Effects of pioglitazone and metformin on β-cell function in nondiabetic subjects at high risk for type 2 diabetes

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Rasouli N, Kern PA, Reece EA, Elbein SC. Effects of pioglitazone and metformin on β-cell function in nondiabetic subjects at high risk for type 2 diabetes. Am J Physiol Endocrinol Metab 292: E359–E365, 2007. First published September 12, 2006; doi:10.1152/ajpendo.00221.2006.—Thiazolidinediones (TZDs) and metformin decreased the incidence of diabetes in subjects at risk for developing diabetes and improved peripheral or hepatic insulin sensitivity, respectively. Whether they also directly improved β-cell function is not clear. In vitro studies showed improved β-cell function in response to TZDs and metformin; however, the effects of TZDs or metformin on β-cell function in humans are still uncertain. We hypothesized that both TZDs and metformin directly affect β-cell function. We evaluated β-cell function and insulin sensitivity (Si) in subjects with impaired glucose tolerance or a history of gestational diabetes using oral and intravenous glucose tolerance tests in addition to the glucose-potentiated arginine stimulation test. In contrast to metformin, pioglitazone improved Si, glucose tolerance, and insulin-independent glucose disposal [glucose effectiveness (SG)]. Neither pioglitazone nor metformin significantly improved β-cell compensation for insulin resistance [disposition index (DI)], but the change in DI significantly correlated with baseline Si. Insulin secretion in response to arginine at maximally potentiating glucose levels (AIRmax) tended to increase after metformin and to decrease after pioglitazone, however, when adjusted for Si, the changes were not significant. Our results demonstrate that, in nondiabetic subjects at risk for diabetes, pioglitazone, but not metformin, significantly improved glucose tolerance by improving Si and SG. We did not find any evidence that either pioglitazone or metformin improved β-cell function. Improved β-cell compensation was observed primarily in the subgroup of subjects that had the lowest Si at baseline.

Subjects with type 2 diabetes (T2DM) are characterized by chronic insulin resistance with poor β-cell compensation. Insulin secretory compensation fails progressively as insulin resistance worsens, leading to impaired glucose tolerance (IGT) and, subsequently, T2DM (18). Several interventions have been attempted in IGT subjects with the goal of preventing T2DM. Improved insulin sensitivity (Si) with lifestyle modification (weight loss) and metformin decreased the incidence of diabetes (19, 29). Thiazolidinediones (TZDs) also decreased the incidence of diabetes in subjects at risk for developing T2DM (19a, 35). However, it is unclear whether these interventions prevented or delayed diabetes by enhancing Si or whether they also directly improved β-cell function. In vitro studies have demonstrated that rosiglitazone preserved β-cells by reducing insulin demand, decreasing amyloid formation, and reducing the rate of β-cell apoptosis (9). Treatment of Zucker diabetic fatty rats with troglitazone lowered islet fat content and preserved β-cell function, presumably by reducing lipotoxicity (13, 27). However, another study suggested that TZDs failed to protect against fatty acid-mediated cytotoxicity in pancreatic β-cells (33). In vitro studies also support a protective role for metformin. In a recent study of islets isolated from type 2 diabetic patients (21), 24-h incubation with metformin was associated with increased insulin content, increased number and density of mature insulin granules, improved glucose-stimulated insulin release, and increased insulin mRNA expression. Another study (14) showed decreased amyloid deposition and preservation of β-cell mass with metformin treatment.

The effect of metformin or TZDs on β-cell function in intact humans is still uncertain, because the accurate assessment of β-cell function in vivo is complex (9). Furthermore, most studies of insulin sensitizers have been performed in diabetic subjects, where differentiation of the direct effects on β-cell function from indirect benefits of glycemic control is difficult. Previous studies of the effects of TZDs on β-cell function in nondiabetic subjects (4–7, 35) have shown an improvement in β-cell function; however, troglitazone was used in most of these studies (4–7). Troglitazone belongs to the TZD family but has been removed from the market. Whether the beneficial effect of troglitazone on β-cell function was a class effect is unknown. Treatment of Latino subjects with a history of gestational diabetes (GDM) with pioglitazone improved β-cell compensation for insulin resistance (35), but this result has not been confirmed in other ethnicities. The effects of metformin on β-cell function in humans have not been reported previously.

