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# Enhanced renoprotective effects of ultrahigh doses of irbesartan in patients with type 2 diabetes and microalbuminuria

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**Enhanced renoprotective effects of ultrahigh doses of irbesartan in patients with type 2 diabetes and miroalbuminuria.**

*Background.* The purpose of this study was to evaluate the renoprotective effect as reflected by short-term changes in albuminuria of ultrahigh doses of irbesartan in type 2 diabetic patients with microalbuminuria.

*Methods.* This double-masked randomized crossover trial included 52 (41 males) hypertensive type 2 diabetic patients with microalbuminuria on ongoing antihypertensive medication. At inclusion, previous antihypertensive treatment was discontinued and replaced with bendroflumethiazide, 5 mg once daily, for the entire study. Following 2 months wash-out (baseline), patients were treated randomly with irbesartan 300, 600, and 900 mg once daily, each dose for 2 months. End points evaluated at the end of each study period included urinary albumin excretion rate (UAE) (mean of three 24-hour collections), 24-hour ambulatory blood pressure, and glomerular filtration rate (GFR) [chromium 51 ethylenediaminetetraacetic acid ($^{51}$Cr-EDTA)].

*Results.* Baseline values were: 24-hour UAE [geometric mean (95% CI)] 134 (103 to 170) mg/24 hours, ambulatory blood pressure [mean (SD)] 140 (10)/77 (7) mm Hg, and GFR 103 (19) mL/min/1.73 m$^2$. All doses of irbesartan significantly reduced UAE, ambulatory blood pressure, and GFR from baseline. Reductions in UAE from baseline were 52% (46% to 57%), 49% (43% to 54%), and 59% (54% to 63%) with increasing doses of irbesartan ($P < 0.01$). UAE was reduced significantly more by irbesartan 900 mg compared with lower doses with an additional reduction in UAE of 15% (2% to 26%) by irbesartan 900 mg compared with 300 mg ($P = 0.02$). The greater reduction in albuminuria by irbesartan 900 vs. 300 mg was more pronounced in patients with UAE during irbesartan 300 mg above vs. below the median [31% (18% to 42%) vs. −9% (−25% to 6%), respectively ($P < 0.05$)].

With increasing doses systolic ambulatory blood pressure was reduced from baseline by 8 (4 to 12), 9 (5 to 13), and 9 (5 to13) mm Hg, and diastolic ambulatory blood pressure by 6 (4 to 7), 7 (6 to 9), and 7 (6 to 9) mm Hg (NS between doses).

*Conclusion.* Ultrahigh dosing of irbesartan (900 mg once daily) is generally safe and offers additional renoprotection independent of changes in systemic blood pressure and GFR in comparison to the currently recommended dose of 300 mg.

**Key words:** type 2 diabetes, microalbuminuria, irbesartan, angiotensin II receptor blockade, hypertension, renin, aldosterone.

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At present more than 170 million people worldwide have diabetes and the number is expected to double within the next 20 years mainly due to an epidemic increase in the prevalence of type 2 diabetes [1]. Diabetes is associated with an increased occurrence of cardiovascular disease and approximately 40% of all diabetic patients are at risk of developing diabetic nephropathy which has become the leading cause of end-stage renal disease (ESRD) in the Western world [2]. Therefore, the early identification and subsequent end-organ protective treatment of all patients at risk are of outmost importance. In this regard patients with persistent microalbuminuria [urinary albumin excretion (UAE) between 30 and 300 mg/24 hours] have a 10 to 20 times increased risk of developing diabetic nephropathy as compared to patients with normoalbuminuria [2]. In addition, the occurrence of microalbuminuria is associated with a highly increased risk of premature death due to cardiovascular disease [3].

Reduction of UAE by blockade of the renin-angiotensin-aldosterone system (RAAS) has emerged as a key treatment goal for both reno- and cardiovascular protection [4–10]. Data from the large clinical Irbesartan in Patients with Type 2 Diabetes and Microalbuminuria (IRMA2) Study [11] firmly demonstrated that treatment with the angiotensin II receptor blocker (ARB) irbesartan reduces UAE and the risk of progression to overt diabetic nephropathy in patients with type 2 diabetes and persistent microalbuminuria. Furthermore, in type 2 diabetic patients with more advanced renal disease, ARBs have been shown to reduce the risk of reaching the combined renal end point of doubling in serum creatinine, ESRD, or death [12, 13]. Since 2002, ARBs have consequently been recommended as first-line therapy in hypertensive type 2 diabetic patients with microalbuminuria

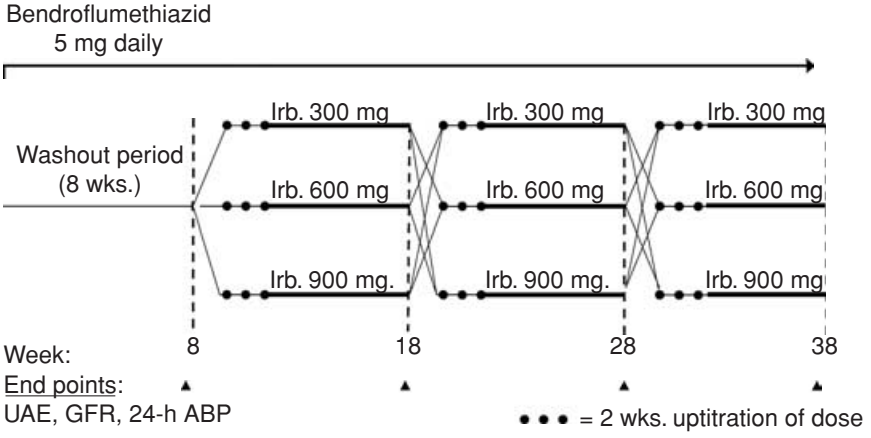


**Fig. 1. Crossover study evaluating the efficacy and safety of irbesartan (Irb) 300, 600. and 900 mg once daily in 52 type 2 diabetic patients with microalbuminuria.** The study consisted of an initial 8-week washout period with discontinuation of all previous antihypertensive medication and with initiation of bendroflumethiazide 5 mg daily in all patients. Following the washout period, patients were treated in random order with irbesartan 300, 600, and 900 mg once daily. Each treatment period consisted of an initial 2 weeks' titration period with irbesartan 300 mg followed by 8 weeks with daily doses of irbesartan of 300, 600, and 900 mg, respectively. End points were evaluated after the initial washout period (baseline and at the end of each of the three treatment periods).

or clinical albuminuria according to guidelines from the American Diabetes Association [14].

