

Intrauterine Diabetes Exposure and the Risk of Renal Disease in Diabetic Pima Indians

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The association between the diabetic intrauterine environment and renal disease was examined cross-sectionally in 503 Pima Indians with type 2 diabetes. Subjects were selected from participants in an ongoing study of diabetes and its complications in the Gila River Indian Community of Arizona. Subjects' exposure to diabetes in utero was established from periodic examinations conducted as part of the study. The prevalence of elevated urinary albumin excretion (UAE) (albumin-to-creatinine ratio ≥ 30 mg/g) was 40% (83 of 207) in the offspring of nondiabetic mothers, 43% (105 of 246) in the offspring of prediabetic mothers (i.e., women who were not diabetic at the time of the pregnancy but who developed diabetes after the pregnancy), and 58% (29 of 50) in the offspring of mothers who had diabetes during pregnancy. After controlling for age, sex, duration of diabetes, HbA_{1c}, and mean arterial pressure in the offspring in a logistic regression analysis using generalized estimating equations, maternal diabetes during pregnancy was strongly associated with elevated UAE. The odds of elevated UAE in the offspring of mothers who had diabetes during pregnancy was 3.8 times (95% CI 1.7–8.4) that of the offspring of prediabetic mothers; the odds of elevated UAE in the offspring of nondiabetic and prediabetic mothers were similar (odds ratio of 0.94; 95% CI 0.59–1.5). We concluded that exposure to a diabetic intrauterine environment increases the risk of elevated UAE in diabetic Pima Indians. The effect of this exposure appears to be independent of other susceptibility factors that lead to nephropathy in diabetes. *Diabetes* 47:1489–1493, 1998

D iabetic pregnancy increases the risk of intrauterine death, prematurity, and perinatal mortality, and has lasting effects on anthropomorphic and metabolic development. Freinkel (1) proposed that fetal exposure to fuel-mediated alterations in maternal metabolism in a pregnancy complicated by diabetes may do the greatest harm to poorly replicating, terminally differen-

tiated cells, perhaps by reducing the number of such cells or by altering their functional capacity.

In the kidney, nephron development begins during the fifth week of gestation and continues into the 3rd trimester (2,3). Induction of ~60% of the normal complement occurs during the 3rd trimester (3). Under normal circumstances, new nephron formation ceases between 28 and 36 weeks of gestation (2,3). Because additional nephrons do not form after birth, damage that occurs as a result of exposure to an abnormal intrauterine environment may have long-lasting effects that are more likely to become clinically apparent when the individual is exposed to diseases that further damage the kidney, such as diabetes.

In our recent study of the relationship between birth weight and renal disease in diabetic Pima Indians (4), the risk of elevated urinary albumin excretion (UAE) was found to be over six times higher in offspring exposed to diabetes in utero than in unexposed offspring of nondiabetic mothers. Because susceptibility to diabetic nephropathy and diabetes in Pima Indians may be inherited from the same genetic locus or loci (5), the relationship between maternal diabetes during pregnancy and diabetic renal disease in the offspring could be due to inherited susceptibility factors rather than to an effect of exposure to the diabetic intrauterine environment per se.

The present study examined the relationship between diabetes exposure in utero and renal disease in Pima Indians with type 2 diabetes. To differentiate the possible effects of susceptibility factors for renal disease inherited jointly with susceptibility for diabetes from the effect of the intrauterine environment on diabetic offspring, the unexposed offspring of nondiabetic mothers were divided into two groups: a prediabetic group that included those diabetic subjects whose mothers developed diabetes after pregnancy and were presumed to have transmitted the same susceptibility to renal disease as did the diabetic mothers, and a nondiabetic group that included those diabetic subjects whose mothers did not develop diabetes and were less likely to have transmitted susceptibility to renal disease to their offspring.

RESEARCH DESIGN AND METHODS

Pima and the closely related Tohono O'odham Indians from the Gila River Indian Community are participating in a longitudinal diabetes study (6). Since 1965, each member of the Community age 5 years or older has been asked to have a research examination every 2 years. Each examination includes an oral glucose tolerance test, with the glucose concentration determined in venous plasma drawn after an overnight fast and 2 h after a 75-g oral carbohydrate load. In addition, a 75-g oral glucose tolerance test is administered to women after the 24th week of each pregnancy. Diabetes and impaired glucose tolerance are defined on the basis of the results of these tests, according to criteria of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus (7). The date of diagnosis was determined from these examinations or from a review of clinical records if diabetes had been diagnosed in the course of routine medical care.

