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What is the effect of rosiglitazone treatment on insulin secretory function in insulin-resistant individuals? It depends on how you measure it

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Abstract

The goal of this study was to compare methods used to quantify the effect of rosiglitazone (RSG) on insulin secretory function, particularly estimates based on changes in fasting plasma glucose and insulin concentration vs daylong insulin responses to meals. To do this, we compared these measures of insulin secretion before and 3 months after RSG treatment in insulin-resistant individuals, subdivided into nondiabetic subjects (n = 29) and patients with type 2 diabetes mellitus (2DM) (n = 22). Insulin resistance was quantified by determining the steady-state plasma glucose concentration during the insulin suppression test and insulin secretory function by homeostasis model assessment of β-cell function (HOMA-β) and the total integrated daylong plasma insulin responses to mixed meals (insulin area under the curve). Baseline fasting and daylong plasma glucose concentrations were higher ($P < .001$) in patients with 2DM, associated with lower HOMA-β values ($P < .001$). However, neither fasting nor daylong insulin concentrations after mixed meals differed in the 2 groups. Insulin sensitivity improved ($P < .001$) after RSG administration, with decreases of 31% ± 23% and 21% ± 14% in steady-state plasma glucose concentration in nondiabetic and diabetic subjects, respectively. Although fasting and daylong plasma glucose and insulin concentrations fell ($P < .001$) in both groups of RSG-treated individuals, HOMA-β decreased in nondiabetic subjects and did not change in those with 2DM. In conclusion, RSG administration improved insulin sensitivity in both groups, associated with lower fasting and daylong glucose concentrations. Fasting and daylong insulin concentrations were also lower in both groups of RSG-treated subjects, but the values of HOMA-β indicated either a decrease (nondiabetics) or no change (diabetics) in insulin secretory function. These results suggest that measurements of HOMA-β may not provide a complete view of insulin secretory function, either when comparing diabetic with nondiabetic individuals or when assessing the response to RSG treatment in insulin-resistant individuals.

1. Introduction

In a recent study focused on the durability of glycemic control with monotherapy [1], homeostasis model assessment of β-cell function (HOMA-β) was used to compare the changes in insulin secretion in response to 3 different glucose-lowering drugs.

The HOMA-β provides a surrogate estimate of insulin secretion based on changes in fasting plasma glucose and insulin concentrations [2]. In the case of rosiglitazone (RSG), it was suggested that its therapeutic effect was associated with significant increase in HOMA-β. Because thiazolidinedione compounds have been shown [3,4] to improve insulin sensitivity in both nondiabetic individuals and patients with type 2 diabetes mellitus (2DM), the conclusion that the improvement in glycemic control in RSG-treated patients was associated with an increase in HOMA-β was surprising. Furthermore, this conclusion seemed to be discordant with our finding that, using the graded intravenous glucose infusion technique, RSG-induced improvement in insulin resistance in nondiabetic individuals was associated with a *decrease* in glucose-stimulated insulin secretion [5]. An explanation for the apparent disparity in views as to the effect of RSG on insulin secretory function was not obvious to us; nor was the physiologic implication of the conclusion that insulin secretory function, as estimated by HOMA-β,

Institutional approval: The study was approved by Stanford University Human Subjects Committee; and all subjects gave written informed consent for participation in the study.

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was increased in RSG-treated patients. Consequently, the study to be presented was initiated to pursue this apparent conflict by quantifying the effects of RSG administration in insulin-resistant individuals, both nondiabetic or with 2DM, and comparing estimates of insulin secretory function obtained with HOMA-β to the more direct measure of the daylong insulin concentrations elicited by mixed meals.

2. Methods

The Committee on Human Research at Stanford University approved this study, and subjects gave written informed consent for participation in the study. The study volunteers were identified from the San Francisco Bay Area on the basis of their response to newspaper advertisements describing our interest in the relationship between RSG treatment and changes in insulin metabolism. Volunteers enrolled in this study were in apparently good general health, other than 2DM, without clinical or laboratory evidence of anemia or liver, kidney, or cardiovascular disease.

