Preservation of Pancreatic β-Cell Function and Prevention of Type 2 Diabetes by Pharmacological Treatment of Insulin Resistance in High-Risk Hispanic Women

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Type 2 diabetes frequently results from progressive failure of pancreatic β-cell function in the presence of chronic insulin resistance. We tested whether chronic amelioration of insulin resistance would preserve pancreatic β-cell function and delay or prevent the onset of type 2 diabetes in high-risk Hispanic women. Women with previous gestational diabetes were randomized to placebo (n = 133) or the insulin-sensitizing drug troglitazone (400 mg/day; n = 133) administered in double-blind fashion. Fasting plasma glucose was measured every 3 months, and oral glucose tolerance tests (OGTTs) were performed annually to detect diabetes. Intravenous glucose tolerance tests (IVGTTs) were performed at baseline and 3 months later to identify early metabolic changes associated with any protection from diabetes. Women who did not develop diabetes during the trial returned for OGTTs and IVGTTs 8 months after study medications were stopped. During a median follow-up of 30 months on blinded medication, average annual diabetes incidence rates in the 236 women who returned for at least one follow-up visit were 12.1 and 5.4% in women assigned to placebo and troglitazone, respectively ($P < 0.01$). Protection from diabetes in the troglitazone group (*1*) was closely related to the degree of reduction in endogenous insulin requirements 3 months after randomization, (*2*) persisted 8 months after study medications were stopped, and (*3*) was associated with preservation of β-cell compensation for insulin resistance. Treatment with troglitazone delayed or prevented the onset of type 2 diabetes in high-risk Hispanic women. The protective effect was associated with the preservation of pancreatic β-cell function and appeared to be mediated by a reduction in the secretory demands placed on β-cells by chronic insulin resistance.
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Type 2 diabetes frequently results from progressive failure of pancreatic β-cells in a setting of chronic insulin resistance (1–3).Whether β-cell defect and insulin resistance are simply coincident or are causally related is unknown. The distinction has important implications for the prevention of type 2 diabetes with interventions that ameliorate insulin resistance. If type 2 diabetes develops when preprogrammed β-cell failure occurs in people who also happen to be insulin resistant, then treatment of insulin resistance will only delay the inevitable onset of β-cell failure and hyperglycemia. If, on the other hand, insulin resistance causes or accelerates β-cell failure, then treatment of resistance could preserve β-cell function and truly prevent diabetes over relatively long periods of time.

Hispanic women with gestational diabetes mellitus (GDM) are at high risk for type 2 diabetes (4). They have insulin resistance and poor β-cell compensation for that resistance during the index pregnancy (5). Poor β-cell compensation for insulin resistance predicts the development of diabetes after pregnancy (6). Moreover, one additional pregnancy increases the risk of diabetes after GDM (7). Because pregnancy induces insulin resistance (8,9), this last observation suggests that insulin resistance causes diabetes by inducing β-cell dysfunction in susceptible women. If so, treatment of insulin resistance should preserve β-cell function and delay or prevent diabetes.

Thiazolidinedione drugs reduce insulin resistance. We initially tested the effects of one thiazolidinedione, troglitazone, on insulin resistance and β-cell function in Hispanic women with recent GDM (10,11). Treatment for 3 months increased S_I by 88%. Insulin output decreased proportionally, so that less endogenous insulin was required to maintain stable glucose tolerance. In other words, short-term treatment reduced the secretory demands placed on β-cells by insulin resistance. The present study tested whether chronic treatment of insulin resis-

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AIRg, acute insulin response to intravenous glucose; DI, disposition index; GDM, gestational diabetes mellitus; HR, hazard ratio; IVGTT, intravenous glucose tolerance test; K_g, glucose disappearance rate; OGTT, oral glucose tolerance test; S_I, insulin sensitivity; TRIPOD, Troglitazone in Prevention of Diabetes.

tance can preserve β-cell function and delay or prevent type 2 diabetes.

RESEARCH DESIGN AND METHODS

Subjects. Between August 1995 and May 1998, chart reviews and patient interviews identified women from Los Angeles County Women's and Children's Hospital who were ≥18 years of age, had GDM (1) in the previous 4 years, and were willing to use effective contraception. They were offered participation in the Troglitazone in Prevention of Diabetes (TRIPOD) (12) study if they had no evidence of chronic disease; a serum alanine aminotransferase concentration <1.5 times the laboratory upper normal; no diabetes (1); and a sum of five oral glucose tolerance test (OGTT) plasma glucose concentrations ≥625 mg/dl (34.7 mmol/l), predicting a 70% risk of diabetes in the next 5 years (4). Enrollment continued until 266 women had been randomized. This sample size was projected to provide power ≥0.8 to detect a ≥20% difference in cumulative diabetes incidence rates between treatment groups at a median follow-up of 42 months (12). Participants gave written informed consent for participation in the institutional review board–approved study.

Study protocol. Enrolled subjects received dietary advice and were advised to walk for 30 min 3 days each week. They had a frequently sampled intravenous glucose tolerance test (IVGTT) within 4 weeks of the screening OGTT, then they were randomized to receive troglitazone 400 mg/day or placebo in a double-blind fashion. Follow-up visits were scheduled every 3 months for pill counts and measurement of fasting plasma glucose. OGTTs were scheduled annually. Diet and exercise recommendations were repeated at OGTTs. IVGTTs were scheduled 3 months after randomization.

