

Irbesartan Treatment Reduces Biomarkers of Inflammatory Activity in Patients With Type 2 Diabetes and Microalbuminuria

An IRMA 2 Substudy

Frederik Persson,¹ Peter Rossing,¹ Peter Hovind,¹ Coen D.A. Stehouwer,² Casper Schalkwijk,² Lise Tarnow,¹ and Hans-Henrik Parving^{1,3}

The impact of irbesartan treatment on biomarkers of low-grade inflammation, endothelial dysfunction, growth factors, and advanced glycation end products (AGEs) during the Irbesartan in Patients with Type 2 Diabetes and Microalbuminuria (IRMA 2) study was evaluated. IRMA 2 was a 2-year multicenter, randomized, double-blind trial in patients comparing irbesartan (150 or 300 mg once daily) versus placebo. The primary end point was onset of overt nephropathy. A subgroup ($n = 269$, 68%) was analyzed for biomarkers at baseline and after 1 and 2 years. High-sensitivity C-reactive protein (hs-CRP), interleukin (IL)-6, fibrinogen, adhesion molecules, transforming growth factor- β , and AGE peptides were assessed. Irbesartan treatment yielded significant changes in hs-CRP (based on generalized estimating equation regression coefficient) with a 5.4% decrease per year versus a 10% increase per year in the placebo group ($P < 0.001$). Fibrinogen decreased 0.059 g/l per year from baseline versus placebo's 0.059 g/l increase per year ($P = 0.027$). IL-6 showed a 1.8% increase per year compared with placebo's 6.5% increase per year ($P = 0.005$). Changes in IL-6 were associated with changes in albumin excretion ($P = 0.04$). There was no treatment effect on the other biomarkers. Irbesartan (300 mg once daily) reduces low-grade inflammation in this high-risk population, and this may reduce the risk of micro- and macrovascular disease. *Diabetes* 55:3550–3555, 2006

From the ¹Steno Diabetes Center, Gentofte, Denmark; the ²Department of Medicine, University Hospital Maastricht, Maastricht, the Netherlands; and the ³Diabetes Center, Institution for Faculty of Health Sciences, Aarhus, Denmark.

Address correspondence and reprint requests to Frederik Persson, Steno Diabetes Center, Niels Steensensvej 2, DK-2820 Gentofte, Denmark. E-mail: frip@steno.dk.

Received for publication 16 June 2006 and accepted in revised form 21 August 2006.

H.-H.P. has been an advisor for Merck, Pfizer, Novartis, and Sanofi-Aventis, has received research grant support from Sanofi-Aventis, and owns stock in Merck and Novo Nordisk.

AGE, advanced glycation end product; AT₁, angiotensin II type 1; GEE, generalized estimating equation; hs-CRP, high-sensitivity C-reactive protein; IL, interleukin; IRMA 2, Irbesartan in Patients with Type 2 Diabetes and Microalbuminuria; RAAS, renin-angiotensin-aldosterone system; REVERSAL, Reversal of Atherosclerosis with Aggressive Lipid Lowering; sICAM-1, soluble intercellular adhesion molecule-1; sVCAM-1, soluble vascular cell adhesion molecule-1; TGF, transforming growth factor.

DOI: 10.2337/db06-0827. Clinical trial reg. no. NCT00317915, clinicaltrials.gov.

© 2006 by the American Diabetes Association.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

In diabetes, the activity of the renin-angiotensin-aldosterone system (RAAS) is elevated in circulation and various tissues and organs (1–3). The enhanced RAAS activity plays an important role in the hemodynamic and the nonhemodynamic pathogenetic mechanisms involved in kidney and cardiovascular disorders.

Although interventions that diminish RAAS activity in diabetes have been shown to be beneficial (4–6), the mechanisms remain incompletely understood. In this context, it is relevant that the pathogenesis of micro- and macroalbuminuria in diabetes may involve endothelial dysfunction, low-grade inflammation, growth factors such as transforming growth factor (TGF)- β , and advanced glycation end product (AGE) peptides (7,8) and that all of these have been postulated to be affected by interventions in the RAAS (7,9–14). In addition, these mechanisms, especially endothelial dysfunction and low-grade inflammation, may also be important in explaining the link between microalbuminuria and risk of fatal and nonfatal cardiovascular disease (15–17).

The Irbesartan in Patients with Type 2 Diabetes and Microalbuminuria (IRMA 2) study showed that inhibiting the angiotensin II type 1 (AT₁) receptor with 300 mg irbesartan once daily protected against developing overt nephropathy (18), and other trials have shown that blocking the AT₁ receptor in patients with type 2 diabetes and diabetic nephropathy reduces progression of nephropathy and some cardiovascular outcomes, particularly hospitalization for heart failure (19,20). In 60 mostly nondiabetic patients with established coronary artery disease, irbesartan treatment has shown beneficial effect on markers of low-grade inflammation and endothelial dysfunction (9). However, the impact of blocking the RAAS on markers of inflammation, endothelial dysfunction, TGF- β , and AGE peptides in patients with type 2 diabetes with microalbuminuria has not been investigated.