Patients with 2DM were either hyperglycemic in the absence of treatment with any glucose-lowering agent (n = 8) or receiving pharmacologic treatment (n = 14). The latter group was willing to stop their antihyperglycemic medication for a minimum of 1 month before participating in any experimental measurement. The nondiabetic individuals did not have a history of diabetes and had a fasting plasma glucose concentration of less than 7.0 mmol/L.

Subjects meeting these general criteria were admitted to the General Clinical Research Center (GCRC) of Stanford Medical Center for assessment of their degree of insulin sensitivity as quantified by a modification [6] of the insulin suppression test (IST) introduced and validated by our research group [7,8]. As shown previously, the results provided by the IST are highly correlated (r >0.90) with the hyperinsulinemic-euglycemic clamp technique [8]. The IST was performed after an overnight fast and involved a 180-minute intravenous infusion of octreotide (0.27 μg/[m^2 min]), insulin (32 mU/[m^2 min]), and glucose (267 mg/[m^2 min]) by an indwelling catheter placed in a superficial antecubital vein. Venous blood samples were obtained every 30 minutes for the first 150 minutes, then every 10 minutes during the last 30 minutes of the infusion for measurement of plasma glucose and insulin concentrations [6-8]. The mean value of these 4 measurements was used to calculate the steady-state plasma glucose (SSPG) and steady-state plasma insulin (SSPI) concentrations. Under these experimental circumstances, the SSPI concentrations were comparable in all individuals; and the SSPG concentrations provided a direct measure of insulin-mediated glucose disposal: the higher the SSPG concentration, the more insulin-resistant the individual.

Volunteers whose SSPG concentration was greater than 7.90 mmol/L were classified as being insulin resistant, a cut point that has been associated with an increased risk of developing various age-related diseases in apparently healthy individuals [9,10]. The 51 individuals meeting this criterion were further divided into those whose fasting plasma glucose concentration was less than 7.0 mmol/L (nondiabetic) (n = 29) or at least 7.0 mmol/L (diabetic) (n = 22).

Volunteers meeting these criteria were readmitted to the GCRC after an overnight fast for a daylong meal tolerance test in which plasma glucose and insulin concentrations were determined before and after test breakfast and lunch meals [11]. Each meal had, as percentage of daily calories, 15% protein, 42% carbohydrate, and 43% fat. Breakfast was given at 8:00 AM and contained 20% of the daily caloric intake, and lunch was given at noon and contained 40% of the daily caloric intake. Each subject's daily caloric intake was calculated by the Mifflin-St Jeor formula [12]. Blood was drawn at hourly intervals before and after the test meals from 8:00 AM to 4:00 PM. Glucose and insulin areas under the curve were calculated by the trapezoidal method to evaluate the daylong insulin and glucose responses to the test meals.

Measurements of SSPG concentration were used as the measure of insulin-mediated glucose uptake; and HOMA-β ([20*fasting insulin {in microunits per milliliter}]/[glucose {in millimoles per liter} − 3.5]) and the insulin area under the curve (AUC) were used to assess insulin secretory function.

After collection of the baseline data, participants were treated with RSG 4 mg/d for 4 weeks and then 8 mg/d for 8 weeks. Volunteers were seen every 2 weeks during the 12-week treatment period, monitored for medication compliance, and counseled by certified diabetes educators to maintain the same level of physical activity and dietary intake during the study. After completion of 12 weeks of RSG therapy, participants were readmitted to the GCRC to repeat the insulin suppression and meal tolerance tests.

