Rosiglitazone Improves Glomerular Hyperfiltration, Renal Endothelial Dysfunction, and Microalbuminuria of Incipient Diabetic Nephropathy in Patients

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Microalbuminuria, an early feature of diabetic nephropathy, indicates intrarenal endothelial damage. In type 2 diabetes, microalbuminuria is strongly related to insulin resistance. We therefore investigated whether rosiglitazone, an insulin-sensitizing drug that is known to improve endothelial dysfunction, was able to improve intrarenal endothelial dysfunction and microalbuminuria. Nineteen type 2 diabetic patients participated in this double-blind cross-over trial. Nine patients with newly diagnosed disease without microalbuminuria were randomized to a treatment with rosiglitazone or nateglinide, each for 12 weeks. Ten patients with microalbuminuria were randomized to rosiglitazone or placebo, each for 12 weeks in addition to their previous antidiabetic medication. After each treatment, glomerular filtration rate (GFR), renal plasma flow, and filtration fraction were measured before and after blockade of nitric oxide (NO) by intravenous administration of *N*-monomethyl-L-arginine-acetate (L-NMMA). Ten healthy subjects served as control subjects. Type 2 diabetic patients at baseline showed glomerular hyperfiltration compared with healthy control subjects. Rosiglitazone reduced elevated GFR and filtration fraction toward control primarily in patients with microalbuminuria (GFR: 133.4 ± 9.8 vs. 119.6 ± 8.7 ml/min; filtration fraction: 23.2 ± 1.7 vs. 20.5 ± 1.6% before and after rosiglitazone, respectively; control subjects: GFR 111.7 ± 8.6 ml/min, filtration fraction 20.4 ± 1.5%). Rosiglitazone improved intrarenal NO bioavailability in type 2 diabetes toward control as shown by infusion of L-NMMA. Rosiglitazone reduced albumin excretion in type 2 diabetes with microalbuminuria from 116.5 ± 31 to 40.4 ± 12 mg/day. Rosiglitazone ameliorated glomerular hyperfiltration in early type 2 diabetes, improved NO bioavailability, and lessened renal end-organ damage in type 2 diabetes with microalbuminuria. *Diabetes* 54:2206–2211, 2005

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GFR, glomerular filtration rate; L-NMMA, *N*-monomethyl-L-arginine-acetate; MAP, mean arterial pressure; PAH, para-aminohippurate; RPF, renal plasma flow.

Diabetic nephropathy is a worldwide public health concern of increasing proportions. It has become the most common single cause of end-stage renal disease in the U.S. and Europe (1). Previous studies (2,3) found that ACE inhibitors and angiotensin receptor blockers retard diabetic nephropathy. These agents improve endothelial function, change renal glomerular hemodynamics, and reduce microalbuminuria. The degree of microalbuminuria determines the progression of diabetic nephropathy. It may reflect the renal manifestation of a global vascular dysfunction (4). Microalbuminuria is a marker of inflammation and an independent risk factor for cardiovascular mortality (5). It is strongly related to insulin resistance (6).

In a previous study (7), we demonstrated that insulin-sensitizing thiazolidinediones were effective in correcting endothelial dysfunction in the forearm of type 2 diabetic patients. Because of the potential relevance for diabetic nephropathy, we proceeded to the present studies of the kidney. We asked three questions: *1*) Is it possible to show an effect of thiazolidinediones to improve endothelial dysfunction in the kidney? *2*) Are any such effects associated with hemodynamic changes in the kidney? *3*) Do these agents decrease microalbuminuria?