Women who became pregnant during the trial were given the option of learning their treatment status. One placebo-treated patient did so and was followed off medication for the duration of the study. All other women who became pregnant remained blinded to their assigned treatment and had study medication resumed after delivery and completion of breastfeeding. Testing for diabetes was done at least 4 months after delivery and at least 1 month after completion of breastfeeding.

Diabetes by criteria of the American Diabetes Association (1) was the primary study end point. The trial was scheduled to continue until 1 August 2000 but was terminated on 24 March 2000, when troglitazone was withdrawn from the market after reports of hepatotoxicity in patients taking the drug. At that time, 79% of 105 subjects active in TRIPOD had not reached their annual OGTT visit for the year 2000. They were notified of their treatment status, asked to discontinue study medications, and scheduled for an end-of-trial OGTT. Testing was completed by 30 June 2000. Women who did not develop diabetes during the trial were asked to return 8 months posttrial for an OGTT and an IVGTT.

Clinical testing protocols. OGTTs and IVGTTs were initiated between 7:00 and 9:00 A.M., after an 8- to 12-h overnight fast. For OGTTs, subjects drank 75 g dextrose. Venous blood was sampled from an indwelling catheter before and 30, 60, 90, and 120 min after the dextrose ingestion. For IVGTTs, dextrose (300 mg/kg body wt) was injected into an antecubital vein. Tolbutamide (125 mg/m^2 body surface area; Orinase Diagnostic, Pharmacia Upjohn, Peapack, NJ) was injected 20 min later. Twenty-two arterialized venous blood samples were drawn and placed on ice before and up to 240 min after the dextrose injection. Plasma was separated within 20 min and stored at −80°C.

Laboratory methods. Glucose was measured by glucose oxidase (Glucose Analyzer II; Beckman, Brea, CA). Insulin was measured by a radioimmunoassay (Linco, St. Charles, MO) that provided <0.2% cross-reactivity with proinsulin.

Data analysis. Whole-body insulin sensitivity (S_I) was calculated from IVGTTs using the Bergman minimal model (13). Glucose disappearance (K_g) during IVGTTs was calculated as 100 × fractional glucose disappearance rate 10–40 min after the glucose injection. Areas under the glucose and insulin curves were calculated using the trapezoid rule. Insulin concentrations during IVGTTs were analyzed in two ways. The total area under the insulin curve from 0 to 240 min was used to assess total posthepatic insulin output by β-cells. At baseline, this measure was highly correlated with the OGTT insulin area from 0 to 120 min ($r = 0.73$). The acute insulin response to intravenous glucose (AIRg) (the incremental insulin area 0–10 min after the glucose injection) was used as a sensitive measure of β-cell well-being (14,15), reflecting a combination of β-cell mass (16) and function (17). The product of AIRg and S_I (the "disposition index" [DI]) was used as a measure of the ability of β-cells to compensate for insulin resistance (11,18,19).

Statistical analyses addressed the following four questions: *1*) Did troglitazone reduce the incidence of diabetes? *2*) What early metabolic changes were associated with protection from diabetes? *3*) Did troglitazone prevent or only mask deterioration to diabetes? *4*) Did troglitazone preserve β-cell function? All analyses were conducted with subjects assigned to their initial treatment group, using all available data for the period of follow-up relevant to the particular analysis. Continuous variables were compared between treatment groups or subgroups by t tests if normally distributed in their raw or transformed state. Otherwise, nonparametric Wilcoxon tests were used. Specifically, baseline insulin concentrations, areas under the insulin curves, S_I, and AIRg were natural-log transformed, and DI was square-root transformed before intergroup comparisons. Changes between baseline and follow-up measurements were compared between groups by Wilcoxon's nonparametric methods, using actual differences between baseline and 3 months on trial or, for later time points, rates of change between baseline and the specific time in question. Categorical variables were compared by χ^2 or Fisher's exact test. Cumulative diabetes incidence rates were calculated by life table analysis. Average annual diabetes incidence rates were calculated by person-years. Incidence rates were compared between treatment groups or subgroups by log-rank tests. Hazard ratios (HRs) of diabetes were calculated by Cox proportional hazards regression analysis without and with adjustment for differences ($P < 0.15$) in baseline variables (listed in Table 1) and four on-trial variables—rate of weight change, average pill compliance, pregnancy (yes/no), and fraction of months on hormonal contraception. Assumption of the proportional hazard in the Cox model was not violated by testing the interaction between treatment and follow-up time ($P > 0.6$). Statistical analyses were conducted with SAS (SAS Institute, Cary, NC). All statistical tests were two-sided, and statistical significance was accepted for $\alpha < 0.05$. Data are presented as the means ± SD or median (range) in tables and text and the means ± SE in figures.

For questions 1 and 2, primary analyses were conducted on data collected before unblinding on 24 March 2000. Secondary analyses were conducted using all end-of-trial data up to 30 June 2000. For question 2, subgroups of placebo and troglitazone-treated women were created by dividing each treatment group into tertiles according to 0- to 3-month changes in six IVGTT variables: S_I, AIRg, β-cell compensation for insulin resistance (DI), total posthepatic insulin output (area under the IVGTT insulin curve), fasting glucose, and K_g. Diabetes rates were compared by log-rank tests among subgroups for each variable to identify variables for which differences in early changes were associated with differences in long-term diabetes rates. When a difference in diabetes rates was found among tertiles for a given variable in the troglitazone group, subgroups in which protection had occurred were identified by comparing the diabetes rate in each tertile for the variable in question to the rate in the placebo-treated subjects who returned for follow-up.

