Placental Structure in Type 1 Diabetes
Relation to Fetal Insulin, Leptin, and IGF-I

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OBJECTIVE—Alteration of placental structure may influence fetal overgrowth and complications of maternal diabetes. We examined the placenta in a cohort of offspring of mothers with type 1 diabetes (OT1DM) to assess structural changes and determine whether these were related to maternal A1C, fetal hematocrit, fetal hormonal, or metabolic axes.

RESEARCH DESIGN AND METHODS—Placental samples were analyzed using stereological techniques to quantify volumes and surface areas of key placental components in 88 OT1DM and 39 control subjects, and results related to maternal A1C and umbilical cord analytes (insulin, leptin, adiponectin, IGF-I, hematocrit, lipids, C-reactive protein, and interleukin-6).

RESULTS—Intervillous space volume was increased in OT1DM (OT1DM 250 ± 81 cm^3 vs. control 217 ± 65 cm^3; $P = 0.02$) with anisomorphic growth of villi ($P = 0.025$). The placentas showed a trend to increased weight (OT1DM 690 ± 19 g; control 641 ± 22 g; $P = 0.08$), but villous, nonparenchymal, trophoblast, and capillary volumes did not differ. Villous surface area, capillary surface area, membrane thickness, and calculated morphometric diffusing capacity were also similar in type 1 diabetic and control subjects. A1C at 26–34 weeks associated with birth weight ($r = 0.27$, $P = 0.03$), placental weight ($r = 0.41$, $P = 0.0009$), and intervillous space volume ($r = 0.38$, $P = 0.0024$). In multivariate analysis of cord parameters in OT1DM, fetal IGF-I emerged as a significant correlate of most components (intervillous space, villous, trophoblast, and capillary volumes, all $P < 0.01$). By contrast, fetal insulin was only independently associated with capillary surface area (positive, $r^2 = 6.7\%$; $P = 0.02$).

CONCLUSIONS—There are minimal placental structural differences between OT1DM and control subjects. Fetal IGF-I but not fetal insulin emerges as a key correlate of placental substructural volumes, thereby facilitating feedback to the placenta regarding fetal metabolic demand. ***Diabetes*** **58:2634–2641, 2009**

Maternal diabetes is associated with adverse consequences to mother and baby, with increased risks of perinatal morbidity and mortality, in particular in association with fetal macrosomia. The Pedersen hypothesis (1) proposed that maternal hyperglycemia drives increased transplacental glucose transfer and thereby compensatory fetal hyperinsulinemia and induction of fetal growth. Although the fetal consequences of maternal glycemia are clearly recognized, there is still uncertainty about the role of the placenta in determining these outcomes. Specifically, the nature and scale of attendant structural change within the type 1 diabetic placenta remains contentious. Notably, the respective contribution of placental structural differences and how these relate to fetal hormonal axes in the attainment of enhanced placental and fetal growth is also unknown, even in control populations.

Classically, older histological studies of type 1 diabetic placentas have described grossly abnormal placentas that are enlarged, thick, and plethoric, with abnormalities of villous maturation (2). These changes would all support the increased incidence of placental-related complications observed in diabetic pregnancy (3). However, other historical series have not detected significant differences (2), and more recent stereological studies continue to differ with either no disparity in placental composition (4,5) or isolated changes including increases in capillary volume and surface area (6,7), increased villous surface area (8), increased total diffusive conductance (9), and increased intervillous and trophoblast volume (7,10). This lack of consistency may reflect a combination of small series, grouping of different classes of maternal diabetes, differences in glycemic control between individual patients, recent improvements in antenatal care, and differing methodology.

To date, studies in diabetes have also largely used fetal macrosomia as a surrogate of maternal glycemia and excessive transplacental glucose transfer (7,10) rather than assessment of the fetal hormonal response including hyperinsulinemia. Certainly, fetal hyperinsulinemia has an independent positive association with birth weight and placental weight in offspring of mothers with type 1 diabetes (OT1DM) (11). IGF-I and IGF-II also influence feto-placental growth. IGF-I has strong correlations to both birth weight and placental weight in control subjects and OT1DM (11–13). The role of IGF-II is less clear in human studies and is likely modified by circulating IGF-II receptor (12). Adiponectin, although not directly associated with birth weight, does correlate with placental weight and contributes to the matching of fetal and placental growth in control subjects and OT1DM (11,14). Finally, leptin also correlates with placental weight in control subjects and OT1DM and has recently been proposed as an in utero signal of nutrient availability (11,15). Collectively, these fetal hormone axes may therefore facilitate enhanced growth of the fetus and compensatory changes within the placenta, including structural modification, particularly in response to an excessive glucose supply as seen in diabetic pregnancy. To address this potential interaction of maternal environment, fetal hor-

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mones, and placental structure, we have examined placentas in relation to birth weight, neonatal adiposity, and fetal hormonal indexes, in particular those of insulin and IGF-I in OT1DM.