Despite the proven benefit of treatment with irbesartan in preventing diabetic nephropathy the optimal renoprotective dose remains unknown. Inadequate dosing may in part be responsible for the development and progression of diabetic renal disease in some patients. In the IRMA2 study, irbesartan 300 mg once daily was superior to irbesartan 150 mg once daily in reducing UAE and preventing the development of overt diabetic nephropathy [11]. However, 2.5% per year of patients developed diabetic nephropathy despite treatment with irbesartan 300 mg once daily in addition to conventional blood pressure–lowering agents. Doses of irbesartan above 300 mg daily have not been evaluated. Consequently it remains unknown if the full renoprotective potential is reached by currently recommended doses, which are based on the blood pressure–lowering effects in patients with primary hypertension [15]. However, higher doses may be needed for a more complete end-organ protection in patients with diabetic renal disease.

The aim of the present study was therefore to evaluate the safety and antiproteinuric efficacy of irbesartan by doses exceeding the present maximum recommended dose of 300 mg once daily in patients with type 2 diabetes and microalbuminuria.

## METHODS

### Patients

All patients were recruited from the Steno Diabetes Center. Patients included in the study were type 2 diabetic patients [World Health Organization (WHO) criteria], with microalbuminuria (persistent UAE between 30 and 300 mg/24 hours, ~ 20 to 200 μg/min) on ongoing antihypertensive medication implying that patients were not excluded if UAE increased above 300 mg/24 hours after withdrawal of previous treatment. Patients were excluded if they had a known nondiabetic kidney or renal tract disease, serum potassium values >4.6 mmol/L, systolic blood pressure <100 mm Hg during antihypertensive treatment or systolic blood pressure persistently >180 mm Hg and/or diastolic blood pressure >105 mm Hg upon discontinuation of previous antihypertensive treatment.

### Design

The study consisted of an 8-week washout period of all previous antihypertensive medication followed by a double-masked randomized crossover trial with three 10-week treatment periods (Fig. 1). Randomization was concealed with computer-generated envelopes. The code was not broken until all data were entered into a database, which was locked for editing.

*The washout period.* Eight weeks prior to randomization all previous antihypertensive medication was discontinued and treatment with bendroflumethiazide 5 mg once daily was initiated in all patients and continued at 5 mg once daily throughout the entire study. During the 8-week washout period, blood pressure was monitored at home by the patients two times daily three times a week with an automatic blood pressure measuring device to ensure that blood pressure did not exceed the safety limit of the study (>180/105 mm Hg).

*Double-blind crossover periods.* All treatment periods were of 10 weeks' duration and consisted of an initial 2-week dose titration period with irbesartan 300 mg once daily followed by 8 weeks' treatment with irbesartan 300, 600, and 900 mg once daily, respectively. All patients received treatment with irbesartan 300, 600, and 900 mg once daily in random order.

For safety reasons arterial blood pressure, plasma potassium, and plasma creatinine were measured 4 weeks after the beginning of each treatment period (2 weeks after the full dose of the treatment period was reached).

End points of the study were evaluated after the washout period (baseline) and at the end of each three treatment periods with irbesartan. The primary end

point was UAE measured as geometric mean from three consecutive 24-hour urinary collections. Secondary end points included 4-hour UAE measured during the clearance procedure, fractional clearance of albumin, ambulatory blood pressure, and glomerular filtration rate (GFR).

The local ethical committee approved the study, and all patients gave their informed consent to participate in the study after the nature of the study had been explained. The study was performed in accordance with the Helsinki Declaration.

### Laboratory procedures

UAE in the 24-hour and 4-hour urine samples was determined by turbidimetry (Hitachi 912 system) (Roche Diagnostics, Mannheim, Germany). Urinary sodium, potassium, creatinine, and urea excretion were determined from the 24-hour urine samples (Hitachi 912 system). To eliminate changes in UAE caused by variation in plasma albumin and GFR, we determined the fractional clearance of albumin (θalbumin) during the 4-hour clearance procedure as [UAE]/[(plasma albumin concentration) × (GFR)], where UAE was measured in the 4-hour urine collection during GFR determination.

Blood pressure was measured by a 24-hour ambulatory blood pressure device (Takeda TM2421) (A & D Medical, Tokyo, Japan). Blood pressure was measured every 15 minutes during the day (7:00 a.m. to 11:00 p.m.) and every 30 minutes during the night (11:00 p.m. to 7:00 a.m.). Values were averaged for each hour before calculating mean day, night, and 24-hour ambulatory blood pressure values.

GFR was measured after a single intravenous injection of 3.7 MBq chromium 51 ethylenediaminetetraacetic acid ($^{51}$Cr-EDTA) at 8:30 a.m. by determining the radioactivity in venous blood samples taken 180, 200, 220, and 240 minutes after injection [16]. The results were standardized for 1.73 $m^2$ body surface area, using the patient's surface area at the start of the study. The mean day-to-day coefficient of variation is 4% in our laboratory.

From venous samples, hemoglobin concentration (Sysmex SF3000) (Sysmex Corporation, Kobe, Japan), and plasma potassium, sodium, creatinine, and cholesterol concentrations were determined (Hitachi 912 system), and hemoglobin $A_{1c}$ ($HbA_{1c}$) was measured by high-performance liquid chromatography (HPLC) (normal range 4.1% to 6.4%) (Tosoh Automated Glycohemoglobin Analyser) (Tosoh Bioscience, Minato, Japan). Blood samples for plasma renin activity and aldosterone concentrations were taken after 30 minutes of supine rest. Plasma renin activity was measured by a method, based on determining by radioimmunoassay, the amount of angiotensin I generated, as previously described [17]. Plasma aldosterone was measured using a commercially available radioimmunoassay (Coat-a-Count) (Diagnostic Products Corporation, Los Angeles, CA, USA).

