The Effect of Rosiglitazone on Urine Albumin Excretion in Patients With Type 2 Diabetes Mellitus and Hypertension

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**Background:** Thiazolidinediones are antidiabetic agents that improve insulin sensitivity (IS). Accumulating data indicate that these agents provide beneficial effects beyond glycemic control, such as improvement in vascular function. The aim of this study was to determine the effect of rosiglitazone on urine albumin excretion (UAE) in patients with type 2 diabetes mellitus (DM) and hypertension.

**Methods:** The study involved 20 subjects with type 2 DM who were already on 15 mg glibenclamide daily but were achieving poor glycemic control and who had either poorly controlled or newly diagnosed hypertension. In these patients, rosiglitazone (4 mg daily) was added to the existing therapeutic regimen for 26 weeks. At baseline and the end of the treatment, subjects gave a 24-h urine collection for direct measurement of albumin and a spot specimen for determination of the albumin-to-creatinine ratio (ACR). Subjects also had a hyperinsulinemic euglycemic clamp and an ambulatory blood pressure (BP) monitoring.

**Results:** At the end of the study, UAE was significantly reduced versus baseline, as measured either directly in the 24-h collection (22.4 ± 4.6 v 13.8 ± 3.0 mg/day, P < .05) or with ACR (20.9 ± 3.8 v 14.0 ± 2.8 mg/g, P < .05). The percentage changes in UAE (ΔALB for the 24-h collection and ΔACR for ACR) correlated with the respective changes in IS (r = −0.64, P < .01 for ΔALB and r = −0.48, P < .05 for ΔACR), systolic BP (r = 0.63, P < .01 and r = 0.58, P < .01 respectively), and diastolic BP (r = 0.56, P < .05 and r = 0.50, P < .05 respectively).

**Conclusions:** In this study, treatment of type 2 diabetic hypertensive patients with rosiglitazone significantly decreased UAE. Lowering of BP and improvement of IS should play roles in this UAE reduction. Am J Hypertens 2005;18:227–234 © 2005 American Journal of Hypertension, Ltd.

**Key Words:** Rosiglitazone, urinary albumin excretion, electrolytes, type 2 diabetes mellitus, hypertension.
nephropathy. The prevalence and the severity of microalbuminuria increase with the duration and the severity of type 2 DM and hypertension. The great majority of individuals with type 2 DM with microalbuminuria are also hypertensive; thus, from a practical point of view, the patient with type 2 DM, nephropathy, and hypertension represents a unified problem. Apart from predicting diabetic renal disease progression, microalbuminuria is a prognostic marker of CVD and mortality in both diabetic and nondiabetic individuals; therefore, its presence is a sign for aggressive intervention to reduce all cardiovascular risk factors.

Thiazolidinediones (TZD) constitute a new class of agents for the treatment of type 2 DM that improve insulin action and thus reduce hyperglycemia. The TZD decrease IR through activation of the peroxisome proliferator–activated receptor gamma, which is involved in the regulation of genes controlling glucose and lipid metabolism.

Accumulating data indicate that TZD possess vasculoprotective properties, such as control of vascular cell proliferation and migration after injury, decrease of the plasma in C-reactive protein levels and PAI-1 activity, decrease of the intima-media thickness of the carotid arteries, restoration of blunted endothelium-mediated vasodilation in insulin-resistant states, and others.

With regard to microalbuminuria, before this study was designed there were published data indicating a favorable effect of TZD from studies in animal models and in patients with type 2 DM and microalbuminuria, but mostly not hypertension, without parallel reliable measurements of IR or blood pressure (BP). Therefore, the primary aim of this study was to determine the effect of the TZD rosiglitazone on urine albumin excretion in relation to IR and BP changes in patients with type 2 DM and hypertension. In addition, this study aimed to evaluate the effect of rosiglitazone on renal function and serum electrolytes.