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HbA_{1c}, total glycosylated hemoglobin; HbA_{1c}, glycosylated hemoglobin, fraction c; MAP, mean arterial pressure; UAE, urinary albumin excretion.

TABLE 1
Clinical and demographic features of the 503 diabetic Pima Indians (197 men, 306 women) in the study population, according to the mother's diabetes status during pregnancy

	Nondiabetic	Prediabetic	Diabetic
<i>n</i>	207	246	50
Age (years)	44 ± 15 (14–77)	38 ± 10 (16–68)	24 ± 9 (12–50)
Duration of diabetes (years)	7.4 ± 7.6 (0–27.5)	7.7 ± 7.5 (0–32.9)	5.1 ± 6.5 (0–28.7)
MAP (mmHg)	93 ± 14 (39–143)	92 ± 13 (63–152)	87 ± 14 (59–123)
HbA _{1c} (%)	8.4 ± 2.7 (3.1–16.1)	9.1 ± 2.6 (4.2–15.5)	8.8 ± 2.9 (4.6–15.3)
BMI (kg/m ²)	36 ± 8 (19–72)	36 ± 8 (21–64)	34 ± 7 (24–56)
Urinary albumin excretion (mg/g)	21 (undetectable–13,526)	20 (undetectable–12,603)	37 (undetectable–6,354)

Data are means ± SD (range) or median (range).

Blood pressure was measured to the nearest 2 mmHg with a mercury sphygmomanometer while subjects rested in the supine position. Systolic and diastolic blood pressure were measured at the first and fourth Korotkoff sounds, respectively. Mean arterial blood pressure (MAP) was calculated as the diastolic pressure plus one-third of the pulse pressure. Hypertension was defined according to Joint National Committee on Detection, Evaluation, and Treatment of High Blood Pressure criteria as a systolic pressure ≥ 140 mmHg, a diastolic pressure ≥ 90 mmHg, or treatment with antihypertensive medicine (8).

Urine specimens collected at the end of the 2-h glucose tolerance test were assayed for albumin with a nephelometric immunoassay (9). Creatinine concentration was measured in the same specimen using a modification of the picrate method of Jaffe proposed by Chasson et al. (10), and an albumin-to-creatinine ratio (milligram of albumin/gram creatinine) was computed. Elevated UAE was defined as an albumin-to-creatinine ratio ≥ 30 mg/g (11). The same urine specimens were also tested for total protein by the Shevky-Stafford method (12), and a protein-to-creatinine ratio (gram protein/gram creatinine) was computed. Proteinuria was defined as a protein-to-creatinine ratio ≥ 0.5 g/g, equivalent to a total protein excretion rate of ≥ 500 mg or more per day (13–15).

Plasma glucose concentrations were measured with an autoanalyzer using the potassium ferricyanide method. The stable fraction of total glycosylated hemoglobin (HbA_{1c}) was measured until 31 December 1989 by agar gel electrophoresis (16), after which HbA_{1c} was measured by high-performance liquid chromatography (17). Among 133 subjects (not all from the present study) in whom both variables were measured, the correlation between the two measures was very high ($r = 0.92$, $P = 0.0001$) (4). The linear regression formula from this comparison ($HbA_{1c} = [0.99 \times HbA_1] - 1.535$) was used to estimate HbA_{1c} in the 77 subjects of the present study in whom HbA₁ alone was measured.

This study included 503 diabetic subjects whose UAE was measured and whose mother's diabetes status in pregnancy was known. The most recent examination with the required data was used. Subjects were divided into three groups based on their mother's diabetes status at the time of the pregnancy and at follow-up (18). The offspring of nondiabetic women were those whose mother had normal glucose tolerance at the time of pregnancy (no previous 2-h postload plasma glucose concentration ≥ 140 mg/dl), had a documented normal oral glucose tolerance test 4 weeks after delivery, and did not subsequently develop diabetes. The offspring of prediabetic women were those whose mother had normal glucose tolerance at the time of pregnancy and 4 weeks after delivery, but who subsequently developed diabetes. The offspring of diabetic women were those whose mother already had diabetes at the time of pregnancy or developed it during pregnancy. Potential subjects were excluded if their nondiabetic mother had been followed for <5 years after the index pregnancy, if their mother had a history of impaired glucose tolerance (2-h postload plasma glucose concentration of 140–199 mg/dl), or if their mother's onset of diabetes could not be dated relative to the index pregnancy. Fathers of the offspring were also tested for diabetes at the research examinations described above. All offspring and their parents were at least half Pima or Tohono O'odham by heritage.

