

The Paradox of the Low-Renin State in Diabetic Nephropathy

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Abstract. Although diabetic nephropathy is often a low renin state, the renin system appears to be implicated in its pathogenesis. In this study, it was hypothesized that the low plasma renin activity (PRA) is misleading, masking and perhaps reflecting an activated intrarenal renin system. PRA and renal vascular responses (inulin and para-aminohippurate clearance) to graded doses of an angiotensin II (AngII) antagonist, irbesartan, were assessed in eight healthy volunteers and 12 patients with type 2 diabetes mellitus and nephropathy on a 10 mmol Na intake, to activate the renin system. Basal PRA was suppressed in type 2 diabetes mellitus compared with the healthy subjects (0.58 ± 0.14 versus 1.58 ± 0.28 ng/L per s, mean \pm SEM; $P < 0.01$). Despite the low PRA, renal perfusion rose more in response to irbesartan in type 2 diabetes mellitus (714 ± 83 to 931 ± 116 ml/min; $P = 0.002$) than

normal (624 ± 29 to 772 ± 49 ml/min; $P = 0.008$). The youngest patients were hyperfiltrating and showed the largest rise in renal plasma flow in response to irbesartan, whereas renal plasma flow rose less and GFR fell in patients with low basal GFR. PRA rose in response to irbesartan more gradually in the patients with type 2 diabetes mellitus, but ultimately matched the normal response. To account for the apparent paradox of a heightened renal hemodynamic response to an AngII antagonist in the face of a low PRA in type 2 diabetes mellitus, and the rise in PRA following the AngII antagonist, it is proposed that there is increased intrarenal AngII production in type 2 diabetes mellitus. This increase could account for suppressed circulating renin, the exaggerated renal vasodilator response to irbesartan, and the therapeutic effectiveness of interrupting the renin system in diabetic nephropathy.

Early studies on plasma renin activity (PRA) in diabetes mellitus reflected interest in the concomitant hypertension that occurs frequently, especially with diabetic nephropathy (1). Rather than elevated PRA levels, which might have accounted for the hypertension, renin suppression was frequently observed (1,2). The low renin state has been attributed to sclerotic renal arterioles and glomeruli, possibly amplified by functional factors such as autonomic neuropathy and sodium retention (3–5).

Strong evidence indicates that angiotensin-converting enzyme (ACE) inhibition delays nephropathy in type 1 diabetic patients (6), and perhaps in type 2 diabetic patients (7–9). The responsible mechanism goes beyond BP reduction (6), possibly reflecting a pathogenetic role for the local renin-angiotensin system, but that is the subject of substantial debate (10–12). Renin suppression in diabetic nephropathy suggests that the benefit conferred by ACE inhibition might reflect alternative pathways. On the other hand, a dissociation between reduced PRA and evidence suggesting well-maintained or even increased renal tissue renin levels has been described in animal diabetes models (12–18).

The development of blockers that act at the angiotensin receptor level (19) provides a more specific probe for exploring control mechanisms. In this study we examined the renal hormonal and hemodynamic response in patients with type 2 diabetes mellitus and heavy proteinuria to graded doses of a new angiotensin II (AngII) antagonist, irbesartan, which induces prolonged specific blockade at the AT₁ receptor site (19). Our observations indicate that the low PRA in patients with diabetes and nephropathy is misleading, indeed paradoxical: The intrarenal renin system is activated, and regulating renal perfusion, renal function, renin release, and influencing the pathogenesis of the nephropathy.

Materials and Methods

Subjects and Protocols

The 12 patients with type 2 diabetes mellitus and nephropathy ranged in age from 36 to 61 yr (53 ± 2 yr). The 30 age-matched healthy subjects averaged 51.4 ± 3.7 yr and had a mean body mass index (BMI) of 25.9 ± 1.3 kg/m². The subset of eight healthy subjects who received irbesartan ranged in age from 24 to 64 yr (44 ± 5 yr). The healthy subjects and patients were similar in height: the increased BMI in type 2 diabetes mellitus (40.3 ± 3.5 kg/m²) reflected a mean body weight of 115 ± 9 kg. Type 2 diabetes mellitus was diagnosed according to accepted guidelines (20). The entry criteria for diabetic nephropathy were identical to those of the Collaborative Study Group. All had nephropathy with protein excretion that ranged from 0.5 to 11.4 g/24 h (mean 2.8 ± 0.8 g/24 h). The diabetic patients excreting <1 g/24 h of protein also had documented diabetic retinopathy. When these criteria were applied by the Collaborative Study Group, all but two of the patients had predominate diabetic glomerular sclerosis.

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Diabetes duration averaged 11.6 ± 2.3 yr. Three had been treated with oral agents and nine used insulin. HbA1c ranged from 7.4 to 11.8% (reference $<6.1\%$) with an average of $9.8 \pm 0.4\%$. Glucose excretion in 24-h urine samples on study days averaged only 6 g/d, and fasting blood sugar averaged 9.5 ± 0.7 mmol/L on the morning of the first study day, and 8.8 ± 1.1 mmol/L at the end of the protocol. Baseline mean arterial BP (MAP) was 98.9 ± 3.9 mmHg in the diabetic patients compared with 81.6 ± 3.9 mmHg in the healthy individuals ($P = 0.008$). Seven of the subjects with diabetes were hypertensive and six were on antihypertensive therapy.

