Long-Term Follow-Up of Infants of Mothers With Type 1 Diabetes

Evidence for hereditary and nonhereditary transmission of diabetes and precursors

PETER A.M. WEISS, MD
HEINZ S. SCHOLZ, MD
JOSEF HAAS, PHD
KARL F. TAMUSSINO, MD
JOCHEN SEISSLER, MD
MARTIN H. BORKENSTEIN, MD

OBJECTIVE — To estimate the impact of type 1 diabetes during pregnancy on transgenerational genetically caused and/or fuel-mediated amplification of types 1 and 2 diabetes and to estimate the impact of elevated amniotic fluid insulin levels.

RESEARCH DESIGN AND METHODS — A total of 75 white offspring of type 1 diabetic mothers and 49 control subjects of similar age and pubertal stage were examined at 5–15 years of age. All offspring had an oral glucose tolerance test. Glucose, insulin, and C-peptide were measured at 0, 30, 60, and 120 min after loading. Lipids and autoimmune antibodies were measured in fasting plasma.

RESULTS — Of the 75 offspring, 4 (5.3%) had overt diabetes, and 16 of 71 (22.5%) had autoimmune antibodies. Offspring of diabetic mothers had significantly higher BMI; symmetry indexes; cholesterol, glucose, insulin, and C-peptide levels; and insulin resistance than control subjects. With the exception of cholesterol, these values were significantly elevated in offspring who had elevated amniotic fluid insulin levels (>8 μU/ml, >48 pmol/l) during pregnancy compared with normoinsulinemic offspring and control subjects.

CONCLUSIONS — Offspring of type 1 diabetic mothers have an increased risk for diabetes later in life. The relative risk for type 1 and type 2 diabetes is 71.6 and 3.2, respectively. Type 2 diabetes–associated risk factors, such as high BMI; elevated glucose, insulin, and C-peptide levels; and insulin resistance, are related to the fetal metabolic experience in utero, as reflected by amniotic fluid insulin concentration.

Diabetes Care 23:905–911, 2000

The estimated number of adults with diabetes worldwide in 1995 was 135 million, with 51 million in developed countries. These numbers are estimated to increase to 300 and 72 million, respectively, by 2025 (1).

A genetically supported mode of transmission is known for type 1 and type 2 diabetes. Additionally, type 2 diabetes can be transmitted from mother to offspring by a nongenetic fuel-mediated mechanism (2,3). This is supported by cross-sectional epidemiologic studies showing that individuals with type 2 diabetes are more likely to have a diabetic mother than a diabetic father (4,5) and by follow-up studies in whites (6) and Pima Indians (7,8). Transgenerational transmission of diabetes has also been demonstrated in rats (9).

Only 2 studies, both from the Diabetes in Pregnancy Center at Northwestern University, have addressed the impact of fetal hyperinsulinism on diabetes-associated problems in adolescents (10,11). Both studies found a correlation between elevated amniotic fluid insulin levels and obesity and between the amniotic fluid insulin level and glucose tolerance in offspring of diabetic mothers (10,11). These studies did not distinguish between gestational (type 2–like) diabetes and type 1 diabetes. Thus, the genetic divergence makes it difficult to determine whether metabolic impairment in offspring is genetically transmitted or fuel-mediated. In the present study, we examined fuel-mediated transmission of diabetes to offspring of type 1 diabetic mothers, because a genetic predisposition to type 2 diabetes is less likely in this group. Because type 2 diabetes in a white population usually becomes manifest after 20 years of age (12), we looked for metabolic variations that antedate and are highly predictive of subsequent type 2 diabetes years before the clinical diagnosis (13). We also looked at the correlation of these data with fetal exposure to insulin in utero, as indicated by the amniotic fluid insulin concentration.

RESEARCH DESIGN AND METHODS

Study subjects and methods

Between 1982 and 1991, 210 viable newborns were delivered of mothers with type 1 diabetes at our institution. We considered the mothers to have type 1 diabetes because they had acute onset of insulin-dependent diabetes at a mean age of 11.6 ± 7.4 years. The mean C-peptide level (136 values in 51 mothers between 5 and 41 weeks' gestation) was 0.3 ± 0.2 ng/ml (0.1 ± 0.07 nmol/l). Moreover, insulin-requiring type 2 diabetes at this age is very rare in our population. All mothers were white. We contacted 136 of the mothers (74 mothers were not trace-

From the Departments of Obstetrics and Gynecology (P.A.M.W., H.S.S., J.H., K.F.T.) and Pediatrics (M.H.B.), University of Graz, Graz, Austria; and the Diabetes Research Center (J.S.), Heinrich Heine University Düsseldorf, Düsseldorf, Germany.

Address correspondence and reprint requests to Peter A.M. Weiss, MD, Associate Professor, Department of Obstetrics and Gynecology, University of Graz, Auenbruggerplatz 14, A-8036 Graz, Austria.

Received for publication 22 December 1999 and accepted in revised form 13 March 2000.

Abbreviations: ANOVA, analysis of variance; GADA, GAD antibodies; IA-2A, receptor-type protein tyrosine phosphatase antibodies; ICA, islet cell antibodies; I/G, insulin/glucose; IGT, impaired glucose tolerance; JDF, Juvenile Diabetes Foundation; OGTT, oral glucose tolerance test; RR, relative risk; RU, relative unit; SI, symmetry index.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Table 1—*Characteristics and metabolic data of control subjects*

