Renal Findings in Patients with Short-Term Type 2 Diabetes

C.K. Keller, K.H. Bergis, D. Fliser, and E. Ritz

C.K. Keller, D. Fliser, E. Ritz, Department of Internal Medicine, Heidelberg, Germany
K.H. Bergis, FIDAM Forschungsinstitut der Diabetes Akademie, Bad Mergentheim, Germany
(J. Am. Soc. Nephrol. 1996; 7:2627-2635)

ABSTRACT

Under semiambulatory conditions, 85 consecutive patients with the diagnosis of Type 2 diabetes of short duration (excluding patients with islet cell antibodies or maturity onset diabetes of the young) were admitted to a self-control training program and were examined in this study. A comprehensive renal assessment was performed, including evaluation of albumin excretion rate (AER), renal hemodynamics, blood pressure (BP) profile, and indicators of genetic risk. AER ≥ 30 mg/24 h was found in 13 (15%) of patients; in two of these patients, AER was ≥ 300 mg/24 h. By logistic regression, high HbA1, current smoking, and BP parameters were significantly correlated with an increased risk of microalbuminuria (MA). In a multiple linear regression model, accounting for 57% of total variance, HbA1, ERPF, and current smoking were significantly correlated with AER. Median GFR (Cr-inulin clearance) 136 mL/min per 1.73m²; range, 94 to 194) and ERPF (Cr-para-aminohippuric acid clearance) 733; range, 451 to 1328) were significantly higher in patients than in control subjects (upper 95th percentile, 131 and 706 mL/min per 1.73m², respectively). In a multiple linear regression model, explaining 27% of total variance, age, AER, gender, and fasting blood glucose were significantly correlated with GFR. According to the criteria of average daytime BP ≥ 135/85 mm Hg or 24-h BP ≥ 130/80 mm Hg, 60% of patients were hypertensive (HT). Sixty-one percent of all patients (including 50% of the untreated normotensive patients) were "nondippers", i.e., < 15% nighttime decrease of mean arterial pressure. Either HT or non-dipping was found in 79% of all patients, so that only 21% had a completely normal blood pressure profile. Ninety-four percent of untreated hypertensive patients had no MA. First-degree relatives of patients with MA compared with patients without MA had more frequent cardiovascular events (69% versus 31%). The risk of MA in diabetic patients with positive family history was amplified by poor glycemic control. MA, but not hypertension, was marginally related to Km of Na+/Li+ countertransport. It was concluded that (1) microalbuminuria is found in 15% of patients newly presenting with Type 2 diabetes; (2) a high proportion of patients exhibit hyperfiltration; (3) according to ambulatory BP only, 21% of patients have a completely normal circadian BP profile; (4) a family history of cardiovascular events interacts with glycemic control to increase the risk of MA.

Key Words: Type 2 diabetes, albuminuria, glomerular filtration, cardiovascular risk

In the past, it had been assumed that renal involvement in Type 2 diabetes was rare (1). More recently, a high frequency of renal involvement has been firmly established (2); this was observed in Pima Indians with Type 2 diabetes (3), as well as in black and Caucasian patients with Type 2 diabetes (4). Indeed, the cumulative risks of nephropathy, i.e., albuminuria and renal failure, are quite comparable in Type 1 and Type 2 diabetes (5).

Microalbuminuria has been well recognized as an important risk factor for cardiovascular events in Type 2 diabetes (6,7); it is less predictive of subsequent nephropathy than Type 1 diabetes, and this may be explained—at least in part—by (1) the competing risk of cardiovascular death (6,7), and (2) the frequent presence of potential confounding factors (e.g., heart failure, hypertensive renal damage, or superimposed primary renal diseases) (8). Nevertheless, microalbuminuria predicts development and progression of nephropathy in patients with Type 2 diabetes as well (9).

Some studies noted a very high prevalence of albuminuria at the time of diagnosis of Type 2 diabetes (10-13). For several reasons the interpretation of these findings is difficult, however, and the results require confirmation in different populations.

