

ORIGINAL ARTICLE

Renal and Retinal Effects of Enalapril and Losartan in Type 1 Diabetes

Michael Mauer, M.D., Bernard Zinman, M.D., Robert Gardiner, M.D.,
 Samy Suissa, Ph.D., Alan Sinaiko, M.D., Trudy Strand, R.N.,
 Keith Drummond, M.D., Sandra Donnelly, M.D., Paul Goodyer, M.D.,
 Marie Claire Gubler, M.D., and Ronald Klein, M.D., M.P.H.

ABSTRACT

BACKGROUND

Nephropathy and retinopathy remain important complications of type 1 diabetes. It is unclear whether their progression is slowed by early administration of drugs that block the renin–angiotensin system.

METHODS

We conducted a multicenter, controlled trial involving 285 normotensive patients with type 1 diabetes and normoalbuminuria and who were randomly assigned to receive losartan (100 mg daily), enalapril (20 mg daily), or placebo and followed for 5 years. The primary end point was a change in the fraction of glomerular volume occupied by mesangium in kidney-biopsy specimens. The retinopathy end point was a progression on a retinopathy severity scale of two steps or more. Intention-to-treat analysis was performed with the use of linear regression and logistic-regression models.

RESULTS

A total of 90% and 82% of patients had complete renal-biopsy and retinopathy data, respectively. Change in mesangial fractional volume per glomerulus over the 5-year period did not differ significantly between the placebo group (0.016 units) and the enalapril group (0.005, $P=0.38$) or the losartan group (0.026, $P=0.26$), nor were there significant treatment benefits for other biopsy-assessed renal structural variables. The 5-year cumulative incidence of microalbuminuria was 6% in the placebo group; the incidence was higher with losartan (17%, $P=0.01$ by the log-rank test) but not with enalapril (4%, $P=0.96$ by the log-rank test). As compared with placebo, the odds of retinopathy progression by two steps or more was reduced by 65% with enalapril (odds ratio, 0.35; 95% confidence interval [CI], 0.14 to 0.85) and by 70% with losartan (odds ratio, 0.30; 95% CI, 0.12 to 0.73), independently of changes in blood pressure. There were three biopsy-related serious adverse events that completely resolved. Chronic cough occurred in 12 patients receiving enalapril, 6 receiving losartan, and 4 receiving placebo.

CONCLUSIONS

Early blockade of the renin–angiotensin system in patients with type 1 diabetes did not slow nephropathy progression but slowed the progression of retinopathy. (ClinicalTrials.gov number, NCT00143949.)

From the Departments of Pediatrics (M.M., A.S., T.S.) and Medicine (M.M.), University of Minnesota, Minneapolis; Samuel Lunenfeld Research Institute, Mount Sinai Hospital, University of Toronto, Toronto (B.Z., S.D.); the Departments of Medicine (R.G.), Epidemiology and Biostatistics (S.S.), and Pediatrics (K.D., P.G.), McGill University, Montreal; Hôpital Necker–Enfants Malades, Paris (M.C.G.); and the Department of Ophthalmology and Visual Sciences, University of Wisconsin School of Medicine and Public Health, Madison (R.K.). Address reprint requests to Dr. Mauer at the Department of Pediatrics, University of Minnesota, 420 Delaware St. SE, MMC 491, Minneapolis, MN 55455, or at mauer002@umn.edu.

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DIABETIC NEPHROPATHY, RESPONSIBLE for more than 45% of cases of end-stage renal disease in the United States,¹ may be structurally advanced once albuminuria becomes detectable.^{2,3} Blockers of the renin-angiotensin system are more effective than other antihypertensive agents in slowing nephropathy progression in patients who have proteinuria, diabetes mellitus, and a reduced glomerular filtration rate (GFR),⁴⁻⁶ and such blockers can also decrease proteinuria in patients with diabetes.⁷ Although the reduction of proteinuria in patients with diabetes has been associated with a reduction in the rate of decline in GFR in small studies,⁸ this association has not been systematically tested; in addition, proteinuria reduction is not a generally accepted surrogate for hard clinical end points such as end-stage renal disease.⁹ Intensive multifactorial intervention in patients with type 2 diabetes with microalbuminuria nearly halved the progression of proteinuria but did not alter the rate of GFR decline.^{10,11}

In the Renin-Angiotensin System Study (RASS), we asked whether blockade of the renin-angiotensin system before the onset of albuminuria in patients with type 1 diabetes could slow progression of the early histologic lesions of diabetic nephropathy. RASS was based on the concept that slowing the structural changes responsible for renal dysfunction in diabetes^{2,3} would delay or prevent clinical diabetic nephropathy.

