Course of Renal Function in Type 2 Diabetic Patients With Abnormalities of Albumin Excretion Rate

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Heterogeneity in renal structure has been described in type 2 diabetic patients with both microalbuminuria and proteinuria; in fact, only a subset of type 2 diabetic patients have the typical diabetic glomerulopathy. However, it is currently unknown whether abnormalities in albumin excretion rate (AER) have a different renal prognostic value depending on the underlying renal structure. Aims of this study were: *1*) to study the course of renal function in type 2 diabetic patients with altered AER; *2*) to evaluate the relationship between the course of glomerular filtration rate (GFR) and renal structure; and *3*) to evaluate the relationship between the course of GFR and baseline AER levels, metabolic control, and blood pressure levels during a follow-up period of 4 years. A total of 108 type 2 diabetic patients, 74 with microalbuminuria (MA) and 34 with proteinuria (P), were recruited into a prospective study that encompassed: *1*) a baseline kidney biopsy with morphometric measurements of glomerular parameters; *2*) intensified antihypertensive treatment for an average 4-year period (blood pressure target <140/90 mmHg); and *3*) determinations of GFR at baseline and every 6 months. Mean (± SD) GFR significantly decreased from baseline in both MA (–1.3 ± 9.4 [95% CI –3.51 to +0.86], $P < 0.05$) and P (–3.0 ± 13.0 ml · min^{-1} · 1.73 m^{-2} per year [–7.71 to +1.61], $P < 0.01$). However, the changes in GFR were quite heterogeneous. Thus, on the basis of percent GFR change per year from baseline (Δ%GFR), both MA and P patients were defined as progressors or nonprogressors when they were below or above the median, respectively. Baseline parameters of glomerular structure had a strong influence on the course of GFR. Indeed, the odds ratios of being progressors significantly increased across the quartiles of baseline glomerular basement membrane (GBM) width and mesangial fractional volume [Vv(mes/glom)], being 2.71 and 2.85 higher, respectively, in the fourth quartile than in the first quartile ($P < 0.01$ for both). Conversely, nonprogressors outnumbered progressors in the first quartile of GBM width (odds ratio: 2.14, $P < 0.05$) and in the first quartile of Vv(mes/glom) (odds ratio: 2.28, $P < 0.01$). Baseline albumin excretion rate (AER) did not influence Δ%GFR; in fact, the number of progressors did not increase across quartiles of baseline AER among either MA or P. Similarly, mean blood pressure levels during follow-up (and intensified antihypertensive therapy) did not affect the course of GFR: the number of progressors and nonprogressors did not change across quartiles of mean blood pressure. In contrast, HbA_{1c} during follow-up had an impact on Δ%GFR: the odds ratio for being a progressor increased across quartiles of HbA_{1c}, particularly for the highest quartile (HbA_{1c} >9.0%). In conclusion, the course of renal function is heterogeneous in type 2 diabetic patients with microalbuminuria or proteinuria. In fact, a subset of patients has a rapid decline in GFR over a 4-year follow-up period; these patients have more advanced diabetic glomerulopathy and worse metabolic control than the remaining patients, whose GFR remains stable. These two cohorts are otherwise undistinguishable as regards the degree of AER at baseline and tight blood pressure control. Kidney biopsy has an important prognostic role in these patients. Thus, tight blood pressure control, when not associated with satisfactory glycemic control, is unable to prevent rapid GFR decline in type 2 diabetic patients with typical diabetic glomerulopathy. *Diabetes* 49:476–484, 2000

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Received for publication 19 February 1999 and accepted in revised form 17 November 1999.

AER, albumin excretion rate; ESRD, end-stage renal disease; GBM, glomerular basement membrane; GFR, glomerular filtration rate; Δ%GFR, percent GFR change from baseline per year; MBP, mean blood pressure; OR, odds ratio; Vv(mes/glom), mesangial fractional volume.

Type 1, and particularly type 2, diabetes are the leading causes of end-stage renal disease (ESRD) in the U.S. and Europe (1,2). The earliest manifestation of renal disease in type 1 and 2 diabetes is the occurrence of slightly elevated albumin excretion rate (AER), called microalbuminuria. Once this develops, 50–80% of patients with type 1 diabetes eventually progress to clinical proteinuria and to ESRD (3). The cumulative risk of overt nephropathy is 20% in Caucasian type 1 diabetic patients at 20 years since onset of the disease (4). Humphrey et al. (5)

showed that the cumulative incidence of renal failure was 10.7% at 10 years and 16.8% at 15 years in Caucasian type 2 diabetic subjects during a 10 year follow-up. Hasslacher et al. (6) among German type 2 diabetic patients and Kunzelman et al. (7) among Pima Indian type 2 diabetic patients found rates of cumulative incidence of ESRD comparable with or even higher than those in type 1 diabetes. On the contrary, Fabre et al. (8) reported that only 1 of 543 type 2 diabetic patients developed renal failure and 43 developed overt nephropathy during a prolonged period of follow-up. Also, Mogensen (9) observed the occurrence of overt nephropathy and/or renal failure only in 20% of type 2, as compared with 80% of type 1, diabetic patients with microalbuminuria.

The reasons for these discrepancies are not clear. It can be postulated that renal failure does not supervene in type 2 diabetic patients, because of premature mortality due to cardiovascular events.

Alternatively, it has been proposed that the pathogenetic mechanisms underlying abnormalities in renal function are not the same in type 1 and type 2 diabetic patients.

With regard to this latter hypothesis, it should be pointed out that AER >30 μg/min is accompanied by the accumulation of extracellular matrix in the glomerulus in most type 1 diabetic patients (10). Mesangial expansion is the renal structural parameter that correlates best with all functional abnormalities in Caucasian type 1 diabetic patients (11). On the contrary, heterogeneous patterns of renal lesions and glomerular filtration rate (GFR) have been observed in Caucasian type 2 diabetic patients, both those with microalbuminuria and those with proteinuria (12–16). More particularly, we have recently observed by light microscopy that only a subgroup of Caucasian type 2 diabetic patients with microalbuminuria has the typical diabetic glomerulopathy (12). Osterby et al. (16) showed that diabetic glomerulopathy was more advanced in type 1 than in type 2 diabetic patients with proteinuria. Significantly advanced diabetic glomerulopathy was also observed in proteinuric, but not in microalbuminuric, type 2 diabetic Pima Indians (17).