Pancreatic β-cells express peroxisome proliferator-activated receptor-γ (PPARγ) (33), and AMP-activated kinase regulates glucose-stimulated insulin secretion in β-cells (20). Because TZDs function through activation of PPARγ (36) and the beneficial effects of metformin are linked to the activation of AMP kinase (37), we hypothesized that both TZDs and metformin directly affect β-cell function. In this study, we evaluated β-cell function using oral and intravenous glucose tolerance tests in addition to glucose-potentiated arginine stimulation tests in subjects at high risk for T2DM (IGT or GDM) who were treated with metformin or pioglitazone. Subjects with IGT or history of GDM have insulin resistance and impaired β-cell function (10, 31), but without overt hyperglycemia. Because the glucose-lowering effect of pioglitazone or met-
in good health were recruited by local advertisement and underwent an initial 75-g oral glucose tolerance test (OGT). Subjects were included if they were glucose intolerant (fasting glucose <126 mg/dl and the 2-h post-challenge glucose between 140 and 199 mg/dl) or had a history of GDM within the past 5 yr (n = 5). Two of the subjects with IGT also had a history of GDM within the past 5 yr. GDM subjects were included if they were treated with insulin during pregnancy or provided a letter from their community physician indicating the diagnosis of GDM. Subjects were 25–65 yr with a body mass index of 27–38 kg/m². Subjects with a history of coronary artery disease or the concomitant use of fibrates, angiotensin-converting enzyme inhibitors, and angiotensin II receptor blockers were excluded. All subjects were weight stable for ≥3 mo. Subjects were started on a 35% fat eucaloric diet 2 wk prior to randomization and were maintained on this diet throughout the study. Total body fat content and fat distribution were determined by dual-energy X-ray absorptiometry (DEXA) and computed tomography (CT) scan, respectively. Abdominal subcutaneous and visceral adipose tissue were determined from the cross-sectional fat distribution using the Slicomatic program (Tomovision, Montreal, QC, Canada) to analyze the CT scan images at the level of the umbilicus. The range of Hounsfield units used to assess the quantity of fat was −250 to −40.

All subjects underwent an insulin-modified, frequently sampled intravenous glucose tolerance test (FSIGT), followed by an arginine stimulation test, as described below. Subjects were then randomized to metformin (500 mg twice daily) or pioglitazone (30 mg once daily) for 2 wk, followed by 8 wk at a maximum dose (1,000 mg of metformin twice daily or 45 mg of pioglitazone daily). This study included subjects from the previous study (26) in addition to the new recruitment. IGT and GDM subjects were randomized separately into treatment groups. Compliance and laboratory tests were monitored three times during the followup. The oral and intravenous glucose tolerance tests, the arginine stimulation test, DEXA, and CT scan were repeated after 10 wk of treatment.

Subtracted using intravenous arginine bolus superimposed on a short hyperglycemic plateau, as described previously (32). At the conclusion of the FSIGT, a second glucose bolus of D20 (50 g) was given, followed by a variable glucose infusion, to achieve and maintain a glucose level ≥25 mmol/l (450 mg/dl) for ≥30 min, at which time 5 g of arginine was administered intravenously for 30 s. Samples for insulin measurement were taken at 2, 3, 4, 5, 7, and 10 min after arginine injection. The AIRmax was calculated as mean insulin excursion above prearginine baseline for 2–10 min after arginine bolus. From this test one can derive a maximal insulin response, which correlated well with β-cell mass in previous studies in baboons (23).

Because the magnitude of the insulin response is determined in part by the prevailing SI (17), we also determined the disposition index (DI), which is calculated as the product of SI and AIRg (DI = SI × AIRg). The calculation of this parameter is based on the described hyperbolic relationship between SI and the insulin secretory response (17). Because AIRmax is related to SI in a hyperbolic manner, we also calculated the product of SI × AIRmax, which we refer to as DImax (30).

