The course of diabetic glomerulopathy in patients with type I diabetes: A 6-year follow-up with serial biopsies

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Diabetic nephropathy is a severe complication and few studies have described the early morphological changes over time. Two kidney biopsies were performed, within a 6-year interval, in 29 primarily normoalbuminuric patients, aged 24 years at the second biopsy. These were examined with light and electron microscopy. Glomerular filtration rate, and effective renal plasma flow were determined with inulin and para-aminohippurate clearances. Urinary albumin excretion rate and the 24 ambulatory blood pressure were determined. Ten patients had developed microalbuminuria and/or hypertension; of these, six were treated with antihypertensive medication for 2 years or more. Significant increases were found in night time diastolic blood pressure and decreases in systolic and diastolic dipping. The glomerular volume, mesangial volume, mesangial matrix volume fraction and foot process width increased significantly. The group that was treated later for complications had the worst long-term metabolic control, thicker basement membranes and larger mesangial matrix and volume at the first biopsy, than the persistent normoalbuminuric group. During the follow-up, the untreated group with complications and the persistent normoalbuminuric group showed an increase in morphological parameters, whereas no progression occurred in the treated patients who also improved their metabolic control. In conclusion, the morphological parameters deteriorated in the normoalbuminuric patients and in those with complications, but were unchanged in the small antihypertensive-treated group with improved metabolic control.

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The well-known report by Kimmelstiel and Wilson in 1936 on glomerular changes in advanced diabetic nephropathy (DN) was the first description of this pathological finding. In the 1970s, Ruth Østerby described the earliest changes in the glomeruli of diabetic patients, with thickening of the basement membrane.[1] During the past 30 years, more morphological changes have been reported, including the increase in the mesangial matrix and mesangial volume fractions,[2–7] which resulted in the concept of diabetic glomerulopathy (DG). The patients with DG developing microalbuminuria (MA) may change spontaneously,[8] with better metabolic control[9,10] and/or the introduction of antihypertensive treatment (AHT).[11]

Today, DN is the most prevalent disease in Sweden and the Western world causing end-stage renal disease.[12,13] The mortality rate among patients with diabetes on renal replacement therapy is high.[12,13]

In a group of normoalbuminuric (NA) and normotensive (NT) adolescents, we studied the relationships between long-term renal function, the ambulatory blood pressure (AMBP), metabolic control and kidney morphology.[6,14,15] The cohort included 46 patients who have now been followed for about 6 years since the first biopsy; 29 have had a second biopsy. We have previously reported the 19 patients who remained normoalbuminuric and normotensive (called NANT) until the second biopsy.[16] The present report concerns the morphological and clinical changes during a 6-year follow-up period in the entire rebiopsied group.

RESULTS

Ten of the 29 (16 males) rebiopsied patients had developed hypertension (HT) and/or MA and six of them were on AHT for 2 years or more.

There was no significant difference in metabolic control in the 29 patients, as determined by the mean yearly hemoglobin A_{1c} (HbA_{1c}) and the actual HbA_{1c} at the times of the biopsies (Table 1) or urinary albumin excretion rate (UAE) between the first and second biopsy (6 vs 5.5 μg/min). The daytime blood pressure (BP) was unchanged, but significant changes in the night time diastolic BP, systolic and diastolic dipping occurred between the first and second biopsies (Table 1).

Changes in renal morphology in all rebiopsied patients

In the whole group of 29 patients, the GV in absolute terms and in relation to BSA increased significantly between the biopsies (Table 1, Figure 1a). The basement membrane thickness (BMT) showed a tendency, although not significant, to increase between the biopsies, but when related to BSA, it did not change (Table 1, Figure 1b). The V_V (matrix/glom) (mesangial matrix volume fraction) and V_V (mes/glom) (mesangial volume fraction/glomerulus) increased significantly from the first to the second biopsy (Table 1, Figure 1c and d). We found significant increases in the foot process width (Table 1, Figure 1e) while the total slit pore length was unchanged (Table 1).

Renal function

No changes occurred in the glomerular filtration rate (GFR), effective renal plasma flow (ERPF), and filtration fraction (FF) from the first to the second biopsy (Table 1). Neither did the mean previous GFR (137 (18) and 135 (17) ml min^{-1} per 1.73 m^2), the mean previous ERPF (637 (85) and 635 (94) ml min^{-1} per 1.73 m^2), or the mean previous FF (21.8 (3) and 21.7 (3)%) before the first and second biopsy, respectively). GFR and FF were significantly higher than in the controls matched for age and gender (GFR $P<0.001$ on both occasions, FF $P<0.0001$ and 0.003). Their ERPF values were similar on both biopsies and in the controls.

Thirteen patients on the first biopsy and 11 patients on second biopsy were still hyperfiltrating, although some were treated with AHT. No differences were noted in kidney function between the normoalbuminuria (NA), AHT, and MA/HT groups. The five ACE-inhibitor (ACE-I)-treated patients showed a tendency to fall in GFR from mean 152 ml min^{-1} per 1.73 m^2 on the first biopsy to mean