Recently, the Diabetic Retinopathy Candesartan Trials (DIRECT; ClinicalTrials.gov numbers, NCT00252733, NCT00252720, and NCT00252694) reported that angiotensin-receptor blockade reduced the rate of retinopathy development in normotensive patients with type 1 diabetes and normoalbuminuria who did not have diabetic retinopathy¹² but not in patients with mild-to-moderate diabetic retinopathy. Our study was designed to assess the effect of renin-angiotensin system blockade with either an angiotensin-converting-enzyme (ACE) inhibitor or an angiotensin-receptor blocker (ARB) on both renal and retinal morphologic features in normotensive patients with type 1 diabetes and normoalbuminuria.¹³

METHODS

The authors designed the study, wrote and made the decision to submit the manuscript for publication, and vouch for the completeness, accuracy, and integrity of the data and data analyses. Data

gathered at the three study centers were forwarded to the data center based at McGill University, where all analyses were done under an author's supervision. There were no confidentiality agreements between the authors or their institutions and the sponsors (Merck [United States] and Merck Frosst [Canada]), who provided partial support for this study and donated the study drugs, nor did these sponsors have any role in the study design, data accrual, data analysis, or manuscript preparation. The study was approved by the relevant institutional review boards, and written informed consent was obtained from each participant. The study was overseen by a data and safety monitoring board of the National Institutes of Health.

STUDY DESIGN

RASS¹³ was a 5-year, multicenter, randomized, double-blind, placebo-controlled investigator-initiated trial comparing effects of the ACE inhibitor enalapril (Vasotec, Merck) and the ARB losartan (Cozaar, Merck) with those of placebo on early renal structural changes from diabetic nephropathy in type 1 diabetes. The prespecified primary study end point was a change in the fraction of glomerular volume occupied by mesangium (the mesangial fractional volume).^{2,14} Secondary renal end points included changes in other glomerular, vascular, tubular, and interstitial variables and changes in the albumin excretion rate and GFR. Shortly after RASS began, we added a study with an a priori end point of a progression of diabetic retinopathy of two steps or more.¹³ Patients were randomly assigned to one of three groups with the use of computer-generated blocks of six and stratified according to center and sex: those receiving enalapril, 10 mg daily; losartan, 50 mg daily; or daily placebo. During the study, doses were doubled because of new data indicating greater reduction in proteinuria with higher doses.¹⁵ Patients received the doubled dose of the study drugs for an average of 2.9 ± 0.9 years.

STUDY PATIENTS

Exclusion criteria were hypertension (blood pressure exceeding 135/85 mm Hg or receipt of antihypertensive medications), an albumin excretion rate above 20 μg per minute, pregnancy, failure to take at least 85% of placebo pills during a 2-week run-in period, and a GFR of less than 90 ml per minute per 1.73 m² of body-surface area (<80 ml per minute if the patient had a strictly vegan diet).¹⁶ Patients for whom fundus photographs

were taken at baseline (within 1 year after randomization) and who did not have proliferative diabetic retinopathy were included in the diabetic retinopathy studies.

The duration of type 1 diabetes among the study patients ranged from 2 to 20 years. Patients 18 years of age or older were recruited from diabetes clinics and by means of local advertising; the Minnesota and Montreal centers also enrolled 32 patients (11% of the total 285 patients enrolled) who were 15 to 17 years of age, from the Natural History of Diabetic Nephropathy Study.¹⁷ Of the 1065 patients with type 1 diabetes screened, 707 declined to participate, 73 were ineligible, and 285 were randomly assigned to one of the three study groups (Fig. 1). There were no demographic differences between the patients who agreed to participate and those who declined (see Table 1 in the Supplementary Appendix, available with the full text of this article at NEJM.org).¹³

FOLLOW-UP MEASURES

Patients were followed for 5 years. Pill counts and measurements of blood pressure, albumin excretion rate, and glycated hemoglobin level were obtained quarterly; GFR was assessed annually.¹³