Statistical analysis. Repeated-measures ANOVA models were used to analyze continuous data measured at baseline and after 10 wk of treatment with either metformin or pioglitazone. The trends of the treatments groups over time were compared by means of the group-by-time interaction. If the test for interaction was not significant, the time effect from the repeated-measures ANOVA was used to evaluate the change observed in baseline and 10-wk measures. In the case of a significant interaction test, paired t-tests were used to compare the change in baseline and 10-wk measures for each group separately. Continuous variables that were not normally distributed (SI, AIRg, AIRmax, DI, and DImax) were log transformed. Data are presented in tables, figures, and text as means ± SD in their original scales. All reported P values are two sided. A P value of 0.05 was accepted as statistically significant. The study was designed to examine the effects of metformin and pioglitazone on β-cell function, using the DI as the primary indicator. Our data revealed a baseline DI of 748 ± 528 (n = 50). Assuming α = 0.05, and an expected SD of change of 500, our study had 82% power to detect a change in DI of 300 (40%).

RESULTS

Table 1 contains the baseline characteristics of the 50 subjects (26 metformin and 24 pioglitazone) who completed the study. Seven subjects were African-Americans, one was Hispanic, and the remainder were Caucasian. Five subjects (2 in pioglitazone group) had a history of GDM within the past 5 yr, with normal glucose tolerance subsequently. Glucose-tolerant subjects with a history of GDM were younger than IGT subjects (32.6 ± 4.7 vs. 47.2 ± 7.8 yr old, P < 0.01). The baseline characteristics of the subjects did not differ by treatment group.

Body composition. In contrast to subjects in the metformin group, who lost weight during the drug treatment phase (1.2 ± 2.3 kg, P = 0.02), subjects in the pioglitazone group gained 2.5 ± 2.4 kg (P < 0.01), with a slight increase in the total percent body fat (Table 1). The calculated fat mass (body weight × %body fat) increased after pioglitazone (from 36.4 ± 8.2 to 38.0 ± 8.8 kg, P < 0.01) and decreased after metformin treatment (from 38.0 ± 9.0 to 37.3 ± 8.4 kg, P = 0.03). Subjects treated with pioglitazone, subcutaneous fat area measured by CT scan increased from 452 ± 116 to 481 ± 121 cm², P < 0.01, without a significant change in visceral fat area. However, metformin therapy decreased both subcutaneous (from 450 ± 109 to 431 ± 111 cm², P = 0.01) and visceral fat area (182 ± 80 to 167 ± 75 cm², P < 0.01). Both pioglitazone and metformin treatment decreased the visceral to subcutane-
A Variable & Meformin & Polo glutazone & P value‡ & Interaction P value†
& Sex (F/M) & 22/4 & 21/3 & &
& Age & 46.0 ± 8.7 & 45.5 ± 9.1 & &
& BMI, kg/m² & 33.2 ± 3.4 & 32.3 ± 3.8 & &
& Weight, kg & 93.9 ± 18.6 & 92.1 ± 17.8 & &
& Body fat, % & 40.6 ± 6.3 & 40.5 ± 6.1 & &
& Fasting glucose, mg/dl & 90.1 ± 9.9 & 92.1 ± 12.1 & &
& 2-h Glucose, mg/dl & 156.0 ± 30.6 & 162.5 ± 26.8 & &
& Hb Atc, % & 5.5 ± 0.6 & 5.5 ± 0.5 & &
& Cholesterol, mg/dl & 190.0 ± 40.6 & 200.3 ± 52.7 & &
& LDL, mg/dl & 110.5 ± 41.3 & 111.8 ± 41.3 & &
& HDL, mg/dl & 52.9 ± 17.8 & 50.7 ± 8.0 & &
& Triglyceride, mg/dl & 131.7 ± 73.7 & 178.6 ± 101.6 & &
& SI, min & 14.0 ± 4.7 & 11.2 ± 5.3 & &
& AIRg, min−1·μU·ml−1·10−4 & 1.42 ± 0.73 & 1.43 ± 0.57 & &
& Insulinogenic index & 1.05 ± 0.64 & 1.10 ± 1.13 & &
& AIRp & 483 ± 352 & 418 ± 342 & &
& DI & 823 ± 479 & 666 ± 565 & &
& AIRmax & 185 ± 114 & 204 ± 131 & &
& DLmax & 306 ± 129 & 320 ± 214 & &