RESULTS

Question 1: Did troglitazone reduce the incidence of diabetes? A total of 133 women were randomized to each treatment. Randomized groups were balanced for all of the variables listed in Table 1 ($P \geq 0.10$; data not shown). A total of 30 women failed to return for any follow-up, 11 in the placebo group and 19 in the troglitazone group. Compared with the 236 women who returned for follow-up, these 30 women had significantly higher BMI (32.7 ± 6.7 vs. 30.5 ± 5.7 kg/m^2, $P = 0.04$), lower S_I (1.72 ± 1.3 vs. 2.45 ± 1.73 × 10^{-4} min^{-1} per μU/ml; $P = 0.004$), and higher insulin area on OGTTs (13.7 ± 7.8 vs. 10.1 ± 6. × 10^3 μU/ml × min; $P = 0.004$) and IVGTTs (14.3 ± 8.6 vs. 10.0 ± 5.6 × 10^3 μU/ml × min; $P = 0.004$). Otherwise, they were similar to women who returned for follow-up. Baseline variables for women who failed to return for follow-up did not differ significantly between the 11 women assigned to placebo and the 19 women assigned to troglitazone.

Baseline characteristics were similar in the 122 women randomized to placebo and the 114 randomized to troglitazone who returned for at least one follow-up visit, with three exceptions: S_I was lower and IVGTT fasting insulin and total insulin area were higher in the women randomized to placebo (Table 1). Annual dropout rates were similar (13.4 and 16.3%, respectively; $P = 0.44$), and none of the variables listed in Table 1 differed significantly between women who did or did not drop out or between dropouts in placebo and troglitazone groups (data not shown). Median follow-up during the blinded trial was

TABLE 1
Baseline characteristics of women who returned for at least one follow-up visit after randomization to placebo or troglitazone

	Placebo group	Troglitazone group	*P*
n	122	114	
Clinical characteristics			
Age (years)	34.3 ± 6.5	34.9 ± 6.6	0.52
BMI (kg/m^2)	30.3 ± 5.3	30.6 ± 6.1	0.63
Waist-to-hip circumference ratio	0.86 ± 0.05	0.85 ± 0.06	0.19
Using hormonal contraceptives*	48%	43%	0.41
OGTT†			
Fasting glucose (mg/dl)	98.1 ± 9.1	98.7 ± 10.2	0.64
2-h glucose	154.0 ± 24.0	154.4 ± 27.0	0.97
Total glucose area (mg/dl × min × 10^{-3})‡	18.7 ± 2.0	18.9 ± 2.0	0.25
Impaired glucose tolerance§	72%	69%	0.66
Fasting insulin (μU/ml)	16.0 ± 7.5	17.0 ± 10.8	0.82
Total insulin area (μU/ml × min)‡	10,209 ± 5536	9,902 ± 6543	0.55
IVGTT‖			
Fasting glucose (mg/dl)	94.3 ± 10.4	94.8 ± 10.2	0.71
K_g (min^{-1} × 100)¶	1.48 ± 0.40	1.42 ± 0.39	0.23
Fasting insulin (μU/ml)	18.7 ± 9.7	16.6 ± 9.5	0.05
S_i (min^{-1} per μU/ml × 10^{-4})#	2.28 ± 1.75	2.64 ± 1.70	0.05
AIR_g (μU/ml × min)**	569 ± 527	454 ± 360	0.18
Total insulin area (units/ml × min)‡	10,686 ± 5686	9,273 ± 5434	0.03
DI (S_I × AIRg)††	983 ± 697	976 ± 717	0.99

Data are means ± SD. *P* values by two group *t* test for means, χ^2, or Fisher's exact test for proportions. *Combination oral contraceptives or depo-provera; †75-g OGTT; ‡calculated by trapezoid method using data from entire duration of test; §plasma glucose 140–199 mg/dl 2 h after 75-g oral glucose load; ‖IVGTT, as described in RESEARCH DESIGN AND METHODS; ¶fractional glucose disappearance rate 10–40 min after glucose injection; #calculated by the Bergman minimal model (11); **incremental insulin area 0–10 min after the glucose injection; ††a measure of β-cell compensation for insulin resistance (9,14,15). To convert values for glucose to mmol/l, multiply by 0.05551. To convert values for insulin to pmol/l, multiply by 6.0.

similar in placebo and troglitazone groups (28.1 and 30.9 months, $P = 0.47$). The groups were also similar in compliance with study medications (87 ± 10 vs. 85 ± 16%; $P = 0.30$), the fraction of women using hormonal contraception (56 vs. 46%; $P = 0.23$), the fraction that became pregnant (8.2 vs. 7.1%, $P = 0.81$), and weight gain (1.6 ± 4.8 vs. 1.9 ± 4.0 kg/year; $P = 0.60$).

