The offspring of Pima Indians with early onset type 2 diabetes are at high risk for developing diabetes at an early age. This risk is greater among those whose mothers were diabetic during pregnancy. To define the metabolic abnormalities predisposing individuals in these high-risk groups to diabetes, we conducted a series of studies to measure insulin secretion and insulin action in healthy adult Pima Indians. In 104 normal glucose-tolerant subjects, acute insulin secretory response (AIR) to a 25-g intravenous glucose challenge correlated with the age at onset of diabetes in the mother ($r = 0.23, P = 0.03$) and, in multiple regression analyses, the age at onset of diabetes in the father ($P = 0.02$), after adjusting for maternal age at onset and after allowing for an interaction between these terms. In contrast, insulin action (hyperinsulimemic glucose clamp) did not correlate with the age at onset of diabetes in the parents. To determine whether early onset diabetes in the parents affected insulin secretion in the offspring across a range of glucose concentrations, responses to a stepped glucose infusion were measured in 23 subjects. Insulin secretion rates were lower in individuals whose mothers had developed diabetes before 35 years of age ($n = 8$) compared with those whose parents remained nondiabetic until at least 49 years of age ($n = 15$) (average insulin secretory rates: geometric mean [95% CI] 369 [209–652] vs. 571 [418–780] pmol/min, $P = 0.007$). Finally, the AIR was lower in individuals whose mothers were diabetic during pregnancy ($n = 8$) than in those whose mothers developed diabetes at an early age but after the birth of the subject ($n = 41$) (740 [510–1,310] vs. 1,255 [1,045–1,505] pmol/L, $P < 0.02$). Thus, insulin secretion is lower in normal glucose tolerant offspring of people with early onset type 2 diabetes. This impairment may be worsened by exposure to a diabetic environment in utero. *Diabetes* 50:1828–1833, 2001

Type 2 diabetes is common among the Pima Indians of Arizona (1). In this population, individuals at a particularly high risk include those whose parents developed diabetes at an early age (2) and those whose mothers were diabetic during pregnancy (3,4). The mechanisms causing these individuals to be at increased risk for diabetes are unknown.

Substantial evidence indicates that genetic factors contribute to the high prevalence of diabetes in Pima Indians. Diabetes is a familial trait in this population, as it is in others (2,5). Moreover, segregation analyses are consistent with a major gene affecting the age at onset of diabetes (2). Risk factors for developing diabetes among the Pima Indians include obesity, insulin resistance, and a low acute insulin response (AIR) to an intravenous glucose challenge (6,7). These traits are also familial (8–11). Thus, genetic transmission of a prediabetic trait, such as insulin resistance or impaired insulin secretion, could contribute to the higher risk for diabetes in the offspring of individuals with early onset diabetes.

Environmental or acquired factors also contribute to the high prevalence of diabetes in Pima Indian. Exposure to a diabetic environment in utero is associated with a substantially higher risk of diabetes in offspring than would be expected based on genetics alone (3,4). Among 20- to 24-year-old Pima Indians, 45% of individuals whose mothers were diabetic during pregnancy developed diabetes compared with only 9% of 20- to 24-year-olds whose mothers became diabetic subsequent to delivery (3). Furthermore, Pima Indians who were born after their mother developed diabetes have a 3.7-fold higher risk of developing diabetes than their full siblings who were born before their mother developed diabetes (4). These findings indicate that in utero exposure to a diabetic environment increases the risk for developing diabetes beyond that attributable to genetic factors.

To investigate the metabolic factors increasing the risk for diabetes in the offspring of Pima Indians with early onset diabetes, we conducted a series of studies to measure insulin secretion and insulin action in these individuals. First, measures of insulin secretion and insulin action in normal glucose-tolerant adult Pima Indians were compared with the age at onset of diabetes in their parents. Second, insulin secretory responses to graded glucose infusions were compared in the offspring of women with early onset diabetes and the offspring of those who developed diabetes at a later age. Finally, the effects of expo-
sure to a diabetic environment in utero were examined by comparing the metabolic characteristics of the offspring of women who were diabetic during pregnancy with those of the offspring of women who had developed diabetes at an early age but after the birth of the subject.