Antihypertensive therapy, particularly using ACE inhibitors, has been shown to curb GFR decline in Caucasian type 2 diabetic patients with micro- or macroalbuminuria (18,19). Little information is available in Caucasian type 2 diabetic patients with abnormalities of AER on the course of GFR in relationship with morphometric parameters of glomerular structure.

The aim of the present study was to evaluate the course of kidney function in Caucasian type 2 diabetic patients with microalbuminuria or proteinuria in relation to baseline renal functional and structural parameters and to the patterns of blood glucose and blood pressure control during a 4-year follow-up period.

RESEARCH DESIGN AND METHODS

Subjects and procedures. This study was approved by the Ethical Committee of the University of Padova and Genova and by National Health Ministry. A total of 125 consecutive Caucasian type 2 diabetic patients living in northeast Italy were recruited. The inclusion criteria were the following: *1*) onset of diabetes between the ages of 40 and 70 years, *2*) duration of diabetes >2 years, *3*) no insulin therapy in the first 2 years after onset, *4*) blood pressure levels repeatedly >140/90 mmHg at 3–5 days after washout from antihypertensive therapies, *5*) serum creatinine concentrations <2 mg/dl, and *6*) no contraindication to kidney biopsy. The geometric mean of three AER 24-h measurements was evaluated 3–5 days after washout from antihypertensive therapies. A total of 86 patients had AER in the range of microalbuminuria (20–199 μg/min), and 39 patients were in the range of proteinuria (>199 μg/min). At recruitment into the study, 84 microalbuminuric patients were treated with ACE inhibitors. Two microalbuminuric patients were not receiving any treatment, but had arterial hypertension. All 39 proteinuric patients received ACE inhibitors before recruitment. One had unilateral hydronephrosis due to nephrolitiasis and was ineligible for the study. One patient had plasmocitoma and one had membraneous glomerulonephritis, cryoglobulinemia, and chronic C hepatitis; both patients were excluded. All the patients received ACE inhibitors again 10–15 days after discontinuing previous treatments. Blood pressure was measured every month, and the antihypertensive regimen was confirmed or changed every 2 months. Four patients died from myocardial infarction and one from stroke after 1 year of follow-up. Four patients had untoward side effects after the administration of ACE inhibitors. Five patients were unwilling to participate in the study. Two more patients had myocardial infarctions but remained in the study. One patient died because of renal cancer and one because of ESRD, but they were eligible for the study because these events occurred after 2 years of follow-up. Therefore, 108 patients eventually were included in the study, in which they had: *1*) at least 2 years of follow-up, during which they had intensified antihypertensive therapy aimed at a blood pressure target <140/90 mmHg and associated metabolic control using oral antidiabetic agents and insulin, aiming at an HbA_{1c} <7.5%; and *2*) baseline kidney biopsy and measurements of GFR, AER, HbA_{1c}, and mean blood pressure levels at baseline and every 6 months. The patients were admitted to the Department of Internal Medicine, University of Padova. Renal biopsies from 27 kidney donors, similar to diabetic patients with regard to sex (16 males; 11 females) and age (mean ± SD: 57 ± 5 years), were kindly provided by M. Mauer, University of Minnesota. Arterial hypertension (20) and diabetes (21) were diagnosed as described elsewhere. AER was measured by immunoturbidimetric technique (22) in the absence of urinary infections. Mean arterial pressure was calculated as diastolic pressure (fifth Korotkoff sound), plus one-third of pulse pressure (mean blood pressure [MBP]). BMI was computed as weight divided by square of height (kg/m^2). Overnight fasting blood was withdrawn for measurement of HbA_{1c} (23) and serum creatinine (24). GFR was determined by modeling analysis of plasma decay of ^{51}Cr-labeled ethylenediaminetetra-acetate from frequent blood samples over 300 min, after abrupt injection of the tracer, as described elsewhere (25).

Biopsy studies. Percutaneous renal biopsies were performed under ultrasound guidance. Tissue was examined under a dissecting microscope to insure adequate numbers of glomeruli. Three 1-mm cores containing glomeruli were placed in 2.5% glutaraldehyde in Milloning buffer embedded in Polybed 812 and processed for electron microscopy as previously described (10,11). One 1-mm core was processed for immunofluorescence. The rest of the core was placed in Zenker's fixative and processed for light microscopy.

Electron microscopy. Sections 1 μm thick were cut and stained with toluidine blue to permit random selection of the centermost, intact glomeruli at least one tubular diameter from the edge of the tissue. Globally sclerotic glomeruli were excluded. Three glomeruli were analyzed from each biopsy. Ultrathin sections were examined with a Hitachi H600 electron microscope (Hitachi, Tokyo); glomeruli were photographed at a magnification of ×3,900 to produce photomontages of the entire glomerular profile, defined as the circumscribed, minimal convex polygon enclosing the glomerular tuft (the tuft comprises the capillaries, the mesangium, and the intervening urinary space) (10,11). The montages were used to estimate mesangial fractional volume [Vv(mes/glom)], superimposing a double lattice square grid with equally spaced coarse points 60 mm apart and equally spaced fine points 30 mm apart. Vv(mes/glom) was estimated by counting the number of fine points falling on the mesangium in relation to the number of coarse points counted to determine points hitting the glomerular tuft. The transition between the peripheral capillary area and the mesangium was determined on the basis of the widening of the distance and disappearance of the parallelism between the endothelial and epithelial cells. The coefficient of variation among the three glomeruli in the patients presented here was on average 14%; however, it tended to be higher in patients with proteinuria. To estimate glomerular basement membrane (GBM) width, another set of micrographs—photographed at ×12,000 by entering the glomerulus at its lowest segment and systematically sampling about 20% of the glomerular profile—was used and the orthogonal intercept method was applied (26). The measurements were made at each point that a line of the grid intercepted an endothelial/peripheral GBM interface. The intercept was measured on a line orthogonal to the edge of the epithelial aspect of the peripheral GBM. The number of measurements performed in each biopsy to estimate GBM width was not less than 150. The coefficient of variation among the three glomeruli in the patients presented here is 13%.