RESEARCH DESIGN AND METHODS

Recruitment and collection of cord blood. Recruitment, which began in January 1999 and ended in May 2001, took place in eight hospital-based antenatal centers in Scotland. A total of 250 women with type 1 diabetes consented to participate in the study (a 94% participation rate of those enrolled in and planning to deliver in the centers), and cord blood samples were obtained from 200 subjects (80%). No differences in gestation at delivery, maternal age at delivery, years of diabetes, fetal sex, or maternal A1C (where available) were found between those with and without cord samples.

A detailed sampling protocol was placed in all centers with local training to ensure standardization. Given the effects of delayed cord clamping on stereological parameters (16) and to maximize cord blood available for collection, on immediate delivery of the fetus, two disposable cord clamps were placed at 10 cm from the umbilicus and a further two disposable cord clamps were placed at 30 cm from the umbilicus. This allowed an isolated loop of cord to be sampled for cord blood, facilitating the short median collection time for cord samples and a constant volume of fetal blood in the placenta. On delivery of the placenta, a fifth clamp was placed at 1 cm from the chorionic plate, with trimming of the cord to that level. To minimize effects of sample hemolysis on insulin levels, samples were included only if collected from the cord within 20 min and frozen within 60 min. The 200 samples were therefore restricted to those in whom *1*) there was no evidence of hemolysis of cord blood (17 excluded); *2*) cord blood had been collected within 20 min (12 exclusions: median [interquartile range] collection time for remaining samples 2 min [1–7]); *3*) cord blood centrifuged and plasma frozen within 60 min (17 exclusions: time from collection to freezing for remaining samples, 17 min [11–26]); *4*) antenatal glucocorticoids not administered in the 24 h before birth (15 excluded); *5*) children delivered before 33 weeks' gestation (5 excluded); and finally *6*) placental tissue sampled appropriately at time of birth (59 excluded). A total of 89 patients met these selection criteria.

A convenience sample of control mothers, with no history of obstetric or metabolic disease and with negative routine screening for gestational diabetes (national guidelines available at http://www.sign.ac.uk/guidelines/fulltext/55/section8.html), were recruited from routine obstetric follow-up clinics after the 34th week of pregnancy in the same centers at the same time. Of 145 women who gave initial consent, cord samples were attempted in 75 and obtained in 70. Thirty-nine collections met the above restriction criteria.

Data on clinical outcome, including caesarean section, intercurrent medical conditions, and hypertensive conditions of pregnancy, were obtained by case note review. Gestational ages were calculated from estimated dates of delivery from chart review. This date was derived from dates of last menstrual period (LMP), where available, or by ultrasound if there was either conflict with dates as assessed by LMP (>6 days) or LMP was unavailable.

Weight was measured at birth and, for offspring born between 33 and 42 weeks of gestation, further expressed as an SD score (17). Skinfold thickness at subscapular and triceps was measured using Holtain calipers by pediatricians at each site and using a centrally agreed protocol, which was available in writing at the time of measurement. Skinfolds were not measured in all subjects. There were no significant differences in baseline demographic or biochemical measures between those with and without skinfold measurements in either control subjects or OT1DM (data not shown). All mothers gave informed consent, and the local ethical committees approved protocols.

Stereological analysis. On completion of the cord-sampling procedure and after delivery of the placenta, the placental membranes were trimmed, the umbilical cord shortened to within 1 cm of the chorionic plate, and any large maternal clots removed. Placentae were then weighed to the nearest 1 g. The feto-placental index (birth weight [grams] divided by placental weight [grams]) was calculated for each delivery. Samples of placenta were then taken in a systematic random fashion. Specifically, the placenta was cut with a sharp knife into a series of parallel slices 1–2 cm thick. The slices were then diced into smaller blocks 2–3 cm wide and fixed by immersion in 4% formaldehyde for 24 h and subsequently embedded in paraffin wax. Placental blocks were cut into 3-μm sections and stained with hematoxylin and eosin. These were used to estimate the volume fractions and surface areas as previously described, with avoidance of section edges (10). Placentae from OT1DM and control subjects were treated in an identical manner.

All estimates were made at the light microscope level by a combination of point and intersect counting and computer-assisted length measurements using the CAST system (version 2.0; Olympus, Glostrup, Denmark). Fields of view were selected in a systematic random fashion and analyzed as previously described (10) (Fig. 1). In brief, volume densities of intermediate and terminal villi and of the villous trophoblast and capillaries were estimated by test point counting. Villous and capillary surface densities were estimated by intersect counting. For each placenta, ~200 events were counted across 20 systematic random fields of view per section. To convert volume and surface densities into absolute values (in cm^3 and cm^2), the volume of each placenta was taken as the reference volume. Volumes were calculated from trimmed placental weights assuming a specific gravity of 1.05 g cm^{-3} (18,19). The intersections of the test lines with the villous surface also provided random start points for the measurement of orthogonal intercepts across the villous membrane, from the microvilli to the nearest capillary lumen. Harmonic means of the intercept lengths were calculated and then converted to diffusion distances. Given that there are standard values for physiological constants of oxygen in red cells, plasma, and tissues, including the villous membrane, the overall morphometric diffusing capacity for oxygen of the villous membrane of each placenta was able to be derived from the formula described by Laga et al. (18). This is based on the Fick equation and provides an estimate of the maximal theoretical diffusion capacity of the placenta.