Data are presented in tables and text as means ± SD in their original scales. All reported P values are two sided. BMI, body mass index; Hb Atc, glycosylated hemoglobin; SI, glucose effectiveness; Sg, insulin sensitivity; AIRp, acute insulin response to glucose; DI, disposition index; AIRmax, maximal acute insulin response; DLmax, product of Sg × AIRmax. *Time effect was evaluated using a paired t-test because of significant group-by-time interaction; †test of the group-by-time interaction from the RM-ANOVA; ‡repeated-measures ANOVA was used to evaluate the time effect, unless noted otherwise.

β-CELL FUNCTION AND INSULIN SENSITIZERS

Table 1. Baseline characteristics of subjects and the effects of different treatments

![Graphs A, B, C, D](http://aspendo.physiology.org/)

A: Mean glucose and insulin responses during oral glucose tolerance testing. Left: glucose (A) and insulin (C) responses before and after pioglitazone. Right: glucose (B) and insulin (D) responses before and after metformin. Pioglitazone reduced the area under curve (AUC) of glucose by 18% (P = 0.001) and AUC of insulin by 44% (P = 0.008).
11.3 ± 9.6 to 7.7 ± 5.5 μU/ml, P < 0.01 after metformin), but
the change in the 2-h insulin level only reached significance
after pioglitazone treatment (from 122.2 ± 109.9 to 49.5 ±
49.0 μU/ml, P < 0.01 in pioglitazone group, and from 102.3 ±
91.0 to 83.7 ± 67.1 μU/ml, P = 0.07 in metformin group; Fig.
1). Likewise, the area under curve and mean of glucose and
insulin levels during the OGT decreased significantly only after
pioglitazone treatment (Fig. 1).

We evaluated β-cell function in response to pioglitazone and
metformin using the insulinogenic index, which is the ratio of
insulin: glucose increments above the baseline at 30 min during
the OGT test (Insulin30–0/Glucose30–0). Neither metformin nor
pioglitazone changed the insulinogenic index (Table 1). Like-
wise, other OGT-derived indexes of β-cell function (insulin:
glucose area under curve; insulin: glucose increment above
baseline) did not change after either treatment. In summary,
despite significant improvement in glucose tolerance after
pioglitazone, the insulin secretion adjusted for the concomitant
insulin resistance (as a marker of β-cell function) did not
improve after either treatment.

Intravenous glucose tolerance test. FSIGTs were performed
as another method to evaluate β-cell function in response to
different insulin sensitizers. As shown in Table 1, AIRg in-
creased slightly after metformin and decreased after pioglita-
zone. Neither the changes in AIRg after pioglitazone nor
metformin were significant (Table 1). Since insulin secretion
should be considered in the context of SI, we calculated the DI
(DI = S1 × AIRg) as an indicator of β-cell compensation for
insulin resistance. As shown in Table 1, DI did not change in
either group. In the pioglitazone group, DI did not increase
significantly despite a 45% improvement in SI. Thus, pioglita-
zone improved SI but did not specifically alter
β-cell compensation. However, the change in DI correlated significantly with baseline SI (r = 0.38, P = 0.05, Fig. 2), suggesting that the most insulin-resistant subjects demonstrated the largest in-
crease in DI.

Arginine stimulation test. We assessed β-cell function using a 5-g intravenous arginine bolus superimposed on a hypergly-
cemic plateau of >450 mg/dl, which represents the maximum
glucose potentiation effect for arginine-induced acute insulin
release (AIRmax) (32). AIRmax is a measure of maximal β-cell
reserve. AIRmax tended to increase after metformin treatment
(185 ± 114 to 221 ± 137, P = 0.08) but decreased with
pioglitazone treatment from 204 ± 131 to 159 ± 89 (P < 0.01;
Table 1). The two groups were significantly different with
respect to the change in AIRmax, but when normalized to the
change in SI (AIRmax × S1), which we denote as DImax, neither

treatment resulted in a change (Table 1).

