

# Smoking and Progression of Diabetic Nephropathy in Type 1 Diabetes

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**OBJECTIVE**— Cigarette smoking contributes to development of diabetic nephropathy. However, long-term studies on the effect of smoking on decline in kidney function in diabetic nephropathy are lacking. We assessed the impact of smoking on progression of diabetic nephropathy in type 1 diabetic patients enrolled in a prospective observational cohort study started in 1983.

**RESEARCH DESIGN AND METHODS**— We identified all albuminuric type 1 diabetic patients ( $n = 301$ ) followed for at least 3 years, median (range) 7 years (3–14), who underwent at least yearly measurement of glomerular filtration rate (GFR) by the <sup>51</sup>Cr-EDTA plasma clearance technique ( $n = 8$ , range 3–24). In total, 192 men and 109 women were included (age [mean  $\pm$  SD]  $36 \pm 11$  years, duration of diabetes  $22 \pm 8$  years); 271 patients were treated with antihypertensive drugs, predominantly ACE inhibitors in 179 patients. Patients were classified as smokers if they smoked more than one cigarette per day during a portion of or the entire observation period. Blood pressure, albuminuria, HbA<sub>1c</sub>, and serum cholesterol were measured every 3–4 months during the study.

**RESULTS**— In all 301 patients, the mean (SE) rate of decline in GFR ( $\Delta$ GFR) was  $4.0$  ( $0.2$ )  $\text{ml} \cdot \text{min}^{-1} \cdot \text{year}^{-1}$  during the investigation period. No difference in  $\Delta$ GFR was demonstrated between nonsmokers ( $n = 94$ ),  $\Delta$ GFR  $4.5$  ( $0.4$ ), ex-smokers ( $n = 31$ ),  $\Delta$ GFR  $3.1$  ( $0.7$ ), and smokers ( $n = 176$ ),  $\Delta$ GFR  $3.9$  ( $0.3$ )  $\text{ml} \cdot \text{min}^{-1} \cdot \text{year}^{-1}$ , respectively (NS). Adjustment for other risk factors for progression of diabetic nephropathy did not alter the results: smoking was not associated with  $\Delta$ GFR, whereas blood pressure, albuminuria, HbA<sub>1c</sub>, and serum cholesterol were demonstrated to be independent progression promoters.

**CONCLUSIONS**— In our study, smoking was not associated with decline in kidney function in type 1 diabetic patients with diabetic nephropathy.

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**D** iabetic nephropathy is a progressive disease characterized by persistent albuminuria, elevated arterial blood pressure, and relentless decline in glomerular filtration rate (GFR) (1) ranging from 2 to 20  $\text{ml} \cdot \text{min}^{-1} \cdot \text{year}^{-1}$  (2–4). It is the leading cause of end-stage renal disease and develops in  $\sim 35\%$  of type 1 diabetic patients (5,6). Several modifiable factors, i.e., arterial blood pressure, albuminuria, glycemic control, and serum cholesterol, have been identified as so-

called progression promoters (7). Because these progression promoters can only explain a part of the observed variation in the rate of decrease in GFR (7), identification of other potentially modifiable risk factors for loss of kidney function is therefore of utmost importance. Cigarette smoking has been associated with development of persistent microalbuminuria (8–10) as well as overt nephropathy in diabetic patients (10–16). However, whether smoking tobacco promotes pro-

gression of loss of kidney function in diabetic kidney disease has scarcely been studied. To evaluate the impact of cigarette smoking on decline in kidney function, we analyzed data from a large long-term prospective observational study in a consecutive cohort of 301 type 1 diabetic patients with diabetic nephropathy at the Steno Diabetes Center.

## RESEARCH DESIGN AND METHODS

### Patients

The patients and procedures have been described in detail previously (7). In brief, since 1983 at the Steno Diabetes Center, all type 1 diabetic patients with nephropathy have had their kidney function monitored with one yearly determination of GFR. We consecutively included all type 1 diabetic patients who had a minimum follow-up of 3 years ( $n = 301$ ). The recruitment period for our prospective cohort study ended in 1997. At the end of the study, 221 patients remained in the study, 18 patients had progressed to end-stage renal failure, 40 patients had died, and 22 patients had moved away from Copenhagen. Smoking history was assessed using a standardized questionnaire and information from the individual patient records. Patients were classified as smokers, ex-smokers, and nonsmokers. Smokers were defined as patients smoking more than one cigarette per day during a portion of or the entire observation period. Ex-smokers were defined as subjects who quit smoking before entering the study and remained nonsmokers throughout the study. A total of 12 patients who stopped smoking during the study period were classified as smokers. Nonsmokers were patients who reported never having smoked.