Light microscopy. Two-micrometer thick periodic acid Shiff (PAS) stained light microscopy sections were independently and blindly (regarding knowledge of GFR course) evaluated by P.F. and by Prof. Michael Mauer. In this cohort of patients, we did not find cases of any definable nondiabetic renal disease.

Statistical analysis. The Statistical Package for Social Science (SPSS, Chicago) was used to perform statistical analysis. The data are presented as means ± SD

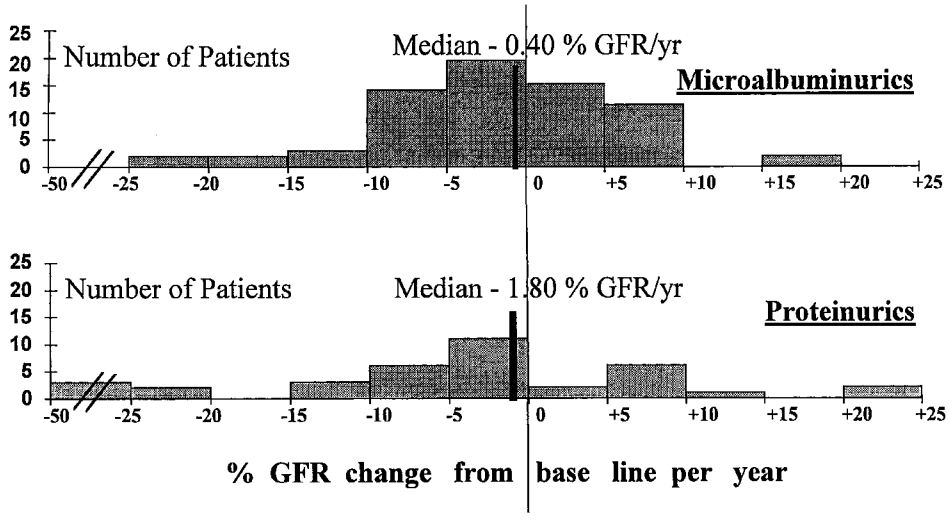

FIG. 1. The absolute number of the overall 108 microalbuminuric and proteinuric type 2 diabetic patients, evaluated according to Δ%GFR. The percentage decrement from median was –0.40% in 74 microalbuminuric and –1.80% in 34 proteinuric patients. The patients with Δ%GFR below and above the median value were defined as progressors and nonprogressors, respectively, among microalbuminuric and proteinuric patients.

and 95% CI, except for AER, for which medians and ranges are given. AER values were log-transformed before analysis. Kidney function and glomerular structure in diabetic and control subjects were compared by analysis of variance and an unpaired, two-sided Student's t test, after Bonferroni correction. To account for the variable numbers and timing of follow-up examinations, changes in group mean GFR were assessed with a mixed-effect model, which combines spline fitting for each serially measured parameter with follow-up times as random effects (27). To assess the degree of statistical differences between dichotomized measures of GFR (progressors and nonprogressors) and development rates of ESRD or proteinuria, χ^2 analysis was used. Univariate and multivariate logistic regression analyses were performed to calculate odds ratios (ORs) (adjusted for age, duration of the disease, and sex) between quartiles of follow-up HbA_{1c} and blood pressure, baseline AER, GBM width, and Vv(mes/glom) in relation to dichotomized measures of percent GFR change from baseline per year (Δ%GFR). The Δ%GFR below and above median was used to divide patients into progressors and nonprogressors, respectively. More particularly, a 2×2 table was plotted with progressor and nonprogressor in rows and first versus second, first versus third, and first versus fourth quartiles in columns. Positive ORs indicate prevalence of progressors; negative ORs indicate prevalence of nonprogressors. Multivariate regression analysis was also performed using Δ%GFR as a continuous variable in relation to clinical-structural parameters, without dichotomous distinction between progressors and nonprogressors.

RESULTS

Course of GFR. The average duration of the follow-up was 4.28 years (range 2.0–6.0) in microalbuminuric and 3.41 years (range 2.0–6.0) in proteinuric patients. Mean (± SD) GFR significantly decreased from baseline both in microalbuminuric (–1.3 ± 9.4 [95% CI –3.51 to 0.86], $P < 0.05$) and proteinuric (–3.0 ± 13.0 ml · min^{-1} · 1.73 m^{-2} per year [–7.71 to 1.61], $P < 0.01$) type 2 diabetic patients. The absolute GFR change from baseline was then expressed as Δ%GFR. The plot of the individual Δ%GFR values showed that courses of GFR were scattered in a wide range, which encompassed both negative and positive values in microalbuminuric and proteinuric type 2 diabetic patients (Fig. 1). The microalbuminuric and proteinuric patients were subdivided into progressors (below median: –0.4 Δ%GFR among microalbuminuric and –1.8 Δ%GFR among proteinuric patients, respectively) and nonprogressors (above median Δ%GFR) (Fig. 1). Progressors were older than nonprogressors among proteinuric, but not among microalbuminuric, patients. There were more men in the proteinuric than in the microalbuminuric groups, but no differences in sex were found between progressors and nonprogressors (Table 1). Duration of diabetes was also similar in the two cohorts of patients (Table 1). The average HbA_{1c} levels were similar at baseline and during the follow-up period in progressors and nonprogressors (Table 2).

