122 ± 10	74 ± 7	67 ± 12
		Third	7.5 ± 1.1	111 ± 34	126 ± 13‡	79 ± 9	76 ± 12
Δ			0	−14	4	5	9

Data are means ± SE. *$P < 0.05$, no retinopathy vs. all other groups; †$P < 0.05$, proliferative group vs. all others (ANOVA, Newman-Keul's posthoc test); ‡$P < 0.01$, all groups (ANOVA); Δ, change from first to third trimester; BP, blood pressure.

TABLE 5
Initial grading of retinopathy and clinical finding during pregnancy

Grade	Progression	No change	Improvement	All
1	15	27	0	42
2	8	20	8	36
3	1	7	6	14
4	1	2	1	4
5	0	2	1	3
6	0	4	0	4
All	25	62	62	103

Data are *n*.

relation is possible because serum and vitreous IGF-I concentrations have been shown to correlate, probably due to leakage of IGF-I from the blood stream (10). Considering the established adverse effects of exogenous IGF-I on the diabetic microcirculation and the benefit of pituitary ablation on proliferative diabetic retinopathy, a causal effect of the increased serum IGF-I is possible. The question arises, however, whether our sample size is adequate to detect meaningful relations. When calculating the proportion of women with progression in diabetic retinopathy compared with those with no progression, a power of 90 ($\beta = 0.10$) with $\alpha = 0.05$ was found, thus adding adequate power to our conclusions.

We have previously reported that retinopathy is associated with an elevated level of fibroblast growth factor-2 and phosphorylated IGFBP-1 (16,17). Total IGF-I is largely dependent on IGFBP-3 levels, which in turn are regulated by the degree of IGFBP-3 proteolysis during pregnancy. Thus, the effect on the eye may be directly mediated by IGF-I or indirectly by a modifying effect of IGFBP-3. It is still controversial whether the binding affinity of IGF-I to IGFBP-3 is altered by the pregnancy-induced IGFBP-3 proteolysis. We have previously shown that an increasing level of serum IGF-I corresponds to a similar increase of IGFBP-3 despite prominent proteolysis, indicating that binding affinity may be unaltered in pregnancy of type 1 diabetes (18). Noteworthy, serum IGFBP-3 levels showed no correlation with progression of retinopathy in the present study.

The association of IGF-I and retinopathy in type 1 diabetes in nonpregnant women has not been consistent in the published literature. In one study (13), low free IGF-I level was reported in type 1 diabetic patients with retinopathy compared with patients without diabetic retinopathy, whereas another study showed the opposite (14). The latter study concluded that free IGF-I measurements have no clear advantage compared with total IGF-I in that particular setup, because age is an important confounder. IGFBP-1 is known to be an important regulator of free IGF-I. However, in the present study IGFBP-1 showed no correlation to the progression of retinopathy.

In our study, the women with proliferative retinopathy had elevated HbA_{1c} levels, which has been shown to be associated with higher IGF-I in nonpregnant diabetic subjects (19,20). However, we failed to confirm such an association. Further, it seems unlikely that IGF-I was due to differences in age or BMI, because the groups were similar in these respects. As for the role of IGF-I in glycemic control, just one severe incidence of hypoglycemia was observed during pregnancy in a woman delivering a macrosomic neonate. The HbA_{1c} indicated good glycemic control in this population, but one cannot preclude that the number of women is insufficient. Glycemic regulation is associated with measurable retinal change at HbA_{1c} >7.5%, and long-term prospective studies have found the progression to be related to HbA_{1c} independently of the duration of diabetes (21,22)

The bulk of literature points to an overall adverse effect of pregnancy on diabetic retinopathy, which relates to diabetic risk factors. In addition, regression of diabetic retinopathy after pregnancy is common and may cause the determination of which women are at risk of persisting retinopathy to be unclear. We had a very low progression rate during pregnancy (7 of 85), and more women had progression after delivery. This suggests that the time after delivery may need as much focus as the time before delivery.

The finding of an association between increasing serum IGF-I and progression of retinopathy may reflect a possible growth stimulus induced by the pregnant condition per se. This may act on retinal neovascularization as well as on birth weight. In women with type 1 diabetes with increasing macrosomia, higher levels of serum IGF-I seem to imply excessive fetal growth from the start of pregnancy. Other conditions associated with early vigorous fetal growth (e.g., multiple pregnancies) do not present with higher level of IGF-I early in pregnancy (23). Moreover,