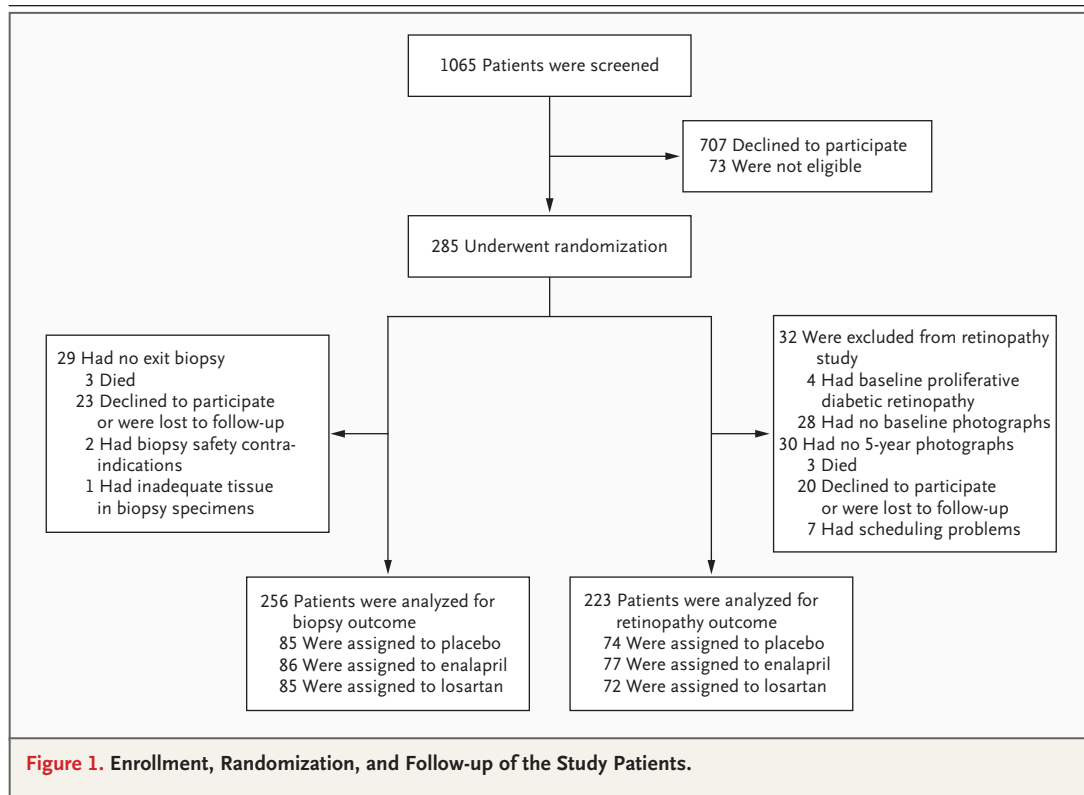
Study drugs were withheld during 18 pregnancies in 14 patients (with 6 pregnancies in 5 patients receiving placebo, 4 pregnancies in 4 receiving enalapril, and 8 pregnancies in 5 receiving losartan).

Glycated hemoglobin was measured with the use of a Diamat analyzer (BioRad) until 2002, when the Tosoh method was introduced (Tosoh Medics). Blood pressure was measured by means of a Dinamap monitor. If hypertension persisted for 2 weeks, medication that does not block the renin-angiotensin system was initiated to achieve a blood-pressure target of less than 130/80 mm Hg.

The GFR was measured according to the io-hexol plasma disappearance method.¹⁸ The baseline albumin excretion rate was expressed as the median of three samples obtained before randomization.¹³ Microalbuminuria was defined as the mean of at least two of three consecutive values between 20 and 200 μg per minute.

RENAL BIOPSY AND MORPHOMETRIC MEASUREMENTS

Percutaneous biopsy¹⁹ was performed before randomization and 5 years later. The presence of at least two glomeruli, for purposes of electron microscopy, was required for randomization. One



baseline biopsy and three 5-year biopsies were repeated because of inadequate tissue; one patient had inadequate tissue twice. Five 5-year biopsy specimens had fixation problems; biopsy was repeated for four of these. Electron microscopy was performed on 3.14 ± 0.53 glomeruli per biopsy (range, 1 through 6; only one biopsy contained just a single glomerulus). All measurements were performed by one observer, who was unaware of the study-drug assignments. Mesangial fractional volumes per glomerulus were estimated by means of point counting, as reported elsewhere.^{3,19,20} The surface area of peripheral glomerular basement membrane per glomerulus and the width of glomerular basement membrane were estimated as previously described.^{3,19} Two observers who were unaware of the study-drug assignments estimated the fraction of each cortical arteriolar wall that was replaced by hyaline, on random light-microscopy slides, and the index of arteriolar hyalinosis was calculated.²¹ The fraction of the volume of the cortex that was interstitium and the fraction of the volume of the cortical tubules that were atrophic were estimated by means of point counting²² by one observer who was unaware of the study-drug assignments.

GRADING OF RETINOPATHY

Stereoscopic fundus photographs were taken at 30 degrees for seven standard Early Treatment Diabetic Retinopathy Study (ETDRS) fields,²³ at baseline and 5 years. These were graded by observers, unaware of the study-drug assignments, at the University of Wisconsin Ocular Epidemiology Reading Center who used the modified Airlie House Classification and the ETDRS severity scale²⁴ (see the Supplementary Appendix). For each eye, the maximum grade in any of the standard fields for each lesion was used in classifying the severity of diabetic retinopathy (see the Supplementary Appendix).¹⁹ If the severity of diabetic retinopathy in an eye could not be graded (as in three instances), the eye was assigned the same grade as the other eye. The diabetic retinopathy grade was derived by concatenating the grades of the two eyes of a patient, with the eye with the higher grade given greater weight. This provided a 15-step diabetic retinopathy severity scale.^{19,23} The primary and secondary analyses reflected an increase on this scale of two or three steps or more, respectively — both clinically meaningful amounts of diabetic retinopathy progression.²⁵

STATISTICAL ANALYSIS

Baseline characteristics were compared with the use of chi-square tests and analysis of variance. Glycated hemoglobin levels and clinic blood pressures during the 5-year follow-up period were compared by means of analysis of variance.