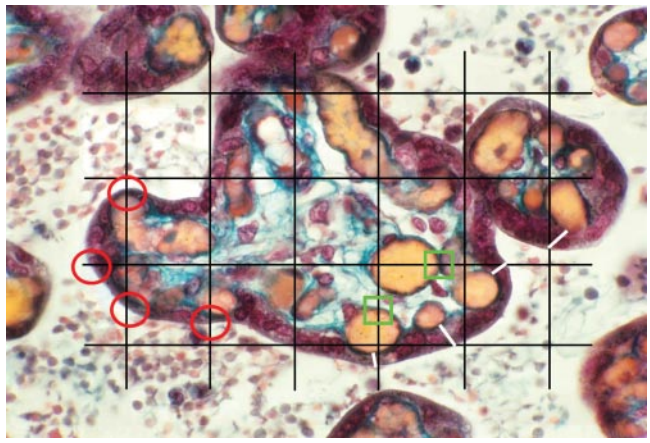

FIG. 1. Stereological methodology. Randomly selected fields of view were overlain with a quadratic test grid. Intersections between the test lines acted as test points and were used for estimating the volume fraction of the villous components by the point counting technique. Intersections between the test lines and the villous (circles) or capillary (squares) surfaces were counted to estimate the surface densities. Intersections of the test lines with the villous surface also acted as random start points for the estimation of villous membrane thickness by measurement of orthogonal intercepts. (A high-quality color digital representation of this figure is available in the online issue.)

Changes of growth or adaptation of villi and the IVS were assessed by the isomorphy coefficient. Briefly, this represents villous surface area, S, raised to the power 3/2, and divided by the volume, V, of either the villous or intervillous compartment (20) and is designed to measure disproportionate growth of villous surface area compared with villous volume. Similarly, the coefficients for capillary surface area relative to villous and capillary volume were also derived by $S^{3/2}/V$. The villous elaboration index, $I = S/V^{0.667}$, was also calculated for each case. This index gives similar information to the villous isomorphy coefficient, with a high value representing increased elaboration of the villous surface (8).

Cord blood assays. Plasma insulin, 32-33 split proinsulin, proinsulin, leptin, IGF-I, adiponectin, plasma total cholesterol, triglycerides, nonesterified fatty acid, VLDL cholesterol, LDL cholesterol, HDL cholesterol, C-reactive protein (CRP), intercellular adhesion molecule (ICAM)-1, and interleukin (IL)-6 were assayed as previously described (13,21–24). In particular, IGF-I was assayed by chemiluminescence immunoassay (Nichols Institute Diagnostics, San Juan Capistrano, CA) using standards referenced to the World Health Organization 1st International Reference Reagent 1988 (IGF-1 87/518). The limit of detection is 1.0 nmol/l. Intra- and interassay coefficients of variation were 5.5–6.8% and 5.4–7.0%, respectively. All lipid assays were carried out at the Biochemistry Department of Glasgow Royal Infirmary, which is a Centers for Disease Control and Prevention (Atlanta, GA) reference laboratory and accredited by Clinical Pathology Accreditation U.K. Maternal A1C was measured centrally by one laboratory.

Statistical analysis. Data were analyzed using standard software (Stata 10; Stata, College Station, TX). In several cases (insulin, leptin, triglycerides, VLDL, nonesterified fatty acids, total-to-HDL cholesterol ratio, CRP, ICAM-1,

TABLE 1
Characteristics of mothers with type 1 diabetes and their singleton offspring versus control mothers and children