The aim of this post hoc analysis from the IRMA 2 study population was to investigate if irbesartan treatment had an impact on the biomarker levels compared with placebo and, in addition, to investigate whether the favorable effect of irbesartan on urinary albumin excretion rate seen in the principal core study could be due to amelioration of one or more biomarkers.

TABLE 1
Baseline characteristics of 269 patients with type 2 diabetes and microalbuminuria

Characteristics	Irbesartan group (300 mg)	Placebo group	<i>P</i>
<i>n</i>	143	126	
Age (years)	57.3 ± 8.0	58.4 ± 9.0	0.30
Male sex	100 (69.9)	84 (66.7)	0.57
Caucasian	138 (96.5)	125 (99.2)	0.22
Known diabetes duration (years)	9.1 (0–36)	9.9 (0–36)	0.35
Smoking	22 (15.4)	24 (19.0)	0.43
BMI (kg/m ²)	29.8 ± 4.4	30.4 ± 4.3	0.24
Systolic blood pressure (mmHg)	154.0 ± 13.0	153.7 ± 14.6	0.85
Diastolic blood pressure (mmHg)	91.7 ± 9.7	89.7 ± 8.4	0.07
A1C (%)	6.9 ± 1.7	7.1 ± 1.6	0.50
LDL cholesterol (mmol/l)	3.4 ± 0.9	3.7 ± 1.0	0.06
HDL cholesterol (mmol/l)	1.1 ± 0.3	1.2 ± 0.3	0.25
Triglycerides (mmol/l)	2.0 (0.3–11.6)	1.8 (0.2–12.1)	0.10
Creatinine (μmol/l)	93.7 ± 14.1	94.6 ± 15.0	0.79
Urinary albumin excretion rate (mg/24h)	74 (29–251)	73 (26–239)	0.89

Data are means ± SD, *n* (%), or median (range).

RESEARCH DESIGN AND METHODS

The IRMA 2 study was a 2-year multicenter, randomized, double-blind trial in patients with type 2 diabetes and microalbuminuria comparing irbesartan (150 or 300 mg once daily) versus placebo on top of conventional antihypertensive treatment (18). A total of 590 randomized patients were followed for a median of 2 years. The patients were randomly assigned to receive 150 mg irbesartan once daily, 300 mg irbesartan once daily, or matching placebo once daily.

In the original study, the primary end point was onset of overt nephropathy (persistent albuminuria >200 μg/min and at least 30% higher than the baseline level). The aim of this post hoc analysis was primarily to evaluate the effect of irbesartan treatment on the biomarker levels and, secondarily, to investigate whether the favorable effect of irbesartan on urinary albumin excretion rate seen in the principal study was associated with changes in one or more biomarkers.

The trial enrolled hypertensive patients, ranging in age from 30 to 70 years, with type 2 diabetes, persistent microalbuminuria (defined as an albumin excretion rate 20–200 μg/min in two of three consecutive, sterile, overnight urine samples), and a serum creatinine concentration of no more than 1.5 mg/dl (133 μmol/l) for men and no more than 1.1 mg/dl (97 μmol/l) for women. Type 2 diabetes was diagnosed according to the criteria of the World Health Organization.

The patients were examined at the time of randomization, 2 and 4 weeks after randomization, and at 3, 6, 12, 18, and 22–24 months. A clinical examination; measurements of blood pressure, urinary albumin excretion, serum creatinine concentration, and HbA_{1c} (A1C) concentration; and other laboratory evaluations were performed at each visit. All assessments of urine and blood were performed at a central laboratory. The urinary albumin concentration was determined by nephelometry (21) and serum creatinine concentration by Jaffe reaction with the use of a Hoffmann-LaRoche kit (22). A1C (normal range 2.7–5.8%) was measured by ion-exchange high-performance liquid chromatography (23).

The target blood pressure 3 months after randomization was <135/85 mmHg. Additional antihypertensive drugs included diuretics, β-blockers, calcium-channel blockers (except dihydropyridines), and α-blockers; ACE inhibitors were not allowed. Patients continued to receive their usual care for diabetes. No restriction on dietary salt or protein was implemented.

Laboratory analyses. A broad panel of biomarkers was chosen in order to assess the impact of irbesartan treatment on low-grade inflammation, endothelial dysfunction, AGE peptides, and TGF-β. Of the 590 subjects randomized, 409 had a follow-up after 2 years. Unfortunately, all samples from the 150 mg irbesartan group were discarded by error. However, there was no significant effect of the irbesartan treatment in the main study in the 150 mg irbesartan group compared with the placebo group. Eventually, samples for assessment of AGE peptides, TGF-β, and biomarkers of low-grade inflammation (high-sensitivity C-reactive protein [hs-CRP], interleukin [IL]-6, and fibrinogen) and endothelial dysfunction (soluble vascular cell adhesion molecule-1 [sVCAM-1], soluble intercellular adhesion molecule-1 [sICAM-1], and soluble E-selectin) were available from a total of 269 patients receiving either placebo or 300 mg irbesartan once daily (68% of these two groups). Samples were only available to allow determination of these biomarkers at baseline and after 1 and 2 years of treatment. It should be mentioned that sICAM-1 also can be

regarded as a marker of low-grade inflammation but in this analysis was considered a marker of endothelial dysfunction.

