Kidney Function During and After Withdrawal of Long-Term Irbesartan Treatment in Patients With Type 2 Diabetes and Microalbuminuria

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OBJECTIVE — Irbesartan is renoprotective in patients with type 2 diabetes and microalbuminuria. Whether the observed reduction in microalbuminuria is reversible (hemodynamic) or persistent (glomerular structural/biochemical normalization) after prolonged antihypertensive treatment is unknown. Therefore, the present substudy of the Irbesartan in Patients with Type 2 Diabetes and Microalbuminuria Study (IRMA-2) investigated the reversibility of kidney function changes after withdrawal of 2 years' antihypertensive treatment.

RESEARCH DESIGN AND METHODS — The substudy included 133 hypertensive type 2 diabetic patients with persistent microalbuminuria in IRMA-2, randomized to double-masked treatment with either placebo, irbesartan 150 mg, or irbesartan 300 mg o.d. for 2 years. Arterial blood pressure, overnight urinary albumin excretion rate, and glomerular filtration rate (GFR) were determined repeatedly.

RESULTS — Baseline characteristics were similar in the placebo, irbesartan 150-mg, and irbesartan 300-mg groups. At the end of the study, mean arterial blood pressure (MABP) was similarly lowered to 105 ± 2 (mean ± SE), 103 ± 2, and 102 ± 2 mmHg, respectively ($P < 0.05$ versus baseline), and urinary albumin excretion rate reduced by 8% (−16 to 27) (NS), 34% (95% CI 8–53), and 60% (46–70) ($P < 0.05$). Rates of decline in GFR were 1.3 ± 0.7, 1.2 ± 0.7, and 1.0 ± 0.8 ml · min^{-1} · 1.73 m^{-2} per month, respectively, during the initial 3 months of the study and 0.3 ± 0.1, 0.3 ± 0.1, and 0.4 ± 0.1 ml · min^{-1} · 1.73 m^{-2} per month in the remaining study period. One month after withdrawal of all antihypertensive medication, MABP remained unchanged in the placebo group, 105 ± 2 mmHg, but increased significantly in the irbesartan groups, to 109 ± 2 and 108 ± 2 mmHg, respectively. Compared with baseline, urinary albumin excretion rate was increased by 14% (−17 to 54) in the placebo group and by 11% (−26 to 65) in the irbesartan 150-mg group but was persistently reduced by 47% (24–73) in the irbesartan 300-mg group ($P < 0.05$). GFR levels increased to baseline values in the placebo group and approached initial levels in irbesartan groups.

CONCLUSIONS — Persistent reduction of microalbuminuria after withdrawal of all antihypertensive treatment suggests that high-dose irbesartan treatment confers long-term renoprotective effects.

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Abbreviations: ARB, angiotensin II receptor blocker; GFR, glomerular filtration rate; IRMA-2, Irbesartan in Patients with Type 2 Diabetes and Microalbuminuria Study; MABP, mean arterial blood pressure; RAAS, renin-angiotensin-aldosterone system; TGF-β, transforming growth factor-β.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Several vasoactive hormones exhibit effects on renal hemodynamics, but angiotensin II is the major intrarenal hormone to regulate glomerular filtration rate (GFR) (1). Angiotensin II plays an important role in the initiation and progression of diabetic and nondiabetic glomerulopathies (2,3), but the recognized role of angiotensin II in the pathogenesis of diabetic renal disease cannot be attributed exclusively to its hemodynamic effects (4). Accumulating data suggest that angiotensin II exerts several nonhemodynamic effects, such as growth stimulation, fibrogenesis, and impairment of endothelial function (4).

Studies in type 2 diabetic patients with incipient and overt diabetic nephropathy have demonstrated that angiotensin II receptor blockers (ARBs) are renoprotective in addition to what might be expected from blood pressure reduction alone (5–7). In the Irbesartan in Patients with Type 2 Diabetes and Microalbuminuria Study (IRMA-2), irbesartan 300 mg o.d. significantly reduced the risk of progression to diabetic nephropathy by 70% and lowered microalbuminuria by 38% in hypertensive type 2 diabetic patients (5). Whether the reduction in microalbuminuria is reversible or persistent after prolonged antihypertensive treatment is unknown. Reversibility would suggest a predominantly renal hemodynamic mechanism of ARBs, whereas persistent reduction of microalbuminuria may imply renal improvement of structural damages and/or biochemical abnormalities. The present IRMA-2 substudy aimed to evaluate these mechanisms by investigation of kidney function changes after withdrawal of 2 years' antihypertensive therapy.

