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Renal functional reserve in IDDM patients

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Summary The aim of this study was to determine whether renal functional reserve (RFR) is altered in insulin-dependent diabetic (IDDM) patients according to the stage of diabetic nephropathy. RFR was examined in 33 IDDM patients in similar glycaemic and metabolic control and compared to 12 healthy control subjects, during eight 1 h clearance periods prior to, during and after a 3-h stimulation by amino acid infusion (4.5 mg · kg^{-1} · min^{-1}). RFR was calculated as the difference between stimulated and baseline glomerular filtration rates (GFR). In 14 early normotensive diabetic patients with normal urinary albumin excretion, mean baseline GFR (133 ± 3 ml · min^{-1} · 1.73 m^{-2}) was higher whereas RFR (10 ± 4 ml · min^{-1} · 1.73 m^{-2}) was lower ($p < 0.05$) than in control subjects (113 ± 4 and 28 ± 2 ml · min^{-1} · 1.73 m^{-2}, respectively). In 10 normotensive patients who had lived with IDDM for 16 years and who had microalbuminuria, baseline GFR and RFR (109 ± 7 and 24 ± 6 ml · min^{-1} · 1.73 m^{-2}, respectively) were similar to those in control subjects. In 9 patients who had suffered IDDM for 23 years and had developed macroalbuminuria and hypertension, baseline GFR (78 ± 8 ml · min^{-1} · 1.73 m^{-2}) was lower than in control subjects ($p < 0.05$) and RFR (8 ± 4 ml · min^{-1} · 1.73 m^{-2}) was not significant. In addition, renal vascular resistance decreased significantly during infusion ($p < 0.05$) in microalbuminuric normotensive patients as well as in control subjects (by 9 ± 4 and 11 ± 4 mm Hg · l^{-1} · min^{-1} · 1.73 m^{-2}, respectively) but not in normoalbuminuric normotensive or macroalbuminuric hypertensive patients. These results indicate that microalbuminuric normotensive patients retain a normal RFR, whereas RFR is reduced or suppressed at two opposite stages of the disease: in normoalbuminuric normotensive patients with a high GFR and in macroalbuminuric hypertensive patients with a decreased GFR. This dissimilar impairment reveals permanent glomerular hyperfiltration in both early IDDM without nephropathy and IDDM with overt diabetic nephropathy, but not in IDDM with incipient nephropathy. [Diabetologia (1998) 41: 86--93]

Keywords Insulin-dependent diabetes mellitus, diabetic nephropathy, renal functional reserve, glomerular filtration rate, amino acid infusion.

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Abbreviations: GFR, glomerular filtration rate; RFR, renal functional reserve; ERPF, effective renal plasma flow; RVR, renal vascular resistance; nA-nT, normoalbuminuric normotensive patients; μA-nT, microalbuminuric normotensive patients; mA-hT, macroalbuminuric hypertensive patients; CEI, converting enzyme inhibitor.

Renal functional reserve (RFR) is usually defined as the difference between basal or "unstimulated"" glomerular filtration rate (GFR) and "stimulated"" GFR increased by stimuli such as oral protein load [1, 2] or amino acid infusion [3--5]. There is general agreement that RFR reflects the capacity of the healthy kidney to achieve a higher degree of function by vasodilation of glomerular arterioles. The reduction or the absence of the so-called functional reserve in renal diseases may imply that the residual nephrons are working at maximum capacity and there is substantial evidence that such a glomerular hyperfiltra-

Table 1. Characteristics of non-albuminuric normotensive (nA-nT), microalbuminuric normotensive (μA-nT) and macroalbuminuric hypertensive (mA-hT) diabetic patients, and of control subjects