RESEARCH DESIGN AND METHODS

A total of 19 type 2 diabetic patients (4 women and 15 men, mean age 57.5 ± 7.6 years, range 49–70 years) were included into this double-blind, randomized, cross-over study. Nine patients had recently diagnosed disease (two women and seven men, mean age 58.4 ± 6.2 years) with no history or evidence of cardiovascular events, diabetic microvascular complications, or microalbuminuria. Mean time span between diagnosis and enrolment in the study was 4.6 ± 0.6 weeks. None of these patients had previously taken antidiabetic medication. Type 2 diabetes in 10 patients with microalbuminuria (two women and eight men, mean age 56.9 ± 8.3 years) had been known for 6.4 ± 2.3 years. Microalbuminuria was defined as urinary albumin excretion between 30 and 300 mg/day (1). These 10 patients had no evidence of cardiovascular events. Patients with microalbuminuria took sulfonylurea alone ($n = 4$), metformin alone ($n = 2$), or a combination of these drugs ($n = 4$). Concomitant disorders of diabetic patients included hypertension ($n = 15$) and hypercholesterolemia ($n = 13$). Hypertension was treated with one or a combination of the following drugs: ACE inhibitors ($n = 6$), angiotensin receptor blockers ($n = 5$), calcium channel blockers ($n = 5$), β-receptor blockers ($n = 4$), and diuretics ($n = 4$). Any concomitant medication was maintained unchanged throughout the study. The control group consisted of ten matched healthy volunteers (two women and eight men, mean age 55.9 ± 8.4 years, range 49–72 years) with normal glucose tolerance and not taking any medication.

The study protocol was approved by the ethics committee of our institution. All participants gave written informed consent before inclusion into the study. The time course of the study protocol is outlined in Fig. 1. All patients had a dietary consultation after the screening examination and were advised