Tight blood pressure control was achieved and maintained in both progressors and nonprogressors. All the microalbuminuric and proteinuric patients were treated with ACE inhibitors (captopril 100–150 mg thrice/day; lisinopril or enalapril 20–40 mg once/day; ramipril 10 mg once/day). In 26%

TABLE 1
Demographic characteristics of type 2 diabetic patients at baseline by group

Type 2 diabetic patients	Number of patients (M/F)	Age (years)	BMI (kg/m^2)	Diabetes duration (years)	Serum creatinine (mg/dl)	Duration of follow-up (years)
Microalbuminuric						
Progressors	20/17	58 ± 6	28.8 ± 9.0	12 ± 6	0.93 ± 0.20	4.02 ± 1.94
Nonprogressors	19/18	58 ± 7	27.9 ± 4.0	10 ± 7	0.95 ± 0.19	4.47 ± 1.92
Proteinuric						
Progressors	11/6	58 ± 8	28.6 ± 4.9	15 ± 5	1.05 ± 0.29	3.44 ± 1.46
Nonprogressors	10/7	52 ± 7	30.0 ± 3.3	12 ± 7	0.94 ± 0.20	3.62 ± 2.06

Data are means ± SD.

TABLE 2
HbA_{1c} and blood pressure levels after 3- to 5-day washout from previous antihypertensive therapies and during the follow-up period in microalbuminuric and proteinuric type 2 diabetic patients divided into progressors and nonprogressors

Type 2 diabetic patients	*n*	Baseline HbA_{1c} (%)	HbA_{1c} during follow-up (%)	Baseline vs. follow-up HbA_{1c}	Baseline blood pressure (mmHg)		Blood pressure during follow-up (mmHg)		Baseline vs. follow-up systolic and diastolic blood pressure (*P*)
					Systolic	Diastolic	Systolic	Diastolic	
Microalbuminuric	74								
Progressors	37	8.13 ± 2.17	7.83 ± 1.44	NS	156 ± 5	92 ± 4	131 ± 3	78 ± 2	<0.01
Nonprogressors	37	8.34 ± 2.23	7.40 ± 1.43	NS	158 ± 6	90 ± 4	132 ± 4	77 ± 2	<0.01
Proteinuric	34								
Progressors	17	9.11 ± 2.44	8.38 ± 1.32	NS	164 ± 7	93 ± 5	133 ± 3	77 ± 2	<0.01
Nonprogressors	17	9.44 ± 2.79	8.00 ± 1.16	NS	161 ± 8	92 ± 5	134 ± 3	76 ± 2	<0.01

Data are means ± SD. Values for HbA_{1c} and blood pressure are the average values of each measurement taken every 6–12 months during the follow-up period.

of the patients, dihydropyridinic calcium antagonists were added to the ACE inhibitors (amlodipine or nifedipine slow-release). Thiazides or furosemide were combined with ACE inhibitors in 74% of the patients. Forty-two percent of the patients were also treated with α- or β-blockers. ACE inhibitors were combined with one of the above-mentioned drugs in 100% of the patients and with two in 59% of the patients. Sixteen percent of the patients were treated with isocaloric, simple sugar–free diet and sulfonylureas. Metformin was used in 66% of the patients and insulin in 48% of the patients. No differences were observed in antihypertensive or metabolic control between progressors and nonprogressors. All the proteinuric patients received insulin, as associated therapy.

Baseline GFR was similar in progressors and nonprogressors among both microalbuminuric and proteinuric patients. Baseline AER was similar in progressors and nonprogressors among both microalbuminuric and proteinuric patients (Table 3). No significant changes of AER from baseline were observed in the last year of follow-up in microalbuminuric progressors (medians 50 vs. 41 μg/min, NS) and nonprogressor patients (medians 39 vs. 33 μg/min, NS). In contrast, during the last year of follow-up, AER was higher than at baseline in proteinuric progressors (medians 1,281 vs. 420 μg/min, $P < 0.01$). No significant changes were observed in proteinuric nonprogressors (medians 411 vs. 371 μg/min, NS). Of the 37 microalbuminuric progressors, 8 developed macroalbuminuria and 1 developed ESRD. Of the 37 microalbuminuric nonprogressors, 3 developed macroalbuminuria and none developed ESRD (Table 3). Six of 17 proteinuric progressors and none of the 17 nonprogressors developed ESRD.

Eight patients with proteinuria and six with microalbuminuria had proliferative retinopathy and were all progressors. Twenty-one patients with proteinuria and 34 with microalbuminuria had background retinopathy and were 61% progressors (33 patients) and 39% nonprogressors (21 patients).

TABLE 3
Renal function in microalbuminuric and proteinuric type 2 diabetic patients divided into progressors and nonprogressors on the basis of the course of GFR

Type 2 diabetic patients	*n*	Baseline GFR (ml · min^{-1} · 1.73 m^{-2})	Absolute change of GFR from baseline (ml · min^{-1} · 1.73 m^{-2}) per year	Baseline AER (μg/min)	Development of macroalbuminuria	Development of ESRD	Regression to	
							Normoalbuminuria	Microalbuminuria
Microalbuminuric								
Progressors	37	107 ± 26	–5.54 ± 6.74	41 (21–199)	8 (22)	1 (3)	0	—
Nonprogressors	37	96 ± 20	+4.08 ± 11.07	33 (23–199)	3 (8)	0	8 (22)	—
Proteinuric								
Progressors	17	89 ± 36	–10.92 ± 9.99	420 (232–3,126)	—	6 (35)	0	0
Nonprogressors	17	104 ± 27	+3.96 ± 5.84	371 (200–2,200)	—	0	0	3 (17)
Statistics								
Microalbuminuric progressors vs. nonprogressors		NS	—	NS	$P < 0.05$	NS	$P < 0.05$	
Proteinuric progressors vs. nonprogressors		NS	—	NS	—	$P < 0.05$	NS	

Data are means ± SD, medians (range), or *n* (%). The rate of development of macroalbuminuria from microalbuminuria and of ESRD and of regression either to normo- or microalbuminuria were expressed as absolute number and percent frequency of patients in whom such an event occurred.