RESEARCH DESIGN AND METHODS

Epidemiological studies of type 2 diabetes have been conducted since 1965 among the Pima and Tohono O’odham Indians of the Gila River Indian Community (1). Approximately every 2 years, community residents over 5 years of age are asked to undergo an examination that includes anthropometric measurements and a 2-h 75-g oral glucose tolerance test (OGTT). Since 1983, more detailed studies of the metabolic predictors of diabetes have been performed on a subset of community members (7). Data from the epidemiological study were used to establish whether the parents of the subjects participating in the detailed metabolic studies had developed diabetes and, if so, the age at onset. Because even mild degrees of glucose intolerance can be associated with impairments in insulin secretion and insulin action (12), only subjects with normal glucose tolerance (13) and detailed metabolic studies were selected from the cohort for further analyses.

In the first analysis, insulin secretion and insulin action were examined in relation to the age at onset of diabetes in the parents. All parents of the individuals in this subset were diabetic or had been examined and found to not have diabetes when they were at least 50 years of age. Only subjects with at least one diabetic parent were included in the analysis. The offspring of mothers who were diabetic before pregnancy and those with incomplete parental information were excluded. A total of 104 offspring from 39 nuclear families (ranging in size from 1 to 3 offspring) met these criteria and were included in this analysis.

Next, a group of unrelated Pima Indians was specifically recruited to study insulin secretion responses to graded glucose infusions. This group included 8 individuals whose mothers had developed diabetes before 35 years of age and 15 individuals whose parents had been tested and were nondiabetic until at least 49 years of age (although 6 mothers and 4 fathers subsequently developed diabetes). These age criteria were chosen based on previous segregation analyses to maximize the likelihood that individuals in these two groups either did or did not have a major gene affecting the age at onset of diabetes (2). None of the individuals in this analysis were the offspring of a diabetic pregnancy.

Finally, the 8 individuals whose mothers were known, based on glucose tolerance testing, to be diabetic before pregnancy were compared with 41 individuals whose mothers had at least one nondiabetic glucose tolerance test after the birth of the subject and who developed diabetes before 40 years of age. The latter criterion was included to minimize potential confounding differences in the age at onset of diabetes of the mothers in the two groups. All studies were approved by the Institutional Review Board of the National Institutes of Health and by the Tribal Council of the Gila River Indian Community, and all subjects gave written informed consent before participation. All subjects were in good health, as determined by a comprehensive medical evaluation including medical history, physical examination, and routine laboratory testing. Subjects were admitted to the Clinical Research Unit of the National Institute of Diabetes and Digestive and Kidney Diseases in Phoenix, Arizona, for 8–15 days and were provided a standard weight-maintaining diet containing 50% of calories as carbohydrate, 30% as fat, and 20% as protein for at least 3 days before metabolic testing. While in the unit, subjects were restricted to sedentary activities.

**Anthropometric measurements.** Fat mass, fat-free mass, and percent fat were derived from body density measurements obtained by underwater weighing, with simultaneous determination of residual lung volume by helium dilution (14) or by dual-energy X-ray absorptiometry using a total-body scanner (DPX-L; Lunar Radiation, Madison, WI) (15). Measurements of body composition from the two methods were made comparable using an equation derived in our laboratory (15).

**Metabolic measurements.** All metabolic testing was performed in the morning after a 12-h overnight fast. At least 3 days in the research ward, a 2-h 75-g OGTT was performed. The AIR was calculated as the mean increase in plasma insulin concentration above basal for samples obtained 3, 4, and 5 min after an intravenous injection of 25 g glucose (7). Approximately 7 days after admission, a 2-step hyperinsulinemic-euglycemic glucose clamp was performed. As detailed elsewhere (7), primed-continuous infusions of regular insulin (Novo Nordisk, Bethesda, MD) were administered at rates of 240 pmol/m² body surface area per min for 100 min and 2,400 pmol/m² per min for an additional 100 min. These infusions produced mean ± SD steady-state plasma insulin concentrations of 720 ± 305 and 13,800 ± 10,402 pmol/l, respectively. Endogenous glucose production was measured for 2 h at baseline and during the low-dose infusion using a primed-continuous infusion of [3-3H]glucose. Glucose disposal rates during the low- and high-dose insulin infusions (MINS and MINS, respectively) were adjusted for differences in steady-state plasma glucose concentrations, expressed per kilogram of estimated metabolic body size (fat-free mass + 17.7), and corrected for endogenous glucose production (assumed to be 0 during the high dose) (16).