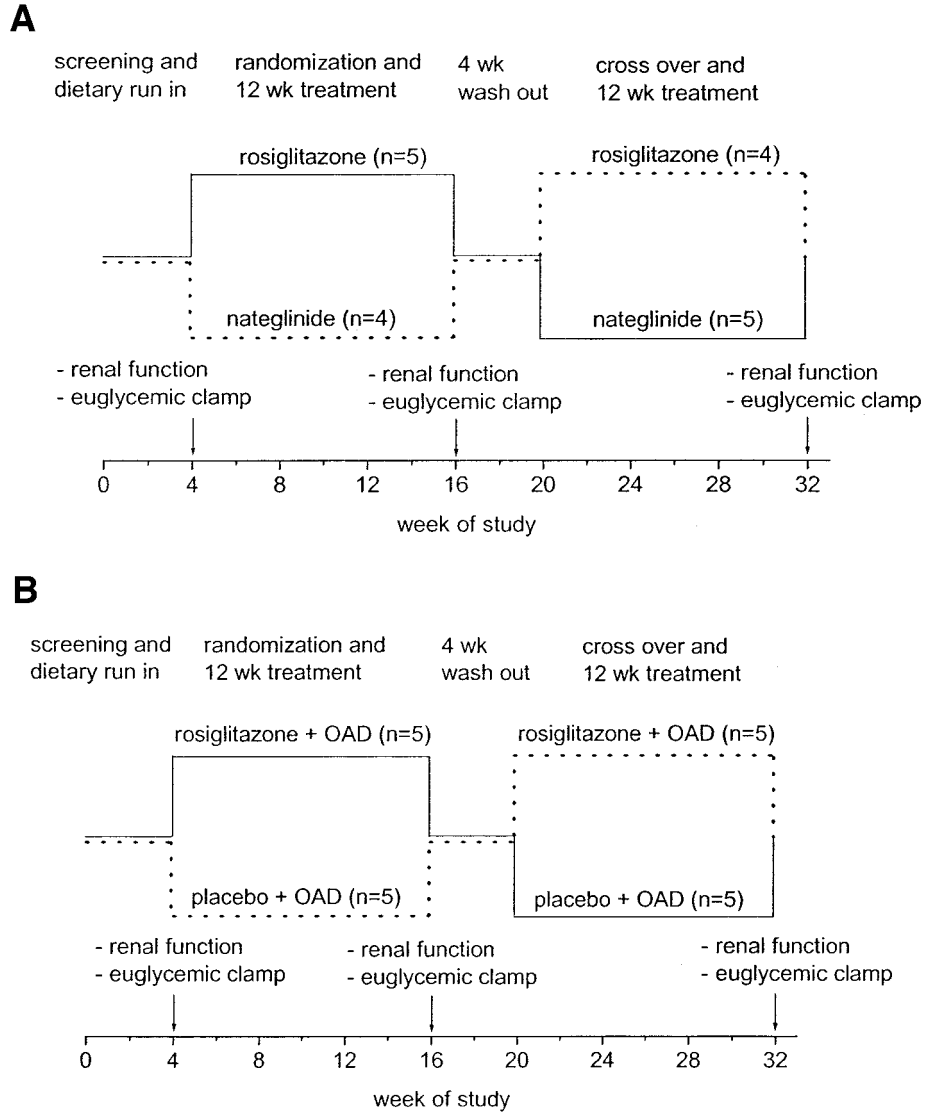

FIG. 1. Flow chart of the study protocol. *A*: Patients without microalbuminuria (n = 9). *B*: Patients with microalbuminuria (n = 10). OAD, previous oral antidiabetic drugs. Renal function: PAH clearance and inulin clearance before and after L-NMMA. OADs of microalbuminuric patients were kept unchanged throughout the study.

to keep their lifestyle habits unchanged throughout the study. Visits for control of compliance and assessment of side effects were conducted every 4 weeks. All measurements were performed once in healthy volunteers.

Tests of renal function. Renal plasma flow (RPF) and glomerular filtration rate (GFR) were determined by constant infusion technique of para-aminohippurate (PAH; Merck, Whitehouse Station, NJ) and inulin (Inutest; Fesenius Kabi, Graz, Austria), respectively, as published by Cole et al. (8). Briefly, after an overnight fast, bolus doses of inulin and PAH were given intravenously, followed by a constant infusion of both agents at a rate calculated to maintain constant blood levels. A steady state between infusion and renal excretion was reached after 150 min of infusion. Blood samples were taken for determination of PAH and inulin, and thereafter *N*-monomethyl-L-arginine-acetate (L-NMMA) (Clinalfa, Läufelingen, Switzerland) was administered intravenously for inhibition of NO synthase at a total dose of 4.25 mg/kg body wt over 30 min, as described by Delles et al. (9). Subsequently, a further blood sample was taken to measure levels of inulin and PAH.

Blood pressure was determined using an automatic device (Dinamap pro 200; Criticon, Tampa, Mexico). Means of three measurements are presented. All participants rested in a supine position and were encouraged to drink water (15 ml/kg) throughout the renal function test. Filtration fraction was calculated as GFR-to-RPF ratio. Renal vascular resistance was calculated as mean arterial pressure (MAP) × (1-hematocrit)/RPF.

Euglycemic-hyperinsulinemic clamp. The euglycemic-hyperinsulinemic clamp technique was applied as described (10). After an overnight fast, blood samples for measurements of baseline plasma glucose and baseline plasma insulin levels were taken. Thereafter, an intravenous insulin infusion was given in descending dosage calculated on the basis of body surface area for 10 min. From the 11th min onwards, insulin was administered at a constant infusion rate of 60 mU/(m^2 min). Twenty percent glucose solution was infused to keep the plasma glucose concentration steady at 5.5 mmol/l (5.3–5.7 mmol/l) over the duration of the clamp procedure. Insulin sensitivity index (M_c) was calculated as the quantity of glucose metabolized divided by the measured plasma insulin concentration, normalized for body weight.

Biochemical analysis. Blood samples for determination of inulin and PAH were centrifuged immediately and stored at −80°C until measurement after completion of the entire study. PAH was measured using the modified Bratton-Marshall reaction (11). Inulin was determined by the anthrone method (12). Each blood sample was measured in triplicate. We used the calculated mean of the three measurements for determination of clearances. Inulin and PAH clearance rates were calculated from serum concentrations and infusion rates.

Cholesterol and triglyceride measurements were done using the CHOD-PAP and GPO-PAP test kit (Roche Diagnostics, Basel, Switzerland). Plasma insulin was measured by enzyme immunosorbent assay (Bio-Source, Fleurus, Belgium). Plasma renin activity was measured by radioimmunoassay (Schering, Berlin, Germany). Blood samples for determination of plasma renin activity were collected from study participants after a resting period of 2.5 h in a supine position. All participants were asked for a 24-h urine collection on the day before each renal function test. We determined urinary protein, albumin, electrolytes, and creatinin.

