

# Low Glomerular Filtration Rate in Normoalbuminuric Type 1 Diabetic Patients

## An Indicator of More Advanced Glomerular Lesions

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Increased urinary albumin excretion rate is widely accepted as the first clinical sign of diabetic nephropathy. However, it is possible that some diabetic patients could first manifest reduced glomerular filtration rate (GFR) or hypertension. Relatively advanced diabetic renal lesions can be present in some diabetic patients with long-standing normoalbuminuria, and this might indicate increased risk of progression to microalbuminuria and then to overt diabetic nephropathy. The aim of this study was to identify a group of normoalbuminuric type 1 diabetic patients with low GFR and compare them with normoalbuminuric patients with normal GFR. Altogether, 105 normoalbuminuric type 1 diabetic patients with at least 10 years of diabetes duration that had a renal biopsy performed for research purposes were studied. Patients were divided according to GFR into groups with normal ( $\geq 90 \text{ ml} \cdot \text{min}^{-1} \cdot 1.73 \text{ m}^{-2}$ ) or reduced ( $< 90 \text{ ml} \cdot \text{min}^{-1} \cdot 1.73 \text{ m}^{-2}$ ) GFR. Clinical and renal structural parameters were compared between these two groups. Glomerular structural parameters were estimated by electron microscopic morphometry. The 23 patients with reduced GFR had more advanced diabetic glomerular lesions. The finding of reduced GFR was much more common among female patients, particularly if retinopathy and/or hypertension were also present. This report confirms that reduced GFR occurs among long-standing normoalbuminuric type 1 diabetic patients and is associated with more advanced diabetic glomerular lesions and, probably, with increased risk of progression. For these reasons, we suggest that regular measurements of GFR be performed in long-standing normoalbuminuric type 1 diabetic female diabetic patients, especially in those with retinopathy or hypertension. *Diabetes* 52:1036–1040, 2003

Diabetic nephropathy affects 25–30% of type 1 diabetic patients and, mainly due to renal failure among type 2 diabetic patients, is the leading cause of end-stage renal disease in the U.S. (1) and Europe (2). Increased albumin excretion rate (AER) has been considered the first clinical sign of diabetic nephropathy in both type 1 and type 2 diabetic patients (3); although microalbuminuria is usually the first manifestation of renal disease, in some patients hypertension or reduction in glomerular filtration rate (GFR) may antedate its development. It has recently been described that mild increases in blood pressure, detectable only by 24-h blood pressure monitoring, precede the development of microalbuminuria in type 1 diabetic patients, and may, therefore, help to identify patients at higher nephropathy risk (4). Decreased GFR has previously been reported in eight normoalbuminuric long-standing type 1 diabetic women (5). In this small study, these women had more advanced glomerular lesions when compared with 19 normoalbuminuric women with normal GFR (5). Although a report from Australia confirmed the idea that reduced GFR may antedate increased AER in type 1 and type 2 diabetic patients (6), renal biopsies were not done. Because our preliminary results indicate that normoalbuminuric patients with more advanced diabetic glomerular lesions appear to be at increased risk of progression to overt diabetic nephropathy (7), normoalbuminuric type 1 diabetic patients with low GFR could represent a subset of patients at increased risk of progression. The present study evaluated the clinical and glomerular structural characteristics of a large cohort of long-standing normoalbuminuric type 1 diabetic patients with a wide range of GFR in order to identify the variables associated with the presence of low GFR in this population and to determine whether the association of this clinical finding with more advanced diabetic glomerulopathy lesions could be confirmed.