In Type 1 diabetes of recent onset, GFR and RPF are usually elevated and return toward the normal range with improved metabolic control (14). The situation in Type 2 diabetes is considerably less clear. Schmitz et al. (15) found that GFR was within the normal range in patients with recently diagnosed Type 2 diabetes, and decreased within the normal range to even lower levels with good metabolic control. In contrast, other authors found glomerular hyperfiltration in a sizeable proportion of Pima Indians (16) and Caucasian patients (17,18) with Type 2 diabetes of recent onset. This issue is of interest because it has been suggested that—at least in Type 1 diabetes—high baseline GFR is predictive of ultimate diabetic nephropathy; this hypothesis has not been confirmed by all authors (19), however.
After the seminal communication of Marañón (20), it has been widely recognized that hypertension may precede the onset of overt diabetes mellitus (21,22) suggesting that the two phenomena represent different facets of the metabolic syndrome. Several studies reported a high prevalence of abnormal ambulatory blood pressure (23,24) and left ventricular hypertrophy (25) in patients with newly diagnosed Type 2 diabetes, but there is little information available on the prevalence of abnormal circadian blood pressure profile.

Finally, it has been postulated that a hereditary predisposition to primary hypertension predisposes also to the development of diabetic nephropathy and that such predisposition is reflected by increased \( \text{Na}^{+}/\text{Li}^{+} \) countertransport in Type 1 and possibly also type 2 diabetes (26,27). Polymorphism of the angiotensin-converting enzyme (ACE) gene has also been proposed as a factor determining the genetic risk of nephropathy, at least in Type 1 diabetes (28,29).

We examined a cohort of patients with recently diagnosed Type 2 diabetes who were consecutively admitted to a diabetes clinic. The aim of the study was to characterize the relationship between albuminuria, renal hemodynamics, blood pressure profile, and indicators of genetic risk.

**PATIENTS AND METHODS**

**Patients**

We approached 92 consecutive patients who were admitted with the diagnosis of Type 2 diabetes of short duration to the Diabetes Clinic at Mergentheim. Instruction in diet and self-control was financed by insurance companies. Comparison with Type 2 diabetes patients insured by these companies showed that our group was a representative sample of younger Type 2 diabetic patients in the work force. All 92 patients consented to participate in the study, which had been approved by the local ethics committee. All patients gave informed consent. In each patient, Type 2 diabetes had been diagnosed by the patient’s private physician less than 24 months before the study. The MODY (maturity onset diabetes of the young) type of diabetes was diagnosed in two patients. In five patients, islet cell antibodies were present (data courtesy of Professor Böhm, Ulm, Germany). The measurements were carried out in all 92 patients, but final statistical analysis was restricted to the 85 remaining patients. (The outcome of analysis for the 92 and 85 patients did not differ significantly.)

The diagnoses of diabetes and of Type 2 diabetes were verified by two additional measurements, i.e., intravenous glucose tolerance test and measurement of C peptide levels in the fasting state and after stimulation with glibenclamide (7 mg) plus a standard meal providing 50 g of carbohydrates, respectively. The diagnosis of diabetes mellitus was made when, during the intravenous glucose tolerance test, venous S-glucose (enzymatic method) at 60 min exceeded 180 mg/dL and/or at 120 min, exceeded 160 mg/dL. The Type 2 diabetes diagnosis was confirmed by fasting C peptide levels measuring in excess of 1.0 nmol/L and/or stimulated levels measuring 2.4 nmol/L or more.

The patients were studied after a 10-day stay in the unit, under semiambulatory conditions. All patients were carefully examined to exclude primary chronic renal disease (ultrasonography, urine bacteriology, microscopic urine analysis). Three of the 85 patients had background retinopathy.

Twenty-four patients had diet treatment exclusively. 42 patients had monotherapy with sulfonylurea (SU), six patients with metformine (M), two patients with acarbose (A), and four patients received SU plus A. Three patients received insulin (I) in the evening. Without any further oral antidiabetic drugs, two patients received I plus SU and two patients received I and SU plus M.

Control subjects for measurements of renal hemodynamics were volunteers (median age, 50.6 yr; range, 23 to 69; 16 men, 13 women) who were studied as outpatients according to the same protocol.

**METHODS**

**Clearance.** Steady-state clearance measurements using inulin and PAH (30) were performed after an overnight fasting period exceeding 10 h. Inulin was measured using a two-step enzymatic assay (31) and PAH using colorimetry (32).

**Glucose tolerance.** Conard’s coefficient of assimilation (33) was measured using an intravenous glucose tolerance test. After an overnight fast, the patients were given an intravenous load of 0.33 g/kg of glucose (40%) over 2 min.