Statistical analyses. All data are reported as means ± SE. Tests for order and sequence effects of study medication yielded nonsignificant results. Comparisons of continuous variables were performed by two-way ANOVA or Student's t test for paired samples. Correlation was calculated using Pearson's correlation coefficient. Statistical significance was considered at the 5% level. All analyses were performed using SPSS version 11.0 for Windows.

TABLE 1
Baseline clinical parameters and baseline renal function of type 2 diabetic patients and healthy control subjects

	Healthy control subjects	Type 2 diabetic patients
n	10	19
BMI (kg/m^2)	27.9 ± 1.1	29.8 ± 1.3
Body surface area (m^2)	2.00 ± 0.05	1.98 ± 0.05
Systolic blood pressure (mmHg)	135.1 ± 3.6	135.3 ± 3.9
Diastolic blood pressure (mmHg)	81.8 ± 3.3	84.0 ± 3.1
MAP (mmHg)	96.3 ± 3.3	99.4 ± 3.4
A1C (%)	5.3 ± 0.3	6.7 ± 0.7*
Fasting plasma glucose (mmol/l)	4.9 ± 0.4	7.4 ± 0.7*
Insulin sensitivity index (M_c) (mg · kg^{-1} · min^{-1})	6.5 ± 2.9	2.2 ± 1.1*
LDL cholesterol (mmol/l)	3.6 ± 0.3	3.0 ± 0.7
HDL cholesterol (mmol/l)	1.5 ± 0.3	1.3 ± 0.3
Triglycerides (mmol/l)	1.6 ± 0.8	1.8 ± 0.9
GFR (ml/min)	111.7 ± 8.6	126.4 ± 7.8
RPF (ml/min)	552.3 ± 45.3	564.5 ± 21.3
Filtration fraction (%)	20.4 ± 1.5	22.4 ± 1.4
Creatinine (μmol/l)	84.1 ± 3.3	83.8 ± 4.8
Urea (mmol/l)	6.0 ± 0.3	5.4 ± 1.5

Data are means ± SE. *$P < 0.01$.

RESULTS

Baseline clinical characteristics and baseline renal function are shown in Table 1. Type 2 diabetic patients had higher HbA_{1c} (A1C) and fasting plasma glucose levels together with markedly reduced insulin sensitivity compared with healthy control subjects. GFR, RPF, and filtration fraction of patients and healthy subjects were not significantly different; however, there was a tendency toward a higher GFR and filtration fraction in diabetic patients. Treatment with rosiglitazone increased insulin sensitivity in type 2 diabetic patients with and without microalbuminuria (Table 2). There were comparable A1C and fasting plasma glucose values between treatments.

Rosiglitazone improved endothelial NO generation in the kidney (Table 3). In healthy control subjects, the response to systemic L-NMMA consisted of an increase in MAP, a decrease in RPF, and a large increase in filtration fraction. The latter two responses were greatly diminished or absent in diabetic patients without and with microalbuminuria. However, in response to rosiglitazone, much of the normal response was restored in both groups of diabetic subjects. The major observed increase of filtration fraction after rosiglitazone in response to L-NMMA suggests that rosiglitazone restored NO availability in the glomerulus, i.e., it ameliorated renal endothelial function.

A mirror image of these changes was observed in the baseline hemodynamic state in diabetic patients after the 12-week treatment of rosiglitazone (Table 4): the filtration fraction decreased significantly, MAP tended to fall, RPF did not change, and GFR decreased. We found a significant inverse correlation between the changes of filtration fraction induced by L-NMMA and the corresponding changes of filtration fraction induced by rosiglitazone ($r = -0.67$; $P < 0.05$), indicating that improvement of renal endothelial function by rosiglitazone probably contributed to baseline renal hemodynamics.

