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Glomerular size-selective dysfunction in NIDDM is not ameliorated by ACE inhibition or by calcium channel blockade

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Glomerular size-selective dysfunction in NIDDM is not ameliorated by ACE inhibition or by calcium channel blockade.

Background. In patients with insulin-dependent diabetes mellitus (IDDM) and overt nephropathy glomerular barrier size-selectivity progressively deteriorates with time and is effectively improved by angiotensin converting enzyme (ACE) inhibition. Whether similar glomerular functional changes develop in proteinuric patients with non-insulin–dependent diabetes mellitus (NIDDM), and whether antihypertensive agents can favorably affect glomerular filtration of macromolecules in these patients, has not been documented yet.

Methods. We investigated renal hemodynamics and fractional clearance of neutral dextrans of graded sizes, in nine proteinuric patients with NIDDM and renal biopsy findings of typical diabetic glomerulopathy. Six healthy volunteers served as controls. We also investigated the effects of an ACE inhibitor and of a calcium channel blocker, both given in doses targeted to achieve a comparable level of systemic blood pressure control, on glomerular hemodynamics and sieving function. Theoretical analysis of glomerular macromolecule transport was adopted to evaluate intrinsic glomerular membrane permeability properties.

Results. Fractional clearance of large macromolecules (42 to 66 Å in radius) was significantly higher in diabetic patients than in controls, and the distribution of membrane pore radii was calculated to be shifted towards larger pore sizes in diabetics (mean radius increased from 55 to 60 Å). Despite effective blood pressure control, neither antihypertensive affected glomerular hemodynamics to any significant extent. Fractional clearance of dextrans, as well as of albumin and IgG, and total urinary proteins were not modified by either treatments.

Conclusions. These data indicate that patients with NIDDM and overt nephropathy develop abnormalities in size-selective function of the glomerular barrier and, at variance to IDDM, such changes were not ameliorated either by ACE inhibition or calcium channel blockade.

Key words: dextran, glomerular sieving, proteinuria, non-insulin–dependent diabetes mellitus, angiotensin converting enzyme, blood pressure.

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Studies of the glomerular sieving functional properties in patients with insulin-dependent diabetes mellitus (IDDM), clinical proteinuria and biopsy findings of diabetic-type glomerulopathy (overt nephropathy) provided solid evidence of loss of size-selective properties of the glomerular membrane, which appears a major determinant of abnormal filtration of plasma proteins [1–4]. Whether charge-selective dysfunction also occurs and to what extent it may play a role in abnormal permeability to albumin and other proteins is less clear, partly due to technical difficulties in measuring glomerular charge-selective function in humans [4, 5]. In diabetic as in nondiabetic proteinuric nephropathies, plasma proteins, ultrafiltered in exuberant amount and reabsorbed by the proximal tubule, up-regulate inflammatory and vasoactive genes whose protein products trigger an interstitial inflammatory reaction that culminates in renal scarring [6, 7].

Theoretical analysis of glomerular sieving coefficients of neutral test macromolecules indicated that in patients with IDDM, who were studied early in the course of the disease, angiotensin converting enzyme (ACE) inhibition reduced proteinuria by reducing the size of large unselective pores assumed to perforate the glomerular membrane [8]. IDDM patients benefitted from ACE inhibition even when they were in an advanced stage of the nephropathy. Data are indeed available showing the amelioration of glomerular barrier function and protein excretion even independently from changes in systemic blood pressure and renal hemodynamic parameters [9]. Moreover, experimental and clinical evidence has accumulated in recent years to indicate that a reduction of the urinary protein excretion rate predicts a slower rate of glomeru-

lar filtration rate (GFR) decline in patients with IDDM and overt nephropathy [10]. The results of several clinical studies have consistently indicated that among other antihypertensives ACE-inhibitors, in addition to blood pressure control, reduce urinary protein excretion and prevent renal injury and progression to end-stage renal failure [11]. On the other hand, agents that fail to reduce urinary proteins (namely nifedipine and other dihydropyridinic calcium channel blockers) were unable to block renal disease progression; instead their use appears to be consistently associated with a fast rate of GFR decline [12].

At variance with IDDM, information on glomerular permselective function in patients with non-insulin–dependent diabetes mellitus (NIDDM) with overt nephropathy are missing, and the only available study was done in a group of Pima Indian Americans who happened to have a rather preserved GFR [13]. One explanation for the lack of data on this condition is that precise characterization of the nature of renal disease in NIDDM with overt nephropathy poses some difficulties. In a recent paper we found rather heterogeneous renal biopsy findings in NIDDM that included at least three distinct patterns of injury classified as diabetic-type glomerulopathy, prevailing nephroangiosclerosis-type changes and lesions of nondiabetic glomerulopathy superimposed to classical features of diabetes [14]. These diverse patterns of lesions could potentially account for different manifestations of glomerular barrier dysfunction and different responses to antihypertensive therapies.

The purpose of the present study was to investigate renal hemodynamics and glomerular barrier function in Caucasian proteinuric NIDDM patients with biopsy evidence of pure diabetic-type glomerular structural lesions. We also explored the possibility of modulating intrinsic glomerular barrier properties by either an ACE inhibitor or by a calcium channel blocker given in doses targeted to achieve a comparable level of systemic blood pressure control.