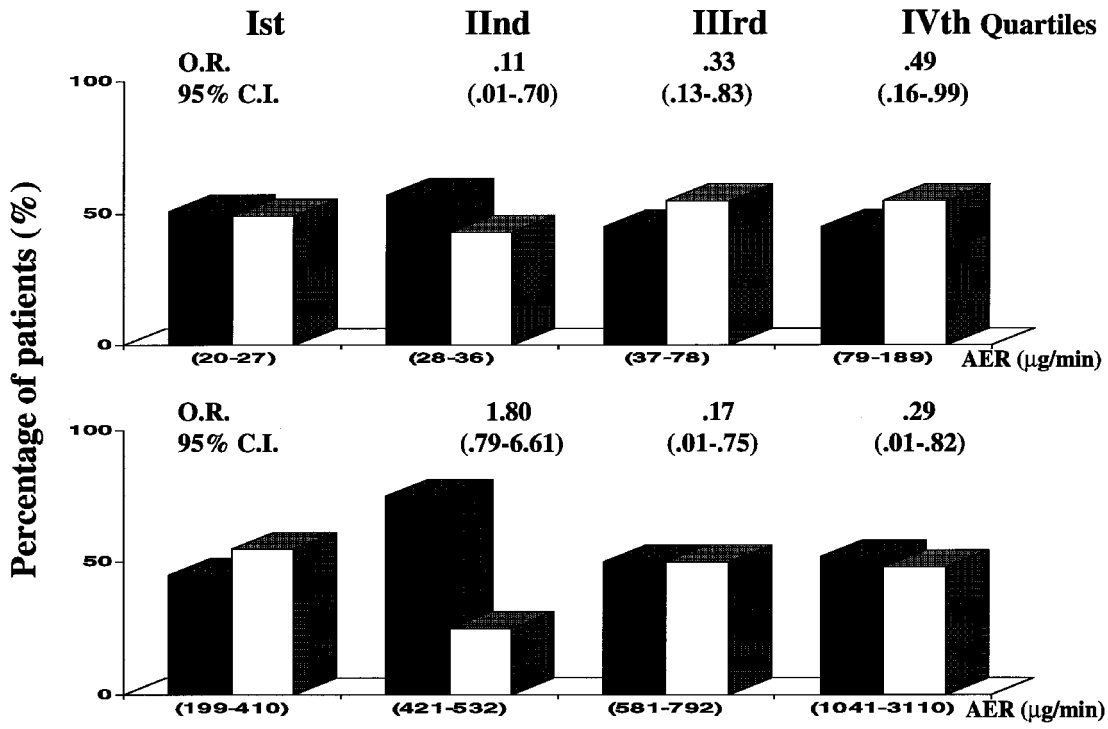

FIG. 2. Proportion of the 74 microalbuminuric (*top*) and 34 proteinuric (*bottom*) type 2 diabetic patients in each quartile of baseline values of AER who progressed (■) or did not progress (□) according to the definition in the text. The ORs and 95% CIs for being a progressor are indicated on the top of the figure as a positive value. Values below the columns indicate the range of values of AER in each quartile.

Dichotomous analysis of the course of GFR in relationship with structural and functional renal parameters. ORs for being a progressor were computed according to quartiles of distribution of baseline AER. The risk of developing a decline of GFR (number of progressors) did not increase across quartiles of baseline AER in either microalbuminuric or proteinuric patients (Fig. 2). However, patients who had a significant increase of AER during the follow-up period were more frequently found among proteinuric progressors than among proteinuric nonprogressors. This was not the case among microalbuminuric patients. To further elucidate the relationship between the course of GFR and other clinical and morphological parameters, we pooled the microalbuminuric and proteinuric patients. ORs for being a progressor were computed according to quartiles of distribution of average HbA_{1c} during follow-up. The risk of being a progressor increased across quartiles of HbA_{1c}, especially in the fourth quartile, with HbA_{1c} levels >9% (χ^2: $P < 0.01$) (Fig. 3). Concomitantly, the number of nonprogressors decreased across quartiles of HbA_{1c} levels and was significantly lower in the first and the second quartiles with HbA_{1c} levels <8.0% (Fig. 3). The risk of developing a decline of GFR did not increase across quartiles of the MBP levels during the follow-up period (Fig. 4). The OR for becoming a progressor increased across the quartiles of GBM width and Vv(mes/glom) values: the OR was 2.71 higher in the fourth quartile (highest value) than in the first quartile (lowest value) of GBM width ($P < 0.01$) and was 2.85 higher in the fourth quartile than in the first quartile for Vv(mes/glom) ($P < 0.01$) (Figs. 5 and 6). Conversely, the number of nonprogressors was higher than that of progressors in the first quartiles of GBM width (OR: 2.14, $P < 0.05$) and Vv(mes/glom), (OR: 2.28, $P < 0.01$) (Fig. 5 and 6). Progressors had higher GBM width and Vv(mes/glom) than nonprogressors in both microalbuminuric and proteinuric patients (Table 4). These parameters were significantly greater in diabetic patients than in control subjects (Table 4). However, there was a substantial overlap of values between diabetic patients and control subjects as shown in Figs. 5 and 6. The first quartile of distribution in diabetic patients (lowest degree of severity of GBM thickening) fell into the normal range for GBM width (Fig. 5), whereas the third and fourth

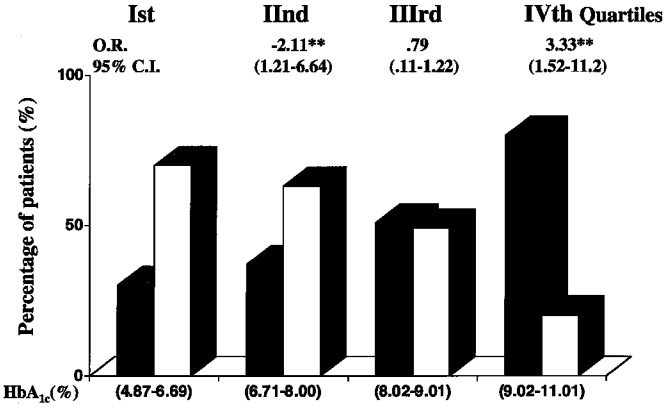

FIG. 3. Proportion of the overall 108 microalbuminuric and proteinuric type 2 diabetic patients in each quartile of mean follow-up values of HbA_{1c} who progressed (■) or did not progress (□) according to the definition in the text. The ORs and 95% CIs for being a progressor are indicated at the top of the figure as positive values and for being a nonprogressor as negative values. Values below the columns indicate the range of values of HbA_{1c} (%) in each quartile. $^{**}P < 0.01$.