Diabetic nephropathy was diagnosed clinically if the following criteria were fulfilled: persistent albuminuria  $>300$  mg/24-h in at least two of three consecutive 24-h urine collections, presence of diabetic retinopathy, and absence of any clinical or laboratory evidence of other kidney or renal tract disease (17). A total of 271 patients were treated with antihy-

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**Abbreviations:** GFR, glomerular filtration rate.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Table 1—Characteristics of 301 type 1 diabetic patients with diabetic nephropathy at baseline

	Nonsmokers	Ex-smokers	Smokers	P
n	94	31	176	
Sex (men/women)	53/41	24/7	115/61	NS
Height (cm)	172 ± 8	173 ± 10	173 ± 9	NS
Age (years)	35 ± 11	40 ± 11	36 ± 11	0.048
Duration of diabetes (years)	22 ± 8	25 ± 9	22 ± 8	NS
Retinopathy (simplex/proliferative)	26/68	8/23	65/111	NS
Systolic blood pressure (mmHg)	143 ± 19	145 ± 23	137 ± 17	<0.05
Diastolic blood pressure (mmHg)	87 ± 10	87 ± 7	84 ± 9	<0.05
Albuminuria (μg/min)*	714 (45–4,821)	623 (109–3,987)	618 (61–6,176)	NS
HbA <sub>1c</sub> (%)	9.5 ± 1.5	9.1 ± 1.6	9.3 ± 1.4	NS
GFR (ml · min <sup>-1</sup> · 1.73 m <sup>-2</sup> )	86 ± 30	80 ± 24	92 ± 27	<0.05

Data are n, means ± SD, for medians (range). \*Some patients with previously persistent albuminuria receiving antihypertensive treatment had baseline albuminuria <200 μg/min.

antihypertensive medication, predominantly ACE inhibitors in 179 patients. Patients were classified as taking a class of antihypertensive agent if the drug was prescribed for more than 50% of the individual follow-up time. A total of 17% of the patients received monotherapy, 47% received two agents, 30% received three agents, and 6% were treated with four or more antihypertensive drugs. During the entire follow-up period, we strived to keep blood pressure lower than 140/90 mmHg. A total of 30 patients remained normotensive (with arterial blood pressure below 140/90 mmHg) without antihypertensive treatment before and throughout the observation period. The local ethical committee approved the experimental design.

### Procedures

The measurement of GFR was performed 3–24 times (median 8) in each patient during a follow-up period of 3–14 years (median 7). GFR was measured after a single intravenous injection of 3.7 MBq <sup>51</sup>Cr-EDTA by determination of the radioactivity in venous blood samples taken 180, 200, 220, and 240 min after the injection (18). The mean variability in GFR of each patient from day to day was 4%. Results were standardized for 1.73 m<sup>2</sup> body surface, using the patients' surface area at the start of the study throughout.

All patients visited the outpatient clinic every 3–4 months during the study. At each visit to the out patient clinic, blood glucose concentration, HbA<sub>1c</sub>, albuminuria, blood pressure, and body weight was monitored, and the insulin dose and antihypertensive treatment were adjusted.

Albuminuria was measured in urine samples collected during the 4-h clearance period (7) and at each visit to the out patient clinic in 24-h urine collections an average of three to four times per year. HbA<sub>1c</sub> was measured from venous blood samples by isoelectric focusing and high-performance liquid chromatography (7). The normal range was 4.1–6.4%. Serum cholesterol was measured with standard laboratory techniques every second year. Arterial blood pressure was measured at each visit to the out patient clinic and during GFR investigation with a standard mercury sphygmomanometer and appropriately sized cuff. The measurements were performed twice, on the right arm after an at least 10-min rest in the sitting position, and averaged. Diastolic blood pressure was recorded at the disappearance of Korotkoff sounds (phase V). Arterial hypertension was diagnosed according to the World Health Organization criteria (≥160/95 mmHg) until 1995 and thereafter according to the American Diabetes Association criteria ≥140/90 mmHg (19). Retinopathy was assessed at least yearly after pupillary dilation by ophthalmoscopy; beginning in 1991, retinopathy was assessed by funduscopy and graded as nil, simplex, or proliferative diabetic retinopathy.