METHODS

Study population

Study patients were selected among a pool of 52 NIDDM patients (30 males and 22 females) referred to the Unit of Nephrology and Dialysis of the Azienda Ospedaliera, Ospedali Riuniti di Bergamo from April 1983 to November 1991 who had a renal biopsy performed to investigate the causes of persistent clinical proteinuria and/or renal insufficiency [14]. Among these, 12 subjects satisfied the inclusion/exclusion criteria detailed in the study protocol and 9 (8 males and 1 female) provided their informed consent according to the Declaration of Helsinki. The patients had newly diagnosed or previously treated mild-to-moderate hypertension and diabetic nephropathy diagnosed on the basis of the following: (*1*) urinary protein excretion rate $\geq$ 0.5 g/24 hr on at least three consecutive occasions before study entry; (*2*) changes in creatinine clearance $<$ 30% over the last three months; and (*3*) biopsy-proven diabetic glomerulopathy. At entry HbA_{1c} was $<$10% and all the patients had clinically stable diabetes (that is, changes in $HbA_{1c} < \pm 30\%$ as compared to HbA_{1c} values four months before selection), stable body weight (changes in body weight $< \pm 5$ kg as compared to the body wt 6 months before selection) and were on constant treatment with diet and/or oral antidiabetic agents and/or insulin for at least two months. Serum potassium ranged between 3.5 and 5.0 mM/liter. Pregnant or potentially child-bearing women with no effective contraception treatment and nursing women were excluded. Patients with urinary tract infection, secondary hypertension, renovascular disease, solitary kidney or renal disease other than diabetic glomerulopathy, heart failure (NYHA class III-IV), atrio-ventricular block grade 2–3, stroke or acute myocardial infarction in the last three months, symptomatic coronary ischemic disease, liver or hematologic disease, collagen vascular disease, cancer, chronic treatment with cimetidine, steroidal and nonsteroidal treatment in the two months before screening evaluation, known or suspected intolerance to the study drugs, and any condition that in the investigator's judgment could interfere with the results of the study were excluded. In particular, all potentially eligible patients had an ultrasound evaluation performed before study entry and those with echographic evidence of a postvoiding urine retention suggestive for obstructive uropathy and/or bladder (dysautonomic) dysfunction were not included.

Pathological evaluations of kidney biopsies before entering into the study revealed typical findings of diabetic glomerulopathy consisting of marked glomerular hypertrophy associated with glomerulosclerosis, both of the diffuse and nodular types, and diffuse arteriolar hyalinosis. Global glomerulosclerosis was observed in most patients (affecting on average 33% of glomeruli). All specimens exhibited thickening of glomerular capillary wall and of Bowman's capsule, interstitial fibrosis and thickening of tubular basement membrane. Electron microscopy examinations displayed typical changes of diabetic nephropathy characterized by a marked and diffuse thickening of glomerular basement membrane and diffused sclerotic changes. Six male normotensive volunteers with no sign of renal disease served as healthy controls (Table 1). All subjects gave informed consent before being allowed to enter the study.

Study design

This was a double-blind sequential study (Fig. 1) with a placebo run-in phase followed by two treatment periods,

Table 1. Main clinical and laboratory findings in healthy controls and in NIDDM patients at baseline evaluation

Parameter	Healthy controls	NIDDM
Age *year*	26 (24–27)	64 (51–72)[b]
Sex *M/F*	6/0	8/1
Diabetes duration *year*	—	12.6 (1–25)
Hypertension duration *year*	—	3.6 (0.3–11)
SBP *mm Hg*	128 ± 9	167 ± 11[b]
DBP *mm Hg*	69 ± 7	99 ± 7[b]
Body mass index kg/m^2	23.0 ± 2.7	28.5 ± 4.3[a]
Serum creatinine *mg/dl*	1.0 (0.9–1.1)	1.3 (1.1–3.9)
Creatinine clearance $ml/min/1.73m^2$	120 (105–151)	66 (29–99)[b]
Urinary protein excretion rate *g/24 hr*	ND	5.6 (0.7–13.3)[b]

Data are mean ± SD or median and range in parenthesis. Abbreviations are: ND, not detectable; SBP, systolic blood pressure; DBP, diastolic blood pressure.
[a] $P < 0.05$ vs. healthy controls
[b] $P < 0.01$ vs. healthy controls

with the ACE inhibitor perindopril and the calcium channel blocker nitrendipine, given in a randomized, cross-over design. Selected patients stopped previous antihypertensive therapy before study entry and during the four-week placebo run-in period were given a tablet of placebo twice daily (at 8:00 a.m. and at 8:00 p.m.).

At the end of the placebo run-in phase, patients with diastolic blood pressure (DBP) > 90 and ≤ 110 mm Hg (mean of 3 consecutive measurements, Korotkoff phase V, taken 2 minutes apart in the sitting position after 5 min rest), which satisfied all the selection criteria of the study protocol, including a placebo tablet compliance >80%, had a baseline evaluation of renal hemodynamics and glomerular size-selectivity using solute clearance technique. Patients were then randomly allocated to a 10-week treatment period with an ACE inhibitor (perindopril, 2 mg/day at 8:00 a.m. and identical placebo at 8:00 p.m.) or with a calcium channel blocker (nitrendipine, 10 mg/day at 8:00 a.m. and 10 mg at 8:00 p.m.). Two weeks after randomization, patients with DBP > 90 mm Hg had the dose of the study drugs increased as follows: 4 mg/day perindopril at 8:00 a.m. and identical placebo at 8:00 p.m.; 20 mg/day nitrendipine at 8:00 a.m. and 20 mg/day at 8:00 p.m. After completion of the first 10-week treatment period, patients underwent a second clearance study and then crossed over to the other study drug for an additional 10-week treatment period. The study drugs were given and titrated as described above. A final clearance study was then performed at completion of the second treatment period. The decision to prolong the treatment periods up to 10 weeks the clearance studies permitted to be performed at a time when, on the basis of previously available data [8, 9], the potential residual effect of the first versus the second treatment period was completely exhausted since at least four weeks, thus minimizing the risk of a carryover effects still present at the end of the treatment period. Urine collections for evaluation of 24-hour urinary protein excretion rate were performed at 8 and at 10 weeks of each treatment period, and the mean of two determinations was used for statistical analysis.

The study protocol was approved by the Italian Health Authorities (Ministero della Sanità, Direzione Generale del Servizio Farmaceutico) and by the Ethical Committee of the Regione Lombardia.