The difference between the 5-year and baseline values of the prespecified primary study end point, mesangial fractional volume, was used to compute change over time. Mean changes between the enalapril or losartan group and the placebo group were first compared by simple linear regression. Multiple linear regression analyses accounted for the baseline mesangial fractional volume, duration of type 1 diabetes, age at diabetes onset, sex, glycated hemoglobin level, systolic blood pressure, diastolic blood pressure, GFR, and albumin excretion rate as covariates. Such analyses, used to improve precision of the estimates, were the prespecified approach to analysis. They were also used to assess all secondary structural outcomes.¹³

For the secondary outcomes related to albumin excretion rate and GFR, the value at the time of the 5-year biopsy and the mean of all values over the 5-year period were analyzed with the use of multiple linear regression, with the baseline value of each end point as the only covariate. The Kaplan–Meier approach and the log-rank test were used to estimate and compare the cumulative incidences of microalbuminuria.

Logistic-regression analysis was used to estimate the odds ratios of the secondary outcomes of diabetic retinopathy progression by two or three steps or more. Odds ratios were estimated separately for the losartan and enalapril groups, relative to the placebo group, and were adjusted for baseline characteristics, center, and baseline grade of diabetic retinopathy according to the 15-step severity scale. To assess the independent effect of blood pressure, we used blood-pressure measurements during the 5-year period as a post hoc predictor of the odds of having a progression of diabetic nephropathy by two steps or more or by three steps or more, after adjustment for age, sex, and center. Study group was added to the model to quantify the change in the odds ratio in association with blood pressure.

A sensitivity analysis was performed for the primary renal and the diabetic retinopathy end points, with the use of multiple imputation techniques to assess effects of patients excluded for

Table 1. Baseline Characteristics of All 285 Patients, According to Study Group.*

Characteristic	Enalapril (N=94)	Losartan (N=96)	Placebo (N=95)
Age (yr)	30.6±10.0	29.3±10.2	29.1±9.1
Diabetes duration (yr)	11.7±4.9	10.7±4.8	11.2±4.5
BMI†‡	25.6±3.4	26.1±4.0	25.4±3.7
Male sex (%)	48	46	45
White race (%)‡	98	96	100
Glycated hemoglobin (%)	8.6±1.6	8.7±1.7	8.3±1.4
Blood pressure (mm Hg)			
Systolic	120±13	120±11	119±11
Diastolic	71±8	70±8	70±8
Median albumin excretion rate (µg/min)	5.1	5.5	4.8
Glomerular filtration rate (ml/min/1.73 m ² of body-surface area)	129±20	131±18	126±22

* Plus-minus values are means ±SD. GFR denotes glomerular filtration rate.

† The body-mass index (BMI) is the weight in kilograms divided by the square of the height in meters.

‡ Race was self-reported.

not having both the baseline and 5-year biopsy data or diabetic retinopathy grades, respectively. Assessment of the effect of doubling the dose during the study was performed by adding a term in the multiple regression analysis for the time from randomization to dose doubling, as well as for the time from randomization to the first fundus photography, the latter only for diabetic retinopathy analyses.

We calculated that a sample size of 86 patients per group would be required for the study to have a statistical power of 80% to detect a 50% reduction in the change in mesangial fractional volume over the 5-year period, with a significance level of 5% that was reduced to 2.5% to allow for the two contrasts of the primary analysis (losartan vs. placebo and enalapril vs. placebo).¹³ The sample-size calculation was based on available data from 21 patients meeting the study's entry criteria, in whom the mean change in mesangial fractional volume per glomerulus over the 5-year period was 0.0533 and the standard deviation was 0.0557 after regression on the baseline values of mesangial fractional volume, GFR, albumin excretion rate, and diabetes duration. In anticipation of a 10% dropout rate, we enrolled 95 patients per group. Data were entered at the data center based

at McGill University, managed with the use of Paradox software, and analyzed with SAS software (version 9.1), with investigators and participants kept unaware of the results until the final analyses were completed.

RESULTS

Of the 285 patients who underwent randomization, 256 (90%) had renal biopsy completed at both baseline and 5 years (Fig. 1). There were no differences in baseline characteristics between the three groups (Table 1) among the patients who had data from both biopsies (Table 2 in the Supplementary Appendix), or between those with and those without data from both biopsies (Table 3 in the Supplementary Appendix). The overall rate of medication adherence was approximately 85%, and the overall rate of visit attendance exceeded 93%, with both rates being similar across all three groups ($P=0.87$ and $P=0.92$, respectively).