	Control mothers	Mothers with type 1 diabetes	*P**
n	39	88	
Age (years)	28.4 ± 6.0	30.0 ± 5.0	0.13
Duration of diabetes (years)	—	13.1 ± 7.9	
Parity			
0	15 (38.5)	38 (43)	
1	19 (49)	37 (42)	
>1	5 (12)	13 (15)	0.78
Maternal A1C (weeks)			
5–12	—	7.8 ± 1.2	
16–24	—	6.8 ± 0.9	
26–34	—	7.0 ± 1.0	
35–40	—	6.7 ± 1.0	
Children (male/female)	19/20	42/46	0.92
Gestational age at delivery (weeks)	40.2 ± 1.2	37.8 ± 1.4	<0.001
Mode of delivery			
Vaginal	25 (64)	27 (31)	
Elective caesarean section	8 (21)	35 (40)	
Emergency caesarean section	6 (15)	26 (30)	0.002
Birth weight (kg)†			
Male	3.77 ± 0.52	3.81 ± 0.72	0.05
Female	3.42 ± 0.51	3.66 ± 0.62	<0.001
Z weight‡	0.37 ± 0.18	1.78 ± 1.5	<0.001
Placental weight (g)	641 ± 22.3	690 ± 19.0	0.08
Fetal-to-placental weight ratio	5.7 ± 0.14	5.6 ± 0.11	0.45
Cord indices			
n	39	88	
Cord insulin (pmol/l)	22.5 (16.6–39.6)	99.3 (57–219)	<0.001
Proinsulin (pmol/l)	9.3 (6.6–12.6)	13.6 (9.9–22.2)	<0.001
Split 32-33 insulin	10.6 (6.0–21.1)	43.8 (22.5–82.6)	<0.001
Leptin (ng/ml)	9.1 (4.3–18.5)	29.5 (12.8–54.2)	<0.001
IGF-I (mmol/l)	8.13 ± 0.55	8.0 ± 0.31	0.56
Adiponectin (μg/ml)	22.2 ± 5.2	20.0 ± 6.4	0.068
Cortisol (nmol/l)	399.9 (264.3–531.7)	271.1 (195.8–395.0)	<0.001
HDL cholesterol (mmol/l)	0.70 ± 0.16	0.63 ± 0.26	0.16
Triglycerides (mmol/l)	0.46 (0.38–0.58)	0.42 (0.33–0.55)	0.15
Nonesterified fatty acids (mmol/l)	0.25 (0.18–0.35)	0.17 (0.13–0.23)	<0.001
CRP (mg/l)	0.14 (0.12–0.17)	0.17 (0.13–0.24)	0.002
ICAM-1 (ng/ml)	165.6 (143.0–188.2)	179.7 (152.6–201.1)	0.07
IL-6 (pg/ml)	7.35 (2.4–12.4)	4.0 (2.4–9.3)	0.03
Hematocrit (%)	0.51 (0.48–0.54)	0.54 (0.49–0.58)	0.08
Platelets (10^9/l)	245 (113–300)	229 (185–262)	0.42
Offspring anthropometry			
n	17	40	
Crown rump length (cm)	33.7 ± 2.4	34.9 ± 2.3	0.079
Crown heel length (cm)	50.3 ± 3.0	50.9 ± 2.4	0.43
Triceps skinfold thickness (mm)	5.85 ± 2.4	7.66 ± 3.2	0.04
Subscapular skinfold thickness (mm)	5.53 ± 2.1	7.44 ± 2.2	0.004
Total skinfold thickness (mm)	11.38 ± 4.2	15.10 ± 5.0	0.01

Data are means ± SD, *n* (%), or medians (interquartile range). Unadjusted values are given as means ± SD or medians (interquartile range). **P* = value of significance in unpaired *t*, χ^2, or Mann-Whitney test, as appropriate. †Birth weights given as unadjusted *P* value for difference dependent on maternal diabetes status adjusted for gestational age at delivery. ‡*Z* weight is SD score compared with standard values for gestational age, sex, and maternal parity. A subset of offspring had detailed anthropometry performed. Cord hormonal profiles of singleton OT1DM vs. control offspring are shown.

and IL-6), measures were not normally distributed, and unadjusted values are presented as medians (interquartile range) and, for normally distributed variables, means ± SD. Variables were logarithmically transformed to obtain normal distributions. Intergroup differences were assessed by unpaired *t* test after checking homogeneity of variance by means of the *F* test, ANOVA, or, where further predictor variables were included, by general linear models. Exploration of more complex statistical models did not suggest alternative relationships. Spearman correlation coefficients are reported. Stepwise logistic regression was performed using an α of $P \leq 0.1$ for adding or removing predictors from the model. Statistical significance was determined at $P < 0.05$.

RESULTS

Fetal cord analytes and placental structure in OT1DM versus control subjects. Maternal and fetal characteristics of this cohort have been previously described and are included in Table 1. Maternal type 1 diabetes was associated with marked increases in standardized birth weight and absolute values of cord insulin, proinsulin, and split 32-33 insulin (21); cortisol; leptin; and CRP, with reductions in adiponectin, HDL cholesterol,

TABLE 2
Placental stereology of mothers with type 1 diabetes versus control mothers

