RESEARCH ARTICLE

Consecutive measurements show association of IGF-1 with fetal growth in type 1 diabetic pregnancy

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Highlights

- Low fetal growth was associated with low levels of IGF-1
- Sub-optimal fetal growth may be identified and addressed in late 2nd trimester by consecutive IGF-1 measurements

Abbreviations

AER: Albumin excretion rate

AASI: Ambulatory arterial stiffness index

hPGH: Human placental growth factor

IGF-1: Insulin-like growth factor-1

MTHFR: Methylene-tetrahydro-folate-reductase

PAPP-A: Pregnancy-associated plasma protein-A

SGA: Small for gestational age

T1DM: Type 1 diabetes mellitus

Abstract

Background: Abnormal fetal growth can lead to adverse outcomes in pregnancy as well in later postnatal life and is more prevalent in diabetic pregnancies. These are usually associated with large for gestational age neonates, an issue that has already been broadly examined. On the other side, diabetes in pregnancy can also relate to intrauterine growth restriction and small for gestational age neonates. During pregnancy, maternal IGF-1 is secreted excessively and additionally produced by placenta, which regulates transport of nutrients to the fetus acting through an IGF-receptor and accordingly affects its growth. As type 1 diabetes carries a higher risk of adverse events associated with fetal growth there is a natural focus on possible links between IGF-1 and fetal growth.

Aim: The present study investigated the time-course relationship between the maternal serum IGF-1 and the birthweight as an obstetrical outcome in type 1 diabetic pregnancies with various levels of background risk.

Methods: 130 pregnant women with type 1 diabetes were consecutively recruited for measurement of growth factors, genes affecting coagulation, evaluated for diabetes status, and perinatal outcome. The birthweight z-score was computed and grouped into tertiles for analysis of association with repeated measurements of IGF-1. Diurnal blood pressure was measured by monitor. Retinopathy grade was evaluated by two specialists independently, blinded of the clinical data. Blood samples for IGF-1 during pregnancy were drawn from week 6 and every 4th week until week 30, then every 2 nd week. Genomic DNA was extracted from peripheral blood.

Results: The median of the lowest tertile of birthweight z-score was 0.4 (-1 - +1.3) and included more women with micro/macroalbuminuria than the middle and upper tertile; however, ¾ had normoalbuminuria and no further sign of surplus vasculopathy compared to the other tertile groups. The lowest birthweights were associated with a lower rise in IGF-1 from week 22 to 32. Neither glycemic control, genetics, grade of retinopathy, renal function nor vascular resistance indices in diurnal blood pressure were different between the tertile groups.

Conclusion: Our main finding is that lower IGF-1 levels are associated with subsequent lower birthweight in diabetic pregnancy and is displayed markedly at the end of 2nd trimester. We hypothesize that the relative low birthweight, despite being within the limits of appropriate for gestational age may display inappropriate growth if not outright growth-restriction. We were able to discern different levels of growth at a critical point in pregnancy where ultrasound may pick up different levels of growth patterns and optimized care can be commenced.

Key words: IGF-1, fetal growth, type 1 diabetes, pregnancy, albuminuria, vasculopathy

Introduction

Abnormal fetal growth can lead to adverse outcomes in pregnancy as well in later postnatal life and is more prevalent in diabetic pregnancies than in non-diabetic ones. It is usually associated with neonates who are larger for gestational age and this link has already been broadly examined. On the other side, diabetes in pregnancy can also relate to intrauterine growth restriction and small for gestational age (SGA) neonates, especially in women with pregestational type 1 diabetes (T1DM). The etiology may be vasculopathy resulting in placental dysfunction and, hence, restricted fetal growth but the detailed mechanism remains unclear[1].

SGA fetuses are at three to four times higher risk for stillbirth than appropriate weight fetuses, which signifies that early prediction of SGA and intrauterine growth restriction is crucial in prevention[2,3]. For this reason, researchers are trying to find possible

biomarkers with a strong predictive value. Pregnancy-associated plasma protein-A (PAPP-A) and human placental growth factor (hPGH) were linked to SGA but showed little clinical value when evaluated alone[2]. Insulin-like growth factor-1 (IGF-1) was proposed as a possible marker for several pregnancy outcomes, including preeclampsia and SGA. The IGF-1 values are lower in the cord blood of SGA neonates, when investigating non-diabetic pregnancies[4,5]. There is a natural focus on possible links in T1DM as these pregnancies carries a U-shaped risk profile for adverse events associated with fetal growth.