Table 1
Baseline characteristics of the study groups

Variable	Nondiabetes (n = 29)	2DM (n = 22)	P value
Age (y)	53 ± 8	54 ± 8	.82
White, n (%)	19 (66)	16 (73)	.58
Women, n (%)	18 (62)	6 (27)	.01
Postmenopausal, n (%)	15 (83)	3 (50)	.14[b]
HRT, n (%)	11 (73)	2 (67)	1.0[b]
Body mass index (kg/m^2)	29.5 ± 3.7	29.4 ± 4.5	.98
SSPG (mmol/L)	12.7 ± 2.5	16.2 ± 3.0	<.001
Fasting glucose (mmol/L)	5.7 ± 0.6	8.8 ± 2.1	<.001
Fasting insulin[a] (pmol/L)	144 ± 68 115 (90-181)	176 ± 69 169 (117-219)	.07
HOMA-β[a]	198 ± 115 160 (115-262)	104 ± 55 94 (61-129)	<.001
Glucose AUC (mmol/[L 8 h])	919 ± 117	1428 ± 297	<.001
Insulin AUC[a] (pmol/[L 8 h])	3431 ± 2027 2750 (1908-4434)	3062 ± 1238 2807 (2186-3862)	.46

Data are number (percentage) of subjects or mean ± SD. Fasting insulin, HOMA-β, and insulin AUC were log-transformed for statistical analyses. Proportions were compared by χ^2 test; and means, by Student unpaired t test. HRT indicates postmenopausal women on hormone replacement therapy.

[a] Mean ± SD and median (interquartile range).

[b] Proportions were compared by Fisher exact test.

Data were analyzed using SPSS software, version 16.0 for Windows (SPSS, Chicago, IL). Summary statistics are expressed as number (percentage) of subjects, mean ± SD, or median (interquartile range). Fasting insulin, HOMA-β, and insulin AUC were log-transformed to improve normality for statistical analyses. Baseline characteristics of the 2 groups were compared by χ^2 test, Fisher exact test, or Student unpaired t test. Within each group, the effect of RSG treatment was evaluated by Student paired t test. A P value $\leq .05$ was considered statistically significant.

3. Results

Baseline demographic and metabolic characteristics of the 2 groups are compared in Table 1. There were proportionally more women in the nondiabetic group, but the 2 groups did not significantly differ in terms of proportion of women who were postmenopausal or on hormone replacement therapy. Furthermore, the 2 groups were similar in age, racial background, or adiposity as assessed by body mass index. By selection, patients with 2DM had higher fasting and daylong plasma glucose concentrations and were more insulin resistant as assessed by SSPG concentration. Values of HOMA-β were significantly lower in patients with 2DM, whereas the total daylong insulin response to the mixed meals (insulin AUC) was not significantly different in the 2 experimental groups.

All subjects completed the study, without any adverse events. Both groups gained a modest amount of weight, with the magnitude being somewhat greater in the patients with diabetes (1.7 ± 2.2 kg, $P = .001$) than in the nondiabetic group (1.0 ± 2.9 kg, $P = .07$).

Fig. 1 compares the daylong changes in plasma glucose and insulin concentrations after mixed meals in the 2 experimental groups, before and after treatment with RSG. Fig. 1A demonstrates that although baseline plasma glucose concentrations were not elevated in the nondiabetic group, plasma glucose concentrations were modestly