### Statistical analysis

Before the present study, we calculated the SD (log scale 0.1771) of the mean difference in UAE rate in three consecutive 24-hour urine samples collected twice within 3 months in 36 diabetic patients with diabetic nephropathy. On the basis of these data, a sample-size calculation revealed a necessary minimum of 50 patients to detect a 15% difference in change in UAE rate between any two dose levels (alpha = 0.05 and beta = 0.8). Results are expressed as mean (SE) unless otherwise stated. UAE, fractional clearance of albumin, renin, and aldosterone were logarithmically transformed before analysis due to its skewed distribution and are given as geometric mean with 95% confidence intervals. Comparisons of clinical end points, including UAE, ambulatory blood pressure, and GFR between each treatment period, were performed using linear mixed models [18]. The adapted model was one with fixed effects of treatment level, visit and carryover (i.e., treatment level in the previous period) and a random effect of person included to account for the person dependencies in data. For the simplest models the *P* value and effects correspond to results obtained from paired *t* test and two-way analysis of variance (ANOVA), but these models allow for more elaborate exploration of the material. Tests for presence of effects were performed as likelihood-ratio tests, and final estimates were reported as Restricted Maximum Likelihood (REML) estimates. Linear regression analysis was performed by the least square method. The software used was R version 2.0.1 (*http://www.r-project.org*) and SPSS 13.0 (SPSS, Chicago, IL, USA).

## RESULTS

A total of 58 patients were included in the study. Two patients were excluded during the initial 2-month washout period due to increase in systolic blood pressure above 180 mm Hg. Among the remaining 56 patients who were randomized to treatment with irbesartan, four patients were excluded due to adverse clinical events, which were not considered related to the study medication as described in the safety section below. A total of 52 patients completed the trial and were included in the present analysis. Baseline characteristics of these patients are shown in Table 1.

### Efficacy

UAE, fractional clearance of albumin, 24-hour blood pressure, and GFR were all significantly reduced from baseline by all three doses of irbesartan as shown in Table 2. No significant carryover or treatment sequence effects were found for any of these end points.

**Table 1.** Baseline characteristics of 52 type 2 diabetic patients with microalbuminuria randomized to treatment with irbesartan 300, 600 and 900 mg once daily

| | $N = 52$ |
|---|---|
| Age *years*[a] | 58 (10) |
| Known diabetes duration *years*[a] | 13 (8) |
| Males/females | 41/11 |
| Body mass index $kg/m^2$[a] | 33 (5) |
| Diabetic retinopathy % | |
| Nil | 36 |
| Simplex | 58 |
| Proliferative | 6 |
| Insulin treatment% | 74 |
| Low-dose aspirin% | 94 |
| Lipid-lowering treatment%[b] | 87 |

[a]Values are mean (SD): [b]Predominantly statins.

## UAE

The geometric mean (95% CI) of 24-hour UAE was 134 mg/24 hours (103 to 170) corresponding to 93 μg/min (72 to 118). Reductions of 24-hour UAE from baseline were 52% (46% to 57%), 49% (43% to 54%), and 59% (54% to 63%) for irbesartan 300, 600, and 900 mg daily, respectively ($P < 0.01$). Four-hour UAE and fractional clearance of albumin were reduced to a similar extent by the three doses (Table 2). The reductions from baseline in UAE and fractional clearance of albumin were similar for irbesartan 300 and 600 mg, whereas significantly greater reductions were obtained by irbesartan 900 mg (Table 2). The effect of increasing the dose of irbesartan from 300 to 900 mg was an additional reduction in 24-hour UAE of 15% (95% CI 2% to 26%) from 64 mg/24 hours (49 to 84) to 54 mg/24 hours (43 to 68), in 4-hour UAE of 20% (8% to 31%) from 42 μg/min (31 to 57) to 34 μg/min (26 to 44) and in fractional clearance of albumin of 15% (2% to 26%) ($P < 0.05$ for all). Compared to 600 mg, the effect of 900 mg was an additional reduction in 24-hour UAE of 20% (7% to 31%) from 68 mg/24 hours (53 to 86) to 54 mg/24 hours (43 to 68), 4-hour UAE of 24% (6% to 38%) from 45 μg/min (35 to 60) to 34 μg/min (26 to 44), and in fractional clearance of albumin of 22% (4% to 37%) ($P < 0.05$ for all).

Patients with the greatest additional antiproteinuric effects of the highest dose (reductions in 24-hour UAE above the median of 15% when irbesartan was increased from 300 to 900 mg) were characterized by higher levels of UAE both at baseline and during treatment with irbesartan 300 mg as compared with patients having reductions in 24-hour UAE below the median. No other significant differences were found between these two groups, including gender distribution, known diabetes duration, and body mass index (BMI). In addition, the two groups had similar levels of systemic blood pressure, GFR and aldosterone at baseline and during treatment with irbesartan 300 mg and comparable reductions in blood pressure

**Table 2.** Effects on urinary albumin excretion (UAE), fractional clearance, glomerular filtration rate (GFR), and ambulatory blood pressure of ultrahigh doses of irbesartan in 52 patients with type 2 diabetes and microalbuminuria

| | | Reduction from baseline by irbesartan | | | Reduction by irbesartan 900 mg vs. lower doses | |
|---|---|---|---|---|---|---|
| | Baseline | 300 mg once daily | 600 mg once daily | 900 mg once daily | 900 vs. 300 mg once daily | 900 vs. 600 mg once daily |
| 24-hour UAE | 93 μg/min (72 to 118)[a,b] | 52% (46 to 57) | 49% (43 to 54) | 59% (54 to 63) | 15% (2 to 26)[c] | 20% (8 to 31)[c] |
| 4-hour UAE | 84 μg/min (61 to 117)[a] | 50% (37 to 60) | 47% (33 to 57) | 60% (50 to 68) | 20% (8 to 31)[c] | 24% (6 to 38)[c] |
| θalbumin | 21 (15 to 33)[a] | 48% (33 to 59) | 43% (24 to 57) | 55% (42 to 65) | 15% (2 to 26)[c] | 22% (4 to 37)[c] |
| Glomerular filtration rate mL/min/1.73 $m^2$ | 103 (3) | 4 (1 to 7) | 7 (4 to 11) | 8 (5 to 11) | 4 (1 to 7)[c] | 1 (−2 to 4) |
| Systolic blood pressure mm Hg | | | | | | |
| 24 hours | 140 (2) | 8 (4 to 12) | 9 (5 to 13) | 9 (5 to 13) | 1 (−3 to 5) | 0 (−4 to 5) |
| Day | 146 (2) | 9 (5 to 13) | 10 (6 to 14) | 10 (6 to 14) | 1 (−3 to 6) | 0 (−4 to 4) |
| Night | 127 (2) | 5 (1 to 10) | 6 (1 to 11) | 6 (1 to 11) | 1 (−5 to 7) | 0 (−6 to 6) |
| Diastolic blood pressure *mm Hg* | | | | | | |
| 24 hours | 77 (1) | 6 (4 to 7) | 7 (6 to 9) | 7 (6 to 9) | 2 (−1 to 3) | 0.5 (−1 to 2) |
| Day | 81 (1) | 7 (5 to 8) | 8 (6 to 9) | 8 (7 to 10) | 2 (−1 to 3) | 0.5 (−1 to 2) |
| Night | 70 (1) | 4 (2 to 6) | 5 (3 to 8) | 6 (4 to 8) | 2 (0 to 4)[c] | 0.5 (−2 to 3) |