Functional studies

Each patient underwent solute clearance studies at the end of the placebo run-in phase and after completion of each treatment period. Exactly the same procedure was used for each patient in all functional evaluations. Clearance studies were also performed in a group of six volunteers, with normal blood pressure and no evidence of renal disease, who served as healthy controls. The morning of the clearance studies, patients were admitted to a Metabolic Ward and submitted a 24-hour urine collection for the evaluation of creatinine clearance, and urinary urea, sodium, and total protein excretion. They were fasting and without antidiabetic treatment from the evening before. The last dose of placebo (end of the washout period) and of the study drugs (end of the two treatment periods) was administered at 8:00 a.m. Blood glucose was checked every 10 minutes and regular insulin was infused intravenously whenever required to maintain blood glucose concentration between 80 and 120 mg/dl for at least two hours. Constant diuresis was induced by oral water loading. As soon as target blood glucose and steady state diuresis were achieved, inulin, para-aminohippurate (PAH) and neutral dextrans clearances were performed during an euglycemic clamp, as described in details previously [9, 15]. Briefly, primed infusion of inulin and para-aminohippurate (PAH) was immediately followed by a slow intravenous administration (about 15 min) of Dextran-40 (130 mg/kg, Rheomacrodex, Pharmacia, Upsala, Sweden). A sustained infusion of inulin and PAH was continued throughout the study to maintain constant plasma concentrations. After an equilibration period of about 60 minutes, three exactly timed urine collections 30 minutes apart were made by spontaneous voiding. To avoid loss of free PAH in glycosuric urine, samples were collected over 10 μl of 4 M NaOH [16]. Blood samples were collected at the beginning and at the end of each clearance period. Urine and plasma samples obtained during the first clearance period were used to determine fractional clearance of dextran molecules of graded size (effective molecular radius ranging from 26 to 66 Å). The same urine and plasma samples were used for the determination of albumin and IgG concentrations. An ultrasound evaluation was performed at the end of each clearance study to verify that in no case could the data be affected by incomplete bladder emptying.

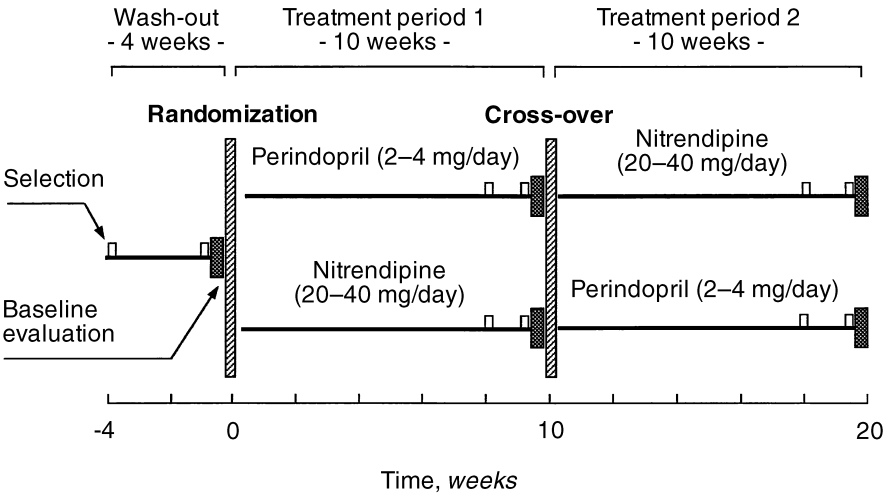

Fig. 1. Schematic representation of the study protocol. A description of the study design is in the text. Symbols are: (□) 24-hour protein excretion; (▨) clearance study.

Analytical methods

Inulin and PAH concentrations in plasma and urine samples were determined using previously described colorimetric assays [9, 17]. GFR was calculated as the average of the three inulin clearances. Effective renal plasma flow (ERPF) was calculated from average PAH clearance assuming a renal extraction coefficient for PAH of 0.9 for healthy controls and of 0.7 for proteinuric patients with reduced GFR, as reported previously [18]. Separation of graded-size dextran molecules and inulin in plasma and urine samples was performed by gel permeation chromatography on a Sephacryl S-300 column (1.6 × 95 cm). Column calibration was performed using dextran standards of known molecular weight (Pharmacosmos, Viby Sj., Denmark). Molecular radius of individual dextran fractions was calculated according to direct measurements of dextran reported by Oliver et al [19] that relate effective molecular radii and mean molecular weight of neutral dextran. Dextran and inulin concentrations were assayed in the eluted fractions using colorimetric methods previously described [9, 17, 20]. Urine-to-plasma concentration ratio for inulin was calculated from inulin concentrations in eluted fractions. Elution volume of fractions containing inulin (molecular radius of 11 to 13 Å) were identified by periodic separation of a standard solution of inulin. Fractional clearance of dextran macromolecules was computed as

$$\vartheta_D = (U/P)_D/(U/P)_{IN}$$

where $(U/P)_D$ and $(U/P)_{IN}$ are the urine-to-plasma concentration ratios of dextran and inulin, respectively.

Albumin and IgG concentrations in plasma and urine samples were determined using a sensitive enzyme-linked immunosorbent assay (ELISA). Briefly, diluted samples were placed in polystyrene wells coated with antibodies to human albumin (Sigma Chimica, Milan, Italy) or IgG (Boehringer Mannheim, Milan, Italy). Goat antihuman albumin (American Qualex, La Miranda, CA, USA) or IgG (Sigma Chimica) conjugated with alkaline phosphatase was added, followed by alkaline phosphatase substrate (Sigma Chimica), and optical density of wells read using a micro-ELISA reader. Fractional clearances of albumin and IgG were calculated using the same formula employed for dextran. Other laboratory measurements were performed using routine laboratory techniques.