RESEARCH DESIGN AND METHODS

— The IRMA-2 study protocol has been described in detail previously (5). In brief, 590 hypertensive type 2 diabetic patients with microalbumin-

Table 1—*Baseline characteristics of the patients*

	Placebo	Irbesartan 150 mg o.d.	Irbesartan 300 mg o.d.
n	48	42	43
Sex (male/female)	35/13	33/9	31/12
Age (years)	57 ± 9	57 ± 9	55 ± 9
BMI (kg/m^2)	31 ± 5	30 ± 4	30 ± 5
Known diabetes duration (years)	7 ± 6	8 ± 6	8 ± 7
Systolic blood pressure (mmHg)	154 ± 16	153 ± 14	153 ± 14
Diastolic blood pressure (mmHg)	91 ± 9	90 ± 9	92 ± 9
Urinary albumin excretion (μg/min)	46 (21–159)	60 (19–243)	51 (21–174)
GFR (ml · min^{-1} · 1.73 m^{-2})	108 ± 28	117 ± 20	113 ± 23
HbA_{1c} (%)	7.1 ± 1.7	7.2 ± 1.7	7.1 ± 1.7
Serum cholesterol level (mmol/l)	5.7 ± 1.1	5.9 ± 1.1	5.9 ± 1.1
Serum creatinine level (μmol/l)	97 ± 17	97 ± 9	97 ± 17

Data are means ± SE or median (range). NS between all treatment groups.

uria were included in this multinational, randomized, double-masked, placebo-controlled study of irbesartan (150 and 300 mg o.d.) and were followed for 24 months. The primary outcome was time to onset of diabetic nephropathy, defined as persistent albuminuria in overnight specimens with a urinary albumin excretion rate >200 μg/min and ≥30% increase from baseline level. Target trough blood pressure was <135/85 mmHg 3 months after randomization. Additional antihypertensive treatment used included diuretics, β-blockers, calcium-channel blockers (except dihydropyridines), and α-blockers. These agents were added if target blood pressure was not reached 3 months after randomization.

The present substudy was prespecified in the main study protocol. A total of 11 centers capable of measuring GFR were invited to the substudy. All 133 patients from these 11 centers participated in the substudy (Table 1). Mean arterial blood pressure (MABP), urinary albumin excretion rate, and GFR were measured at baseline, after a single-blind 3-week run-in period, at 3 months, at 24 months, and 1 month after withdrawal of all antihypertensive treatment.

A total of 18 patients in the substudy did not complete 24 months of double-blind treatment for various reasons. In 91 patients who accepted discontinuation of antihypertensive medication, treatment was withdrawn and measurements were repeated after 1 month. During the withdrawal phase, patients were followed by weekly visits in the outpatient clinic and antihypertensive treatment was restarted if blood pressure exceeded 165/95 mmHg. A total of 15 patients did not complete the 1-month withdrawal phase due to increasing blood pressure >165/95 mmHg or development of peripheral edema (Fig. 1).

Trough office blood pressure (Korotkoff phase I/V) was measured using an appropriate cuff with a sphygmomanometer in the sitting position after at least 10 min of rest. Two readings were recorded 2 min apart, and the average value was used for calculation.

The urinary albumin concentration was determined by nephelometry (8).

GFR was measured after a single intravenous injection of 5 MBq ^{51}Cr-EDTA at 8:00 A.M. by determining the radioactivity in venous blood samples taken 180, 200, 220, and 240 min after the injection, considering sex and body weight of the patient (9–11). The results were standardized for 1.73 m^2 body surface area.

Statistical analysis

Results are presented as mean ± SD or mean ± SE. One-way ANOVA was used to test for differences between treatment groups. The level of urinary albumin excretion was log-transformed before analysis. Pairwise comparisons were performed using Student's *t* test. A *P* value <0.05 indicated statistical significance. All statistical tests were two sided.

