Thiazolidinediones Improve Beta-Cell Function in Type 2 Diabetic Patients

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ABSTRACT

Thiazolidinediones (TZDs) improve glycemic control and insulin sensitivity in patients with type 2 diabetes mellitus (T2DM). There is growing evidence from in vivo and in vitro studies that TZDs improve pancreatic β-cell function. The aim of this study was to determine whether TZD-induced improvement in glycemic control is associated with improved β-cell function.

We studied 11 normal glucose tolerant and 53 T2DM subjects (age=53±2 y; BMI=29.4±0.8 kg/m²; fasting plasma glucose [FPG] =10.3±0.4 mM; HbA1c=8.2±0.3 %). Diabetic patients were randomized to receive placebo or TZD for 4-months. Subjects received: (i) 2-hour OGTT with determination of plasma glucose, insulin and C-peptide concentrations, (ii) 2-step euglycemic insulin (40 and 160 mU·m⁻²·min⁻¹) clamp with [3-³H]glucose. T2DM were then randomized to receive 4 months of treatment with pioglitazone (45 mg/d), rosiglitazone (8 mg/d), or placebo. Pioglitazone and rosiglitazone similarly improved FPG, mean PG during OGTT, HbA1c, and insulin-mediated total body glucose disposal (Rd) and decreased mean plasma FFA during OGTT (all p < 0.01, ANOVA). The insulin secretion/insulin resistance (disposition) index (ΔISR[AUC]/Δglucose[AUC]/IR) was significantly improved in all TZD-treated groups: +1.8±0.7 (PIO + drug naïve diabetics), +0.7±0.3 (PIO + sulfonylurea-treated diabetics), and 0.7±0.2 (ROSI + sulfonylurea-withdrawn diabetics) versus -0.2±0.3 in the two placebo groups (p<0.01, all TZDs vs placebo, ANOVA). Improved insulin secretion correlated positively with increased body weight, fat mass and Rd, and inversely with decreased plasma glucose and FFA during the OGTT. In T2DM patients, TZD treatment leads to improved β-cell function, which correlates strongly with improved glycemic control.
INTRODUCTION

Thiazolidinediones (TZDs), working through the peroxisome proliferator activator receptor gamma (PPARγ) (1), have proven to be very effective in improving glycemic control in type 2 diabetic patients (2,3). It generally is believed that the major effect of the TZDs is mediated via their insulin sensitizing effects on muscle and liver (4-9). Because of the paucity of PPARγ in muscle and liver (10), the insulin sensitizing effect of the TZDs is believed to result secondarily to alterations in FFA metabolism and fat topography, i.e. decreased muscle/liver triglyceride, fatty acyl CoA, diacylglycerol, and ceramide concentrations (11,12) and to their effect on circulating adipocytokines, i.e. increased plasma adiponectin and decreased plasma resistin (11-15).

In recent years, a significant body of evidence has accumulated to indicate that TZDs also may have important effects on the pancreatic beta cells. In animal models of type 2 diabetes improved insulin secretory capacity, preservation of beta cell histology, and prevention of beta cell apoptosis have been demonstrated with a variety of TZDs (18-25). Unger has provided strong evidence in the Zucker Diabetic Fatty rat that the beneficial effect of the TZDs on beta cell function is mediated via amelioration of lipotoxicity (18-20), and similar results have been reported by others (23-25). Rosiglitazone also has been shown to prevent the impairment in human beta cell function induced by fatty acids (26). However, a direct effect of the TZDs can not be excluded since PPARγ have been demonstrated on pancreatic beta cells (27).

In humans with impaired glucose tolerance, troglitazone has been shown to slow the progression to type 2 diabetes by preventing the decline in beta cell function, as well as by improving
insulin sensitivity (28-30). Both troglitazone and rosiglitazone also have been shown to restore normal oscillatory insulin secretion in individuals with IGT (28,31). Similarly, both troglitazone (TRIPOD) (29,32) and pioglitazone (PIPOD) (33) reduce the development of diabetes in Latino women with a history of gestational diabetes mellitus by improving insulin sensitivity and preventing the progressive deterioration of beta cell function, as assessed by the acute insulin response to intravenous glucose. We have shown that pioglitazone enhances insulin secretion in type 2 diabetic patients, as assessed by the ΔI/ΔG ratio during the oral glucose tolerance test (34). Most recently, pioglitazone has been shown to augment insulin secretion in obese subjects with combined IFG/IGT (35), while rosiglitazone reduced the insulin secretory rate during graded glucose infusion in normal glucose tolerant, insulin-resistant individuals (36). Despite this mounting evidence, suggesting an important effect of TZDs on insulin secretion, a systematic examination of beta cell function in type 2 diabetic patients using state-of-the-art techniques has not yet been performed.

In the present study, we report the first comprehensive assessment of beta cell function in type 2 diabetic individuals using state-of-the-art methods to quantitate both insulin secretion and insulin sensitivity in the same individual.

**METHODS**

**Experimental Design:** We examined insulin secretion during the OGTT in 53 type 2 diabetic and 11 normal glucose tolerant subjects matched for age, gender, ethnicity and BMI, while simultaneously taking into account changes in insulin sensitivity. Only diabetic subjects who were healthy, as assessed by medical history, physical examination, routine screening blood tests, urinalysis and EKG, were studied. Other than sulfonylureas, no subject was taking any medication known to affect glucose or lipid metabolism. Body weight was stable for at least 3 months prior to study and no
subject was participating in a heavy exercise program prior to study. Subjects were asked to consume a weight maintaining diet containing 50% carbohydrate, 30% fat, and 20% protein for 3 days prior to each study.

During the 7-10 day period prior to randomization, all subjects had the following: (i) determination of HbA1c, fasting plasma glucose, insulin, FFA and lipid concentrations; (ii) oral glucose tolerance test with tritiated H₂O to measure fat mass and fat free mass; (iii) two-step euglycemic insulin (40 and 160 mU m⁻² min⁻¹) clamp with [3-³H]-glucose. Diabetic subjects then were randomized to the following protocols: (i) 9 drug naïve subjects were treated with pioglitazone, 45 mg/d; (ii) 20 diabetic subjects previously treated with a sulfonylurea were randomized in double blind fashion to receive pioglitazone, 45 mg/d (n=10), or placebo (n=10), with unchanged sulfonylurea dose; (iii) 24 diabetic patients discontinued their sulfonylurea for 6 weeks and then were randomized in double blind fashion to receive rosiglitazone, 8 mg/d (n=12), or placebo (n=12). During the 4-6 week period following sulfonylurea withdrawal, the FPG concentration changed by less than 10% in all diabetic subjects.

Following initiation of treatment, subjects returned to the Clinical Research Center every two weeks for 4 months. During each visit an interim medical history, pill count, body weight, and fasting plasma glucose concentration was obtained. HbA₁c was determined every 4 weeks. After 4 months of treatment with pioglitazone, rosiglitazone, or placebo all of the above studies were repeated. All studies were performed on the Clinical Research Center at 8 AM following a 10-12 hour overnight fast. For subjects who were taking a sulfonylurea, the medication was omitted on the morning of the OGTT and the insulin clamp study.

**Oral Glucose Tolerance Test:** At 8 AM a catheter was placed into an antecubital vein for the withdrawal of all blood samples. Blood for fasting plasma glucose, FFA, insulin, and C-peptide
determinations was obtained at -30, -15, and 0 minutes. At time zero subjects ingested 75 grams of glucose in an orange-flavored solution and an intravenous bolus of tritiated water (100 uCi) was given. Following glucose ingestion blood samples for measurement of plasma glucose, FFA, insulin, and C-peptide concentrations were obtained every 15 minutes for 2 hours. Samples for determination of $^{3}$H$_{2}$O radioactivity were obtained at 90, 105, and 120 minutes.