Characteristic	nA-nT	μA-nT	mA-hT	Control subjects
Age (years)	30.7 ± 2.0	31.6 ± 2.2	40.4 ± 3.7[a, b]	27.7 ± 1.5
Duration of diabetes (years)	2.1 ± 0.4	17.3 ± 2.6	23.4 ± 3.0	–
BMI (kg/m^2)	22.6 ± 0.7	22.1 ± 0.3	23.9 ± 0.7[a]	20.6 ± 0.4
Protein intake (g per day)	81.0 ± 6.4	84.4 ± 7.9	82.8 ± 6.8	77.8 ± 11.9
Fasting P-glucose (mmol/l)	6.6 ± 0.5	7.1 ± 0.5	7.7 ± 0.5	–
Insulin dosage (IU per day)	26 ± 4	39 ± 4	35 ± 2	–
Fasting P-C peptide (ng/ml)	0.45 ± 0.43	< 0.10	< 0.10	–
Glycated Hb (%)	7.7 ± 0.4	8.4 ± 0.4	9.2 ± 0.2	–
Serum creatinine (μmol/l)	85 ± 2	89 ± 3	125 ± 11	78 ± 3
Creatinine clearance ($ml \cdot min^{-1} \cdot 1.73\ m^{-2}$)	139 ± 4	126 ± 5	102 ± 9	129 ± 5
P-renin (ng/l)	10.3 ± 1.8	11.1 ± 3.4	16.0 ± 3.0	12.4 ± 2.2
P-aldosterone (ng/dl)	9.2 ± 1.1	11.6 ± 2.5	8.4 ± 2.1	14.7 ± 1.7

Values are means ± SEM
[a] $p < 0.01$ vs control subjects
[b] $p < 0.05$ vs nA-nT values

tion may induce a relentless functional decline and a progression toward renal failure regardless of the primary disease [1, 6].

The issue of whether insulin-dependent diabetic (IDDM) patients have a normal response and RFR deserves cautious attention and interpretation [7] for two main reasons. First, because RFR is dependent on the baseline GFR and therefore, on the stage of diabetic nephropathy; in particular, stimulated GFR and RFR would be assumed to be diminished in the case of supranormal GFR which is a frequent hallmark of early IDDM. And second, because both baseline GFR and RFR also strictly depend on recent dietary intakes and prevailing metabolic control of diabetes mellitus. These reasons are likely to explain why the results of previous RFR studies, that have used either an oral protein load or an amino acid infusion in partial or heterogeneous groups of diabetic patients with dissimilar protein intakes and metabolic control conditions, seem, above all, conflicting. According to groups and conditions, the reported increases in stimulated GFR were likely to be either not significant with no RFR [1, 8--13], or significant but lower than in control subjects with a reduced RFR [14], or similar to that in control subjects with a normal RFR [8, 11, 13, 15--17].

This remarkable inconsistency between previous results and the fact that no study so far has precisely considered the response to stimulation according to the degree of renal functional impairment prompted us to examine separately and to compare the ability of diabetic patients at the different stages of the disease to demonstrate a functional reserve. The aim of this study was therefore to assess, under similar metabolic conditions, the renal response of IDDM patients to amino acid infusion and to compare their ability to increase GFR with respect to the stage of diabetic nephropathy as determined by the prevailing levels of proteinuria and blood pressure.

Subjects and methods

Study subjects. The study (previously approved by the ethical review board of the Centre Hospitalier Universitaire de Toulouse, France) was carried out in 33 IDDM patients and 12 healthy control subjects. Diabetic patients were distributed among three groups according to the stage of renal involvement and nephropathy [18, 19].

Fourteen patients (11 males, 3 females) had suffered diabetes for approximately 2 years (Table 1). They had normal urinary albumin excretion (2 ± 5 mg/day) and were normotensive (mean arterial pressure: 77 ± 2 mm Hg). These normoalbuminuric and normotensive patients (nA-NT) were considered to be in the "silent"" stage of preclinical renal involvement. Plasma glucose concentration in fasting patients on insulin therapy, and gross renal function, as evaluated by serum creatinine level and 24-h creatinine clearance, were normal (Table 1). They exhibited none of the extrarenal complications of diabetes.

Ten patients (8 males, 2 females) had lived with IDDM for approximately 17 years (Table 1). They exhibited microalbuminuria (99 ± 20 mg/day) and had normal mean arterial pressure (85 ± 3 mm Hg). These microalbuminuric normotensive patients (μA-nT) were considered to be in the "incipient"" stage of preclinical renal involvement. Fasting plasma glucose concentration and gross renal function were normal (Table 1). Seven patients out of 10 had a low grade retinopathy, 1 had a lower limb arterial insufficiency and 1, a peripheral neuropathy. Five patients were usually maintained on stable doses of a converting enzyme inhibitor (CEI: 3 with enalapril 5 or 10 mg per day, 1 with perindopril 4 mg per day and one with fozinopril 10 mg per day).