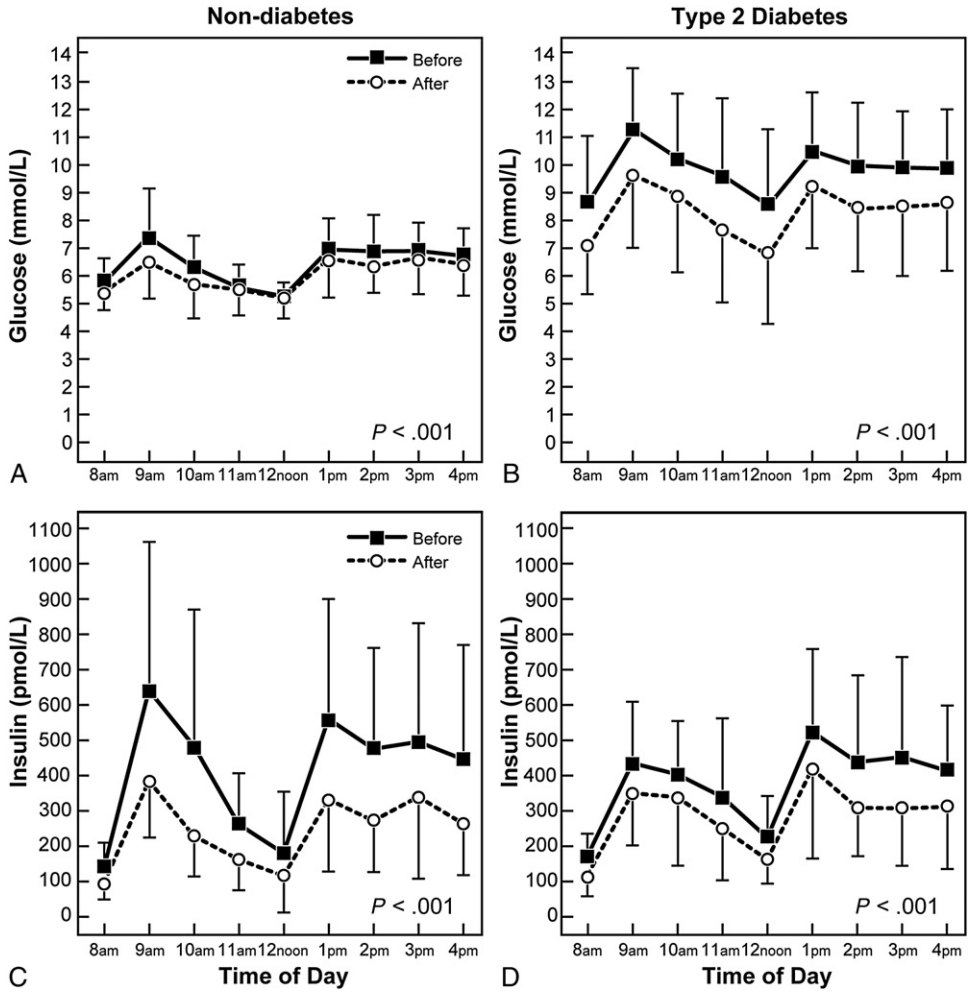

Fig. 1. Mean (SD) daylong plasma glucose and insulin concentrations in nondiabetic individuals (A and C) and in patients with diabetes (B and D) before (closed square) and after (open circle) RSG treatment. Within each group, the effect of treatment was evaluated by comparing baseline and after-treatment AUCs by Student paired t test.

Table 2
Rosiglitazone treatment-associated changes in the measures of insulin secretion and insulin resistance

Variable	Nondiabetes (n = 29)			2DM (n = 22)		
	Before	After	*P* value	Before	After	*P* value
SSPG (mmol/L)	12.7 ± 2.5	8.8 ± 3.5	<.001	16.2 ± 3.0	12.8 ± 4.2	<.001
Fasting glucose (mmol/L)	5.7 ± 0.6	5.3 ± 0.5	<.001	8.8 ± 2.1	7.4 ± 2.0	<.001
Fasting insulin[a] (pmol/L)	144 ± 68	100 ± 41	<.001	176 ± 69	121 ± 51	<.001
	115 (90-181)	90 (68-140)		169 (117-219)	111 (94-141)	
HOMA-β[a]	198 ± 115	165 ± 87	.008	104 ± 55	102 ± 52	.70
	160 (115-262)	139 (92-221)		94 (61-129)	98 (52-136)	
Insulin AUC[a] (pmol/[L 8 h])	3431 ± 2027	2056 ± 925	<.001	3062 ± 1238	2378 ± 1010	<.001
	2750 (1908-4434)	1718 (1300-2803)		2807 (2186-3862)	2195 (1701-2864)	
Glucose AUC (mmol/[L 8 h])	919 ± 117	852 ± 104	<.001	1428 ± 297	1205 ± 326	<.001

Data are mean ± SD. Fasting insulin, HOMA-β, and insulin AUC were log-transformed for statistical analyses. Within each group, means were compared by Student paired *t* test.