In insulin secretion responses to a graded glucose infusion were measured in a group of individuals using a sequential euglycemic-hyperinsulinemic step-hyperglycemic protocol. For the first 100 min of this study, insulin action was measured during an infusion of insulin at a rate of 240 pmol/m² body surface area per min. Glucose disposal during the phase was calculated as described above. The insulin infusion was then discontinued, and plasma insulin concentrations were allowed to return to basal levels over 60 min. This was followed by a stepped glucose infusion administered at rates of 11, 17, 22, 33, and 44 pmol·kg⁻¹·min⁻¹ (2, 3, 4, 6, and 8 mg·kg⁻¹·min⁻¹) for 40 min per step. Three blood samples for measurement of insulin, C-peptide, and glucose concentrations were obtained at baseline and during the last 10 min of each glucose step. The insulin secretion rate (ISR) was derived by deconvolution assuming a two-compartmental model of C-peptide clearance kinetics (17–19). At baseline and during each glucose infusion period, the average glucose concentration and ISR were calculated.

**Statistical analysis.** Data were analyzed using the procedures of the SAS software (SAS Institute, Cary, NC). Plasma insulin concentrations (the log10-transformed to normalize their distributions before parametric analyses. Pearson's correlation coefficients were calculated to determine the relation between selected variables. The relation of AIR (adjusted for age, sex, percent body fat, and MINS) to the age at onset of diabetes in the parents was analyzed using multivariate linear regression models that included both maternal and paternal age at onset and an interaction term between maternal and paternal age at onset (i.e., maternal age at onset × paternal age at onset). Because many of the subjects in this analysis were full siblings, the effect of parental age at onset of diabetes on AIR was also examined using a general estimating equation procedure to control for the nonindependence of family members. Changes in glucose, insulin, and ISR during the graded glucose infusion were compared in the offspring of mothers with early onset diabetes (<35 years of age) and in those whose mothers and fathers remained nondiabetic until at least 49 years of age using repeated measures analysis of variance (ANOVA). Average ISRs over the entire glucose infusion procedure were compared between groups using ANOVA after adjustment (using linear regression) for average glucose concentrations. Differences between the group of subjects whose mothers were diabetic during pregnancy and the group of subjects whose mothers subsequently developed diabetes were tested using Student's t test or the nonparametric Wilcoxon's rank-sum test. Group differences were also tested after adjusting the dependent variables for age, sex, percent body fat and, for measures of insulin secretion, insulin action (MINS) using multivariate linear regression analyses. Data are expressed as means ± SD (or geometric means ± 10% CI for log-transformed variables), and P values <0.05 are considered significant.

**RESULTS**

**Effects of parental age at onset of diabetes.** Parental age at onset of diabetes was not related to body composition or insulin action in 104 subjects with normal glucose tolerance (58 men and 46 women, age 29 ± 10 years, body fat 33 ± 10%). Log-transformed AIR, adjusted for age, sex, percent body fat, and insulin action, correlated with the age at onset of diabetes in the mother (r = 0.23, P = 0.03, among 85 subjects whose mothers had diabetes) but not with the age at onset of diabetes in the father (r = −0.02, among 72 subjects whose fathers had diabetes). In 64 subjects in whom the age at onset of diabetes was known in both parents, offspring whose mother's and father's age at onset of diabetes were both above the median (>41 and >46 years of age, respectively) had the highest AIR (geometric mean 1,530 pmol), whereas those with at least one parent whose age at onset of diabetes was below the median (early onset diabetes) had a lower AIR (geometric mean 1,040 and 1,280 pmol in those with maternal and paternal early onset diabetes, respectively) (Fig. 1). However, having two parents with early onset diabetes did not seem to further impair the AIR (geometric mean 1,155 pmol).
Although there were no significant differences between groups when subjects were classified according to median age at onset of diabetes of their parents in this manner, multiple regression analyses, in which parental age at onset of diabetes was treated as a continuous variable, showed that both maternal and paternal age at onset were related to adjusted AIR \( (P = 0.06 \text{ and } P = 0.02, \text{ respectively}) \), and a significant interaction was observed between the age at onset of diabetes in the mother and the age at onset of diabetes in the father \( (P = 0.02) \). Similar results were obtained using the general estimating equation procedure to control for family membership. These results suggest that both maternal and paternal age at onset of diabetes affect AIR in the offspring and that the effects depend on the age at onset of diabetes in the other parent.