Nine patients (6 males, 3 females) had suffered IDDM for approximately 23 years. They had developed macroalbuminuria (1779 ± 432 mg/day) and hypertension (mean arterial pressure: 111 ± 5 mm Hg). These macroalbuminuric and hypertensive diabetic patients (mA-hT) were considered to be in the "overt"" stage of clinical nephropathy. Serum creatinine level was slightly elevated and creatinine clearance, slightly reduced (Table 1). All mA-hT patients had retinopathy, 1 had coronary arterial disease, 1 had a lower limb arterial insufficiency, and 3 of them, peripheral neuropathy. All of them were usually maintained on stable doses of CEI (7 with enalapril 5 to 15 mg per day, 1 with ramipril 5 mg per day and 1 with fozinopril 10 mg per day).

All diabetic patients had been trained with the same educational programme and were on intensified insulin therapy with either multiple daily injections (24 patients) or continuous s.c. insulin infusion with an external pump (9 patients). Daily insulin doses are reported in Table 1. All patients were deprived of all medication including CEI therapy, but not insulin, for at least 1 week prior to the day of the study.

Control subjects were 12 (6 males, 6 females) normoalbuminuric (8 ± 2 mg/day) and normotensive (mean arterial pressure: 80 ± 2 mm Hg) healthy volunteers who had given their informed consent. They were deprived of all medication and had a normal renal function (Table 1).

Diabetic patients and control subjects were personally provided with advice from a dietician to follow an identical isocaloric diet (comprising 50% carbohydrates, 4 to 6 g NaCl and 1.3 g per kg body weight protein daily content) at least 1 week before the studies. Daily intakes were controlled by electrolyte and urea nitrogen urinary excretion on 24-h samples prior to the study and precise protein intake was calculated from urea nitrogen urinary excretion, weight and Maroni's formula [20] (Table 1).

Study protocol. Renal functional reserve studies were performed on recumbent diabetic patients and control subjects. In order to achieve the required glycaemic stability, the patients were admitted to the hospital on the day before the test. Continuous s.c. insulin infusion was given with an external pump (Microjet Quark U100; Ames Dpt, Bayer Diagnostics, Miles, Elkart, Ind., USA) during the night and throughout the test. Blood glucose level was monitored every hour (One Touch II; Lifescan, Johnson and Johnson Co, Milpitas, Calif., USA) and the rate of insulin infusion was adapted to maintain the glycaemic level between 5.5 and 9.9 mmol/l. On the morning of the study, a loading dose of 1000 mg polyfructosan S (inulin, Inutest; Laevosan Gesellschaft, Linz, Austria) and 700 mg *p*-amino hippurate (PAH; Nephrotest; Biol. Arbeitsgem. GmbH, Lich, Germany) was administered i.v. Then, inulin (9 mg/min) and PAH (5.2 mg/min) were administered i.v. in a 5% dextrose solution to maintain plasma concentrations at 200 mg/l and 20 mg/l respectively throughout the study. A second indwelling venous cannula was used to deliver a 154 mmol/l NaCl infusion in the other arm at a constant rate of 4 ml/min. After a 60-min equilibration period, a steady state of urine flow was obtained and a series of eight 60-min standard clearance periods were performed. After completion of two baseline periods, the NaCl solution was replaced by a 7.4% amino acid solution infused at the same rate of 4 ml/min for 3 h. The solution was made up of Azonutril 25 (Roger Bellon Laboratories, Neuilly sur Seine, France) diluted v/v with sterilized distilled water and conveying 300 mg/min of a mixture of 20 l-amino acids, i.e. approximately 4.5 mg amino acids · min^{-1} · kg^{-1}. Three clearance periods were performed during amino acid infusion. Then, the amino acid solution was replaced by the NaCl solution and three additional periods were obtained. Blood samples were taken at the midpoint of each clearance period and urine samples were collected by spontaneous voiding. Blood pressure and heart rate were monitored every 15 min during the study (Dynamap 1846 SX; Critikon, Tampa, Fla., USA).

Laboratory procedures and calculations. Plasma and urine levels of inulin and PAH were determined immediately, without any storage, by photocolorimetric methods. Creatinine levels were determined by the Jaffé reaction with picric acid using a kinetic method (Merckotest kits; Merck, Darmstadt, Germany). All absorbances were read on a Beckman DU 40 spectrophotometer (Fullerton, Calif., USA). Active renin concentration (RIA with IRMA kits; Pasteur-Sanofi, Marne-la-Coquette, France) and aldosterone concentration (RIA with ALDO II kits; Sorin Biomedica, Saluggia, Italy) were measured in plasma samples from the baseline clearance periods before amino acid infusion.