Treatment with rosiglitazone was followed by 60% reductions of albuminuria and proteinuria in diabetic patients with microalbuminuria (Table 2). In these patients, we found a relation between the degree of reduction in urinary albumin excretion and the degree of decrease of GFR (Fig. 2) by rosigliatzone. The improvement after rosiglitazone occurred in a comparable manner in patients receiving ACE inhibitors or angiotensin receptor blockers as baseline therapy. We did not find significant correlation between changes of microalbuminuria and changes of blood pressure, insulin sensitivity, fasting plasma glucose, or A1C. However, there was an association between filtration fraction and M_c, considering all diabetic patients at baseline ($r = -0.6$; $P < 0.01$).

DISCUSSION

The present studies were undertaken to answer the question whether rosiglitazone would improve intrarenal endothelial function and renal hemodynamics in diabetic

TABLE 2
Clinical characteristics of type 2 diabetic patients with and without microalbuminuria after rosiglitazone or an alternative treatment

	Without microalbuminuria		With microalbuminuria	
	Nateglinide	Rosiglitazone	Placebo + previous oral antidiabetic drug	Rosiglitazone + previous oral antidiabetic drug
A1C (%)	6.3 ± 0.3	6.2 ± 0.3	6.8 ± 0.2	6.6 ± 0.2
Fasting plasma glucose (mmol/l)	6.9 ± 0.6	6.2 ± 0.4	7.9 ± 0.7	7.0 ± 0.7
Insulin sensitivity index (M_c) (mg · kg^{-1} · min^{-1})	2.4 ± 0.4	3.7 ± 0.3*	2.0 ± 0.4	2.9 ± 0.3*
Systolic blood pressure (mmHg)	127.9 ± 4.0	123.0 ± 2.7	140.4 ± 3.7	137.4 ± 3.3
Diastolic blood pressure (mmHg)	77.8 ± 3.4	76.1 ± 4.0	84.6 ± 3.4	79.7 ± 2.4
MAP (mmHg)	94.8 ± 3.2	92.6 ± 3.3	103.0 ± 3.4	99.8 ± 3.1
Urinary albumin excretion (mg/24 h)	5.6 ± 1.4	6.6 ± 1.9	116.5 ± 31.1	40.4 ± 12.3*
Urinary protein excretion (mg/24 h)	106.3 ± 21.0	97.5 ± 20.1	316.57 ± 56.4	106.5 ± 24.0*
Urinary sodium excretion (mmol/24 h)	203.8 ± 19.9	204.7 ± 19.0	200.6 ± 19.1	202.4 ± 17.8
Urinary urea excretion (mmol/24 h)	441.9 ± 46.9	435.1 ± 25.6	438.5 ± 27.8	432.3 ± 29.1
Plasma renin (pg/ml)	20.8 ± 7.3	23.4 ± 6.3	45.6 ± 15.4	47.0 ± 15.1
C-reactive protein (mg/l)	2.4 ± 1.1	1.5 ± 0.8	2.4 ± 0.3	1.8 ± 0.9
Creatinin (μmol/l)	82.4 ± 5.5	84.7 ± 6.7	85.3 ± 5.0	85.7 ± 4.9

Data are means ± SE. *$P < 0.01$ vs. nateglinide or placebo.

TABLE 3
Effects of L-NMMA on renal function and hemodynamic parameters (expressed as percentage change of each parameter)

		Without microalbuminuria		With microalbuminuria	
	Control subjects	Nateglinide	Rosigliatzone	Placebo + oral antidiabetic drug	Rosiglitazone + oral antidiabetic drug
GFR	3.8 ± 5.9	−4.7 ± 5.4	2.1 ± 4.4	−3.6 ± 3.4	−2.3 ± 3.1
RPF	−10.9 ± 2.5*	−4.1 ± 3.4*	−13.1 ± 4.3*	−2.8 ± 6.6	−9.6 ± 3.1*
Filtration fraction	17.4 ± 4.2*	−1.1 ± 2.5†	16.5 ± 5.9*	−0.8 ± 3.1†	11.9 ± 3.8*
MAP	9.9 ± 4.7*	4.6 ± 1.8*	7.6 ± 1.6*	4.8 ± 1.9*	6.5 ± 2.1*
Renal vascular resistance	21.0 ± 4.3*	16.5 ± 3.9*	26.9 ± 5.7*	14.7 ± 3.1*	20.4 ± 3.2*