[a] Data are mean ± SD and median (interquartile range).

but significantly ($P < .001$) lower throughout the day after RSG administration in this group (change in glucose AUC, −6.5% ± 7.1%). Not surprisingly, the data in Fig. 1B indicate that the magnitude of improvement in daylong glucose concentrations was accentuated in RSG-treated patients with 2DM (change in glucose AUC, −15.9% ± 9.3%).

Panels C (nondiabetics) and D (diabetics) of Fig. 1 display plasma insulin concentrations throughout the day in response to mixed meals, before and after administration of RSG. Daylong plasma insulin concentrations were significantly lower ($P < .001$) in both study groups after RSG treatment. In contrast to the effect of RSG on daylong plasma glucose concentrations, the decline in insulin AUC was particularly dramatic in the nondiabetic individuals (−35.1% ± 16.7%) as compared with patients with 2DM (−24.6% ± 13.5%).

The actual changes in the experimental variables that resulted from RSG administration to the 2 groups are compared in Table 2. It can be seen that SSPG concentrations decreased significantly ($P < .001$) in both groups after RSG administration, associated with lower fasting plasma glucose and insulin concentrations as well as daylong plasma glucose and insulin concentrations. In contrast, the changes in HOMA-β after RSG treatment varied as a function of experimental group, decreasing in the nondiabetic group (−11.4% ± 26.5%) and not changing in patients with 2DM (1.1% ± 26.9%). Thus, despite a significant decrease in both fasting and daylong plasma insulin concentrations in these subjects, HOMA-β remained unchanged.

4. Discussion

This study was initiated to understand the basis for the conclusion in the A Diabetes Outcome Progression Trial (ADOPT) study [1] that the improvement in glycemic control achieved with RSG, a drug that enhances insulin sensitivity and results in a *decrease* in ambient plasma insulin concentrations, was associated with an *increase* in insulin secretory function as assessed by HOMA-β. Perhaps the best way to begin this discussion is to compare baseline HOMA-β values of the 2 experimental groups. Specifically, mean HOMA-β values were approximately 50% lower in patients with 2DM, consistent with the view that these patients have a marked defect in insulin secretory function [13]. However, fasting plasma insulin concentrations tended ($P = .07$) to be higher in patients with 2DM; and there was no difference between the daylong insulin responses (insulin AUC) in the 2 experimental groups. Thus, despite HOMA-β values indicating markedly impaired insulin secretory function in patients with 2DM, measurements of daylong plasma insulin concentrations after mixed meals demonstrate that the pancreatic β-cells of these individuals are capable of secreting abundant amounts of insulin. It has been argued that despite the similarity in daylong insulin concentrations, patients with 2DM are "relatively" insulin deficient in that their fasting and daylong insulin concentrations should be higher given their degree of hyperglycemia. Indeed, it is precisely this line of reasoning that accounts for why HOMA-β values are so much lower in patients with 2DM. The HOMA-β is derived from a formula based on a putative "appropriate" relationship between fasting plasma insulin and glucose concentrations. Using this formula, if the average plasma insulin concentrations were twice as high (352 vs 176 pmol/L) in patients with 2DM, they would have had a HOMA-β value of 185, comparable to the value of 198 seen in the nondiabetic group. It can be seen from Fig. 1D that plasma insulin concentrations after mixed meals in patients with 2DM before they were treated with RSG reached levels that are approximately twice as high than this hypothetical value of 352 pmol/L, indicating that the ability of their β-cells to secrete substantial amounts of insulin is retained.

Several implications arise from the observation that patients with 2DM retain the capacity to substantially increase insulin secretion in response to mixed meals. At the simplest level, it seems that the physiologic implications of the phrase "a defect in β-cell function" will vary with the experimental method used to determine it. Although patients

with 2DM are deemed to be insulin deficient on the basis of a low value for HOMA-β, they have not lost the ability to secrete substantial amounts of insulin when assessed by insulin AUC. More importantly, it appears that a low HOMA-β value in a patient with 2DM is simply the manifestation of a fasting plasma insulin concentration that an arbitrary formula has deemed to be low in relationship to the coexisting fasting plasma glucose concentration. Indeed, the very nature of the formula for HOMA-β ensures that all patients with fasting hyperglycemia will be considered to have an insulin secretory defect.

