Rosiglitazone Reduces Glucose-Stimulated Insulin Secretion Rate and Increases Insulin Clearance in Nondiabetic, Insulin-Resistant Individuals

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Compensatory hyperinsulinemia permitting insulin-resistant individuals to maintain normal glucose tolerance is associated with a left shift in the glucose-stimulated insulin secretion rate (GS-ISR) dose-response curve and decrease in the insulin metabolic clearance rate (I-MCR). To see whether these changes would reverse with improvement in insulin sensitivity, 14 nondiabetic insulin-resistant subjects received rosiglitazone for 12 weeks (4 mg daily for 4 weeks and then 8 mg daily for 8 weeks). Insulin-mediated glucose uptake was quantified by measuring the steady-state plasma glucose concentration during the insulin suppression test. GS-ISR and I-MCR were determined during a 240-min graded intravenous glucose infusion. I-MCR was also calculated during the insulin suppression test. After rosiglitazone treatment, insulin sensitivity improved with significant fall in steady-state plasma glucose (means ± SE from 13.5 ± 0.62 to 9.8 ± 1.02 mmol/l, \( P < 0.001 \)). In response, the integrated GS-ISR decreased by 21% (\( P < 0.001 \)), with a right shift in the dose-response curve. Calculated I-MCR increased by 34% (\( P = 0.008 \)) during the insulin suppression test and by 21% (\( P = 0.03 \)) during the graded glucose infusion. In conclusion, enhanced insulin sensitivity in rosiglitazone-treated nondiabetic insulin-resistant individuals was associated with a shift to the right in the GS-ISR dose-response curve and an increase in I-MCR.

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In insulin-resistant individuals, glucose homeostasis can be maintained if the defect in insulin action is adequately compensated for by an increase in insulin concentration. This is achieved by a shift to the left of the glucose-stimulated insulin secretion rate (GS-ISR) dose-response curve, as well as by a decrease in the insulin metabolic clearance rate (I-MCR) (1,2). Therefore, for any given plasma glucose concentration, there is more insulin secreted by pancreatic β-cells and less insulin cleared in insulin-resistant individuals when compared with their insulin-sensitive counterparts. Although the ensuing hyperinsulinemia is capable of preventing gross decompensation of glucose tolerance, it increases the likelihood that insulin-resistant/hyperinsulinemic individuals will develop a variety of clinical syndromes including cardiovascular disease, essential hypertension, polycystic ovary syndrome, nonalcoholic fatty liver disease, and certain forms of cancer (3–9).

Studies in nondiabetic, insulin-resistant obese individuals have demonstrated that GS-ISR and the I-MCR can return toward normal when insulin sensitivity improves with weight loss (10–12). However, because obesity may be independently associated with an increase in insulin secretion (13) and a decrease in insulin clearance (14), the impact of improving insulin sensitivity apart from weight loss is unknown. The current study was initiated to understand whether the changes in GS-ISR and I-MCR, already noted when weight loss occurs in overweight insulin-resistant subjects, would also occur when enhanced insulin sensitivity was produced by pharmacological means. To accomplish this goal, we evaluated the effects on both GS-ISR and I-MCR of treating 14 insulin-resistant, nondiabetic volunteers with rosiglitazone, a compound known to improve insulin sensitivity (15).

RESEARCH DESIGN AND METHODS

Participants consisted of 11 women and 3 men from the San Francisco Bay area who volunteered for this study in response to a newspaper advertisement. They were nondiabetic (16), predominantly overweight, and insulin resistant as defined by the insulin suppression test described below. More than 85% of the individuals were white with a mean ± SD age of 54 ± 10 years and BMI of 29.3 ± 4.3 kg/m². Participants were in good general health, with a normal history and physical examination. They were not taking any medications known to affect carbohydrate or lipid metabolism and had normal
erythrocyte count and chemical laboratory screening. All gave informed written consent for the study that was approved by the institutional review board at Stanford University.

All study participants underwent three separate procedures at baseline and after 12 weeks of rosiglitazone treatment. These included the insulin suppression test, meal tolerance test, and the graded glucose infusion test, all of which are described below.