Eligibility criteria included serum creatinine concentration <229 $\mu\text{mol/L}$, and 24-h protein excretion exceeded 500 mg in all three collections in patients with diabetic retinopathy. The only concurrent drug use was a stable dose of aspirin <325 mg/d. Antihypertensive medications were discontinued 10 d before the study in the type 2 diabetes mellitus patients treated for hypertension. A 10-mmol sodium diet was initiated before admission, to control BP. After an outpatient evaluation, subjects were admitted to our metabolic ward, where written consent was obtained.

Protocol

During hospitalization, blood glucose concentration was controlled by diet and the original treatment, either oral agents or insulin. The diet contained 1600 to 2500 calories, depending on BMI and glycemic control, 10 mmol sodium, 80 to 100 mmol potassium, and 2500 ml of water. Daily 24-h urine collections were taken for measurement of sodium, potassium, creatinine, glucose, and protein. When the morning spot urine sodium was <10 mmol/L, the physiologic study was initiated.

On each physiologic study day, an intravenous catheter was placed in each arm, for infusion and for blood sampling. The subjects were supine and fasting for at least 8 h. Fasting blood glucose was checked at the start and completion of study. Oral agents were withheld that morning, and those on insulin received half their usual dose.

The physiologic studies were 5 h in duration. All subjects underwent an identical protocol including the amount of fluid infused. At 7 a.m., each study began with a bolus and then 60-min infusion of para-aminohippurate (PAH) and inulin, followed by a single oral irbesartan dose. Renal plasma flow (RPF) measurements were made at baseline and at 45-min intervals. PRA was measured at baseline and 90 and 180 min, 24 and 48 h after irbesartan. BP was recorded by an automatic recording device (Dinamap, Critikon, Tampa, FL) at 5-min intervals during the infusion and every 6 h thereafter. The electrocardiogram was monitored continuously.

Each subject received three ascending doses of irbesartan, from 37.5 to 300 mg, each dose separated by a rest day. If a dose induced an excessive BP fall, or a rise in serum creatinine exceeding 0.3 mg/dl, drug administration was discontinued.

Renal Clearance Studies

PAH (Merck, Sharp & Dohme, West Point, PA) and inulin (Inutest Polyfructosan; Fresenius Pharma, Austria) clearances were assessed as described (21). In brief, a control blood sample was obtained followed by loading doses of PAH (8 mg/kg) and inulin (50 mg/kg). A constant infusion was initiated immediately with an IMED pump (IMED Corp., San Diego, CA) at a rate of 12 mg/min (PAH) and 30 mg/min (inulin) that achieved plasma PAH concentration at which PAH clearance represents about 90% of RPF. Likewise, at the inulin levels achieved, inulin clearance reflects GFR. PAH and inulin clearances were calculated as a metabolic clearance rate from their plasma concentration and infusion rates. The filtration fraction (FF) was

calculated by dividing the GFR by the RPF and multiplying the result by 100. Renal vascular resistance (RVR) was estimated by dividing the calculated MAP by the RPF, and multiplying this result by 100, expressed as units of mmHg/min per 100 ml.

Laboratory Procedures

Blood samples were collected on ice, spun immediately, and the plasma was frozen until assay. Serum and urine sodium and potassium levels were measured by flame photometry. Serum creatinine, PAH, and inulin were measured by autoanalyzer. Inulin samples were treated to an additional glucose oxidase step (22) to rid the sample of glucose. Samples were then incubated at 37 and 60°C for 30 and 15 min, respectively, to destroy the hydrogen peroxide produced and the remaining glucose oxidase. PRA and aldosterone were assayed by RIA techniques (21).

Statistical Analyses

Group means are presented with the SEM as the index of dispersion. Statistical probability in two-sample data analyses was assessed by the paired or unpaired *t* test for normally distributed data. When indicated by a skew distribution, as in the case of baseline PRA and for variables estimated as ratios (FF), nonparametric (signed rank or Mann–Whitney rank sum) tests were used. To analyze three or more variables, one-way ANOVA was used. Because age has an independent influence on RPF, GFR, and FF, we also used analysis of covariance (ANCOVA) by least squares for age adjustment of the renal hemodynamic data. When appropriate, a Kruskal–Wallis ANOVA was used. Statistical probability was also assessed by linear regression analysis. The null hypothesis was rejected when the *P* value was <0.05 .

Results

The patients with type 2 diabetes mellitus averaged 53 ± 2 yr of age, similar to the healthy subjects (Table 1). Mean serum creatinine concentration in type 2 diabetes mellitus, 106 ± 18 $\mu\text{mol/L}$, was normal (Table 1), but there was a wider range in the patients, from 62 to 221 $\mu\text{mol/L}$. Average protein intake was 0.99 g/kg with a range of 0.53 to 1.47 g/kg.

Table 1. Demographics and baseline renal hemodynamic state^a

Parameter	Healthy Subjects	Type 2 Diabetes Mellitus
<i>n</i>	30	12
Age (yr)	51.4 ± 3.7	53.0 ± 2.0
Height (cm)	174 ± 2	171 ± 3
Weight (kg)	73.8	115 ± 9^b
BMI (kg/m ²)	24.7 ± 0.6	40.3 ± 3.5^b
Baseline MAP (mmHg)	81.6 ± 3.9	98.9 ± 3.9^c
Serum creatinine ($\mu\text{mol/L}$)	97 ± 3	106 ± 18
Serum K ⁺ (mEq/L)	4.4 ± 0.2	4.6 ± 0.1
Clearance PAH (ml/min)	595 ± 24	622 ± 65
Clearance inulin (ml/min)	116 ± 3	114 ± 10

^a BMI, body mass index; MAP, mean arterial pressure; PAH, para-aminohippurate.