	Control mothers	Mothers with type 1 diabetes	*P**
n	39	88	
Volume fractions			
Intervillous space volume fraction ($cm^2 \times cm^3$)	0.4 ± 0.1	0.4 ± 0.10	0.06
Villous volume fraction ($cm^2 \times cm^3$)	0.4 ± 0.1	0.4 ± 0.1	0.83
Nonparenchymal volume fraction ($cm^2 \times cm^3$)	0.2 ± 0.1	0.2 ± 0.1	0.58
Trophoblast volume fraction ($cm^2 \times cm^3$)	0.2 ± 0.0	0.2 ± 0.0	0.01
Capillary volume fraction ($cm^2 \times cm^3$)	0.1 ± 0.0	0.1 ± 0.0	0.28
Compartment volumes			
Intervillous space volume (cm^3)	216.7 ± 65.2	250.3 ± 81.2	0.02
Villous volume (intermediate + terminal villi) (cm^3)	217 ± 70.3	231.6 ± 95.8	0.39
Nonparenchymal (stroma + fibrin) (cm^3)	130.2 ± 59.6	131.9 ± 65.1	0.90
Trophoblast volume (cm^3)	49.7 ± 20.0	48.1 ± 23.3	0.72
Capillary volume (cm^3)	24.3 ± 16.2	27.9 ± 19.9	0.32
Surface areas			
Villous surface area (m^2)	4.0 ± 7.2	12.7 ± 6.8	0.34
Capillary surface area (m^2)	14.4 ± 9.1	13.7 ± 9.7	0.73
Villous membrane thickness (μm)	9.3 ± 1.2	9.2 ± 1.0	0.59
Morphometric diffusing capacity ($cm^2 \cdot min^{-1} \cdot kPa^{-1}$)	2.7 ± 1.6	2.5 ± 1.6	0.50
Morphometric diffusing capacity per birth weight ($cm^2 \cdot min^{-1} \cdot kPa^{-1} \cdot kg^{-1}$)	0.8 ± 0.4	0.7 ± 0.4	0.30
Isomorphic change			
Villous coefficient (cm^3/cm^3)	239,317 ± 125,212	192,225 ± 99,827	0.025
Intervillous space coefficient (cm^3/cm^3)	275,676 ± 215,189	220,532 ± 192,795	0.154
Capillary surface area relative to villous volume	250,770 ± 172,032	219,690 ± 177,986	0.36
Capillary surface area relative to capillary volume	2,338,266 ± 1,033,081	1,854,403 ± 921,906	0.010
Capillary volume relative to villous volume	0.1 ± 0.04	0.1 ± 0.0	0.28
Villous elaboration index	3,759 ± 1,212	3,226 ± 1,138	0.019

Data are means ± SD. *P = value of significance in unpaired t test.

nonesterified fatty acids, and IL-6 (Table 1). All differences remained significant after adjustment for sex and mode of delivery. There was a trend toward increased placental weight in OT1DM strengthened by adjustment for sex and gestation at delivery ($P = 0.06$). Feto-placental index did not differ (Table 1).

Analysis of placental stereological data demonstrated that intervillous space volume fraction and absolute intervillous space volume were increased in OT1DM (Table 2). Adjustment for mode of delivery, sex, gestational age at birth, and smoking status strengthened this difference for both ($P = 0.028$ and $P = 0.008$, respectively). The contribution of trophoblast to villous structure was reduced in OT1DM ($P = 0.01$); however, this was not associated with an overall reduction in trophoblast volume or villous surface area (Table 2). Absolute values of capillary volume and surface area were similarly unaltered by the presence of maternal diabetes.

Assessment of the complexity of the villous trees using the villous isomorphy coefficient and the elaboration index demonstrated that branching was reduced in OT1DM ($P = 0.025$ and $P = 0.019$, respectively). With respect to capillary development, the ratio of capillary surface area relative to capillary volume was also reduced in OT1DM ($P = 0.01$). The villous isomorphy coefficient and elaboration index were unrelated to gestational age, mode of delivery, sex, and smoking status. Capillary development, as related to capillary surface area relative to capillary volume, however, was related to gestational age and adjustment attenuated the OT1DM-specific difference ($P = 0.24$).

Placental composition, relationship with birth weight, and adiposity. Assessment of the relationship of birth weight to placental structure demonstrated a strong association with placental weight (control subjects, $r = 0.62$, $P < 0.0001$; OT1DM, $r = 0.62$, $P < 0.0001$), with additional strong correlations of birth weight to the individual placental components, including villous volume (control subjects, $r = 0.34$, $P = 0.03$; OT1DM, $r = 0.24$, $P = 0.03$), intervillous space volume (control subjects, $r = 0.58$, $P = 0.001$; OT1DM, $r = 0.44$, $P < 0.0001$), and nonparenchymal volume (control subjects, $r = 0.24$, $P = 0.13$; OT1DM, $r = 0.42$, $P < 0.0001$) in control subjects and OT1DM. All relationships were strengthened after standardizing birth weight for gestational age and sex ($P < 0.05$ for controls and OT1DM for all). Capillary volume ($r = 0.23$, $P = 0.03$) and capillary surface area ($r = 0.21$, $P = 0.05$) were associated with standardized birth weight in OT1DM only. For the subset with neonatal anthropometry, total skinfold thickness was associated with placental weight (control subjects, $r = 0.36$, $P = 0.15$; OT1DM, $r = 0.31$, $P = 0.05$) and capillary volume (control subjects, $r = 0.13$, $P = 0.62$; OT1DM, $r = 0.32$, $P = 0.046$), with no relationship seen with other placental components.