**Insulin suppression test.** Resistance to insulin-mediated glucose uptake was estimated by a modification (17) of the original insulin suppression test (18), which has a high correlation (r >0.9) with the euglycemic clamp technique, independent of glycemic status and adiposity (19). After an overnight fast, an intravenous catheter was placed in each of the subject’s arms. One arm was used for the administration of a 150-min infusion of octreotide (0.27 μg · m⁻² · min⁻¹), insulin (32 mU · m⁻² · min⁻¹), and glucose (207 mg · m⁻² · min⁻¹); the other arm was used for collecting blood samples. Blood was drawn at 10-min intervals from 150 to 180 min of the infusion to determine the steady-state plasma insulin (SSPG) and insulin concentrations.

Because steady-state plasma insulin concentrations are similar in all subjects, the SSPG concentration provides a direct measure of the ability of insulin to mediate glucose uptake by adipose (fat) and muscle tissue. At the end of each infusion period, subjects received graded intravenous infusions of glucose at progressively increasing rates (1, 2, 3, 4, 6, and 8 mg · kg⁻¹ · min⁻¹) during the meal tolerance test (20). To evaluate the basic metabolic effects of rosiglitazone treatment, baseline measurements were made of plasma glucose, insulin, and free fatty acid (FFA) concentrations at hourly intervals for 5 h in response to breakfast and lunch given at 8 A.M. and 12 P.M., respectively. Breakfast comprised 20% and lunch 40% of the individual’s daily caloric requirements. The macronutrient content of each meal (as percentage of total calories) consisted of 43% carbohydrate, 42% fat, and 15% protein.

**Graded glucose infusion test.** To determine baseline values for GS-ISR and I-MCR, subjects received graded intravenous infusions of glucose at progressively increasing rates (1, 2, 3, 4, 6, and 8 mg · kg⁻¹ · min⁻¹) as previously described (1,14). Each glucose infusion rate was administered for a total of 40 min. Glucose, insulin, and C-peptide concentrations were measured at fasting and then 10, 20, 30, and 40 min into each glucose infusion period. The last two values at the end of each infusion period were averaged and used as the mean for that infusion.

Intravenous insulin secretion rates (ISRs) were derived by deconvolution of peripheral plasma C-peptide concentrations, using a two-compartment model of C-peptide kinetics and standard parameters for C-peptide clearance estimated for each subject based on body surface area and age (21). For each subject, the mean ISR before and during the six glucose infusion periods was plotted against the corresponding mean glucose to construct a dose-response relationship. The best-fit line (using Systat Table Curve 2D version 5.0; Systat Software, Richmond, CA) was then drawn through the data to allow comparison of ISRs at the same glucose level. The ISR at molar increments of plasma glucose from 5 to 9 mmol/l was therefore obtained by interpolation.

Metabolic clearance rate for insulin (I-MCR) was calculated in two ways as previously described (1). First, insulin clearance was estimated from the steady-state conditions of the insulin suppression test by dividing the insulin infusion rate by steady-state plasma insulin concentration. This calculation yields an estimate of exogenous insulin clearance. Second, I-MCR for endogenous insulin was estimated by calculating the ratio of the total production of insulin to the area under the peripheral insulin curve during the graded glucose infusion. Both calculations of I-MCR were adjusted for body surface area.

After these baseline measurements, subjects were treated with rosiglitazone for 12 weeks (4 mg orally per day × 4 weeks, then twice daily × 8 weeks). Subjects were seen every 2 weeks during the treatment period, at which time they were weighed, asked about their tolerance, reminded to maintain a consistent diet and activity level, and encouraged to maintain compliance with the medication. All 14 subjects completed the 3-month rosiglitazone treatment period without incident, and liver function tests remained within the normal range. At the end of the treatment period, they were reappotted to the General Clinical Research Center, and all of the baseline measurements were repeated.

Statistical analyses were performed using SPSS (version 12 for Windows; SPSS, Chicago, IL). Data are expressed as means ± SE. In most instances, statistically significant differences after rosiglitazone treatment were assessed by Student’s paired, two-tailed t-test. To evaluate the effect of rosiglitazone treatment on measurements obtained during the meal tolerance test and graded glucose infusion, the total integrated area under the curve (AUC) was calculated using the trapezoidal rule. Differences in GS-ISR at molar increments of glucose were assessed by two-way repeated-measure ANOVA.