IGF-1 is known as an anabolic single-chain polypeptide, similar to proinsulin, but including a D domain with a carboxyterminal end, which does not occur in insulin. Its levels in the blood stream are mainly an effect of growth-hormone dependent production and release by the liver. IGF's bioavailability is modulated by specific IGF-binding proteins, lifelong programming of this system by the intrauterine hormonal and in part by the nutritional environment. This provides, too, a crucial link between small size at birth and cardiovascular disease in adulthood proposed by the Barker hypothesis. During pregnancy in T1DM IGFs are associated with fetal overgrowth and vasculopathy, in particular retinopathy. The dilemma arises when accelerated growth of the fetus is diagnosed caused by fetal hyperinsulinemia despite good glycemic regulation, whereas fetal growth restriction is often seen with prominent vasculopathy during pregnancy. IGF-binding protein proteolysis increases with the subsequent presence of proteolyzed binding protein fragments in the circulation, and these themselves are modulated by phosphorylation, all in effect regulating the bioavailability of IGFs. The modulating effects together with macrosomia were previously published by this group, but not so the low birthweight cases that, too, can be observed in diabetic pregnancy[6-8]. Interestingly, PAPP-A is one of the proteolytic substances of IGF-binding proteins, whose 1st trimester level seems to relate to fetal growth in normal and diabetic pregnancy[9]. Above its numerous functions of maintaining homeostasis, IGF-1 affects glucose and lipid metabolism[10]. In pregnancy, IGF-1 is additionally produced by placenta and by acting through an IGF-receptor regulating transportation of nutrients to the fetus and accordingly affecting its growth[11]. Thus, intrauterine growth is in part determined by IGF-1 levels and is moderated with glucose-insulin axis[10].

Albumin excretion rates (AER) and ambulatory arterial stiffness index (AASI) are used to detect incipient or overt microangiopathy. Similarly, increased homocysteine levels are observed in vasculopathy and preeclampsia in T1DM[12]. Accumulating evidence points at genetic factors in the genesis of preeclampsia with reduced activity of the methylene-tetrahydro-folate-reductase (MTHFR) gene and concurrent increased homocysteine[13]. Similarly, diabetic retinopathy and ocular thrombosis are associated to the MTFHR mutation and Factor V Leiden. The progression of retinopathy during pregnancy is linked to IGF-1 and fibroblast growth factors and its growth stimulus on retinal vessels with neovascularization[34]. Again, the modification of IGF-binding proteins by phosphorylation plays a part in accelerating diabetic retinopathy during pregnancy together with genetic background in susceptible populations[8,14].

The aim of the present study was to investigate the time-course relationship between the maternal serum IGF-1 and the birthweight as an obstetrical outcome in T1DM pregnancies with various levels of background risk. In accordance, we included pregnancies of women with different levels of AER and preeclampsia. Further, we ascertained on various modalities to identify potential modulators and confounders, which could exert their effect during pregnancy and as maternal basal characteristics. These included genetic factors, incipient vasculopathy by diurnal blood pressure evaluation, serum and urine markers, endocrine regulation and fetal vascular evaluation.

Methods

One-hundred-and-thirty pregnant women with pregestational T1DM were consecutively recruited for measurement of growth factors, genes affecting coagulation, evaluated for deterioration of diabetes status, and adverse perinatal outcome from 1994-1998. The study was part of an evaluation of nephropathy and retinopathy, approved by the local Ethical Committee (jr. no. 1992/2523, 1998/4147, and 2026-99) and by the Danish Data Protection Agency (no. 1-16-02-92-16) and performed in concordance with the Helsinki II declaration. All women gave their informed consent.