Glomerular filtration rate (GFR) was equated with the clearance of inulin (C In) and effective renal plasma flow (ERPF) with the clearance of PAH (C PAH). RFR was equated with the difference between the higher GFR measured during amino acid infusion and baseline GFR. The filtration fraction was calculated as the ratio of C In to C PAH. Renal blood flow (RBF) was calculated as the ratio of ERPF to 1-haematocrit, and renal vascular resistance (RVR) was calculated as the ratio of mean arterial pressure mm Hg to RBF l/min. All rates of filtration, flow and excretion as well as RVR are expressed per 1.73 m^2 of body surface area. All fractional data are expressed as percentages.

Statistical analysis. Reported data before (baseline) and after (recovery) amino acid infusion represent the mean of that obtained during two and three consecutive clearance periods, respectively. Reported data during amino acid infusion (stimulation) represent the values obtained during the period of the higher level of GFR stimulation because RFR was calculated as the difference between the stimulated higher GFR and baseline GFR. This period of stimulated higher GFR was more often than not during the last hour of amino acid infusion in diabetic patients (30 times out of 33) as well as in control subjects (11 times out of 12). The values reported represent means ± SEM. The results were processed by repeated variance analysis measurements. Differences between values obtained during baseline, stimulation and recovery periods, and between nA-nT, μA-nT, mA-hT patients and control subjects were analysed using the Scheffe *F*-test for multiple comparisons. Differences inside groups of diabetic patients were analysed using the non-parametric Mann-Whitney U test. Best-fit linear regression was evaluated using the least-squares method. Results with $p < 0.05$ were considered statistically significant. Analysis of covariance was used to further evaluate the relative importance of independent variables on the dependent variable RFR. A significant *p* value for the correlation between RFR and a variable would indicate that the latter affects RFR after adjusting for the contribution of the other variables.

Results

Mean baseline GFR in nA-nT patients was 133 ± 3 ml · min^{-1} · 1.73 m^{-2} and significantly higher ($p < 0.05$) than in control subjects (113 ± 4 ml · min^{-1} · 1.73 m^{-2}) (Fig. 1). It ranged from 118 to 149 ml · min^{-1} · 1.73 m^{-2} and it could be considered that 5 patients out of 14 had a supranormal GFR that exceeded the value of basal controls by more than two standard deviations, i.e. higher than 140 ml · min^{-1} · 1.73 m^{-2}. Mean baseline GFR did not differ between μA-nT patients (109 ± 7, range 72 to 135 ml · min^{-1} · 1.73 m^{-2}) and control subjects because only 2 patients out of 10 had a GFR lower than that of control subjects by more than two standard deviations, i.e. lower than 86 ml · min^{-1} · 1.73 m^{-2}. In contrast, mean GFR in mA-hT patients (78 ± 8, range 38 to 107 ml · min^{-1} · 1.73 m^{-2}) was sig-

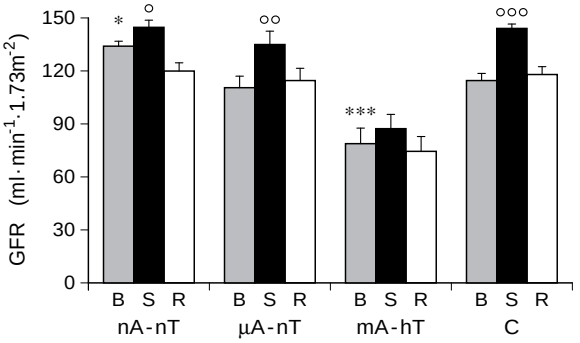

Fig. 1. Glomerular filtration rate (GFR) before (baseline, B), during (stimulation, S) and after (recovery, R) amino acid infusion in 14 normoalbuminuric normotensive (nA-nT), 10 microalbuminuric-normotensive (µA-nT) and 9 macroalbuminuric hypertensive (mA-hT) diabetic patients, and 12 control subjects (C). Values represent means ± SEM.
* $p < 0.05$, *** $p < 0.001$ for differences with control subjects' baseline values ^{o} $p < 0.05$, ^{oo} $p < 0.01$, ^{ooo} $p < 0.001$ for differences between stimulated and respective baseline values