	Mean ± SD (range)	Percentiles				
		3rd	10th	50th	90th	97th
BMI	17.3 ± 2.9 (12.7–24.2)	13.0	14.0	16.6	22.0	24.0
Glucose (mg/dl)						
G_0	86 ± 8 (66–105)	70	78	86	95	104
G_{30}	122 ± 22 (69–175)	78	90	124	149	163
G_{60}	106 ± 25 (66–180)	68	78	101	141	154
G_{120}	100 ± 17 (64–132)	66	73	104	119	128
G_{sum}	413 ± 54 (304–531)	340	377	420	537	575
Insulin (μU/ml)						
I_0	9.6 ± 4.7 (3.1–23.1)	4.2	4.8	9.4	15.0	20.8
I_{30}	45.3 ± 29.7 (6.6–136.0)	9.1	15.4	34.2	87.6	120.5
I_{60}	39.8 ± 25.4 (9.6–129.0)	10.0	13.6	34.8	74.5	95.5
I_{120}	33.3 ± 20.4 (4.9–79)	5.3	10.4	28.0	62.2	75.6
I_{sum}	128.1 ± 71.8 (43.9–342.7)	46.4	52.9	116.2	233.3	294.6
C-peptide (ng/ml)						
C_0	1.24 ± 0.49 (0.4–2.4)	0.50	0.70	1.10	2.0	2.3
C_{30}	3.56 ± 1.41 (1.6–8.1)	1.70	2.1	3.2	5.1	7.3
C_{60}	4.03 ± 1.51 (1.8–7.2)	1.9	2.1	3.8	6.4	6.8
C_{120}	3.63 ± 1.55 (0.6–7.5)	0.8	1.9	3.5	5.9	6.5
C_{sum}	12.46 ± 4.36 (6.2–22.6)	6.5	7.2	12.3	18.1	22.0
Insulin/glucose quotient (μU/ml/mg/dl)						
I/G_0	0.112 ± 0.05 (0.040–0.246)	0.046	0.058	0.105	0.181	0.232
I/G_{30}	0.366 ± 0.22 (0.083–1.015)	0.095	0.135	0.305	0.774	0.824
I/G_{60}	0.374 ± 0.23 (0.119–1.229)	0.120	0.139	0.310	0.637	0.860
I/G_{120}	0.331 ± 0.19 (0.046–0.718)	0.063	0.112	0.273	0.618	0.688
I/G_{sum}	1.183 ± 0.62 (0.429–3.058)	0.452	0.493	0.967	2.048	2.418
Lipids (mg/dl)						
$Cholesterol_0$	160 ± 23.2 (114–208)	119	126	164	184	205
HDL_0	54 ± 13.5 (22–86)	28	38	55	72	78
LDL_0	92 ± 20.2 (51–137)	58	65	94	124	132
$Triglycerides_0$	67 ± 31.5 (21–156)	27	33	63	124	141
$Sum\ lipids_0$	373 ± 61.9 (254–515)	276	288	375	456	505

Data are means ± SD and percentiles. BMI, glucose (G), insulin (I), and C-peptide (C) in plasma and I/G quotient at 0, 30, 60, and 120 min after glucose loading (1.75 g/kg), and fasting lipids in 49 offspring (20 males, 29 females) of healthy mothers at the age of 5–15 years.

able), and a total of 75 offspring of diabetic mothers were available for examination at 5–15 years of age. We contacted the first 12 metabolically healthy mothers (negative oral glucose tolerance test [OGTT]) (14) who delivered at our clinic during the same years to act as control subjects. Of these patients, 49 of 120 were available for examination.

Type 1 diabetic mothers were treated during pregnancy with 3–5 split doses of human insulin, aiming to keep preprandial blood glucose values <100 mg/dL (5.5 mmol/l) and postprandial levels <130 mg/dl (7.2 mmol/l) (15). The mean insulin requirements in the 20th and 38th–40th weeks were 63 and 90 U/24 h, respectively, and the mean maternal blood glucose levels were 100.2 mg/dl (5.5 mmol/l) and 94.0 mg/dl (5.2 mmol/l), respectively. Mean HbA_{1c} levels decreased from 8% at the onset of pregnancy to 5.1 and 5.0% in the 20th and 38th–40th weeks, respectively (15). Amniotic fluid insulin levels at 31 ± 2 weeks were available for 59 of the 75 patients (79%) (16).

Of the 75 type 1 diabetic mothers, 12 (16%) had a family history of maternal or paternal diabetes. Of these 12 mothers' parents, 7 (9.3%) (5 mothers and 2 fathers) had insulin-dependent diabetes at <20 years of age (probable type 1 diabetes) and 5 (6.7%) (2 mothers and 3 fathers) had manifestation at >50 years of age and oral or dietary treatment (probable type 2 diabetes). Of the control mothers, 3 (6.1%) of 49 had a maternal history of probable type 2 diabetes.

Height and weight were noted for all offspring to calculate the BMI (kg/m^2) and the symmetry index (SI) ([observed weight/median]/[observed height/median] for age of control subjects) (10,17), and pubertal stage was assessed (18). All offspring underwent an OGTT. Of the offspring of diabetic mothers, 4 had overt diabetes and only 1 of these had an OGTT. Data of overtly diabetic offspring were not included in the metabolic analyses. After an overnight fast, an oral glucose load of 1.75 g/kg (maximum dose 75 g) dissolved in 200 ml of water was administered (11). Venous plasma was drawn 0, 30, 60, and 120 min after the oral challenge to determine glucose, insulin, and C-peptide levels. Plasma glucose was measured enzymatically with a hexokinase method (Gluco-quant; Boehringer Mannheim, Mannheim, Germany). Plasma samples for insulin and C-peptide measurement were deep-frozen and analyzed at the end of the study. Insulin was measured with a double-antibody radioimmunoassay (Pharmacia RIA 100 Kit; Pharmacia, Uppsala, Sweden) and C-peptide with a competitive protein binding radioimmunoassay (RIA-gnost hC-Peptid; Behring, Marburg, Germany). Cholesterol, HDL, and triglycerides were measured enzymatically in fasting blood samples (test set; Boehringer Mannheim). LDL was calculated from cholesterol and HDL.