RESULTS — Baseline characteristics did not differ between treatment groups. In the placebo, irbesartan 150-mg, and irbesartan 300-mg groups, MABP was similarly lowered from 112 ± 1 (mean ± SE), 111 ± 1, and 112 ± 2 mmHg to 105 ± 2, 103 ± 2, and 102 ± 2 mmHg, respectively, after 24 months of treatment ($P < 0.05$ vs. baseline). Urinary albumin excretion decreased by 8% (95% CI −16 to 27) (NS), 34% (8–53), and 60% (46–70) in the placebo, irbesartan 150-mg, and irbesartan 300-mg groups, respectively ($P < 0.05$). Rates of decline in GFR were 1.3 ± 0.7, 1.2 ± 0.7, and 1.0 ± 0.8 ml · min^{-1} · 1.73 m^{-2} per month in the placebo, irbesartan 150-mg, and irbesartan 300-mg groups, respectively, during the initial 3 months of the study. The sustained decreases in GFR were 0.3 ± 0.1, 0.3 ± 0.1, and 0.4 ± 0.1 ml · min^{-1} · 1.73 m^{-2} per month in the three groups, respectively, during the remaining study period (Fig. 2). One month after withdrawal of all antihypertensive medication, MABP was unchanged in the placebo group, 105 ± 2 mmHg, but increased significantly in the irbesartan groups, to 109 ± 2 and 108 ± 2 mmHg, respectively ($P < 0.01$). Urinary albumin excretion rate increased by 13% (−10 to 42) (NS) in the placebo group, 68% (21–133) ($P < 0.05$) in the irbesartan 150-mg group, and 26% (−14 to 87) (NS) in the irbesartan 300-mg group. Comparing data to baseline levels, urinary albumin excretion rate was insignificantly increased by 14% (−17 to 54) in the placebo group and by 11% (−26 to 65) in the irbesartan 150-mg group but remained persistently reduced by 47% (24–63) in the irbesartan 300-mg group ($P < 0.05$ vs. baseline). The persistent reduction in the irbesartan 300-mg group, as compared with baseline, was highly significantly different from irbesartan 150 mg ($P < 0.01$). GFR levels increased to baseline values, 109 ± 5 ml · min^{-1} · 1.73 m^{-2}, in the placebo group but only approached initial levels in the irbesartan groups, 107 ± 6 and 108 ± 6 ml · min^{-1} · 1.73 m^{-2}, respectively.

Hyperfiltration, defined as GFR exceeding normal values + 2 SD (12), was present at baseline in 30% of the investigated patients. Data are analyzed disregarding treatment groups due to limited numbers of patients with hyperfiltration. No significant differences in rate of decrease in GFR between treatment groups were found (data not shown). Mean GFR at baseline in hyperfiltering patients was 139 ± 3 ml · min^{-1} · 1.73 m^{-2}, compared with 101 ± 2 ml · min^{-1} · 1.73 m^{-2} in patients with normofiltration ($P < 0.01$).