To determine whether maternal age at onset of diabetes affected insulin secretory responses in the offspring over a range of glucose concentrations, insulin secretion rates were measured during graded glucose infusions in 23 Pima Indians, for whom physical and metabolic characteristics are summarized in Table 1. Subjects were divided into two groups: those whose mothers developed diabetes before 35 years of age \((n = 8)\) and those whose parents were both known to be nondiabetic until at least 49 years of age \((n = 15)\). Mean age, percent body fat, glucose tolerance, and insulin action were similar in the two groups. Insulin secretion rates increased with progressively increasing insulin action were similar in the two groups. Insulin secretion rates increased with progressively increasing glucose concentrations; however, this increase was less pronounced in the offspring of women who developed diabetes before 35 years of age \((n = 8)\) and those whose parents were both known to be nondiabetic until at least 49 years of age \((n = 15)\). Mean age, percent body fat, glucose tolerance, and insulin concentrations during the OGTT were also similar between the two groups, and there were no differences in insulin action between the two groups, as judged by the \( M \) values during the euglycemic clamp. The mean AIR was \(-40\%\) lower in the offspring of women who were diabetic during pregnancy compared with that of the offspring of women who subsequently developed diabetes, even after adjusting for age, sex, percent body fat, \( M_{\text{low}} \), and age at onset of diabetes in the mother \((P = 0.018, \text{Table 2})\).

Data on paternal diabetes status were available for only 4 of the 8 individuals whose mothers were diabetic during pregnancy (although it was available for all of the 41 control subjects). Consequently,

**Table 2.** There were no differences in the mean age at onset of diabetes in the mothers \((31 \pm 6 \text{ vs. } 34 \pm 6 \text{ years, } P = 0.17 \text{ in the groups with and without in utero exposure to diabetes, respectively})\). Mean BMI, percent body fat, glucose tolerance, and insulin concentrations during the OGTT were also similar between the two groups, and there were no differences in insulin action between the two groups, as judged by the \( M \) values during the euglycemic clamp. The mean AIR was \(-40\%\) lower in the offspring of women who were diabetic during pregnancy compared with that of the offspring of women who subsequently developed diabetes, even after adjusting for age, sex, percent body fat, \( M_{\text{low}} \), and age at onset of diabetes in the mother \((P = 0.018, \text{Table 2})\). Data on paternal diabetes status were available for only 4 of the 8 individuals whose mothers were diabetic during pregnancy (although it was available for all of the 41 control subjects). Consequently,

**Effects of a diabetic in utero environment.** The physical and metabolic characteristics of the adult offspring of women who were diabetic during pregnancy and women who developed diabetes after pregnancy are compared in
it was not possible to control for possible paternal influences in these analyses.

**DISCUSSION**

Among the Pima Indians of Arizona, the offspring of individuals with early onset type 2 diabetes and the offspring of women who were diabetic during pregnancy are at increased risk for developing diabetes (2–4). The present study was undertaken to characterize the metabolic abnormalities underlying the increased risk for diabetes in offspring. Our analyses indicate that the AIR to an intravenous glucose challenge is related to the age at onset of diabetes in the parents. Thus, individuals whose parents had early onset diabetes manifest an impairment in insulin secretion relative to those whose parents developed diabetes at a later age. Using a stepped glucose infusion to define the dose-response relationship between plasma glucose and insulin secretion, we confirmed that insulin secretory rates were lower across a range of glucose concentrations in individuals whose mothers had early onset diabetes than in those whose parents developed diabetes at a later age. Finally, we observed that the mean AIR to intravenous glucose was lower in the offspring of women who were diabetic during pregnancy compared with the offspring of women who had also developed diabetes at an early age but after the birth of the subject.