Intercorrelation of in vivo tests of insulin secretion. No single
test accurately assesses β-cell function in vivo. Whether
different measurements of β-cell function correlate with each
other is unclear (9). Hence, we examined the correlation of
insulin secretion measures in response to oral and intravenous

glucose and to arginine at maximally potentiating glucose
levels (AIRmax) in our study population. Although the mea-
sures were significantly correlated (Fig. 3), no single measure

Fig. 2. The change in disposition index (DI) correlated significantly with
baseline insulin sensitivity (S1) in pioglitazone-treated subjects (n = 24).

Fig. 3. Intercorrelation between different methods of measuring insulin secre-
cion. Insulinogenic index derived from oral glucose tolerance test correlated
significantly with the acute insulin response to glucose (AIRg), an index of
first-phase insulin secretion (r = 0.5), and the maximal acute insulin response
during glucose-potentiated arginine stimulation test (AIRmax), an index of
β-cell mass (r = 0.54). The correlation between AIRg and AIRmax was weaker
but still significant (r = 0.40). Insulinogenic index: the incremental rise of
insulin from basal to 30 min divided by incremental rise of glucose from basal
to 30 min.
could account for >25% any other measure (r < 0.5). Thus, the incomplete correlations support the hypothesis that each assessment of insulin secretion measures a different aspect of β-cell function.

DISCUSSION

The progression from normal glucose tolerance to IGT and diabetes involves a defect in the ability of the islet β-cell to increase insulin secretion to adequately compensate for decreases in S1 (25, 34). Because the prevalence of obesity and, hence, insulin resistance is increasing (8, 12, 24), a recent focus has centered on ways to prevent the development of diabetes. In recent trials, insulin sensitizers have been used to prevent or delay diabetes (5, 19), but whether insulin sensitizers caused any improvement in β-cell function is uncertain.

To address this question, we randomized subjects with IGT or a history of GDM to treatment with either metformin or pioglitazone for 10 wk and assessed glucose tolerance, β-cell function, and S1. In contrast to the metformin group, subjects in the pioglitazone group gained weight and expanded their subcutaneous fat depot, with a slight decrease in V/SQ ratio. Despite the weight gain, pioglitazone, but not metformin, improved both S1 and glucose disposal independent of insulin (S0,C). Although pioglitazone improved S1 by 45%, neither AIRg nor DI changed, suggesting that pioglitazone did not have a direct effect on insulin secretion or β-cell compensation. These findings are in agreement with previously reported results of the effects of rosiglitazone on β-cell function (15).

The relationship between measures of insulin secretion and insulin action (such as AIRg and S1) has been shown in cross-sectional studies to describe a hyperbolic curve when S1 is graphed on the abscissa and insulin secretion on the ordinate (17). Appropriate compensation of insulin secretion for changes in S1 remain on the same curve, such that DI (S1 × AIRg) remains constant. In contrast, with a deterioration of glucose tolerance, the curve is shifted downward and to the left, such that AIRg is lower for each measure of S1. Improved glucose tolerance is expected to move the curve upward and to the right, such that insulin secretion is higher for each value of S1. In the present study, IGT subjects treated with pioglitazone remained on the same hyperbolic curve. The curve is indeed lower than that of normal individuals described previously (17), consistent with the IGT state. The improvement in glucose tolerance would have been expected instead to show improved DI and movement to a higher curve. This contradiction between improved glucose tolerance and the lack of improvement in DI suggests that either the hyperbolic relationship between S1 and AIRg is not perfect or factors other than β-cell compensation are responsible for the improved glucose tolerance. Indeed, S0,C improved significantly with pioglitazone and appears to account for the improvement in glucose tolerance beyond the effects of pioglitazone on S1.

In agreement with the FSIGT, we did not detect any change in insulin secretion during an OGT test (measured by insulinogenic index) after either drug. Interestingly AIRmax, as an indicator of maximal response of the β-cell (32), decreased after pioglitazone but increased after metformin. However, neither change was significant when we adjusted for S1.