During blinded treatment, average annual diabetes incidence rates in women who returned for follow-up were 12.1 and 5.4% in placebo and troglitazone groups, respectively. Life table analysis confirmed a significantly lower cumulative incidence of diabetes in the troglitazone group (Fig. 1). Their HR for diabetes was 0.45 (95% CI 0.25–0.83) and was unchanged (HR = 0.44) by adjustment for differences in baseline and on-trial characteristics. The risk reduction in women randomized to troglitazone remained robust (HR = 0.54, 95% CI 0.32–0.92) when the 30 women without follow-up were assigned the diabetes rate observed in the placebo group and a median follow-up of 30 months. The risk reduction was also robust (HR = 0.51, 95% CI 0.28–0.95) when only women who had impaired glucose tolerance at baseline were considered. Inclusion of data obtained after the blind was broken resulted in a median follow-up of 31 months in each treatment group. Diabetes rates were 12.1 and 6.0% per year in women who returned for follow-up. The HR for diabetes in the troglitazone group was 0.50 (95% CI 0.28–0.89) without and 0.44 with adjustment for differences in baseline and on-trial characteristics. Thus, troglitazone reduced the incidence of diabetes in women who returned for follow-up by at least 50%.

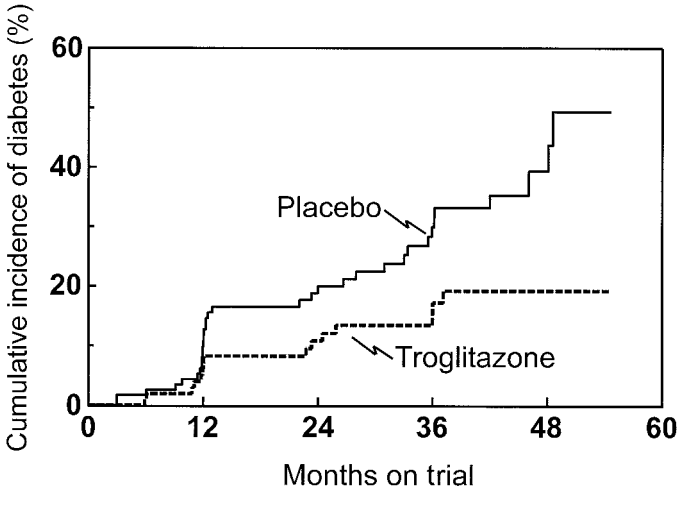

FIG. 1. Cumulative incidence rates of type 2 diabetes in women who returned for at least one follow-up visit after randomization to placebo or troglitazone. The rate in the troglitazone group was significantly lower than the rate in the placebo group ($P = 0.009$).

During the course of the blinded trial, nine women had study medication discontinued when serum transaminase concentrations exceeded three times the upper normal limit with no clinical explanation. One of these women dropped out of the trial for personal reasons. The other eight resumed their assigned study medication after transaminase levels returned to less than twice the upper normal limit. When the blind was broken, it was revealed that six of the nine women had been assigned to troglitazone.

Question 2: What early metabolic changes were associated with protection from diabetes? Mean changes between baseline and 3 months on trial are shown in Table 2. Among women in the placebo group, the risk of diabetes

TABLE 2
Metabolic parameters from IVGTTs at baseline and 3 months on trial

	Placebo group (n = 109)			Troglitazone group (n = 108)		
	Baseline	3 months	P	Baseline	3 months	P
Fasting glucose (mg/dl)	94.3 ± 10.4	97.2 ± 14.5	0.009	94.5 ± 10.0	91.0 ± 10.4	<0.0001
K_g ($min^{-1} \times 100$)	1.49 ± 0.40	1.48 ± 0.41	0.99	1.43 ± 0.41	1.46 ± 0.48	0.53
S_I (min^{-1} per μU/ml × 10^{-4})	2.34 ± 1.80	2.17 ± 1.48	0.17	2.60 ± 1.67	3.76 ± 2.27	<0.0001
Insulin area (μU/ml × min)	10518 ± 5,544	10701 ± 5,673	0.90	9,402 ± 5,504	6,551 ± 3,644	<0.0001
AIRg (μU/ml × min)	542 ± 499	534 ± 460	0.91	461 ± 357	406 ± 294	0.10
DI	995 ± 709	969 ± 720	0.35	987 ± 720	1321 ± 852	<0.0001

Data are means ± SD from all women who had IVGTTs at both times. P values are for difference from zero of change in each group between baseline and 3 months, by Wilcoxon's signed-rank test.

was unrelated to differences in any of these early changes ($P > 0.10$). Among women in the troglitazone group, protection from diabetes was not associated with differences in early changes in fasting glucose levels, K_g, AIRg, or DI ($P > 0.10$ for each). By contrast, rates differed according to early changes in S_I and insulin output (Table 3). For S_I, protection from diabetes was limited to the two-thirds of women with the greatest increase from baseline (S_I tertiles 2 and 3, Table 3). For insulin output, protection was limited to one-third of women with the greatest reduction from baseline (insulin area tertile 3, Table 3).

Figure 2*A* displays schematically the response of pancreatic β-cells to increased S_I observed in our short-term study of troglitazone in women with a history of GDM (11). On average, total insulin output decreased proportionally to increased S_I. The fact that two-thirds of troglitazone-treated women in the present study had a protective increase in S_I, while only one-third had the most protective decrease in insulin output (Table 3), suggested a more complex series of events than depicted in Fig. 2*A*. Thus, we examined in sequence the relative importance of increased S_I and reduced insulin output at 3 months to subsequent protection from diabetes in the troglitazone group.