Blood samples during pregnancy were drawn from week 6 every 4th week until week 30, then every 2nd week until birth and then at 3 months post-partum. From all participating women sufficient clinical data was gathered several times each trimester on insulin dose, HbA1c, weekly glucose measurements and 24-h urine albumin excretion. No woman had a history of or delivered due to cervical incompetence, and only pregnancies with delivery after gestational week 32 are presented. Complete blood sample measurements were available in 130 women from week 14 to 32. Further 24 women were excluded as 19 had incomplete sampling between week 14 to 32 and five delivered before week 32 and, thus, our study group comprised of 130/159 (82%) of the eligible women. Non-fasting blood samples were obtained after informed consent was given, blood was centrifuged, and serum was pipetted off and frozen at -20 C for later analysis.

Preterm delivery was defined as delivery before 36 weeks of gestation. The birthweight z-score was computed by subtracting the observed birthweight and the expected birthweight for the same gender and gestational age (50 % percentile) divided by the standard deviation (SD). The expected weights were calculated from the birthweight charts endorsed by the Danish Health and Medicines Authority[15]. The z-scores were grouped into tertiles for analysis of association with repeated measurements of IGF-1. The reason to split the birthweight into tertiles was to detect growth inhibition in the cohort; the birthweight distribution being non-Gaussian and expected skewed towards macrosomia. The department's policy on deliveries in T1DM was to induce delivery in week 37–38 and all women were instructed to measure frequent glucose at home and administrating insulin 4–6 times daily, aiming at normoglycemia with HbA1c below 6.5 %.

A portable monitor (SpaceLab 90207; Redmond, WA) was used to measure diurnal blood pressure once every trimester and after delivery and from these multiple diurnal recordings ambulatory arterial stiffness index (AASI) was calculated. AASI was defined as one minus the regression slope of diastolic on systolic blood pressure[16]. Increased blood pressure of 140/90 mmHg or greater and AER in excess of 300 mg was defined as preeclampsia in individuals who were normohypertensive before week 20 and, concomitantly, AER in excess of 300 mg per day in previously normoalbuminuric women. AER was measured by 24-h collection of urine and grouped into normo- (<30 mg/24h), micro- (30-299 mg/24h) and macroalbuminuria (>300 mg/24h).

Women came for ophthalmologic examination with visual acuity testing and fundus photography before pregnancy, once in each trimester, and post-partum. Changes in retinopathy was registered after grading the photograph of each eye, which was assigned an overall retinopathy grade following the principles used in the Wisconsin Epidemiological Study of Diabetic Retinopathy.

Measurements of umbilical blood flow was done routinely from week 32 and commenced before if indicated. The last pulsatility index was one performed within one week before delivery, registered and stored as a photograph. The flow measurements were done by the associate professor in charge of the out-patient pregnant diabetes ward and, in his absence, by the registrar or consultant. Delivery was induced in week 37 to 38 if cesarean was not planned.

Serum total IGF-1

Serum total IGF-1 was measured with a non-competitive time-resolved immunofluoro-metric assay based on monoclonal antibodies and performed in micro test wells[17]. IGF-1 antibodies were immobilized on the solid matrix and the detection limit was 0.0025 µg/l for the IGF-1 assay. The operating range included upwards of 2.5 µg/l (IGF-1). All clinically relevant serum concentrations could be measured in one final dilution (1:1,066 for IGF-1) after ethanol extraction. The inter-assay variation was <10%. Samples for IGF-1 were drawn at gestational weeks 6, 10, 14, 18, 22, 26, 30, 32, 34, 36, and 38 and in the women who came for check-up 3 to 6 months post-partum. In total 870 of 910 (96%) possible blood samples were obtained from weeks 6–34. Blood samples were measured in 29, 58, 92, and 35 % of possible samples in week 6, 10, 34, and post-partum, respectively.

MTFHR and Factor V Leiden

Genomic DNA was extracted from peripheral blood leukocytes from frozen samples, spotted on Whatman filter paper (Struers KEBO Lab. Rødovre, Denmark) and extracted[18]. The MTHFR 677C→T polymorphism was detected[19]. Polymerase chain reaction was carried out in a volume of 25 µl. We used 3 µl of the supernatant from the women blood spots as a polymerase chain reaction template. The methods of for detection of Factor V Leiden (1691G→-A) variant were performed according Gaustadnes et al.[20]. The background population consisted of samples drawn from Guthrie cards submitted to Statens Serum Institute, Copenhagen, in a cohort of children in Denmark[20]. The incidence for MTHFR (677C→T) and Factor V Leiden was 50 % (n = 1084) and 7 % (n = 4188) in the background population.