Placental composition and relationship with fetal hormones. Analysis of the associations of fetal hormonal axes to placental compartments demonstrated that insulin, in addition to known associations with birth weight ($r = 0.42$, $P < 0.0001$) and placental weight ($r = 0.44$, $P < 0.0001$) in OT1DM, demonstrated OT1DM-specific correlations to villous volume ($r = 0.23$, $P = 0.03$), intervillous space volume ($r = 0.25$, $P = 0.02$), nonparenchymal volume ($r = 0.23$, $P = 0.03$), and capillary volume ($r = 0.27$, $P = 0.009$). Cord IGF-I levels were associated with birth weight in control subjects and OT1DM (control subjects, $r = 0.32$, $P = 0.04$; OT1DM, $r = 0.48$, $P < 0.0001$) but only correlated with placental weight and substruc-

TABLE 3
Multivariate analysis of independent correlates of placental structure in OT1DM and control subjects

	Control mothers				Mothers with type 1 diabetes			
	Associate	β ± SE	*P*	Variance (%)	Associate	β ± SE	*P*	Variance (%)
Intervillous space volume	IGF-I	5.8 ± 3.3	0.09	8.4	IGF-I	9.8 ± 2.8	<0.001	22.6
					Leptin	35.7 ± 15.3	0.022	
					Adiponectin	−2.2 ± 1.3	0.081	
Villous volume	—	—	—	—	IGF-I	14.4 ± 3.3	<0.001	18.6
Nonparenchymal (stroma + fibrin)	—	—	—	—	Mode of delivery	14.7 ± 5.5	0.009	7.9
Trophoblast volume	Mode of delivery	−5.1 ± 2.5	0.05	18.4	IGF-I	2.5 ± 0.8	0.005	9.3
	Adiponectin	−1.1 ± 0.6	0.10					
Capillary volume	Leptin	10.7 ± 6.3	0.10	7.8	IGF-I	2.1 ± 0.7	0.005	9.0
Villous surface area	—	—	—	—	IGF-I	0.6 ± 0.3	0.021	6.3
Capillary surface area	Leptin	6.5 ± 3.6	0.08	8.8	Insulin	5.2 ± 2.1	0.017	6.7
Villous membrane thickness	Mode of delivery	−0.3 ± 0.2	0.06	10.0	—	—	—	—
Theoretical diffusing capacity	—	—	—	—	IGF-I	0.15 ± 0.06	0.012	7.4
Capillary surface area relative to villous volume					Insulin	74,051 ± 39,656	0.06	4.0
Capillary surface area relative to capillary volume					Mode of delivery	−161,606 ± 87,257	0.068	
					Insulin	560,045 ± 238,033	0.021	
					Leptin	−334,678 ± 200,058	0.098	8.1

Stepwise regression with log(insulin), log(leptin), adiponectin, IGF-I, mode of delivery, and sex was performed with an α of $P \leq 0.1$ for adding or removing predictors from the model. Variance (%) explained by the model.

tural indexes in OT1DM, specifically, placental weight ($r = 0.49$, $P < 0.0001$), villous volume ($r = 0.23$, $P = 0.03$), intervillous space volume ($r = 0.35$, $P < 0.001$), and nonparenchymal volume ($r = 0.29$, $P = 0.005$), with a weaker relationship to capillary volume ($r = 0.18$, $P = 0.08$). Cord leptin values demonstrated associations with birth weight (control subjects, $r = 0.48$, $P = 0.002$; OT1DM, $r = 0.40$, $P < 0.0001$) and placental weight (control subjects, $r = 0.40$, $P = 0.01$; OT1DM, $r = 0.33$, $P = 0.0019$) in control subjects and OT1DM but were only related to intervillous space volume (control subjects, $r = 0.30$, $P = 0.07$; OT1DM, $r = 0.32$, $P = 0.002$). Assessment of maternal glycemic state in OT1DM demonstrated that maternal A1C at 26–34 weeks was associated with birth weight ($r = 0.27$, $P = 0.03$), placental weight ($r = 0.41$, $P = 0.0009$), intervillous volume ($r = 0.38$, $P = 0.0024$), and nonparenchymal volume ($r = 0.25$, $P = 0.05$). First- and third-trimester maternal A1C demonstrated similar relationships with intervillous volume ($r = 0.34$, $P = 0.059$; $r = 0.31$, $P = 0.03$, respectively) but not with any of the other placental parameters including placental weight. Cord adiponectin and cortisol were unrelated to birth weight or placental parameters, and no consistent associations were observed for fetal inflammatory indexes, fetal hematocrit or fetal lipids, and placental composition. Isomorphy coefficients were also unrelated to birth weight, placental weight, or cord analytes in control subjects or OT1DM.