**Euglycemic Insulin Clamp:** At 8 AM catheters were inserted into an antecubital vein for the infusion of all test substances and retrogradely into a vein on the dorsum of the hand for withdrawal of all blood samples. The hand then was inserted into a heated box (65°C) for arterialization of the venous blood. At -180 minutes (-120 for nondiabetic subjects) a prime (25 uCi x FPG/100) – continuous (0.25 uCi/min) infusion of [3-$^{3}$H]-glucose (New England Nuclear, Boston, MA) was begun. Blood samples for determination of plasma insulin, glucose, and FFA concentrations and tritiated glucose radioactivity were obtained at -30, -20, -10, -5, and 0 minutes. At time zero a prime-continuous insulin (40 mU•m$^{-2}$•min$^{-1}$) infusion was begun and the plasma glucose concentration was measured every 5 minutes for two hours. After two hours, the insulin infusion rate was increased to 160 mU•m$^{-2}$•min$^{-1}$ for 100 minutes. In nondiabetic subjects the plasma glucose concentration was maintained at the fasting level by the periodic adjustment of a 20% glucose infusion based upon the negative feedback principle (37). In diabetics, after the start of insulin infusion, no glucose was infused until the plasma glucose concentration declined to 100 mg/dl, at which level it was maintained. During the insulin clamp plasma samples for determination of tritiated glucose radioactivity were obtained every 15 minutes from 0-90 minutes, every 5-10 minutes from 90-120 minutes, every 15 minutes from 120-190 minutes, and every 5-10 minutes from 220-240 minutes.
The study protocol was approved by the Institutional Review Board of the University of Texas Health Science Center and all subjects gave their informed written consent prior to participation.

**Analytical Techniques.** Plasma glucose was measured by the glucose oxidase reaction (Beckman Glucose Analyzer, Fullerton, CA). Plasma insulin (Diagnostic Products Corp., Los Angeles, CA) and C-peptide (Diagnostic Systems Laboratories Inc., Webster, TX) concentrations were measured by radioimmunoassay (Linko Research, St. Louis, MO). HbA1c was measured by affinity chromatography (Biochemical Methodology, Drower 4350; Isolab, Akron, OH). Plasma FFA was measured by enzymatic calorimetric quantification (Wako Chemicals GmbH, Neuss, Germany). Plasma total cholesterol and triglycerides were measured enzymatically (Roche Molecular Biochemicals, Indianapolis, IN) on a Hitachi 704 autoanalyzer. HDL cholesterol was measured enzymatically on a Hitachi 704 autoanalyzer after precipitation of chylomicrons and very low-density lipoprotein and LDL cholesterol by phosphotungstic acid. LDL cholesterol was calculated from the Friedwald equation. Plasma 3-$[^3]$H-glucose radioactivity was measured on Somogyi precipitates.

**Data Analysis.** During the baseline period, both the plasma glucose concentration and plasma 3-$[^3]$H-glucose specific activity were stable during the last 30 min of tracer infusion in all subjects. Therefore, the rate of endogenous glucose production (EGP) was calculated as the ratio of the 3-$[^3]$H-glucose infusion rate (DPM$\cdot$min$^{-1}$) to 3-$[^3]$H-glucose specific activity (DPM$\cdot$mg$^{-1}$) (mean of 5 determinations). Under postabsorptive conditions the rate of EGP equals the rate of glucose disposal by all tissues in the body. During the insulin clamp, non-steady state conditions prevail and the rate of glucose appearance (Ra) was calculated using Steele’s equation (38). EGP was then obtained as the difference between the tracer-derived Ra and the exogenous glucose infusion rate. The rate of glucose disappearance (Rd) during the insulin clamp was determined by adding the rate of residual EGP to the
exogenous glucose infusion rate during the last 30 minutes of the insulin clamp, and was expressed per kg of fat free mass (FFM). The basal hepatic insulin resistance index was calculated as the product of basal rate of EGP and the fasting plasma insulin (FPI) concentration. Over the range of plasma insulin concentrations that typically are seen under basal conditions (55±3 to 75±4 to 110±4 pmol/L), there is a linear relationship between the increase in the plasma insulin concentration and the decrease in hepatic glucose production (r=0.92, p<0.001) (39).

Insulin secretion rate (ISR) was calculated from the plasma C-peptide concentrations by deconvolution, as previously described (40). Because the beta cell responds to an increment in plasma glucose concentration by an increment in plasma insulin concentration, we calculated ΔI/ΔG and ΔISR/ΔG (41). Since the severity of insulin resistance also influences beta cell function (42-44), we calculated the insulin secretion/insulin resistance (disposition) index as ΔISR/ΔG ÷ 1/TGD, where TGD equals the rate of insulin-mediated glucose disposal during the euglycemic clamp (41).

Parameters of β-cell function (45) were derived from mathematical analysis of plasma glucose and C-peptide concentrations during the OGTT, according to a previously developed model (46,47). According to this approach, glucose-stimulated insulin secretion is the sum of two components. The first is proportional to the rate of change of plasma glucose concentration during the OGTT and is denoted as rate-sensitivity. The second component, which represents the dependence of insulin secretion on the absolute glucose concentration at any time-point and is characterized by a dose-response relationship, is denoted as glucose sensitivity. The dose-response relationship is modulated by a third factor, called the potentiation factor, which incorporates glucose-mediated and non-glucose-mediated potentiation, i.e., by non-glucose substrates and gastrointestinal hormones (46-48).

Potentiation is a time-dependent phenomenon (45-47) and the potentiation parameter used for the
present analysis is the ratio of the potentiation factor at the end of the OGTT (100-120 min) to the one at the beginning of the OGTT (0-20 min).

Areas under the glucose, FFA, and insulin concentration curves were calculated by the trapezoidal rule. Data are given as the mean ±SEM. Group values were compared by ANOVA and Bonferroni-Dunn post hoc analysis. Associations between variables were tested with Spearman rank correlations. The contribution of multiple factors to the measured variables was tested by multivariate analysis, using mixed models with both continuous (e.g., age and BMI) and nominal (e.g., sex, familial diabetes, and ethnicity) variables. P value <0.05 was considered to be statistically significant.
RESULTS

Clinical Characteristics (Table 1)

Subjects: Clinical and laboratory characteristics are shown in table 1. NGT subjects were well matched to the diabetic groups with respect to age, gender, ethnicity and BMI. Amongst the diabetic groups there were no significant differences with the exception of HbA1c which was slightly lower in pioglitazone-drug naive diabetic group. Within the two sulfonylurea-treated groups there were slightly more males. NGT subjects had a higher HDL concentration, but similar values of total cholesterol and triglycerides. All other clinical and laboratory parameters were similar.

Body Weight and Composition: After 4 months there were no significant changes in body weight, BMI or fat mass from baseline in either of the placebo groups. In contrast, in the diabetic patients who received thiazolidinedione (TZD) treatment for 4 months (i.e., drug naive diabetics treated with pioglitazone, sulfonylurea-treated diabetics receiving pioglitazone, sulfonylurea-withdrawn diabetics treated with rosiglitazone) body weight and BMI increased by 3.5±0.6 kg and 1.22±0.20 kg/m², respectively. In placebo-treated diabetics neither body weight (+0.2±0.4 kg) nor BMI (+0.08±0.14 kg/m²) changed significantly (p<0.001, TZD vs placebo for both body weight and BMI). The increase in body weight primarily was accounted for by an increase in fat mass (3.1±0.5 kg versus 0.1±0.3 kg in TZD and placebo treated groups, respectively; p<0.0001).