Statistical analysis

Statistics were performed with the software IBM SPSS statistics 24. If continuous data followed Gaussian distribution, the difference between two means was tested with Student's t-test; otherwise, Mann-Whitney's U-test was used. For evaluation of categorical variables, $\chi 2$- test was used. The variable AER was normalized by logarithm computing. Comparison of the difference between several means was tested with analysis of variance (ANOVA); if non-Gaussian distributed, Kruskal-Wallis' test was used. If the ANOVA test was $p < 0.05$, Student-Newman-Keul's post-hoc test was applied. Linear regression analysis was performed with IGF-1 as dependent variable and as independent variables z-scores, body weight, age, duration of diabetes, HbA1c, AER, and preeclampsia. Repeated measures analysis of variance (two-way-ANOVA) was used for comparison of IGF-1 taken over time with z-score tertiles as group variable and co-variants of body weight, HbA1c, creatinine, and duration of diabetes/log AER were added; first, duration of diabetes and later replaced with log AER and adding HbA1c and creatinine. The reason to do separately analysis is that the former constitute static personal characteristics while the other mirrors physiologic dynamics of pregnancy. Finally, all were added to the model. Values are stated as mean ± SD or, if non-Gaussian, as median (range). The level of significance was two-sided p-value of 0.05.

Results

The included women had a mean age of 28 ± 4 with the mean duration of diabetes of 12 ± 8 years. The body weight was 67 ± 11 kg. Blood pressure in 1st trimester was 120/72 ± 9/6 mmHg. Creatinine in 1st trimester was 59 ± 10 µmol/l, creatinine clearance 140 ± 27 ml/min, and Hb1Ac 7.4 ± 1.1 %. Cholesterol levels were lowest in the highest birthweight group. No differences between the tertile groups were seen in accordance to any other parameters in 1st trimester (Table 1). The dynamic changes in the clinical variables during pregnancy showed slightly higher systolic and diastolic blood pressure in the lowest birthweight group compared to the other two groups. Besides that, no clinical significant differences were found between the birthweight groups in body weight, glycemia levels, creatinine clearance, and triglyceride. Serum creatinine was lowest in the lower birthweight group in 3rd trimester.

Table 1: Maternal characteristics in 130 pregnant women with type 1 diabetes

	Lowest birthweight tertile (n=44)	Middle birthweight tertile (n=43)	Highest birthweight tertile (n=43)	p-value (ANOVA)
Age (years)	28 ±4	28 ±4	28 ±4	0.73
Duration of diabetes (years)	12 ±8	11 ±8	13 ±7	0.52
Parity n (%)				
- 1st	22 (50)	22 (51)	20 (47)	
- 2nd	13	12	16	0.8
- >2nd	9	9	7	
Body weight (kg)				
- 1st trim	66 ±13	68 ±10	67 ±9	0.55
- 2nd trim	69±12	72±10	72±10	0.26
- 3rd trim	74±12	78±12	79±10	0.052
Albuminuria n (%)				
- Normo-	31 (70)	35 (81)	41 (95)	
- Micro-	9	4	2	**0.032**
- Macro-	4	4	0	
Retinopathy n (%)				
- No	18 (41)	22 (51)	16 (37)	
- Simplex	22 (50)	18 (42)	26 (60)	0.36
- Proliferative	4 (9)	3 (7)	1 (3)	
MTFHR mutation n (%)				
- Heterozygicity	17 (39)	23 (50)	18 (43)	0.49
- Homozygocity	4 (9)	3 (7)	2 (3)	
Factor V Leiden no.	1	6	0	-
HbA1c (%)				
- Pregravid	8.3 ±1.5	7.9 ±1.5	7.5 ±1.4	0.17
- 1st trim	7.7 ±1.2	7.3 ±1	7.2 ±1	0.054
- 2nd trim	7.1 ±1	7.1 ±1	7 ±0.8	0.95
- 3rd trim	7.5 ±1.1	7.4 ±1.2	7.1 ±1.1	0.26
- 3 months post-partum	8.9 ±1.4	8.6 ±1.5	8.6 ±1.2	0.72
Creatinine (µmol/l)				
- 1st trim	58 ±15	58 ±10	57 ±8	0.81
- 2nd trim	55±9	59±9	58±10	0.064
- 3rd trim	58±10*	61±10	62±9	**0.045**
Creatinine clearance (ml/min)				
- 1st trim	125 ±28	131 ±28	133 ±25	0.3
- 2nd trim	122±30	123±27	124±28	0.97
- 3rd trim	116±30	118±30	118±30	0.93
Cholesterol (mmol/l)				
- 1st trim	5±1	4.9±0.8	4.5±0.71*	**0.02**
- 2nd trim	6.3±1.3	6.1±1	6±1	0.27
- 3rd trim	6.8±1.5	6.9±1.2	6.5±1.3	0.44
Triglyceride (mmol/l)				
- 1st trim	1.23±0.54	1.12±0.51	1.18±0.69	0.67
- 2nd trim	2.26±1.03	2.31±0.81	2.39±1.1	0.82
- 3rd trim	3.22±1.23	3.36±1.19	3.76±2.11	0.27
Sysdiu (mmHg)				
- 1st trim	123 ±9	120 ±10	117 ±7	0.051
- 2nd trim	122±11	119±9	118±6	0.11
- 3rd trim	128±13	123±13	125±12	0.55
Diadiu (mmHg)				
- 1st trim	74 ±7	71 ±7	70 ±5	0.061
- 2nd trim	74±7*	70±5	70±5	**0.031**
- 3rd trim	79±9	76±9	77±7	0.38
AASI 1st trimester				