Theoretical analysis of glomerular membrane transport

To investigate membrane permeability properties we analyzed glomerular sieving coefficients of test macromolecules using a mathematical model of glomerular size-selectivity described in details previously [9, 21, 22]. This model allows separation of the effects of membrane permeability changes from glomerular hemodynamic alterations on glomerular filtration of neutral test macromolecules. According to this model the glomerular membrane was assumed to be perforated by cylindrical pores having a lognormal distribution of their radii that is fully described by two parameters: the mean (u) and standard deviation (s) of the corresponding normal distribution. Thus, u is a measure of the mean and s of the spread of the pore-size distribution. Besides these two parameters, the model is based on another freely adjustable parameter, the ultrafiltration coefficient (K_f), the product of hydraulic membrane permeability and glomerular membrane-filtering surface area. We calculated K_f as extended to all glomerular population in both kidneys using the model of glomerular ultrafiltration previously described [23]. The intrinsic membrane permeability parameters were calculated, as shown in detail previously [9, 17], minimizing the sum of squared errors (χ^2) between experimental and calculated sieving coefficients,

in healthy controls and patients, during each individual clearance study. Using preliminary analysis we also performed these calculations using the log-normal plus shunt model described in details previously [22]. This model considers the glomerular capillary wall to be perforated by restrictive pores having log-normal distribution of their radii and by a small fraction of large nonselective pores that act as a shunt pathway. The accuracy of the two models in simulating experimental data, estimated using the average sum of squared errors, was higher for the log-normal distribution that for the log-normal plus shunt model (data not shown). For this reason we adopted the log-normal distribution model for our theoretical analysis.

Sample size estimation and statistical analysis

The primary efficacy variable of the study was the fractional clearance of the largest neutral dextrans (in particular 60 Å radius dextran), which most reliably reflect glomerular barrier size-selectivity to plasma macromolecules. Then we predicted that the pattern of glomerular barrier dysfunction and response to ACE inhibitor therapy in NIDDM patients with typical glomerulopathy would have reflected the pattern previously observed in IDDM patients with a comparable degree of GFR depression and urinary proteins excretion, and a similar pattern of glomerular histologic changes. On the basis of this hypothesis, we predicted that in our series of NIDDM patients baseline fractional clearance of neutral dextrans with radii of 60 Å would have ranged from 0.02 $\pm$ 0.01 to 0.04 $\pm$ 0.01 and would have decreased by 60% after ACE inhibitor therapy [9]. Power analysis based on these assumptions revealed that four to nine patients (according to the given range in neutral dextran fractional clearances) were to be included to achieve a β-error of 0.10 with an α-error of 0.05.

Data are expressed as mean $\pm$ standard deviation (SD) or median and range as specified. Results of individual groups were compared using two-tailed Student's *t*-test for unpaired data or one-way analysis of variance, as appropriate. Statistical analysis was performed using the software package StatView (Abacus Concepts Inc., Berkeley, CA, USA). Due to their skewed distribution, urinary protein excretion rate, albumin and IgG fractional clearance values were log-transformed before statistical analysis. The statistical significance level was defined as $P < 0.05$. The Grizzle approach was used to investigate the possibility of a carryover effect from the first to the second treatment periods of the study [24]. An analysis restricted to the first treatment period was planned if a carryover effect could not be excluded by the above approach.

RESULTS

All nine patients with baseline evaluation entered and completed the crossover study. Baseline characteristics of patients and of healthy controls are shown in Table 1. Within the limits related to difficulties in precisely establishing disease onset in NIDDM, patients enrolled in this study had long-standing diabetes and a relatively good metabolic control, on the basis of their baseline levels of HbA1c (Table 2). All patients reported a history of hypertension and were found to have mild-to-moderate hypertension at baseline evaluation, after one month washout from previous antihypertensive therapy. The degree of renal dysfunction at study entry was heterogeneous, with some patients having near-normal creatinine clearances and others more advanced renal insufficiency. However, all patients had clear evidence of severe glomerular barrier dysfunction, as documented by high urinary protein excretion rates (in the nephrotic ranges in seven out of nine patients) and protein-creatinine ratios (P/C) in spot morning urine collections (Table 2). Grizzle analysis of response to perindopril and nitrendipine in NIDDM patients [24] excluded the possibility of a carryover effect between the two treatment periods on the main efficacy variable of the study (60 Å dextran fractional clearance) and on the other efficacy variables, including mean sitting systolic (SBP) and diastolic (DBP) blood pressures, protein/creatinine ratio, 24-hour urinary protein excretion rate and GFR measured at the end of each treatment period.

Mean sitting SBP and DBP measured before drug administration at the end of the 10-week treatment periods were significantly lowered by both perindopril and nitrendipine. Actually, by the end of the perindopril treatment, SBP and DBP were on average 19 and 11 mm Hg lower than at baseline evaluation. Similarly, corresponding blood pressure reductions after nitrendipine treatment averaged 17 and 13 mm Hg, respectively, for SBP and DBP. Comparable values were observed for SBP and DBP at the end of perindopril or nitrendipine treatment (Table 2).

Renal hemodynamic parameters measured during clearance studies are reported in Table 3. During the clearance studies the euglycemic condition was satisfactorily maintained in all individual patients. Actually, blood glucose averaged 92 $\pm$ 21, 88 $\pm$ 17 and 102 $\pm$ 22 mg/dl, respectively, during functional evaluation at baseline and at the end of perindopril and nitrendipine treatments. As expected, GFR and ERPF were both significantly lower in diabetic patients, at baseline evaluation, than in normal controls. GFR and ERPF did not differ significantly at the end of both antihypertensive treatments. Actually, GFR showed a tendency to decrease with both perindopril and nitrendipine treatments, but the differences did not reach statistical sig-

Table 2. Main clinical parameters in NIDDM patients at baseline and at the end of treatment period with perindopril or nitrendipine