The three study groups had similar glycated hemoglobin levels ($P=0.54$) (Fig. 1 in the Supplementary Appendix) and insulin doses ($P=0.29$) during the 5-year period. The clinic-obtained systolic and diastolic blood pressures (mean ±SD) during the study were lower in the enalapril group ($113±9/66±6$ mm Hg) and the losartan group ($115±8/66±6$ mm Hg) than in the placebo group ($117±8/68±5$ mm Hg) ($P<0.001$ for the two systolic and $P≤0.02$ for the two diastolic comparisons, respectively). (See Table 4 in the Supplementary Appendix for further details on blood pressure.) Hypertension developed in nine patients in the placebo group, three in the enalapril group, and four in the losartan group ($P=0.04$).

The prespecified primary study end point, change in mesangial fractional volume between baseline and 5 years, increased by 0.016 units in the placebo group ($P=0.004$) and 0.026 units in the losartan group ($P<0.001$) but did not change significantly (0.005 units) in the enalapril group (Table 2). The change associated with placebo was not significantly different from that with either enalapril ($P=0.16$) or losartan ($P=0.17$). Nor did the findings change after inclusion of the time to the doubling of the study drug and after the use of multiple imputation to account for patients with missing second biopsy specimens. The results for secondary renal structural end points were generally similar (Table 5 in the Supplementary Appendix).

Table 2. Effects of Enalapril and Losartan on Change in the Mesangial Fractional Volume, Albumin Excretion Rate, and Glomerular Filtration Rate, According to Study Group.*

End Point	Enalapril	Losartan	Placebo
Mesangial fractional volume			
Mean at baseline	0.201±0.044	0.189±0.041	0.187±0.045
Mean change at 5 yr	0.005±0.050	0.026±0.054	0.016±0.048
Change vs. placebo			
Mean difference	-0.011	0.010	0 (reference)
P value	0.16	0.17	
Adjusted change vs. placebo			
Mean difference	-0.006	0.008	0 (reference)
P value	0.38	0.26	
Albumin excretion rate (μg/min)			
Mean at baseline	6.3±4.6	6.5±6.7	6.4±6.2
Mean over 5-yr period	7.7±15.5	10.6±17.6	6.5±5.9
Change vs. placebo			
Mean difference	1.3	4.0	0 (reference)
P value†	0.47	0.03	
Mean at 5 yr	6.9±7.8	14.0±36.1	5.3±3.9
Change vs. placebo			
Mean difference	1.0	8.0	0 (reference)
P value†	0.74	0.007	
GFR (ml/min/1.73 m ² of body-surface area)			
Mean at baseline	129±20	131±18	126±22
Mean over 5-yr period	124±18	125±17	125±18
Change vs. placebo			
Mean difference	-2.6	-2.4	0 (reference)
P value‡	0.11	0.14	
Mean at 5 yr	123±20	121±21	120±22
Change vs. placebo			
Mean difference	0.4	-1.5	0 (reference)
P value‡	0.88	0.54	

* Plus–minus values are means ±SD. The change in the mesangial fractional volume is the fraction of glomerular volume occupied by mesangium at 5 years minus that at baseline, calculated for 86 patients receiving enalapril, 85 receiving losartan, and 85 receiving placebo. The adjusted difference was calculated with the use of data adjusted for mesangial fractional volume at baseline, blood pressure, glycated hemoglobin value, glomerular filtration rate (GFR), albumin excretion rate, age at diabetes onset, diabetes duration, and sex. The albumin excretion rate and glomerular filtration rate were assessed during the 5-year period and at 5 years, for 94 patients receiving enalapril, 96 receiving losartan, and 95 receiving placebo.

† These analyses were adjusted for the baseline albumin excretion rate.

‡ These analyses were adjusted for the baseline GFR.

The albumin excretion rate increased significantly from baseline only in the losartan group ($P=0.04$). As compared with placebo, the 5-year average rate was higher by $4.0 \mu\text{g}$ per minute with losartan ($P=0.03$) but was not significantly higher with enalapril ($P=0.47$) (Table 2). The albumin

excretion rate at 5 years was higher with losartan than with placebo, by $8.0 \mu\text{g}$ per minute ($P=0.007$), but not with enalapril ($P=0.74$). The microalbuminuria 5-year cumulative incidence was higher with losartan than with placebo (17% vs. 6%, $P=0.01$ by the log-rank test) but was not signifi-

cantly higher with enalapril (4% vs. 6%, $P=0.96$ by the log-rank test) (Fig. 2). The GFR decreased similarly in all three groups over the 5 years: by 6.6 to 8.9 ml per minute ($P<0.002$ for all three) (Table 2, and Fig. 2 in the Supplementary Appendix).