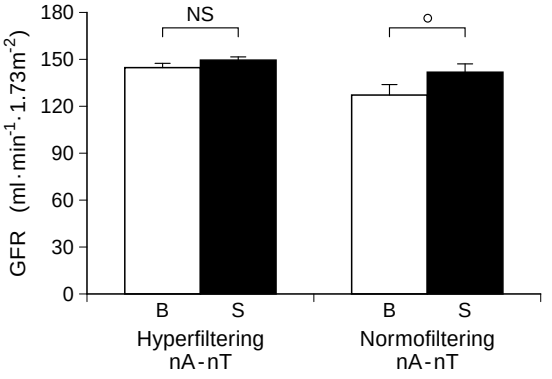

Fig. 2. Glomerular filtration rate (GFR) before (baseline, B) and during (stimulation, S) amino acid infusion in 5 hyperfiltering and 9 normofiltering nonalbuminuric normotensive diabetic patients (nA-nT). Values represent means ± SEM.
NS not significant, ^{o} $p < 0.05$ for differences between stimulated and respective baseline values

nificantly lower than in control subjects ($p < 0.001$) (Fig. 1).

Stimulated GFR values during amino acid infusion were significantly higher than respective baseline values in nA-nT patients (143 ± 5 ml · min^{-1} · 1.73 m^{-2}, $p < 0.05$), µA-nT patients (133 ± 8 ml · min^{-1} · 1.73 m^{-2}, $p < 0.01$) and control subjects (142 ± 3 ml · min^{-1} · 1.73 m^{-2}, $p < 0.001$), but not in mA-hT patients (87 ± 8 ml · min^{-1} · 1.73 m^{-2}) (Fig. 1). Amino acid-induced stimulation was fully reversible as evidenced by the fact that there was no difference between recovery and baseline GFR values. Linear regression analysis indicated that stimulated GFR was significantly and positively correlated with baseline GFR in diabetic patients ($y = 0.94\,x + 21.17$, $r = 0.84$, $p < 0.001$) as well as in control subjects ($y = 0.58\,x + 76.37$, $r = 0.85$, $p < 0.001$).

RFR was similar in µA-nT patients (24 ± 6 ml · min^{-1} · 1.73 m^{-2}) and control subjects (28 ± 2 ml · min^{-1} · 1.73 m^{-2}) while representing 24 ± 6 and 25 ± 3 % of respective baseline GFR (Fig. 1). In contrast, RFR in nA-nT patients (10 ± 4 ml · min^{-1} · 1.73 m^{-2}) was significantly lower ($p < 0.05$) while representing only 8 ± 3 % of corresponding baseline GFR, and RFR was absent in mA-hT patients. RFR was significantly and inversely correlated to baseline GFR in control subjects ($y = -0.42\,x + 76.37$, $r = 0.76$, $p < 0.01$) indicating that the lower the GFR, the higher the RFR. Such a correlation could not be detected in diabetic patients ($r = 0.10$). Analysis of covariance revealed that patients' RFR was not affected by age ($p = 0.93$), sex ($p = 0.56$), diabetes duration ($p = 0.66$), daily insulin dosage ($p = 0.97$), urinary albumin excretion rate ($p = 0.42$), mean arterial pressure ($p = 0.73$) and baseline GFR ($p = 0.19$). In contrast, there was a significant "group effect"" ($p < 0.05$) demonstrating that the major factor influencing RFR was the distribution of patients according to the usual bioclinical criteria. In particular, RFR appeared to be positively and independently associated as a categorical covariate with the microalbuminuric normotensive patient group.

In-depth analysis of our glomerular data also revealed two interesting points. 1) The effects of amino acid stimulation were inversely dependent on baseline GFR in nA-nT patients. Stimulated GFR (149 ± 7 ml · min^{-1} · 1.73 m^{-2}) was not significantly higher than baseline GFR (145 ± 2 ml · min^{-1} · 1.73 m^{-2}) and RFR was absent in the 5 nA-nT hyperfiltering patients (Fig. 2). In contrast, stimulated GFR (140 ± 6 ml · min^{-1} · 1.73 m^{-2}) was significantly higher ($p < 0.05$) than baseline GFR (126 ± 2 ml · min^{-1} · 1.73 m^{-2}) in the 9 normofiltering nA-nT patients whose RFR was thus 14 ± 6 ml · min^{-1} · 1.73 m^{-2}. And, 2) in µA-nT patients, no difference in results was detected between those whose usual CEI therapy had been interrupted 1 week before the study and those who had never been submitted to CEI: neither baseline GFR (109 ± 7 vs 109 ± 13 ml · min^{-1} · 1.73 m^{-2}) nor RFR (29 ± 7 vs 19 ± 9 ml · min^{-1} · 1.73 m^{-2}) differed significantly between the 5 CEI-previously treated and the 5 CEI-untreated µA-nT patients respectively.