To examine the relationship between early changes in S_I (horizontal arrow, Fig. 2*A*) and subsequent protection from diabetes, the troglitazone group was divided into nonresponders (S_I tertile 1, Table 3) who were not protected from diabetes compared with the placebo group and responders (S_I tertiles 2 and 3) who were protected. At baseline, these two subgroups did not differ significantly for any of the variables listed in Table 1 (all $P > 0.10$, data not shown). At 3 months (Table 4), rates of compliance with study medications were similarly high. Selection dictated that S_I increased in the responders but not in the nonresponders. Responders also had a slightly greater mean decrease in fasting glucose and AIRg and a much greater decrease in total insulin area compared with nonresponders. The decrease in AIRg in responders was proportionally less than their increase in S_I, so DI increased. During the blinded trial, annual diabetes rates were 9.8% in nonresponders and 3.0% in responders ($P = 0.02$). The unadjusted HR for diabetes in responders versus nonresponders was 0.30 (95% CI 0.10–0.88) and decreased to 0.19 after adjustment for differences in baseline and on-trial characteristics. Inclusion of data obtained after the blind was broken yielded annual diabetes rates of 9.5 and 3.4% ($P = 0.03$) and an adjusted HR of 0.19 in responders.

To examine the importance of an early reduction in total insulin output (vertical arrow, Fig. 2*A*), the 73 troglitazone responders were divided into two subgroups, one in which changes in IVGTT insulin area at 3 months decreased into the small and nonprotective range (i.e., in tertiles 1 and 2, Table 3) and the other in which changes in insulin area decreased into the large and protective change (tertile 3, Table 3). At baseline, glucose variables were similar ($P > 0.15$), but the subgroup destined for a large reduction in insulin area had a lower S_I (1.33 ± 0.66 vs. 3.35 ± 1.39 min^{-1} per μU/ml × 10^{-4}; $P < 0.0001$), higher IVGTT insulin area (13,989 ± 5,625 vs. 6,103 ± 1,957 μU/ml × min; $P < 0.0001$), and higher BMI (32.0 ± 5.6 vs. 29.2 ± 6.0 kg/m^2, $P = 0.05$) than women destined to have a small reduction in insulin area. At 3 months (Table 5), the subgroup with a large reduction in insulin area had a greater reduction in fasting glucose but no greater increase in K_g. Surprisingly, S_I had increased by a similar

TABLE 3
Average annual incidence rates of diabetes in tertiles of the troglitazone group defined by changes in S_I or by changes in IVGTT insulin area between baseline and 3 months on trial

	Tertile 1	Tertile 2	Tertile 3	P
Change in S_I				
Median	−0.09	0.99	2.28	
Range	−2.13 to 0.44	0.54 to 1.41	1.43 to 7.67	
Annual diabetes incidence	9.8%	1.1%*	4.8%†	0.04
Change in IVGTT insulin area				
Median	−40	−1,813	−5,315	
Range	4,487 to −1,180	−1,238 to −3,053	−3,160 to −19,364	
Annual diabetes incidence	7.2%	7.8%	0.9%‡	0.05

Data are from 108 women randomized to troglitazone who had IVGTTs at baseline and 3 months on trial. P values among subgroups by log-rank test. †$P < 0.05$, *$P < 0.01$, ‡$P < 0.001$ vs. diabetes incidence in placebo group (log-rank test).