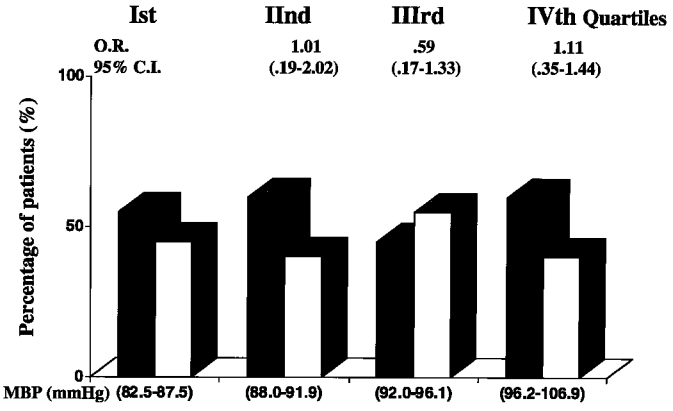

FIG. 4. Proportion of the overall 108 microalbuminuric and proteinuric type 2 diabetic patients in each quartile of mean follow-up values of MBP who progressed (■) or did not progress (□) according to the definition in the text. The ORs and 95% CIs for becoming a progressor are indicated at the top of the figure. Values below the columns indicate the range of values of MBP in each quartile.

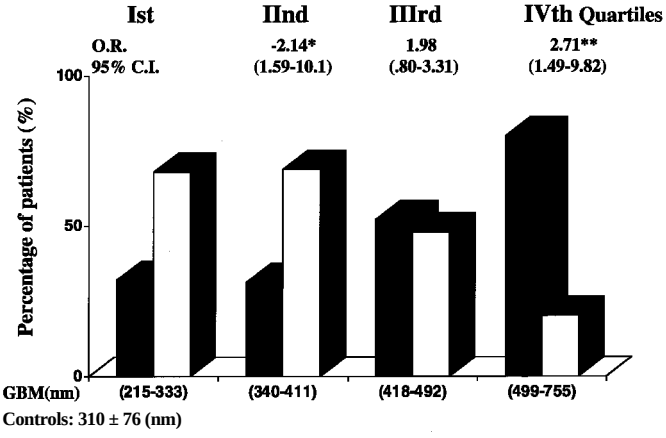

FIG. 5. Proportion of the overall 108 microalbuminuric and proteinuric type 2 diabetic patients in each quartile of baseline values of GBM width who progressed (■) or did not progress (□) according to the definition in the text. The ORs and 95% CIs for becoming a progressor are indicated at the top of the figure as positive values and for becoming a nonprogressor as negative values. Values below the columns indicate the range of values of GBM in each quartile and the mean plus 2 SD in 27 control subjects. *P < 0.05, **P < 0.01.

quartiles (greatest GBM width) were completely above the normal range. Only a small fraction of the values observed in diabetic patients in the second quartile overlapped with those of control subjects (Fig. 5). The number of progressors sharply increased and that of nonprogressors decreased in the third quartile of GBM width only.

With regard to Vv(mes/glom), both first and second quartiles (lowest degree of severity of mesangial volume expansion) fell into the normal range (Fig. 6). Most of nonprogressors had Vv(mes/glom) values in first and second quartiles (41 nonprogressors vs. 17 progressors). Number of progressors sharply increased (three times as much as that of nonprogressors) in the third quartile of distribution of Vv(mes/glom), just above the upper limit in normal control subjects (37 progressors vs. 13 nonprogressors) (Fig. 6). Nine and 8 patients were progressors (13 microalbuminuric and 4 proteinuric), although they had Vv(mes/glom) in first and second lowest quartiles (Fig. 6).

Analysis of GFR course as a continuous variable in relationship with structural and functional renal parameters. Multivariate regression analysis showed that there was a significant relation between Δ%GFR values as a continuous variable and the other clinical and renal morphological parameters, irrespective of the definition of progressors and nonprogressors, in the overall population of microalbuminuric and proteinuric patients (Multiple r value: 0.56; F value: 3.50; P < 0.004). The individual coefficients of linear correlation indicated that there was no significant relation between Δ%GFR and MBP levels and AER, at baseline and during follow-up, and baseline GFR and diabetes duration. There was a trend toward a significant relation with HbA_{1c} levels during follow-up (t value: 1.87; P < 0.07) and GBM width at baseline (t value: 2.02; P < 0.056). The only significant relation was with Vv(mes/glom) (t value: 2.63; P < 0.01).

DISCUSSION

This study demonstrates that the course of renal function is heterogeneous among microalbuminuric and proteinuric Caucasian type 2 diabetic patients, who are otherwise two homogeneous groups in terms of abnormalities of baseline AER, GFR, and blood pressure values. The cohort of microalbuminuric and proteinuric patients with more advanced glomerulopathy at baseline is more likely to rapidly lose renal function. This subgroup of patients has worse metabolic control and is less responsive to tight blood pressure control, even using ACE inhibitors.

The definition of two different cohorts of patients—progressors and nonprogressors on the basis of differences in GFR course—was further supported by our results on the rate of change of category of AER. In fact, a greater number of patients progressed from microalbuminuria to clinical proteinuria among progressors, and a greater number of patients showed regression to normoalbuminuria or microalbuminuria among nonprogressors. However, the level of AER, not only

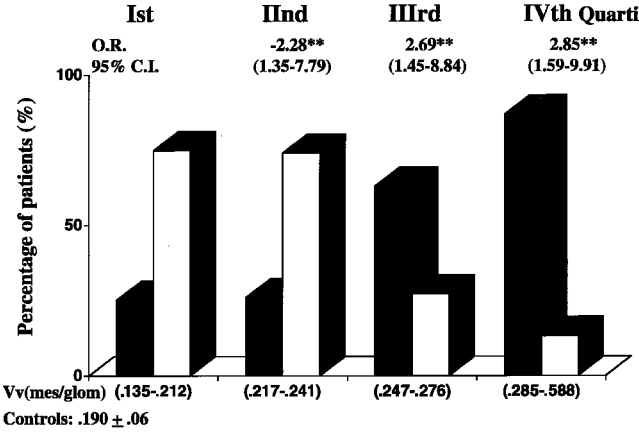

FIG. 6. Proportion of the 108 microalbuminuric and proteinuric type 2 diabetic patients in each quartile of baseline values of Vv(mes/glom) who progressed (■) or did not progress (□) according to the definition in the text. The ORs and 95% CIs for being a progressor are indicated at the top of the figure as positive values and for being a nonprogressor as negative values. Values below the columns indicate the range of values of Vv(mes/glom) for each quartile and the mean plus 2 SD in 27 control subjects. **P < 0.01.