Statistical analysis. The crude (unadjusted) relationship between maternal diabetes and elevated UAE in offspring was examined by computing the prevalence of elevated UAE in each category of maternal diabetes status. The relationship was also evaluated by logistic regression to control for the effects of potentially confounding variables. These variables included age, sex, duration of diabetes, HbA_{1c}, and MAP. Models that included variables for maternal (or parental) hypertension and proteinuria were also presented, since hypertension and proteinuria both aggregate in families and each is related to the development of renal disease in Pima Indians (19,20). Because observations within families were not independent of one another (an assumption of conventional regression techniques), the logistic regression analyses were conducted using binomial generalized estimating equations (Statistical Package for Interactive Data Analysis; Sta-

tistical Computing Laboratory, Eastwood, Australia), which allow for lack of independence among observations (21,22). Continuous variables included in the regression models were centered at their mean values, and categorical variables were coded so that the largest category was the reference group. Product terms of independent variables did not improve the regression models and therefore were not included. Appropriateness of the logistic models was evaluated by the goodness-of-fit statistic proposed by Lemeshow and Hosmer (23).

Two indicator variables were used to model the effects of maternal diabetes: an indicator for a nondiabetic mother was given a value of "1" if the mother never developed diabetes or "0" otherwise; an indicator for a diabetic mother was given a value of "1" if the mother had diabetes at the time of the pregnancy or "0" otherwise. Thus when both indicator variables were included as predictors in the model, the reference group for each contrast was the group of subjects with prediabetic mothers (those who developed diabetes after the index pregnancy). Given our hypothesis that a diabetic intrauterine environment predicts diabetic renal disease independent of susceptibility factors inherited jointly with the susceptibility for diabetes, the subjects with prediabetic mothers, and not those with nondiabetic mothers, were the more appropriate reference group. Covariate-adjusted prevalences and 95% CIs of elevated UAE according to maternal diabetes status were estimated from the fitted regression models after setting the values of the covariates to the sample means (24).

RESULTS

Of the 503 diabetic subjects in the present study, 207 were offspring of 131 nondiabetic mothers, 246 were offspring of 131 prediabetic mothers, and 50 were offspring of 42 diabetic mothers. Two mothers were counted as diabetic for their last offspring and prediabetic for all others. Elevated UAE was found in 217 (43%; 92 men, 125 women) of the diabetic subjects. Clinical and demographic features of the study population are shown in Table 1.

The crude (unadjusted) prevalence of elevated UAE was similar in the offspring of nondiabetic and prediabetic mothers (40 and 43%, respectively), but was higher in the offspring of mothers with diabetes during pregnancy (58%). Table 2 shows the crude prevalence according to maternal diabetes status, stratified by age, sex, duration of diabetes, HbA_{1c}, hypertension, and father's diabetes status at the time of pregnancy. In each stratum, the prevalence of elevated UAE was higher in subjects exposed to diabetes in utero than in the other two groups. The offspring of diabetic fathers had a lower prevalence of elevated UAE than the offspring of nondiabetic fathers, regardless of the mother's diabetes category, but the number of fathers known to have diabetes was small and the diabetes status in the majority of fathers was unknown.

When examined in a logistic regression analysis using generalized estimating equations to account for the inclusion of siblings, maternal diabetes during pregnancy was strongly associated with the prevalence of elevated UAE, adjusting for age, sex, duration of diabetes, HbA_{1c}, and MAP in the offspring (Table 3, Model A). The odds of elevated albumin

TABLE 2

Number of offspring and prevalence of elevated UAE in the diabetic offspring according to the mother's diabetes status during pregnancy and category of selected covariates