In offspring of type 1 diabetic mothers, islet cell antibodies (ICA), GAD antibodies (GADA), and receptor-type protein tyrosine phosphatase antibodies (IA-2A) were measured as markers for type 1 diabetes. ICA were measured by the standard immunofluorescence method using sections of frozen human group 0 pancreas, GADA, and IA-2A with a radiobinding assay. Cutoff for positivity was ≥1 Juvenile Diabetes Foundation (JDF) units in ICA and ≥0.9 relative units (RUs) in GADA and IA-2A. In the International Diabetes Workshop ICA Proficiency Programs, values of 90–100% for sensitivity and 100% for specificity were achieved. The assays for the detection of GADA and IA-2A were evaluated in the IDW Combined Autoantibody Workshop. The disease sensitivity of the GADA assay was 81%, and the specificity was 99%. The IA-2 antibody assay had a diagnostic sensitivity of 74% and a specificity of 99% (19).

Data were expressed as means with SD or medians with percentiles. Analysis of variance (ANOVA), the Wilcoxon Mann-Whitney test, the Fisher's exact test, and the

Table 2—*Demographic data of control subjects and type 1 diabetic patients (case subjects)*

	Control subjects	Diabetic patients	*P*
n	49	71	—
Sex (M/F)	20/29	42/29	NS
Maternal age (years)	26.2 ± 4.6	27.4 ± 6.1	NS
Parity	1.9 ± 0.8	1.9 ± 1.2	NS
Miscarriage	0.28 ± 0.57	0.45 ± 0.73	NS
Maternal height (cm)	164 ± 5.3	164 ± 5.2	NS
Prepregnacy weight (kg)	60 ± 9.7	65 ± 5.2	NS
Maternal weight gain (kg)	13.1 ± 5.0	12.1 ± 5.3	NS
Maternal BMI	22.5 ± 3.3	23.9 ± 5.0	NS
Birth weight (g)	3,334 ± 689	3,109 ± 569	NS
Birth weight >90th percentile	9 (18.4)	8 (10.6)	NS
Birth weight >4,000 g	7 (14.3)	4 (5.3)	NS
Gestational age (weeks)	39.5 ± 2.3	38.4 ± 1.9	0.005
Adolescents age (years)	10.8 ± 2.7	10.3 ± 2.7	NS
Adolescents height (cm)	142.3 ± 17.6	142.4 ± 17.2	NS
Adolescents weight (kg)	36.3 ± 13.0	40.4 ± 18.0	NS

Data are *n*, means ± SD, or *n* (%).

Spearman's 2-tailed correlation were used as appropriate with Statistical Product and Service Solution software (SPSS, Chicago). Differences were considered statistically significant at $P < 0.05$.

RESULTS

Control subjects

Postload glucose, insulin, and C-peptide levels and the I/G quotient in control subjects are shown in Table 1. There was no correlation between the glucose or lipid levels and age. In contrast, insulin, C-peptide, and I/G quotients correlated at all time points and in sum with age (sum insulin, $R = 0.553$, $P = 0.0001$; sum C-peptide, $R = 0.595$, $P = 0.0001$; sum I/G quotient I/G, $R = 0.593$, $P = 0.0001$). As expected, BMI also increased with age ($R = 0.448$, $P = 0.0014$).

There were significant differences in glucose-insulin homeostasis between male ($n = 20$) and female ($n = 29$) subjects, adjusted for age, height, weight, and BMI. During OGTT, male and female subjects had identical glucose levels at 0 and 120 min, but the glucose peak at 30 min was higher in female subjects (127 ± 20 vs. 114 ± 23 mg/dl, $P = 0.036$). Insulin levels, C-peptide levels, and I/G quotients were significantly higher in female than in male subjects and most distinct at 60 min after loading (insulin, 27.3 ± 15.7 vs. 48.5 ± 27.4 μU/ml, $P = 0.003$; C-peptide, 3.2 ± 1.1 vs. 4.6 ± 1.5 ng/ml, $P = 0.002$; I/G, 0.262 ± 0.128 vs. 0.454 ± 0.246, $P = 0.003$).

Control subjects and offspring of diabetic mothers

Maternal demographic data and fetal outcome were similar in offspring of type 1 diabetic mothers and control subjects. There were no differences in maternal age, parity, previous pregnancy losses, height, prepregnancy weight, weight gain during pregnancy, BMI, and macrosomic newborns (20). Similarly, there were no statistically significant differences in age, pubertal stage, height, weight, or sex in offspring of diabetic mothers and control subjects. The only statistically significant difference was a shorter gestational period in infants of diabetic mothers (Table 2).

Amniotic fluid insulin was documented at a mean gestational age of 31 ± 2 weeks in 59 of 75 (79%) diabetic mothers. The mean amniotic fluid insulin level was 10.5 ± 7.1 μU/ml (63.0 ± 42.6 pmol/l), the median amniotic fluid insulin 8.2 μU/ml (49.2 pmol/l), and the range 2.0–34.9 μU/ml (12.0–209.4 pmol/l). Only 25 of 59 (42.4%) of amniotic fluid insulin levels were >10 μU/ml (>60.0 pmol/l, >99th percentile of normal) (21).

Overt diabetes and autoimmune antibodies in offspring

Of diabetic mothers, 4 offspring (5.3%, 3 females, 1 male; aged 11.4, 10.7, 9.0, and 15.7 years) had overt diabetes with mani-

Table 3—*Metabolic data of offspring of diabetic mothers and control subjects*

		Offspring	
	Control subjects	With antibodies	Without antibodies
n	49	16	55
BMI	17.3 ± 2.9	17.3 ± 4.1	19.4 ± 4.7*
Glucose (mg/dl)			
G_0	86 ± 8	87 ± 7	89 ± 7*
G_{30}	122 ± 22	129 ± 25	135 ± 27†
G_{60}	106 ± 25	107 ± 27	112 ± 32
G_{120}	100 ± 17	102 ± 15	104 ± 19
G_{sum}	413 ± 54	425 ± 54	439 ± 63*
Insulin (μU/ml)			
I_0	9.6 ± 4.6	9.7 ± 4.4	14.1 ± 9.6†
I_{30}	45.3 ± 29.7	35.9 ± 12.4	71.3 ± 41.7†
I_{60}	39.8 ± 25.4	34.3 ± 15.8	55.6 ± 36.3*
I_{120}	33.3 ± 20.4	30.2 ± 17.4	48.6 ± 49.9*
I_{sum}	128 ± 72	110 ± 40	190 ± 111†
C-peptide (ng/ml)			
C_0	1.2 ± 0.5	1.2 ± 0.5	1.7 ± 0.8†
C_{30}	3.6 ± 1.4	3.8 ± 1.4	6.1 ± 2.9†
C_{60}	4.1 ± 1.5	4.5 ± 2.1	5.7 ± 2.8†
C_{120}	3.6 ± 1.5	3.6 ± 1.3	4.8 ± 2.9*
C_{sum}	12.5 ± 4.4	13.0 ± 4.4	18.2 ± 8.1‡
Sum I/G	1.183 ± 0.625	1.007 ± 0.369	1.614 ± 0.861*
Sum lipid	373 ± 62	388 ± 64	420 ± 72‡