Glycemic Control: In both placebo-treated groups the HbA1c increased by a mean of 1.0±0.3% (p<0.0001). In the three TZD-treated groups there was a significant decrease in HbA1c versus baseline (p<0.01-0.001) and versus the respective placebo groups (p<0.001). The mean HbA1c decrease in the three TZD-treated groups was 1.6±0.2%. The change in FPG (Table 2) paralleled the change in HbA1c in all groups.
**Plasma Lipids** Plasma HDL cholesterol was significantly lower at baseline in T2DM compared to NGT subjects and there was a tendency for the HDL cholesterol to increase in the three TZD-treated groups compared to the placebo-treated groups (0.04±0.03 vs -0.03±0.03 mM, p=0.08). In the two pioglitazone treated groups, there was a significant decrease in plasma triglyceride concentration (p<0.01). Rosiglitazone did not alter the plasma triglyceride concentration. Total and LDL-cholesterol decreased slightly, but not significantly in the pioglitazone-treated groups and increased slightly but not significantly in the rosiglitazone treated groups.

**Metabolic Characteristics (Figure 1 and Table 2)**

**Oral Glucose Tolerance Test** Before treatment the fasting plasma glucose, insulin, C-peptide, and FFA concentrations during the OGTT were similar in the two placebo and three thiazolidinedione-treated groups. After 4 months of TZD treatment, the fasting plasma glucose concentration declined significantly in all three TZD groups compared to placebo (Δ = -2.3±0.4 vs Δ = 1.1±0.7 mM, p<0.001-0.0001 for TZD vs placebo and vs baseline). The incremental area under the plasma glucose curve also decreased significantly in the TZD-treated groups (Δ = -0.07±0.02 mol/l•120 min, p < 0.001 vs baseline) and rose slightly in the two placebo-treated groups (Δ = 0.01±0.03 mol/l•120 min, p<0.05 vs TZD groups).

There were no significant changes in fasting plasma insulin or C-peptide concentrations in any group. There also were no significant changes in the mean plasma insulin or C-peptide concentrations or the incremental areas under the plasma insulin or C-peptide curves in any of the five treatment (TZDs or placebo) groups.

The fasting plasma FFA concentration did not change significantly in either placebo group (mean Δ = +34±46 µmol/l), while the fasting FFA declined in all three TZD treated groups (mean Δ = -
139±32 µmol/l). The mean plasma FFA concentration (AUC) decreased significantly in all three TZD-treated groups (mean Δ = -15.0±2.7 mol/l•120min, p < 0.0001 vs baseline) and increased slightly in the two placebo-treated groups (Δ = +7.6±4.1 mmol/l•120min, p < 0.0001 vs TZD group).

**Basal and Insulin-Stimulated Glucose Disposal (Rd)** Prior to treatment, the basal rate of tissue glucose uptake (which equals the rate of endogenous glucose production) and the insulin-stimulated Rd during the insulin clamp were similar in all 5 diabetic groups. Compared to normal glucose tolerant subjects, all diabetic subjects in the present study were very resistant to the action of insulin (p<0.001). In all three TZD-treated groups, insulin sensitivity during the insulin clamp improved significantly, especially during second insulin step clamp (p<0.05-0.001). No change in insulin sensitivity was observed in either placebo-treated group.

Basal EGP was unchanged in the two placebo groups and declined slightly, although not significantly in the three TZD-treated groups. The basal hepatic insulin resistance index (EGP x fasting plasma insulin) improved significantly (p<0.05-0.01) in all three TZD-treated groups.

**Beta Cell Function (Table 3)**

During the OGTT the ratio of the incremental AUC of insulin to the incremental AUC of plasma glucose concentration increased significantly in all three TZD treated groups and did not change significantly in the two placebo groups (7.3±3.1 vs -9.4±6.7, p<0.03 for TZD vs placebo and vs baseline). Similarly, the incremental AUC of insulin secretion rate (ISR) to the incremental AUC of plasma glucose concentration increased significantly (p<0.01-0.001 for TZD vs baseline and vs respective placebo control). Because insulin secretion increases progressively with worsening severity of insulin resistance (IR) (31-34, 40,41), we also calculated in secretion/insulin resistance (disposition) index: ΔI(AUC)/ΔG(AUC) ÷ IR and ΔISR(AUC)/ΔG(AUC) ÷ IR, where IR=1/(Rd/I), and Rd is the insulin-mediated rate of glucose disposal measured during the first step euglycemic insulin clamp and I
is the steady state plasma insulin concentration during the insulin clamp. When insulin secretion is accessed using $\Delta ISR (AUC)/\Delta G (AUC) \div IR$ or $\Delta I(AUC)/\Delta G(AUC) \div IR$, TZD therapy markedly increased beta cell function in drug naive (placebo), sulfonylurea-treated (pioglitazone), and sulfonylurea withdrawn, diet-treated (rosiglitazone) diabetic subjects ($p<0.001$ for TZD vs baseline and vs respective control placebo control group) (Figure 2). Placebo treatment had no effect on $\Delta ISR(AUC)/\Delta G (AUC) \div IR$ or $\Delta I (AUC)/\Delta G (AUC) \div IR$ (Figure 2).

Using a previously validated model of beta cell function (36,37), we examined the effect of TZD therapy on the three major dynamic parameters of beta cell function: beta cell glucose sensitivity, beta cell rate sensitivity, and potentiation. At baseline, all parameters of beta cell function were markedly and significantly reduced in diabetics compared to NGT subjects. Both pioglitazone and rosiglitazone increased beta cell glucose sensitivity by 1.5-2 fold in drug naive diabetic subjects ($p<0.001$ vs baseline and respective placebo control). The increase in beta cell glucose sensitivity when pioglitazone was added to sulfonylurea-treated diabetics ($p<0.001$ vs baseline and vs respective control group) was virtually identical to that observed in drug naive patients. Placebo treatment had no significant effect on beta cell glucose sensitivity. Similarly, rate sensitivity increased 2-3 fold in both pioglitazone-treated groups while no effect was observed in the placebo groups. Rosiglitazone-treated diabetic subjects did not show any change in rate sensitivity. However, prior to the start of rosiglitazone, this parameter was significantly higher than the baseline value of rate sensitivity in the two pioglitazone-treated groups. Following 4 months of pioglitazone, the value for rate sensitivity had increased to the baseline value observed in the rosiglitazone group. Neither TZD nor placebo treatment had any effect on beta cell potentiation.
Correlation Analyses Between Metabolic and Clinical Parameters (Table 4)

Prior to TZD/placebo treatment, $\Delta \text{ISR(AUC)}/\Delta \text{G(AUC)} \div \text{IR}$ was related to the fasting plasma glucose concentration and to the 2-hour plasma glucose concentration during the OGTT, and the typical hyperbolic plot was observed. Log transformation of the variables yielded a strong linear relationship between $\Delta \text{ISR} (\text{AUC})/\Delta \text{G} (\text{AUC}) \div \text{IR}$ and both the fasting plasma glucose concentration ($r=0.83$, $p<0.0001$) and the 2-hour plasma glucose concentration during the OGTT ($r=0.89$, $p<0.0001$) (Figure 3). This strong correlation indicates that glucose tolerance is a direct and linear functions of beta cell function. Significant correlations were observed between the change, after TZD/placebo treatment, in $\Delta \text{ISR(AUC)}/\Delta \text{G(AUC)} \div \text{IR}$ and the changes in body weight and percent fat mass, as well as with the change in plasma FFA concentration (AUC) during OGTT ($r=-0.33$, $p<0.01$) (Figure 6).