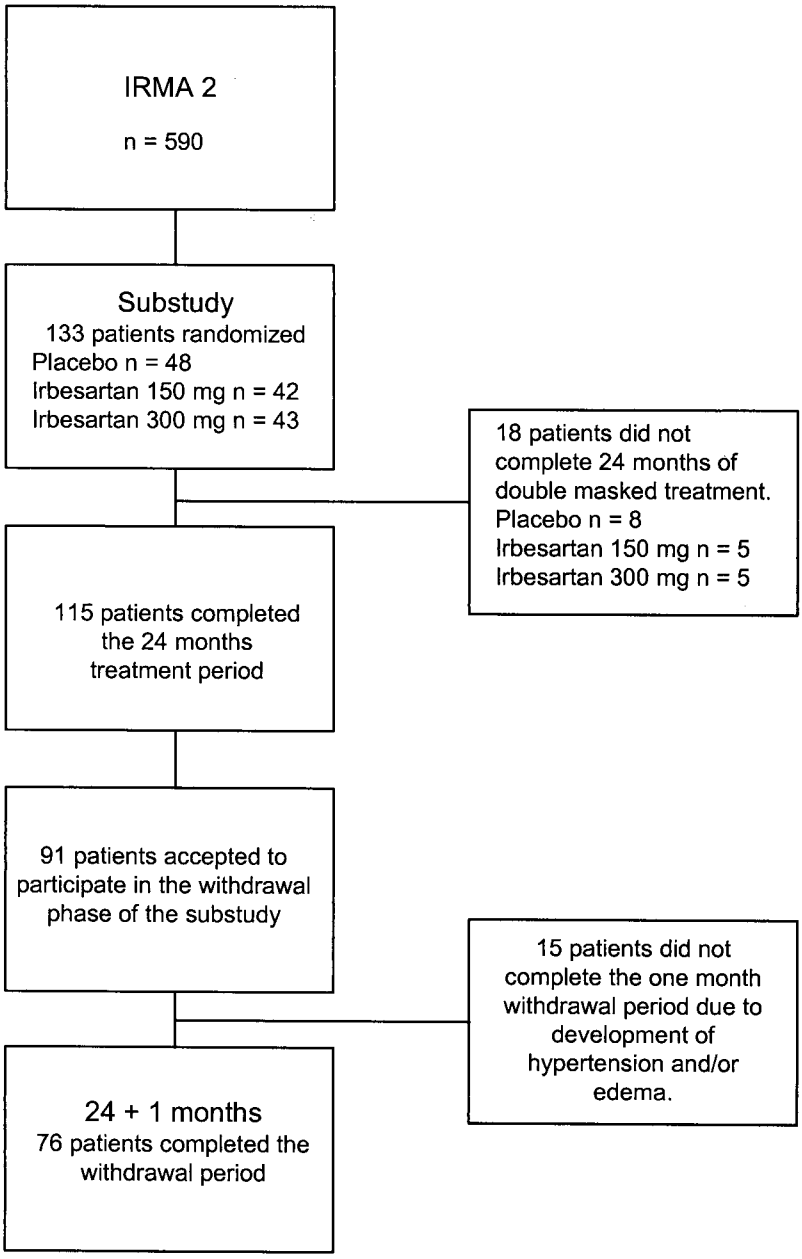

Figure 1—*Profile of the study.*

Rates of decline in GFR were 3.1 ± 0.7 and 0.3 ± 0.4 ml · min^{-1} · 1.73 m^{-2} per month in hyperfiltering and nonhyperfiltering patients, respectively ($P < 0.01$), during the initial 3 months of the study. The sustained decrease in GFR was 0.6 ± 0.1 and 0.3 ± 0.1 ml · min^{-1} · 1.73 m^{-2} per month, respectively, in the remaining study period ($P = 0.05$) (Fig. 3). No significant differences in MABP and urinary albumin excretion rate between the two groups were found. After withdrawal of treatment, GFR increased from 95 ± 4 to 98 ± 4 ml · min^{-1} · 1.73 m^{-2} in normofiltering patients and 118 ± 3 to 125 ± 3 ml · min^{-1} · 1.73 m^{-2} in hyperfiltering patients (Fig. 3).

At baseline, plasma renin levels were 22 ± 2 (geometric mean ± SE), 21 ± 2, and 20 ± 2 mIU/l in the placebo, irbesartan 150-mg, and irbesartan 300-mg groups, respectively. At the end of the study, levels were unchanged in the placebo group, 27 ± 3 mIU/l (NS), but dose-dependently increased in irbesartan 150- and 300-mg groups, to 45 ± 9 and 68 ± 15 mIU/l, respectively ($P < 0.05$ vs. baseline). Plasma angiotensin II levels were 8 ± 1, 7 ± 1, and 8 ± 1 pmol/l, respectively, at baseline. At the end of study, angiotensin II levels were unchanged in the placebo group, 9 ± 1 pmol/l, but significantly increased in the irbesartan 150- and 300-mg groups, to 14 ± 2 and 17 ± 2 pmol/l, respectively ($P < 0.05$ vs. baseline).

Of the 133 patients included in the present substudy, nephropathy developed in 10 patients: 4 patients randomized to placebo and 6 patients in the irbesartan 150-mg group. Considering the group with hyperfiltration, one patient progressed to diabetic nephropathy.