- 1st trim	0.3 ±015	0.33 ±0.15	0.3 ±0.2	0.77
- 2nd trim	0.32±0.17	0.26±0.13	0.31±0.16	0.24
- 3rd trim	0.36±0.18	0.28±0.17	0.32±0.17	0.27

MTFHR: methylene-tetrahydro-folate-reductase, trim: trimester, Diadiu: Diastolic diurnal blood pressure, Sysdiu: Systolic diurnal blood pressure, AASI: Ambulatory arterial stiffness index. Data are given as mean ±SD, median (range) or numbers (percentage of column)

*: Post-hoc test indicates this value is different from the (two) others ($p<0.05$)

The lowest tertile of birthweight z-score included more women with micro / macroalbuminuria than the middle and upper tertile; however, ¾ had normoalbuminuria and no further sign of surplus vasculopathy compared to the other tertile groups (Table 1). Neither grade of retinopathy, MTFHR mutation, Factor V Leiden, renal function nor AASI in diurnal blood pressure were different between the tertile groups.

IGF-1 rose steeply from gestational week 26 to 36 and turned out different between the tertile groups in gestational week 30 to 34. IGF-1 values showed a tendency to higher levels at the 1st than at the beginning of the 2nd trimester. It also showed a difference in 3 months post-partum but it was measured in only 48 (37 %) women (Table 2, Fig.1). IGF-1 and z-scores at week 32 and 34 stayed significantly associated at regression analysis with adjustment for body weight, age, duration of diabetes, HbA1c, AER, and preeclampsia (wk. 32 and 34, $p<0.04$ and $p<0.02$, respectively). The lowest birthweights were associated with a lower rise in IGF-1 from week 22 to 32 ($p<0.001$, Fig. 1).