Data for mean arterial pressure, ERPF and filtration fraction are presented in Table 2. Baseline arterial pressure in mA-hT patients was significantly higher than in control subjects ($p < 0.001$). Mean pressure was not significantly altered in diabetic patients or control subjects during amino acid infusion. Although higher in nA-nT and µA-nT patients, and lower in mA-hT patients, baseline ERPF was not significantly different from that in control subjects. During amino acid infusion, ERPF did not change in mA-hT patients whereas it increased in nA-nT and µA-nT

Table 2. Effects of amino acid infusion (Stimulation) on mean arterial pressure, effective renal plasma flow (ERPF) and filtration fraction in IDDM patients and control subjects (C)

	Baseline	Stimulation	Recovery
Mean arterial pressure (mm Hg)			
nA-nT	77 ± 2	78 ± 5	76 ± 2
μA-nT	85 ± 3	81 ± 3	83 ± 3
mA-hT	111 ± 5[b]	114 ± 5[b]	108 ± 4[b]
C	80 ± 2	78 ± 6	80 ± 2
ERPF (ml · min^{-1} · 1.73 m^{-2})			
nA-nT	596 ± 37	630 ± 46	534 ± 30
μA-nT	566 ± 39	626 ± 66	489 ± 46
mA-hT	487 ± 53	487 ± 51	441 ± 49
C	533 ± 31	584 ± 29	536 ± 19
Filtration fraction (%)			
nA-nT	23.3 ± 1.3	24.0 ± 1.6	22.9 ± 0.9
μA-nT	19.7 ± 1.4	22.1 ± 1.0	24.1 ± 1.5
mA-hT	16.5 ± 1.4[a]	18.2 ± 1.2[a]	17.8 ± 2.5
C	22.0 ± 1.4	24.3 ± 1.3	21.8 ± 0.7

Values are means ± SEM
[a] $p < 0.05$, [b] $p < 0.001$ vs control subjects

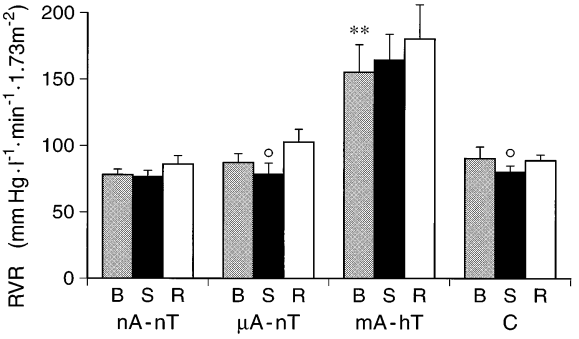

Fig. 3. Renal vascular resistance (RVR) before, during and after amino acid infusion. B, S, R, nA-nT, μA-nT, mA-hT and C are as in Figure 1. Values represent means ± SEM.
** $p < 0.01$ for differences with control subjects' baseline values ° $p < 0.05$ for differences between stimulated and respective baseline values

patients as well as in control subjects although the difference with the respective baseline values was not significant. Baseline filtration fraction did not differ between nA-nT patients, μA-nT patients and control subjects, and was significantly lower in mA-hT patients ($p < 0.05$). The consistent tendency of filtration fraction to increase during stimulation was not significant in either diabetic patients or control subjects.

Although slightly lower, calculated baseline RVR in nA-nT patients (78 ± 5 mm Hg · l^{-1} · min^{-1} · 1.73 m^{-2}) did not differ significantly from RVR in μA-nT patients and control subjects which were similar (88 ± 6 and 90 ± 7 mm Hg · l^{-1} · min^{-1} · 1.73 m^{-2}, respectively) (Fig. 3). RVR was significantly higher ($p < 0.01$) in mA-hT patients (155 ± 21 mm Hg · l^{-1} · min^{-1} · 1.73 m^{-2}). RVR was decreased by amino acid infusion in both μA-nT patients (by 9 ± 4 mm Hg · l^{-1} · min^{-1} · 1.73 m^{-2}, $p < 0.05$) and control subjects (by 11 ± 4 mm Hg · l^{-1} · min^{-1} · 1.73 m^{-2}, $p < 0.05$. In striking contrast, RVR was not significantly altered by stimulation in nA-nT (-2 ± 4 mm Hg · l^{-1} · min^{-1} · 1.73 m^{-2}) or mA-hT patients ($+ 8 \pm 9$ mm Hg · l^{-1} · min^{-1} · 1.73 m^{-2}).