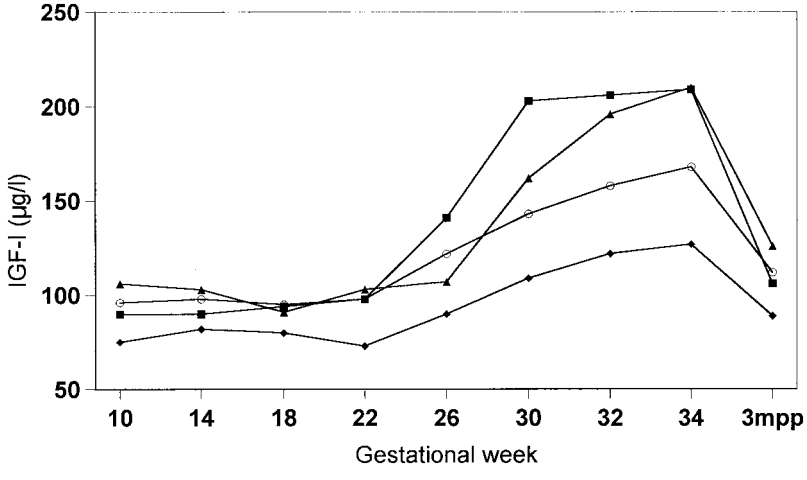

FIG. 1. Serum IGF-I in pregnant women with type 1 diabetes in relation to change of retinal grading. ○, no change ($n = 62$); ◆, improvement of retinal grading ($n = 16$); ▲, progression during pregnancy ($n = 7$); ■, progression of retinopathy during and after pregnancy ($n = 18$); 3mpp, 3 months postpartum. $P < 0.01$ for all groups, weeks 14–34, two-way ANOVA. Data are given as mean.

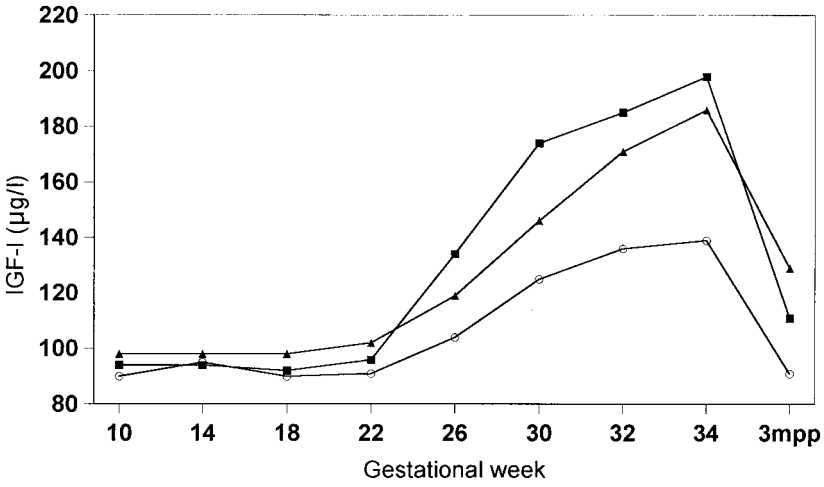

FIG. 2. Serum IGF-I in pregnant women with type 1 diabetes in relation to birth weight ratio. ○, birth weight ratio <1.18 (n = 32); ▲, birth weight ratio 1.18–1.4 (n = 28); ■, birth weight ratio >1.4 (n = 40); 3mpp, 3 months postpartum. P < 0.01 for all groups, weeks 14–34, two-way ANOVA. Data are given as mean. Three women were excluded due to deliveries before week 30.

maternal levels of serum IGF-I were similar in normal, small-for-gestational-age, and multiple pregnancies, respectively (23,24).

In conclusion, our data show that the presence of retinopathy during pregnancy in type 1 diabetes does not increase the risk of progression in retinopathy when good glycemic control is achieved. Further, we report a stepwise increase in serum IGF-I with an association to progression of retinopathy during and after pregnancy.

ACKNOWLEDGMENTS

This study was supported by grants from the Medical Research Council (no. 970592), the Novo Foundation, the Aage Louis-Hansen Memorial Foundation, the Nordic Insulin Foundation, the Eva and Henry Frænkels Memorial Foundation, and the Aarhus University-Novo Nordisk Center for Research in Growth and Regeneration (no. 9600822).

We thank Inga Bisgaard, Kirsten Nyborg, Joan Hansen, and Susanne Sørensen for excellent technical assistance.