Methods
Study Population
The present study was conducted in the same diabetic hypertensive patients and with the same protocol as a study aiming to evaluate the effects of rosiglitazone on BP, which have been published elsewhere. At baseline, 24 subjects (12 men and 12 women) were evaluated. All had type 2 DM and were already on treatment with 15 mg of glibenclamide daily but with poor glycemic control. Among them, 12 had a previous diagnosis of essential hypertension and were receiving antihypertensive treatment but were not having their BP controlled. The rest had newly detected hypertension and were not taking antihypertensive medication. The patients volunteered for the study after receiving information about it. Two of them subsequently refused to undergo the second clamp test and one could not undergo it because of difficulties in intravenous access. One subject refused the second ambulatory blood pressure monitoring (ABPM). Therefore, the final study group included 20 subjects with complete data sets.

The study was approved by the review board of the Division of Medicine of Aristotle University of Thessaloniki, and participants provided informed consent before enrollment.

Study Protocol
To establish either the inadequate control of a previously diagnosed hypertension, or the recent diagnosis of mild hypertension, subjects were initially evaluated at the Hypertension Outpatient Clinic at three separate visits with use of the threshold of 140/90 mm Hg. On the initial visit participants had a screening physical examination and laboratory tests to exclude subjects with congestive heart failure, coronary artery disease, renal failure, liver disease, or history of malignancy.

Participants were admitted to our clinical research laboratory where at 07:00 (day 1) without morning medication and after 12-h fast blood samples were drawn for fasting plasma glucose and insulin, glycated hemoglobin (HbA1c), renal function tests, serum electrolytes and routine laboratory parameters. On this day subjects had the 24-h urine collection and the ABPM, as described below. On the next morning (day 2), subjects came back to the laboratory to have their insulin sensitivity determined with the clamp technique. On day 3, subjects gave a random first-voided urine specimen to determine the albumin-to-creatinine ratio (ACR).

After these tests, rosiglitazone (4 mg once daily) was added. Absolutely no change was made in the pre-existing sulphonylurea or antihypertensive treatment throughout the study. Subjects were strictly advised to keep their physical activity and dietary habits unchanged during treatment. All subjects were evaluated every 2 months with a physical examination and routine laboratory tests. After 26 weeks of treatment they again underwent all of the previously mentioned tests. To avoid any seasonal variation in BP that could influence the results, all subjects were evaluated within a 2-month period in the spring and autumn.

Urinary Albumin Excretion Measurements
Urinary albumin was measured in two ways. The first was the direct measurement of albumin in a 24-h urine collection performed on day 1 with a nefelometric method using the Behring Nefelometer 100 (Dade Behring Inc., Deerfield, IL). In addition, ACR was measured in a random, first morning-voided urine specimen with a DCA 2000 Analyzer (Bayer Corp., Elkhart, IN), in which albumin was measured with an immunoturbidimetric method.

Insulin Sensitivity Measurements
Insulin sensitivity (IS) was estimated using the hyperinsulinemic euglycemic clamp technique, as described elsewhere. Briefly, two intravenous lines were placed, one into a hand or wrist vein by retrograde cannulation for blood sampling and the other into an antecubital vein for...
insulin and glucose infusion. The cannulated hand remained heated at 65°C throughout the study. After a 10-min priming infusion, insulin infusion was held constant at 0.6 mmol · m⁻² · min⁻¹ for 110 min. Glucose concentration was clamped at the euglycemic level through a variable infusion of a 20% dextrose solution. The average value of the glucose infusion rate during the final 40 min of the study (M-value) represented IS and was further normalized with body weight (Mbw). The mean of three insulin measurements at 80, 100, and 120 min represented steady-state insulin, which was used to standardize M-values for each subject.

We also measured IR with the Homeostasis Model assessment (HOMA) method, according to the following model:

\[ \text{HOMA-IR} = \frac{(\text{fasting glucose} \cdot \text{fasting insulin})}{22.5} \]

A mean HOMA-IR value was calculated from day 1 and day 2 values for each subject.