Analyses of the biomarkers were all (except fibrinogen) performed at a central lab by C.S. hs-CRP was determined by enzyme immunoassays (normal range 0.13–3.0 mg/l) as previously described (24). Commercially available enzyme-linked immunosorbent assay kits were used for measurements of plasma sVCAM-1 (normal range for assay 538–1,286 ng/ml), sICAM-1 (98–647 ng/ml), soluble E-selectin (Diaclone), and IL-6 (Quantikine High Sensitivity; R&D Systems, Oxon, U.K.).

Total TGF-β was measured by an ELISA Development system (R&D Systems). AGE peptide measurement was performed with a simple analytical procedure as described by Wrobel et al. (25). Fibrinogen was analyzed at Bio Analytical Research Corporation (Ghent, Belgium) using the Clauss method (STA Compact).

Demographic and clinical data were analyzed for variance by independent samples *t* test. Urinary albumin excretion rate and non-normally distributed biomarker levels were log transformed before analysis. (Fibrinogen levels were normally distributed as opposed to all other biomarkers; hence, it is expressed in absolute changes versus change in percentage.) For hs-CRP, we also looked at how many subjects shifted risk category according to American Heart Association guidelines (low, <1; average, 1–2; and high, >2 mg/l) (26).

We used generalized estimating equation (GEE) analyses (27) when evaluating the changes in biomarkers with time in each group and the effect of time and treatment in the whole group. This is a regression model for evaluation of repeated measurements evaluating time-dependent changes and associations between variables over time. In additional models, we adjusted for baseline values of sex, age, duration of diabetes, creatinine clearance, presence of any retinopathy, and smoking status (yes/no); for values at baseline and during follow-up of urinary albumin excretion, mean arterial blood pressure, A1C, weight, cholesterol, presence of any retinopathy, and smoking status (yes/no) were adjusted. For log-transformed variables, the GEE regression coefficient was back transformed and expressed in percent. GEE correlations were also used to investigate possible associations between changes in albuminuria and changes in biomarkers. Urinary albumin excretion was considered the dependent variable, and biomarker levels, duration of diabetes, mean blood pressure, A1C, age, sex, smoking status, and retinopathy were treated as independent variables. GEE analyses were performed with Stata (version 8.0). All other calculations were made using SPSS (version 13.0; SPSS, Chicago, IL). Adjustment for multiple comparisons was not performed, but all performed analyses are presented. A *P* value <0.05 was considered significant in two-sided tests.

RESULTS

The clinical characteristics of the 269 subjects included in this substudy are given in Table 1. The two subgroups were well matched for all demographic and clinical baseline data. When comparing the substudy population (*n* = 269) with the 590 subjects included in the principal study, no significant differences were found in any of the baseline

TABLE 2
Biomarkers at baseline and after 2 years of treatment with 300 mg irbesartan ($n = 143$) or placebo ($n = 126$) in patients with type 2 diabetes and microalbuminuria

Marker type	Baseline geometric mean	1-year geometric mean	2-year geometric mean	Change per year*	Treatment effect†	<i>P</i>
Low-grade inflammation						
hs-CRP (mg/dl)						
Placebo	2.96 (2.43–3.61)	4.14 (3.40–5.04)	3.45 (2.80–4.25)	10.0 (–0.04 to 21.0)	–24.9 (–35.6 to –12.3)	<0.001
Irbesartan	3.13 (2.62–3.74)	2.57 (2.16–3.06)	2.84 (2.39–3.39)	–5.4 (–11.5 to 1.2)		
IL-6 (pg/ml)						
Placebo	3.12 (2.80–3.48)	3.74 (3.35–4.18)	3.49 (3.06–3.97)	6.5 (0.4–13.1)	–13.6 (–22.0 to –4.3)	0.005
Irbesartan	2.98 (2.65–3.36)	2.83 (2.48–3.23)	3.13 (2.80–3.50)	1.8 (–3.1 to 7.0)		
Fibrinogen (g/l)						
Placebo	3.52 (3.37–3.67)	3.58 (3.39–3.79)	3.65 (3.48–3.83)	0.059 (–0.028 to 0.15)	–0.16 (–0.30 to –0.018)	0.027
Irbesartan	3.49 (3.34–3.63)	3.32 (3.15–3.50)	3.41 (3.27–3.55)	–0.059 (–0.13 to 0.015)		
Endothelial dysfunction						
sICAM-1 (ng/ml)						
Placebo	557 (529–588)	581 (552–612)	575 (541–610)	1.5 (–0.3 to 3.3)	–1.1 (–4.1 to 2.0)	0.48
Irbesartan	576 (550–603)	578 (553–605)	576 (551–603)	0.1 (–1.2 to 1.4)	–0.8 (–3.4 to 1.8)	0.52
sVCAM-1 (ng/ml)						
Placebo	956 (910–1004)	985 (938–1034)	1,010 (963–1,059)	2.7 (1.2–4.2)	–3.8 (–9.4 to 2.3)	0.22
Irbesartan	919 (884–956)	946 (911–983)	951 (915–989)	1.7 (0.6–2.8)		
Soluble E-selectin (ng/ml)						
Placebo	94 (84–104)	98 (88–110)	102 (91–115)	3.0 (–0.3 to 6.4)		
Irbesartan	97 (86–109)	97 (87–110)	97 (86–109)	–0.5 (–3.1 to 2.2)		
Growth factor						
TGF- β (ng/ml)						
Placebo	5.71 (4.95–6.60)	6.45 (5.65–7.40)	6.26 (5.54–7.07)	5.3 (–2.1 to 13.4)	4.4 (–1.6 to 8.2)	0.48
Irbesartan	6.25 (5.50–7.10)	6.45 (5.66–7.35)	6.00 (5.30–6.80)	–2.9 (–9.2 to 3.8)		
Advanced glycation						
AGE peptides (%)						
Placebo	3.75 (3.49–4.04)	3.65 (3.36–3.91)	3.69 (3.42–3.98)	–0.4 (–3.9 to 3.1)	3.5 (–2.2 to 9.5)	0.23
Irbesartan	3.46 (3.29–3.65)	3.60 (3.41–3.81)	3.76 (3.52–4.01)	4.1 (1.4–7.0)		