Data are *n* or means ± SD. Difference versus control subjects: *$P < 0.05$, †$P < 0.01$, ‡$P < 0.001$. Glucose (G), insulin (I), and C-peptide (C) in plasma and I/G quotient at 0, 30, 60, and 120 min after glucose loading (1.75 g/kg) in 16 offspring of type 1 diabetic mothers with autoimmune antibodies and in 55 without autoimmune antibodies at 5–15 years of age.

Table 4—*Metabolic data of offspring of control subjects and offspring of type 1 diabetic mothers*

	Control subjects	Total ODM	ODM	
			Low (<8 μU/ml) AFI levels	High (≥8 μU/ml) AFI levels
n	49	71	28	28
AFI (μU/ml)	—	—	5.3 ± 1.6	15.7 ± 6.5*
BMI	17.3 ± 2.9	18.9 ± 4.6†	16.0 ± 2.6	20.7 ± 4.6*
BMI ≥22 (≥90th percentile)	5	21	1	13*
Lipids (mg/dl)				
Cholesterol	160 ± 23	178 ± 29‡	176 ± 28	174 ± 32
HDL	54 ± 13	56 ± 19	62 ± 21	53 ± 18
LDL	93 ± 20	107 ± 26‡	105 ± 23	106 ± 26
Triglyceride	67 ± 31	73 ± 47	60 ± 30	75 ± 35
Lipids sum	373 ± 62	413 ± 71‡	404 ± 64	407 ± 73
Glucose (mg/dl)				
G_0	86 ± 8	88 ± 7	87 ± 7	89 ± 7
G_{30}	122 ± 22	133 ± 26†	127 ± 24	137 ± 27
G_{60}	106 ± 25	111 ± 31	99 ± 26	116 ± 29§
G_{120}	100 ± 17	103 ± 18	101 ± 14	102 ± 17
G_{sum}	413 ± 54	435 ± 61†	412 ± 46	445 ± 55§
Insulin (μU/ml)				
I_0	9.6 ± 4.7	13.1 ± 8.9†	9.6 ± 4.1	15.3 ± 11.5§
I_{30}	45.3 ± 29.7	63.3 ± 39.9‡	49.7 ± 25.7	69.2 ± 35.0§
I_{60}	39.8 ± 25.4	50.8 ± 33.9	34.3 ± 16.5	57.4 ± 34.9‖
I_{120}	33.3 ± 20.4	44.5 ± 45.2	27.4 ± 16.5	51.0 ± 51.6§
I_{sum}	128.1 ± 71.8	171.7 ± 105.0†	121.0 ± 45.8	192.9 ± 109.5‖
C-peptide (ng/ml)				
C_0	1.2 ± 0.5	1.6 ± 0.7‡	1.3 ± 0.4	1.8 ± 0.9§
C_{30}	3.6 ± 1.4	5.5 ± 2.8¶	4.7 ± 2.2	6.2 ± 3.0§
C_{60}	4.0 ± 1.5	5.4 ± 2.7‡	4.3 ± 1.6	6.0 ± 3.2§
C_{120}	3.6 ± 1.5	4.5 ± 2.7†	3.3 ± 1.2	5.1 ± 3.1‖
C_{sum}	12.4 ± 4.4	17.0 ± 7.7¶	13.6 ± 4.2	19.1 ± 9.0‖
I/G quotient				
I/G_0	0.112 ± 0.050	0.147 ± 0.095†	0.109 ± 0.044	0.170 ± 0.122§
I/G_{30}	0.366 ± 0.220	0.466 ± 0.259†	0.388 ± 0.176	0.508 ± 0.256
I/G_{60}	0.374 ± 0.225	0.443 ± 0.241	0.349 ± 0.135	0.487 ± 0.250‖
I/G_{120}	0.331 ± 0.191	0.416 ± 0.367	0.270 ± 0.132	0.492 ± 0.466§
I/G_{sum}	1.183 ± 0.625	1.475 ± 0.815†	1.115 ± 0.391	1.657 ± 0.940§

Data are *n* or means ± SD. Difference ODM versus control subjects: †$P < 0.05$; ‡$P < 0.01$; ¶$P < 0.001$. Difference ODM high amniotic fluid insulin levels versus ODM low amniotic fluid insulin levels: §$P < 0.05$; ‖$P < 0.01$; *$P < 0.001$. Amniotic fluid insulin (AFI), BMI, glucose (G), insulin (I), and C-peptide (C) in plasma and I/G quotient at 0, 30, 60, and 120 min after glucose loading (1.75 g/kg). ODM, offspring of diabetic mothers.

festation at 3.0, 10.1, 4.6, and 15.7 years of age, respectively. The last case was diagnosed with the study OGTT (venous plasma glucose levels of 287 [15.9], 401 [22.3], 486 [27.0], and 466 [25.9] mg/dl [mmol/l] at 0, 30, 60, and 120 min, respectively). All overtly diabetic offspring were lean (BMI 14.9–21.5), of whom 3 had lean mothers (BMI 19.8–20.8) and the last case had an obese mother (BMI 34.1). Amniotic fluid insulin levels were available in the first 3 cases (7.4, 26.4, and 10.6 μU/ml [44.4, 158.4, and 63.6 pmol/l]) and showed no trend.