None of the beta cell indices, determined from the model of beta cell function, were correlated with changes in clinical parameters such as body weight or percent fat mass. When the dynamic parameter of beta cell glucose sensitivity (36,37) is plotted against the 2-hour plasma glucose concentration during the OGTT and against $\Delta \text{ISR(AUC)}/\Delta \text{G(AUC)} \div \text{IR}$, a strong linear relationship is observed (figure 4). The improvements in beta cell glucose sensitivity and rate sensitivity were correlated with the reduction in fasting plasma glucose concentration and with the improvement in insulin secretion, insulin sensitivity, and $\Delta \text{ISR(AUC)}/\Delta \text{G(AUC)} \div \text{IR}$ (figure 5).

DISCUSSION

The thiazolidinediones were developed as insulin sensitizers and they have proven to be very efficacious in improving glycemic control and reducing the HbA$_{1C}$ (2-9). Although the primary insulin sensitizing action of the TZDs was believed to be exerted on muscle, they also have been shown to improve hepatic sensitivity to insulin (5,14,15) and to inhibit hepatic gluconeogenesis (49,50). Most
recently, evidence has accumulated to suggest that the TZDs also may enhance beta cell function. In rodent models of type 2 diabetes improved insulin secretion and beta cell histology have been demonstrated following chronic TZD treatment (18,22-25). In humans with type 2 diabetes mellitus (5-9,34) and impaired glucose tolerance (28-30,32), TZD treatment has been associated with either no change or a modest reduction in the plasma insulin response to both oral and intravenous glucose. The decline in plasma insulin response generally has been interpreted to represent the normal beta cell response to improved insulin sensitivity and/or reduced plasma glucose concentration (42-44,52,53). However, several studies in man suggest that the TZDs exert beneficial effect on beta cell function. Using a graded glucose infusion in individuals with impaired glucose tolerance, Cavaghan et al (28) demonstrated that troglitazone did not change or increased slightly the slope of the line relating the plasma insulin response to the increment in plasma glucose concentration. In a more recent study from the same laboratory (36) the investigators reported a decrease (rightward shift) in the slope of the line relating the insulin secretory rate (ISR) to the increase in plasma glucose concentration during a stepped intravenous glucose infusion in normal glucose tolerant, insulin resistant subjects. The authors interpreted the difference between their most recent results (36) and their previous report (28) to the different study populations. Thus, in the NGT insulin- resistant subjects beta cell function is intact and the improvement in insulin sensitivity unloads the beta cell leading to a reduction in the elevated ISR. In their previous study (28) of IGT subjects, beta cell function was more severely compromised at the start of TZD therapy and the improvement in insulin sensitivity was accompanied with no change or a slight increase in ISR. Troglitazone (28) also has been shown to normalize the abnormal oscillatory insulin secretory response to glucose that was present in subjects with IGT.

Because TZDs improve insulin sensitivity and lower the plasma glucose concentration, both of which would be expected to reduce the plasma insulin response, one cannot utilize the absolute plasma insulin concentration to assess the beta cells’ response to a hyperglycemic challenge. Moreover, the
beta cell responds to an increment in plasma glucose concentration ($\Delta G$) with an increment in plasma insulin response ($\Delta I$) (45). In a recent study, we evaluated $\Delta I/\Delta G$ from 0-120 minutes in poorly controlled type 2 diabetics who were treated with pioglitazone for 4 months (34). A highly significant, dose response (15 to 30 to 45 mg/day of pioglitazone) related increase in $\Delta I/\Delta G$ from 0-120 minutes was observed in diabetic patients treated with pioglitazone compared to placebo. Because insulin sensitivity was not assessed independently in this previous study, the complete impact of pioglitazone on beta cell function could not be evaluated. In fact, no previous study in type 2 diabetic subjects has examined the effect of TZDs on the ISR while simultaneously taking into account the glucose stimulus ($\Delta G$) and the severity of insulin resistance.

In the present study we have examined the effect of two different thiazolidinediones, pioglitazone and rosiglitazone, on beta cell function during an OGTT, using two independent methods. The first approach used $\Delta I/\Delta G$ and $\Delta ISR/\Delta G$ from 0-120 minutes of the OGTT and related $\Delta I/\Delta G$ and $\Delta ISR/\Delta G$ to the change in insulin sensitivity, which was quantitated on a separate day using the euglycemic insulin clamp technique. The second approach employed a mathematical model of beta cell function (46,47) to provide quantitative information about the three dynamic parameters - glucose sensitivity, rate sensitivity, and potentiation - that determine beta cell function (45). To examine whether prior treatment influenced the response to TZD treatment, we studied drug naïve, sulfonylurea-treated, and sulfonylurea-withdrawn type 2 diabetic patients. Using both approaches ($\Delta I/\Delta G_{0-120} \div $IR and the model of beta cell function), we observed that both pioglitazone and rosiglitazone significantly improved beta cell function in drug naïve, sulfonylurea-treated, and sulfonylurea-withdrawn type 2 diabetic subjects.

Following 4 months of treatment, insulin sensitivity improved similarly in all TZD-treated groups and remained unchanged in both placebo-treated groups (Table 2). When the incremental insulin secretory response (AUC) per incremental plasma glucose response (AUC) from 0-120 minute
was expressed in relationship to the severity of insulin resistance ($\Delta ISR/\Delta G_{0-120^{+}}IR$), both pioglitazone and rosiglitazone were found to enhance beta cell function by a similar amount in drug naive and sulfonylurea-withdrawn diabetic subjects, respectively (Figure 2). The magnitude of improvement in $\Delta ISR/\Delta G +IR$ following pioglitazone treatment in drug naive diabetics was approximately 2-fold greater ($\Delta = 1.4$ vs $0.7$, $p<0.05$) than in sulfonylurea-treated patients (Figure 2). It is noteworthy that the duration of diabetes in the sulfonylurea-treated diabetics was significantly longer than in the drug naïve group (6 vs 2 years, $p<0.01$). Sulfonylurea-treated diabetic patients also had a higher starting HbA$_{1c}$ (Table 2). These observations suggest that the beneficial effects of pioglitazone on beta cell function may diminish as a function of diabetes duration and severity of hyperglycemia or more likely, some combination of the two. Nonetheless, a beneficial effect of the TZDs on beta cell function was observed in diabetic subjects with moderately poor to very poor glycemic control (HbA$_{1c}$ ranging from 7.9 ± 0.4 % in drug naive patients to 9.1 ± 0.3% in sulfonylurea-treated diabetics) (Table 1). Since the duration of the present study was only 4 months, it remains to be determined whether this beneficial effect on beta cell function will be maintained after several years of TZD therapy.

We also evaluated the effect of TZD treatment on beta cell function utilizing a novel beta cell model (46,47) which allows quantitation of the three major dynamic parameters that influence insulin secretion (45). Following 4 months of pioglitazone or rosiglitazone treatment, beta cell glucose sensitivity, i.e. the ability of the beta cell to respond to a given change in plasma glucose concentration, improved by ~2-2.8 fold in drug naïve, sulfonylurea-treated, and sulfonylurea-withdrawn diabetic patients (Figure 2). To the best of our knowledge, this represents the first demonstration that, in type 2 diabetic individuals, TZDs enhance beta cell sensitivity to glucose. Beta cell rate sensitivity, i.e. the ability of the beta cell to respond to the rate of change in plasma glucose concentration, increased 2-3 fold in both pioglitazone-treated groups. Although rosiglitazone did not increase rate sensitivity, the starting value in this group (113 pmol•m$^{-2}•$mM$^{-1}$) was similar to the post-treatment values in both
pioglitazone-treated diabetic groups (Table 3). Beta cell potentiation, i.e. the ability of the beta cell to respond to non-glucose stimuli, was not influenced by TZD treatment. The insulin secretion/insulin resistance index (ΔISR/ΔG+IR) provides the same basic information as the glucose sensitivity parameter derived from the beta cell model. We found a very strong correlation between ΔISR/ΔG+IR and glucose sensitivity (r=0.78, p<0.0001) (Figure 4), providing further validation of the model (46) employed to examine the dynamic parameters of beta cell function.