	Baseline	Perindopril	Nitrendipine
SBP *mm Hg*	167 ± 11	148 ± 10[a]	150 ± 7[a]
DBP *mm Hg*	99 ± 7	88 ± 3[a]	86 ± 5[a]
Mean AP *mm Hg*	122 ± 8	108 ± 4[a]	107 ± 5[a]
HbA1c %	6.4 ± 1.3	6.1 ± 1.2	6.2 ± 1.6
Total proteins *g/24 hr*	5.6 (0.7–13.3)	5.0 (0.5–11.2)	5.2 (0.4–10.5)
P/C ratio	4.9 (0.4–16.5)	3.3 (0.3–7.0)[b]	3.5 (0.3–7.1)[c]
θ Albumin $*10^{-3}$	2.4 (0.1–6.9)	2.3 (0.1–6.9)	2.4 (0.1–6.8)
θ IgG $*10^{-3}$	0.84 (0.03–4.02)	0.59 (0.01–3.38)	0.60 (0.01–3.30)
Urea excretion *g/24 hr*	22.1 ± 5.1	24.3 ± 4.8	22.6 ± 7.6
Sodium excretion *mEq/24 hr*	189 ± 90	172 ± 37	199 ± 80

Data are mean ± SD or median and range in parentheses. Abbreviations are: AP, arterial pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure; θ Albumin, fractional clearance of albumin; θ IgG, fractional clearance of IgG.

[a] $P < 0.01$ vs. baseline

[b] $P > 0.7$ vs. baseline

[c] $P > 0.8$ vs. baseline

Table 3. Renal hemodynamic parameters in healthy controls and in NIDDM patients at baseline and at the end of the two treatment periods

	Controls	Baseline	Perindopril	Nitrendipine
GFR *ml/min/1.73 m²*	99.6 ± 13.4[b]	44.9 ± 19.9	38.2 ± 17.0	39.7 ± 17.2
ERPF *ml/min/1.73 m²*	617 ± 99[a]	432 ± 196	386 ± 156	505 ± 275
FF %	16 ± 3[a]	11 ± 4	10 ± 4	9 ± 4
RVR *mm Hg/ml/min/1.73 m²*	7.3 (5.8–8.8)[a]	22.1 (7.1–48.7)	22.5 (11.0–55.3)	22.9 (5.2–82.5)

Data are mean ± SD or median (range). Abbreviations are: GFR, glomerular filtration rate; ERPF, effective renal plasma flow; FF, filtration fraction; RVR, renal vascular resistances.

[a] $P < 0.05$ vs. patients at baseline

[b] $P < 0.01$ vs. patients at baseline

nificance. Differences in ERPF and filtration fraction values at the end of the two treatment periods, as compared to baseline evaluation, were only numerical and did not reach statistical significance. Baseline renal vascular resistances (RVR) were significantly higher in patients, as compared to controls, and were not significantly affected by the two study drugs.

At the end of the treatment periods, total urinary protein excretion, as well as fractional clearance of albumin and IgG, were not significantly modified either by the ACE-inhibitor or the Ca channel blocker (Table 2). The mean P/C tended to decrease with both treatments but the reduction did not reach statistical significance. Similarly, neither treatment significantly affected urea and Na^+ excretion (Table 2). Fractional clearance of neutral dextran molecules of radii ranging from 26 to 66 Å measured in normal controls and in diabetics are reported in Figures 2 and 3. Sieving coefficients of dextran molecules smaller than 40 Å in radius were comparable in diabetic patients, at baseline evaluation, to those measured in healthy controls. However, for molecules of 42 Å in radius and larger, the fractional clearance measured in diabetic patients was significantly elevated above the control group. The effects of perindopril and nitrendipine treatments on fractional clearances of dextran molecules are reported in Figure 3. Neither drug was capable of modifying dextran fractional clearance to any significant extent for the entire range of molecular radii investigated.

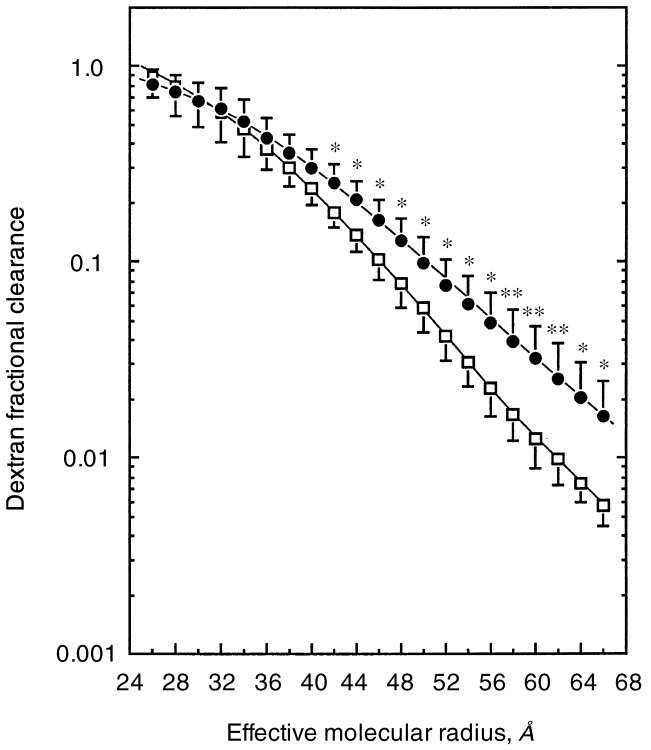

Fig. 2. Fractional clearance of neutral dextrans in normal controls and in diabetic patients at baseline evaluation. Symbols are: (●) NIDDM baseline; (□) healthy controls; $*P < 0.05$ vs. healthy controls; $**P < 0.01$ vs. healthy controls.