Autoimmune antibodies were detected in 16 of 71 offspring without overt diabetes (22.5%, 9 males and 7 females). Of this group, 14 had ICA with a concentration of 1–40 JDF U, 3 had GADA with a concentration of 1.0–47.3 RUs, and 1 offspring had IA-2A (80 RU), ICA (40 JDF U), and GADA (47.3 RU). Glucose tolerance was normal in the 16 offspring with antibodies (Table 3). Antibody-positive cases had significantly lower mean insulin, C-peptide, and I/G quotients than 55 antibody-negative cases adjusted for demographic and anthropometric factors of mothers and offspring, suggesting onset of impaired β-cell function (Table 3). Offspring with antibodies showed similar metabolic data as control subjects. Antibody-negative offspring differed more distinctly from control subjects than the overall study group (Tables 3 and 4). Mean ± SD amniotic fluid insulin levels in antibody-positive and antibody-negative cases were 11.7 ± 9.1 and 10.2 ± 6.4 μU/ml (70 ± 55 and 61 ± 38 pmol/l), respectively (NS).

Comparison of offspring data in control subjects and offspring of type 1 diabetic mothers

Offspring of type 1 diabetic mothers had a higher BMI and higher cholesterol, LDL, postload glucose, insulin, and C-peptide levels than control subjects. Insulin resistance (as judged by I/G quotients) was also increased (Table 4).

When taking the 97th percentile of control subjects as the cutoff (Table 1), infants of diabetic mothers had a relative risk (RR) of 7.8 for elevated BMI (2.0 vs. 14.1%, $P < 0.03$), 5.7 for elevated cholesterol (4.1 vs. 19.7%, $P < 0.02$), 4.8 for elevated LDL (4.1 vs. 16.9%, $P < 0.05$), 7 for elevated glucose levels 60 min after stimulation (2.0 vs. 12.7%, $P < 0.05$), 7.9 for elevated fasting insulin (2.0 vs. 14.1%, $P < 0.03$), 6.8 for elevated C-peptide 30 min after stimulation (4.1 vs. 22.5%, $P < 0.01$), and 7.9 for elevated I/G quotients in the fasting state (2.0 vs. 14.1%, $P < 0.03$).

These metabolic differences between offspring of type 1 diabetic mothers and control subjects were more distinct in male than in female subjects (Fig. 1). Although glucose levels showed only a statistically significant difference at 30 min after loading, insulin, C-peptide, insulin resistance as judged by the I/G quotient, and the sums of these parameters were significantly elevated at most postload time points (Table 4 and Fig. 1).

Amniotic fluid insulin concentration: correlation with metabolic values

After stratification according to high and low amniotic fluid insulin levels (3 values of overtly diabetic offspring were excluded), there was no difference in maternal data and age of the offspring. Lipid levels were similar in offspring of diabetic mothers with low and high amniotic fluid insulin levels. All other metabolic data of offspring with high amniotic fluid insulin levels were elevated, most of them signifi-

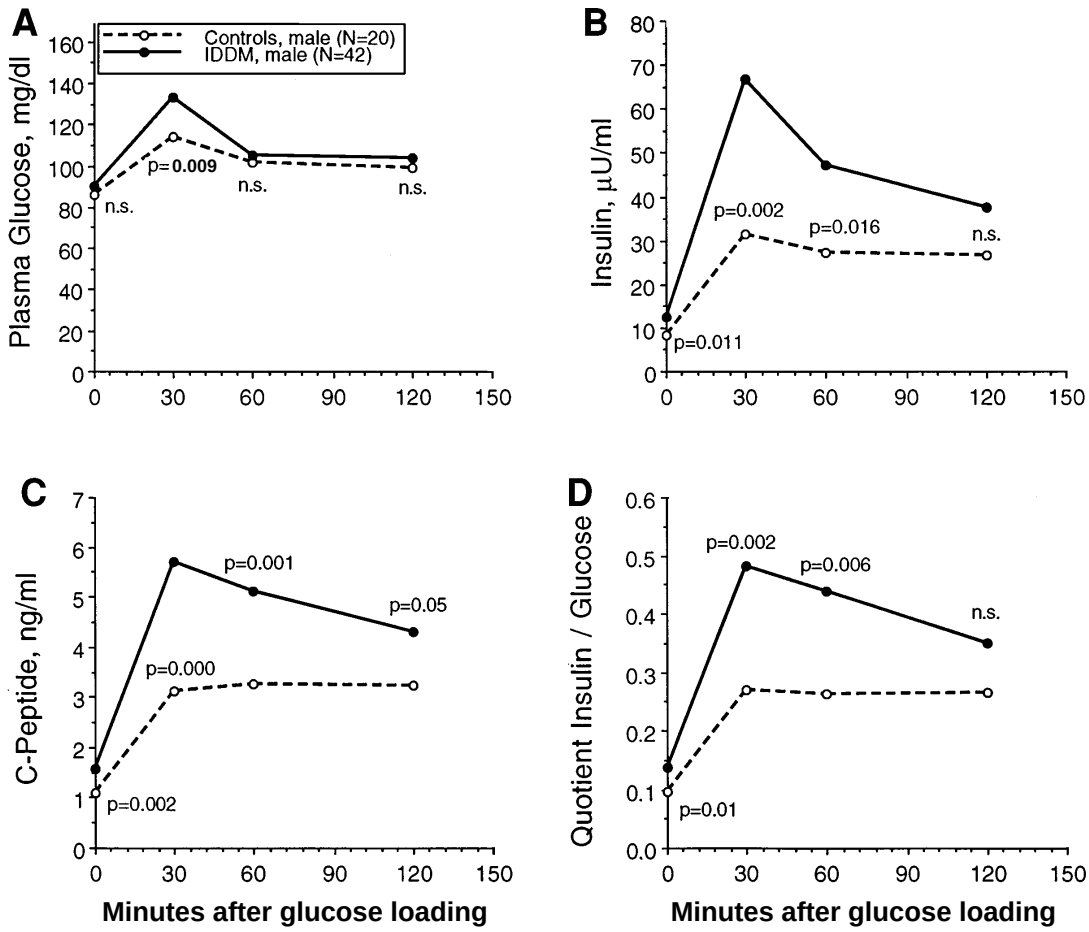

Figure 1—*Glucose (*A*), insulin (*B*), and C-peptide (*C*) levels in plasma and the I/G quotient (*D*) after glucose loading in 20 male control subjects and 42 male offspring of type 1 diabetic mothers.*

cantly so (Table 4). The RR for an obese offspring was 13 in cases with elevated amniotic fluid insulin levels (odds ratio 23.4, CI 2.8–10.30, $P = 0.0004$) (Table 4).