### Ambulatory BP Monitoring

Ambulatory BP was monitored with a SpaceLabs 90207 device (SpaceLabs, Redmond, WA) on day 1 at baseline and at the end of the study. After blood sampling, subjects took their morning antihypertensive medication (if any), and ABPM started at 8:00. The ABPM was assessed for 24 h, using the first hour to enable patients become comfortable with the equipment and excluding its data from the analysis. The monitor recorded BP three times per hour between 08:00 and 24:00 and hourly between 24:00 and 08:00. The subjects took their full antihypertensive medication (if any) and sulphonylurea as usual during the ABPM.

### Biochemical Analyses

All biochemical parameters were measured with a Roche/Hitachi 912 automatic analyzer (Roche Diagnostics, Basel, Switzerland). Glucose measurements during the clamp were performed with a HemoCue B-Glucose analyzer (HemoCue AB, Ängelholm, Sweden), and deuterated analysis was made with the Roche/Hitachi 912 analyzer. Measurements of HbA₁c were made by high-performance liquid chromatography (Menarini Diagnostics, Florence, Italy). Plasma insulin concentration was determined by radioimmunoassay (Diasorin, Saluggia, Italy). Fasting plasma glucose and insulin values for each subject represent the mean of two fasting morning values on day 1 and day 2 of the evaluation.

### Statistical Analysis

Statistical power was determined using a change in IS with the clamp and a change in BP as primary end-points. Analysis was performed using the Statistical Package for Social Sciences (SPSS), version 11 (SPSS Inc., Chicago, IL). Baseline demographic data are expressed as mean ± SD and all remaining data as mean ± SE. For the comparison between baseline and the end of the study, the Student t test for paired variables and Wilcoxon signed ranks test were used where appropriate, depending on normality of distribution. Bivariate correlation coefficients (r) were calculated using the Pearson product formula. A P value < .05 (two-tailed) was considered to be statistically significant.

### Results

Background demographic data for all subjects are shown in Table 1. As described elsewhere in detail, at the end of the study IS as measured with the clamp was significantly increased (eg, mean Mbw/I from 33.9 ± 2.6 to 41.9 ± 3.2 μmol · min⁻¹ · kg⁻¹, P < .001) and HOMA-IR index decreased (from 6.34 ± 0.39 to 4.40 ± 0.33, P < .001) compared with baseline. There were also reductions in fasting glucose (169.1 ± 7.3 v 135.8 ± 5.6 mg/dL, P < .001) and HbA₁c levels (8.15 ± 0.24 v 7.24 ± 0.19%, P < .001). Ambulatory BP showed small but significant reductions both in the total population (135.3 ± 1.8 v 129.9 ± 1.7 mm Hg, P < .001 for systolic BP [SBP] and 76.0 ± 1.6 v 71.9 ± 1.6 mm Hg, P < .001 for diastolic BP [DBP]) and in subgroups (Table 2).

At the end of the treatment, rosiglitazone was associated with significant reductions in UAE, regardless of the method used to measure it. Albumin in the 24-h urine collection was decreased from 22.4 ± 4.6 to 13.8 ± 3.0 mg/day (P < .05) and ACR in the spot specimen from

### Table 1. Background characteristics of the study subjects

<table>
<thead>
<tr>
<th></th>
<th>All subjects</th>
<th>Men</th>
<th>Women</th>
<th>Subjects without antihypertensive treatment</th>
<th>Subjects with antihypertensive treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>20</td>
<td>9</td>
<td>11</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Age (y)</td>
<td>63.8 ± 6.4</td>
<td>64.7 ± 7.0</td>
<td>63.1 ± 6.0</td>
<td>62.8 ± 5.6</td>
<td>64.9 ± 7.2</td>
</tr>
<tr>
<td>Duration of DM (y)</td>
<td>9.8 ± 6.4</td>
<td>13.0 ± 7.0</td>
<td>7.3 ± 4.7</td>
<td>11.0 ± 7.7</td>
<td>8.7 ± 5.1</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>74.7 ± 9.1</td>
<td>77.6 ± 9.1</td>
<td>72.4 ± 8.8</td>
<td>72.5 ± 6.4</td>
<td>76.9 ± 11.1</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.4 ± 2.9</td>
<td>25.7 ± 1.8</td>
<td>28.8 ± 3.0</td>
<td>26.6 ± 2.3</td>
<td>28.2 ± 3.3</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>0.93 ± 0.07</td>
<td>0.95 ± 0.06</td>
<td>0.91 ± 0.08</td>
<td>0.94 ± 0.07</td>
<td>0.92 ± 0.08</td>
</tr>
</tbody>
</table>

**BMI** = body mass index; DM = diabetes mellitus.

Data are expressed as means ± SD.
Twenty-four-hour urine albumin excretion (UAE), albumin-to-creatinine ratio (ACR), and ambulatory blood pressure levels before and after rosiglitazone treatment in the study subgroups.