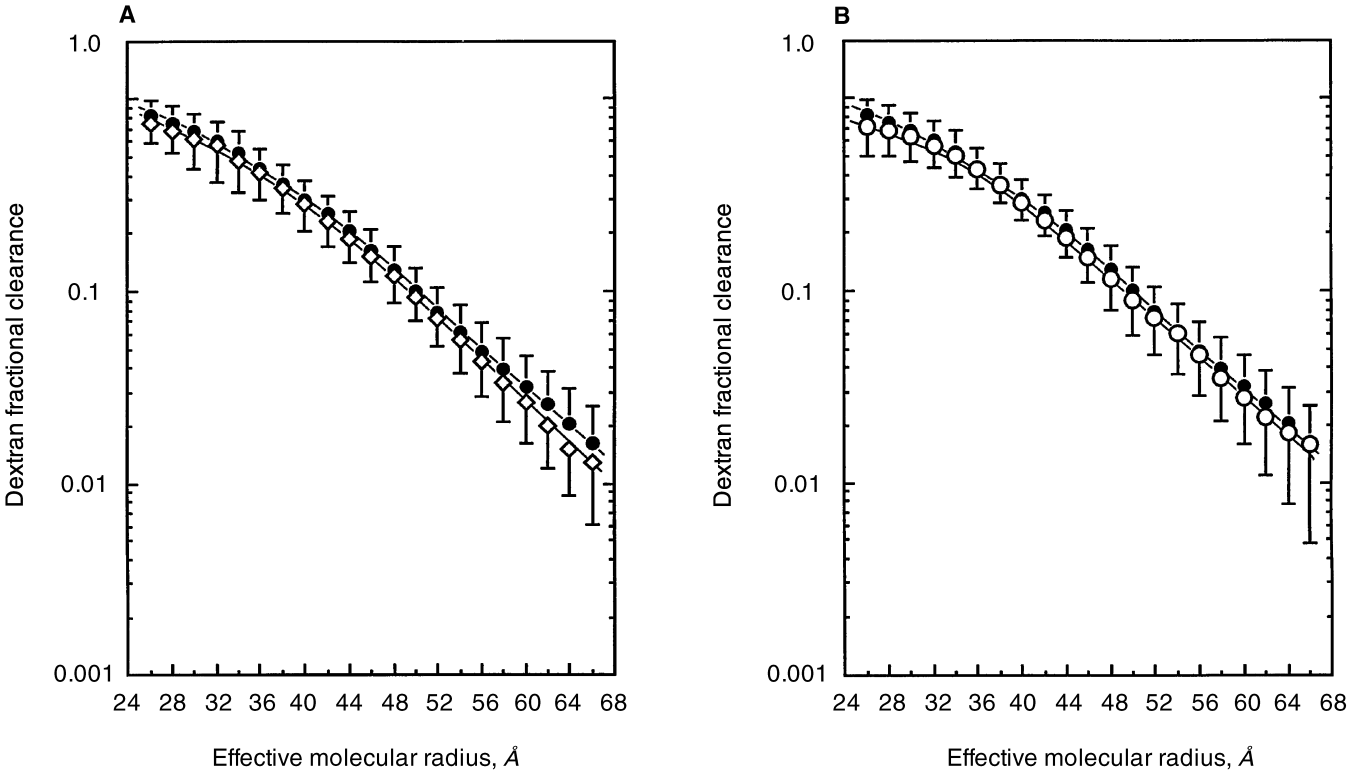

Fig. 3. Fractional clearance of neutral dextrans in diabetic patients at the end of the treatment period with perindopril (*A*) and nitrendipine (*B*). Symbols are: (●) NIDDM baseline; (◇) NIDDM + perindopril; (○) NIDDM + nitrendipine.

Table 4. Computed glomerular ultrafiltration coefficient and membrane parameters of the lognormal pore-size distribution

	ΔP *mm Hg*	K_f *ml/min/mm Hg/1.73 m²*	u *Å*	s	r* (5%) *Å*	r* (1%) *Å*	χ^2
Controls	35	11.4 ± 1.9	55.1 ± 4.0	1.16 ± 0.03	77.1 ± 2.3	85.4 ± 3.8	0.38 (0.06–1.71)
	40	7.3 ± 0.4	56.1 ± 3.7	1.15 ± 0.03	76.9 ± 2.2	84.7 ± 3.7	0.67 (0.06–2.17)
Basal	40	2.8 ± 1.2[c]	60.8 ± 6.3	1.16 ± 0.05	84.8 ± 6.1[a]	94.0 ± 8.3[a]	0.39 (0.07–2.75)
	45	2.1 ± 0.9[d]	61.2 ± 6.1	1.16 ± 0.05	84.6 ± 6.0[b]	93.5 ± 8.1[b]	0.46 (0.06–2.88)
Perindopril	35	3.5 ± 1.7	57.9 ± 8.3	1.17 ± 0.08	83.5 ± 6.6	93.3 ± 11.7	0.47 (0.13–1.00)
	40	2.4 ± 1.1	59.6 ± 7.1	1.16 ± 0.07	83.1 ± 6.6	90.4 ± 10.1	0.54 (0.09–1.26)
Nitrendipine	35	3.7 ± 1.9	59.3 ± 5.8	1.17 ± 0.06	85.3 ± 6.9	95.2 ± 10.9	0.51 (0.08–0.84)
	40	2.5 ± 1.2	60.6 ± 5.3	1.16 ± 0.06	84.8 ± 6.8	93.9 ± 10.6	0.40 (0.07–0.90)

Data are mean ± SD, or median (range). Abbreviations are: ΔP, transmembrane pressure difference; K_f, ultrafiltration coefficient; u, mean radius of membrane pore radii distribution; s, standard deviation of log normal distribution; r* (5% and 1%), membrane pore radius parameters.

[a] $P < 0.05$ vs. controls (ΔP = 35 mm Hg)
[b] $P < 0.05$ vs. controls (ΔP = 40 mm Hg)
[c] $P < 0.01$ vs. controls (ΔP = 35 mm Hg)
[d] $P < 0.01$ vs. controls (ΔP = 40 mm Hg)

To definitely rule out any possibility of a confounding carryover effect between the two treatment periods, we also compared data at baseline and at the end of the first treatment period only. Even in this case none of the above parameters describing renal hemodynamics and glomerular barrier size-selectivity was significantly affected by perindopril or nitrendipine.