### RESEARCH DESIGN AND METHODS

**Patients.** A total of 105 normoalbuminuric type 1 diabetic patients that had research kidney biopsies performed as part of their evaluation for pancreas transplantation ( $n = 69$ ) or as participants in a study of the natural history of diabetic nephropathy or in studies of renal structure and function in type 1 diabetic sibling pairs ( $n = 36$ ) were studied. These studies were performed between 1971 and 1999. Eligibility criteria for the present study included type 1 diabetes according to World Health Organization criteria (8) with a duration of at least 10 years, normoalbuminuria, serum creatinine  $< 2.0 \text{ mg/dl}$  (176.8

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AER, albumin excretion rate; GBM, glomerular basement membrane; GCRC, general clinical research center; GFR, glomerular filtration rate; HPLC, high-performance liquid chromatography; MC, mesangial cell; MM, mesangial matrix; Sv(PGBM/glom), surface density of the peripheral GBM per glomerulus; Vv(MC/glom), fractional volume of the glomerulus occupied by MC; Vv(Mes/glom), fractional volume of the glomerulus occupied by mesangium; Vv(MM/glom), fractional volume of the glomerulus occupied by MM.

TABLE 1  
Demographic and clinical characteristics in long-standing normoalbuminuric type 1 diabetic patients

	Normal GFR group	Low GFR group	<i>P</i>
<i>n</i>	82	23	
Sex (male/female)	36/46	4/19	0.021
Age (years)	35.2 ± 9.5	36.0 ± 8.0	NS
Age at diabetes onset (years)	12.9 ± 7.0	11.3 ± 7.9	NS
Diabetes duration (years)	22.2 ± 9.6	24.6 ± 8.0	NS
HbA <sub>1c</sub> (%)	8.5 ± 1.6	8.4 ± 1.1	NS
Serum creatinine (mg/dl)*	0.84 ± 0.17	0.96 ± 0.21	0.005
SBP (mmHg)	119.8 ± 10.9	116.8 ± 12.9	NS
DBP (mmHg)	71.2 ± 7.8	71.7 ± 7.3	NS
MBP (mmHg)	87.6 ± 8.2	86.5 ± 8.4	NS
Hypertension (yes/no)	27/55	11/12	NS
Antihypertensive treatment (yes/no)	13/69	9/14	0.022
ACEI or AIIRB (yes/no)	4/78	3/20	NS
GFR (ml · min <sup>-1</sup> · 1.73 m <sup>-2</sup> )†	121.2 ± 16.6	75.9 ± 11.7	ND
AER (μg/min)	7.7 (2.8–19.9)	7.7 (2.0–17.6)	NS
Retinopathy (none/background/proliferative)	33/31/15	2/11/10	0.006

Data are means ± SD, *n*, and median (range). ACEI, ACE inhibitor; AIIRB, angiotensin II type 1 receptor blocker; DBP, diastolic blood pressure; MBP, mean blood pressure; SBP, systolic blood pressure. \*To convert values to μmoles/l, multiply by 88.4; †to convert values to ml/s, multiply by 0.01667.

μmol/l), and absence of clinical or morphologic evidence of other glomerulopathies. Patients known to be microalbuminuric before antihypertensive therapy was started were not eligible for this study. Reference values for glomerular structure were derived from 76 age- and sex-matched normal, living kidney transplant donors. These studies were approved by the committee for the Use of Human Subjects in Research of the University of Minnesota. Informed consent was obtained from all participants before each study. The patients were admitted in the general clinical research center (GCRC) at the University of Minnesota where renal function studies and percutaneous kidney biopsy were performed.

**Kidney function studies.** Blood pressure was measured by trained observers using an oscillometric automatic monitor while the patients were in the GCRC. The mean value of multiple measurements was used to calculate systolic and diastolic blood pressure. Mean blood pressure was calculated as (2 × diastolic blood pressure + systolic blood pressure)/3. Hypertension was defined as blood pressure levels ≥130/85 mmHg (9) or use of antihypertensive medication. HbA<sub>1c</sub> was measured by high-performance liquid chromatography (HPLC). Serum and urinary creatinine were measured by Jaffé reaction. AER was assessed in three 24-h sterile urine collections by a fluorimetric immunoassay (10). Normoalbuminuria was defined as AER <20 μg/min in at least two of three consecutive measurements. The median AER value for each patient was used for the analyses. GFR was estimated by iohalamate clearances using four timed urine and blood collections (HPLC) (36 patients) or by the mean of two or three 24-h creatinine clearances, with urine carefully collected under direct supervision of the GCRC nursing staff (69 patients). We have previously demonstrated that these GCRC creatinine clearances are highly correlated with classical inulin clearances performed during the same GCRC admission (*r* = 0.92; *P* < 0.001) (11). Bland-Altman plots indicated no trend or deviation, and there was no significant difference between the values obtained by these two methods.