Amniotic fluid insulin concentration correlated with the offspring's BMI ($R = 0.563$, $P < 0.001$) and SI ($R = 0.473$, $P < 0.001$); postload glucose levels at 60 min ($R = 0.315$, $P = 0.018$); insulin levels at 0 ($R = 0.289$, $P = 0.03$), 60 ($R = 0.395$, $P = 0.003$), and 120 min ($R = 0.322$, $P = 0.016$); C-peptide levels at 0 ($R = 0.344$, $P = 0.009$), 60 ($R = 0.390$, $P = 0.003$), and 120 min ($R = 0.416$, $P = 0.001$); and with the I/G quotient at 0 ($R = 0.274$, $P = 0.041$), 60 ($R = 0.271$, $P = 0.043$), and 120 min ($R = 0.356$, $P = 0.008$). Of lipids, only triglycerides correlated with amniotic fluid insulin concentration ($R = 0.284$, $P = 0.034$).

There was no significant correlation between amniotic fluid insulin concentration and the sum of plasma glucose during OGTT, but there was a correlation with the sums of insulin ($R = 0.387$, $P = 0.003$), C-peptide ($R = 0.399$, $P = 0.002$), and the I/G quotient in plasma ($R = 0.306$, $P = 0.023$).

CONCLUSIONS — This is the first study of the impact of the fetal metabolic experience in utero, as reflected by amniotic fluid insulin levels, on amplification of types 1 and 2 diabetes in offspring of type 1 diabetic mothers.

Offspring of mothers with type 1 diabetes have been reported to have a 1.4–3.2% cumulative inheritance of type 1 diabetes by age 20 (22,23) that increases to 6.3% at 35 years (23). In our series, 4 of 75 (5.3%) infants of type 1 diabetic mothers had overt diabetes at a mean ± SD age of 10.3 ± 2.7 years (range 5–15). In the area of southern Germany bordering Austria, which has a similar population and lifestyle, the population-based prevalence of type 1 diabetes in children aged 0–14 years was 0.074% (24). Thus, based on our data, the RR for type 1 diabetes in offspring of type 1 diabetic mothers is 71.6.

Autoimmune antibodies are a marker of diabetes manifestation within the next 1–10 years (25). The predictive power is associated with the level of the antibodies. The risk of overt diabetes increases from 18% at an ICA level of ≥4 JDF U to 44–68% at ICA levels ≥20 JDF U (26,27). In the present series, only 1 offspring had an ICA level of 40 JDF U (he also had 47.3 RU GADA and 80 RU IA-2A), and 7 had an ICA level of 3 JDF U and 6 of 1 JDF U. There are too few data in the literature to calculate the risk for diabetes on the basis of these levels. But, antibody-positive subjects in our series had also significantly lower fasting and postload insulin levels than antibody-negative offspring (Table 3). Although we did not measure antibodies in control subjects, the prevalence of ICA in normal schoolchildren is 1.05% in southern Germany (28).

Neither overtly diabetic offspring nor nondiabetic antibody-positive offspring showed a trend in amniotic fluid insulin levels. This suggests genetic, rather than fuel-mediated, transmission of type 1 diabetes. Why only antibody-negative offspring had metabolic alterations similar to the prediabetic stage of type 2 diabetes is unclear. It is possible that autoimmune antibodies precipitate predominantly in those with genetic predisposition to type 1 diabetes and who, consequently, are less prone to type 2 diabetes and associated risk factors.

Considering the prevalence of antibodies in the nondiabetic offspring of type 1 diabetic mothers in our series and the age-related increase during the natural history of type 1 diabetes (23), the prevalence of type 1 diabetes in our cohort is likely to approach 8.4% by age 35 years.

Elevated glucose levels, BMI, postprandial and fasting insulin concentrations, and insulin resistance are highly predictive of subsequent type 2 diabetes years before the clinical diagnosis (13,29,30). The natural history seems to be obesity combined with insulin resistance, leading to compensatory hyperinsulinemia to maintain normoglycemia followed by impaired glucose tolerance (IGT). Type 2 diabetes develops when β-cells become exhausted or fail to maintain the increased level of insulin secretion (31,32).

Elevated BMI, which is an indicator of a network of mutually interrelated functions indicating past insulin resistance, is a significant predictor of diabetes in white Europeans with normal or impaired glucose tolerance (33,34). In the present series, the mean BMIs and SIs of infants of type 1 diabetic mothers were significantly higher than those of control subjects and significantly correlated with fasting and postload insulin levels ($R = 0.635$, $P < 0.001$ and $R = 0.650$–0.497, $P < 0.001$, respectively). The RR of an elevated BMI was 13 in offspring with high amniotic fluid insulin levels (>8 μU/ml, >48 pmol/l) compared with those at low amniotic fluid insulin levels (Table 4).

A linear association exists between insulin levels and the incidence of forthcoming IGT and type 2 diabetes (36). Fasting insulin levels and 2-h postload insulin levels in the highest quartile have an RR for an 8-year incidence of type 2 diabetes of 6.6 and 11.6 compared with the remaining 3 quartiles combined (35,36). Thus, hyperinsulinemia is the key feature of the susceptibility to type 2 diabetes also underlying other relevant prediabetic disorders (6,29,31,34).