### Table 2

<table>
<thead>
<tr>
<th>Subjects without antihypertensive treatment (n = 10)</th>
<th>Subjects with antihypertensive treatment (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Men</strong> (n = 9)</td>
<td><strong>Women</strong> (n = 11)</td>
</tr>
<tr>
<td><strong>Baseline</strong></td>
<td><strong>Week 26</strong></td>
</tr>
<tr>
<td>24-Hour UAE (mg/day)</td>
<td>25.5 ± 7.1</td>
</tr>
<tr>
<td>ACR (mg/g)</td>
<td>3.7 ± 1.0</td>
</tr>
<tr>
<td>Ambulatory SBP*</td>
<td>139.0 ± 2.1</td>
</tr>
<tr>
<td>Ambulatory DBP*</td>
<td>77.9 ± 1.7</td>
</tr>
</tbody>
</table>

*Ambulatory blood pressure data from Sarafidis PA, et al, with permission.

### Table 3

<table>
<thead>
<tr>
<th>Data are expressed as means ± SE.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ACR (mg/g)</strong></td>
</tr>
<tr>
<td><strong>Ambulatory SBP</strong></td>
</tr>
<tr>
<td><strong>Ambulatory DBP</strong></td>
</tr>
</tbody>
</table>

### Figure 1

**FIG. 1** Twenty-four-hour urine albumin excretion (UAE) and albumin-to-creatinine ratio (ACR) before and after rosiglitazone treatment in the total study group. Data are means ± SE.

20.9 ± 3.8 to 14.0 ± 2.8 mg/g (P < .05) (Fig. 1). After dividing whole group into subgroups the downward trend persisted; however the reduction was significant only for men and for patients without antihypertensive treatment (Table 2). At baseline, eight of 20 subjects had UAE > 30 mg/day in the 24-h collection, whereas at the end of the study only three subjects had these values. With the use of ACR, at baseline five subjects were classified as having microalbuminuria, and at week 26 only two subjects. Albumin measurement in the 24-h collection showed strong correlations with ACR (r = 0.86, P < .001 for baseline and r = 0.91, P < .001 at final evaluation).

As shown in Fig. 2, the percentage changes in UAE (ΔALB for the 24-h collection and ΔACR for ACR) were inversely correlated with the respective changes in IS represented by the M_ho/I index (r = -0.48, P < .05 for ΔALB and r = -0.45, P < .05 for ΔACR) and positively correlated with the change in HOMA-IR (r = 0.64, P < .01 and r = 0.51, P < .05, respectively). Moreover, they were also correlated with the respective changes in mean ambulatory SBP (r = 0.63, P < .01 for ΔALB and r = 0.58, P < .01 for ΔACR) and DBP (r = 0.56, P < .05 and r = 0.50, P < .05 respectively). No significant correlations between changes in UAE and changes in fasting glucose or HbA1c were observed.

Renal function was not altered during rosiglitazone treatment, inasmuch as no significant changes in creatinine clearance, blood urea nitrogen and serum creatinine were noted. Similarly, no changes in uric acid, serum sodium, potassium, and phosphorus levels were noted. However, serum chloride showed a significant reduction, whereas serum calcium and magnesium showed significant increases (Table 3). At the end of the study, no patient had any of the these parameters outside normal limits.