^b $P < 0.001$ versus healthy subjects.

^c $P \pm 0.008$ versus healthy subjects.

Similarly, although mean GFR did not differ significantly from normal in the diabetic patients, GFR varied more, ranging from 52 to 172 ml/min, with a coefficient of variation of 32% (Figure 1). GFR in healthy subjects showed the anticipated negative relation to age (Figure 1A) ($r = -0.54$, $P < -0.05$). In type 2 diabetic patients, the relation of age to GFR differed substantially; GFR in the younger diabetic patients was well above the normal relationship, but was reduced in the older patients (Figure 1A). There was a correlation between age and both GFR ($r = -0.62$; $P = 0.003$) and duration of diabetes ($r = 0.84$; $P < 0.01$). The relation between age and renal perfusion in the healthy subjects ($r = -0.75$; $P < 0.001$) and in the diabetic patients ($r = -0.77$; $P = 0.003$) resembled the relationships for inulin clearance (Figure 1B).

PRA in the diabetic patients at baseline (0.58 ± 0.14 ng/L per s) was significantly less than normal (1.58 ± 0.28 ng/L per s; $P = 0.0079$) (Table 2). In five of the 12 diabetic patients, the PRA was below the lowest normal value, and all but one of the values was below the normal mean value.

Baseline RPF and GFR in the eight healthy subjects who received irbesartan were 629 ± 29 and 113 ± 3 ml/min, respectively. The AngII antagonist then induced a dose- and time-related rise in RPF (Figure 2). At each dose, RPF rose

Table 2. Plasma renin activity and aldosterone^a

Parameter	Healthy Subjects	Type 2 Diabetes Mellitus
<i>n</i>	8	12
PRA basal (ng/L per s)	1.58 ± 0.28	0.58 ± 0.14^b
PRA peak (ng/L per s)	9.47 ± 0.53	6.61 ± 1.03^c
Aldosterone basal (ng/dl)	25.2 ± 4.7	20.3 ± 6.8
Aldosterone at PRA peak (ng/dl)	13.3 ± 2.9	8.7 ± 1.0
Na (mmol/24 h) ^d	20 ± 4	26 ± 6

^a PRA, plasma renin activity.

^b P versus healthy subjects = 0.008.

^c $P < 0.05$ versus healthy subjects.

^d Sodium excretion reflects the 24-h collection completed the morning of the study.

progressively to a zenith at 90 min, well maintained over the 3.5 to 4 h assessed. The dose–response relationship peaked at 150 mg of irbesartan (Figure 2). GFR did not change at any irbesartan dose. At the peak RPF response to 150 mg of irbesartan, GFR was unchanged (114 ± 3 ml/min versus 118 ± 4 ml/min; $P < 0.4$).