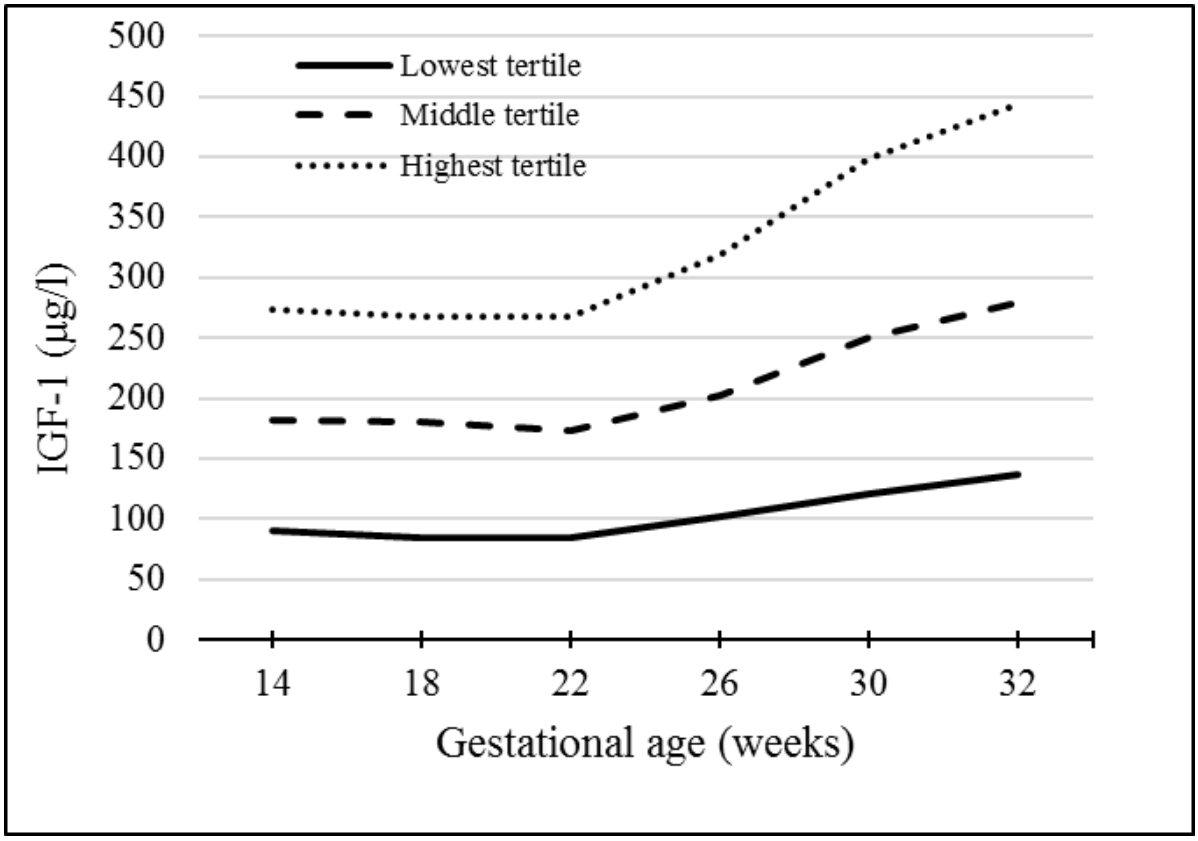
Table 2: Serum IGF-1 in 130 pregnant women with type 1 diabetes

Gestational week (n)	Lowest birthweight tertile	Middle birthweight tertile	Highest birthweight tertile	p-value (ANOVA)
6 (40)	100 (43-141)	99 (54-130)	101 (46-189)	0.63
10 (81)	98 (23-134)	105 (49-160)	93 (56-173)	0.98
14 (130)	90 (30-179)	92 (39-247)	92 (33-168)	0.11
18 (130)	84 (28-201)	97 (44-240)	87(35-181)	0.43
22 (130)	84 (29-158)	90 (45-241)	94 (35-240)	0.39
26 (130)	102 (15-204)	100 (50-291)	117 (44-578)	0.1
30 (130)	121 (19-268)	129 (57-321)	148 (66-713)*	0.028
32 (130)	137 (17-326)	142 (45-449)	165 (80-597)*	0.022
34 (113)	135 (33-336)	164 (77-361)	207 (70-534)*	0.007
36 (80)	146 (65-301)	180 (67-357)	181 (96-605)	0.07
3 months post-partum (48)	79 (49-194)	127 (88-199)*	100 (64-148)	0.01

Data are given as median (range). *: Post-hoc test indicates this value is different from the (two) others ($p<0.05$)

The incidences of preeclampsia (21 %) and preterm delivery (29 %) were similar in all three groups (Table 3). The median birthweight was 3715 g delivered at the median gestation of 36 weeks. The last umbilical pulsatility index measured before delivery was higher in the lowest birthweight group compared to the two other groups while umbilical pH was lowest in the middle birthweight group; however, both measurements were incomplete as only 70 and 56 % of the data was registered, pulsatility index and umbilical pH, respectively.