Routine chemistry was measured using an autoanalyzer (Hitachi 705, Boehringer Mannheim, Germany); the glycosylated fraction of hemoglobin (HbA1) was measured by affinity chromatography with boric acid (Abbott IMX; Abbott Park, IL); the upper 95% confidence interval was 6%. C-peptide was measured using a commercial RIA (Sorin Biomedica Deutschland, Düsseldorf, Germany); the stimulated normal range was 1.7 to 3.5 nmol/L; thrombomodulin levels were measured in citrate plasma samples, using a commercial enzyme immunoassay (Asserachrom Co., Lyon, France; normal values, < 50 ng/mL). In serum samples, apolipoproteins A1 and B were measured by kinetic nephelometry.

**Urinary albumin.** One 24-h urine sample was collected on 80 mL of 0.3% EDTA and 0.3% NaN3 for measurement of albumin excretion (day-to-day variability CV, 5%). In addition, albumin/creatinine ratio was measured in two morning urine samples (the correlation to 24-h albumin excretion rate was \( r = 0.98 \)). Categorization as microalbuminuria from 24-h collections was confirmed in all patients by morning urine measurements. Urine albumin and alpha-1-microglobulin were measured using kinetic nephelometry (Beckmann Protein Array System). Dako rabbit anti-alpha-1-MG A 262; antibody against albumin from Beckmann Array kit *MA*); the detection threshold was 2 mg/L and 4 mg/L for albumin and alpha-1-microglobulin, respectively, and CV of replicate measurements was < 5% and 7%, respectively.

Na+/Li+ countertransport. Countertransport rates and kinetics (\( V_{\text{max}} \) and \( K_m \)) were measured according to the methods of Canessa et al. (34) and Rutherford et al. (35). Twenty milliliters of blood was drawn into vacutainers coated with Li-heparinate and centrifuged for 10 min at 3000 rpm at 4°C. Plasma anduffy coats were removed and the remaining erythrocytes were incubated in a loading solution containing 240 mM LiCl for 6 h at 37°C in a gently shaking water bath. After removal of the loading solution, the erythrocytes were washed three times to remove extracellular Li+. Loaded erythrocyte suspension (0.5 mL) was added to efflux solution (2 mL) containing either Na (for stimulation) or Mg (for inhibition of countertransport). Na, K-ATPase and Na, K-cotransport were inhibited by addition of bumetanide (0.02

2628 Volume 7 • Number 12 • 1996
mM) and ouabain (0.1 mm) to the efflux media. After incubation for 0 and 60 min at three Na concentrations (150 mM, 100 mM, and 50 mM) at 37°C with gentle shaking, efflux was stopped by immersion into ice water.

Samples were centrifuged immediately for 10 min at 3000 rpm and 4°C. Intracellular Li was calculated after hemolysis of 100 μL of erythrocytes in 9.9 mL of distilled water. Mean intracellular Li was 5.6 mM (range, 4.3 mM to 7.6 mM), i.e., at concentrations saturating the countertransport system.

\[ \frac{(Li_{80} - Li_{0}) \times (100 - Hct/5)}{Hct/5} \]  

where Li = Li concentration (mmol/L) in supernatant after 0 or 60 min, and Hct = hematocrit (%).

For the calculation of \( V_{max} \) and \( K_m \), the Eadie-Hofstee transformation of the Michaelis-Menten equation was used. Insertion/deletion (I/D) polymorphism of the ACE gene according to Schmid et al. (29).

Ambulatory blood pressure measurements. Casual blood pressure was measured in the clinics ward (36,37), using extra cuff size if upper-arm circumference exceeded 32 cm. To determine the tiobrachial blood pressure ratio, we measured brachial systolic pressure (both arms) after 5 min in supine position; systolic BP of the posterior tibial arteries of both sites was measured with Doppler ultrasound (normal 0.80 to 1.20). The 24-h blood pressure profile was examined using a Spacelabs 90207 monitor (Redmond, WA). Patients wrote a protocol to document their activities and time of bed rest. In the hospital, bed rest was standardized from 2200 h until 0600 h.

Forty-eight patients had no antihypertensive medication, 40 patients were on antihypertensive drugs, 25 on monotherapy (12 on ACE inhibitors, seven on beta-adrenoreceptor antagonists, two on diuretics, and four on calcium channel blockers) and 12 were on combination therapy (seven with ACE inhibitors).