The correlation between adjusted AIR and the age at onset of diabetes in the mothers could be caused by an inherited impairment in insulin secretion in the offspring of women with early onset diabetes, or it could reflect a unique effect of the maternal-fetal environment. The finding that paternal age at onset of diabetes was associated with insulin secretory function in the offspring after adjusting for the maternal effect is consistent with the former interpretation. Thus, individuals who had at least one parent with an age at onset of diabetes below the median had a lower AIR than individuals who had two parents with an age at onset of diabetes above the median (Fig. 1). The interaction of maternal and paternal age at onset is evident in that the effect of early onset parental diabetes is not additive—the AIR in those with two parents with early onset diabetes was comparable with that in those who had just one parent with early onset diabetes. One possible explanation for this is that the selection criteria for inclusion in these analyses biased the data against demonstrating an additive effect, i.e., those individuals manifesting a more severe impairment in insulin secretion may have been excluded because they had already developed impaired glucose tolerance or diabetes.

Segregation analyses suggest that a major gene contributes to the risk of developing type 2 diabetes by influencing the age at onset of the disease in Pima Indians (2).

**TABLE 2**

Comparison of the offspring of diabetic pregnancies and those whose mothers developed diabetes after pregnancy.

<table>
<thead>
<tr>
<th>Maternal diabetes during pregnancy</th>
<th>Yes</th>
<th>No</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>8</td>
<td>41</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>22 ± 3</td>
<td>27 ± 6</td>
<td>0.03</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>4/4</td>
<td>24/17</td>
<td>0.7</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>34 ± 8</td>
<td>33 ± 6</td>
<td>0.8</td>
</tr>
<tr>
<td>Mothers’ age at onset of diabetes (years)</td>
<td>31 ± 6</td>
<td>34 ± 6</td>
<td>0.17</td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td>6.4 ± 1.1</td>
<td>6.4 ± 0.6</td>
<td>0.8</td>
</tr>
<tr>
<td>Fasting</td>
<td>215 (155–300)</td>
<td>220 (200–260)</td>
<td>0.8</td>
</tr>
<tr>
<td>Insulin (pmol/l)</td>
<td>790 (510–1,240)</td>
<td>910 (755–1,105)</td>
<td>0.5</td>
</tr>
<tr>
<td>M (µmol/kg · metabolic body size · min⁻¹)</td>
<td>15 (13–19)</td>
<td>15 (14–16)</td>
<td>0.6</td>
</tr>
<tr>
<td>AIR (pmol/l)</td>
<td>740 (510–1,310)</td>
<td>1,255 (1,045–1,505)</td>
<td>0.018</td>
</tr>
</tbody>
</table>

Data are means ± SD or geometric means (95% CI). M, glucose disposal rate at mean plasma insulin concentrations of 720 pmol/l (M_{low}) and 13,800 pmol/l (M_{high}) expressed as micromole per kilogram of estimated metabolic body size (kg fat free mass + 17.7) per min. AIR, acute insulin response expressed as the mean of the change in plasma insulin levels relative to basal value from the 3rd to the 5th min after the intravenous glucose injection. AIR is adjusted for age, sex, percent body fat, M_{low}, and age of onset of diabetes in the mother.
These models predict a greater genetic component among those with early onset diabetes compared with those with later onset diabetes (2). Because insulin secretion is a familial trait in Pima Indians (10,11) and because defects in the acute insulin response predict diabetes in this population and others (7,20,21), it is possible that inherited defects in insulin secretion could account for the familial nature of early onset type 2 diabetes in Pima Indians. The significant association of AIR with age at onset of diabetes in the parents observed in the present study is consistent with this hypothesis. Studies in other populations support this as well. European subjects who had a first-degree relative with type 2 diabetes had reduced first-phase insulin secretion (22,23), as did insulin-resistant offspring of diabetic patients (24). Furthermore, recent studies in maturity-onset diabetes have firmly established that mutations in genes that affect pancreatic β-cell development or function can cause diabetes (25–27). Thus, our findings of an association between age at onset of diabetes in the parents and insulin secretion in the offspring suggest a possible physiological mechanism for a major gene affecting the age at onset of diabetes in this population. However, the possibility that shared behavioral and/or environmental factors are responsible for this association cannot be excluded.