### RESULTS

Despite a modest weight gain of 1.6 ± 0.59 kg, the results in Table 1 show that SSPG concentration fell significantly (P < 0.001) after rosiglitazone treatment (from 13.5 ± 0.62 to 9.8 ± 1.02). The improvement in insulin sensitivity (lower SSPG concentration) in response to the administration of rosiglitazone was associated with lower fasting and daylong glucose, insulin, and FFA concentrations (during the meal tolerance test) as quantified in Table 1 and illustrated in Fig. 1. These data indicate that fasting plasma glucose, insulin, and FFA concentrations decreased by 7% (P = 0.004), 30% (P = 0.005), and 28% (P = 0.018), respectively. It is apparent from Fig. 1 that this improvement lasted throughout the day in response to breakfast and lunch, with decreases of greater magnitude (P < 0.001) in the daylong total integrated glucose (9%), insulin (41%), and FFA (41%) responses.

Figure 2 displays the glucose, insulin, and C-peptide responses to progressive increases in the glucose infusion rate during the graded glucose infusion study. These

<p>| TABLE 1 |</p>
<table>
<thead>
<tr>
<th>Summary of changes after 12 weeks of rosiglitazone</th>
<th>Rosiglitazone (n = 14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>29.3 ± 1.1</td>
</tr>
<tr>
<td>Insulin suppression test</td>
<td>13.5 ± 0.62</td>
</tr>
<tr>
<td>SSPG (nmol/l)</td>
<td>5.6 ± 0.18</td>
</tr>
<tr>
<td>Meal tolerance test</td>
<td>152 ± 21</td>
</tr>
<tr>
<td>Fasting insulin (pmol/l)</td>
<td>697 ± 67</td>
</tr>
<tr>
<td>Fasting FFA (µmol/l)</td>
<td>513 ± 1.7</td>
</tr>
<tr>
<td>Glucose AUC (mmol/l, 8 h)</td>
<td>3,318 ± 382</td>
</tr>
<tr>
<td>Insulin AUC (µmol/l, 8 h)</td>
<td>3,594 ± 248</td>
</tr>
<tr>
<td>C-peptide AUC (µmol/l, 4 h)</td>
<td>3,594 ± 248</td>
</tr>
<tr>
<td>Glucose AUC (µmol/l, 4 h)</td>
<td>1,129 ± 226</td>
</tr>
<tr>
<td>Insulin secretion rate AUC (µmol/min, 4 h)</td>
<td>6.4 ± 0.71</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SE (n = 14).
results demonstrate that all curves were shifted to the right after rosiglitazone treatment, reflecting a decrease in each of the measured values at any given glucose infusion rate. The AUC values quantified in Table 1 reveal similar results to the meal tolerance test. The plasma glucose concentration again showed the least change with only a 5% decrease in the integrated response ($P = 0.03$). However, there was a much greater decrease ($P = 0.001$) in the AUC for insulin (35%) and C-peptide (20%).

Figure 3A illustrates the ISR at each glucose infusion rate, which also decreased by 21% ($<0.001$) when the AUCs were compared before and after rosiglitazone (see also Table 1). To compare ISR at the same plasma glucose level, instead of at the same glucose infusion rate, ISR was plotted against molar increments in plasma glucose from 5 to 9 mmol/L (Fig. 3B). Even with this standardization, the GS-ISR remained lower ($P = 0.03$ by ANOVA) and shifted to the right, reflecting a fundamental change in $\beta$-cell responsiveness to glucose.

Because the relative decrease in peripheral insulin concentrations in rosiglitazone-treated individuals was greater than the decrease in GS-ISR, it seemed likely that I-MCR had also increased in response to treatment. This value was calculated in two ways as described in RESEARCH DESIGN AND METHODS, and the results are shown in Fig. 4. Figure 4A displays the I-MCR values based on dividing the insulin infusion rate by the steady-state plasma insulin concentration during the insulin suppression test; the I-MCR was

FIG. 1. Daylong glucose (A), insulin (B), and FFA (C) concentrations during the meal tolerance test before ( ) and after ( ) rosiglitazone treatment. Breakfast was given at 8:00 A.M. and lunch at 12:00 P.M.