### Table 1. Patient characteristics

<table>
<thead>
<tr>
<th>Albumin Excretion</th>
<th>All Patients (N = 85)</th>
<th>&lt;30 mg/day (N = 72)</th>
<th>&gt;30 mg/day (N = 13)</th>
<th>Normal Range</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td>49 (26 to 69)</td>
<td>49 (26 to 69)</td>
<td>42 (30 to 65)</td>
<td></td>
</tr>
<tr>
<td><strong>Gender (%)</strong></td>
<td>63 m, 22 f (74/26)</td>
<td>53 m, 19 f (74/26)</td>
<td>10 m, 3 f (27/3)</td>
<td></td>
</tr>
<tr>
<td><strong>Diabetes Duration (months)</strong></td>
<td>12 (2 to 24)</td>
<td>13.5 (2 to 24)</td>
<td>7 (2 to 22)</td>
<td></td>
</tr>
<tr>
<td><strong>BMI (kg/m²)</strong></td>
<td>29.6 (22.6 to 54.1)</td>
<td>29.4 (22.6 to 54.1)</td>
<td>30.1 (23.7 to 35.4)</td>
<td>&lt;26 kg/m²</td>
</tr>
<tr>
<td><strong>Waist/Hip Ratio</strong></td>
<td>1.02 (0.85 to 1.17)</td>
<td>1.02 (0.85 to 1.17)</td>
<td>1.02 (0.92 to 1.13)</td>
<td>&lt;1.25</td>
</tr>
<tr>
<td><strong>HbA1 (%)</strong></td>
<td>9.1 (4.7 to 14.7)</td>
<td>8.9 (4.7 to 14.3)</td>
<td>11.2* (8 to 14.7)</td>
<td>&lt;6%</td>
</tr>
<tr>
<td><strong>Conard's Assimilation Coefficient</strong></td>
<td>0.77 (0.3 to 2.66)</td>
<td>0.76 (0.4 to 2.66)</td>
<td>0.83 (0.3 to 1.54)</td>
<td>1.0 to 2.0</td>
</tr>
<tr>
<td><strong>Thrombomodulin (ng/mL)</strong></td>
<td>21.9 (0 to 87.5)</td>
<td>21.9 (0 to 81)</td>
<td>17.5 (3.13 to 87.5)</td>
<td>&lt;50 ng/mL</td>
</tr>
<tr>
<td><strong>Serum Creatinine (mg/dL)</strong></td>
<td>0.93 (0.65 to 1.23)</td>
<td>0.9 (0.65 to 1.23)</td>
<td>1 (0.72 to 1.18)</td>
<td>0.5 to 0.9</td>
</tr>
<tr>
<td><strong>Urea Excretion (g/kg per day)</strong></td>
<td>0.34 (0.02 to 0.91)</td>
<td>0.34 (0.02 to 0.91)</td>
<td>0.30 (0.09 to 0.67)</td>
<td>0.19 to 0.34 g/kg per d</td>
</tr>
<tr>
<td><strong>Sodium Excretion (mmol/24 h)</strong></td>
<td>255 (100.1 to 645)</td>
<td>254 (100.1 to 645)</td>
<td>257 (160 to 476)</td>
<td>&lt;220 mmol/day</td>
</tr>
<tr>
<td><strong>Hypertension</strong></td>
<td>51 of 85 (60%)</td>
<td>42 of 72 (58.3%)</td>
<td>9 of 13 (69%)</td>
<td></td>
</tr>
<tr>
<td><strong>Current Smokers</strong></td>
<td>23 of 85 (27%)</td>
<td>1 of 72 (22%)</td>
<td>7 of 13* (53%)</td>
<td></td>
</tr>
</tbody>
</table>

*Values are given as median and range.  
*P* < 0.0005 difference between microalbuminuric and normal albuminuric patients.  
*Criteria of German High Blood Pressure League (RR ≥ 130/80 mm Hg, average 24-h BP or antihypertensive therapy (39).
Albuminuria