Data are mean (95% CI). *Regression coefficients for the effect of time from GEE analysis in the treatment and placebo groups. All biomarker values (except fibrinogen) were log transformed due to skewed distribution, and regression coefficients were back transformed and given as change in percent per year (gram per liter per year for fibrinogen). †Regression coefficients for the effect of treatment from GEE analysis in all subjects. All biomarker values (except fibrinogen) were log transformed due to skewed distribution, and regression coefficients were back transformed and given as treatment effect in percent (gram per liter for fibrinogen).

characteristics, except for A1C, which was lower in the substudy population (7.0 vs. 7.3%, $P = 0.036$).

The biomarker results are shown in Table 2. There were no significant differences in the level of the markers of inflammation, endothelial dysfunction, AGE peptides, and TGF- β between the groups at baseline. During the 2 years of follow-up, hs-CRP levels in the 300 mg irbesartan group changed (based on GEE regression coefficients) -5.4% (95% CI -11.5 to 1.2) per year from baseline geometric mean of 3.13 mg/l (2.62 – 3.74) versus the 10% (-0.04 to 21.0) increase per year from baseline geometric mean 2.96 mg/l (2.43 – 3.61) in the placebo group. The effect of treatment (based on the GEE regression coefficient) was -24.9% (-35.6 to -12.3) during follow-up compared with placebo ($P < 0.001$). For IL-6, there was a 1.8% (-3.1 to 7.0) increase per year in the 300 mg irbesartan group from baseline 2.98 pg/ml (2.65 – 3.36) compared with placebo's 6.5% (0.4 – 13.1) increase per year from 3.12 pg/ml (2.80 – 3.48). The treatment effect during follow-up was -13.6% (-22.0 to -4.3) ($P = 0.005$). Fibrinogen decreased in the treatment group -0.059 g/l (-0.13 to 0.0159) per year from baseline 3.49 g/l (3.34 – 3.63) versus the placebo group's 0.059 g/l (-0.028 to 0.15) per year increase from baseline 3.52 g/l (3.37 – 3.67). The treatment effect during follow-up was -0.16 g/l (-0.30 to -0.018) ($P = 0.027$). The treatment effect on all the markers of inflammation remained significant after adjustment for baseline values of sex, age, known duration of diabetes, creatinine clearance, and current levels of arterial blood pressure, A1C, cholesterol, and weight. There was significant sex difference in hs-CRP levels at baseline but no sex difference in treatment effect (data not shown). As seen in Table 2, there was no significant treatment effect on any of the other biomarkers.

For hs-CRP, we found that 32 treated subjects (23%) and 17 placebo subjects (14%) shifted to a lower American Heart Association risk category, whereas 32 subjects (26%) in the placebo group and 19 subjects (13%) in the treatment group shifted to a higher category ($P = 0.005$). hs-CRP was higher in women compared with men, but there was no interaction with treatment effect.

We found an association between the change in albuminuria during the 24 months and the change in IL-6 levels ($P = 0.04$). A doubling of IL-6 levels was associated with an 8.8% (95% CI 0.3 – 19) increase in urinary albumin excretion after adjustment for baseline values of age, sex, duration of diabetes, creatinine clearance, smoking status, and retinopathy and baseline and follow-up levels of markers of inflammation, arterial blood pressure, A1C, weight, and cholesterol. If also adjusted for treatment, the association with IL-6 was weakened ($P = 0.056$). The interaction term of treatment and IL-6 was not significant ($P = 0.38$).