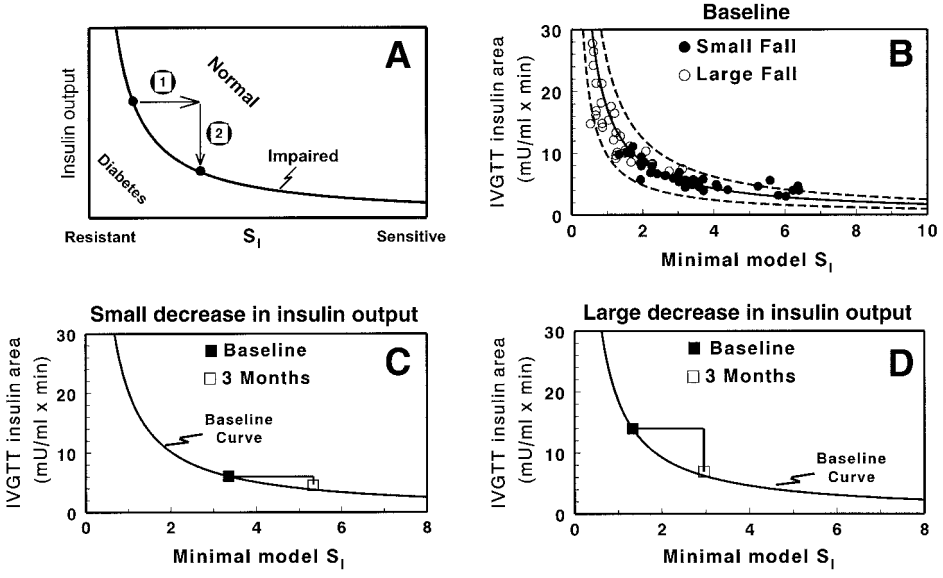

FIG. 2. ***A*: Schematic diagram of mechanism for protection from diabetes tested under question 2. Curved line is hyperbolic relationship between insulin output and S_I at baseline. Arrow no. 1 represents an intervention-induced increase in S_I. Arrow no. 2 represents a reciprocal reduction in total insulin output, as seen in a previous short-term study of women with recent GDM (11). *B*: S_I and total insulin output (IVGTT insulin area) at baseline in each of 73 troglitazone-treated women who increased S_I into the protective range after 3 months on trial. ●, women destined to have a small decrease in IVGTT insulin area 3 months after randomization (i.e., women in tertiles 1 and 2 for change in insulin area, Table 3). ○, women destined to have a large decrease in insulin area (women in tertile 3 for change in insulin area). ——, hyperbola y = k/x, where "k" is the mean of the products of S_I and insulin area for each of the 73 women (11,18,19). ——, 95% CIs. *C and D*: Means of S_I and IVGTT insulin area at baseline and 3 months later in each of the two subgroups.**

amount in the two subgroups. AIRg decreased slightly more and DI rose slightly more in the subgroup with a large decrease in insulin area. Diabetes rates during the trial differed considerably between these two subgroups. Responders with a small early decrease in insulin output developed diabetes at 5.8% per year, whereas none of the responders with a large early decrease in insulin output developed diabetes ($P = 0.01$ between groups). The HR for diabetes could not be calculated between these subgroups due to the lack of events in the latter group. Inclusion of data collected after the blind was broken yielded annual diabetes rates of 5.7 and 1.0% in responders with small and large decreases in insulin area, respectively. The HR for diabetes in the latter compared with the former subgroup was 0.14 (95% CI 0.02–1.22, $P = 0.08$) and remained at 0.13 after adjustment for differences in baseline and on-trial variables.

The large difference in insulin output for the same change in S_I in the two subgroups of troglitazone responders is explained in Fig. 2*B–D*. At baseline (Fig. 2*B*), responders destined for a small decrease in insulin output were relatively insulin sensitive and on a flat part of the sensitivity-output curve. Responders destined for a large decrease were relatively insulin resistant and on a steep part of the same curve. When S_I was increased by approximately the same amount in the two subgroups (Fig. 2*C–D*), β-cells reduced their insulin output to remain on the baseline sensitivity-output curve. The resulting decrease in insulin output was more than fourfold greater in the group that was on the steep part of the curve at baseline.

Thus, protection from diabetes required an increase in S_I early on but was most prominent in women who responded to that increase with a large reduction in insulin output from β-cells.

Question 3: Did troglitazone prevent or only mask deterioration to diabetes? A total of 102 women completed the trial without diabetes. Of these, 40 who had received placebo during the trial and 44 who had received troglitazone returned for posttrial testing at medians of 8.5 and 7.7 months, respectively, after stopping study medications (53 and 54 months after randomization). Weight gain during the posttrial period averaged 0.4 ± 3.5 and 0.6 ± 2.8 kg in the troglitazone and placebo groups, respectively ($P = 0.70$). At the time of posttrial testing, six (15%) of the placebo group and one (2.3%) of the troglitazone group had developed diabetes. Average annual incidence rates of diabetes during the posttrial period were 21.2 and 3.1%, respectively ($P = 0.03$). The posttrial HR for diabetes in women who had received troglitazone was 0.13 (95% CI 0.02–1.14) without and 0.08 with adjustment for differences in baseline and on-trial characteristics. Thus, protection from diabetes in the troglitazone group persisted long after the drug was stopped, suggesting that the drug

TABLE 4
Changes between baseline and 3 months in the troglitazone nonresponders and responders

	Non-responders*	Responders†	*P*
n	35	73	
Fasting glucose (mg/dl)	−1.0 (−26.0, 50.0)	−4.0 (−26.0, 10.0)‡	0.008
K_g (min^{-1}/100)	−0.01 (−0.53, 0.75)	0.06 (−1.69, 1.75)	0.12
S_I (min^{-1} per μU/ml × 10^{-4})	−0.09 (−2.13, 0.44)	1.41 (0.54, 7.67)‡	<0.000
Total insulin area (μU/ml × min)	−287 (−7,517, 4,487)	−2,717 (−19,364, 985)‡	<0.000
AIRg (μU/ml × min)	18 (−769, 341)	−15 (−926, 364)	0.04
DI	−6 (−1,010, 625)	477 (−1,822, 2,732)‡	<0.000
Pill compliance (% of prescribed)§	87 (20, 100)	90 (46, 99)	0.78

Data are median (minimum, maximum). *P* values for difference between groups, by Wilcoxon's rank-sum test. *Tertile 1 for change in S_I, Table 3; †tertiles 2 and 3 for change in S_I, Table 3; ‡$P < 0.01$ for change from baseline within a group by Wilcoxon's signed-rank test; §during first 3 months on trial.