### Statistical analysis

Results are expressed as means and SE; means and SD are used for descriptive information. Albuminuria is given as median (range) and logarithmically transformed before analysis because of the positively skewed distribution. In each patient, all measurements performed during the entire follow-up period were used

to calculate mean values. Linear regression analysis, least-squares method, was used to determine the slope of GFR for each patient. In normally distributed variables, comparison between groups was performed by an unpaired Student's *t* test. In non-normally distributed continuous variables, Mann-Whitney *U* test was used for comparison between groups. A  $\chi^2$  test was used to compare frequencies.

A multiple linear regression analysis with backward selection was performed with decline in GFR as a dependent variable, including smoking status and all variables with *P* < 0.10 in univariate analyses. The effect of smoking on rate of decline in GFR was evaluated in a univariate ANOVA with independent progression promoters as covariates. All calculations were performed with commercially available software (SPSS version 10.0; SPSS, Chicago, IL).

**RESULTS** — The characteristics of the patients, divided according to smoking status at baseline, are shown in Table 1. The demographic data of patients in the nonsmoking, ex-smoking, and smoking groups were alike, apart from patients in the ex-smoking group being slightly older (*P* = 0.048), with a lower baseline GFR (*P* < 0.05) than current smokers. Furthermore, the level of blood pressure was significantly lower in the smoking group at baseline, as well as during follow-up, compared with the other groups (*P* < 0.05; Table 1 and 2). The median (range) tobacco consumption was 20 (3–60) cigarettes per day in the current smokers and had been 20 (3–50) cigarettes per day in the ex-smokers.

Based on level of albuminuria during

Table 2—Clinical and laboratory data during the follow-up period of 301 type 1 diabetic patients with diabetic nephropathy

	Nonsmokers	Ex-smokers	Smokers	P
n	94	31	176	
Rate of decline in GFR during entire observation period (ml · min <sup>-1</sup> · year <sup>-1</sup> )	4.5 ± 0.2	3.1 ± 0.7	3.9 ± 0.3	NS
Adjusted rate of decline in GFR during entire observation period (ml · min <sup>-1</sup> · year <sup>-1</sup> )†	4.4 ± 0.4	3.4 ± 0.6	4.0 ± 0.3	NS
Systolic blood pressure (mmHg)	144 ± 1.4	146 ± 2.7	141 ± 1.0	<0.05
Diastolic blood pressure (mmHg)	85 ± 0.7	82 ± 0.9	83 ± 0.5	<0.01
Albuminuria (μg/min)*	485 (12–2,271)	419 (11–2,099)	546 (26–5,152)	NS
HbA <sub>1c</sub> (%)	9.3 ± 0.1	9.1 ± 0.2	9.3 ± 0.1	NS
Serum cholesterol (mmol/l)	5.7 ± 0.1	5.8 ± 0.2	5.6 ± 0.1	NS
Antihypertensive treatment (nil/non ACE-I/ACE-I)	8/29/57	1/9/21	21/54/101	NS
Observation time (years)	5.6 (3.1–14.0)	7.0 (3.3–13.4)	7.6 (3.0–14.0)	NS

Data are n, means ± SE or medians (range). In each patient, all measurements performed during the entire follow-up period were used to calculate mean/median values. \*In some patients with previously persistent albuminuria receiving antihypertensive treatment, albuminuria was <200 μg/min; †adjusted for differences in other independent progression promoters (blood pressure, albuminuria, HbA<sub>1c</sub>, and serum cholesterol).

follow-up compared with baseline values, patients were divided into progressors (with an increase in albuminuria) or non-progressors. There was no difference in the prevalence of nonsmokers, ex-smokers, and smokers comparing patients who progressed versus patients who did not progress in the level of albuminuria (NS).