Figure 1. IGF-1 values during pregnancy in 130 women with type 1 diabetes by birthweight tertiles.



Lowest birthweight tertile (full line), middle birthweight tertile (interrupted line), highest birthweight tertile (dotted line). IGF-1 from gestational week from 22 to 32, $p < 0.001$ (all tertiles, 2-way ANOVA).

Table 3: Obstetrical outcomes in 130 pregnant women with type 1 diabetes

	Lowest birthweight tertile (n=44)	Middle birthweight tertile (n=43)	Highest birthweight tertile (n=43)	p-value (ANOVA)
Preeclampsia n (%)	7 (16)	10 (23)	10 (23)	0.62
Preterm delivery n (%)	9 (20)	11 (26)	17 (40)	0.13
Last umbilical pulsatiliy index[a]	1.25±0.48*	1.04±0.23	1.03±0.27	0.022
Umbilical pH[b]	7.27±0.08	7.22±0.1*	7.28±0.09	0.045
Birthweight (g)	3070 (1640-3975)	3840 (2650-4435)	4415 (3370-5445)	0.001
Gestational age (weeks)	37 (32-40)	36 (32-38)	36 (32-38)	0.02
Birthweight z-score	0.4 (-1 - +1.3)	1.9 (1.3 – 2.5)	3.1 (2.5-4.8)	0.001

Data are given as as mean ±SD, median (range) or numbers (percentage of column), a: data from 91 of 130 women, b: data from 73 of 130 women

Discussion

Our main finding is that lower IGF-1 levels are associated with subsequent lower birthweight in pregnancy with diabetes and is displayed markedly at the end of the 2nd into 3rd trimester (Fig. 1). We hypothesize that the relative low birthweight, despite being within the limits of appropriate for gestational age percentiles, may display inappropriate growth if not outright growth-restriction. Most pregnancies with T1DM display the potential of fetal macrosomia and timed delivery is common to avoid adverse outcomes, even in uneventful pregnancies with good glycemic control. This

discrepancy underlines the importance to detect the seemingly harmless appropriate growth. One may wonder which factors may limit the full potential growth before growth restriction can be detected. Teramo et al. introduced a novel approach for growth in neonates in T1DM suggesting that growth restriction may be present when birthweight z-score was below -0.6 SD[21]. Our group of the lowest tertile includes this range of z-scores to some degree (Table 3). Our approach was not population-based as the above example but pragmatic for evaluation of repeated sampling. The observed changes in levels of IGF-1 may help in solving the enigma on correlations between birthweight and IGF-1 in pregnancies in T1DM as well as non-diabetes[22-24]. Thus, 2nd trimester's increasing IGF-1 levels were reported in normal pregnancies and in pregnancies complicated by diabetes mellitus, multiple fetuses, and growth restriction but with no differentiation to identify macrosomia or lower birthweight, if not outright growth restriction[25,26]. We were able to discern different levels of growth at a critical point in pregnancy where ultrasound may pick up different levels of growth patterns and optimized care can commence. Nevertheless, the lower tertile group hints at a different growth path associated with IGF-1, suggesting that growth is non-optimal and maybe restricted as we would expect macrosomia in the women with normoalbuminuria and satisfactory glycemic control. Therefore, we agree on a re-appraisal of different birthweight standards in T1DM pregnancies[21].

IGF-1 promotes growth of the fetus, which often results in excess (i.e. macrosomia) in T1DM; the observation is that birthweight in glycemic well-regulated T1DM pregnancy deviates further from the norm than approaching that of non-diabetic neonate. The dilemma suggests that glycemia cannot be solely responsible for neither macrosomia nor low birthweight despite less adverse perinatal outcome. Growth-promoting and -restricting factors, which act during pregnancy, have neonatal weight as the ultimate end-point in a long chain of sequence. These includes binding protein modulations and proteolysis as well as placental growth hormone (hPGH) and probably fibroblast growth factor[14]. The endogenous GH is replaced during pregnancy by hPGH, which points at placentation as key feature for which only scarce and indirect data are available. For this, PAPP-A and ultrasound were found promising in early prediction of SGA but so far has no practical value[2]. Higgins et al. showed that IGF-1 was lower in T1DM pregnancies than in non-diabetic controls, which may be a further indication on vasculopathy and placentation issues in T1DM compared to non-diabetic women. Similarly, it implies that both birthweight and IGF-1 have different levels as well as different directions with respect to diabetic versus non-diabetic pregnancies[27]. Some point out that the cause is an altered regulation of IGF-1 effects, probably via the placental growth hormone and proteolysis of IGF-binding proteins that in T1DM pregnancies usually imply a higher risk of macrosomia[22]. However, we have no data on these aspects and so far only the total IGF-1 and IGF-2 in maternal and fetal serum has been linked to growth[5-7,22,24,27-31].