REFERENCES

1. Lundbæk K, Christensen NJ, Jensen VA, Johansen K, Olsen TS, Hansen AaP, Ørskov H, Østerby R: Diabetes, diabetic angiopathy and growth hormone. *Lancet* 2:131–133, 1970
2. Merrimee TJ, Zapf J, Froesch R: Insulin-like growth factors: studies in diabetics with and without retinopathy. *N Engl J Med* 309:527–530, 1983
3. Hopp H, Vollert W, Ebert A, Weitzel H, Glockner E, Jahrig D: Diabetic retinopathy and nephropathy-complications in pregnancy and labor (in German) *Geburtshilfe Frauenheilkd* 55:275–279, 1995
4. Rosenn B, Miodovnik M, Kranias G, Khoury J, Combs CA, Mimouri F, Siddiqi TA, Lipman MJ: Progression of diabetic retinopathy in pregnancy: association with hypertension in pregnancy. *Am J Obstet Gynecol* 166: 1214–1218, 1992
5. Horvat M, Maclean H, Goldberg L, Crock GW: Diabetic retinopathy in pregnancy: a 12-year prospective survey. *Br J Ophthalm* 64:398–403, 1980
6. Early Treatment For Diabetic Retinopathy Study Group: Grading diabetic retinopathy from stereoscopic color fundus photographs: an extension of the modified Airlie House classification: ETDRS report number 10. *Ophthalmology* (Suppl. 5):786–806, 1991
7. Poulsen PL, Bek T, Ebbehøj E, Hansen KW, Mogensen CE: 24-h ambulatory blood pressure and retinopathy in normoalbuminuric IDDM patients. *Diabetologia* 41:105–110, 1998
8. Hansen KW, Schmitz A, Pedersen MM: Ambulatory blood pressure measurement in type 2 diabetic patients: methological aspects. *Diabet Med* 8:567–572, 1991
9. Frystyk J, Dinesen B, Ørskov H: Non-competitive time-resolved immunofluorometric assays for determination of human IGF-I and -II. *Growth Reg* 5:169–176, 1995
10. Grant M, Russell B, Fitzgerald C, Merimee TJ: Insulin-like growth factors in vitreous: studies in control and diabetic subjects with neovascularization. *Diabetes* 35:416–420, 1986
11. Hyer SL, Sharp PS, Sleightholm M, Burrin JM, Kohner EM: Progression of diabetic retinopathy and changes in serum IGF-I during continous subcutaneous insulin infusion. *Horm Metab Res* 21:18–22, 1989
12. Chantalau E: Evidence that upregulation of serum IGF-I concentration can trigger acceleration of diabetic retinopathy. *Br J Ophthalmol* 82:725–730, 1998
13. Feldmann B, Lang GE, Arnavaz A, Jehle PM, Böhm BO, Lang GK: Lower concentration of bioavailable IGF-I in patients with diabetic retinopathy (Verminderte Serumspiegel von freiem bioverfügbarem IGF-I bei Patienten mit diabetischer Retinopathie) (in German). *Ophthalmologe* 96:300–305, 1999
14. Janssen JAMJL, Jacobs ML, Derkx FHM, Weber RFA, Van der Lely AJ, Lamberts SW: Free and total IGF-I, IGFBP-1, and IGFBP-3 and their relationships to the presence of diabetic retinopathy and glomerular hyperfiltration in insulin-dependent diabetes mellitus. *J Clin Endocrinol Metab* 82:2809–2815, 1997
15. Wang Q, Dills DG, Klein R, Klein BEK, Moss SE: Does insulin-like growth factor I predict incidence and progression of diabetic retinopathy? *Diabetes* 44:161–164, 1995
16. Hill DJ, Flyvbjerg A, Arany E, Lauszus FF, Klebe JG: Increased levels of serum fibroblast growth factor-2 (FGF-2) in diabetic pregnant women with retinopathy. *J Clin Endocrinol Metab* 82:1452–1457, 1997
17. Gibson JM, Westwood M, Lauszus FF, Klebe JG, Flyvbjerg A, White A: Phosphorylated insulin-like growth factor binding protein-1 is increased in pregnant diabetic subjects. *Diabetes* 48:321–326, 1999
18. Lauszus FF, Klebe JG, Flyvbjerg A: A prospective study of the association between maternal serum insulin-like growth factor I and -II in diabetic pregnancy and macrosomia. *Obstet Gynecol* 97:734–741, 2001
19. Bereket A, Lang CH, Blethen SL, Fan J, Frost RA, Wilson TA: Effect of insulin on IGF system in children with new onset IDDM. *J Clin Endocrinol Metab* 80:1312–1317, 1990
20. Bereket A, Lang CH, Blethen SL, Fan J, Frost RA, Wilson TA: IGFBP-3 proteolysis in children with IDDM: a possible role for insulin in the regulation of IGFBP-3 proteases activity. *J Clin Endocrinol Metab* 80: 2282–2288, 1995
21. Klein R, Klein BEK, Moss SE, Davis MD, DeMets DL: The Wisconsin Epidemiologic Study of Diabetic Retinopathy. II. Prevalence and risk of diabetic retinopathy when age at diagnosis is less than 30 years. *Arch Ophthalmol* 102:520–526, 1984
22. Chase HP, Barg SK, Jackson WE, Thomas MA, Harris S, Marshall G, Chews MJ: Bloodpressure and retinopathy in type 1 diabetes. *Opthalmology* 97:155–159, 1990
23. Langford KS, Nicolaides KH, Jones J, Abbas A, McGregor AM, Miell JP: Serum IGFBP-3 levels and IGFBP-3 protease activity in normal, abnormal, and multiple pregnancy. *J Clin Endocrinol Metab* 80:21–27, 1995
24. Holmes RP, Holly JM, Soothill PW: A prospective study of maternal serum insulin-like growth factor I in pregnancies with appropriately grown or growth restricted fetuses. *Br J Obstet Gynaecol* 105:1273–1278, 1998