During the investigation period, the mean (±SE) rate of decline in GFR in all 301 patients was 4.0 ± 0.2 ml · min<sup>-1</sup> · year<sup>-1</sup>. No difference in the rate of decline in GFR was demonstrated between nonsmokers (n = 94), mean (95% CI) 4.5 ml · min<sup>-1</sup> · year<sup>-1</sup> (3.7–5.4), ex-smokers (n = 31), 3.1 ml · min<sup>-1</sup> · year<sup>-1</sup> (1.6–

4.7), and smokers (n = 176), 3.9 ml · min<sup>-1</sup> · year<sup>-1</sup> (3.3–4.5) (NS). Analyzing the impact of smoking status on rate of decline in GFR in men and women separately did not alter the results (NS).

Variables with a P value <0.10 in univariate analyses for correlation with rate of decline in GFR were examined in combination by multiple linear regression analysis. Smoking was not associated with rate of decline in GFR, whereas blood pressure, albuminuria, HbA<sub>1c</sub>, and serum cholesterol were demonstrated to be independent progression promoters ( $r^2_{adj} = 0.29$ ,  $P = 0.001$ ). The change in slope (ml · min<sup>-1</sup> · year<sup>-1</sup>) (95% CI) corresponding to changes in the independent

variables were as follows: mean arterial blood pressure (per 10 mmHg), 1.11 (0.50–1.72),  $P < 0.001$ ; albuminuria (log<sub>10</sub>), 2.39 (1.40–3.38),  $P < 0.001$ ; HbA<sub>1c</sub> (1%), 0.72 (0.34–1.10),  $P < 0.001$ ; and serum cholesterol (1 mmol/l), 0.70 (0.30–1.00),  $P = 0.001$ . For nonsmokers versus ex-smokers and smokers, the coefficient was -0.52 ml · min<sup>-1</sup> · year<sup>-1</sup> (0.36 to -1.4;  $P = 0.25$ ). Adjustment for differences in blood pressure among groups was performed in a univariate ANOVA, the rate of decline in GFR was 4.1 (0.3) ml · min<sup>-1</sup> · year<sup>-1</sup> in nonsmokers, 3.1 (0.7) ml · min<sup>-1</sup> · year<sup>-1</sup> in ex-smokers, and 4.1 (0.3) ml · min<sup>-1</sup> · year<sup>-1</sup> in the smoking group (NS) (see Fig. 1).

The rate of decline in GFR was 3.0 ml · min<sup>-1</sup> · year<sup>-1</sup> in smokers below versus 3.7 ml · min<sup>-1</sup> · year<sup>-1</sup> in smokers above the median number (n = 20) of cigarettes smoked per day ( $P = 0.3$ ). However, in heavy smokers, mean arterial blood pressure was higher (102.7 vs. 99.4 mmHg,  $P = 0.01$ ) and glycemic control was poorer (9.5 vs. 9.0%) than in the non-heavy smokers. Adjusted for differences in blood pressure and glycemic control among groups, univariate ANOVA showed a rate of decline in GFR of 3.7 (0.5) ml · min<sup>-1</sup> · year<sup>-1</sup> in smokers with tobacco consumption below the median number of cigarettes per day and 3.2 (0.4) ml · min<sup>-1</sup> · year<sup>-1</sup> in the heavy smokers ( $P = 0.44$ ). When all patients who were smokers were evaluated in a multiple linear regression analysis, the co-