**Retinal studies.** Retinopathy was assessed by funduscopy in 102 patients and classified as absent, background, or proliferative.

**Renal structural studies.** Percutaneous kidney biopsy was performed with ultrasound guidance under local anesthesia.

#### Morphometric analyses

**Tissue processing.** Electron microscopy tissues were processed as detailed elsewhere (12,13). A calibration grid was photographed with each glomerulus, and 10–20 evenly spaced micrographs were obtained at 11,000× for measurement of glomerular basement membrane (GBM) width and for mesangial composition. Micrographs at 3,900× were constructed into a montage of the entire glomerular profile for measurements of the fractional volume of the glomerulus occupied by mesangium [Vv(Mes/glom)] and the surface density of the peripheral GBM per glomerulus [Sv(PGBM/glom)].

**EM measurements.** The presence of at least two nonsclerotic glomeruli per biopsy in the EM blocks was an entry criterion for this study. However, in most cases, three glomeruli were used. Glomeruli were evaluated for: GBM width, by the orthogonal intercept method (14); Sv(PGBM/glom), by intercept counting (12,13); and Vv(Mes/glom) and mesangial components, by point counting (15). Grid points falling on mesangial cell (MC) and mesangial matrix

(MM) were noted and the fractional volume of the glomerulus occupied by MC [Vv(MC/glom)] and MM [Vv(MM/glom)] were calculated (15).

**Statistical analyses.** Results are presented as mean ± SD. AER is presented as median and range. Values for AER were logarithmically transformed before analysis. Normoalbuminuric type 1 diabetic patients were categorized according to GFR into the normal GFR group (GFR ≥90 ml · min<sup>-1</sup> · 1.73 m<sup>-2</sup> body surface area, the lower limit of the normal range at the Fairview-University of Minnesota Medical Center) or the low GFR group (GFR <90 ml · min<sup>-1</sup> · 1.73 m<sup>-2</sup>). Pearson's linear correlation was used to test associations between GFR and structural parameters. Unpaired *t* tests were used to compare continuous variables between the low GFR and the normal GFR groups. Discrete variables were compared by χ<sup>2</sup>. ANOVA followed by Fisher's least significant difference procedure was used to compare continuous variables between control subjects and normal GFR and low GFR groups. Values for *P* < 0.05 were considered statistically significant.

## RESULTS

A total of 105 patients (65 women) were studied. Age was 35.4 ± 9.1 years, age at diabetes onset 12.6 ± 7.2 years, and diabetes duration 22.7 ± 9.3 years. HbA<sub>1c</sub> at the time of biopsy was 8.4 ± 1.5%. Median AER was 7.7 μg/min (2.0–19.9). Retinopathy was present in 67 patients (64%); 25 (24%) had proliferative changes. Hypertension was present in 38 (36%) patients; 22 were receiving antihypertensive drugs at the time of the studies, and 7 were receiving ACE inhibitors or angiotensin II type 1 receptor blockers (AIIRBs).