In the same model, the coefficients for hs-CRP and fibrinogen were nonsignificant. Only 18 of 269 patients in the substudy reached the primary end point in the original study (macroalbuminuria), 5 in the irbesartan group and 13 in the placebo group ($P < 0.05$). Thus, the substudy was not powered to assess if the treatment effect on biomarkers can explain effects on the development of persistent macroalbuminuria or on the incidence of cardiovascular events.

DISCUSSION

In this post hoc analysis of a randomized, double-blind, placebo-controlled trial, we found that treatment with 300

mg irbesartan once daily yielded significant reductions of hs-CRP and fibrinogen compared with an increase in the placebo group after 2 years. In addition, treatment attenuated the increase with time in IL-6. Since low-grade inflammation is thought to be involved in the pathogenesis of micro- and macroalbuminuria and as interventions in the RAAS affect progression of disease (e.g., the main IRMA 2 study), we hypothesize that the beneficial impact on inflammatory markers that we find could at least in part explain the effect of irbesartan treatment on the development of macroalbuminuria in the main study. In addition, we investigated a broad panel of other biomarkers associated with progression of nephropathy and cardiovascular disease and saw no significant impact on markers of endothelial dysfunction, TGF- β , and AGE peptides.

Preventing progression of microalbuminuria to overt nephropathy is possible through aggressive treatment with irbesartan and by reducing other risk factors. It is not yet known whether this renoprotective treatment also yields cardiovascular protection by reduction of low-grade inflammation, a risk factor for cardiovascular disease. To our knowledge, no studies of the effect of irbesartan treatment on markers of inflammation and endothelial dysfunction have been performed in patients with type 2 diabetes and microalbuminuria. In a 6-month placebo-controlled study with another angiotensin receptor blocker, losartan (50 mg daily), in 80 subjects with type 2 diabetes and microalbuminuria, no change in plasma hs-CRP was found, despite a significant reduction in mean albumin excretion rate in the losartan group (12). There is scarce literature on the effects of blocking the AT₁ receptor in other similar high-risk populations. In a recent study by Ridker et al. (28) in 1,668 patients with stage-two hypertension, valsartan monotherapy resulted in 13.3% lower levels of hs-CRP compared with valsartan/hydrochlorothiazide combination therapy. In patients with coronary artery disease, Schieffer et al. (9) treated 21 subjects with 300 mg irbesartan once daily and 27 subjects with 20 mg ramipril once daily for 3 months. In the irbesartan group, there was a significant reduction in serum IL-6 and hs-CRP levels. Schram et al. (10) found that 12 months of aggressive antihypertensive therapy based on hydrochlorothiazide, candesartan, or lisinopril reduced levels of sVCAM-1 and sICAM-1, whereas von Willebrand factor and hs-CRP were unaffected. The 70 subjects with type 2 diabetes had hypertension and most had normoalbuminuria (14 subjects with microalbuminuria); thus, this population may not have been at high risk of imminent progression to diabetic nephropathy.

In a study with 199 hypertensive nondiabetic subjects, Fliser et al. (11) found that olmesartan reduced serum levels of hs-CRP and IL-6 significantly after 6 weeks. With the exception of the losartan study (12), our findings regarding markers of low-grade inflammation seem to be consistent with previous findings with similar compounds in other high-risk populations, but our analyses comprise a much longer follow-up and a larger number of subjects.

Blocking the AT₁ receptor in this population may influence low-grade inflammation through several pathways (29). Angiotensin II increases smooth muscle lipoxygenase activity, which can increase inflammation and the oxidation of LDL cholesterol (30). Angiotensin II also elicits IL-6 production by smooth muscle cells (31). During the 2-year treatment period, there was no significant treatment effect on markers of endothelial dysfunction (sICAM-1, sVCAM-1, and soluble E-selectin) or AGE peptides and

TGF- β levels. Gasic et al. (32) found that blocking the RAAS in a different way using an ACE inhibitor (fosinopril) decreased levels of sVCAM-1 in borderline hypertensive patients with type 2 diabetes and microalbuminuria, and Andersen et al. (33) found that 2 months of treatment with losartan or enalapril in 16 patients with type 1 diabetes and overt diabetic nephropathy reduced levels of sVCAM-1 but did not affect levels of von Willebrand factor or hs-CRP. In a study (13) with 33 normotensive subjects with stable coronary artery disease, 24 weeks of irbesartan treatment showed a 36% reduction in soluble VCAM-1 levels. It thus seems as if there may be an initial decrease in sVCAM-1 levels after onset of treatment, which can be seen as early as after 12 weeks. In our study, the first measurement after initiation of treatment was after 1 year. It was thus not possible to assess if there was an earlier effect of irbesartan treatment on the sVCAM-1 levels.