θalbumin denotes fractional clearance of albumin. Baseline values are mean (SE) and reductions are expressed as mean difference (95% CI). All parameters were significantly changed from baseline by all three doses of irbesartan.
[a]Geometric mean (95% CI); [b]Corresponds to 134 (103 to 170) mg/24 hours; [c]$P < 0.05$ for comparison between two groups.

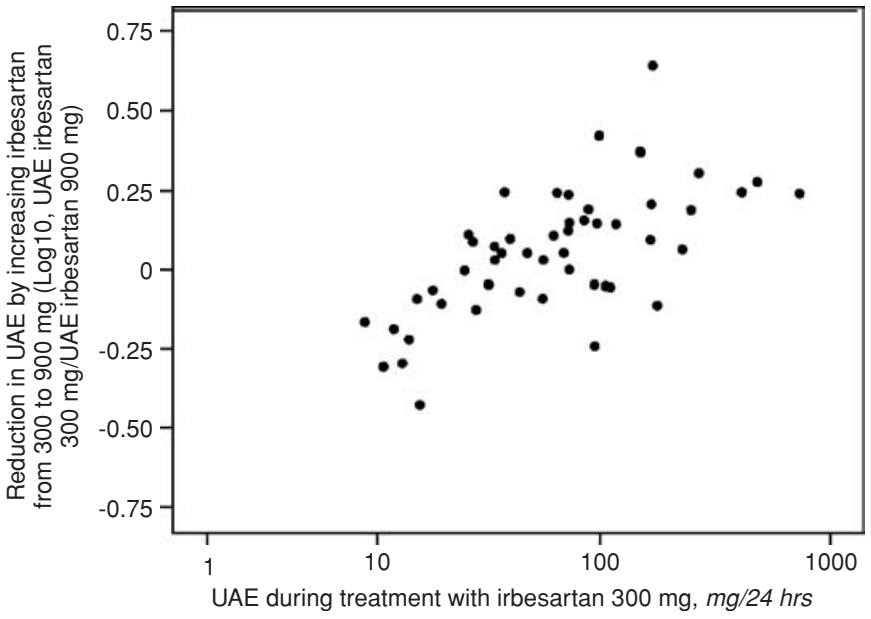


**Fig. 2. Linear regression analysis between urinary albumin excretion rate (UAE) during conventional treatment with irbesartan 300 mg once daily, and the relative change in UAE (log10) when irbesartan was increased from 300 to 900 mg once daily ($r$=0.66, $P$<0.001).** The figure illustrates that the beneficial impact on UAE of 900 mg irbesartan increases with higher levels of UAE during treatment with irbesartan 300 mg.

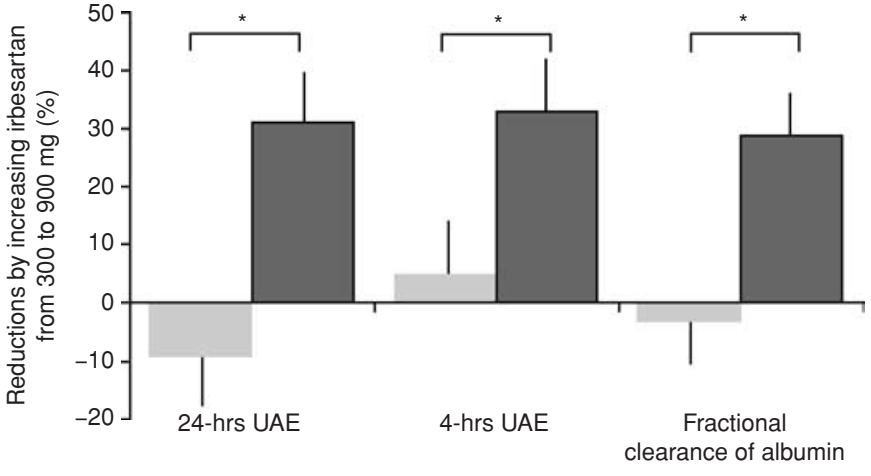


**Fig. 3. Reduction in urinary albumin excretion (UAE) rate and fractional clearance of albumin by increasing the dose of irbesartan from the currently recommended dose of 300 mg daily to 900 mg daily in 52 type 2 diabetic patients with microalbuminuria.** Patients have been stratified according to the median level of 24-hour urinary albumin excretion (71 mg/24 hours) during treatment with irbesartan 300 mg daily. Results for patients with 24-hour UAE above the median during treatment with irbesartan 300 mg once daily are shown by the black bars, whereas the results for patients below the median are shown by the gray bars. *$P$ < 0.05. Bars indicate 95% CIs of the mean difference.

GFR, and aldosterone when irbesartan was increased from 300 to 900 mg.