Altogether, 23 (22%) patients were in the low and 82 (78%) patients were in the normal GFR groups. There were more women in the low GFR than in the normal GFR group (*P* = 0.021, Table 1). Age, age at diabetes onset, and diabetes duration were not different between low and normal GFR patients. HbA<sub>1c</sub> was similar in low and normal GFR patients. Serum creatinine was higher in the low than in the normal GFR group (*P* = 0.005). The prevalence of hypertension was the same in both groups (Table 1); however, when defined as blood pressure values ≥140/90 mmHg or use of antihypertensive drugs, the prevalence was greater in the low GFR group (*P* < 0.001). A similar proportion of hypertensive patients in the low and normal GFR groups was receiving renin-angiotensin system blockers (*P* = 0.39). AER was not different between groups. The

TABLE 2

Glomerular structural characteristics in long-standing normoalbuminuric type 1 diabetic patients and nondiabetic control subjects

	Control	Normal GFR	Low GFR	<i>P</i>
GBM width (nm)	331.5 ± 45.7	469.4 ± 84.2	544.5 ± 140.7	<0.001
Vv(Mes/glom)	0.20 ± 0.03	0.28 ± 0.06	0.34 ± 0.08	<0.001
Vv(MM/glom)	0.09 ± 0.02	0.15 ± 0.04	0.20 ± 0.06	<0.001
Vv(MC/glom)	0.08 ± 0.02	0.08 ± 0.02	0.10 ± 0.02	<0.001
Sv(PGBM/glom) ( $\mu\text{m}^2/\mu\text{m}^3$ )	0.126 ± 0.018	0.116 ± 0.019	0.094 ± 0.021	<0.001

Data are means ± SD.

prevalence of any retinopathy was more frequent in low versus normal GFR patients ( $P = 0.003$ ), as was proliferative retinopathy ( $P = 0.016$ ).

Low GFR was present in 31% of patients with any degree of retinopathy (Table 1). This increased to 39% if the patient was also female and to 50% if hypertension was also present. Only 14% of men with any degree of retinopathy ( $P < 0.03$  vs. women with retinopathy) had low GFR, whereas the number of normoalbuminuric men with both retinopathy and hypertension ( $n = 6$ ) was too small for statistical comparison. Fifty-six percent of normoalbuminuric women with proliferative retinopathy had low GFR. Finally, 41% of patients on antihypertensive medications had low GFR vs. 17% of those not on these agents (Table 1). When patients on antihypertensive medications were excluded, the sex differences remained ( $P = 0.016$ ); retinopathy was more common in the low GFR group ( $P = 0.048$ ), and a similar trend for differences in retinopathy grade was found ( $P = 0.061$ ).

Compared with control values, GBM width was increased by 42% in the normal and by 64% in the low GFR groups (Table 2). Similarly, Vv(Mes/glom) was increased by 40% in the normal and by 70% in the low GFR groups, whereas Vv(MM/glom) was increased by 67% in the normal and by 122% in the low GFR groups. Sv(PGBM/glom) was significantly decreased in both normal (by 8%) and low (by 25%) GFR groups (Table 2). These group differences were significant ( $P < 0.005$  for all comparisons between control subjects and the normal GFR group and  $P < 0.001$  between control subjects and the low GFR group). Structural lesions were more advanced in low versus normal GFR patients ( $P < 0.001$  for all comparisons) (Table 2). All of these group differences, other than for Vv(MC/glom), were confirmed when patients on antihypertensive medications were excluded from the analyses (data not shown). Considered as a single group of type 1 diabetic patients, there were inverse correlations between GFR and Vv(Mes/glom) ( $r = -0.34$ ,  $P = 0.005$ ), Vv(MM/glom) ( $r = -0.34$ ,  $P < 0.0001$ ), Vv(MC/glom) ( $r = -0.25$ ,  $P = 0.009$ ), and GBM width ( $r = -0.22$ ,  $P = 0.027$ ). GFR was directly correlated with Sv(PGBM/glom) ( $r = 0.40$ ,  $P < 0.001$ ). These relations between structural parameters and GFR were not seen among the 82 normal GFR patients. However, in the low GFR group, GFR correlated inversely with Vv(Mes/glom) ( $r = -0.63$ ,  $P = 0.0002$ ), Vv(MM/glom) ( $r = -0.51$ ,  $P = 0.014$ ), and Vv(MC/glom) ( $r = -0.50$ ,  $P = 0.015$ ) and directly with Sv(PGBM/glom) ( $r = 0.56$ ,  $P = 0.005$ ).