Covariate category	Nondiabetic		Prediabetic		Diabetic	
	<i>n</i>	Prevalence (%)	<i>n</i>	Prevalence (%)	<i>n</i>	Prevalence (%)
Age (years)						
<40	92	26.1	153	36.6	47	57.5
40	115	51.3	93	52.7	3	66.7
Sex						
M	91	41.8	89	50.6	17	52.9
F	116	38.8	157	38.2	33	60.6
Duration of diabetes (years)						
<5	112	25.0	123	34.2	34	41.2
5–9	25	32.0	46	34.8	6	83.3
10	70	67.1	77	61.0	10	100.0
HbA _{1c} (%)						
<9	121	31.4	110	27.3	26	46.2
9	86	52.3	136	55.2	24	70.8
Hypertension						
No	122	28.7	177	33.3	38	47.4
Yes	85	56.5	69	66.7	12	91.7
Father's diabetes status						
Nondiabetic	52	36.5	50	42.0	8	100.0
Diabetic	22	22.7	35	28.6	12	33.3
Unknown	133	44.4	161	46.0	30	56.7
Total	207	40.1	246	42.7	50	58.0

excretion in offspring of diabetic mothers was 3.8 times (95% CI 1.7–8.4) that of offspring of prediabetic mothers who later developed diabetes. The odds in offspring of nondiabetic mothers was similar to that in offspring of prediabetic mothers (OR = 0.94; 95% CI 0.59–1.5). The predicted prevalence of elevated UAE by maternal diabetes status, setting the covariates in Table 3, Model A equal to their sample means, is shown in Fig. 1. When variables for maternal hypertension and maternal proteinuria were added to the model (Table 3, Model B), the odds of elevated UAE in offspring of diabetic mothers was 3.4 times (95% CI 1.3–8.5) that of offspring of prediabetic mothers; the odds in the offspring of nondiabetic and prediabetic mothers remained similar (OR = 1.2; 95% CI 0.73–2.1). The predicted effect of each of the maternal variables was small. Only three (6%) offspring of diabetic mothers were 40 years old. When the analysis was restricted to the 292 subjects <40 years old, the conclusions were unchanged (data not shown).

The effect of paternal variables could not be effectively analyzed because of sparse data. Nevertheless, when variables for the presence of hypertension and proteinuria in at least one parent were substituted for variables for maternal hypertension and maternal proteinuria, maternal diabetes during pregnancy was still associated with the prevalence of elevated UAE. The odds of elevated UAE were 7.2 times higher (95% CI 1.6–33.3) in offspring of diabetic mothers than in offspring of prediabetic mothers. Although the odds were greater than in the previous models, the greatly reduced number of offspring of diabetic mothers (*n* = 19, 11 cases) in this subset affected the precision of the point estimate, as reflected by the wider CI. The odds in the offspring of nondiabetic and prediabetic mothers remained similar (OR = 1.1; 95% CI 0.53–2.2).

TABLE 3

Estimated adjusted effects (ORs and 95% CIs) of maternal diabetes during pregnancy and other factors on the prevalence of elevated UAE: results of binomial generalized estimating equations, fitting two models (A and B), where Model B includes maternal hypertension and proteinuria variables

Model covariates	Odds ratio	95% CI	<i>P</i> value
Model A (<i>n</i> = 503)			
Age (per 5 years)	1.0	0.92–1.1	0.69
Sex (M/F)	1.1	0.73–1.7	0.61
Duration of diabetes (per 5 years)	1.5	1.2–1.8	<0.001
HbA _{1c} (per 2%)	1.5	1.3–1.8	<0.001
MAP (per 10 mmHg)	1.4	1.2–1.7	<0.001
Nondiabetic mother (yes/no)	0.94	0.59–1.5	0.79
Diabetic mother (yes/no)	3.8	1.7–8.4	0.001
Model B (<i>n</i> = 447)			
Age (per 5 years)	1.0	0.90–1.1	0.88
Sex (M/F)	1.0	0.66–1.6	0.89
Duration of diabetes (per 5 years)	1.5	1.3–1.8	<0.001
HbA _{1c} (per 2%)	1.6	1.4–1.9	<0.001
MAP (per 10 mmHg)	1.4	1.2–1.7	<0.001
Maternal hypertension (yes/no)	0.93	0.57–1.5	0.77
Maternal proteinuria (yes/no)	1.3	0.73–2.4	0.37
Nondiabetic mother (yes/no)	1.2	0.73–2.1	0.44
Diabetic mother (yes/no)	3.4	1.3–8.5	0.01

Odds ratio for the number of units shown in parentheses.