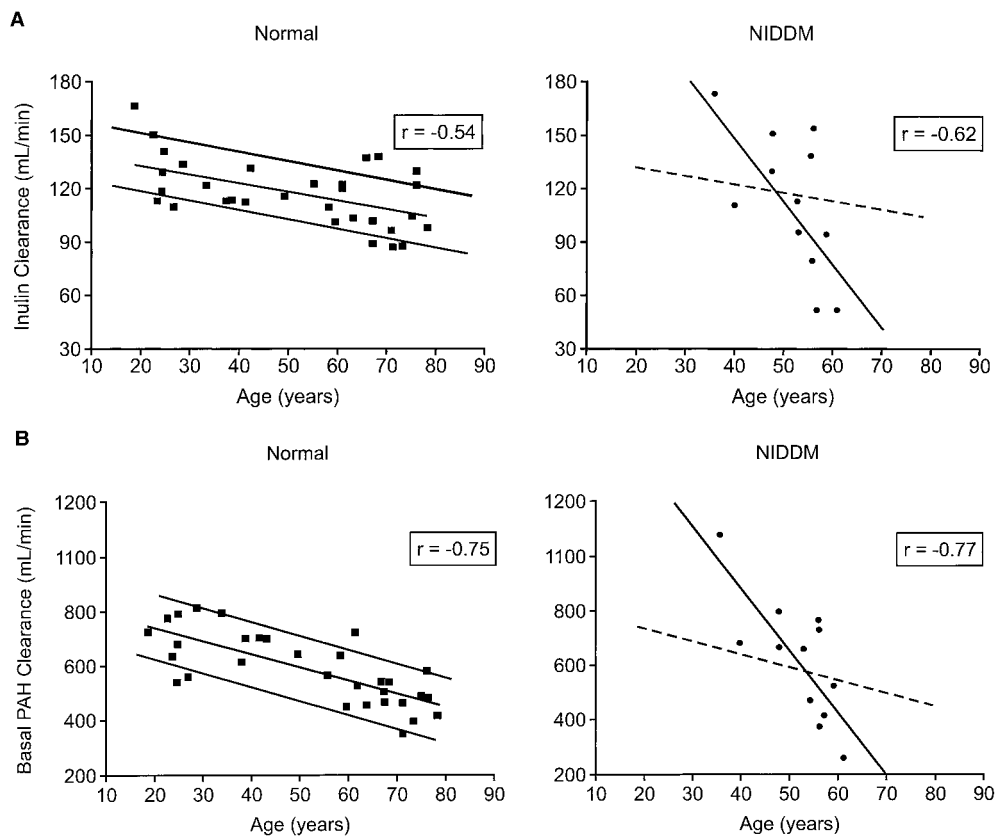


Figure 1. (A) Relation between age and inulin clearance as an index of GFR in healthy subjects (left) and patients with type 2 diabetes mellitus (right). In the healthy subjects, the line of best fit and the 95% confidence interval are shown. In patients with type 2 diabetes mellitus (right), the regression relationship in healthy subjects is shown as a dashed line, and the steeper regression relationship in the patients is shown as a solid line. (B) Relation between para-aminohippurate (PAH) clearance as an index of renal plasma flow (RPF) and age in the same healthy subjects and patients with diabetes as shown in A. Because the relationships are identical to the those in Panel A, the data suggest that the range of glomerular filtration shown in A is hemodynamically driven.

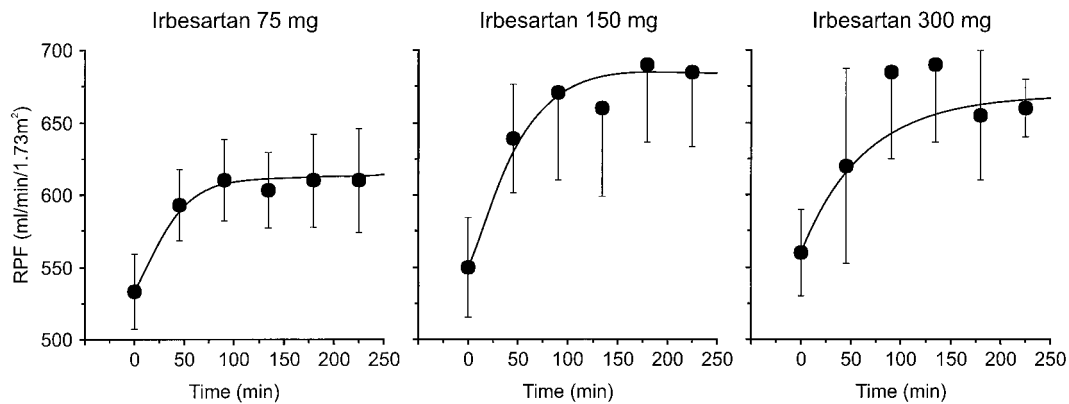


Figure 2. Relation between irbesartan dose, time, and RPF in healthy subjects. Note that about 90 min was required for complete expression of the renal vasodilator response, and that the 150 mg dose lies at the top of the dose–response relationship for renal perfusion.

The renal response in diabetic patients was enhanced at each irbesartan dose (Figure 3) with a time course identical to the healthy subjects. However, there was a striking difference in the renal functional response between the two groups. GFR rose significantly in the nine diabetic patients that received the 150-mg irbesartan dose, from 123 ± 12 to 159 ± 17 ml/min ($P < 0.01$). This rise in GFR occurred despite the decrease in MAP from 90 ± 4.2 to 85 ± 5.8 mmHg and decrease in RVR from 13.7 ± 1.7 to 9.9 ± 1.0 mmHg/min per 100 ml (Table 3).

The magnitude and direction of the renal hemodynamic response to irbesartan in the type 2 diabetic patients was influenced strongly by age, presumably reflecting duration and stage of disease. Figure 4, A and B, plots the relation between basal RPF and the RPF response to irbesartan and basal GFR and the GFR response to irbesartan. Both for RPF ($r = 0.88$; $P < 0.001$) and for GFR ($r = 0.75$; $P > 0.01$), the basal level was a strong predictor of the response. The higher the basal level of GFR and RPF, the larger the renal vasodilator response to irbesartan. In those patients in whom basal perfusion was

lowest, irbesartan failed to induce a vasodilator response at any dose, and GFR fell. Data on the response to the 37.5-mg dose are represented, because two of the patients with the lowest basal GFR showed a rise in serum creatinine and blood urea nitrogen with this dose, and additional doses were not administered. The relationship between basal level and response, however, was evident for each of the irbesartan doses. FF fell in all of the subjects after irbesartan, from 19.3 ± 0.2 to 14.0 ± 0.1 in the four patients in the lowest tertile, and from 18.2 ± 0.2 to 17.5 ± 0.2 in the patients with the highest baseline renal perfusion levels. The trends were similar with higher irbesartan doses. RVR also showed a downward trend with time and dose in all subjects, although there were no significant differences between the two groups or the basal values and nadir (Table 3).

PRA rose rapidly in the healthy subjects, reaching a zenith at the lowest irbesartan dose, and earliest time point (Figure 5). At the peak response—defined as the highest PRA level achieved in each individual—PRA had risen from 1.58 ± 0.28 to 9.47 ± 0.31 ng/L per s ($P < 0.001$). In type 2 diabetes