Compliance to study medication was acceptable; by the end of the study, an average of 81% of the irbesartan was taken in the 150-mg group and 89% of the irbesartan was taken in the 300-mg group.

CONCLUSIONS — The present 2-year substudy of the IRMA-2 trial demonstrated a highly significant sustained reduction in urinary albumin excretion rate, even after withdrawal of all antihypertensive treatment in the irbesartan 300-mg o.d. group, whereas the irbesartan 150-mg o.d. group returned to baseline. This difference occurred although the regain in MABP and GFR between the two irbesartan groups was nearly identical. Furthermore, a dose-dependent reduction in urinary albumin excretion rate during irbesartan treatment was demonstrated. Finally, the initial rate of decrease in GFR was significantly greater than the sustained decrease in GFR. These differences were particularly prominent, comparing patients with hyperfiltration and normofiltering patients.

Changes in kidney function after withdrawal of long-term antihypertensive treatment has previously been investigated in patients with incipient and overt diabetic nephropathy (13–16). In microalbuminuric diabetic patients, withdrawal of perindopril or nifedipine after 12 months' treatment was associated with an increase in urinary albumin excretion rate to levels exceeding baseline values for both drugs (13). Our group demonstrated a significant increase in urinary albumin excretion rate after cessation of long-term ACE inhibitor therapy in type 1 diabetic patients with microalbuminuria (15). Similarly, in patients with overt diabetic nephropathy, we found significant

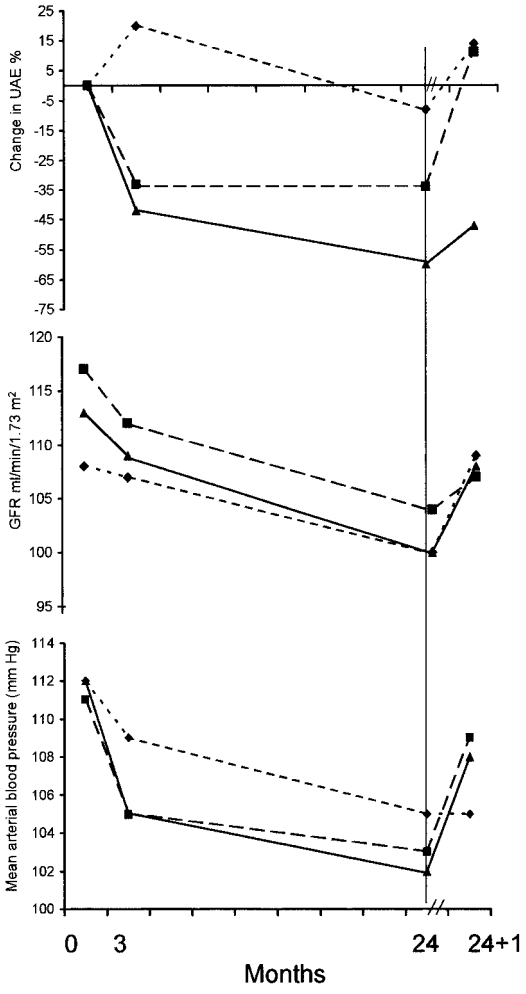

Figure 2—*Changes in urinary albumin excretion rate in relation to baseline* (top)*, cross-sectional values of GFR* (middle)*, and MABP* (bottom) *during treatment with placebo (◆), irbesartan 150 mg (■), and irbesartan 300 mg (▲) and 1 month after withdrawal of all antihypertensive treatment.*

increases in urinary albumin excretion rate after stopping long-term antihypertensive treatment (14,16). Significant increase in urinary albumin excretion rate suggests that systemic and renal hemodynamic mechanisms are primarily responsible for reduction of urinary albumin excretion in these studies.

Baseline albuminuria predicts the rate of decline in GFR in patients with diabetic and nondiabetic renal disease (17,18). The initial reduction in albuminuria after initiation of antihypertensive treatment is predictive of the long-term efficacy of subsequent renoprotection in patients with diabetic and nondiabetic renal disease (19,20). Finally, residual albuminuria during treatment has been shown to predict rate of decline in GFR (21). Therefore, renoprotective therapy should aim to achieve the maximal antiproteinuric effect in addition to reduction of blood pressure (18).