In stepwise regression models (Table 3), cord IGF-I was associated with intervillous volume in control subjects and OT1DM, with further contributions of IGF-I to nonparenchymal volume, trophoblast volume, capillary volume, villous surface area, and theoretical diffusion capacity in OT1DM ($P < 0.05$ for all). In contrast, insulin was only positively associated with capillary surface area, an association that was restricted to OT1DM ($P = 0.017$). Inclusion of maternal A1C at 26–34 weeks attenuated this association with insulin, and IGF-I became the sole associate of capillary surface area ($\beta = 0.87 \pm 0.4$, $P = 0.022$, $r^2 = 8.9\%$). Notably, A1C at 26–34 weeks was not related to any other placental parameters and did not alter the noted associations of IGF-I demonstrated in Table 3 and indeed made IGF-I the sole associate of nonparenchymal volume in OT1DM ($\beta = 5.2 \pm 2.7$, $P = 0.06$, $r^2 = 6.1\%$). For the isomorphic variables, insulin was associated with capillary surface area relative to both villous volume and capillary volume (Table 3), an effect that was partially explained by maternal A1C.

To assess the role of fetal hypoxia in determining placental structure, the surrogate marker of fetal hematocrit was included in the models. This did not attenuate the associations of IGF-I with placental structure in OT1DM. However, in control subjects, fetal hematocrit demonstrated positive associations with intervillous volume ($P = 0.023$), which was independent of sex ($P = 0.06$), and leptin ($P = 0.036$), with IGF-I dropping out of the model. Fetal hematocrit was also associated in control subjects with capillary volume ($P = 0.043$), which was independent

of the previously observed relationship with leptin ($P = 0.013$) (Table 3).

DISCUSSION

To our knowledge, this is the largest series examining placental stereology in a contemporary cohort of women with type 1 diabetes. It gives an important opportunity both to examine the influence of maternal diabetes on placental structure and to assess whether hormones in the fetal circulation (particularly insulin and leptin, which are markedly raised in this cohort) correlate with placental substructure. We find that, overall, there are relatively few systematic differences between placentas from mothers with diabetes and control subjects. In particular, the key measures that might be expected to influence substrate diffusion across the placenta (villous surface area, capillary surface area, villous membrane thickness) are not altered. In keeping with this, the placental morphometric diffusing capacity was also not different between diabetic and control pregnancies.

Historically, a range of abnormalities have been described in the placenta in type 1 diabetes, including increased placental size in conjunction with fetal macrosomia. In the oldest series, gross pathology including an increased rate of placental infarcts was described, as well as villous immaturity. In the series from the 1990s, microscopic morphology was often described as normal, particularly where maternal diabetes was well controlled (25). Increases in capillary volume and surface area (6,7), villous surface area (8), increased total diffusive conductance (9), and intervillous and trophoblast volume (7,10) were described and their presence or absence often ascribed to the degree of maternal diabetes control. In our series, we did not observe these changes with the exception of an increase in intervillous space volume, which classically indicates a deficiency of terminal villi and persisting villous immaturity. Consistent with this and previous reports (20), we observe a reduction in the villous coefficient and elaboration index, measures of the complexity of the villous tree that would impinge upon the intervillous space, suggesting that the observed villous growth is anisomorphic. Importantly, however, despite this reduction in villous development, there is no overall impact on villous volume or surface area, primarily due to an increased total placental volume in OT1DM. One might speculate that the reduction in villous development and increased placental growth are compensatory changes, but it is not possible to determine the primary direction of this relationship. Appropriately, we demonstrate that individual placental components, including villous volume, all correlate with birth weight.

Despite these limited changes, the inconsistent nature of the studies to date, combined with the major findings of our series, supports the view that there are minimal changes in placental structure inherent in contemporary diabetic pregnancy. This is of interest, as the overall hormonal environment on the fetal side of the circulation is markedly abnormal, with median fetal insulin and leptin levels four and three times higher, respectively, than control subjects. It is therefore striking that these significant increases in insulin and leptin do not appear to be driving any consistent change in placental structure. Only IGF-I displayed a consistent relationship with the volumes of the intervillous space, villous trophoblast, and placental capillaries.