FIG. 2. Plasma glucose (A), insulin (B), and C-peptide (C) concentrations during the graded glucose infusion before ( ) and after ( ) rosiglitazone.

FIG. 3. ISR at each glucose infusion rate (A), which also decreased by 21% ($<0.001$) when the AUCs were compared before and after rosiglitazone (see also Table 1). To compare ISR at the same plasma glucose level, instead of at the same glucose infusion rate, ISR was plotted against molar increments in plasma glucose from 5 to 9 mmol/L (Fig. 3B). Even with this standardization, the GS-ISR remained lower ($P = 0.03$ by ANOVA) and shifted to the right, reflecting a fundamental change in $\beta$-cell responsiveness to glucose.
with the decrease in GS-ISR (insulin during the meal tolerance test tended to correlate possible link between changes in the metabolic variables one of these relationships that provided any insight into a changes in GS-ISR and I-MCR after treatment. The only glucose, insulin, and FFAs were significantly related to the whether the rosiglitazone-induced changes in daylong curves when compared with insulin-sensitive individuals (1). Therefore, at any given glucose concentration, insulin-resistant individuals secreted more insulin than insulin-sensitive individuals. Furthermore, there is evidence that the improvement in insulin sensitivity associated with significant weight loss returns GS-ISR toward normal (10–12). The notion that a similar change would also occur when insulin sensitivity improved after thiazolidinedione administration is consistent with results of studies showing that plasma insulin concentrations in response to various glucose challenges decrease in subjects treated with these compounds (22–27). The results of the current study provide direct evidence that this is the case, showing that the GS-ISR dose-response curve shifted to the right in response to enhanced insulin sensitivity after 3 months of rosiglitazone treatment. Therefore, individuals after rosiglitazone treatment secreted less insulin at any given glucose concentration compared with before treatment (Fig. 3B). The similarity of the changes in GS-ISR after weight loss provides evidence that enhanced insulin sensitivity, per se, is responsible for the adaptive pancreatic β-cell response.

To the best of our knowledge, this is the first time that the dose-response relationship between glucose and insulin secretion rate has been shown to dynamically change in response to improvement in insulin sensitivity after rosiglitazone treatment. As mentioned, other studies on this issue have mainly been performed in obese individuals after dramatic weight loss (10–12). Although they showed a normalization of insulin secretion rate, these studies could not separate the contribution from insulin sensitivity versus weight as both simultaneously improved. This issue is important because obesity has been implicated to increase insulin secretion (13,28,29). In addition, methods to estimate GS-ISR were different from the current study and used single glucose injection (10,12) or fixed glucose infusion (11) with subsequent quantification of C-peptide response. Unlike the graded glucose infusion, these techniques do not allow construction of a dose-response curve between glucose and insulin secretion rate at various glucose concentrations.

In addition to changes in GS-ISR, I-MCR also increased when determined during both exogenous insulin infusion (insulin suppression test) and endogenous insulin production (graded glucose infusion). Although I-MCR has been shown to be decreased in insulin-resistant individuals (1), studies have not always differentiated the contribution from insulin resistance versus obesity (30–33), which may independently lower insulin clearance (14). Individuals in this study, if anything, gained weight yet increased insulin clearance, suggesting that changes in insulin resistance can independently impact I-MCR.

Although both exogenous (I-MCR_{ex}) and endogenous (I-MCR_{end}) calculations of I-MCR increased after rosiglitazone treatment, the relative increase from baseline was higher with the first method. This difference may only reflect the fact that I-MCR_{end} was calculated under non–steady-state conditions. During steady-state conditions, the two are closely related as follows: I-MCR_{end} = I-MCR_{ex}/(1 - E_{h}), where E_{h} signifies the hepatic extraction ratio of insulin (34,35). Therefore, I-MCR_{end} which reflects clearance of insulin secreted into the portal circulation, is influenced by extensive first-pass metabolism by the liver before reaching the systemic circulation. In contrast, I-MCR_{ex} represents the clearance of exogenously infused insulin when endogenous secretion is suppressed by octreotide. Therefore, the portal and arterial concentra-