Of the 285 patients who underwent randomization, 32 were excluded from the diabetic retinopathy study (Fig. 1): 28 had photos taken too late to qualify as baseline photos (>1 year after randomization), and 4 had proliferative diabetic retinopathy. Of the remaining 253 participants, 223 (88%) completed the diabetic retinopathy studies; 122 had baseline photographs taken before randomization and 101 had them taken within 4.8 ± 4.8 months after randomization. There were no significant differences at baseline between the patients with and those without both baseline and 5-year photographs (Table 6 in the Supplementary Appendix) or among the patients that had both (Table 7 in the Supplementary Appendix). At baseline, 34% of patients had no diabetic retinopathy (level 10 in both eyes), 40% had minimal nonproliferative diabetic retinopathy (level 21 in one or both eyes), 18% had early nonproliferative diabetic retinopathy (levels 31 through 37 in the worse eye), and 9% had moderate-to-severe nonproliferative diabetic retinopathy (levels 41 through 53 in the worse eye). Baseline distributions of diabetic retinopathy severity scores among groups were not significantly different (Fig. 3 in the Supplementary Appendix). A total of 94% of the patients with diabetic retinopathy progression of two steps or more or three steps or more had no or minimal nonproliferative dia-

betic retinopathy (levels 10 through 37) at baseline, with 7% occurring in patients with more severe retinopathy (levels 40 through 53). This pattern did not vary significantly among groups. One patient in the placebo group and one in the enalapril group required laser therapy.

A progression in diabetic retinopathy of two steps or more occurred in 38% of patients receiving placebo but only 25% of those receiving enalapril ($P=0.02$) and 21% of those receiving losartan ($P=0.008$) (Table 3). The odds of progression of two steps or more was reduced by 65% with enalapril (odds ratio vs. placebo, 0.35; 95% confidence interval [CI], 0.14 to 0.85) and by 70% with losartan (odds ratio vs. placebo, 0.30; 95% CI, 0.12 to 0.73) (Table 3). Results were similar for progression of three steps or more (Table 4). These effects remained even after adjustment for the mean of all blood-pressure measurements obtained during the 5-year study, time to first retinal photograph, and time to doubled drug dose and also after multiple imputation analyses accounting for patients lacking second photographs.

ADVERSE EVENTS

Serious adverse events were few and similar among the three groups (Table 4). There were three deaths: one from ketoacidosis in the enalapril group, one from traumatic cerebral hemorrhage in the losartan group, and one from hypoglycemia in the placebo group. There were two perinephric hematomas and one large bladder clot, but no permanent sequelae. Similar numbers of participants had hypoglycemia or ketoacidosis, or both, in the three groups. Chronic cough occurred in 12 patients receiving enalapril, 6 receiving losartan, and 4 receiving placebo (Table 4); 2 of the patients in the enalapril group discontinued the drug for this reason. Transient hyperkalemia occurred in one patient in the enalapril group, and transient elevation of the serum creatinine level occurred in one patient in the losartan group, with neither requiring discontinuation of the study medication (Table 4).

DISCUSSION

Mesangial fractional volume, the primary pre-specified renal end point in RASS, is the variable most closely correlated with reduction of GFR in diabetic nephropathy.¹⁴ Despite normal blood

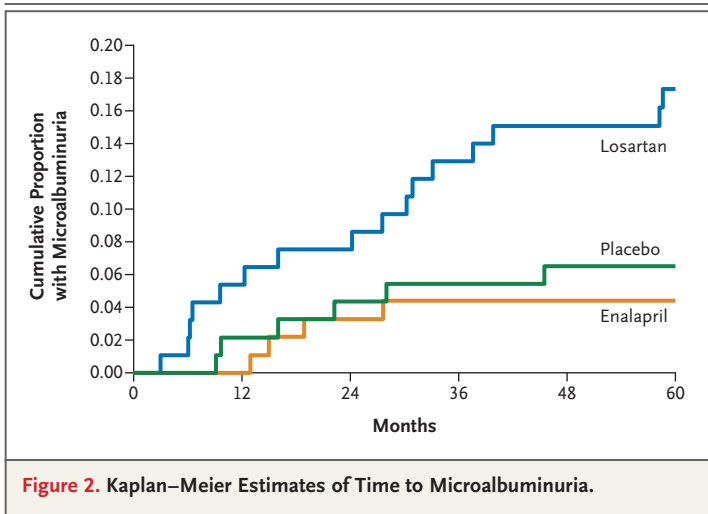


Table 3. Effects of Enalapril and Losartan on Retinopathy, as Measured by the Odds Ratio of Progression, during the Five-Year Follow-up Period.

Progression	No. of Events <i>no./total no. (%)</i>	Adjusted Odds Ratio (95% CI)*	P Value
By two steps or more			
Placebo	28/74 (38)	Reference	Reference
Enalapril	19/77 (25)	0.35 (0.14–0.85)	0.02
Losartan	15/72 (21)	0.30 (0.12–0.73)	0.008
By three steps or more			
Placebo	21/74 (28)	Reference	Reference
Enalapril	15/77 (19)	0.41 (0.16–1.05)	0.06
Losartan	9/72 (12)	0.21 (0.07–0.62)	0.005

* The odds ratio was adjusted for baseline characteristics, center, and baseline grade on the 15-point diabetic retinopathy severity scale.