There were no significant correlations between AER and GFR. AER in the 105 normoalbuminuric type 1 diabetic patients was weakly related to GBM width ( $r = 0.27$ ,  $P = 0.005$ ) but was not statistically related to other structural variables (data not shown). AER also correlated with GBM

width in the 82 normal GFR patients ( $r = 0.36$ ,  $P = 0.001$ ). There were no significant correlations between AER and structural variables in the low GFR group.

## DISCUSSION

Long-standing type 1 diabetic patients with normal AER are still at risk of developing clinically significant nephropathy (16,17). It is therefore important to identify markers of increased nephropathy risk among these patients. One possibility is to perform kidney biopsies in such patients, given that those with more advanced glomerulopathy are more likely to develop abnormalities in AER (7). However, this is not very practical in most clinical settings. Thus, we examined whether reduced GFR can be predictive of more advanced underlying glomerular lesions.

We previously reported that reduced GFR in eight normoalbuminuric long-standing type 1 diabetic women was associated with worse diabetic glomerular lesions (5). Shortly thereafter, a small group of normoalbuminuric long-standing type 1 and type 2 diabetic largely female patients with reduced GFR was described (6). A similar prevalence of reduced GFR was reported among long-standing normoalbuminuric and normotensive type 1 diabetic patients in Brazil (18). Reduced GFR has also been observed in normoalbuminuric type 2 diabetic patients in Denmark (19), but the higher prevalence of hypertension among type 2 diabetic patients could have accounted for some GFR loss.

However, other investigators (20) did not encounter reduced GFR in normoalbuminuric type 1 diabetic patients. Based on this paucity of information and these conflicting results, the present study was undertaken using a much larger cohort of patients and confirmed that reduced GFR occurs among normoalbuminuric long-standing type 1 diabetic patients. This, and the earlier studies (5,6), showed a marked predominance of this phenomenon in women. Our earlier report (5) suggested that, at least in part, the sex effect could be related to the self-selection of a low protein diet among female patients, but this was not investigated in the present cohort.

As noted, a large cross-sectional study did not observe reduced GFR among normoalbuminuric type 1 diabetic patients (20). However, diabetes duration was shorter (20), mean of 14 years in this study versus 23 years in the present study. Also, patients with diabetes duration as brief as 1 year were included in the former (20) versus a minimum of 10 years in the present study. Moreover, only 29% of the patients in the study of Hansen et al. (20) were women as compared with 62% in the current study. Finally, patients on antihypertensive drugs were excluded from this earlier study (20), whereas our report indicates that

low GFR is much more common among normoalbuminuric patients who are on these medications. Thus, the differences in the findings of the present and the earlier report of Hansen et al. (20) are best explained by the marked differences in inclusion criteria and patient demographics. These differences are unlikely to be explained by differences in GFR methodology. Although inulin clearance is considered to be the gold standard for GFR estimates, iothalamate clearances are highly correlated with the inulin method (21). Creatinine clearances with home urine collections are generally considered to provide a less precise estimate of true GFR, at least in part due to collection inaccuracies. However, as already described, multiple supervised clinical research center creatinine clearances are highly correlated with inulin clearances (11) and show no deviation or trend in Bland-Altman analysis. Moreover, 96% of the low GFR group had creatinine clearance measurements. There were very strong correlations between this GFR estimate and renal structure within this group, further supporting the validity of this carefully performed measure as an indicator of underlying renal pathology.