PPARγ receptors have been found to be expressed in human beta cells, but their expression is low (26,27). Although a direct effect of the TZDs to enhance insulin secretion by pancreatic beta cells cannot be excluded, the paucity of pancreatic PPARγ receptors suggests an indirect effect of the TZDs to enhance insulin secretion. Studies in rodent models of type 2 diabetes have demonstrated that accumulation of triglycerides and metabolites of FFA within the beta cells play a causal role in the development of impaired insulin secretion (18-21). Conversely, treatment with TZDs has been shown to mobilize fat out of the beta cells and enhance beta cell function in these animal models of type 2 diabetes (18-25). Recent studies in humans who are genetically predisposed to develop type 2 diabetes have demonstrated that lipid infusion to increase the plasma FFA concentration impairs insulin secretion (53,54). Conversely, a reduction in plasma FFA concentration with acipimox in these genetically predisposed individuals improves both first and second phase insulin release (20,55).

Consistent with these in vivo observations, prolonged exposure of human pancreatic islets in vitro has been shown to have cytostatic and pro-apoptotic effects which can be prevented completely by inhibition of upstream caspases and prevented partially by inhibition of ceramide synthesis or serine protease activity (56). In the present study we observed an inverse correlation between the change in ΔISR/ΔG+IR and the decrement in plasma FFA (ΔAUC) during the OGTT (r=-0.33, p<0.01) (Figure 6). This observation suggests that part of the beneficial effect of TZDs on beta cell function may be
related to reversal of lipotoxicity (11, 19). The reductions in fasting plasma FFA \( (r=0.42, p<0.001) \) and mean FFA during the OGTT \( (r=0.49, p<0.0002) \) also were correlated with the decrement in HbA1c.

TZD treatment usually is associated with weight gain (2-4). Nonetheless, glycemic control improves and the greater the weight gain, the greater is the decrease in HbA1c (5-9,11). This apparent paradox is explained by the TZD-induced alteration in fat topography, which is characterized by a reduction in visceral, hepatic, and muscle fat and an increase in subcutaneous fat (5,9,11,14,15). In the present study, tissue fat distribution was not assessed. However, body weight and fat mass increased following TZD treatment (Table 1) and, as in previous studies, the increases in body weight \( (r=-0.57, p<0.0001) \) and fat mass \( (r=-0.56, p<0.0001) \), were correlated inversely with the decrement in HbA1c. The changes in body weight \( (r=0.38, p<0.01) \) and fat mass \( (r=0.37, p<0.01) \) also were positively correlated with the improvement in beta cell function, as assessed by the change in \( \Delta \text{ISR} / \Delta \text{G÷IR} \).

Because the increases in body weight and fat mass parallel the remodeling of fat, one could hypothesize that a similar redistribution of fat out of the beta cell contributes to TZD-related improvement in beta cell function.

We also observed significant correlations between the change in \( \Delta \text{ISR (AUC)}/\Delta \text{G (AUC)} \div \text{IR} \) following TZD treatment and the decreases in HbA1c \( (r=0.27, p<0.05) \), FPG \( (r=-0.47, p<0.001) \), and 2-hour plasma glucose concentration during the OGTT \( (r=-0.51, p<0.001) \).

These observations can be interpreted in one of two ways. In these insulin resistant type 2 diabetic subjects, beta cell function, i.e. insulin secretory rate, is an important determinant of glycemic control. Conversely, it could be argued that hyperglycemia inhibits beta cell function, i.e. glucotoxicity (57), and that the TZD-induced reduction in day-long plasma glucose concentration that results from enhanced tissue sensitivity to insulin is responsible for the improvement in beta cell function.

Diabetic subjects in all five treatment groups were markedly resistant to insulin-stimulated glucose disposal (Table 2). Following TZD treatment, a significant increase in Rd was observed in all
TZD-treated groups. Changes in insulin secretion, both ΔISR (AUC)/ΔG (AUC) (r=0.34, p<0.02 vs Rd-s1, r=0.38, p<0.006 vs Rd-s2) and beta cell glucose sensitivity (r=0.31, p<0.03 vs Rd-s1, r=0.32, p<0.03 vs Rd-s2)), were correlated to the changes in insulin sensitivity following TZD treatment. The mechanism via which the improvement in insulin sensitivity results in or is associated with an improvement in beta cell function remains to be determined. It commonly is stated that insulin resistance, by placing an increased demand on the beta cell to augment its insulin secretion, leads to an acceleration of beta cell failure. However, this descriptive explanation does not address the causality of the association. Our results suggest two potential explanations, which are not mutually exclusive.

First, TZD treatment leads to enhanced adipocyte sensitivity to the antilipolytic effect of insulin (6,9,11) and the resultant decline in plasma FFA concentration (Table 1), in combination with altered (reduced) tissue fat content (5,9,11,14,15), leads to improvements in both beta cell function and tissue sensitivity to insulin, i.e. amelioration of lipotoxicity (11,19). Second, the reduction in mean plasma glucose concentration following TZD treatment leads simultaneously to enhanced beta cell function and to increased insulin sensitivity, i.e. amelioration glucotoxicity (57). Alternative explanations also can be invoked. Thus, it is possible that TZDs alter the release of inflammatory and insulin-sensitizing adipocytokines (i.e. tumor necrosic factor α, adiponectin, resistin, interleukin 6, and others) from fat cells, and that alterations in these adipocytokines simultaneously influence both beta cell function and tissue sensitivity to insulin in a positive manner (reviewed in ref #11). Thiazolidinedione treatment has been shown to decrease plasma TNFα and resistin concentrations and to increase plasma adiponectin levels in association with enhanced muscle and hepatic insulin sensitivity (14,15,58).

It also is possible that thiazolidinediones exert a direct effect on the pancreatic beta cells to enhance insulin secretion. Peroxisome proliferator-activated receptor γ is expressed in all three human pancreatic endocrine cell types: beta, alpha, and delta (26). However, exposure of these nondiabetic human islets to rosiglitazone had no effect on either basal or glucose-stimulated insulin secretion.
Nonetheless, these short term TZD incubation studies would not exclude an antiapoptotic effect, which has been observed with chronic \textit{in vivo} rosiglitazone treatment of Zucker Diabetic Fatty rats (21). Consistent with this possibility, addition of troglitazone to cultured islets from Zucker fatty rats improves glucose-stimulated insulin secretion (19) and PPAR\textsubscript{\(\gamma\)} activation directly induces the expression of the Glut2 glucose transporter and glucokinase, critical regulators of glucose-stimulated insulin secretion in cultured primary rat islets and insulinoma cell lines (59,60). Although targeted elimination of PPAR\textsubscript{\(\gamma\)} in beta cells does not inhibit the \textit{in vivo} response to intravenous glucose or lead to an impairment in glucose homeostasis in insulin resistant mice, the stimulatory effect of troglitazone on isolated islets is completely eliminated in these PPAR\textsubscript{\(\gamma\)} deficient beta cell mice (61). Thus, a direct effect of the TZDs on the beta cell, directed via the PPAR\textsubscript{\(\gamma\)} receptor, to augment insulin secretion and prevent beta cell apoptosis cannot be excluded.

It should be noted that, although the improvement in glycemic control (\(\Delta\text{THB}A_{1c} = 1.3 – 2.3\%\)) was considerable, the increase in beta cell function (to 15-20% of values in healthy controls) (Table 3) was relatively small. This is explained by the observation that the improvement in glycemic control (FPG and 2-hour PG during OGTT) and insulin secretory response are log related. Therefore, even a small increase in beta cell function would be expected to exert a major beneficial effect on blood glucose levels and this effect would be further amplified in the presence of only mild to modest increments in insulin sensitivity (Table 2). The log relationship between plasma glucose concentration and both insulin secretion and insulin sensitivity predicts that, especially at extremes of beta cell function and insulin resistance, small (beneficial) changes in either parameter will exert a profound effect (reduction) on the plasma glucose concentration.