Because 2-h insulin levels may decrease just before diabetes becomes manifest (33), we estimated the risk for diabetes on the basis of fasting insulin levels. In the present series, 28 of 71 (39.4%) offspring of type 1 diabetic mothers had fasting insulin levels above the highest quartile of control subjects. Considering the predictive power (33), the RR for type 2 diabetes in this series is 2.7 and 3.8 in cases with low and elevated amniotic fluid insulin levels, respectively. Considering the 4.4–4.9% prevalence of type 2 diabetes in those >30 years of age in Germany (37), the future prevalence of type 2 diabetes in our cohort is estimated as ~13 and 18% of the offspring of mothers with low and elevated amniotic fluid insulin levels, respectively.

Subjects with IGT have been reported to have an RR of 5.4 for developing type 2 diabetes within 3 years (33). Silverman et al. (11) found an association between amniotic fluid insulin concentration and IGT in offspring of combined type 1 and gestational diabetic patients. We found a lower proportion of IGT in our series of offspring of type 1 diabetic women than Silverman et al. did in infants of type 1 and gestational diabetic women combined. In the present series, 3 offspring of type 1 diabetic mothers (4%), but none of the control subjects, had IGT, but this difference was not significant. Although the difference may not be statistically significant, IGT is highly unusual in white children of this age (11).

The lower rate of IGT in our series may be explained by a lower mean age of our children (10.3 vs. 12.3 years). Also, Silverman et al. (11) found a low prevalence of IGT in children <10 years of age, with a much higher prevalence in those ≥10 years (11) but used a higher amniotic fluid insulin cutoff value (>16.7 μU/ml, 100 pmol/l) than we did (>8 μU/ml, >48 pmol/l).

In the present series, the amniotic fluid insulin concentration correlated with triglycerides; 60-min postload glucose levels; and with postload insulin, C-peptide levels, and the I/G quotients of the offspring. This suggests an influence of fuels on the transmission of the metabolic disturbances known to antedate type 2 diabetes.

In summary, offspring of type 1 diabetic mothers had a much higher rate of type 1 diabetes than control subjects, with a potential of further genetically caused cumulation with increasing age. Additional cumulation is supported by the natural history of diabetes manifestation and by autoimmune antibodies in the absence of diabetes combined with relatively attenuated insulin secretion. On the basis of the literature, the RR of type 1 diabetes was 71.6, and the end point may be calculated as 8.4% by age 35.

Offspring of type 1 diabetic mothers also had a higher incidence of risk factors highly predictive for type 2 diabetes, particularly elevated insulin levels. On the basis of the literature, the estimated RR is 3.2, and the incidence for type 2 diabetes at an age of ≥30 years is ~15%.

Diabetes-associated risk factors correlated with the amniotic fluid insulin concentration, whereas no correlation was found with maternal anthropometric factors, family history, or birth weight. Thus, exaggerated type 2 diabetes susceptibility in offspring of type 1 diabetic mothers, judged by diabetes-associated risk factors, is probably fuel-mediated and not genetic. Follow-up of the offspring beyond the age of 30 years will be necessary to prove the calculated diabetes prevalence.

Acknowledgments — We are indebted to Dr. Boyd E. Metzger, Northwestern University, Chicago, and Dr. David J. Pettitt, Sansum Medical Research Institute, Santa Barbara, California, for critical readings of the manuscript and scientific advice. Moreover, we express our gratitude to the children and parents who participated in the study.

References

1. King H, Aubert RE, Herman WH: Global burden of diabetes, 1995–2025: prevalence, numerical estimates, and projections. *Diabetes Care* 21:1414–1431, 1998
2. Freinkel N: Banting Lecture 1980: of pregnancy and progeny. *Diabetes* 29:1023–1035, 1980
3. Freinkel N: Pregnant thoughts about metabolic control and diabetes. *N Engl J Med* 304:1357–1359, 1981
4. Alcolado JC, Alcolado R: Importance of maternal history of non-insulin dependent diabetic patients. *BMJ* 302:1178–1180, 1991
5. Thomas F, Balkau B, Vauzelle-Kervrodan F, Papoz L, CODIAB-INSERM-ZENECA Study Group: Maternal effect and familial aggregation in NIDDM: the CODIAB Study. *Diabetes* 43:63–66, 1994
6. Plagemann A, Harder T, Kohlhoff R, Rohde W, Dörner G: Glucose tolerance and insulin secretion in children of mothers with pregestational IDDM or gestational diabetes. *Diabetologia* 40:1094–1100, 1995
7. Pettitt DJ, Aleck KA, Baird HR, Carraher MJ,