Several previous studies (4, 6, 7, 35) have examined the effects of TZDs on β-cell function in individuals at high risk for diabetes. Cavaghan et al. (6) and Ehrmann et al. (7) studied carefully the effect of troglitazone on β-cell function in subjects with IGT or polycystic ovary disease using OGT test, FSIGT, graded glucose infusion, and oscillatory glucose infusion. They reported improvements in troglitazone-treated subjects both in DI and insulin secretory response to a graded glucose infusion and oscillatory glucose test. Buchanan et al. (4) and Xiang et al. (35) found a significant improvement in DI in Latino subjects with a history of GDM treated with either troglitazone or pioglitazone (5, 35), thus suggesting that TZDs improved β-cell compensation.

Our results are not consistent with the earlier studies of Buchanan et al. (5) in Hispanic women or Cavaghan et al. (6) and Ehrmann et al. (7) using troglitazone. Whereas Xiang et al. (35) saw a 60% improvement in DI in nondiabetic subjects treated with pioglitazone for 1 yr, we saw no significant change in DI after 10 wk of treatment despite having 80% power to detect a comparable change. This difference could have resulted from the difference in the ethnicity of the two study populations. The prevalence of diabetes is significantly higher in the Latino population (11), and the Latino subjects with former GDM may have been more insulin-resistant than our subjects. In support of this hypothesis, the change in DI was negatively correlated with the baseline S1 among our subjects treated with pioglitazone. Previous studies have also shown an improvement in DI principally among the subgroup of subjects that had the lowest tertile of S1 (5) or among the subgroup of subjects that had an increased S1 (35). On the basis of such findings, Buchanan et al. (5) and Xiang et al. (35) concluded that the protective benefits of TZDs on β-cell function were most likely indirect and mediated through improved S1 and resultant β-cell rest.

Our findings also failed to confirm previous work from Cavaghan et al. (6) and Ehrmann et al. (7), who also found an improved insulin secretory response adjusted for S1 after troglitazone. This discrepancy could reflect the difference in the methods used to evaluate β-cell function, which do not necessarily correlate well. Alternatively, the beneficial effects of troglitazone on β-cell function may not be generalizable to other TZDs. In addition to AIRg and DI, we examined the response of the maximal insulin secretory response, AIRmax, to metformin and pioglitazone. In contrast to the anticipated increase in AIRmax after pioglitazone, we in fact observed a decrease. We found no change when this value was normalized to the S1 (DImax); thus, the decrease in AIRmax was appropriate for the improvement for S1, but no improvement in β-cell function was observed. Interestingly, metformin, which we viewed as less likely to impact β-cell function, caused a slight increase in AIRmax, and the difference in response between pioglitazone and metformin groups was also significant. To our knowledge, the effect of metformin on the β-cell has not been studied previously.

Our study does have some limitations. Unlike some previous studies (4, 6, 7, 35), we chose to directly compare subjects to baseline after treatment with metformin or pioglitazone, without an untreated control group or control period. Although study enrollment might have caused changes in the parameters measured, the lack of any effect on glucose tolerance, DI, or S1 in the metformin group argues against effects related to time but not treatment. Our study population was primarily female, with four male subjects in metformin and three male subjects...
in the pioglitazone group. Hence, we lacked adequate power to determine a sex effect. Conceivably, a different conclusion might have been reached with more male representation, although the studies of Buchanan et al. (5) and Xiang et al. (35) were entirely in women.

The accurate assessment of β-cell function in vivo is complex, and the present standard tests evaluate different aspects of β-cell function. We analyzed the correlation between insulin secretion in response to oral and intravenous glucose in addition to a nonglucose stimulant. We found only a 50% correlation between different tests. Previous studies in diabetic subjects (9) reported no correlation between A1C and the insulogenic index.

In summary, our results demonstrate that, in nondiabetic subjects at risk for diabetes, pioglitazone, but not metformin, significantly improved glucose tolerance, but this effect appeared to occur mainly by improving $S_1$ and $S_2$ rather than an improvement in insulin secretion. We did not find any evidence that either pioglitazone or metformin improved β-cell function. What improvements in β-cell compensation for insulin resistance that we observed were primarily in the subgroup of subjects who had the lowest $S_1$ at baseline, consistent with other studies.

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