Turning now to the question of the effect of RSG treatment, insulin sensitivity improved in both groups. However, there is an obvious disparity between the 2 groups regarding the method of assessing insulin secretory function. Focusing first on insulin AUC, daylong insulin responses to mixed meals were lower in RSG-treated individuals, both nondiabetic and diabetic. In the case of HOMA-β, the value was lower after RSG treatment in nondiabetic persons and was unchanged in those with 2DM. This finding is not consistent with the results of Kahn et al [1], who indicated that HOMA-β significantly increased in RSG-treated patients with 2DM. The clinical characteristics of the study populations in the 2 studies seem reasonably comparable, with almost identical mean values for fasting plasma glucose and insulin concentrations. Perhaps the most likely reason for the apparent difference in findings is that the much greater number (~1500) of patients in the study by Kahn et al [1] provided statistical significance to the relatively modest increase (~15%) in HOMA-β they reported.

To address a more fundamental question, it seems imperative to examine the pathophysiologic implications concerning the effect on RSG treatment that evolve with each of the methods used to assess insulin secretory function. Insulin sensitivity improved in association with RSG treatment in both groups, associated with lower daylong plasma glucose and insulin concentrations. The simplest physiologic conclusion for these findings is that RSG-induced insulin sensitivity was responsible for the improvement in level of glycemia, decreasing the degree of compensatory hyperinsulinemia seen before treatment. Thus, the lower values of insulin AUC are a reflection of the improvement in insulin sensitivity. A lower value for HOMA-β, as seen in RSG-treated, nondiabetic individuals, is usually interpreted to reflect an insulin secretory defect, an unlikely event to be associated with the improved insulin sensitivity and lower daylong glucose concentrations seen in these individuals.

Given the fact that daylong insulin concentrations in response to meals were significantly lower in RSG-treated persons with 2DM, it seems paradoxical that their HOMA-β values were essentially identical before and after treatment. The fact that HOMA-β did not change in RSG-treated patients, whereas it did in the nondiabetic individuals, is because HOMA-β does not provide an actual measure of insulin secretion; its values are simply the reflection of an a priori view of how high the fasting plasma insulin concentration *should be* at a given fasting plasma glucose concentration. Thus, the treatment-associated decline in fasting plasma insulin concentration was comparable in the 2 groups (~30%); but the fall in fasting plasma glucose concentration was approximately twice as great in the patients with 2DM. These differences, and the nature of the formula for HOMA-β, explain why in one instance (nondiabetic individuals), it appeared as if insulin secretory function had deteriorated (lower values for HOMA-β) in response to RSG-induced improved insulin sensitivity and glycemia, whereas in the case of those with 2DM, insulin secretory function was unchanged.

In conclusion, the results of this study confirm previous publications showing that administration of thiazolidinedione compounds to insulin-resistant individuals leads to lower plasma glucose and insulin concentrations, in association with improved insulin sensitivity [3,4]. Regarding the goals of this study, measurement of fasting and daylong insulin responses (insulin AUC) to mixed meals provides a different view of insulin secretory function in RSG-treated individuals, both diabetic and nondiabetic, than does calculation of HOMA-β. Obviously, the data we have presented in this manuscript can only serve to point out that the physiologic interpretation of how RSG affects insulin secretory function will differ dramatically as a function of which one of the 2 methods we have discussed is used. To extend these observations, it is necessary to incorporate additional methods of measuring insulin secretory function. In this context, we have just initiated a study in which we plan to compare insulin secretory function with 4 different methods: HOMA-β; daylong insulin responses to meals; hyperglycemic clamp [14]; and the glucose infusion method introduced by Polonsky and associates [15]. We believe that the results of these new studies will provide important information as how best to assess the impact of various interventions on insulin secretory function.

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