Insulin has classically been proposed as a mediator of enhanced placental development in type 1 diabetes and was thought to underlie the reported increases in proliferation rates of trophoblast, villous stromal cells, and villous capillaries. However, insulin is not usually transported across the villous membrane, and its effects may be different on the fetal and maternal side of the placenta. In the first trimester, insulin receptors are localized to the intervillous surface of the villous trophoblast, with expression predominantly on the surfaces of sprouting segments of the villous tree (26). In contrast, by the third trimester, the highest immunoreactivity for the insulin receptor is found in the fetal villous endothelium, in particular, in segments with capillary sprouting (27). Further analysis of the transcriptional profile and phosphorylation status of first- and third-trimester isolated trophoblast and endothelial cells in response to hyperinsulinemia suggests that there is a spatio-temporal shift in insulin response (28), leading to the hypothesis that there is a predominant effect of maternal insulin in early pregnancy and fetal insulin in later pregnancy (27). Fetal insulin levels would be predicted to contribute to control of villous differentiation in accordance with fetal growth and nutritional needs in later pregnancy. In the current study, we demonstrate that fetal insulin is an independent associate of capillary surface area and of capillary surface area relative to villous and capillary volume. Our observed relationship with capillary development would be consistent with insulin receptor localization to endothelial cells and the ability of fetal insulin to enhance capillary development, including longitudinal growth within a fixed villous volume (6). It is notable, however, that there appears to be little impact of fetal insulin on trophoblast volumes independent of the effects of IGF-I. In this analysis, we rely on cord measures of insulin and IGF-1. It is possible that fetal hyperinsulinemia earlier in pregnancy is acting; however, if hyperinsulinemia is established earlier in pregnancy, it is likely that cord insulin will also be increased. This is apparent in the strong relationship of cord insulin and IGF-1 to measures such as birth weight (13) and our biologically plausible association of fetal insulin with placental capillary volumes.

The relationship of circulating fetal IGF-I to many of the placental components is striking. Cord IGF-I is strongly associated with birth weight and placental weight (12), and IGF-I deletion or reduced receptor expression in humans are both associated with a reduction in birth weight and placental weight (29,30). Conversely, prolonged administration of exogenous IGF-I to growth-restricted fetuses substantially increases body and placental weight (31). In short-term studies, exogenous fetal IGF-I increases placental amino acid transfer and uptake and decreases proteolysis, facilitating organ-specific and placental growth (32). These relationships are in contrast to those observed in mice, suggesting species specificity, where deletion of IGF-I or its receptor IGF-Ir is associated with a significant reduction in fetal but not placental weight (33). Therefore, although IGF-I may directly be enhancing placental growth, via receptors expressed in trophoblast and endothelium (34–36), alternative indirect mediators like adiponectin, which have been implicated in the matching of fetal and placental weight, may contribute (11,14). However, cord levels of IGF-I and adiponectin are not correlated, and adiponectin was not an independent associate in the multivariate models. Fetal lipids, in particular HDL, is associated with IGF-1 in fetal and adult life

(37,38). However, we did not demonstrate any relationship of fetal lipids with placental parameters (data not shown) despite recent recognition that placental endothelial cells efficiently transport cholesterol (39). Similarly, although inflammatory signals, including IL-6, can increase IGF-I expression (40), we did not observe an independent effect of inflammatory mediators on placental parameters (data not shown). Lastly, inclusion of fetal hematocrit, an index of fetal hypoxia, did not alter these relationships but did demonstrate the expected positive association with capillary volume in multivariate models. Overall, our results would support the hypothesis that, in humans, IGF-I has a direct effect on placental development.

Leptin has also been proposed as a regulator of placental growth. In human trophoblast cells in vitro, exogenous leptin treatment has mitogenic and antiapoptotic effects, while inhibition of endogenous placental leptin expression reduces cell proliferation and increases apoptotic cell number and caspase-3 activity (41). Furthermore, leptin stimulates activity of the amino acid transporter system A in human placental villous fragments at term (42,43). Lastly, leptin can induce angiogenesis in primary cultures of endothelial cells (44). Despite these effects, leptin infusion does not induce significant placental growth in sheep (45), and in the current study, leptin did not show consistent relationships with placental components in the control subjects. This limited effect may reflect differential leptin receptor expression, as although leptin receptors have been localized to umbilical endothelial cells and trophoblast (46,47), they have not been demonstrated on placental endothelium, which may explain our observed lack of association of fetal leptin with stereological parameters. Although leptin exerted a small influence on intervillous space volume independent of IGF-I in the OT1DM placentas, leptin is markedly raised in OT1DM, suggesting that the ability of leptin to induce placental angiogenesis and growth may be limited.

Maternal A1C is associated with both birth weight and placental weight in this series. It is notable, however, that maternal diabetes and indeed maternal A1C are not related to most of the placental stereological parameters with the exception of intervillous space volume. This would suggest that excess maternal nutrient supply may not be primarily responsible for changes in placental development, and, where present, such changes and increases in placental weight in general follow increased fetal weight. A notable feature of this study is the close matching of fetal growth with placental size, including placental components. It would appear that the fetus is capable of modulating placental transport in response to metabolic demands and nutrient supply. The signals that facilitate this feedback loop are largely unknown; however, this study suggests that IGF-I, and to a lesser extent insulin and leptin, all contribute and facilitate varying degrees of morphological alternation.

In conclusion, we demonstrate that maternal type 1 diabetes is associated with minimal changes in placental structure and that the changes that do occur relate principally to villous maturity. Second, we identify that fetal IGF-1 is the principal correlate of placental substructure and that the effect of insulin is limited to capillary development. Lastly, we identify that fetal leptin has a limited positive effect on placental angiogenesis and, in conjunction with IGF-I and insulin, contributes to the metabolic feedback from the fetus to the placenta regarding its metabolic demands.

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