TABLE 4
Renal structure at baseline in patients with type 2 diabetes according to group and in 27 control subjects

	n	GBM width (nm)	Vv(mes/glom)
Microalbuminuric type 2 diabetic patients			
Total group	74	418 ± 80	0.249 ± 0.057
Progressors	37	458 ± 107	0.267 ± 0.066
Nonprogressors	37	389 ± 54	0.235 ± 0.047
Proteinuric type 2 diabetic patients			
Total group	34	512 ± 86	0.300 ± 0.08
Progressors	17	539 ± 102	0.326 ± 0.101
Nonprogressors	17	460 ± 66	0.258 ± 0.052
Control subjects	27	310 ± 38	0.190 ± 0.03
Statistics (ANOVA and Bonferroni *t* test)			
Microalbuminuric progressors vs. nonprogressors		$P < 0.05$	$P < 0.02$
Macroalbuminuric progressors vs. nonprogressors		$P < 0.01$	$P < 0.02$
Microalbuminuric vs. control subjects		$P < 0.01$	$P < 0.01$
Macroalbuminuric vs. control subjects		$P < 0.05$	$P < 0.05$

Data are means ± SD.

at baseline but also during the follow-up period, poorly predicted the outcome of kidney function among microalbuminuric patients, as microalbuminuric progressors had GFR loss while AER remained, on average, unchanged. On the other hand, among proteinuric progressors, a rapid worsening of renal function, as testified by a greater GFR decline, was associated with an increase in AER values. The term "nonprogressors" must be cautiously interpreted, however, on the basis of the results of the current study, as the follow-up period lasted only 4 years. Thus, we cannot rule out the possibility that advanced diabetic glomerulopathy may later also develop in the cohort of nonprogressors, eventually resulting in a significant loss of GFR and/or ESRD.

We used morphometric analysis to evaluate two important parameters of diabetic glomerulopathy, i.e., GBM width and Vv(mes/glom). These parameters were significantly higher in microalbuminuric and proteinuric patients than in control subjects. Moreover, most microalbuminuric and proteinuric patients had some evidence of either proliferative or background diabetic retinopathy, among both progressors and nonprogressors.

Thus, on average, some degree of diabetic glomerulopathy and retinopathy was found in most of the type 2 diabetic patients with abnormalities of AER. These observations suggest that the metabolic challenge of diabetes leads, always on average, to diabetic renal structural lesions in the overall population of type 2 diabetic patients with abnormalities of AER, in a manner resembling that shown in type 1 diabetes. These data confirm the view of Ritz and Stefanski (1), who cited a large monograph by Ditscherlein (28) that reported 400 autopsies in which a gross qualitative difference of renal morphology between type 1 and 2 diabetes was not noted. Similar conclusions were also drawn by Olsen and Mogensen (29) in a recent review on this issue. In a cross-sectional study in Japanese type 2 diabetic patients with proteinuria, Hayashi et al. (30) found changes similar to those that characterize the diabetic glomerulopathy seen in type 1 diabetic patients: basement membrane thickening and increase in the mesangium and mesangial matrix, expressed as fraction of the glomerular volume. More recently, Pagtalunan et al. (17) found that Pima Indians with type 2 diabetes and microalbuminuria exhibited moderate increases in glomerular and mesangial volume when compared with those with early diabetes, but they could not be distinguished from subjects who remained normoalbuminuric after an equal duration of diabetes. These latter authors also suggested that a decrease in podocyte number per unit of glomerular volume could contribute to the progression of diabetic nephropathy.

The values of GBM width and Vv(mes/glom), we observed in the current study, were broadly dispersed in a wide range. In fact, the first and/or the second lowest quartiles of distribution of such parameters in micro- and macroalbuminuric patients overlapped on the normal range.

This was not the case in previous reports concerning type 1 diabetic patients, in whom >90% of the patients have GBM width and Vv(mes/glom) values above the mean plus two standard deviations of control subjects when AER is >30 µg/min (10,11,31).

A close correlation between diabetic glomerulopathy and decline in GFR was observed in the current study, as previously shown by Osterby et al. (16) in a series of 20 Caucasian type 2 proteinuric diabetics. However, these authors (16) also pointed out in this retrospective study that type 2 diabetic patients with proteinuria, at variance with those with type 1 diabetes with similar abnormalities of AER, still have normal GFR, on average.

All together, these observations suggest that the point at which the association between renal structure and function is loose, in type 2 diabetic patients, is with respect to AER. This does not mean that the magnitude of AER is not an important parameter to describe the severity of renal involvement in the natural history of any individual patient with type 2 diabetes. In fact, significantly greater increases of AER were observed in the progressor than in the nonprogressor proteinuric patients, although baseline AER was similar in the two subgroups. However, progression of renal damage in terms of GFR decline is better predicted by glomerular structural parameters than by baseline levels of AER.

Our findings demonstrate that mesangial expansion is also a crucial lesion in Caucasian type 2 diabetes, as it has been

previously shown in Caucasian type 1 diabetes (11). Indeed, values of Vv(mes/glom) immediately above the normal range are associated with a sharp increase in the number of patients with rapid decline of GFR. It has been postulated that Vv(mes/glom) is closely related to filtration surface per glomerulus; in turn, this might explain why Vv(mes/glom) is strongly linked to GFR values.