TABLE 5
Changes between baseline and 3 months in troglitazone responders according to magnitude of decrease in IVGTT insulin area during first 3 months

	Small decrease*	Large decrease†	*P*
n	42	31	
Fasting glucose (mg/dl)	−3.0 (−15.0, 10.0)‡	−7.0 (−26.0, 1.0)‡	0.007
K_g (min^{-1}/100)	−0.02 (−0.66, 1.75)	0.17 (−1.69, 1.19)	0.30
S_I (min^{-1} per μU/ml × 10^{-4})	1.49 (0.54, 7.69)‡	1.34 (0.54, 4.40)‡	0.63
Total insulin area (μU/ml × min)	−1591 (−3053, 985)‡	−6011 (−19364, −3160)‡	<0.000
AIRg (μU/ml × min)	−3 (−424, 364)	−126 (−926, 255)‡	0.06
DI	428 (−727, 2416)‡	689 (−1822, 2732)‡	0.08
Pill compliance (% of prescribed)§	93 (46, 99)	90 (62, 98)	0.54

Data are median (minimum, maximum). *Responders with change in IVGTT insulin area in tertiles 1 and 2, Table 3; †Responders with change in IVGTT insulin area in tertile 3, Table 3; ‡$P < 0.01$ for change from baseline within a group by Wilcoxon's signed-rank test; §during first 3 months on trial.

had fundamentally altered the underlying metabolic changes that lead to diabetes.

Question 4: Did troglitazone preserve β-cell function? Of the 84 women who returned for posttrial OGTTs, 40 from the placebo group and 40 from the troglitazone group had IVGTTs at means of 8.5 ± 2.0 and 8.5 ± 2.3 months after completing the trial. At baseline, none of the characteristics listed in Table 1 had differed significantly between these groups (all $P \geq 0.15$, data not shown). At posttrial testing, S_I in the placebo group had decreased 30% from baseline ($P = 0.006$), AIRg had decreased 35% ($P = 0.045$), and DI had decreased 39% ($P < 0.0001$). In the troglitazone group S_I, AIRg, and DI had changed <3% from baseline. OGTT plasma glucose levels and interrelated changes in S_I and AIRg between baseline and posttrial testing are depicted in Fig. 3. The placebo group had increasing glucose levels and decreasing β-cell compensation for increasing insulin resistance. The troglitazone group had stable glucose levels and stable β-cell compensation for stable insulin resistance.

DISCUSSION

The TRIPOD study yielded four findings relevant to the pathogenesis and prevention of type 2 diabetes. First, administration of an insulin-sensitizing drug reduced the incidence of diabetes by >50% in high-risk Hispanic women. Second, protection from diabetes required an initial increase in S_I but was most prominent in women who responded to that increase with a large reduction in insulin output. Third, protection from diabetes persisted for at least 8 months after the drug was stopped, indicating that the intervention changed the natural history of deterioration to diabetes rather than just masking that deterioration through acute effects on circulating glucose levels. Fourth, the intervention preserved pancreatic β-cell function. Taken together, these findings provide strong support for the concept that type 2 diabetes results from progressive β-cell dysfunction that is caused at least in part by high secretory demands place on β-cells by chronic insulin resistance. Reducing those demands can preserve β-cell function and prevent type 2 diabetes for at least 4–5 years.

S_I failed to increase in one-third of women placed on troglitazone. They were not protected from diabetes relative to the placebo group. The nonresponders had no baseline characteristics that distinguished them from women who responded to drug with an increase in S_I. Pill counts, an indirect measure of pills actually taken, suggested similar compliance in nonresponders and responders. Thus, we found no clinical or metabolic characteristics that could be used to distinguish women who

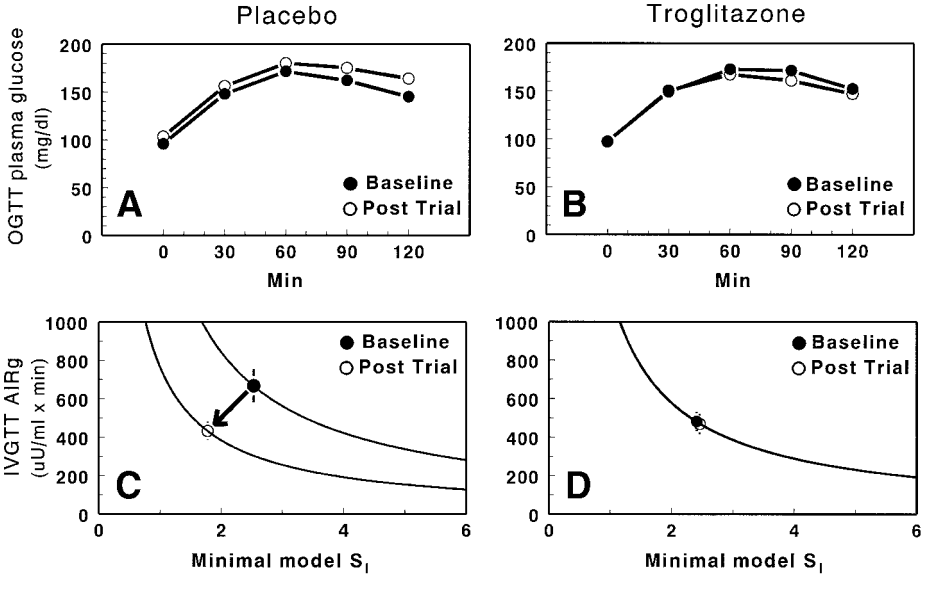

FIG. 3. *A* and *B*: OGTT plasma glucose concentrations at baseline and 8 months posttrial in women who participated in posttrial testing after completing the blinded trial without diabetes. The change in total glucose area between baseline and posttrial testing was significantly different between groups ($P = 0.03$). *C* and *D*: IVGTT AIRg plotted against S_I at baseline and 8 months posttrial. Curved lines are y = k/x, where "k" is product of AIRg and S_I, representing β-cell compensation for insulin resistance. β-cell compensation decreased 39% in the placebo group and 3% in the troglitazone group ($P = 0.01$ between groups).

would from women who would not benefit from treatment. Whether differences in drug metabolism, peroxisome proliferator–activated receptor-γ (20), or the etiology of insulin resistance could distinguish between these groups remains to be determined.