Discussion

This study documents specific characteristics of the renal functional reserve induced by an amino acid infusion in IDDM patients at different stages of the disease. Our observations provide evidence that, compared with healthy control subjects, the ability to vasodilate and demonstrate an RFR is reduced in non-albuminuric normotensive patients, normal in microalbuminuric normotensive patients and absent in macroalbuminuric hypertensive patients, all in similar metabolic control and with similar sodium and protein intakes.

Differences in experimental conditions between subjects were suppressed as far as possible in the study protocol to prevent any influence on the results while meeting clinical requirements. Sodium and protein intakes were standardized, and CEI therapy was interrupted at least 1 week before the day of the study. Infusion rates were similar for all subjects. The result was that protein intake, baseline urine flow rate, urinary sodium excretion rate as well as plasma renin and aldosterone levels did not differ significantly between groups of patients and between patients and healthy control subjects. In addition, no difference in results was detected between μA-nT patients whose CEI therapy had been interrupted 1 week before the study and those who had never been submitted to CEI. Finally, analysis of covariance clearly establishes that the major factor influencing renal reserve in our study is the stage of renal involvement as determined classically by the prevailing levels of proteinuria and blood pressure.

A renal reserve exists but is reduced in non-albuminuric-normotensive patients with recently diagnosed diabetes considered as a unique group with significantly elevated mean basal GFR. A supranormal GFR is a constant feature of early experimental diabetes in streptozotocin-treated rats [21, 22], alloxan diabetic rats [23], pancreatectomized dogs [24], and strains of spontaneously diabetic rats [25]. Numerous studies have also detected a frequent GFR increment in newly diagnosed and/or short-term human diabetes [26--31]. Whatever the haemodynamic and humoral mechanisms of glomerular hyperfiltration [32--35], our data demonstrate that the RFR reduction in nA-nT patients directly results from the supranormal baseline GFR since: 1) Amino acid infusion was unable to elicit any additional renal vasodilation as demonstrated by the fact that it did not significantly

alter the mean renal resistance. 2) The response to stimulation differed strikingly between nA-nT patients according to basal GFR value: the low mean RFR of the whole group is clearly due to the 5 patients with supranormal basal GFR who were deprived of renal reserve. 3) The stimulated GFR during amino acid infusion did not differ between nA-nT patients and control subjects demonstrating that the overall ability of the kidney to vasodilate is not really increased in early diabetes. Part of this ability is merely brought into play in the basal condition, thus narrowing the gap between baseline and stimulated values and decreasing the renal reserve at this silent stage of the disease.

In microalbuminuric normotensive patients, basal GFR and RVR, stimulated GFR, and RFR mean values were not significantly different from those in control subjects since only in 2 patients was GFR slightly decreased. This similarity indicates that whole kidney glomerular hyperfiltration could no longer be detected in the patients who had lived with diabetes for approximately 17 years and were considered to be in the incipient stage of diabetic nephropathy. It also establishes that the kidney's ability to vasodilate and demonstrate RFR is not reduced. Covariance analysis confirmed that RFR is positively and independently associated as a categorical covariate with the μA-nT patient group. Despite the general acceptance of the existence of glomerular structural changes at this stage, it has been emphasized that these lesions can be regularly observed in the kidneys of patients with long-standing diabetes who have little or no clinical disease, and that these lesions are indicative of chronic diabetes but do not necessarily denote significant renal dysfunction [36, 37]. Our results tie up with this interpretation and clearly demonstrate that there is no relative hyperfiltration or irreversible glomerular functional impairment at this incipient microalbuminuric stage of diabetic nephropathy.