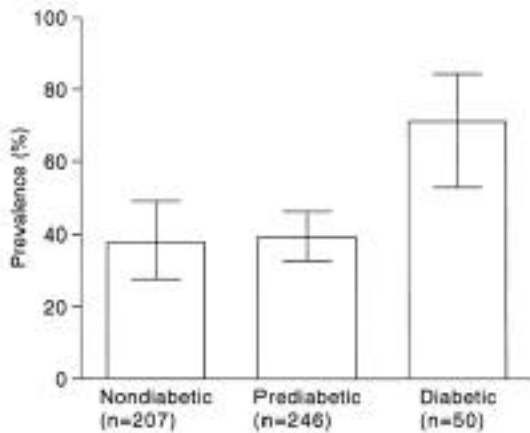


FIG. 1. Predicted prevalence (95% CIs) of elevated UAE (albumin-to-creatinine ratio ≥ 30 mg/g), by maternal diabetes status, adjusted for age, sex, duration of diabetes, HbA_{1c}, and MAP (Table 3, Model A). Values of the covariates were set to their sample means.

DISCUSSION

Exposure to a diabetic intrauterine environment is a strong risk factor for renal disease in diabetic Pima Indians. The risk of elevated UAE was nearly four times as high in offspring of diabetic mothers as in offspring of nondiabetic or prediabetic mothers. If the relationship between diabetic renal disease in offspring and maternal diabetes status was due entirely to susceptibility factors inherited jointly with the susceptibility for diabetes (5), we would expect the prevalence in offspring of prediabetic mothers to be similar to that in offspring of diabetic mothers, since all of these mothers subsequently developed diabetes. The prevalence was substantially higher, however, in offspring of diabetic mothers, suggesting that intrauterine exposure to diabetes plays an important role in the development of diabetic renal disease.

Susceptibility factors for renal disease may be inherited independently of diabetes, or there may be environmental risk factors that cluster within families (19,20,25–33). In Pima Indians, hypertension and proteinuria cluster in families, and each is related to the development of renal disease (19,20). Accordingly, the effect of diabetes exposure in utero on the prevalence of elevated UAE was examined by controlling for the effects of maternal hypertension and maternal proteinuria in one model and parental hypertension and parental proteinuria in another. In these analyses, the relationship between intrauterine diabetes exposure and elevated UAE persisted, affirming an effect of the intrauterine environment independent of the effects of other currently recognized susceptibility factors for renal disease that cluster in families.

Renal agenesis is a well-recognized manifestation of fetal exposure to diabetes (34,35), but less severe manifestations of renal injury associated with fetal exposure have not been described in humans. In sheep, exposure to hyperglycemia causes an increase in glomerular filtration rate in the fetus and induces glycosuria, diuresis, and natriuresis (36). The extent to which these functional changes might result in damage to developing glomeruli, however, is unknown. Nevertheless, our findings are compatible with the hypothesis that a fuel-mediated teratogenic effect (1) of exposure to diabetes in utero may cause glomerular damage that leads, in turn, to earlier development and more rapid progression of renal disease in adulthood once diabetes develops. Exposure

to maternal diabetes in utero was not associated with a higher prevalence of elevated UAE in nondiabetic offspring (data not shown), suggesting that the nephrotoxic effects of intrauterine exposure to diabetes typically do not lead to progressive renal injury unless other diseases that damage the kidney supervene.

Cross-sectional studies are subject to a number of limitations. In the present study, the effect of maternal diabetes during pregnancy on the prevalence of renal disease in diabetic offspring could have been under- or overestimated if postnatal survival or study participation among offspring differed according to maternal diabetes status. In addition, cross-sectional measures of time-dependent variables, such as blood pressure and level of glycemia, may be unreliable proxies for relevant past exposures. In controlling for these covariates, the validity of the study will be reduced to the extent that the previous covariate exposures of subjects are not reflected by the current measurements.

Brenner and Chertow (37) proposed that nephron development is impaired in people of low birth weight because of a critical shortage of the fuels necessary for fetal development. Consistent with this hypothesis, the prevalence of elevated UAE was over twice as high in diabetic Pima Indians of low birth weight than in those of normal birth weight (4). The results of the present study suggest that exposure to an overabundance of fuels, as occurs in the diabetic intrauterine environment (1), may also injure the developing nephron, since the risk of elevated UAE was nearly four times as high in subjects exposed to diabetes in utero than in those exposed to a normal intrauterine environment. Nevertheless, the mechanism by which the diabetic intrauterine environment damages the kidney is unknown. Its effect, however, may be considerable, since maternal diabetes during pregnancy is believed to lead to a vicious cycle in which each successive generation has a higher risk of having diabetes by the time it reaches child-bearing age than the preceding generation (38). Given the heightened risk of elevated UAE associated with exposure to maternal diabetes during pregnancy, this vicious cycle could also lead to a corresponding increase in the incidence of diabetic renal disease in successive generations.

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