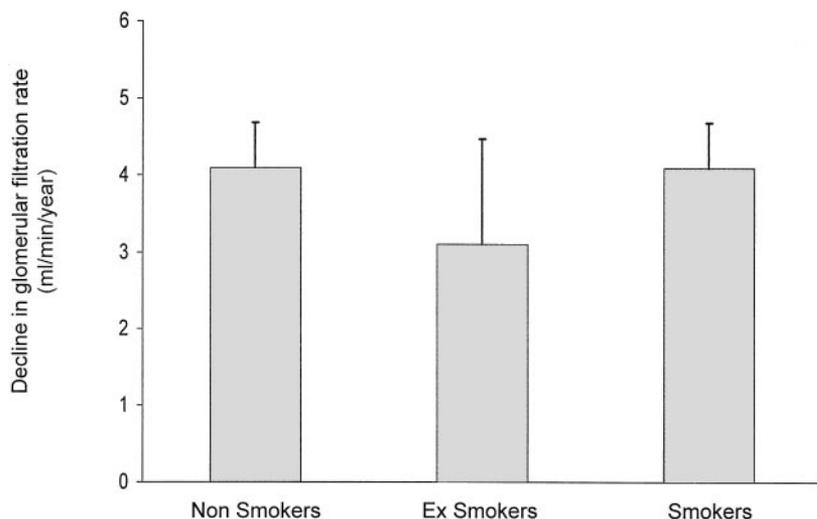


Figure 1—Impact of smoking status on decrease in GFR in 301 type 1 diabetic patients with diabetic nephropathy, adjusted for difference in blood pressure between groups, NS (ANOVA). Error bars represent 95% CIs.

efficients found for the total cohort were not altered substantially.

When the 271 patients on antihypertensive therapy were evaluated separately, smoking had no adverse effect on rate of decline in GFR when patients were stratified according to type of antihypertensive treatment (predominantly ACE inhibitors versus other blood pressure-lowering agents) (NS). Adjustment for blood pressure did not change results.

**CONCLUSIONS**— In our long-term prospective observational cohort study on progression of diabetic nephropathy in type 1 diabetic patients, we found no association between smoking status and rate of decline in GFR measured with a valid plasma clearance technique. A multiple linear regression analysis showed that blood pressure, albuminuria, HbA<sub>1c</sub>, and serum cholesterol acted as independent progression promoters, i.e., risk factors for losing filtration power. However, even after adjustment for these progression promoters, no impact of smoking status on the progression of diabetic nephropathy was demonstrated. The mean rate of decline in GFR in our study was 4.0 ml · min<sup>-1</sup> · year<sup>-1</sup>, which is considerably less than the rate of decline in GFR reported in previous studies dealing with the natural history of diabetic nephropathy: 12 ml · min<sup>-1</sup> · year<sup>-1</sup> (range 2–20) (2–4).

In 1978, Christiansen (11) from the Steno Diabetes Center was the first to report an association between cigarette smoking and proteinuria. Prospective studies have supported the concept that cigarette smoking is of importance in the development of microalbuminuria (8–10,20). Although some cross-sectional studies initially failed to demonstrate a relationship between smoking and overt diabetic nephropathy (21,22), several other cross-sectional (12–14) as well as prospective studies (15,16,20) have found an association. Therefore, the available data suggest an association between cigarette smoking and development of diabetic nephropathy.

Recently, the Reduction of Endpoints in NIDDM with the Angiotensin II Antagonist Losartan (RENAAL) study of 272 smokers and 1,241 nonsmokers with type 2 diabetes and nephropathy found a hazard ratio (95% CI) of 1.04 (0.82–1.31) of doubling of serum creatinine (personal communication, William F. Keane). The

impact of cigarette smoking on decline in kidney function in overt diabetic nephropathy in type 1 diabetes has scarcely been studied. Sawicki et al. (23) suggested that smoking worsens kidney function as measured by creatinine clearance in a 1-year study of 93 type 1 diabetic patients. However, blood pressure was higher in smokers than in nonsmokers at baseline (148/88 vs. 140/86 mmHg) as well as during follow-up (143/84 vs. 137/85 mmHg). Furthermore, albuminuria was not taken into account in the statistical analyses, despite the fact that in smokers at follow-up, the level of albuminuria was almost twice as high as in nonsmokers (2,547 vs. 1,592 mg/24-h). Because both blood pressure and albuminuria act as risk factors for losing filtration power, a considerable bias is at hand.

Another study including 16 type 1 diabetic patients with diabetic nephropathy followed for 6 years with creatinine clearance assessed twice per year showed, in patients smoking more than 10 cigarettes per day (*n* = 8), that the rate of decline in creatinine clearance was significantly higher than in nonsmokers (14.9 vs. 10.3 ml · min<sup>-1</sup> · year<sup>-1</sup>) (24). However, arterial blood pressure was significantly higher in smokers than in nonsmokers (159/89 vs. 141/82 mmHg). Unfortunately, the levels of albuminuria were not presented (24).