It has been suggested that TGF- β acts as a mediator of renal fibrosis caused in part by angiotensin II; thus, the blocking of the AT₁ receptor was expected to decrease levels of TGF- β (34). Treatment with losartan reduces urinary levels of connective tissue growth factor, a cytokine mediator of the profibrogenic effects of angiotensin II, acting downstream from TGF- β (35). In the present study, there was no effect of irbesartan treatment on plasma TGF- β , although this does not exclude an effect on local renal levels of the biomarker.

Changes in IL-6 were correlated with changes in urinary albumin excretion rate. This suggests that the observed reduction in the increase in IL-6 by treatment is associated with the reduction in urinary albumin excretion, or a possible link between impact on inflammation and development of microvascular lesions, although only demonstrated for one of the markers of inflammation.

In our study, we evaluated the effect of irbesartan treatment on several biomarkers that are closely linked to increased cardiovascular risk in patients with type 2 diabetes (17). The markers of low-grade inflammation (hs-CRP, IL-6, and fibrinogen) were all significantly influenced by the treatment, whereas the markers of endothelial dysfunction, TGF- β , and the AGE peptides were unaffected.

The 2003 statement from the American Heart Association/Centers for Disease Control and Prevention regarding the association between markers of inflammation and cardiovascular disease concludes that the current evidence has been derived from observational studies and post hoc analyses and that there is additional need for randomized trials where the intervention is intended to directly alter inflammation (26). This is the first major intervention study in patients with type 2 diabetes and microalbuminuria but still only a post hoc study. The results must be interpreted with caution, but the interesting effect on markers of inflammation should spark new trials with change in markers of inflammation as the primary end point.

A meta-analysis of IRMA 2, the IDNT (Irbesartan in Diabetic Nephropathy Trial), and the RENAAL (Reduction of Endpoints in NIDDM with the Angiotensin II Antagonist Losartan) study showed a relative risk reduction of fatal and nonfatal cardiovascular events when comparing angiotensin receptor blockers to conventional antihypertensive treatment (36). Whether the reduction in low-grade inflammation we have found translates into fewer cardiovascular events in this population remains to be seen; however, recently published statin trials support that

hypothesis, although one has to be cautious when comparing studies with different treatment in other patient populations. In the Reversal of Atherosclerosis with Aggressive Lipid Lowering (REVERSAL) trial, reduction in CRP levels during statin treatment was associated with slower rate of progression of atherosclerosis (37), and in the PROVE IT-TIMI (Pravastatin or Atorvastatin Evaluation and Infection Therapy: Thrombolysis in Myocardial Infarction) 22 trial, the group achieving CRP <2 mg/l had better clinical outcomes (38). The magnitude of change in our study is in line with the changes in the REVERSAL trial; the treatment effect on hs-CRP in our study is ~25%, whereas it was 22% in the REVERSAL population, leading to lower rate of progression in atheroma volume. Larger trials with sufficient follow-up are needed to investigate such hypotheses in patients with type 2 diabetes and micro- and/or macroalbuminuria. It is a limitation that not all of the 590 subjects randomized in the primary study were available for analysis in this substudy where only 68% of the patients in the placebo and the 300 mg irbesartan group had available samples for biomarker analyses, but the substudy population did not differ from the primary study population. In the RENAAL study, it was found that losartan had different treatment effects in diverse ethnic populations (39). As 97% of the included subjects in the IRMA 2 study were Caucasian, the effect in different ethnic groups could not be evaluated in our study. Also it could have been of great interest to genotype the study population, since irbesartan is metabolized in large part by the cytochrome P450 CYP2C9 and since 15–20% of Caucasians have CYP2C9 2* and 3*, resulting in decreased enzyme activity (40). Since no DNA samples were taken, this was not possible.

In conclusion, treatment with 300 mg irbesartan once daily during 24 months reduces low-grade inflammation in this high-risk population. This may contribute to the effect on the risk of microvascular disease, as changes in IL-6 were associated with changes in albuminuria. Additional studies are needed to test the hypothesis that this reduces the risk of cardiovascular disease.

ACKNOWLEDGMENTS

The authors thank Harry Twaalfhoven and Rob Barto from the Department of Clinical Chemistry, VU University Medical Centre, Amsterdam, the Netherlands, for expert technical assistance.

REFERENCES

1. Parving H-H, Mauer M, Ritz E: Diabetic nephropathy. In *Brenner and Rector's The Kidney: 7th edition*. Brenner BM, Ed. WB Saunders, Boston, MA, 2004, p. 1777–1818
2. Strain WD, Chatarvedi N: The renin-angiotensin-aldosterone system and the eye in diabetes. *J Renin Angiotensin Aldosterone Syst* 3:243–246, 2002
3. Volpe M, Savoia C, De Paolis P, Ostrowska B, Tarasi D, Rubattu S: The renin-angiotensin system as a risk factor and therapeutic target for cardiovascular and renal disease. *J Am Soc Nephrol* 13:S173–S178, 2002
4. Heart Outcomes Prevention Evaluation (HOPE) Study Investigators: Effects of ramipril on cardiovascular and microvascular outcomes in people with diabetes mellitus: results of the HOPE study and MICRO-HOPE substudy. *Lancet* 355:253–259, 2000
5. Lacourciere Y, Nadeau A, Poirier L, Tancrede G: Captopril or conventional therapy in hypertensive type II diabetics: three-year analysis. *Hypertension* 21:786–794, 1993
6. Agardh C-D, Garcia-Puig J, Charbonnel B, Angelkort B, Barnett AH: Greater reduction of urinary albumin excretion in hypertensive type II diabetic patients with incipient nephropathy by lisinopril than by nifedipine. *J Hum Hypertens* 10:185–192, 1996
7. Stuveling EM, Bakker SJL, Hillege HL, de Jong PE, Gans ROB, de Zeeuw D:

- Biochemical risk markers: a novel area for better prediction of renal risk? *Nephrol Dial Transplant* 20:497–508, 2005
8. Davis BJ, Forbes JM, Thomas MC, Jerums G, Burns WC, Kawachi H, Allen TJ, Cooper ME: Superior renoprotective effects of combination therapy with ACE and AGE inhibition in the diabetic spontaneously hypertensive rat. *Diabetologia* 47:89–97, 2004
 9. Schieffer B, Bunte C, Witte J, Hoepfer K, Boger RH, Schwedhelm E, Drexler H: Comparative effects of AT1-antagonism and angiotensin-converting enzyme inhibition on markers of inflammation and platelet aggregation in patients with coronary artery disease. *J Am Coll Cardiol* 44:362–368, 2004
 10. Schram MT, van Ittersum FJ, Spoelstra-de Man A, van Dijk RAJM, Schalkwijk CG, IJzerman RG, Twisk JWR, Stehouwer CDA: Aggressive antihypertensive therapy based on hydrochlorothiazide, candesartan or lisinopril as initial choice in hypertensive type II diabetic individuals: effects on albumin excretion, endothelial function and inflammation in a double-blind, randomized clinical trial. *J Hum Hypertens* 19:429–437, 2005
 11. Fliser DM, Buchholz KM, Haller HM, the EUROpean Trial on Olmesartan and Pravastatin in Inflammation and Atherosclerosis (EUTOPIA) Investigators: Antiinflammatory effects of angiotensin II subtype 1 receptor blockade in hypertensive patients with microinflammation. *Circulation* 110:1103–1107, 2005
 12. Tan K, Chow WS, Wong Y, Shiu S, Tam S: Effect of losartan on plasma C-reactive protein in type 2 diabetic patients with microalbuminuria. *Diabetes Care* 25:1254–1255, 2002
 13. Navalkar S, Parthasarathy S, Santanam N, Khan BV: Irbesartan, an angiotensin type 1 receptor inhibitor, regulates markers of inflammation in patients with premature atherosclerosis. *J Am Coll Cardiol* 37:440–444, 2001
 14. Forbes JM, Cooper ME, Thallas V, Burns WC, Thomas MC, Brammar GC, Lee F, Grant SL, Burrell LA, Jerums G, Osicka TM: Reduction of the accumulation of advanced glycation end products by ACE inhibition in experimental diabetic nephropathy. *Diabetes* 51:3274–3282, 2002
 15. Gall M-A, Borch-Johnsen K, Hougaard P, Nielsen FS, Parving H-H: Albuminuria and poor glycemic control predicts mortality in NIDDM. *Diabetes* 44:1303–1309, 1995
 16. Dinneen SF, Gerstein HC: The association of microalbuminuria and mortality in non-insulin-dependent diabetes mellitus. *Arch Intern Med* 157:1413–1418, 1997
 17. Stehouwer CD, Gall MA, Twisk JW, Knudsen E, Emeis JJ, Parving HH: Increased urinary albumin excretion, endothelial dysfunction, and chronic low-grade inflammation in type 2 diabetes: progressive, interrelated, and independently associated with risk of death. *Diabetes* 51:1157–1165, 2002
 18. Parving H-H, Lehnert H, Brochner-Mortensen J, Gomis R, Andersen S, Arner P: The effect of irbesartan on the development of diabetic nephropathy in patients with type 2 diabetes. *N Engl J Med* 345:870–878, 2001
 19. Lewis EJ, Hunsicker LG, Clarke WR, Berl T, Pohl MA, Lewis JB, Ritz E, Atkins RC, Rohde R, Raz I: 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
 20. Brenner BM, Cooper ME, de Zeeuw D, Keane WF, Mitch WE, Parving H-H: Effects of losartan on renal and cardiovascular outcomes in patients with type 2 diabetes and nephropathy. *N Engl J Med* 345:861–869, 2001
 21. Hofman W, Guder W: Preanalytical and analytical factors involved in the determination of urinary immunoglobulin G, albumin, alpha 1-microglobulin and retinol binding protein using the Behring nephelometer system. *Lab Med* 13:470–478, 1989
 22. Seelig HP: [The Jaffe reaction with creatinine: reaction product and general reaction conditions]. *Z Klin Chem Klin Biochem* 7:581–585, 1969 [in German]
 23. Goldstein DE, Little RR, Wiedmeyer HM, England JD, McKenzie EM: Glycated hemoglobin: methodologies and clinical applications. *Clin Chem* 32:B64–B70, 1986