GBM width was also related to loss of renal function, but less closely than Vv(mes/glom). Progressors clearly outnumbered nonprogressors only in the fourth quartile, although the values of GBM width in the second and third quartiles of distribution were already clearly above the normal range.

With regard to the pathogenesis of abnormalities of AER in those patients in whom glomerular structure is less severely altered or normal, we have recently demonstrated that advanced tubulo-interstitial and/or arteriolar lesions might in part explain these abnormalities of renal function (12,13). About 80–100% of type 2 diabetic patients with abnormalities of AER have arterial hypertension and are elderly, at variance with juvenile-onset type 1 diabetic patients, in whom arterial hypertension is shown by only 50% of the patients, despite similar levels of AER (1,18).

Our previous light microscopic analysis showed that 30–40% of type 2 diabetic patients with microalbuminuria had atypical patterns of renal lesions, which were not characteristic of any renal disease other than diabetes (11,13). It can be postulated that a subgroup of type 2 diabetic patients responds to the metabolic challenge of diabetes by developing these atypical patterns of renal lesions. Interestingly, it has been recently observed that intermittent exposure to high glucose enhances cell growth and collagen synthesis in cultured human tubulo-interstitial cells (32). It can be suggested that short-lived, but repeated, excursions in glycemic control may have important pathological effects on the tubulo-interstitium in a cohort of type 2 diabetic patients, whereas sustained hyperglycemia, reflected by worse HbA_{1c} levels, may explain the occurrence of simultaneous lesions at both the glomerular and tubulo-interstital levels in a further subgroup of patients. In this context, age and hypertension may also contribute to a higher rate of glomerular occlusion, an important structural factor, which might lead to loss of GFR without concomitant marked changes of AER (16,31). These findings are in keeping with those of Pinel et al. (33), investigating 22 proteinuric type 2 diabetic patients, who reported nonspecific vascular and glomerular changes in 14% of the patients and early diabetic glomerulopathy with very mild mesangial lesions in an additional 18%. Similar findings have been reported by Gambara et al. (15). Furthermore, our results show that 33% of microalbuminuric and 17% of proteinuric patients had near-normal glomerular structure. These observations resemble those of Parving et al. (14), who found that 11% (4 of 35) of proteinuric type 2 diabetic patients had almost normal glomerular structure, what they called minimal change nephropathy.

The course of renal function in type 2 diabetes has not been described as precisely as in type 1 diabetes. Nelson et al. (34) observed significant GFR changes in proteinuric, but not in microalbuminuric, diabetic Pima Indians. Parving (18) recently reviewed data in literature on GFR decrease in Caucasian proteinuric type 2 diabetic patients during antihypertensive therapy, using either ACE inhibitors or other drugs, and reported an average decline of 4.8 $ml \cdot min^{-1} \cdot year^{-1}$. A GFR decline of 2.3 $ml \cdot min^{-1} \cdot year^{-1}$ was observed by our group in hypertensive type 2 diabetic patients with microalbuminuria during treatment with ACE inhibitors and calcium-channel blockers (19).

Our findings confirm that intensified antihypertensive therapy delays the loss of GFR in hypertensive type 2 diabetic patients. In fact, GFR decline was twice as low in the current study than in the previous report from our group (1.3 vs. 2.3 $ml \cdot min^{-1} \cdot year^{-1}$) in microalbuminuric (19) and proteinuric patients, as compared with the above-mentioned data (3.0 vs. 4.8 $ml \cdot min^{-1} \cdot year^{-1}$) (18).

However, our findings show that intensified antihypertensive therapy does not confer long-term renoprotection in a subgroup of type 2 diabetic patients. This grim long-term prognosis resembles that observed in other specific high-risk populations, such as patients with polycystic kidney or with ACE DD genotype (35,36). Also, in type 2 diabetic Pima Indians with proteinuria, the use of ACE inhibitors did not prevent a decline of GFR that was at least as rapid as in type 1 diabetic patients, despite adequate control of blood pressure (34). Interestingly, diabetic Pima Indians with proteinuria and who show severe loss of GFR have patterns of typical diabetic glomerulopathy similar to those found in the current study in the progressor cohort.

Worse levels of HbA_{1c} in the upper quartile with values >8.5–9.0% were closely associated with a more rapid deterioration of kidney function. A cutoff point of HbA_{1c} levels ~8.0–8.5% has been suggested to be a triggering threshold for the development of microalbuminuria and retinopathy in type 1 diabetic patients (37) and, more recently, in type 2 diabetic patients (38). We suggest that the uniform accomplishment of very strictly controlled blood pressure levels tends to conceal the deleterious effect of hypertension on renal function, highlighting the equally dangerous effect of poor metabolic control.

In conclusion, these findings suggest that the course of renal function is heterogeneous in type 2 diabetic patients and reflects heterogeneous patterns of renal lesions. A subset of patients with microalbuminuria and proteinuria, characterized by typical diabetic glomerulopathy, rapidly lose renal function, despite tight blood pressure control. Thus, abnormalities of AER have a different renal prognostic value depending on the underlying renal structure. Strict glycemic control using intensified insulin therapy with 4–5 daily administrations, in addition to antihypertensive therapy, may be needed to effectively blunt the decline of glomerular function in type 2 diabetic patients with abnormalities of AER.

ACKNOWLEDGMENTS

This work was supported by the National Research Council (CNR) Center for the Study of Ageing, University of Padova, by CNR Progetto Finalizzato Invecchiamento Grants PFI/419,1993,1994,1995,1996, and by CNR 60% grants, University of Sassari and Regione Veneto (responsible investigator for both: R.N.).

The authors gratefully thank Prof. M. Mauer at the University of Minnesota for reading the light microscopy slides along with P.F. and for providing the kidney biopsies from normal kidney donors. The authors are also indebted to Prof. C.E. Mogensen from the University of Aarhus, Denmark, for useful suggestions in the analysis of the results of the present

study. We also acknowledge the collaboration of Prof. Benedetto Salvato, Director of the Centro di Studio per la Fisiologia e Biochimica delle Metalloproteine, in which electron microscopy measurements were performed.

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