pressures and albumin excretion rates, at baseline our patients had structural abnormalities characteristic of diabetic nephropathy.¹⁹ Increased mesangial fractional volume in type 1 diabetes, as confirmed in RASS, results primarily from an increase in mesangial matrix, with a lesser contribution from an increase in the mesangial cellular component.²⁰ Thus, the mesangial fractional volume increased, and all glomerular structural features of diabetic nephropathy, except for mesangial-cell fractional volume, progressed in the placebo group, and neither enalapril nor losartan significantly reduced these rates of progression (Table 5 in the Supplementary Appendix). These structural features do not vary according to age, within the age range of the RASS patients.²⁶ There were also no significant benefits of treatment on albuminuria or reduction of GFR. However, the albumin excretion rate was higher in the losartan group than in the placebo group, during and at the end of the study, and more patients in the losartan group had progression to microalbuminuria. DIRECT also found no benefit of 4.7 years of ARB treatment with candesartan on microalbuminuria incidence in patients with normoalbuminuria and type 1 diabetes or type 2 diabetes but did not find a higher incidence of microalbuminuria among patients receiving candesartan as compared with those receiving placebo.²⁷ Thus, our unexpected and unexplained finding of an increase in microalbuminuria incidence in the losartan group currently lacks confirmation in other randomized controlled trials. Nonetheless, careful monitoring of the albumin excretion rate is rec-

ommended if ARBs are prescribed to such patients. The rate of reduction of GFR was approximately twice that expected among normal people in the age range of our patients,²⁸ but it did not differ significantly among the three study groups. The observed early declines in GFR may be important; a low GFR in patients with type 1 diabetes and normoalbuminuria is associated with worse lesions,²⁹ and progressive reduction of GFR in patients with type 1 diabetes and microalbuminuria is predictive of an increasing albumin excretion rate over time.³⁰

Blockers of the renin–angiotensin system appear to be more effective than other antihypertensive agents in reducing the time to doubling of the serum creatinine level, to dialysis, or to death in patients with elevated serum creatinine levels who also have type 1 diabetes and proteinuria⁴ or type 2 diabetes.^{5,6} Although an ACE inhibitor slowed interstitial expansion in proteinuric type 2 diabetes,³¹ RASS showed that the fractional volume of the interstitium increased by more than 50% in all three study groups (Table 4 in the Supplementary Appendix). Thus, it may be misleading to extrapolate from more advanced stages of diabetic nephropathy to early stages or from type 2 diabetes to type 1 diabetes, especially given the substantial differences in the relation of renal structure to albuminuria³² and the frequent presence of hypertension, obesity, and other risk factors for albuminuria in patients with type 2 diabetes.² Decreased progression of microalbuminuria to proteinuria in patients with diabetes could result from direct effects of ACE

Table 4. Adverse Events, According to Study Group.*

Event	Enalapril		Losartan		Placebo	
	No. of Events	No. of Patients	No. of Events	No. of Patients	No. of Events	No. of Patients
Serious adverse events						
Biopsy-related	3	3	0	0	0	0
Whole body	2	2	1	1	2	2
Cardiovascular system	1	1	5	3	2	2
Digestive system	10	5	13	11	5	3
Endocrine	2	2	1	1	0	0
Hemolymphatic system	1	1	1	1	0	0
Metabolic or nutritional†	23	7	7	7	9	7
Musculoskeletal system	1	1	9	7	7	4
Nervous system	0	0	0	0	3	3
Respiratory system	2	2	1	1	1	1
Skin and appendages	3	3	3	3	4	2
Special senses	0	0	1	1	0	0
Urogenital system	5	3	6	6	4	2
Adverse events						
Biopsy-related	8	8	3	3	3	3
Whole body	26	21	36	26	35	32
Cardiovascular system	24	19	42	32	23	21
Digestive system	104	57	106	52	90	54
Endocrine	6	6	9	8	3	3
Hemolymphatic system	16	12	9	9	6	6
Metabolic or nutritional†	125	37	137	48	133	44
Musculoskeletal system	79	49	89	48	63	41
Nervous system	34	24	36	23	23	17
Respiratory system‡	158	72	148	60	112	59
Skin and appendages	40	29	49	34	53	37
Special senses	27	25	42	26	45	32
Urogenital system	74	34	88	41	70	36

* Serious adverse events and adverse events are mutually exclusive. The events are classified according to the *Coding Symbols for Thesaurus of Adverse Reaction Terms*, fifth edition, of the Food and Drug Administration.

† Among metabolic or nutritional events, for serious adverse events, 12 episodes of hyperglycemia and ketoacidosis occurred in a single patient, and for adverse events, transient hyperkalemia occurred in one patient receiving enalapril and transient elevation of the serum creatinine level occurred in one patient receiving losartan, with neither requiring discontinuation of the study drug.