As illustrated in Figure 2, there was a significant correlation between the level of 24-hrs UAE during treatment with irbesartan 300 mg and the relative reduction of UAE when irbesartan was increased from 300 to 900 mg ($r$ = 0.66, $P$ < 0.001) suggesting that the higher the level of UAE during conventional treatment with irbesartan 300 mg the greater the antiproteinuric response by increasing the dose of irbesartan to 900 mg. As also depicted on Figure 2, the majority of the patients who did not have an additional reduction in UAE when irbesartan was increased from 300 to 900 mg, had UAE levels in the normoalbuminuric range already on 300 mg irbesartan. When stratifying patients above and below the median value of 24-hour UAE of 71 mg/24 hours during treatment with irbesartan 300 mg, patients above the median had an additional reduction in 24-hour and 4-hour UAE and fractional clearance of approximately 30% as compared to the lack of effects in patients below the median (Fig. 3).

There was also a significant correlation between baseline level of 24-hour UAE and the additional reduction in 24-hour UAE by increasing the dose from 300 to 900 mg ($r$ = 0.63, $P$ < 0.03). When stratifying patients above and below the median baseline level of 24-hour UAE of 93 mg/24 hours, the mean reduction (95% CI) in 24-hour UAE upon increasing the dose from 300 to 900 mg were 23% (5% to 38%) vs. 1 (−15% to 14%) ($P$ < 0.05) for patients above vs. below the median, respectively. Similar results were obtained for fractional clearance of albumin.

The intraindividual variation of 24-hour UAE did not correlate to the level of UAE, at any of the dose levels of irbesartan (data not shown).

**Table 3.** Effects on laboratory data of ultrahigh doses of irbesartan in 52 patients with type 2 diabetes and microalbuminuria

| | | Irbesartan | | |
|---|---|---|---|---|
| | Baseline | 300 mg once daily | 600 mg once daily | 900 mg once daily |
| Renin activity n*g/mL/hour* | 5 (4 to 6) | 17 (13 to 23)[a] | 22 (16 to 30)[a,b] | 26 (19 to 35)[a,b] |
| Aldosterone *pg/mL* | 72 (60 to 86) | 67 (55 to 81) | 51 (40 to 67)[a] | 48 (36 to 63)[a] |
| Hemglobin $A_{1c}$% | 8.4 (0.2) | 8.2 (0.2) | 8.3 (0.2) | 8.2 (0.2) |
| Plasma potassium mmol/L | 3.6 (0.04) | 3.9 (0.04)[a] | 3.9 (0.05)[a] | 4.0 (0.05)[a,b,c] |
| Plasma sodium mmol/L | 138.5 (0.3) | 137.5 (0.4)[a] | 137.7 (0.4)[a] | 137.5 (0.4)[a] |
| 24-hour urinary sodium excretion mmol/L | 203 (10) | 213 (12) | 215 (11) | 216 (10) |
| Hemoglobin mmol/L | 8.7 (0.1) | 8.2 (0.1)[a] | 8.2 (0.1)[a] | 8.1 (0.1)[a,b,c] |
| Plasma total cholesterol mmol/L | 4.32 (0.14) | 4.04 (0.14)[a] | 4.18 (0.10)[a] | 4.00 (0.09)[a] |
| Plasma low-density lipoprotein cholesterol | 2.61 (0.11) | 1.88 (0.09)[a] | 2.03 (0.09)[a] | 1.94 (0.09)[a] |
| Plasma high-density lipoprotein cholesterol | 1.21 (0.05) | 1.25 (0.05)[a] | 1.25 (0.05)[a] | 1.24 (0.05)[a] |
| Plasma very low-density lipoprotein cholesterol | 0.84 (0.06) | 0.77 (0.06) | 0.82 (0.06) | 0.75 (0.06)[a] |
| Plasma triglycerides | 2.05 (0.20) | 1.87 (0.18) | 1.94 (0.17) | 1.81 (0.17) |

Values are mean (SE).
[a] $P < 0.05$ vs. baseline; [b] $P < 0.05$ vs. irbesartan 300 mg; [c] $P < 0.05$ vs. irbesartan 600 mg.

### Arterial blood pressure

The mean 24-hour blood pressure was reduced from baseline by 8 (4 to 12) systolic and 6 (4 to 7) mm Hg diastolic during treatment with irbesartan 300 mg with no significant additive effect of increasing the dose further (Table 2). Besides a slightly lower diastolic blood pressure of 2 mm Hg during the night by irbesartan 900 mg as compared with 300 mg, no other significant differences between the three doses were seen in arterial blood pressure during the day or night (Table 2).

### GFR

GFR was reduced from baseline by 4 mL/min/1.73 $m^2$ (95% CI 1 to 7), 7 mL/min/1.73 $m^2$ (4 to 11), and 8 mL/min/1.73 $m^2$ (5 to 11) by increasing doses of irbesartan ($P < 0.05$). There was a significantly greater decrease in GFR of 3 to 4 mL/min/1.73 $m^2$ by the two highest doses as compared with the lowest dose ($P < 0.05$), with no significant difference between the higher doses (Table 2).

### Renin and aldosterone

There was a clear significant dose-dependent increase in plasma renin activity from 5 ng/mL/hour (95%CI 4 to 6) at baseline to 17 ng/mL/hour (13 to 23), 22 ng/mL/hour (16 to 30), and 26 ng/mL/hour (19 to 35) at 300, 600, and 900 mg, respectively ($P < 0.05$). There was a similar graded decrease in plasma aldosterone from 72 pg/mL (60 to 86) at baseline to 67 pg/mL (55 to 81), 51 pg/mL (40 to 67), and 48 pg/mL (36 to 63) with increasing doses of irbesartan (NS for irbesartan 300 mg vs. baseline, and $P < 0.05$ for irbesartan 600 and 900 mg vs. baseline) (Table 3). In linear regression analysis changes in plasma renin activity from baseline to each dose level of irbesartan correlated significantly to changes in UAE ($P$ values <0.05) but changes in plasma renin activity explained only a small proportion of the variation in UAE ($r$ values between 0.10 and 0.15). There were no significant correlations between changes in circulatory aldosterone levels from baseline to each dose level of irbesartan and changes in UAE.

### $HbA_{1c}$, sodium, and lipids

$HbA_{1c}$ was 8.4% (SE 0.2) at baseline and did not change during treatment (Table 3). Plasma sodium decreased slightly from approximately 139 mmol/L at baseline to 138 mmol/L during irbesartan treatment with no significant differences between doses (Table 3). The increase in urinary sodium excretion during irbesartan treatment was not statistically significant from baseline (Table 3). A dose-independent improved lipid profile was obtained during irbesartan treatment with decreased total- and low-density lipoprotein (LDL) cholesterol, and increased high-density lipoprotein (HDL) cholesterol levels (Table 3).