None of the subjects complained of leg edema or symptoms of heart failure. There were no clinical or laboratory findings of anemia in any of the subjects throughout the study period, and no subject had elevation of any liver function test to above normal or a doubling of baseline values.
Discussion

This study was designed to evaluate the effect of add-on treatment with rosiglitazone on UAE in patients with type 2 DM and hypertension and to investigate whether the possible changes in UAE would correlate with changes in IS and BP over time. Another aim was to elucidate whether rosiglitazone treatment would influence renal function and serum electrolytes. The main finding of our study is that rosiglitazone treatment was associated with a significant UAE reduction, regardless of the method used to measure it. This reduction was significantly correlated with an improvement in IS and a decrease in ambulatory SBP and DBP. Renal function was not affected, but serum chloride decreased and serum calcium and magnesium slightly increased.

Although this pilot study is the first to combine direct UAE measurements in 24-h collections and ACR in spot specimens along with the most reliable methods for BP and IS measuring to increase the validity of the results, it has also several limitations that must be acknowledged. Basically it was a nonrandomized, observational, 6-month intervention study. The number of subjects was low, and the small sample size of the subgroups was probably the reason for not detecting significant UAE differences in the subgroups of women and patients with antihypertensive treatment despite the downward trend. Moreover, the study population consisted of patients with established type 2 DM; thus, the population had a relatively high degree of IR. Therefore, it is not known whether a less
Table 3. Renal function, uric acid, and serum electrolytes before and after rosiglitazone treatment

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Week 26</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine clearance (mL/min)</td>
<td>99.2 ± 6.2</td>
<td>105.1 ± 6.6</td>
<td>.22</td>
</tr>
<tr>
<td>Urea nitrogen (mg/dL)</td>
<td>36.2 ± 2.1</td>
<td>39.9 ± 2.6</td>
<td>.08</td>
</tr>
<tr>
<td>Serum creatinine (mg/dL)</td>
<td>0.88 ± 0.05</td>
<td>0.86 ± 0.05</td>
<td>.66</td>
</tr>
<tr>
<td>Uric acid (mg/dL)</td>
<td>5.05 ± 0.26</td>
<td>5.36 ± 0.28</td>
<td>.09</td>
</tr>
<tr>
<td>Sodium (mEq/L)</td>
<td>139.8 ± 0.9</td>
<td>141.7 ± 0.9</td>
<td>.07</td>
</tr>
<tr>
<td>Potassium (mEq/L)</td>
<td>4.51 ± 0.09</td>
<td>4.52 ± 0.10</td>
<td>.91</td>
</tr>
<tr>
<td>Chloride (mEq/L)</td>
<td>107.7 ± 1.3</td>
<td>103.7 ± 1.0</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>Calcium (mg/dL)</td>
<td>9.59 ± 0.11</td>
<td>10.17 ± 0.14</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>Magnesium (mg/dL)</td>
<td>1.83 ± 0.06</td>
<td>2.03 ± 0.07</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>Phosphorus (mg/dL)</td>
<td>3.35 ± 0.16</td>
<td>3.46 ± 0.09</td>
<td>.52</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SE

insulin-resistant group would respond similarly to rosiglitazone treatment.

In the first study to evaluate the effects of a TZD on UAE in humans, troglitazone reduced ACR by approximately 40% in subjects with type 2 DM and microalbuminuria, in contrast to the effect achieved with metformin. Nakamura et al compared the effect of pioglitazone to glibenclamide and voglibose in normotensive subjects with type 2 DM and microalbuminuria. The three drugs produced similar reductions in HbA1c, but only pioglitazone reduced UAE. In two subsequent studies, troglitazone and pioglitazone were again found to reduce UAE in normotensive subjects with type 2 DM and microalbuminuria.

The effect of rosiglitazone on UAE in individuals with type 2 DM was evaluated in two multicenter studies. In the first study, 493 subjects were randomized to 2 or 4 mg rosiglitazone twice daily or placebo for 6 months. In the 4-mg treatment group, ACR showed a reduction of 21.6% compared with baseline, and in microalbuminuric patients the reduction was even greater. Only about 15% of patients in the rosiglitazone groups received antihypertensive therapy and no data on BP changes were presented. In the second study, the effect of 4 mg rosiglitazone twice daily was compared with the effect of glyburide in 121 subjects, 7.5% of whom were hypertensive. After 28 weeks of treatment, both treatment groups showed a significant reduction in ACR of approximately 30%, but after 52 weeks only the rosiglitazone group continued to demonstrate this reduction. This was the case for patients with baseline microalbuminuria, in whom the reduction was approximately 50%. In the rosiglitazone group, ambulatory DBP showed a reduction of 2.3 mm Hg versus baseline, and at week 52 there was a difference of −3.5/−2.7 mm Hg versus the effect achieved with glyburide.