In contrast, macroalbuminuric hypertensive patients had moderate renal insufficiency with significantly lower basal GFR and higher renal resistance representing approximately 172 % of RVR in control subjects. Amino acid infusion did not elicit either a GFR increase or an RVR decrease in mA-hT patients. These patients were significantly older than control subjects. If one agrees that a decline in renal function is found in elderly subjects, this might be responsible, at least in part, for the difference between them and control subjects. In fact, linear regression as well as covariance analysis indicates that renal reserve was not affected by age in our study. Moreover, all of the previous three studies on healthy elderly subjects and non-nephrologically ill elderly patients have consistently demonstrated that an intact RFR can be elicited in humans at an advanced age by either an amino acid infusion [38, 39] or a meat meal [40]. Therefore the difference in age between mA-hT patients and control subjects is not likely to explain the lower RFR in the former. It is worth pointing out that it would have been difficult to predict the likely pathologic lesions of overt nephropathy [36, 37] and the suppressed RFR from a normal serum creatinine level, an almost normal creatinine clearance and a remarkably well preserved mean basal GFR. Several studies by others and ourselves have demonstrated that a proportionally normal RFR could still be elicited in subjects with a lower baseline GFR than that of our mA-hT diabetic patients, for instance in uninephrectomized healthy subjects and kidney transplant recipients [41, 42] or in subjects with non-diabetic chronic renal diseases [1, 43--47]. Whatever the inner pathologic mechanism [48, 49], the glomerular filtration process appears to be impressively well-protected in overt diabetic nephropathy, at the very time when an irreversible glomerular functional impairment is evidenced by the increased resistance and by the suppressed ability to vasodilate and demonstrate an RFR. In this respect, invariability of amino acid-stimulated GFR and absence of an RFR clearly appear to provide more sensitive assessments of the functional renal damage in clinical overt diabetic nephropathy than usual baseline evaluation.

It is generally agreed that the existence of an RFR indicates that all nephrons are not functioning at their maximum capacity and that the kidneys do not exhibit permanent hyperfiltration. Our results clearly indicate that microalbuminuric normotensive diabetic patients with incipient nephropathy retain a normal renal reserve whereas, paradoxically, RFR is reduced or suppressed at two opposite stages of the disease: in normoalbuminuric normotensive patients with supranormal GFR at the stage of silent renal involvement, and in macroalbuminuric hypertensive patients with moderately reduced GFR at the overt nephropathy stage. The main outcome of the study is therefore that RFR in IDDM is dependent on the basal GFR value but in a way which is quite different from that in non-diabetic glomerular diseases. During the preclinical stages, the maximum ability of kidney to vasodilate is neither reduced nor increased but similar to that in healthy subjects as demonstrated by identical amino acid-stimulated GFRs; consequently, RFR is reduced at the silent stage with elevated basal GFR and is intact at the incipient stage with normal GFR. During the clinical stage, the reduced ability of the kidney to vasodilate accounts for an irreversible functional impairment in spite of remarkably well-preserved GFR. These characteristic features of the response to amino acid stimulation also explain why RFR is not correlated to baseline GFR in diabetic patients, unlike in our healthy control subjects or in healthy and diseased subjects who provided an extended range of baseline values for GFR [50].

Whether or not glomerular hyperfiltration expressed by the reduction of the loss of RFR plays a role

in the progression of renal damage is a critical question, especially in patients with early diabetes. It has been reported that early functional changes might relate to the development of glomerulopathy in rats with streptozotocin-induced diabetes [51] as well as in diabetic patients [52--55]. Experimentally, intervention such as uninephrectomy or increasing dietary protein content -- which aggravate glomerular capillary perfusion, pressure and filtration -- accelerate the development of glomerular injury. Furthermore, patients with the highest values for GFR in the early stage of diabetes are more likely to progress to persistent albuminuria or overt nephropathy. An 8-year prospective study in initially normoalbuminuric patients has even revealed initial GFR as the only significant independent predictor for nephropathy [55]. Conversely, an intervention such as dietary protein restriction, which improves the haemodynamic changes, may favourably influence the natural history of the disease. Serial studies of renal function and repeated evaluation of the RFR in diabetic patients who exhibited or did not exhibit glomerular hyperfiltration during the early stage of the disease may allow conclusive new insights to be gained on the subject. Another most interesting group is composed of patients with very long-lasting IDDM who, nevertheless, exhibit no sign of diabetic nephropathy. Whether or not these patients are characterized by an intact RFR is currently being examined.

We conclude that microalbuminuric normotensive diabetic patients retain a normal RFR whereas RFR is reduced in normoalbuminuric normotensive patients with supranormal GFR, and suppressed in macroalbuminuric hypertensive patients with reduced GFR. In particular, the normal response to amino acids in microalbuminuric normotensive patients suggests that the loss of renal functional reserve is not an early phenomenon during diabetic renal disease.

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