A number of potential mechanisms may be involved in the reduction of urinary albumin excretion rate during blockade of the renin-angiotensin-aldosterone system (RAAS). RAAS blockade has been suggested to influence glomerular capillary pressure, glomerular size/charge selectivity, podocyte function, and slit diaphragm proteins such as nephrin, as reviewed by Parving et al. (in press, *Seminars of Nephrology*). Animal studies of diabetic renal disease have demonstrated that increased glomerular capillary pressure and proteinuria may be prevented by ACE inhibitor therapy (2). In accordance, studies of patients with type 2 diabetes have shown that estimated glomerular capillary pressure and efferent arteriolar resistance were increased in patients with elevated urinary albumin excretion rate compared with normoalbuminuric patients, but were decreased by ACE inhibitor therapy (22). Size selectivity of the glomerular capillary wall may be abnormal in early diabetic nephropathy but may be partly restored by ARB treatment (23). This may be related to modulation of podocyte function, which contributes significantly to the permeability properties of the glomerulus (24). Slit diaphragm function depends on proteins such as nephrin (25). Animal studies in diabetic renal disease have suggested that ARBs can normalize nephrin expression (26).

Angiotensin II induces structural glomerular abnormalities by stimulation of cytokines and growth factors such as transforming growth factor-β (TGF-β). Various in vivo experimental models of renal disease, including diabetes, have demonstrated that blockade of the RAAS decreases the expression of TGF-β and

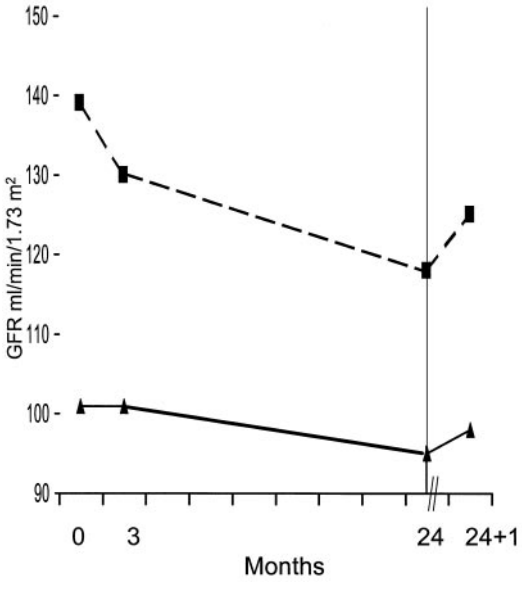

Figure 3—*Cross-sectional values of GFR in patients with hyperfiltration (■) and normofiltration (▲) and 1 month after withdrawal of all antihypertensive treatment.*

matrix proteins (27–30). Similarly, recent human studies demonstrated that plasma and urinary levels of TGF-β were decreased by ARBs (31,32). Furthermore, experimental studies in patients with nondiabetic kidney disease have demonstrated an additional blood pressure–independent reduction in TGF-β during high-dose therapy with ARBs and ACE inhibitors (33). This study shows a clear dissociation between the dose required for maximal blood pressure reduction and the optimal dose of ACE inhibitors and ARBs for inhibition of TGF-β.

Recent data from kidney biopsy studies demonstrated that 2 years of treatment with ACE inhibitors in type 2 diabetic patients with nephropathy improved renal structural abnormalities and was correlated with reduction in proteinuria (34). Similarly, in young type 1 diabetic patients with microalbuminuria, less progression of early glomerulopathy was seen in patients treated with either ACE inhibitors or β-blockers compared with placebo (35). The results from our study may suggest reversal of structural and/or biochemical abnormalities in the glomerular apparatus. However, the exact mechanism involved can only be determined by kidney biopsy studies evaluating the above-mentioned phenomenon quantitatively.