Mühlhauser et al. (20) performed a multicenter study including 636 type 1 diabetic patients free of severe late diabetic complications followed for 6 years. Patients were categorized by a combination of level of proteinuria measured in spot urine samples and serum creatinine in five levels, ranging from normoalbuminuria and normal serum creatinine to renal replacement therapy. Smoking was associated with progression of albuminuria in the entire study population. Only 26 patients (4%) had macroproteinuria or elevated serum creatinine at baseline, and no separate analyses on progression were performed in these patients. Consequently, this study support that cigarette smoking is associated with development of albuminuria, whereas no valid conclusion regarding worsening of kidney function can be made.

In summary, the above-mentioned studies are characterized by a small number of patients with overt diabetic nephropathy (20,23,24), short duration of follow-up (23), inadequate methods of urine collection (20), missing informa-

tion on albuminuria (23,24), and most important, inaccurate methods of estimating the decline in kidney function using serum creatinine (20,23) or creatinine clearance (23,24). To obtain valid determination of the rate of decline in GFR, the following requirements should be fulfilled: the applied GFR method should have good accuracy and precision, repeated measurements of GFR should be performed (approximately every 6–12 months), and the observation period should be extended to at least 2 years (25). These requirements have been fulfilled in our study, in which the follow-up time was 7 years (range 3–14).

Previous studies have demonstrated a dose-dependent relationship between the amount of cigarettes smoked, measured as pack-years, and development of albuminuria (20) and decline in kidney function (23). We found a weak tendency, although not statistically significant, toward faster progression of kidney disease in heavy smokers. However, well established risk factors for progression in diabetic kidney disease, i.e., elevated blood pressure and poor glycemic control, were also present in heavy smokers. In our study, smoking history was assessed using a standardized questionnaire and information from the individual patient records. In a large population-based study from Denmark applying a standardized questionnaire of cigarette consumption, 90% agreement with data on the total tobacco consumption from the Danish custom authorities was demonstrated (26). Although urinary cotinine excretion is reliable to discriminate between smokers and nonsmokers, the correlation between excretion of the metabolite and number of cigarettes consumed per day (ranging from 1 to 40) is poor (*r* = 0.15) (27). Measurement of metabolites such as cotinine was not performed in our study.

The age of the patients in the ex-smoking group were slightly higher and a correspondingly lower baseline GFR was found as compared with the current smokers and the nonsmokers. This confirms previous findings in which increasing age and duration of diabetes were associated with a decrease in the percentage of cigarette smokers (11).

In type 1 diabetes, a renoprotective effect of ACE inhibitors above and beyond the effect of lowering blood pressure has been demonstrated in clinical trials

(28,29). In an observational study of patients with nondiabetic kidney disease, the risk of developing end-stage renal disease was increased in patients on conventional antihypertensive therapy, whereas patients taking ACE inhibitors were protected (30). In our long-term prospective observational study, we could not demonstrate a difference in the rate of decline in GFR in smokers with and without ACE inhibitor therapy.

Previous studies have documented a relationship between the degree of glomerular lesions and GFR and progression of diabetic nephropathy in type 1 and type 2 diabetic patients (31–33). Furthermore, a progressive loss of intrinsic ultrafiltration capacity has been shown as the predominant cause of decreasing GFR in type 2 diabetic patients with nephropathy (34). The combined contribution from previously identified renal structural lesions and the present demonstrated progression promoters explains the vast majority of progression in diabetic nephropathy. Other factors might be of importance for the decline in kidney function, such as triglycerides or tumor necrosis factor- $\alpha$  (35). However, we have previously reported that plasma homocysteine and plasminogen activator inhibitor-1 are not independent predictors of progression in diabetic nephropathy after adjustment for other well established progression promoters (36).

Cigarette smoking is a well established hazard to health, and the relative mortality risk for diabetic patients who smoke is substantially higher than for nonsmoking patients (37,38). Furthermore, the 1-year mortality in patients who have progressed to end-stage renal disease has been demonstrated to be considerably less in ex-smokers than in smokers (39). Even in the absence of convincing data that smoking accelerates disease progression in overt diabetic kidney disease in type 1 diabetes, evidence of increased mortality in smokers as well as reduction in cardiovascular mortality in patients with diabetic nephropathy who cease smoking (39) strongly justify counseling patients to quit smoking. In conclusion, smoking was not associated with progression of kidney disease in type 1 diabetic patients suffering from diabetic nephropathy in our long-term prospective observational cohort study.

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