Recently, several studies (62,63) have demonstrated that certain sulfonylureas (glimepiride > glyburide) possess PPAR\textsubscript{\(\gamma\)} activity and one could question what impact this might have on the present
observations. Several comments are warranted. First, the PPARγ agonistic effects of both glimepiride and glyburide (53) were observed only at concentrations that were more than 10-20 fold greater than the plasma levels required to exert a glucose lowering effect in man. Second, the PPARγ effects were not seen or were quite weak with other sulfonylureas. In the present study, we observed a similar beneficial effect of pioglitazone on beta cell function in drug naïve, sulfonylurea withdrawn, and sulfonylurea-treated type 2 diabetic patients. This argues strongly that the presence or absence of the sulfonylurea (along with any associated PPARγ activity of the sulfonylurea) could not have any impact on the TZD-mediated improvement in beta cell function. Moreover, within the 10 sulfonylurea-treated subjects who received pioglitizone, 8 were on glyburide, 10 were on glipizide and 2 were on chlorpropamide. The improvement in beta cell function ($\Delta$ISR/$\Delta$G÷IR) was observed in all 10 diabetic subjects and was of similar magnitude in each individual despite the different sulfonylureas that were ingested. Since the PPARγ effect was described only with pharmacologic concentrations of glimepiride and glyburide, this further argues against any influence of the sulfonylurea in the present results.

Lastly, we observed a significant improvement in beta cell function ($\Delta$ISR/$\Delta$G÷IR) in the present study, whereas most (29,30,32,36) but not all (28,35) previous studies have reported a decrease in insulin secretion following TZD treatment. Previous investigators have attributed the decline in insulin secretion to the improvement insulin sensitivity and “unloading” or “rest” of the beta cell. Two comments are warranted. First, most of these previous studies did not express the insulin secretory response as a function of the glycemic stimulus or take into account the improvement in insulin sensitivity. The same or even reduced insulin secretory response with a lower glucose stimulus and/or significant reduction insulin resistance would result in augmented beta response as measured by $\Delta$ISR/$\Delta$G÷IR. Second, one needs to take into account where the individual is on the beta cell function
curve at the time of initiation of the thiazolidinedione therapy. We previously have shown that relationship between insulin secretion and plasma glucose concentration resembles an inverted U-shaped curve (41,64-66). If one starts with insulin resistance and normal or only slightly impaired glucose tolerance (implying relatively well preserved beta cell function), one might expect an improvement in insulin sensitivity to be associated with a decline in the elevated plasma insulin response, which represents a compensatory response to the underlying insulin resistance. On the other hand, if one has tipped over the top of Starling’s curve of the pancreas (55) and beta cell function is significantly reduced, one might expect the TZD-induced improvement in insulin sensitivity to be associated with an increase in beta cell function, especially if the TZD exerted a direct effect on the beta cell to enhance insulin secretion (17,18,20,21,23-25).

In summary, the present results demonstrate that chronic treatment of suboptimally controlled type 2 diabetic individuals with both pioglitazone and rosiglitazone improves glycemic control by enhancing insulin sensitivity and augmenting beta cell function. The improvement in beta cell function primarily is related to increased beta cell sensitivity to glucose and was documented by two independent measures, the insulin secretion/insulin resistance index \[\frac{\Delta ISR(AUC)}{\Delta G (AUC)} / IR\] and glucose sensitivity derived from a novel model of beta cell function. The increment in insulin secretion was the major determinant of the decrement in 2 hour plasma glucose concentration during the OGTT and was correlated with the reduction in HbA\textsubscript{1c}, the decrement in plasma FFA concentration during the OGTT, and the improvement in insulin sensitivity.
ACKNOWLEDGMENT

The present work was supported by NIH grant DK 24092, a VA Merit Award, NIH Clinical Research Center grant M01-RR-01346, and grants from Takeda Pharmaceuticals and GlaxoSmithKline. Ms. Lorrie Albarado and Ms. Elva Chapa provided expert secretarial assistance in the preparation of the manuscript.
FIGURE LEGENDS

Figure 1. Plasma glucose (left), insulin (middle), and free fatty acid (FFA) (right) concentrations during the oral glucose tolerance test performed before and after four months in: [A] drug naïve diabetic patients treated with pioglitazone (PIO), [B] sulfonylurea (SU)-treated diabetic patients receiving pioglitazone, [C] sulfonylurea (SU)-treated diabetic patients receiving placebo (PLC), [D] sulfonylurea (SU)-withdrawn diabetic patients receiving rosiglitazone (ROSI), and [E] sulfonylurea (SU) withdrawn diabetic patients receiving placebo (PLC).

Figure 2. Insulin secretion/insulin resistance index \([\Delta \text{ISR (AUC)} / \Delta \text{G (AUC)} \div \text{IR}]\) in non-diabetic controls (open bar), drug naïve diabetic patients before (stippled bar) and after (solid bar) pioglitazone treatment, sulfonylurea-treated diabetic patients before (stippled bar) and after (solid bar) pioglitazone, and sulfonylurea-withdrawn diabetics before (stippled bar) and after (solid bar) rosiglitazone. *p<0.05 and **p<0.02, post vs pre TZD treatment.

Figure 3. Relationship between the log of insulin secretion/insulin resistance index \([\Delta \text{ISR (AUC)} / \Delta \text{G (AUC)} \div \text{IR}]\) and the log of fasting plasma glucose (Glucose\(_0\)) concentration (top) and log of the 2-hour plasma glucose (Glucose\(_{120}\)) concentration during the OGTT in control (open circles) and diabetic (closed circles) subjects prior to TZD treatment.

Figure 4. Relationship between the log of glucose sensitivity (derived from the model) and the log of the insulin secretion/insulin resistance index \([\Delta \text{ISR (AUC)} / \Delta \text{G (AUC)} \div \text{IR}]\) (bottom), log of the fasting plasma glucose (Glucose\(_0\)) concentration (top), and log of 2-hour plasma glucose concentration.
(Glucose_{120}) during the OGTT (middle) in control (open circles) and diabetic (closed circles) subjects prior to TZD treatment.

**Figure 5.** Relationship between the change (post-pre) in ΔISR/ΔG ÷ IR vs the change in 2-hour plasma glucose concentration (top), the change in glucose sensitivity (model derived) (middle), and the change in rate sensitivity (model derived) (bottom) in diabetic patients treated with thiazolidinediones (closed circles) or placebo (open circles).

**Figure 6.** **Top panel:** Relationship between the insulin secretion/insulin resistance index [ΔISR (AUC)/ΔG (AUC) ÷ IR] and the log of the plasma FFA(AUC) during the OGTT in type 2 diabetic patients before (open circles) and after (solid circles) treatment with placebo or thiazolidinediones. **Bottom panel:** Relationship between the change in the insulin secretion/insulin resistance index [ΔISR(AUC)/ΔG(AUC)÷IR] and change in FFA(AUC) in type 2 diabetic patients treated with thiazolidinediones (solid circles) and placebo (open circles).
References:


activity and increases p110 protein levels in skeletal muscle of type 2 diabetic subjects.