Previous studies in type 1 diabetic patients with diabetic nephropathy suggested that the initial decrease in GFR is reversible and due to functional, hemodynamic effects of antihypertensive treatment (16). In contrast, studies in hypertensive type 2 diabetic patients with diabetic nephropathy indicated that the initial steep decrease in GFR is due to an at least partly irreversible effect of antihypertensive treatment (14). In the present study, the initial decrease in GFR was mainly observed in hyperfiltering patients and regained after withdrawal of treatment and, thus, was related to hemodynamic effects of antihypertensive treatment. Furthermore, hyperfiltering patients were characterized by significantly higher sustained rates of decline in GFR compared with normofiltering patients. Elevated sustained rate decline in GFR in the observation period excludes the possibility of a "regression toward the mean phenomenon." Hyperfiltration has primarily been investigated in type 1 diabetes. Longitudinal studies of hyperfiltration as a putative risk factor for development of diabetic nephropathy in normoalbuminuric or microalbuminuric type 1 diabetic patients have reached conflicting results (36–40). Hyperfiltration may, for a period, be associated with elevated rates of decrease in GFR (41), whereas long-term follow-up has found that hyperfiltration does not predict long-term renal outcome (39). In type 2 diabetes, a cross-sectional study in microalbuminuric patients (42) found an incidence of hyperfiltration of 37% in accordance with the present data, but longitudinal studies in type 2 diabetes are not available. Therefore, whether hyperfiltration is a risk factor for development of diabetic nephropathy in type 2 diabetes or a short-term transient phase is unknown. The present data do not indicate a higher risk of progression of diabetic renal disease in hyperfiltering patients, because diabetic nephropathy developed in only one patient in this group.

Antihypertensive medications have been withdrawn in several previous and recent trials in hypertensive diabetic patients, typically in a 1-month wash-out period or placebo run-in phase before the study to assess baseline values (43–46). Similarly, IRMA-2 was preceded by a 4-week wash-out period of previous antihypertensive medication (5). Withdrawal of antihypertensive treatment for 1 month is justified and essential to compare effects of different drugs before and/or after treatment, provided appropriate safety procedures are applied, as in our study.

The present substudy supports the conclusion from the main IRMA-2 study, that irbesartan 300 mg is superior to irbesartan 150 mg for renoprotection. Low-dose irbesartan treatment may be insufficient for renoprotection because of incomplete blockade of the RAAS, as indicated by the dose-dependent increase in plasma renin concentration. However, dose-titration studies of maximal antialbuminuric dose have not been performed; therefore, doses >300 mg may even be more effective.

In summary, the present substudy of the IRMA-2 trial investigated kidney function during and after withdrawal of long-term antihypertensive treatment. Persistent reduction of microalbuminuria after withdrawal of all antihypertensive treatment suggests that high-dose irbesartan therapy confers long-term renoprotective effects that may reflect reversal of renal structural and/or biochemical abnormalities.

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The following individuals participated in the Irbesartan in Patients with Type 2 Diabetes and Microalbuminuria Study (IRMA-2).

Scientific Committee members: Peter Arner, Prof., MD (Chairman); Hans-Henrik Parving, Prof., MD; Jens Bröchner-Mortensen, MD; Ramon Gomis, Prof., MD; Hendrik Lehnert, Prof., MD; Gérald Frangin, MD; and Magali Grégoire, Biochemist.

Data and Safety Monitoring Committee members: Jean-Pierre Boissel, Prof., MD (Chairman); W. Kiowski, Prof., MD; and Louis Monnier, Prof., MD.

The following individuals participated in the Irbesartan GFR substudy.

Argentina: L.I. Juncos, C. Ferrer, Córdoba; Australia: P. Phillips, J. CheeHong Goh, P. Hoadley, A. Smith, Woodville South; Canada: A. Belanger, R. Dumas, N. Kandalaf, Laval PQ; Denmark: H.-H. Parving, S. Andersen, P. Christensen, P. Hovind, H.P. Hansen, Gentofte; K. Kølendorf, Køge; France: H. Affres, A. Prigent, Dr. Yvart, Le Kremlin-Bicêtre; Greece: E. Diamantopoulos, E. Andreadis, M. Kakou, Athens; Croatia: V. Profozic, I. Nazar, G. Roglic, M. Radman, M. Stenzel, J. Vukovic, Zagreb; Italy: F. Quarello, R. Boero, V. Alfieri, A. Pignataro, Torino; South Africa: L. Distiller, B. Kramer, Johannesburg; U.K.: R.L. Kennedy, J. Bell, Sunderland.

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