The results of the theoretical analysis of glomerular hemodynamics and glomerular membrane permeability are summarized in Table 4. This analysis is based on the assumption of the transmembrane hydraulic pressure difference (ΔP), a parameter that cannot be measured directly in humans. In line with previous assumptions [1, 10, 12, 22], we assumed two values of $\Delta P = 35$ and 40 mm Hg for normal controls. For hypertensive diabetic patients, at baseline evaluation, we considered a potential increase in ΔP assuming the values of 40 and 45 mm Hg. Since experimental evidence suggests that antihypertensive treatments used in this study may lower glomerular capillary pressure [25, 26], we assumed $\Delta P = 35$ and 40 mm

Hg for theoretical analysis of fractional clearance data at the end of the treatment periods.

As expected, estimates of K_f were significantly lower in diabetic patients than in normal controls (Table 4). On the contrary, no statistically significant changes in K_f were induced by both antihypertensive treatments. Calculated membrane pore-size distribution parameters (the mean u and the standard deviation s of the log normal probability distribution) for the control group were remarkably similar to those previously reported [12, 22] with an average pore-size of about 55 Å. To quantitate the dimensions of the largest pores, we calculated the parameters r* (5%) and r* (1%). As defined previously [21, 27], by definition 5% and 1% of ultrafiltrate permeates pores larger than r* (5%) and r* (1%), respectively. For healthy controls these two parameters averaged approximately 77 Å and 85 Å, respectively. In line with previous investigations [25, 26], membrane pore parameters did not change importantly for the two assumed values of ΔP. In diabetic patients the mean pore size (u) averaged more than 60 Å; however, the difference above controls did not reach statistical significance. No significant changes were observed for the spread of the pore-size distribution (s). On the contrary, r* (5%) and r* (1%) were significantly elevated in diabetic patients at baseline evaluation than in normal controls. Actually, in diabetic patients r* (5%) and r* (1%) were on average more than 7 Å larger than corresponding radii of normal controls. Neither antihypertensive treatment significantly affected the membrane pore-size distribution parameters [u and s, r* (5%) and r* (1%)]. Values of u, r* (5%) and r* (1%) were numerically lower at the end of perindopril treatment than at baseline evaluation, but the differences did not reach statistical significance.

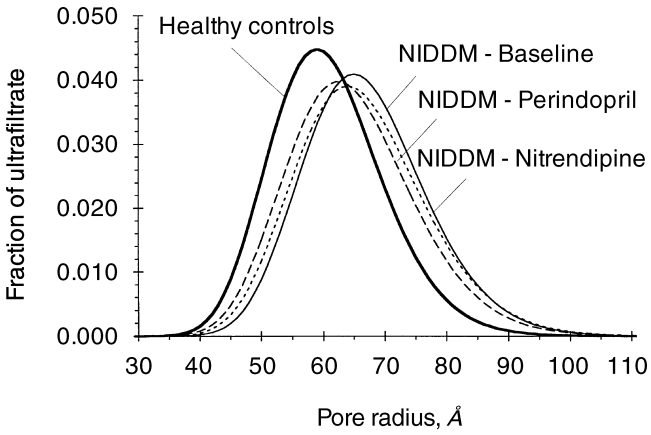

Fig. 4. Fraction of filtrate as a function of pore radii [r^4g (r)] in healthy controls and in diabetic patients at baseline evaluation and at the end of perindopril and nitrendipine treatment.

DISCUSSION

Data are available that IDDM patients with overt nephropathy have a defect in glomerular size-selectivity [1–4]. Studies of this kind in NIDDM patients with overt nephropathy are lacking. Here we investigated renal hemodynamics and glomerular size-selective function in patients with NIDDM and renal biopsy findings of pure diabetic-type glomerulopathy. The results indicated that NIDDM patients with overt nephropathy have lower GFR and ERPF and a decreased ultrafiltration coefficient as compared to healthy controls. By contrast, renal vascular resistances were significantly increased in diabetics as compared to healthy controls. The differences cannot be attributed to age since in normotensive, nondiabetic subjects these parameters are not affected by age [28–30]. We also found a glomerular size-selective dysfunction in NIDDM to the extent that fractional clearance of large dextrans was significantly elevated over control values.

To establish to what extent the altered fractional dextran clearance was due to effective permeability changes or to changes in glomerular hemodynamics, we adopted a theoretical analysis based on the assumption that glomerular membrane is perforated by pores having a log-normal probability distribution of their radii [9, 17, 22]. The agreement between experimental values and theoretical estimates obtained with this distribution was good, as shown by the sum of squared errors (χ^2) reported in Table 4. On average an error of 17% was calculated for the entire sieving curve in the worst cases. As mentioned previously, the log normal distribution fitted the experimental data better than the log normal plus shunt model, and this seems to depend on the shape of the sieving profiles shown in Figures 2 and 3. Actually, sieving coefficients in normal controls and in NIDDM patients decrease linearly for the largest test macromolecules without the tendency to become flat that has been reported for sieving coefficients in nephrotic patients [31]. This sieving profile is better simulated by the assumption of a parallel shunt pathway. Our present results, however, are in line with those obtained by Myers and coworkers in NIDDM, who showed a linear trend for sieving coefficient of the largest dextrans and assumed the log normal distribution of pore radii to simulate dextran fractional clearance [13].

As shown in Figure 4, in the diabetic group distribution function of pore-sizes was on average shifted towards larger pores, as compared to normal controls. We calculated a mean pore-size (u) radius of 5 Å greater in NIDDM patients than in controls (Table 4), and despite the difference it did not reach statistical significance due to the large variability of this parameter in diabetics. Dimensions of the largest pores were also remarkably different

in the two groups. Calculations showed that while 1% of filtrate permeated pores larger than 84 Å in diabetic patients, the corresponding radius for normal controls was only an average of 77 Å. We speculate that in diabetics the circulating plasma proteins are more freely filtered throughout these pores than in controls.