Urinary AER between 30 and 300 mg/24 h (mild albuminuria) were found in nine patients (13%) and albuminuria exceeding 300 mg/24 h in two other patients (2%). Figure 1 shows the distribution of urinary albumin excretion rate. The distribution was unimodal but not normal according to the Shapiro-Wilk test. HbA1 values were significantly ($P < 0.0004$; Wilcoxon test) higher in patients with microalbuminuria (Figure 1). The frequency of hypertension according to the criteria of the German High Blood Pressure League for ambulatory BP measurement (36), i.e., average 24 h BP, $\geq 130/80$ mm Hg or antihypertensive treatment, was not significantly different between patients with microalbuminuria (69%) and patients with normoalbuminuria (58%). The same was true when only patients without ACE inhibitors or without any antihypertensive treatment were considered. The results of multivariate analysis were identical whether patients on ACE inhibitors were excluded or not. Forty-seven percent of the microalbuminuric and 47% of the normoalbuminuric patients were on antihypertensive treatment. One of the three patients with (nonproliferative) retinopathy had microalbuminuria.

Abnormal lipid parameters were noted in a considerable proportion of patients, but no significant difference was noted between patients with or without microalbuminuria, the exception being high-density lipoprotein cholesterol concentration (Table 2). There was also no significant difference in lipid parameters between patients with and without evidence of arterio-occlusive disease (tibiobrachial ratio). Lipid values were consistently higher in women; e.g., total cholesterol, $210 \pm 40$ mg/dL in females versus $182 \pm 39$ mg/dL in men; $P < 0.005$).

**TABLE 2. Lipid parameters (in mg/dL)**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Albumin Excretion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal Range</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>$&lt;220$ mg/dL</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>$&lt;150$ mg/dL</td>
</tr>
<tr>
<td>HDL Cholesterol</td>
<td>$&gt;30$ mg/dL</td>
</tr>
<tr>
<td>LDL Cholesterol</td>
<td>$&lt;150$ mg/dL</td>
</tr>
<tr>
<td>Apolipoprotein A1</td>
<td>$90$ (25 to 556)</td>
</tr>
<tr>
<td>Apolipoprotein B</td>
<td>$79$ (45 to 149)</td>
</tr>
</tbody>
</table>

*Values are given as median and range.

$^a$ $P < 0.05$, difference between microalbuminuric and normalalbuminuric patients.
TABLE 3. Statistical analysis of albuminuria

<table>
<thead>
<tr>
<th>Normalalbuminuria versus Microalbuminuria (logistic regression analysis)</th>
<th>Odds Ratio</th>
<th>Upper/Lower 95% Confidence Limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>HbA1 (%)</td>
<td>3.42&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.81/1.5</td>
</tr>
<tr>
<td>Smoking</td>
<td>26.3</td>
<td>331/2.08</td>
</tr>
<tr>
<td>Nighttime systolic blood pressure</td>
<td>1.32</td>
<td>1.76/1.003</td>
</tr>
</tbody>
</table>

Factors Correlating with Albumin Excretion Rate (multiple linear regression analysis)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Partial $r^2$</th>
<th>Model $r^2$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>ERPF (mL/min per 1.73m&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>0.1479</td>
<td>0.1479</td>
<td>0.0003</td>
</tr>
<tr>
<td>Current smoking</td>
<td>0.0454</td>
<td>0.1934</td>
<td>0.0346</td>
</tr>
<tr>
<td>HbA1 (%)</td>
<td>0.028</td>
<td>0.2217</td>
<td>0.08</td>
</tr>
</tbody>
</table>

<sup>a</sup> Factors included in the model: HbA1, alpha-1-microglobulin excretion rate; 24 h MAP, nighttime systolic blood pressure, percent nocturnal decline in MAP, BMI, Conard's coefficient of assimilation, GFR, ERPF, current smoking, urea excretion rate, duration of hypertension.

<sup>b</sup> Risk rises by a factor of 3.42 for a 1% increment in HbA1.

Median AER in normoalbuminuric patients was 6.6 mg/24 h and the median AER of all patients was 7.05 mg/24 h. Normoalbuminuric patients with AER below the median had significantly lower median HbA1 (8.3%, range, 4.7 to 14.3, versus 9.3%, range, 7 to 13.5; $P < 0.02$) and median nighttime systolic pressure (110 mm Hg, range, 100 to 150 versus 117 mm Hg, range, 93 to 151; $P < 0.01$).