Data are means ± SE. *Indicates a significant change ($P < 0.01$) of the hemodynamic parameter after L-NMMA administration compared with the corresponding baseline parameter. $P < 0.02$ vs. rosiglitazone.

patients with endothelial dysfunction and whether any such improvement would result in a lessening of urinary albumin excretion. To the best of our knowledge, these questions have not been tested in diabetic patients before.

There were several reasons for the present study. First, in previous work (7,13), we and others had been able to show that rosiglitazone corrected endothelial dysfunction of forearm resistance vessels in diabetic patients. One aspect of endothelial dysfunction is increased leakiness of the endothelium. Hence, any improvement of glomerular endothelial function might translate into decreased microalbuminuria. Second, it has been demonstrated that insulin resistance was related to microalbuminuria (6). Accordingly, thiazolidinediones appeared to be candidates to reduce microalbuminuria. Third, low-grade inflammation is associated with diabetic nephropathy (14). Thiazolidinediones are able to reduce systemic inflammation and might therefore improve diabetic nephropathy.

To test these hypothetical suggestions, we used a crossover study design that permitted a comparison of rosiglitazone with other oral antidiabetic agents providing similar glycemic control but not insulin sensitization. According to the data obtained, the diabetic patients tended to have numerically higher mean arterial blood pressure, GFR, and filtration fraction than matched healthy control subjects (Table 1). These changes occurred predominantly in diabetic patients with microalbuminuria (Tables 2 and 4). They also showed quantitatively the largest hemodynamic response to a 12-week treatment with rosiglitazone (Tables 2 and 4).

Significant intrarenal effects of rosiglitazone on endothelial function were demonstrated by our observations after NO blockade with L-NMMA (Table 3). In terms of the RPF and the filtration fraction, these experiments demonstrated that rosiglitazone was able to reestablish the pattern of endothelial response to L-NMMA that had been observed in normal control subjects.

It is now accepted by most that hyperfiltration and the degree of microalbuminuria are indicators of established diabetic nephropathy (15,16). It is also accepted that any reduction of microalbuminuria may be beneficial for kidney function. In several studies (17,18) of diabetic nephropathy, retardation of the progression of nephropathy toward end-stage renal failure has been found associated with preceding reduction of proteinuria. Therefore, the present study suggests that rosiglitazone may be a protective agent in diabetic nephropathy. Since we observed these effects of rosiglitazone in a comparable manner also in diabetic patients receiving ACE inhibitors, rosiglitazone may offer an additive mechanism of protection in diabetic nephropathy. We are not the first group to report a reduction of microalbuminuria in diabetic patients in response to thiazolidinediones. In fact, two previous reports (19,20) have provided comparable observations. However, our study is the first to show renal hemodynamic measurements and tests of renal endothelial function in this context. Accordingly, rosiglitazone ameliorated renal glomerular endothelial dysfunction as evidenced by the changes in filtration fraction (Table 4) and by the improved bioavailability of NO (Table 3). Interestingly, renal hemodynamic changes were correlated with a reduction in microalbuminuria (Fig. 2). A possible explanation of the changes is that increased NO bioavailability lowered intraglomerular capillary pressure and filtration fraction, while some kind of a recovery of the glomerular endothelium might have improved the properties of the filtration barrier. Both together may yield decreased microalbuminuria and perhaps nephroprotection.