<table>
<thead>
<tr>
<th></th>
<th>NGT Subjects</th>
<th>Drug Naive T2DM + Pioglitazone</th>
<th>SU-treated T2DM + Pioglitazone</th>
<th>SU-treated T2DM + Placebo</th>
<th>SU-withdrawn T2DM + Rosiglitazone</th>
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<td>Gender (F/M)</td>
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<td>MA/Cauc</td>
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<td>5/4</td>
<td>5/5</td>
<td>6/4</td>
<td>8/4</td>
<td>11/1</td>
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<tr>
<td>Duration of Diabetes (y)</td>
<td>2 ± 1</td>
<td>6 ± 2</td>
<td>5 ± 2</td>
<td>4 ± 2</td>
<td>2 ± 1</td>
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<td>Age (y)</td>
<td>52 ± 2</td>
<td>50 ± 4</td>
<td>55 ± 4</td>
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<td>56 ± 2</td>
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<td>BMI (kg m⁻²)</td>
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<td>27.4 ± 1.2</td>
<td>28.4 ± 1.2*</td>
<td>28.9 ± 1.3</td>
<td>30.0 ± 1.4*</td>
<td>29.9 ± 1.4</td>
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<td>FFM (kg)</td>
<td>50 ± 4</td>
<td>54 ± 4</td>
<td>54 ± 4</td>
<td>58 ± 4</td>
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<td>Fat mass (%)</td>
<td>33 ± 2</td>
<td>31 ± 2</td>
<td>33 ± 2*</td>
<td>30 ± 2</td>
<td>33 ± 2*</td>
<td>30 ± 2</td>
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<tr>
<td>HbA₁c (%)</td>
<td>5.4 ± 0.1</td>
<td>7.9 ± 0.4</td>
<td>6.6 ± 0.4*</td>
<td>9.3 ± 0.4</td>
<td>7.3 ± 0.6*</td>
<td>8.3 ± 0.4</td>
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<td>TG (mM)</td>
<td>1.8 ± 0.5</td>
<td>1.8 ± 0.2</td>
<td>1.3 ± 0.2*</td>
<td>1.5 ± 0.28</td>
<td>1.1 ± 0.1*</td>
<td>1.4 ± 0.1</td>
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<td>Chol (mM)</td>
<td>5.1 ± 0.4</td>
<td>4.6 ± 0.5</td>
<td>4.2 ± 0.5</td>
<td>4.4 ± 0.1</td>
<td>4.1 ± 0.1</td>
<td>4.4 ± 0.2</td>
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<tr>
<td>HDL (mM)</td>
<td>1.21±0.12</td>
<td>0.93 ± 0.08</td>
<td>0.94 ± 0.07</td>
<td>0.91 ± 0.08</td>
<td>0.92 ± 0.05</td>
<td>0.95 ± 0.05</td>
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<tr>
<td>LDL (mM)</td>
<td>3.32±0.31</td>
<td>2.82 ± 0.37</td>
<td>2.65 ± 0.38</td>
<td>2.80 ± 0.13</td>
<td>2.65 ± 0.14</td>
<td>2.85 ± 0.15</td>
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</table>

**SU = sulfonylurea; T2DM = type 2 diabetes mellitus**

MA=Mexican American; Cauc=Caucasian; NGT=normal glucose tolerant
TGW=triglyceride Chol=total cholesterol
To convert plasma triglyceride from mM to mg/dl divide by 0.01129
To convert total/HDL/LDL cholesterol from mM to mg/dl divide by 0.02586

* P<0.01, pre versus post
+ p<0.001 versus placebo
Table 2 – Metabolic characteristics during OGTT and Insulin Clamp

<table>
<thead>
<tr>
<th></th>
<th>NGT Subjects</th>
<th>Drug Naive T2DM + Pioglitazone</th>
<th>SU-treated T2DM + Pioglitazone + Placebo</th>
<th>SU-withdrawn T2DM + Rosiglitazone + Placebo</th>
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<tr>
<td></td>
<td>Pre Post</td>
<td>Pre Post</td>
<td>Pre Post</td>
<td>Pre Post</td>
</tr>
<tr>
<td>FPG (mmol/l)</td>
<td>5.1 ± 0.1</td>
<td>9.4 ± 0.6*</td>
<td>10.5 ± 0.9</td>
<td>11.2 ± 0.7</td>
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<tr>
<td>AUC_G (mol•l⁻¹•2h)</td>
<td>0.87 ± 0.04</td>
<td>1.83 ± 0.11</td>
<td>1.96 ± 0.10</td>
<td>2.00 ± 0.09</td>
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<tr>
<td>FFA (µmol/l)</td>
<td>741 ± 75</td>
<td>637 ± 74 590 ± 27</td>
<td>656 ± 45 477 ± 35*</td>
<td>736 ± 62</td>
</tr>
<tr>
<td>FFA (AUC) (mmol•l⁻¹•2h)</td>
<td>52 ± 5</td>
<td>60 ± 5 49 ± 3</td>
<td>59 ± 3 41 ± 3*</td>
<td>68 ± 4</td>
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<tr>
<td>FPI (pmol/l)</td>
<td>43 ± 6</td>
<td>83 ± 11 70 ± 11</td>
<td>91 ± 20 86 ± 28 105 ± 13 94 ± 12</td>
<td>105 ± 18</td>
</tr>
<tr>
<td>AUC_F (pmol•l⁻¹•2h)</td>
<td>43 ± 5</td>
<td>22 ± 5 24 ± 5</td>
<td>24 ± 6 25 ± 7 28 ± 4 26 ± 5</td>
<td>25 ± 6</td>
</tr>
<tr>
<td>FCP (pmol/l)</td>
<td>560 ± 130</td>
<td>853 ± 81 682 ± 86</td>
<td>803 ± 124 806 ± 93 758 ± 93 844 ± 176</td>
<td>836 ± 87</td>
</tr>
<tr>
<td>CP (AUC) (nmol•l⁻¹•2h)</td>
<td>283 ± 30</td>
<td>150 ± 15 168 ± 19</td>
<td>150 ± 22 178 ± 50 152 ± 19 170 ± 43</td>
<td>169 ± 23</td>
</tr>
<tr>
<td>Basal Rd=EGP (µmol•min⁻¹•kg⁻¹)</td>
<td>15.3 ± 0.6</td>
<td>15.0 ± 1.0 14.8 ± 0.8</td>
<td>16.1 ± 0.8 15.1 ± 0.4 14.2 ± 0.4 15.2 ± 0.6</td>
<td>17.4 ± 0.8</td>
</tr>
<tr>
<td>Clamp Rd (step 1) (µmol•min⁻¹•kg⁻¹)</td>
<td>51.9 ± 9.9</td>
<td>22.6 ± 2.0 31.9 ± 3.0*</td>
<td>20.5 ± 1.9 21.6 ± 2.4 21.1 ± 1.6 21.6 ± 1.1</td>
<td>16.1 ± 0.9</td>
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<tr>
<td>Clamp GC (step 1) (ml•min⁻¹•kg⁻¹)</td>
<td>7.6±0.9</td>
<td>2.8±0.2 3.9±0.3*</td>
<td>2.5±0.4+ 2.9±0.4 2.8±0.3 2.5±0.2</td>
<td>1.7±0.1</td>
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<tr>
<td>Clamp Rd (step 2) (µmol•min⁻¹•kg⁻¹)</td>
<td>108.5 ± 7.7</td>
<td>54.0 ± 4.6 74.6 ± 7.0*</td>
<td>38.1 ± 6.4 49.6 ± 6.8* 49.5 ± 3.2 41.8 ± 3.4*</td>
<td>34.0 ± 3.6</td>
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<td>Hepatic IR ([pmol/l]x(µmol•min⁻¹•kg⁻¹)</td>
<td>733 ± 74</td>
<td>1101 ± 151 773 ± 104*</td>
<td>1558 ± 335 951 ± 147* 1221 ± 189 1311 ± 244</td>
<td>1587 ± 289</td>
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* p<0.001-0.0001, post vs pre and TZD vs placebo
+p<0.05-0.01, post vs pre and TZD vs placebo
Rd=insulin-stimulated rate of glucose disposal
GC=insulin-stimulated rate of glucose clearance