Renal Hemodynamics

Table 4 shows significantly higher mean rates of GFR and ERPF as well as a tendency for lower renovascular resistance in diabetic patients compared with control subjects of comparable age. The upper limit of the 95% confidence interval in control subjects was 131 mL/min per 1.73m<sup>2</sup> for GFR and 706 mL/min per 1.73m<sup>2</sup> for ERPF, respectively; accordingly, 58% of the diabetic patients had GFR and 61% ERPF above the 95th percentile.

Age was inversely and significantly correlated with GFR and ERPF in controls ($r = -0.55$ and $-0.64$) as well as in diabetic patients ($r = -0.27$ and $-0.39$).

Table 5 lists the factors correlated with GFR and ERPF, respectively, using multiple linear regression analysis. GFR was inversely related to age, positively related to albumin excretion, related to gender (i.e., higher in men) and positively related to fasting blood glucose levels. ERPF was inversely related to age and positively related to albumin excretion and fasting blood glucose. The model explained 27.1% of total variance of GFR and 33.3% of ERPF, respectively.

Blood Pressure

By ambulatory blood pressure measurement, hypertension according to the proposal of the German High Blood Pressure League (36,37), i.e., average 24 h BP above 130/80 mm Hg (or antihypertensive treatment), was present in 60% of patients (Table 1). The proportion was identical if the criterion of average daytime BP ≥ 135/85 mm Hg (or antihypertensive treatment) was used.

By casual BP measurements, the proportion of hypertensive patients was still higher, i.e., 65% according to World Health Organization criteria (BP ≥ 140/90 mm Hg or antihypertensive treatment; Reference 38) and 69% according to the more stringent Joint National Committee criteria (BP ≥ 135/85 mm Hg or antihypertensive treatment; Reference 39). Of the untreated patients, 15 of 48 (32%) were hypertensive by Joint National Committee criteria, but had no antihypertensive treatment.

None of the patients had isolated systolic hypertension, i.e., casual systolic BP ≥ 140 mm Hg, but diastolic BP < 90 mm Hg. An abnormal circadian blood pressure profile, i.e., a nocturnal decrease of BP < 15% and < 10% was noted in 61% and 29% of all patients, respectively (including those on antihypertensive medication). In the 40 normotensive patients without antihypertensive medication, 17 (50%) had nocturnal decline of mean arterial pressure (MAP) by < 15% and five (15%) < 10%. Excluding the seven patients on beta-blockers, the patients with < 10% decrease of nighttime MAP had significantly ($P < 0.001$) higher median nighttime heart rates (i.e., 72 min<sup>-1</sup>; range, 57 to 85, versus 65.5 min<sup>-1</sup>, range, 44–95) and significantly ($P < 0.001$) less decline of nighttime heart rate (9.9%, range, 0 to 24; versus...
There was no significant gender difference in mean 24 h systolic BP variability.

A significant correlation was found between known duration of hypertension and mean nocturnal systolic blood pressure (r = 0.45; P < 0.0001) and mean 24 h systolic BP (r = 0.43; P < 0.0001), but no significant correlation of blood pressure or blood pressure variability.

The median known duration of hypertension in the 51 hypertensive patients was 4.6 yr (range, 0 to 40). A significant correlation was found between known duration of prediabetic hypertension and mean nocturnal systolic blood pressure (r = 0.45; P < 0.0001) and mean 24 h systolic BP (r = 0.43; P < 0.0001), but no significant correlation with albuminuria was found.

The odds ratio for factors increasing the risk of a patient to be hypertensive were analyzed using logistic regression analysis. Age significantly increased the odds ratio. Treating BP as a continuous variable in a multiple linear regression model, only 5% of total variance of all patients (treated and untreated) could be explained by age and Conard's assimilation coefficient.

### Genetic Markers

A significantly higher $K_m$ of $Na^+$/Li$^+$ countertransport rate was found in diabetic patients, compared with control subjects, and there was also a tendency for a higher Li$^+$ transport rate, which did not reach statistical significance (Table 6). Diabetic patients with microalbuminuria tended to have higher $K_m$ than diabetic patients without microalbuminuria.

In this cohort of limited size, allele frequencies for the I/D polymorphism of the ACE gene in microalbuminuric patients (I, 0.5; D, 0.5) and nonmicroalbuminuric patients (I, 0.35; D, 0.65) were not significantly different (Table 7). The values in both groups were in Hardy Weinberg equilibrium.