Although some still question this point (3), this study confirms that normoalbuminuric type 1 diabetic patients have increased Vv(Mes/glom) in addition to Vv(MM/glom) and GBM width and decreased Sv(PGBM/glom). Moreover, the presence of low GFR was associated with worse diabetic glomerular lesions. The statistical differences between the low GFR and the normal GFR normoalbuminuric groups were maintained when patients on antihypertensive medications were excluded from the analyses. This excludes the possibility that our findings were caused by patient misclassification, i.e., the inclusion of patients who would be microalbuminuric if not on antihypertensive drugs, or by grouping errors secondary to GFR having been reduced by these drugs. A relatively high proportion of patients, hypertensive by current standards, was not receiving antihypertensive treatment, and only a small number of patients were on ACE inhibitors. This is because many of these patients were studied when the definition of hypertension was different (22) and when ACE inhibitors were not available or commonly used.

Studies evaluating structural-functional relations in diabetic nephropathy among patients ranging from normoalbuminuria to proteinuria demonstrate that AER, blood pressure, and GFR are strongly correlated with glomerular structure (13,23,24). Thus, patients with worse lesions also have clinical changes of increased AER and blood pressure and reduced GFR. The present study in long-standing normoalbuminuric type 1 diabetic patients confirms the association between GFR and glomerular structural parameters, even in patients with normal AER. Usually, diabetic patients developing diabetic nephropathy will initially present with increased AER followed by or concomitant with increased blood pressure before GFR decline occurs. However, as confirmed here, a significant fraction of patients do not follow this pattern, as they can have reduced GFR and increased blood pressure before AER increases.

Other efforts are being undertaken to identify normoalbuminuric patients at increased diabetic nephropathy risk before microalbuminuria or proteinuria develops.

Thus, Lurbe et al. (4) recently reported that nighttime ambulatory blood pressure values and "nondipper" status were significant predictors of progression from normoalbuminuria to microalbuminuria in adolescent patients with type 1 diabetes. In addition, we agree with the general thesis of the editorial by Ingelfinger (25) accompanying the article of Lurbe et al. (4), which argues that it is important to identify the subset of normoalbuminuric patients at increased nephropathy risk, perhaps as candidates for improved glycemic control and other therapies.

Because it would not be practical to perform renal biopsies in all normoalbuminuric patients, we recommend that long-standing normoalbuminuric type 1 diabetic female patients with retinopathy or hypertension should have GFR measured on a regular basis. As the risk of low GFR in similarly defined men is just over 10%, this recommendation should be considered for men in terms of cost-to-benefit ratio. The importance of identifying such low GFR patients is based on the observation that long-standing normoalbuminuric patients who progress to diabetic nephropathy have worse baseline diabetic glomerular lesions than those remaining normoalbuminuric (7). Similarly, glomerular structure in microalbuminuric type 1 diabetic patients was a predictor of AER after 6 years of follow-up (26). Also, in a cohort of type 1 diabetic adolescents in transition from normoalbuminuria to microalbuminuria, the rate of GFR decline (although still within the normal range) was correlated with GBM width (27). It should be appreciated that the severity of the glomerular structural lesions in the normoalbuminuric patients with low GFR presented here was similar to that of microalbuminuric type 1 diabetic patients with similar diabetes duration (data not presented). Considering these findings, it is not surprising that ~10% of long-standing normoalbuminuric type 1 diabetic patients will progress to diabetic nephropathy (16,17,28,29). Taken together, these studies show a wide range of lesions in normoalbuminuric long-standing diabetic patients and suggest that the severity of these lesions is predictive of progression to microalbuminuria and overt nephropathy (7,27).

The diabetic patients recruited into the studies presented here may not be representative of the entire population of long-standing normoalbuminuric type 1 diabetic patients. However, there is no reason to believe that other patients with the characteristics of the low GFR group, i.e., diabetes duration 10–40 years, female sex, and presence of retinopathy, hypertension, or both, would be different from those in this report. For this reason and those reasons argued above, we recommend that such patients have annual GFR measurements. In the final analysis, however, the true value of this recommendation will only be disclosed by longitudinal studies.

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