AER was the only basal characteristic in our study that was associated to the outcome in the z-score tertiles. We suspected some degree of diabetic microvasculopathy and measured several vascular, renal, ophthalmological, and genetic factors to hint at a possible etiology of lower birthweight. Although we have a modest sample size for several factors to be evaluated simultaneously, the repeated measurements and the strict timing of events should have enabled us to find clinical significant associations with these modifying variables. Nevertheless, we cannot conclude that they do not exert effects or covariate with fetal growth but rather that they may do so at a lower, undetected level. Macroalbuminuria is known to be associated to lower birthweight, also when adjusted for preterm deliveries, preeclampsia and renal insufficiency[32]. Our selection of rather uneventful pregnancies aimed to study physiological phenomena to cast light on the timing of events and possible pathways besides vasculopathy. Even after adjustment with covariates were made in our analysis, the association of low IGF-1 with low

birthweight was still valid; thus, the IGF system is still the prime candidate for the etiology of macrosomia and growth restriction[28-30,33].

Similar glycemic control was achieved in the birthweight tertiles; even undetected fluctuation is not likely to affect the birthweight differences between the groups. However, the lower birthweight tertile group tended to have higher HbA1c outside of pregnancy and during 1st trimester. This corroborates the findings of Skajaa et al. that the women with the highest levels of HbA1c pre- and during pregnancy delivered newborns with the lowest birthweight z-scores and they suggested it was due to poor function of the placenta[34]. They, however, had much higher glycemic levels (HbA1c > 10 %) to trigger significant low birthweight, so it is questionable whether this applies for our cohort.

The repeated sampling proved to be the major strength of the study having complete data from gestational 14 to 32. Possibly larger samples at a singular time points, like week 11-13, are needed, which was the finding in a study of pre-partum prediction of growth restriction[31]. Even though IGF-1 was significantly lower in the SGA group at that early time in pregnancy, after adjustment for maternal characteristics, the association of IGF-1 with fetal growth disappeared[31]. We lack a sufficient sample size in gestational week 11-13 to substantiate these findings but our time-depending results show the end of 2nd / early 3rd trimester to be more promising when differentiating fetal growth (Fig.1).

As for the etiology on the association, the low levels of proteolysis of IGF-binding protein seen early in pregnancy may enable the binding proteins to inhibit the action of IGF-1 in susceptible women. This would account for a possible reduction in growth potential for the early fetus. Later in pregnancy this proteolysis increases 2-3 fold compared to non-pregnant situation in T1DM, which helps to explain prominent macrosomia[6]. We have not accounted for the modification of IGF-binding proteins, which was done in similar studies on macrosomia or multiplets[5,6,26,28]. The focus was instead the timing of events and relation to clinical variables, in particular minute or hidden vasculopathy, in order to pave the way for prediction at a time in pregnancy when appropriate steps can be taken. Similar studies have found variable associations of growth with growth factors and their modulators with similar techniques; however, often with a mixture of sampling time and unpaired measurements over time[17-21]. Most of these studies missed the appropriate time, which very likely have obscured the findings, as the end of 2nd trimester seem to be a window of opportunity. Similarly, hPGH is suggested to cooperate with IGF-1 as growth promoting factor, when one factor cannot account for the entire feature of fetal growth[7]. This study found low growth and IGF-1 well associated but concluded, still, that several more factors most likely were involved. If growth charts and IGF values are adjusted accordingly, the lowest birthweights are potentially predictable and IGF-1 could have early diagnostic value in evaluation of the growth of fetuses in type 1 diabetes.

Conclusion

Lower birthweight was found associated with lower maternal serum IGF-1 in 130 women with type 1 diabetes mellitus

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