### Safety

A total of four patients were excluded due to clinical adverse events during treatment with irbesartan. One 63-year-old woman was excluded due to acute myocardial infarction while receiving irbesartan 300 mg, respectively, and two male patients (70 and 72 years of age, respectively) were excluded due to ischemic stroke while receiving irbesartan 300 and 900 mg, respectively. None of these cardiovascular events were considered due to changes in systemic arterial blood pressure upon treatment with irbesartan since all patients had stable arterial blood pressure between 110 to 170 mm Hg systolic and 60 to 90 mm Hg diastolic before and during the study. One patient discontinued the study medication after 2 weeks on irbesartan 900 mg before any clinical examination was performed due to complaints of dizziness and general discomfort. Among the 52 patients completing the study, seven patients complained of mild and transient dizziness upon irbesartan treatment: one patient during

300 mg, three patients during 600 mg, and three during 900 mg.

There was a significant increase in baseline plasma potassium of 0.3 mmol/L during treatment with irbesartan 300 and 600 mg and by 0.4 mmol/L during treatment with 900 mg (Table 3). However, none of the patients included in the study developed hyperkalemia (plasma potassium >5.5 mmol/L). Plasma hemoglobin decreased significantly from 8.7 mmol/L at baseline to 8.2 mmol/L during treatment with 300 and 600 mg and to 8.1 mmol/L during irbesartan 900 mg (Table 3).

## DISCUSSION

Our randomized double-blind crossover trial of 52 type 2 diabetic patients with microalbuminuria, demonstrates that the full antiproteinuric potential of the ARB irbesartan is not reached at the currently recommended dose of 300 mg once daily. Further increase of the dose up to 900 mg once daily resulted in a more complete RAAS blockade and additional reduction of UAE. The greater reduction of UAE by higher doses of irbesartan was independent of reductions in systemic blood pressure, which was largely unaffected by doses exceeding 300 mg once daily of irbesartan. Our study also suggests that patients with the highest level of UAE during treatment with irbesartan 300 mg are more likely to have additional benefit from increased dosing. We also demonstrated a dose-independent improvement in lipid profiles upon irbesartan treatment with decreased total cholesterol and LDL cholesterol. Since arterial blood pressure, albuminuria, and hyperlipidimia are established surrogate end points for long-term cardiovascular outcome in diabetic patients, our data suggest that blockade of the RAAS may offer cardiovascular protection in these patients.

In our study the effects of each dose level were evaluated after 2 weeks up-titration with irbesartan 300 mg followed by 8 weeks treatment at the full dose. Therefore, if the maximal reduction in UAE and blood pressure is not reached within 8 weeks, our results would underestimate the true treatment effect. However, previous studies of patients with both diabetic and nondiabetic nephropathy have demonstrated that the maximal antiproteinuric and antihypertensive effect by inhibition of the RAAS is reached 3 to 4 weeks after initiation of therapy [19, 20]. Furthermore, the reduction in UAE after 10 weeks of treatment with irbesartan 300 mg in our study was even greater than the 38% reduction obtained after 2 years of treatment with irbesartan 300 mg in the IRMA2 trial [11]. Another potential bias in crossover studies relates to the possible carryover effect, which would occur in the present study, if the effect on the end points evaluated of a given dose persists for more than 10 weeks after discontinuation of the treatment. Such a carryover effect would lead to an underestimation of the treatment effects preferentially of higher doses. However, we could not detect any significant carryover or time-sequence effect in the statistical analysis of the present study or in our previous studies of RAAS inhibition also applying similar treatment intervals [21–23].

Several studies have demonstrated that the baseline level of UAE as well as the initial reduction in albuminuria upon initiation of antihypertensive treatment are strong predictors of the long-term cardiovascular and renal outcome independent of changes in arterial blood pressure (i.e., the greater the short-term reduction in UAE the lower the long-term cardiovascular risk and the slower the progression of renal disease [4–10]). In this regard our data suggest that the full reno- and cardiovascular protective potential of agents blocking the RAAS may not be reached in patients with diabetic renal disease when recommended doses of these agents are extrapolated from their blood pressure–lowering properties, which is currently the case for all angiotensin-converting enzyme (ACE) inhibitors and ARBs used for renoprotection. By exceeding currently recommended maximum dose, our results extend findings in previous dose-response studies of ACE inhibitors and ARBs which has demonstrated that within the currently recommended dose interval higher doses provide greater antiproteinuric effects than lower doses [11, 21, 24, 25]. The only other study of antiproteinuric effects of supramaximal doses of ARBS is a nonrandomized open-labeled study of ten older patients with heavy proteinuria (>1.5 g/day) of different etiology which in line with our findings demonstrated an additional reduction in proteinuria by exceeding the currently recommended maximum dose of the ARB candesartan [26].

Several underlying mechanisms may explain the blood pressure–independent antiproteinuric effects of agents blocking the RAAS seen in our and several previous studies [11–13, 27]. These include reduced intraglomerular hydraulic pressure independent of reduction of systemic blood pressure by vasodilatation preferentially of the postglomerular arterioles [28], and improved perms-elective properties of the glomerular membrane [29]. In addition, ARBs may prevent the occurrence of proteinuria by reducing the loss of glomerular nephrin [30], and by reducing renal levels of prosclerotic cytokines such as transforming growth factor-β (TGF-β) and connective tissue growth factor (CTGF) [31]. Increased RAAS activity and augmented angiotensin II receptor density in the diseased renal tissue together with reduced penetration of the drug may explain that higher doses are needed for complete RAAS blockade in the tissue responsible for antiproteinuric effects as compared to circulatory levels regulating systemic blood pressure.

The additional reduction in plasma aldosterone found in our study by increasing the dose of irbesartan above 300 mg once daily may provide further benefits in

retarding progression of renal disease and reducing cardiovascular risk. There is an accumulating amount of data suggesting that not only angiotensin II but also aldosterone plays an important role for progression of renal and cardiovascular disease [32–35].