TABLE 4
Renal hemodynamic function of type 2 diabetic patients with and without microalbuminuria after rosiglitazone or an alternative treatment

	Without microalbuminuria		With microalbuminuria	
	Nateglinide	Rosiglitazone	Placebo + previous oral antidiabetic drug	Rosiglitazone + previous oral antidiabetic drug
GFR (ml/min)	118.7 ± 7.2	103.2 ± 4.9*	133.4 ± 9.8	119.6 ± 8.7*
RPF (ml/min)	550.3 ± 39.6	554.9 ± 32.0	568.4 ± 30.9	567.5 ± 37.2
Filtration fraction (%)	22.0 ± 1.4	18.9 ± 1.1*	23.2 ± 1.7	20.5 ± 1.6*
Renal vascular resistance (mmHg · min^{-1} · l^{-1})	110.3 ± 8.4	105.9 ± 7.3	108.8 ± 7.6	106.1 ± 9.1

Data are means ± SE. *$P < 0.05$ vs. nateglinide or placebo.

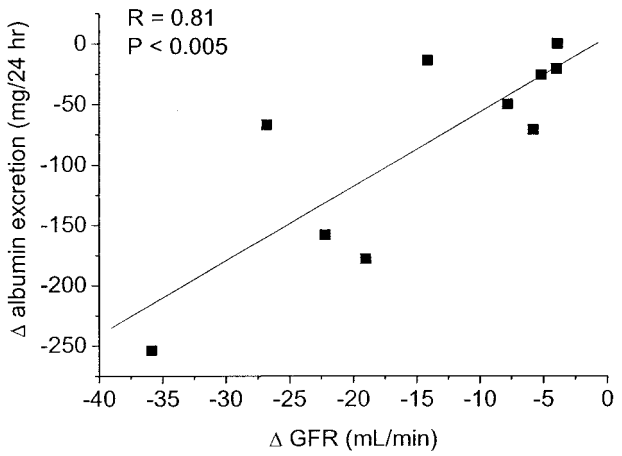

FIG. 2. Association between difference of albumin excretion (albumin excretion after rosiglitazone treatment versus albumin excretion after placebo) and difference of GFR in diabetic patients with microalbuminuria.

Our results are in agreement with animal studies. They demonstrated a decrease of GFR and microalbuminuria in diabetic rats after treatment with troglitazone (21) and a vasodilator action of troglitazone on the postglomerular efferent arteriole (22). The authors suggested a rosiglitazone-induced improvement of NO bioavailability at the efferent arteriole. Experimental data from the single nephron model has demonstrated that NO contributes to efferent arteriolar tone (23). Our conclusion of a decreased efferent NO bioavailability in diabetic subjects that is potentially ameliorated by rosiglitazone appears to be in conflict with studies suggesting a contribution of increased NO to the glomerular hyperfiltration in the early diabetic state (24,25). These studies, however, did not test bioavailable NO (e.g., by assessment of renal hemodynamics). Instead, they demonstrated an increased expression of endothelial NO synthase and an increase of circulating NO degradation products. On the other hand, Erley et al. (26) described a reduced response of RPF to L-NMMA in the diabetic rat, which is in line with our data.

In the literature (27), the changes of diabetic nephropathy are sometimes attributed to hyperglycemia alone. In the present work, significant improvement of renal endothelial function occurred with a thiazolidinedione independent of glycemic control. Further effects of thiazolidinediones like insulin sensitization or reduction of low-grade inflammation, demonstrated by a tendency toward a reduction of C-reactive protein (Table 2), might therefore contribute to the improved NO bioavailability in the present study. Our data do not allow us to exclude the additional possibility that the reduction of systemic arterial blood pressure in response to rosiglitazone could have been transmitted to the glomerular capillary, followed by lessened endothelial dysfunction and contributing to the reduction of microalbuminuria. However, the changes of MAP were small and not significantly correlated with the changes of microalbuminuria. It is more likely that the rosiglitazone-induced changes occurred directly at the glomerular wall and altered glomerular permeability. Recent studies of renal biopsies in patients with type 2 diabetes demonstrated an increased width of the glomerular basement membrane in association with the degree of inflammation (14). Furthermore, there are experimental studies indicating changes of extracellular matrix proteins within the glomerular mesangium after treatment with thiazolidinediones (21), a mechanism that is able to influence glomerular hemodynamics (27). Further studies may be indicated to unravel these issues in patients.

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