Table 7 shows that diabetic patients with microalbuminuria more frequently had first-degree relatives with a history of cardiovascular events than did patients without microalbuminuria. All propositi had information on more than one first-degree relative, the average number of first-degree relatives per propositus being 3 (range, 2 to 6).

Interaction was found between genetic risk, i.e., cardiovascular events in first-degree relatives and glycemic control (Table 8) but interpretation must be cautious in view of the limited sample size.

### DISCUSSION

The present comprehensive clinical investigation in a cohort of patients with confirmed diagnosis of Type 2 diabetes of relatively short known duration and less-than-perfect glycemic control provides information on the interaction between albuminuria, renal hemodynamics, blood pressure, and genetic factors. In agreement with previous observations (10,12,40-42) we found that a considerable proportion of par-

### Table 5. Factors correlated with GFR and ERPF (multiple linear regression analysis)$^a$

<table>
<thead>
<tr>
<th>Factor</th>
<th>Partial $r^2$</th>
<th>Model $r^2$</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>GFR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Albumin excretion (mg/24 h)</td>
<td>0.0968</td>
<td>0.0968</td>
<td>0.0038</td>
</tr>
<tr>
<td>Gender</td>
<td>0.1048</td>
<td>0.2016</td>
<td>0.0015</td>
</tr>
<tr>
<td>Age</td>
<td>0.0371</td>
<td>0.2387</td>
<td>0.0504</td>
</tr>
<tr>
<td>Fasting blood glucose (mmol/L)</td>
<td>0.0326</td>
<td>0.2712</td>
<td>0.0623</td>
</tr>
<tr>
<td>ERPF</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>0.1620</td>
<td>0.1620</td>
<td>0.0001</td>
</tr>
<tr>
<td>Albumin excretion (mg/24 h)</td>
<td>0.1169</td>
<td>0.2790</td>
<td>0.0005</td>
</tr>
<tr>
<td>Fasting blood glucose (mg/dL)</td>
<td>0.0540</td>
<td>0.3330</td>
<td>0.0123</td>
</tr>
</tbody>
</table>

$^a$Factors included in the model: age, gender, MAP, AER, a1-microglobulin excretion rate, fasting glucose, and HbA1.

### Table 6. $Na^+$/Li$^+$ countertransport

<table>
<thead>
<tr>
<th>Group</th>
<th>Transport Rate (µmol/L erythrocytes per h)</th>
<th>$V_{max}$ (µmol/L erythrocytes per h)</th>
<th>$K_m$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Subjects (N = 10)</td>
<td>253 (45 to 1277)</td>
<td>343 (152 to 2034)</td>
<td>85 (85 to 629)</td>
</tr>
<tr>
<td>Diabetic Patients (N = 85)</td>
<td>336 (70 to 1727)</td>
<td>535 (185 to 2829)</td>
<td>97* (11 to 404)</td>
</tr>
<tr>
<td>Without microalbuminuria (N = 72)</td>
<td>334 (111 to 1727)</td>
<td>521 (185 to 2829)</td>
<td>98.5 (117 to 2333)</td>
</tr>
<tr>
<td>With microalbuminuria (N = 13)</td>
<td>344 (70 to 567)</td>
<td>672* (334 to 925)</td>
<td>129.5* (81 to 298)</td>
</tr>
<tr>
<td>With normotension (N = 34)$^b$</td>
<td>319 (111 to 850)</td>
<td>513 (185 to 886)</td>
<td>96.5 (11 to 404)</td>
</tr>
<tr>
<td>With hypertension (N = 51)$^c$</td>
<td>346 (70 to 1727)</td>
<td>580 (286 to 2829)</td>
<td>97 (33 to 298)</td>
</tr>
</tbody>
</table>

$^a$Difference between diabetic patients and control subjects (P < 0.05).

$^b$According to ambulatory BP measuring.

$^c$Difference between normo- and microalbuminuric patients (P < 0.03).
In the past, there have been some arguments regarding whether hyperfiltration, a known feature of incipient Type 1 diabetes, is also present in Type 2 diabetes. There have been reports that GFR was within the normal range (16–18) or above the normal range in a variable proportion of patients (16,18). In this study, we confirm that GFR above the 95th percentile of a control sample is found in 58% of patients with Type 2 diabetes of recent onset. The proportion of patients with abnormal ERPF was even higher, implying that renal vasodilation is more frequent than glomerular hyperfiltration. ERPF was related to fasting blood glucose; this finding is compatible with the notion that increased proximal Na⁺/glucose cotransport activity is involved. As in other studies, GFR and RPF declined with age, but we did not find that the decrease of ERPF with age was greater than that in the control population.