**FIG. 3.** Insulin secretion rate plotted against glucose infusion rate (A) and plasma glucose (B) during the graded-glucose infusion before (●) and after (□) rosiglitazone.

significantly greater after rosiglitazone treatment (0.59 ± 0.05 vs. 0.44 ± 0.02 l·min^{-1}·m^{-2}, P = 0.008). The non–steady-state estimate of I-MCR (Fig. 4B) was derived by calculating the ratio of the total production of insulin to the area under the peripheral insulin curve, and this value was also higher after rosiglitazone treatment (1.36 ± 0.17 vs. 1.12 ± 0.12 l·min^{-1}·m^{-2}, P = 0.03).

Pearson correlation coefficients were calculated to see whether the rosiglitazone-induced changes in daylong glucose, insulin, and FFAs were significantly related to the changes in GS-ISR and I-MCR after treatment. The only one of these relationships that provided any insight into a possible link between changes in the metabolic variables and insulin kinetics was the reduction in the AUC of insulin during the meal tolerance test tended to correlate with the decrease in GS-ISR (r = 0.52, P = 0.057).

**DISCUSSION**

To remain normoglycemic, insulin-resistant individuals need to maintain the degree of hyperinsulinemia required to compensate for the defect in insulin action. Otherwise, type 2 diabetes develops. Although at a cost, most individuals can sustain a level of hyperinsulinemia necessary to continue normal or near-normal glucose tolerance. We have previously shown that nondiabetic insulin-resistant individuals have a left shift in the GS-ISR dose-response curve when compared with insulin-sensitive individuals (1). Therefore, at any given glucose concentration, insulin-resistant individuals secreted more insulin than insulin-sensitive individuals. Furthermore, there is evidence that the improvement in insulin sensitivity associated with...
In this study, Cavaghan et al. (22) evaluated the effects of rosiglitazone in individuals with impaired glucose tolerance. Like our study, insulin sensitivity improved and peripheral insulin concentration decreased in response to oral glucose load. However, absolute GS-ISR did not change. Why the insulin secretion rate did not fall in this study as it did in ours is unclear, but there are three differences in study design that could have contributed to the somewhat discrepant findings. To begin with, Cavaghan et al. (22) selected subjects who met diagnostic criteria for having impaired glucose tolerance, whereas our study population consisted of nondiabetic individuals who demonstrated significant degree of resistance to insulin-mediated glucose disposal. In addition, the thiazolidinedione compounds used in the two studies were not the same, and there may be differences in possible direct actions of the two compounds on β-cell sensitivity to glucose stimulation. Finally, the improvement in insulin sensitivity might differ as a function of the drug used. Because the two studies used different methods to quantify insulin-mediated glucose disposal, it is not possible to directly compare the degree to which insulin sensitivity was enhanced in the two studies. However, there is indirect evidence that the improvement in insulin action may have been greater in the present study. For example, there was a modest but significant reduction in fasting glucose in our study but not in the troglitazone study despite similar baseline fasting glucose levels. Unfortunately, the troglitazone study did not measure FFA concentrations because there may have been a differential decrease here as well. For instance, in a study evaluating the metabolic effects of troglitazone, it was found that only a dosage of 600 mg/day (versus 100, 200, and 400 mg) was able to significantly lower fasting FFA concentrations (23). Cavaghan et al. (22) used a dose of 400 mg/day. Therefore, the differences between rosiglitazone and troglitazone or their relative efficacy may explain the discrepant results between the two studies.

Focusing on the current study, it seems clear that rosiglitazone-induced improvement in insulin sensitivity can decrease the GS-ISR and increase I-MCR, as well as lower daylong glucose, insulin, and FFAs. Given that changes in insulin sensitivity associated with weight loss can also have a similar effect, the common denominator affecting insulin secretion and clearance appears to be insulin sensitivity. Therefore, a number of physiological changes associated with insulin resistance are reversible with improvement in insulin sensitivity, accomplished through pharmacological means as well as diet in nondiabetic, insulin-resistant individuals. Because the prevalence of insulin resistance will likely increase in the midst of the current obesity epidemic (36,37), the future challenge then will be to understand who should receive intervention and how best to intervene.

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