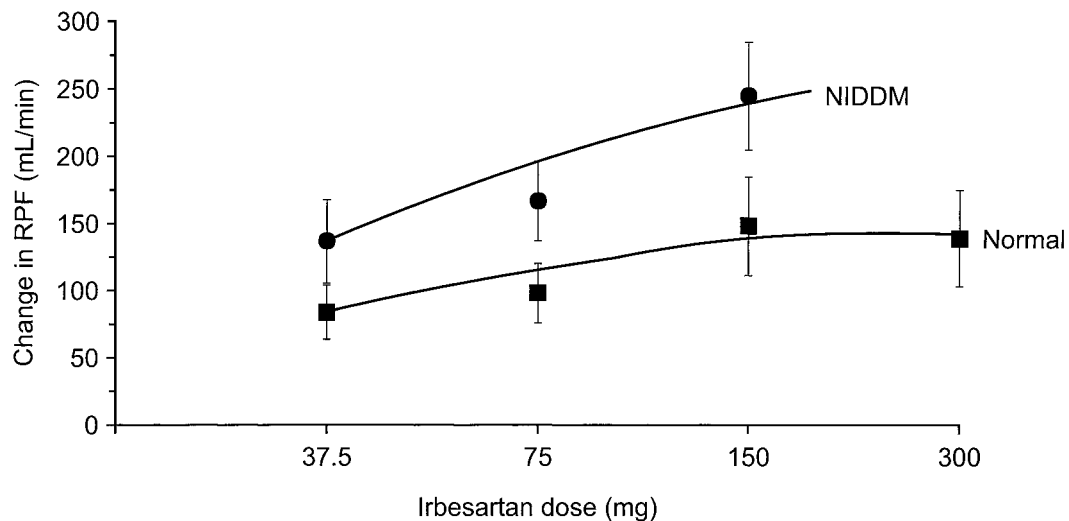


Figure 3. The relation between irbesartan dose and peak RPF response in healthy subjects and patients with type 2 diabetes mellitus. Despite the fact that type 2 diabetic patients with nephropathy is a low-renin state, and that plasma renin activity (PRA) was below normal in these patients with diabetes, the renal vascular response to irbesartan was enhanced significantly ($P < 0.01$).

Table 3. Time course of systemic and renal hemodynamic responses to varying doses of irbesartan in diabetic patients and control subjects^a

Group	Dose	Minutes			
		0	90	180	225
Control subjects	37.5 mg				
	MAP (mmHg)	89 ± 2.1	75 ± 5.7	76 ± 3.9	89 ± 3.5
	GFR (ml/min)	111 ± 2	116 ± 7	116 ± 7	114 ± 7
	RVR (mmHg/min per 100 ml)	15.8 ± 1.2	11.8 ± 1.9	11.5 ± 1.4	12.1 ± 1.4
	75 mg				
	MAP (mmHg)	81 ± 2.3	78 ± 3.0	74 ± 2.6	76 ± 2.4
	GFR (ml/min)	113 ± 5	113 ± 6	118 ± 7	115 ± 5
	RVR (mmHg/min per 100 ml)	13.7 ± 1.1	11.3 ± 0.8	10.8 ± 0.8	11.2 ± 0.7
	150 mg				
	MAP (mmHg)	82 ± 3.0	73 ± 3.1	71 ± 2.6	74 ± 1.8
	GFR (ml/min)	114 ± 3	118 ± 4	118 ± 4	115 ± 5
	RVR (mmHg/min per 100 ml)	13.4 ± 1.1	9.8 ± 0.9	9.5 ± 0.8	10.5 ± 0.8
Type-2 diabetic patients	300 mg				
	MAP (mmHg)	75 ± 3.9	68 ± 3.3	68 ± 3.2	69 ± 2.1
	GFR (ml/min)	118 ± 6	126 ± 4	122 ± 3	115 ± 1
	RVR (mmHg/min per 100 ml)	11.3 ± 0.7	8.7 ± 0.3	9 ± 0	8.7 ± 0.3
	37.5 mg				
	MAP (mmHg)	103 ± 4.2	92 ± 3.2	91 ± 4.7	94 ± 4.4
	GFR (ml/min)	95 ± 12	111 ± 1.6	122 ± 18	119 ± 18
	RVR (mmHg/min per 100 ml)	20.6 ± 3.6	16.1 ± 3.2	15.8 ± 3.1	16.8 ± 3.3
	75 mg				
	MAP (mmHg)	94 ± 4.4	82 ± 4.3	85 ± 4.4	89 ± 4.3
	GFR (ml/min)	121 ± 11	130 ± 14	138 ± 17	135 ± 19
	RVR (mmHg/min per 100 ml)	15.7 ± 1.9	12.1 ± 1.8	11.9 ± 1.5	13.3 ± 1.8
150 mg					
MAP (mmHg)	90 ± 4.2	83 ± 3.2	85 ± 5.8	88 ± 3.7	
GFR (ml/min)	123 ± 11.9	150 ± 20.4	159 ± 17.4	145 ± 18	
RVR (mmHg/min per 100 ml)	13.7 ± 1.7	9.9 ± 1.2	9.9 ± 1.0	10.6 ± 1.1	
300 mg					
MAP (mmHg)	94 ± 2.8	84 ± 15.6	83 ± 14.1	78 ± 17.0	
GFR (ml/min)	151 ± 10	159 ± 7	161 ± 7	166 ± 12	
RVR (mmHg/min per 100 ml)	11.5 ± 0.5	8.5 ± 1.5	8.5 ± 1.5	8.5 ± 1.5	

^a RVR, renal vascular resistance. Other abbreviations as in Table 1.

mellitus, the renin response was sluggish, but PRA rose progressively with dose and duration, from a baseline of 0.58 ± 0.14 to a peak of 6.61 ± 1.03 ng/L per s, still below the normal peak statistically ($P = 0.05$). PRA in the type 2 diabetic patients matched healthy subjects 24 h after the 150-mg irbesartan dose (Figure 5). Baseline aldosterone levels were similar in the two groups (Table 2), and both documented decreased levels corresponding to the peak PRA.