 24. Jager J, Kooy A, Leher P, Bets D, Wulflele MG, Teerlink T, Scheffer PG, Schalkwijk CG, Donker AJ, Stehouwer CD: Effects of short-term treatment with metformin on markers of endothelial function and inflammatory activity in type 2 diabetes mellitus: a randomized, placebo-controlled trial. *J Intern Med* 257:100–109, 2005
 25. Wrobel K, Wrobel K, Garay-Sevilla M, Nava LE, Malacara JM: Novel analytical approach to monitoring advanced glycosylation end products in human serum with on-line spectrophotometric and spectrofluorometric detection in a flow system. *Clin Chem* 43:1563–1569, 1997
 26. Pearson TA, Mensah GA, Alexander RW, Anderson JL, Cannon RO III, Criqui M, Fadl YY, Fortmann SP, Hong Y, Myers GL, Rifai N, Smith SC Jr, Taubert K, Tracy RP, Vinicor F: Markers of inflammation and cardiovascular disease: application to clinical and public health practice: a statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. *Circulation* 107:499–511, 2003
 27. Zeger SL, Liang KY: Longitudinal data analysis for discrete and continuous outcomes. *Biometrics* 42:121–130, 1986
 28. Ridker PM, Danielson E, Rifai N, Glynn RJ, the Val-MARC Investigators: Valsartan, blood pressure reduction, and C-reactive protein: primary report of the Val-MARC Trial. *Hypertension* 48:1–7, 2006
 29. Brasier AR, Recinos A III, Eleftheriades MS: Vascular inflammation and the renin-angiotensin system. *Arterioscler Thromb Vasc Biol* 22:1257–1266, 2002
 30. Ross R: Atherosclerosis: an inflammatory disease. *N Engl J Med* 340:115–126, 1999
 31. Libby P: Inflammation in atherosclerosis. *Nature* 420:868–874, 2002
 32. Gasic S, Wagner OF, Fasching P, Ludwig C, Veitl M, Kapiotis S, Jilka B: Fosinopril decreases levels of soluble vascular cell adhesion molecule-1 in borderline hypertensive type II diabetic patients with microalbuminuria. *Am J Hypertens* 12:217–222, 1999
 33. Andersen S, Schalkwijk CG, Stehouwer CD, Parving HH: Angiotensin II blockade is associated with decreased plasma leukocyte adhesion molecule levels in diabetic nephropathy. *Diabetes Care* 23:1031–1032, 2000
 34. Mezzano SA, Ruiz-Ortega M, Egido J: Angiotensin II and renal fibrosis. *Hypertension* 38:635–638, 2005
 35. Andersen S, van Nieuwenhoven FA, Tarnow L, Rossing P, Rossing K, Wieten L, Goldschmeding R, Parving HH: Reduction of urinary connective tissue growth factor by losartan in type 1 patients with diabetic nephropathy. *Kidney Int* 67:2325–2329, 2005
 36. Pourdjabbar A, Lapointe N, Rouleau JL: Angiotensin receptor blockers: powerful evidence with cardiovascular outcomes? *Can J Cardiol* 18 (Suppl. A):7A–14A, 2002
 37. Nissen SE, Tuzcu EM, Schoenhagen P, Crowe T, Sasiela WJ, Tsai J, Orazem J, Magorien RD, O'Shaughnessy C, Ganz P, the Reversal of Atherosclerosis with Aggressive Lipid Lowering (REVERSAL) Investigators: Statin therapy, LDL cholesterol, C-reactive protein, and coronary artery disease. *N Engl J Med* 352:29–38, 2005
 38. Ridker PM, Cannon CP, Morrow D, Rifai N, Rose LM, McCabe CH, Pfeffer MA, Braunwald E, the Pravastatin or Atorvastatin Evaluation and Infection Therapy-Thrombolysis in Myocardial Infarction (PROVE IT-TIMI 22) Investigators: C-reactive protein levels and outcomes after statin therapy. *N Engl J Med* 352:20–28, 2005
 39. de Zeeuw D, Ramjit D, Zhang Z, Ribeiro AB, Kurokawa K, Lash JP, Chan J, Remuzzi G, Brenner BM, Shahinfar S: Renal risk and renoprotection among ethnic groups with type 2 diabetic nephropathy: a post hoc analysis of RENAAL. *Kidney Int* 69:1675–1682, 2006
 40. Hallberg P, Karlsson J, Kurland L, Lind L, Kahan T, Malmqvist K, Ohman KP, Nystrom F, Melhus H: The CYP2C9 genotype predicts the blood pressure response to irbesartan: results from the Swedish Irbesartan Left Ventricular Hypertrophy Investigation vs Atenolol (SILVHIA) trial. *J Hypertens* 20:2089–2093, 2002