There is consensus that in contrast to Type 1 diabetes, elevated blood pressure is present in a sizeable proportion of newly diagnosed patients with Type 2 diabetes (23,24,40) and even in prediabetic individuals (53,54). Not unexpectedly, the more rigorous the definition of normal blood pressure the higher the prevalence of hypertension in Type 2 diabetes. Approximately 60% of patients had abnormal blood pressure according to ambulatory blood pressure monitoring and an abnormal circadian blood pressure profile was also frequent, similar to that noted in Type 1 diabetes (55). So called “non-dippers” had higher 24-h heart rates, particularly higher nighttime heart rates, pointing to impaired autonomic control. In the general population, men of this age group have more frequently hypertension than women. In contrast, no such gender difference was seen in patients with Type 2 diabetes. AER and microalbuminuria were not predictive of blood pressure, showing that nephropathy is not a major determinant of hypertension at this stage of Type 2 diabetes.

Hypertension is common in the prediabetic stage. It has been shown, at least in Pima Indians, that prediabetic blood pressure is predictive of nephropathy (54). We found no influence of prediabetic blood pressure on microalbuminuria, but it is wise to keep in mind the limited power of our study. Prediabetic blood pressure was a powerful determinant of circadian blood pressure profile, however, long known duration

<table>
<thead>
<tr>
<th>Group</th>
<th>Prevalence of Microalbuminuria</th>
</tr>
</thead>
<tbody>
<tr>
<td>No CV Events in Relatives and HbA1 &lt;8% (N = 12)</td>
<td>0 of 12 = 0%</td>
</tr>
<tr>
<td>Either CV Events in Relatives or HbA1 &gt;8% (N = 52)</td>
<td>1 of 52 = 1.9%&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>CV Events in Relatives and HbA1 &gt;8% (N = 21)</td>
<td>10 of 21 = 48%&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Difference between risk groups (P<4.1 × 10⁻⁵).

deference between risk groups (P<4.1 × 10⁻⁵).

deference between risk groups (P<4.1 × 10⁻⁵).
of prediabetic hypertension predicted higher 24 h and particularly nighttime blood pressures.

In view of the strong genetic component for diabetic nephropathy in Type 1 (50) and Type 2 (51) diabetes, it is attractive to look for genetic markers, e.g., Na+/Li+ countertransport in erythrocytes (34,35) and I/D polymorphism of the ACE gene (28). In this study, we confirmed the observation of Gall (56) that patients with Type 2 diabetes have higher transport rates. In addition, they had higher $K_m$. In contrast, Rutherford (57) found lower $K_m$ and $V_{\text{max}}$ in diabetic patients compared with control subjects. In our study, microalbuminuric patients had higher $K_m$.

This study confirms familial clustering of cardiovascular disease in Type 2 as in Type 1 diabetes (58), and extends the observation by documenting that a family history of cardiovascular disease is related to early microalbuminuria and interacts with glycemia to increase the risk of microalbuminuria.

It is useful to examine the earliest stages of diabetic nephropathy in which many confounding factors of advanced diabetes are still absent. This study documents abnormal renal function and blood pressure profile early on and identifies factors to which these abnormalities are linked. Long-term prospective observations are required in order to find out whether such early abnormalities are predictive of late renal outcome.

ACKNOWLEDGMENTS

This study was supported by a grant from the Else Littauer Foundation, Homburg v.d.H. We thank Professor Böhm (Ulm) for providing urinary albumin, Dr. S. Schmidt (Heidelberg) for genotyping patients with respect to I/D polymorphism, Dr. Boero (Torino) for providing details concerning the Na/Li methodology, and Dr. Messinger (Boehringer Co., Mannheim) for advice with the biostatistical analysis.

REFERENCES


30. Schmid M, Mann JFE, Stein G, Herter M, Nussberger J, Klingbel A, Ritz E: Natriuresis-pressure relationship in...
35. Rutherford PA, Thomas TH, Carr SJ, Taylor R, Wilkin-

45. Rutherford PA, Thomas TH, Carr SJ, Taylor R, Wilkin-