The decrease in levels of cholesterol in our study was obtained despite treatment with lipid-lowering drugs which included mainly statins in the majority of the patients. Changes in cholesterol levels were in the same order of magnitude as reported in a recent open-labeled study of 16,600 type 2 diabetic patients treated with irbesartan 300 mg daily [36].

High-dose irbesartan treatment was generally well tolerated in our study. Three serious cardiovascular events occurred among 56 patients randomized to 30 weeks of irbesartan treatment which corresponds to an annual cardiovascular event rate of approximately 9%. This is in the same order of magnitude as reported previously in type 2 diabetic patients with microalbuminuria [37], and reflects the high cardiovascular risk among these patients rather than adverse events caused by the study medication. The safety of the currently recommended doses of irbesartan up to 300 mg once daily is well documented in diabetic patients [11, 13, 36]. Higher doses have not been evaluated previously in diabetic renal disease but short-term studies of patients with essential hypertension have demonstrated good tolerability and a placebo-like safety of irbesartan in doses up to 900 mg. In these studies side effects were dose-independent [38]. A recent short-term safety study of 12 patients with various forms of chronic renal diseases with severe proteinuria also demonstrated good tolerability of the ARB candesartan in doses five times higher than the currently approved maximum dose [39]. However, before extending the currently recommended dose range of ARBs, the long-term safety still remains to be carefully established.

In our study treatment with irbesartan induced a significant increase in plasma potassium and a reduction in hemoglobin. However, these changes were only marginally greater when exceeding the currently recommended dose of irbesartan. Of note, none of the patients developed hyperkalemia, which may in part be due to the normal levels of GFR among the patients included in the study and due to the concomitant use of diuretics in all patients. Diuretics was also given to control blood pressure and to reduce the risk of cardiovascular events [40] and edema formation during the trial and diminishing the influence of varying dietary salt intake on the effects of irbesartan [41, 42]. The reduction in hemoglobin is in agreement with previous findings [21] and is likely a direct consequence of blocking the actions of angiotensin II, which is known to stimulate erythropoietin synthesis [43]. The decreased GFR during irbesartan treatment represents a beneficial functional hemodynamic consequence of the reduced systemic and glomerular blood pressure. It does not indicate permanent renal function loss as it is fully reversible upon withdrawal of treatment as demonstrated previously [44] and as indicated in our study by the lack of carryover effects on GFR.

## CONCLUSION

Our study has demonstrated that the full antiproteinuric dose or irbesartan is not reached at the recommended dose of 300 mg once daily in type 2 diabetic patients with microalbuminuria. Increasing the dose up to 900 mg once daily leads to a more complete RAAS blockade with additional reduction of albuminuria independent of changes in systemic blood pressure and GFR. Patients with the highest levels of UAE during treatment with irbesartan 300 mg having the poorest renal and cardiovascular prognosis are more likely to have additional benefit from increased dosing. Future studies are needed to evaluate if this finding can be extended to an equal or perhaps even greater additional effect in patients with overt diabetic nephropathy. Additional studies would also be needed to establish if further antiproteinuric effects can be obtained by even higher doses since a plateau of the dose-response curve was not reached in our study even though irbesartan was given in doses well beyond the currently recommended dose.

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## REFERENCES

1. Wild S, Roglic G, Green A, *et al*: Global prevalence of diabetes—Estimates for the year 2000 and projections for 2030. *Diabetes Care* 27:1047–1053, 2004
2. Parving H-H, Mauer M, Ritz E: Diabetic nephropathy (chapter 38), in *Brenner and Rector's the Kidney*, 7th ed., edited by Brenner BM, Philadelphia, WB Saunders, 2004, pp 1777–1818
3. Mogensen CE: Microalbuminuria predicts clinical proteinuria and early mortality in maturity onset diabetes. *N Engl J Med* 310:356–360, 1984
4. Rossing P, Hommel E, Smidt UM, *et al*: Reduction in albuminuria predicts a beneficial effect on diminishing the progression of human diabetic nephropathy during antihypertensive treatment. *Diabetologia* 37:511–516, 1994
5. Apperloo AJ, de Zeeuw D, de Jong PE: Short-term antiproteinuric response to antihypertensive treatment predicts long-term GFR decline in patients with non-diabetic renal disease. *Kidney Int* 45 (Suppl 45):S174–S178, 1994
6. de Zeeuw D, Remuzzi G, Parving H-H, *et al*: Albuminuria, a therapeutic target for cardiovascular protection in type 2 diabetic patients with nephropathy. *Circulation* 110:921–927, 2004