Although these data indicate a beneficial effect of all TZD on UAE, the mechanisms through which this takes place are not yet clear. Strict glycemic control is associated with a reduction in microalbuminuria in individuals with type 2 DM, and many biochemical mechanisms through which chronic hyperglycemia is connected to glomerular dysfunction have been described, such as enhancement of the polyol pathway, increase in diacylglycerol synthesis via protein kinase C (PKC) activation, and others. Fujii et al reported that troglitazone reduced UAE, possibly through PKC inhibition; but in that study, blood glucose levels were not affected by troglitazone. In human studies, the improvement in glycemic control was similar between the different drug groups, but only TZD reduced UAE. Moreover, in our study as in that by Bakris et al, weak or no correlations between changes in UAE and glycemic parameters were found, indicating that the effect of TZD on UAE is rather not mediated by improvement in glycemic control.

Tight BP control is considered even more important than glycemic control in reducing UAE and protecting against renal disease progression. In the studies mentioned above, BP either was not measured or did not show significant changes in comparison to baseline values. However, in all the latter studies, BP changes were in different directions (ie, a decrease in groups given TZD, an increase in the other groups), only clinic BP was measured, and no comparison for BP differences between the groups was performed. Therefore, the possibility that BP differences have contributed to UAE differences cannot be excluded. In our study, strong and persistent correlations between UAE and ambulatory BP reductions were observed, which was the case in the only study that has recorded ambulatory BP to date. Drugs acting on the renin–angiotensin system were included in our patients’ background antihypertensive treatment but were held completely constant throughout the study; thus they must not have played a role in this UAE reduction. Because all TZD have demonstrated a BP-lowering effect, this should be considered an important mechanism for ameliorating UAE.

In various studies, TZD have been shown to possess direct protective effects on vascular cells, such as inhibiting proliferation and migration of vascular smooth muscle cells, decreasing intima-media thickness of the carotid arteries, and suppressing the loss of anionic sites of glomerular basement membranes. Amelioration of mi-
microalbuminuria with TZD via direct vasculoprotective mechanisms cannot be excluded, although the precise mechanisms are unclear.

Microalbuminuria is related to impaired endothelial function$^{26}$ and IR is also associated with endothelial dysfunction, for example, blunted nitric oxide–mediated vasodilation.$^{27}$ A cause–effect relationship between the latter disturbances is difficult to establish, but TZD has been shown to improve endothelial function in insulin-resistant states.$^{27-29}$ Although IR reduction is the primary action of TZD, no study of UAE until the present one included a reliable IS measurement to calculate correlations between those two parameters. The correlations between changes in IS and UAE in our study might reflect the contribution of IS enhancement to UAE reduction, which may be mediated through improvement in endothelial function.

As in other studies$^{12,14}$ renal function was not affected after rosiglitazone treatment. An increase in serum magnesium levels with pioglitazone that is similar to the increase observed in our study has been reported elsewhere.$^{30}$ However, additional data about the effect of TZD on serum electrolytes are missing, and our findings should be considered as initial observations, the validity and clinical importance of which need to be confirmed.

In conclusion, this study demonstrated a significant reduction in UAE after rosiglitazone treatment in patients with type 2 DM and hypertension that was associated with both IR and BP improvement, suggesting that these effects should contribute to the possible renoprotective effect of rosiglitazone. Large-scale ongoing trials are expected to shed more light on the potential benefits of TZD in regard to microalbuminuria and other cardiovascular complications of type 2 DM.

Acknowledgment

Mrs. Anastasia Hersonidou is acknowledged for her work on albumin-to-creatinine ratio measurements.

References