Mean arterial BP (MAP) fell from 91 ± 2 to a nadir of 79 ± 4 mmHg 90 min after irbesartan in the diabetic patients ($P < 0.01$) and was maintained at reduced levels for the remainder of the study. That response reflected a fall in both systolic and diastolic BP. MAP in healthy subjects (81 ± 2 mmHg) also reached a nadir at 90 min of 75 ± 2 mmHg ($P < 0.05$).

Twenty-four-hour urine samples for glucose were collected in the diabetic subjects. Glycosuria ranged from 3 to 726 mg/dl

with a mean \pm SEM of 247 ± 52 mg/dl. There was no correction between level of glycosuria and peak RPF response. The average protein intake for the healthy individuals was 0.96 ± 0.06 g/kg, which was not significantly different from the protein intake of the type 2 diabetic patients, 1.01 ± 0.08 g/kg. There was no correlation of the peak RPF response to protein intake.

There were no clinically important, unanticipated adverse effects of the drug. Serum potassium concentration did not change with irbesartan. Basal serum potassium concentration was 4.5 ± 0.1 mEq/L, and remained unchanged (4.5 ± 0.1 mEq/L) 24 h after 150 mg of irbesartan. In no patient did serum potassium concentration exceed 4.9 mEq following irbesartan. In two patients, protocol design led to irbesartan discontinuation: A serum creatinine rise exceeded $227 \mu\text{mol/L}$ but returned to baseline within 24 h after liberalizing salt intake. In

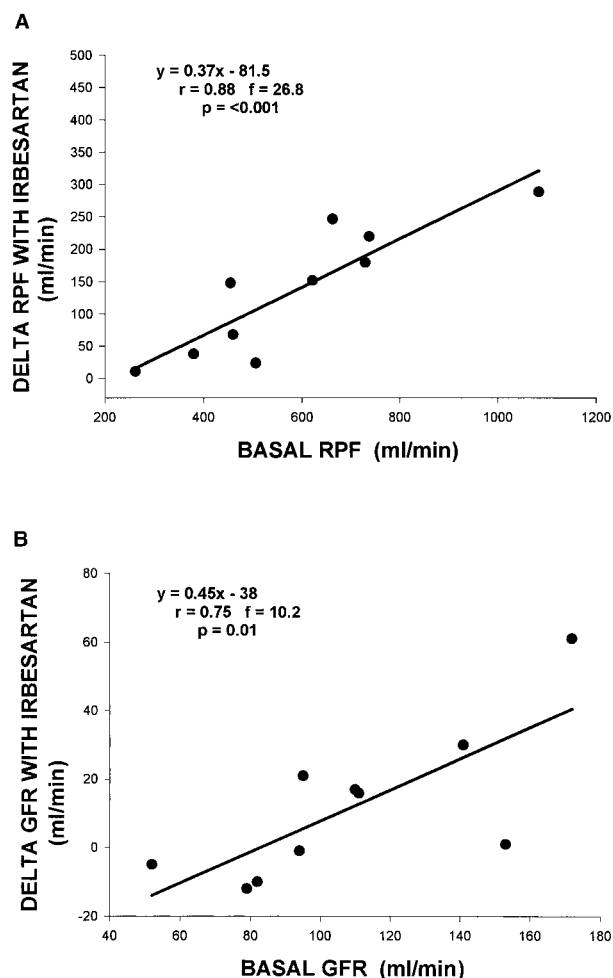


Figure 4. (A) Relation between basal RPF and the change in RPF to 37.5 mg of irbesartan in type 2 diabetic patients. (B) Relation between basal GFR and the response to GFR to 37.5 mg of irbesartan in type 2 diabetes mellitus. The relationships are identical to those in A. The diabetic patients with the highest basal clearance showed the greatest response. Those with lower basal clearances responded very little or had a decrease in GFR in response to the drug.

one additional patient, irbesartan was discontinued after the 37.5-mg dose because BP fell from 140/100 mmHg to a low of 90/55 mmHg. There were no associated symptoms.

Discussion

A dose-related increase in renal perfusion followed irbesartan in healthy subjects in whom the renin system was activated by restriction of salt intake. In diabetic patients with nephropathy, the renal hemodynamic response to the AngII antagonist was enhanced substantially, despite the fact that the low-salt diet had provoked a limited increase in PRA in most of the patients, confirming that this is a low renin state (1,2). Thus, the renal hemodynamic response to the AngII antagonist suggested a different state of the renin system than did a direct measure of PRA.

Our hypothesis that circulating renin might not reflect intra-

renal tissue levels in type 2 diabetes mellitus is suggested by many (12–18), but not all (23), studies in rat models. Our test of that hypothesis was that the AngII antagonist would raise PRA levels substantially in the diabetic patients, if the reduced PRA reflected, at least in part, intrarenal AngII-mediated suppression. Thus, serial measurements of PRA were made over the 48 h after each irbesartan dose, on a constant diet and in recumbency. The striking dose- and time-related renal vasodilator response and rise in PRA after administration of the AngII antagonist in the diabetic patients support the original hypothesis: PRA and the state of the intrarenal renin-angiotensin system are disparate in type 2 diabetic patients.