CP=C-peptide, FPI/FPG=fasting plasma insulin/glucose conc
EGP=endogenous glucose production
NGT=normal glucose tolerant
T2DM = type 2 diabetes mellitus
SU = sulfonylurea
Table 3 – Parameters of beta-cell function

<table>
<thead>
<tr>
<th></th>
<th>NGT Subjects</th>
<th>Drug Naïve T2DM + Pioglitazone</th>
<th>SU-treated T2DM + Pioglitazone</th>
<th>SU-treated T2DM + Placebo</th>
<th>SU-withdrawn T2DM + Rosiglitazone</th>
<th>SU-withdrawn T2DM + Placebo</th>
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<tr>
<td>Basal ISR (pmol/\text{min}^1/\text{m}^2)</td>
<td>73 ± 13</td>
<td>114 ± 11</td>
<td>89 ± 11</td>
<td>106 ± 15</td>
<td>112 ± 36</td>
<td>111 ± 19</td>
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<tr>
<td>Total ISR (nmol/\text{m}^2)</td>
<td>42 ± 5</td>
<td>23 ± 2</td>
<td>28 ± 3</td>
<td>25 ± 3</td>
<td>28 ± 6</td>
<td>29 ± 5</td>
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<tr>
<td>ΔISR (AUC)/ΔG (AUC)</td>
<td>234 ± 48</td>
<td>34 ± 8</td>
<td>40 ± 11</td>
<td>33 ± 9</td>
<td>37 ± 9</td>
<td>45 ± 8</td>
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<td>ΔISR (AUC)/ΔG (AUC) IR</td>
<td>177 ± 30</td>
<td>13 ± 2</td>
<td>28 ± 6*</td>
<td>15 ± 3</td>
<td>24 ± 5*</td>
<td>20 ± 3</td>
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<td>Beta Cell Glucose Sensitivity (pmol/\text{min}^1/\text{m}^2 \cdot \text{mM}^{-1})</td>
<td>22.7 ± 4.3</td>
<td>1.0 ± 0.2</td>
<td>2.8 ± 0.7*</td>
<td>0.7 ± 0.2</td>
<td>1.4 ± 0.5*</td>
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<td>Beta Cell Rate Sensitivity (pmol/\text{m}^2 \cdot \text{mM}^{-1})</td>
<td>118 ± 22</td>
<td>14 ± 3</td>
<td>28 ± 6*</td>
<td>17 ± 4</td>
<td>25 ± 5*</td>
<td>21 ± 5</td>
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<td>Potentiation factor</td>
<td>1400 ± 370</td>
<td>213 ± 48</td>
<td>485 ± 136*</td>
<td>102 ± 49</td>
<td>384 ± 101*</td>
<td>269 ± 92</td>
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<td>2.0 ± 0.4</td>
<td>1.0 ± 0.1</td>
<td>1.2 ± 0.1</td>
<td>1.0 ± 0.1</td>
<td>1.0 ± 0.1</td>
<td>0.9 ± 0.1</td>
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</table>

*p<0.01-0.001 pre versus post and TZD vs placebo

ISR=insulin secretion rate; I=insulin; G=glucose
NGT=normal glucose tolerant
<table>
<thead>
<tr>
<th>Change in</th>
<th>Change in [ΔISR(AUC)/ ΔG(AUC)]</th>
<th>Change in R_{d1}</th>
<th>Change in R_{d2}</th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R</td>
<td>p</td>
<td>R</td>
<td>p</td>
</tr>
<tr>
<td>BW (kg)</td>
<td>0.31</td>
<td>&lt;0.03</td>
<td>0.28</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Fat Mass (kg)</td>
<td>0.30</td>
<td>&lt;0.04</td>
<td>0.29</td>
<td>&lt;0.04</td>
</tr>
<tr>
<td>FPG (mM)</td>
<td>-0.41</td>
<td>&lt;0.005</td>
<td>-0.32</td>
<td>&lt;0.03</td>
</tr>
<tr>
<td>G_{120} (mM)</td>
<td>-0.51</td>
<td>&lt;0.0002</td>
<td>-0.40</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Fasting FFA (mM)</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>-0.44</td>
</tr>
<tr>
<td>Mean FFA_{0-120} (mM)</td>
<td>-0.36</td>
<td>&lt;0.01</td>
<td>ns</td>
<td>-0.27</td>
</tr>
<tr>
<td>FFA (ΔAUC) (mM)</td>
<td>ns</td>
<td>-0.40</td>
<td>-0.40</td>
<td>-0.40</td>
</tr>
<tr>
<td>Rd-s1 (μmol·min^{-1·kg^{-1·ffm}})</td>
<td>0.40</td>
<td>&lt;0.005</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Rd-s2 (μmol·min^{-1·kg^{-1·ffm}})</td>
<td>0.40</td>
<td>&lt;0.004</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>ISR (ΔAUC) (pmol·m^{-2})</td>
<td>-</td>
<td>-</td>
<td>0.45</td>
<td>&lt;0.002</td>
</tr>
<tr>
<td>Glucose sensitivity+</td>
<td>0.63</td>
<td>&lt;0.0001</td>
<td>0.28</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Rate sensitivity+</td>
<td>0.41</td>
<td>&lt;0.004</td>
<td>ns</td>
<td>0.28</td>
</tr>
<tr>
<td>Potentiation+</td>
<td>0.31</td>
<td>&lt;0.03</td>
<td>ns</td>
<td>ns</td>
</tr>
</tbody>
</table>

BW=body weight
FPG=fasting plasma glucose
PG_{120} min=plasma glucose conc at 120 min during the OGTT
Rd-s1 and Rd-s2=insulin-mediated rate of glucose disposal during the first and second steps of the insulin clamp
+ Model-derived parameters of beta cell function (36,37)
Figure 1
Figure 2

TYPE 2 DIABETES MELLITUS

Drug Naïve
SU-Treated
SU-Withdrawn

NGT
*PIO
*PIO
*PLACEBO
*ROSI
*PLACEBO

\( \Delta \text{ISR(AUC)/}\Delta \text{G(AUC)} + \text{IR} \)

* p < 0.05
** p < 0.01

Figure 2
Figure 3

\[ \ln(\text{ISR(AUC)}/\Delta G(\text{AUC}) ÷ \text{IR}) \]

R = 0.83
p < 0.0001

R = 0.89
p < 0.0001

\[ \ln(Glucose_0) \]

\[ \ln(Glucose_{120}) \]
Figure 4

In (glucose sensitivity)

In (Glucose$_0$)

R = 0.77
p < 0.0001

In (Glucose$_{120}$)

R = 0.82
p < 0.0001

ln (△ISR(AUC)/ △G(AUC) ÷ IR )

R = 0.78
p < 0.0001
Figure 5

Change in ISR(AUC)/G(AUC) ÷ IR

Change in Glucose$_{120}$ (mM)

R=0.49  
p<0.0003

Change in Glucose Sensitivity

R=0.56  
p<0.0001

Change in Rate Sensitivity

R=0.32  
p<0.01
Figure 6

\[
\ln(\text{ISR(AUC)}/ \Delta G(\text{AUC}) \div \text{IR}) = \ln(\text{FFA}[\text{AUC}]) \quad (\text{mM})
\]

-4
-2
0
2
4
3
3.5
4
4.5
5

\[ R=0.26 \quad p<0.05 \]

\[
\text{Change in } \frac{\Delta \text{ISR(AUC)}}{\Delta \text{G(\text{AUC})} \div \text{IR}} = \text{Change in } \Delta \text{FFA(\text{AUC})}
\]

-40
-20
0
20
40

\[ R=0.33 \quad p<0.01 \]