Among women who did increase S_I when placed on troglitazone, protection from diabetes was greatest in those who entered the trial with the greatest insulin resistance and hyperinsulinemia. One possible interpretation of this association is that the women who were more insulin resistant and hyperinsulinemic had the best β-cell function to start with. However, Fig. 2*B* indicates that women destined to have small or large reductions in insulin output in response to improved S_I entered the trial with similar β-cell function. That is, they all were on the same curve relating total insulin output to S_I. It is possible that the women differed in the degree to which insulin resistance contributed to their being on that curve by age 35 years. We have no data to refute or support that possibility. All that can be concluded with certainty from TRIPOD is that, due to the tendency of β-cells to autoregulate on a single curve over short periods of time (11), women with the greatest insulin resistance and hyperinsulinemia at entry manifested the greatest β-cell "rest" when insulin resistance was ameliorated. That rest was the variable most closely associated with protection from diabetes, although we cannot prove a cause-effect relation. A biochemical mechanism that could underlie a protective effect of β-cell rest is a reduction in islet amyloid polypeptide, which is cosecreted with insulin (21,22) and diabetogenic when overexpressed in mice under conditions of insulin resistance (23,24). We found no evidence that an initial reduction in glucose levels and, therefore, prevention of glucose toxicity (25) was important in the protection from diabetes observed in this study.

Two observations do not support a direct effect of troglitazone to protect β-cell function and prevent diabetes. First, women who by pill counts appeared to take the medication were not protected from diabetes unless they had an increase in S_I. Second, women who did manifest an increase in S_I were protected from diabetes to the extent that β-cells responded with a reduction in insulin output, a reduction that was readily explainable by autoregulation of β-cell function along the baseline S_I output curve. The first observation could have been due to lack of a direct drug effect on all tissues, including β-cells. However, the second observation could be explained by a direct β-cell effect only if that effect differed in magnitude among subjects who experienced the same improvement in S_I from the drug. Such a disparate direct effect on β-cells compared with muscle and fat is highly improbable. Thus, our results are most consistent with an indirect protective effect that was mediated through improved S_I and resultant β-cell rest rather than a direct effect of troglitazone on β-cells.

The results of TRIPOD have important implications for both the prevention and the etiology of type 2 diabetes. Regarding prevention, our findings support the treatment of insulin resistance to preserve β-cell function. This mechanism may underlie reductions in the incidence of type 2 diabetes reported in response to diet and exercise interventions in the Da Qing IGT and Diabetes Study (26), the Finnish Diabetes Prevention Study (27), and the National Institutes of Health–sponsored Diabetes Prevention Program (28). Pharmacological interventions to unload β-cells, such as other thiazolidinediones or metformin (28), should also preserve β-cell function and delay or prevent diabetes. Given the results of existing studies, we suggest a prevention strategy in which high-risk people are prescribed behavioral interventions initially and advanced to pharmacological treatment if their plasma glucose concentrations continue to increase. To the extent that β-cell rest is important for β-cell preservation, interventions may be most beneficial before clinical hyperglycemia develops, because hyperglycemia itself serves as a chronic stimulus to insulin secretion. However, the long-term benefits and cost-effectiveness of prevention compared with early diagnosis and treatment of type 2 diabetes remain to be determined.

Regarding the pathogenesis of type 2 diabetes, our results define a phenotype in which progressive β-cell dysfunction results from insulin resistance, presumably in susceptible individuals. Elbein et al. (29) reported that β-cell compensation for insulin resistance is highly heritable in families of Caucasian patients with type 2 diabetes. We have made a similar observation for the AIRg in families of Hispanic women with GDM (T.A.B., unpublished observations). These findings suggest important genetic determinants of the tendency for β-cells to fail in the presence of chronic insulin resistance. Identification of the underlying genetic variations should provide important insights into the pathogenesis of β-cell failure in type 2 diabetes. Genetic information should also allow the development of individually targeted and early interventions to preserve β-cell function.

In summary, we observed a >50% reduction in the incidence of type 2 diabetes in young Hispanic women with recent GDM who were treated with an insulin-sensitizing drug. Protection from diabetes persisted 8 months after the drug was stopped, and it was associated with preservation of β-cell compensation for stable insulin resistance. Perhaps most importantly, protection required an improvement in S_I soon after initiation of treatment, but was most closely linked to a reduction in the amount of insulin required from β-cells. Our findings provide strong evidence that insulin resistance contributes to β-cell dysfunction in susceptible individuals by increasing secretory demands on β-cells. Reducing those demands can preserve β-cell function and prevent type 2 diabetes for relatively long periods of time.

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