7. de Zeeuw D, Remuzzi G, Parving H-H, *et al*: Proteinuria, a target for renoprotection in patients with type 2 diabetic nephropathy: Lessons from RENAAL. *Kidney Int* 65:2309–2320, 2004
8. Ibsen H, Olsen MH, Wachtell K, *et al*: Reduction in albuminuria translates to reduction in cardiovascular events in hypertensive patients: Losartan intervention for endpoint reduction in hypertension study. *Hypertension* 45:198–202, 2005
9. Weir MR: Reduction in microalbuminuria: A biomeasure of therapeutic success? *Hypertension* 45:181–182, 2005
10. Remuzzi G, Bertani T: Is glomerulosclerosis a consequence of altered glomerular permeability to macromolecules? *Kidney Int* 38:384–394, 1990
11. Parving H-H, Lehnert H, Bröchner-Mortensen J, *et al*: The effect of irbesartan on the development of diabetic nephropathy in patients with type 2 diabetes. *N Engl J Med* 345:870–878, 2001
12. Brenner BM, Cooper ME, de Zeeuw D, *et al*: Effects of losartan on renal and cardiovascular outcomes in patients with type 2 diabetes and nephropathy. *N Engl J Med* 345:861–869, 2001
13. Lewis EJ, Hunsicker LG, Clarke WR, *et al*: Renoprotective effect of the angiotensin-receptor antagonist irbesartan in patients with nephropathy due to type 2 diabetes. *N Engl J Med* 345:851–860, 2001
14. American Diabetes Association: Diabetic nephropathy. *Diabetes Care* 25:585–589, 2002
15. Reeves RA, Lin CS, Kassler-Taub K, *et al*: Dose-related efficacy of irbesartan for hypertension—An integrated analysis. *Hypertension* 31:1311–1316, 1998
16. Bröchner-Mortensen J, Rödbro P: Selection of routine method for determination of glomerular filtration rate in adult patients. *Scand J Clin Lab Invest* 36:35–45, 1976
17. Derkx FHM, Tantjiong L, Wenting GJ, *et al*: Asynchronous changes in prorenin and renin secretion after captopril in patients with renal-artery stenosis. *Hypertension* 5:244–256, 1983
18. Pinhero JC, Bates DM: *Mixed-Effects Models in S and S-Plus*, New York, Springer, 2000
19. Andersen S, Jacobsen P, Tarnow L, *et al*: Time course of the antiproteinuric and antihypertensive effect of losartan in diabetic nephropathy. *Nephrol Dial Transplant* 18:293–297, 2003
20. Buter H, Navis G, Dullaart RP, *et al*: Time course of the antiproteinuric and renal haemodynamic responses to losartan in microalbuminuric IDDM. *Nephrol Dial Transplant* 16:771–775, 2001
21. Rossing K, Christensen PK, Hansen BV, *et al*: Optimal dose of candesartan for renoprotection in type 2 diabetic patients with nephropathy: A double-blind randomized cross-over study. *Diabetes Care* 26:150–155, 2003
22. Jacobsen P, Andersen S, Jensen BR, *et al*: Additive effect of ACE inhibition and angiotensin II receptor blockade in type I diabetic patients with diabetic nephropathy. *J Am Soc Nephrol* 14:992–999, 2003
23. Rossing K, Jacobsen P, Pietraszek L, *et al*: Renoprotective effects of adding angootensin II receptor blocker to maximal recommended doses of ACE inhibitor in diabetic nephropathy—A randomized double-blind crossover trial. *Diabetes Care* 26:2268–2274, 2003
24. Andersen S, Rossing P, Juhl TR, *et al*: Optimal dose of losartan for renoprotection in diabetic nephropathy. *Nephrol Dial Transplant* 17:1413–1418, 2002
25. Laverman GD, Navis G, Henning RH, *et al*: Dual renin-angiotensin system blockade at optimal doses for proteinuria. *Kidney Int* 62:1020–1025, 2002
26. Weinberg MS, Weinberg AJ, Cord R., *et al*: The effect of high-dose angiotensin II receptor blockade beyond maximal recommended doses in reducing urinary protein excretion. *JRAAS* (Suppl 1):196–198, 2001
27. Viberti G, Wheeldon NM: Microalbuminuria reduction with valsartan in patients with type 2 diabetes mellitus—A blood pressure-independent effect. *Circulation* 106:672–678, 2002
28. Imanishi M, Yoshioka K, Konishi Y, *et al*: Glomerular hypertension as one cause of albuminuria in type II diabetic patients. *Diabetologia* 42:999–1005, 1999
29. Andersen S, Blouch K, Bialek J, *et al*: Glomerular permselectivity in early stages of overt diabetic nephropathy. *Kidney Int* 58:2129–2137, 2000
30. Bonnet F, Cooper ME, Kawachi H, *et al*: Irbesartan normalises the deficiency in glomerular nephrin expression in a model of diabetes and hypertension. *Diabetologia* 44:874–877, 2001
31. MacIsaac RJ, Jerums G, Cooper ME: New insights into the significance of microalbuminuria. *Curr Opin Nephrol Hypertens* 13:83–91, 2004
32. Epstein M: Aldosterone as a mediator of progressive renal disease: Pathogenetic and clinical implications. *Am J Kidney Dis* 37:677–688, 2001
33. Schjoedt KJ, Andersen S, Rossing P, *et al*: Aldosterone escape during angiotensin II receptor blockade in diabetic nephropathy is associated with enhanced decline in GFR. *Diabetologia* 47:A88–A89, 2004
34. Chrysostomou A, Becker G: Spironolactone in addition to ACE inhibition to reduce proteinuria in patients with chronic renal disease. *N Engl J Med* 345:925–926, 2001
35. Rachmani R, Slavachevsky I, Amit M, *et al*: The effect of spironolactone, cilazapril and their combination on albuminuria in patients with hypertension and diabetic nephropathy is independent of blood pressure reduction: a randomized controlled study. *Diabetic Med* 21:471–475, 2004
36. Bramlage P, Pittrow D, Kirch W: The effect of irbesartan in reducing cardiovascular risk in hypertensive type 2 diabetic patients: an observational study in 16,600 patients in primary care. *Curr Med Res Opin* 20:1625–1631, 2004
37. Gaede P, Vedel P, Larsen N, *et al*: Multifactorial intervention and cardiovascular disease in patients with type 2 diabetes. *N Engl J Med* 348:383–393, 2003
38. Simon TA, Gelarden T, Freitag SA, *et al*: Safety of irbesartan in the treatment of mild to moderate systemic hypertension. *Am J Cardiol* 82:179–182, 1998
39. Weinberg AJ, Zappe DH, Ashton M, *et al*: Safety and tolerability of high-dose angiotensin receptor blocker therapy in patients with chronic kidney disease: A pilot study. *Am J Nephrol* 24:340–345, 2004
40. The ALLHAT Officers and Coordinators for the ALLHAT Collaborative Research Group: Major outcomes in high-risk hypertensive patients randomized to angiotensin-converting enzyme inhibitor or calcium channel blocker vs. diuretic: The Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial (ALLHAT). *JAMA* 288:2981–2997, 2002
41. Singer DRJ, Markandu ND, Cappuccio FP, *et al*: Reduction of salt intake during converting-enzyme-inhibitor treatment compared with addition of a thiazide. *Hypertension* 25:1042–1044, 1995
42. Buter H, Hemmelder MH, Navis G, *et al*: The blunting of the antiproteinuric efficacy of ACE inhibition by high sodium intake can be restored by hydrochlorothiazide. *Nephrol Dial Transplant* 13:1682–1685, 1998
43. Freudenthaler SM, Schreeb K, Korner T, *et al*: Angiotensin II increases erythropoietin production in healthy human volunteers. *Eur J Clin Invest* 29:816–823, 1999
44. Andersen S, Brochner-Mortensen J, Parving H-H: Kidney function during and after withdrawal of longterm irbesartan treatment in patients with type 2 diabetes and microalbuminuria. *Diabetes Care* 26:3296–3302, 2003