AngII suppresses renin release via two distinct mechanisms, the “long” and “short” feedback loops (24,25). The long loop reflects the suppressive effect of sodium retention induced by AngII-mediated aldosterone release and renal vasoconstriction, and thus evolves over days. The short loop reflects a direct, local intrarenal effect of AngII on renin release, and responds in minutes (24,25). It has long been recognized that renin system blockade induces an acute reactive renin rise, in part reflecting interruption of the short loop (26). The acute response to renin inhibition in healthy subjects studied under similar conditions includes a remarkable rise in plasma renin mass (27), comparable to levels in this study. Over the irbesartan dose range studied, we found no evidence in the healthy subjects of a dose dependency of the renin response: PRA levels had peaked by the earliest time point sampled, at the lowest irbesartan dose. This response exceeds that induced by the usual provocative stimulus, low-salt diet and quiet standing (21), and suggests that the short feedback loop is the dominant brake on renin release when the system is activated. The aldosterone levels as expected decreased; however, the changes in PRA occurred far too quickly for the long loop to have been crucial.

Christlieb *et al.* (1) were the first to document a persistent reduction in PRA despite provocation in patients with diabetic nephropathy, and suggested that intrarenal events might be responsible. In their study, patients with diabetic nephropathy had a PRA rise provoked by a low salt diet of 0.64 ng/L per s, substantially below normal, similar to our findings in this study. Recent reviews cite several dozen confirming studies (3–5). In their analysis of the pathogenesis of the low renin state, Lush *et al.* identified 23 intrarenal potential targets (4), but the “short feedback loop” was not cited as a possible contributor. The short feedback loop is intact in type 2 diabetes mellitus (28). The rise in PRA following irbesartan in our patients, reflecting responses to single doses at 2-d intervals, fell short of the normal peak response, but was striking nonetheless. The data are compatible with excessive intrarenal AngII generation in type 2 diabetes mellitus. In rat models of diabetes, a low PRA level was associated with normal or elevated renal tissue renin levels, indicating that altered renin release and not reduced production was responsible for the low PRA (13–18). Reactivity of renal vascular and glomerular elements to AngII is downregulated (14,15), which is believed to reflect excessive local AngII levels. In keeping with this finding, our studies in patients with type 2 diabetes mellitus on

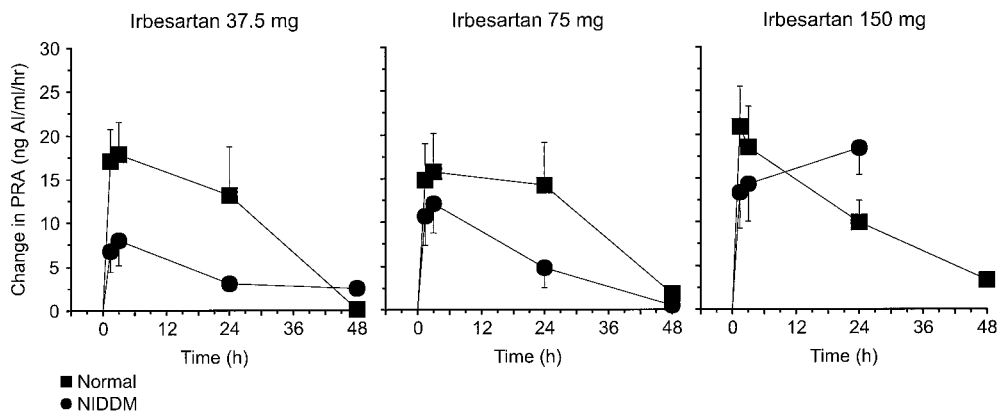


Figure 5. Relation between irbesartan dose, time, and change in PRA in healthy subjects and patients with type 2 diabetes mellitus. Note that the healthy subjects achieve a peak renin response at the lowest dose and earliest time interval studied. The relation of dose and response differs from that of the renal blood supply (Figure 2). In patients with type 2 diabetes mellitus, the PRA rise was blunted and slow, but eventually matched the normal response.

a high salt diet revealed a blunted renovascular response to AngII, which was corrected by ACE inhibition (29). Sustained hyperglycemia can activate the renin system directly in diabetic patients (30). Intracellular signaling interactions downstream from the insulin receptor could account for the associations between renal hemodynamics and angiotensin, as the process was inhibited by an AngII antagonist (31).

Type 2 diabetes mellitus is heterogeneous both in pathogenesis (32) and renal histopathology (33–35). In a recent study that used the same rigorous entry criteria as ours, the prevalence of renal tissue changes incompatible with type 2 diabetes mellitus was <10% (33). Our patients in this study were young, perhaps reflecting exclusion of patients with recent coronary or cerebrovascular disease, or advanced azotemia. However, because of the similar entry criteria, we believe the majority of their renal disease was due to diabetic nephropathy. As we studied a wide age range and documented our findings as a function of age, this is unlikely to be important. Our patients were obese; obesity has an unambiguous influence on renal perfusion (36–38), and obesity complicated adjusting renal perfusion for body size (39,40). As in recent reports on type 2 diabetic patients (41), the renal hemodynamic data were not normalized.