Bennett PH, Knowler WC: Congenital susceptibility to NIDDM: role of intrauterine environment. *Diabetes* 37:622–628, 1988
8. Pettitt DJ, Knowler WC, Bennett PH, Aleck KA, Baird HR: Obesity in offspring of diabetic Pima Indian women despite normal birth weight. *Diabetes Care* 10:76–80, 1987
9. Aerts L, Holemans K, Van Assche FA: Maternal diabetes during pregnancy: consequences for the offspring. *Diabetes Metab Rev* 6:147–167, 1990
10. Metzger BE, Silverman BL, Freinkel N, Dooley SL, Ogata ES, Green OC: Amniotic fluid insulin concentration as a predictor of obesity. *Arch Dis Child* 65:1050–1052, 1990
11. Silverman BL, Metzger BE, Cho NH, Loeb CA: Impaired glucose tolerance in adolescent offspring of diabetic mothers: relationship to fetal hyperinsulinism. *Diabetes Care* 18:611–617, 1995
12. Harris MI, Hadden D, Knowler WC, Bennett PH: Prevalence of diabetes and impaired glucose tolerance and plasma glucose levels in U.S. population aged 20–74 yr. *Diabetes* 36:523–534, 1987
13. McCance DR, Pettitt DJ, Hanson RL, Jacobsson LTH, Bennett PH, Knowler WC: Glucose, insulin concentrations and obesity in childhood and adolescence as predictors of NIDDM. *Diabetologia* 37:617–623, 1994
14. Weiss PAM, Haeusler M, Kainer F, Pürstner P, Haas J: Towards universal criteria for gestational diabetes: relationships between seventy-five and one hundred gram glucose loads and between capillary and venous glucose concentrations. *Am J Obstet Gynecol* 178:830–835, 1998
15. Weiss PAM, Kainer F, Pürstner P, Zehetleitner G, Hüttner U, Haas J: Anti-insulin antibodies and birth weight in pregnancies complicated by diabetes. *Early Hum Dev* 53:145–154, 1998
16. Weiss PAM, Lichtenegger W, Winter R, Pürstner P: Insulin levels in amniotic fluid: management of pregnancy in diabetes. *Obstet Gynecol* 51:393–398, 1978
17. Farquhar JW: Prognosis for babies born to diabetic mothers in Edinburgh. *Arch Dis Child* 44:36–47, 1969
18. Tanner JM: *Growth at Adolescence*. 2nd ed. Oxford, U.K., Blackwell, 1962
19. Verge CF, Stenger D, Bonifacio E, Colman PG, Pilcher C, Bingley PJ, Eisenbarth GS: Combined use of autoantibodies (IA-2 autoantibody, GAD autoantibody, insulin autoantibody, cytoplasmic islet cell antibodies) in type 1 diabetes: Combinatorial Islet Autoantibody Workshop. *Diabetes* 47: 1857–1866, 1998
20. Hohenauer L: Intrauterine Wachstumskurven für den Deutschen Sprachraum. *Z Geburtshilfe u Perinatol* 184:167–179, 1980
21. Weiss PAM, Kainer F, Haeusler M, Pürstner P, Haas J: Amniotic fluid insulin levels in nondiabetic pregnant women: an update. *Arch Gynecol Obstet* 262:81–86, 1998
22. Warram JH, Krolewski AS, Gottlieb MS, Kahn CR: Differences in risk of insulin-dependent diabetes in offspring of diabetic mothers and diabetic fathers. *N Engl J Med* 311:149–152, 1984
23. Lorenzen T, Pociot F, Hougaard P, Nerup J: Long-term risk of IDDM in first-degree relatives of patients with IDDM. *Diabetologia* 37:321–327, 1994
24. Neu A, Kehrer M, Hub R, Ranke MB: Incidence of IDDM in German children aged 0–14 years: a 6-year population-based study (1987–1993). *Diabetes Care* 20:530–533, 1997
25. Turner R, Stratton I, Horton V, Manley S, Zimmet P, Mackay IR, Shattock M, Bottazzo GF, Holman R, U.K. Prospective Diabetes Study (UKPDS) Group: UKPDS 25: autoantibodies to islet-cell cytoplasm and glutamic acid decarboxylase for prediction of insulin requirement in type 2 diabetes. *Lancet* 350:1288–1293, 1997
26. Bosi E, Becker F, Bonifacio E, Wagner R, Collins P, Gale EAM, Bottazzo GF: Progression to type 1 diabetes in autoimmune endocrine patients with islet cell antibodies. *Diabetes* 40:977–984, 1991
27. Robert JJ, Deschamps I, Chevenne D, Roger M, Mogenet A, Boitard C: Relationship between first-phase insulin secretion and age, HLA, islet-cell antibody status, and development of type I diabetes in 220 juvenile first-degree relatives of diabetic patients. *Diabetes Care* 14:718–723, 1991
28. Boehm BO, Manfras B, Seissler J, Schoffling K, Gluck M, Holzberger G, Seidl S, Kuhnl P, Trucco M, Scherbaum WA: Epidemiology and immunogenetic background of islet cell antibody-positive nondiabetic schoolchildren: Ulm-Frankfurt population study. *Diabetes* 40:1435–1439, 1991
29. Haffner SM, Stern MP, Hazuda HP, Mitchell BD, Patterson JK: Increased insulin concentrations in nondiabetic offspring of diabetic parents. *N Engl J Med* 319:1297–1301, 1988
30. Le Stunff C, Bougnères P: Early changes in postprandial insulin secretion, not in insulin sensitivity, characterize juvenile obesity. *Diabetes* 43:696–702, 1994
31. Pettitt DJ, Moli PP, Knowler WC, Mott DM, Nelson RG, Saad MF, Bennett PH, Kottke BA: Insulinemia in children at low and high risk of NIDDM. *Diabetes Care* 16:608–615, 1993
32. Eriksson J, Franssila-Kallunki A, Ekstrand A, Saloranta C, Widen E, Schalin C, Groop L: Early metabolic defects in persons at increased risk for non-insulin-dependent diabetes mellitus. *N Engl J Med* 321:337–343, 1989
33. Charles MA, Fontbonne A, Thibult N, Warnet JM, Rosselin GE, Eschwege E: Risk factors for NIDDM in white population: Paris Prospective Study. *Diabetes* 40:796–799, 1991
34. Ferrannini E, Haffner SM, Mitchell BD, Stern MP: Hyperinsulinaemia: the key feature of a cardiovascular and metabolic syndrome. *Diabetologia* 34:416–422, 1991
35. Haffner SM, Stern MP, Mitchell BD, Hazuda HP, Patterson JK: Incidence of type 2 diabetes in Mexican Americans predicted by fasting insulin and glucose levels, obesity, and body-fat distribution. *Diabetes* 39:283–288, 1990
36. Zimmet PZ, Collins VR, Dowse GK, Knight LT: Hyperinsulinemia in youth is a predictor of type 2 (non-insulin-dependent) diabetes mellitus. *Diabetologia* 35:534–541, 1992
37. Von Ferber L, Salzsieder E, Hauner H, Thoelke H, Koster I, Jutzi E, Michaelis D, Fischer U: Diabetes prevalence from health insurance data: evaluation of estimates by comparison with a population-based diabetes register. *Diabete Metab* 19:89–95, 1993