Similar findings have been reported in IDDM [3, 5] and in nondiabetic proteinuric glomerulopathies [32–35]. Several lines of research have consistently indicated that increased glomerular filtration of albumin and IgG in disease conditions is the consequence of a selective increase in the amount of ultrafiltrate permeating large unrestrictive membrane pores, which collectively form the so-called "shunt pathway." That IDDM and NIDDM share a common pathway of size-selective dysfunction is in harmony with the studies of Myers and coworkers in Pima Indians, who found that glomerular hemodynamic changes and loss of size-selectivity were independent of the type of diabetes [36]. Moreover, comparison of fractional clearance of charged proteins like albumin with that of neutral dextrans can be taken to indicate a concomitant defect of glomerular charge- and size-selective function operating in NIDDM. On the basis of the data recently reported by Blouch et al on sieving coefficient of dextran and ficoll, the 42 to 44 Å dextran molecule is probably the most comparable to albumin in terms of molecular size [31]. Despite the fact that the size of albumin is about 36 Å in radius, it has been shown that elongated dextran molecules permeate the membrane more freely than globular macromolecules [31, 37]. Our present data showing that sieving coefficient of 42 and 44 Å dextran are significantly higher in IDDM patients than in controls (Fig. 2) would indicate that a size-selectivity defect is at least in part responsible for abnormal glomerular filtration of albumin. On the other hand, a charge-selective dysfunction is likely to contribute to the 55 to 75% increase in transmembrane passage of 42 to 44 Å dextran we found in patients, as compared to normal controls. Such an increase alone is too limited to account for the increase in albumin filtration that, on the basis of the observed albumin fractional clearance, exceeded at least two orders of magnitude. Thus, concomitant changes in charge- and size-selectivity function are likely to contribute to albuminuria in NIDDM.

Concerning the effect of ACE inhibition on size-selective properties of glomerular membrane, we and others have consistently found that in patients with IDDM these compounds consistently ameliorated size-selective dysfunction [8, 9] and, as expected, reduced urinary excretion of total proteins and limited fractional clearance of albumin and IgG. At variance, ACE inhibition did not ameliorate glomerular barrier function to any significant extent, nor influence urinary protein excretion in patients with NIDDM taking part in the present study. Failure to detect an effect on test macromolecules sieving profiles cannot be attributed to the small number of patients, since the study was designed *a priori* for a 90% power to detect a 60% reduction in 60 Å neutral dextran fractional clearance (the primary efficacy variable of the study) as statistically significant ($P < 0.05$) at the end of the perindopril treatment as compared to the predicted baseline. Values of 60 Å dextran fractional clearance at baseline were found within the predicted ranges that were assumed for the sample size estimation. After perindopril, the neutral dextran sieving profile was not different from basal levels, indicating that, at variance with IDDM, ACE inhibitors are unable to correct size-selective dysfunction of the glomerular membrane in NIDDM. Failure of ACE inhibitor therapy to significantly affect barrier dysfunction of patients with NIDDM and a renal biopsy finding of diabetic nephropathy are consistent with the negligible effect of perindopril on urinary protein excretion rate. Urinary proteins were quantitated in all patients on two separate occasions, two weeks apart, at the end of each treatment period to minimize variations associated with diet protein intake, blood glucose and blood pressure. Not only was the ACE inhibitor unable to improve dextran sieving coefficient in our setting, but the calcium channel blocker was equally ineffective on sieving coefficient and urinary proteins. Why did neither treatment ameliorate glomerular size-selective function and limit urinary protein excretion in NIDDM as opposed to IDDM and other nondiabetic proteinuric nephropathies [4, 17]? Doses of the ACE inhibitor and duration of treatment were comparable to those used by others in IDDM [8, 9], and were found to be effective in ameliorating size-selectivity and reducing proteinuria. The only possible explanation for this apparent inconsistency is that renal structural changes in our NIDDM patients were very advanced and diffuse, involving thickening of the basement membrane, broadening of the foot processes, and diffuse sclerotic changes. It is tempting to speculate that these type of glomerular structural lesions that develop with time in NIDDM patients can reach an extent that prevents pharmacological treatments from achieving the desired effect on membrane sieving functional properties.

Thus, despite the biopsy finding of pure diabetic nephropathy, the same level of renal insufficiency and the same defect in dextran's clearance, the response to ACE inhibition in NIDDM versus IDDM patients appears to be different. These results may have clinical relevance. The large majority of diabetics with overt nephropathy indeed suffer NIDDM. Today, on the basis of the results of ACE inhibition treatment obtained in IDDM [38], more and more patients with NIDDM are given ACE inhibitors by analogy. However, the few controlled studies in NIDDM patients available thus far, with an adequate follow-up and measured GFR, consistently failed to document any specific effect of ACE inhibition ther-

apy [39–41]. Even studies in hypertensive NIDDM patients at an earlier stage of renal disease (incipient nephropathy) failed to document that ACE inhibitors were better than other therapies [42–44], in contrast with a previous report in which ACE inhibitor therapy prevented a progressive increase in urinary albumin excretion and delayed the onset of overt nephropathy in normotensive microalbuminuric NIDDM patients [45].

In summary, our present data document that NIDDM patients with pure diabetic-type glomerulopathy and overt proteinuria have a pattern of glomerular barrier dysfunction that closely resembles previous findings in IDDM in comparable patient series. In NIDDM, at variance with IDDM, however, glomerular size-selective dysfunction and proteinuria was neither ameliorated by ACE inhibition nor by dihydropyridinic calcium channel blockade. These data provide the physiopathological framework to understand the apparently lower renoprotection offered by ACE inhibitors in NIDDM as compared to IDDM patients, and are in line with other preliminary studies [36, 43]. On the other hand, our findings were obtained in a relatively small number of patients and need confirmation in large scale, long-term clinical trials. In the meantime, differences in the response to ACE inhibitors in NIDDM versus IDDM suggest that it is at least premature to extend the use of ACE inhibitors to all diabetics based on results of trials in IDDM. If one considers the high prevalence of renovascular disease, and the risk of ACE inhibitor-associated acute renal failure and life-threatening hyperkalemia in this setting, the drawbacks of such an approach appear obvious.

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