A slightly greater risk for developing diabetes is conferred by maternal rather than paternal diabetes (3,28,29). Because the offspring of women who were diabetic during pregnancy are at higher risk for diabetes than can be explained based solely on genetics (3,4), we also sought to determine the effects of a diabetic in utero environment on metabolic risk factors for diabetes. Human studies are limited (30,31) and, to date, have not addressed the effects of a diabetic pregnancy on direct measures of insulin secretion and insulin action in offspring. The present results suggest that in utero exposure to a diabetic environment is associated with a defect in the AIR to intravenous glucose, which was ~40% lower in the offspring of women who were diabetic during pregnancy than in the offspring of women who subsequently developed diabetes. This difference cannot be ascribed to an impairment in insulin secretion associated with early onset diabetes because the mean maternal age at onset of diabetes was similar in the two groups and because the effect persisted even after adjusting for maternal age at onset of diabetes. Based, in part, on the observation that the offspring of rats made mildly diabetic during pregnancy have reduced insulin output in response to glucose (32), it has been hypothesized that exposure to hyperglycemia during critical periods of fetal development “programs” the developing pancreas in a way that affects subsequent insulin secretary function. Our findings in the offspring of diabetic women are consistent with this notion.

A significant limitation of the present series of studies is that glucose tolerance tests were not performed during pregnancy in the majority of the mothers. Consequently, it was impossible to examine the relation of maternal glycemia during pregnancy to subsequent insulin secretory function in the offspring. Therefore, it is unknown whether the offspring of mothers with early and late-onset diabetes were exposed to comparable ambient glucose concentrations in utero. It is possible that women predisposed to early onset diabetes might have had higher plasma glucose concentrations during pregnancy, which could have affected fetal β-cell development, than women lacking a predisposition to early diabetes. Transient gestational diabetes or impaired glucose tolerance also would not have been detected in women who did not have a glucose tolerance test during pregnancy. Undiagnosed gestational diabetes could have led to the stronger correlation between AIR in the offspring and the age at onset of diabetes in the mother compared with the father. Thus, an alternative interpretation of the data are that an inherited factor causing early onset diabetes impairs metabolic function but not necessarily insulin secretion. When this factor is present in mothers, the developing fetus can be exposed to higher (although not necessarily diabetic) ambient glucose concentrations that can, in turn, affect subsequent insulin secretory function in the offspring. The impairments of insulin secretion in the offspring of women with early onset diabetes and the offspring of mothers who were diabetic during pregnancy would thus represent different degrees of severity in a spectrum of defects caused by a common underlying abnormality. This mechanism could contribute to the different relation between AIR and age at onset of diabetes in the mothers and fathers as well. Finally, considering the limitations of the present series of studies, it is important to acknowledge that some of the comparisons may have been constrained by small sample sizes, and no attempt was made to account for multiple comparisons. Further studies that include indexes of maternal glycemia during pregnancy will be required to resolve the relative contributions of genetic factors and exposure to hyperglycemia in utero to the increased risk for diabetes in the offspring of individuals with early onset diabetes.

In conclusion, the results of the present analyses indicate that insulin secretory responses to glucose are lower in the offspring of individuals with early onset diabetes. This impairment is worse in individuals exposed to a diabetic environment in utero. These data suggest that strategies aimed at preventing type 2 diabetes might include therapeutic approaches to augment early insulin secretory responses to glucose and the early diagnosis and aggressive treatment of hyperglycemia during pregnancy. Before clinical implementation of these strategies can be recommended, however, trials should be undertaken in carefully selected groups at high risk for developing type 2 diabetes.

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