Obesity may have an influence on the control of the renal circulation that is immediately relevant to this study. We identified blunting of the renal blood flow response to infused AngII with increasing obesity, in a study designed to assess the effect of angiotensinogen (AGT) gene polymorphisms on AngII-mediated control of the renal circulation (40). The diminished response to AngII seen in many hypertensive subjects was to a major degree accounted for by their high BMI. One possibility that we have considered is that this blunted response in hypertension reflects downregulation of renal AngII receptors by chronically elevated intrarenal AngII levels (42). Plasma AGT levels have repeatedly been shown to be strongly positively correlated with BMI (43–45). There are two possible explanations. AGT mRNA is expressed abundantly in adipocytes (46,47). Expression varies with fasting and refeed-

ing in both normal rats and obese mice (46). To close the loop, an association has been demonstrated between AGT gene polymorphisms and body fat distribution in men (48). As an alternative explanation for the relation between obesity and AGT, the insulin resistance that occurs with obesity could play a contributing role. Hepatic AGT production is under the strong influence of plasma insulin, and does not show insulin resistance (49,50). Thus, with the advent of insulin resistance, and the consequent rise in plasma insulin concentration, yet another stimulus to AGT production comes into play. Clearly, the influence of obesity in our findings merits further investigation since obesity may reflect sodium balance and renin-angiotensin activity. Unfortunately, it is extraordinarily difficult to identify individuals with BMI levels to match those in the diabetic patients in this study who are free of carbohydrate intolerance and hypertension.

Reproducibility within subjects on repeated clearance studies on the same or different days has been extremely good (51), reflecting in part the involvement of a single research nurse. The healthy subjects and patients were studied in the same clinical setting, diet, and time interval. A metabolic clearance rate was used to avoid problems related to poor bladder emptying. Plasma samples were treated with glucose oxidase to avoid the influence of plasma glucose on the inulin assay (22). The peak renal vasodilation induced by irbesartan in the healthy control subjects in this study, approximately 140 ml/min per 1.73 m², is essentially identical to the response to irbesartan to two renin inhibitors (52) and to another AngII antagonist (53). The renal responses were coherent with irbesartan dose and with the stage of the diabetic process; misleading conclusions based on clearance techniques are unlikely.

In type 1 diabetes mellitus, an early increase in GFR followed by microalbuminuria, frank proteinuria, and a progressive decline in GFR reflects a series of stages (53). Our original analysis plan assumed a similar sequence in type 2 diabetes mellitus, based on scattered observations (54,55) recently confirmed in a comprehensive study in Pima Indians with type 2 diabetes mellitus (41). The current study, in Caucasians, pro-

vides a strong confirmation. Because the onset of both type 2 diabetes mellitus and proteinuria is clinically silent, we used age as a surrogate. There was a very good correlation between age and known duration of type 2 diabetes mellitus. The magnitude and pattern of the renal hemodynamic response to irbesartan was governed largely by age and stage. Early, when renal perfusion was still hyperdynamic—counter to both intuition and to regression of the mean—the renal vasodilator response was largest. This response suggests that the contribution of AngII was especially substantial early in the course of diabetic nephropathy, when perfusion was luxurious. In the older patients in whom baseline GFR was in the middle or lower end of the continuum sampled, there was a substantial increase in RPF, and a somewhat variable change in GFR in response to irbesartan.

In the oldest patient studied, serum creatinine rose from 230 to 292 $\mu\text{mol/L}$ in the 24 h after the first irbesartan dose (37.5 mg), and the protocol was discontinued. As with ACE inhibitors, caution is appropriate in starting treatment in the azotemic patient as GFR is sustained by an AngII-mediated increase in efferent arteriolar resistance.

The striking renal vasodilator response to irbesartan in the younger patients earlier in the course of diabetic nephropathy presented several surprises. If irbesartan reversed AngII-dependent renal vasoconstriction, it is counterintuitive that this vasoconstriction is maximal at a stage of type 2 diabetes mellitus when basal renal hyperemia is also maximal. The renal vasoconstriction must be more than counterbalanced by the local dilator effects of counterregulatory systems; candidates include nitric oxide and kinins (56–59). Because excessive AngII could participate in the genesis of renal injury, either through its effect on glomerular capillary pressure or through an influence on growth and repair (16), early activation could be important.

Equally surprising was the parallel rise in GFR following irbesartan administration in some patients. GFR was probably flow-dependent in these patients, so that the rise in RPF promoted the GFR rise (56,60–62). Although glomerular hyperfiltration is a marker for subsequent injury, glomerular hypertension rather than hyperfiltration, *per se*, is probably the pathogenetic culprit (63). Glomerular pressure is likely to have fallen sharply after irbesartan, with the decline in arterial BP, efferent arteriolar resistance, and filtration fraction.

Recent observations on the genetics of nephropathy in type 2 diabetes mellitus also favor a pathogenetic contribution of the renin system (64). Overwhelming evidence indicates that ACE inhibitors prevent or delay type 1 diabetic nephropathy (6), and perhaps also in type 2 diabetes mellitus (7–9). The mechanism by which ACE inhibitors confer this protection has been debated, in part because many of these patients have low plasma renin levels, suggestive of an “inactive” renin system (12). Our data suggest a resolution to this paradox. Type 2 diabetic patients have a remarkably robust renal vasodilator and reactive renin response to a highly specific AngII antagonist despite a low PRA, suggestive of increased intrarenal levels of AngII. The increase in renal AngII could explain the low PRA levels, the beneficial effects of ACE inhibition, and ties be-

tween genes governing the renin cascade and diabetic nephropathy. The therapeutic implications of early activation of the renin cascade in type 2 diabetes mellitus are substantial.

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