‡ Among respiratory-system events, chronic cough occurred in 12 patients receiving enalapril (2 of whom discontinued enalapril for this reason), 6 receiving losartan, and 4 receiving placebo.

inhibitors on proteinuria.^{11,33} Thus, despite 8 years of treatment with an ACE inhibitor, 2 months after its discontinuation, the levels of albuminuria no longer differed significantly from that associated with a placebo,³³ suggesting masking of progression of underlying injury. In a small study of patients with type 1 diabetes, measurements of

structural changes from diabetic nephropathy in renal-biopsy specimens were similar in the seven patients receiving an ARB and the three receiving placebo.³⁴

Our large, randomized, double-blind, placebo-controlled trial examined the effects of renin-angiotensin system blockade on early renal struc-

tural changes in normotensive patients with type 1 diabetes and normoalbuminuria. Thus, although the failure to detect benefits of such blockade on structural or functional outcomes from diabetic nephropathy may initially seem at odds with results of other studies, RASS is not comparable to earlier work. Since the patients in our study were selected to have no clinically detectable renal disease at baseline, they most likely included patients who are at low risk for diabetic nephropathy. Moreover, although the rate of change in mesangial fractional volume in the placebo group, 0.016, was significant, the rate was less than the expected rate of 0.053 that was computed on the basis of data from 21 patients with type 1 diabetes who met our entry criteria and had participated in an earlier study.²¹ The effect on the statistical power of the study can be seen from the lower bound of the 95% confidence interval for the difference in the rate of change in mesangial fractional volume, suggesting that the use of enalapril and losartan result in, at most, a reduction in progression of 0.026 and 0.005 units, respectively, as compared with placebo. We estimate that the benefits we may have missed would be at most half to one tenth the rate of increase in mesangial fractional volume required to regularly result in proteinuria.^{3,14} There was no significant influence of the duration of type 1 diabetes on the primary outcome.

Important secondary structural variables, such as interstitial fractional volume,²² also showed no benefit of treatment, despite large increases from baseline in the placebo group. Currently, there are no accurate predictors of diabetic nephropathy risk for patients meeting the entry criteria of the present study. Thus, although a study involving only normotensive patients with type 1 diabetes and normoalbuminuria who were at high risk for nephropathy might have provided different results, such a study is not feasible at present.

Treatment with enalapril and losartan were both associated with a reduction in the progression of diabetic retinopathy by two or three steps or more of approximately 65% and 70%, respectively. These reductions, which are unrelated to glycemia, might be from blood-pressure lowering or direct effects of blockage of the retinal renin-angiotensin system. Earlier trials^{35,36} showed lesser progression of diabetic retinopathy in patients with type 2 diabetes who underwent tight blood-

pressure control, independent of the use of an ACE inhibitor. The severity of diabetic retinopathy at baseline in the normotensive RASS patients correlated with the nighttime systolic blood pressure.³⁷ Although the benefit with regard to diabetic retinopathy remained after adjustment for the lower blood pressures recorded during the study in the enalapril group and the losartan group, as compared with the placebo group, we cannot rule out effects of blood pressure on these diabetic retinopathy outcomes.

Our findings are consistent with those of DIRECT-Prevent¹² of patients with type 1 diabetes who did not have diabetic retinopathy, in which diabetic retinopathy was less likely to develop in those receiving an ARB (candesartan) than in those receiving placebo (hazard ratio, 0.82; 95% CI, 0.67 to 1.00; $P=0.051$). However, our findings are inconsistent with those of the DIRECT-Protect 1,¹² in which there was no benefit of candesartan in patients with nonproliferative diabetic retinopathy (hazard ratio for the development of diabetic retinopathy, vs. placebo group, 1.02; 95% CI, 0.80 to 1.31; $P=0.85$). The reasons for these differences in diabetic retinopathy progression are unknown and not easily explainable by the differences between the RASS and DIRECT-Protect 1 patients in their severity of diabetic retinopathy, blood pressure, glycemia, or diabetes duration at baseline.¹²

The renin-angiotensin system has been implicated in the pathogenesis of diabetic retinopathy.³⁸ Angiotensin II synthesis occurs in ocular areas susceptible to diabetic retinopathy.³⁹ Vitreous levels of vascular endothelial growth factor are increased in the eyes of patients with proliferative diabetic retinopathy⁴⁰ and are correlated with vitreous activity of ACE.⁴¹ Thus, the benefits of enalapril and losartan on diabetic retinopathy in the present study may represent direct effects on the eye, independent of effects of systemic blood pressure.

In summary, we did not detect structural or functional benefits on nephropathy from the blockade of the renin-angiotensin system with an ACE inhibitor or an ARB in normotensive patients with type 1 diabetes and normoalbuminuria. Given the current status of our ability to predict the risk of nephropathy, blockade of the renin-angiotensin system for the primary prevention of diabetic nephropathy in patients with type 1 diabetes is not supported by the present evidence. In contrast, we found beneficial effects

of the ACE inhibitor enalapril and the ARB losartan in reducing the risk of progression of diabetic retinopathy.

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