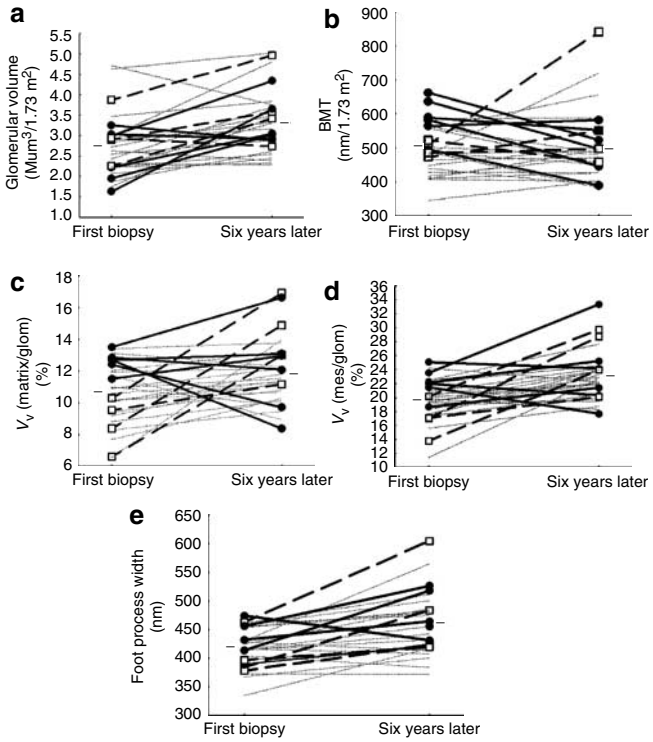

Figure 1 | (**a–e**) Glomerular volume (Mμm^3 per 1.73 m^2), basement membrane thickness (nm per 1.73 m^2), V_v (mesangial matrix volume fraction/glom) (%), V_v (mesangial volume fraction/glom) (%), and foot process width (nm) on the first and second biopsies. The gray dotted lines represent the NANT patients ($n=19$), the circles and thick lines AHT-treated patients ($n=6$). The open rectangles with the long interrupted lines represent the patients who developed MA and/or HT, but had not yet been treated ($n=4$).

Table 1 | Clinical and morphological data of the 29 type I diabetes patients who underwent two biopsies

	First biopsy, *N*=29	Second biopsy, *N*=29	*P*-value
Age (years)	18.6 (15.2–19.3)	24.0 (20.9–26.5)	
Duration (years)	10.7 (8–12.7)	17.2 (14.2–19.4)	
Mean of all yearly HbA_{1c}	7.9 (7.6–8.6)	8.0 (7.6–8.3)	NS
HbA_{1c} at biopsy	7.7 (6.9–8.2)	7.2 (6.7–8.1)	NS
GFR (ml min^{-1} per 1.73 m^2)	134 (24)	132 (24)	NS
ERPF (ml min^{-1} per 1.73 m^2)	622 (134)	649 (160)	NS
FF (%)	22.0 (2.6)	21.0 (3.3)	NS
Daytime systolic BP (mmHg)	129 (9)	126 (10)	NS
Daytime diastolic BP (mmHg)	76 (73–79)	75 (71–79)	NS
Night time systolic (mmHg)	112 (10)	113 (9)	NS
Night time diastolic (mmHg)	58 (56–62)	62 (57–66)	0.023
% Systolic dipping	14 (9–15)	10 (8–12)	0.025
% Diastolic dipping	22 (19–28)	18 (12–23)	0.006
GV (Mμm^3)	2.5 (2.2–3.1)	3.4 (3.0–3.8)	0.00005
GV (Mμm^3 per 1.73 m^2)	2.6 (2.2–3.0)	3.2 (2.8–3.7)	0.0005
BMT (nm)	481 (446–545)	515 (470–592)	0.059
BMT per 1.73 m^2 (nm)	488 (448–563)	482 (446–522)	NS
V_V (matrix/glomerulus) (%)	10.6 (1.9)	11.9 (2.0)	0.014
V_V (mes/glomerulus) (%)	19.5 (17.7–21.5)	23.0 (20.0–24.0)	0.00013
Foot process width (nm)	416 (392–455)	455 (419–484)	0.0007
Slit pore length density (μm^{-2})	0.348 (0.06)	0.287 (0.06)	0.00001
Total slit pore length (m per 1.73 m^2)	0.85 (0.75–1.09)	0.91 (0.74–1.07)	NS

Results are given as mean (s.d.) or median (lower–upper quartiles). NS=not significant.

137 ml min^{-1} per 1.73 m^2 on the second biopsy ($P = 0.15$). The change in GFR between the biopsies was -15 ml min^{-1} per 1.73 m^2 in the ACE-I group vs $+0.3$ ml min^{-1} per 1.73 m^2 in the untreated group ($P = 0.063$). FF did not change in untreated or ACE-I group.

We found positive correlations between the mean FF before the first biopsy and the BMT, V_V (matrix/glom) and V_V (mes/glom) on the first biopsy. However, on the second biopsy, we did not find any correlations between kidney function and the morphometric parameters or in the changes in morphology between the biopsies.

Differences between the NA, AHT, and MA/HT groups

The AHT group had poorer metabolic control before the first biopsy than the NANT group (Table 2). Unlike the latter group, in whom the morphological parameters became more severe, the AHT group showed no further deterioration in morphology and a decrease in HbA_{1c} (Table 2). Before the first biopsy, the MA/HT group had the same level of HbA_{1c} as the NANT group, but between the biopsies, their metabolic control became worse (Table 2).

No significant changes in AMBP occurred in the groups or between the biopsies, but the MA/HT group showed less systolic dipping than the AHT group. We found a decrease in BMT between the biopies in the AHT group and a tendency to an increase in the MA/HT group. On the first biopsy, the V_V (matrix/glom) and V_V (mes/glom) were larger in the AHT group and showed no change during the follow-up, but in the NANT and MA/HT groups, they increased. GV and foot process width increased significantly in the NANT group, but did not change significantly in the AHT or MA/HT group. The total slit pore length did, however, not differ or change between the groups and biopsies.

Single regression analyses of morphology on the second biopsy

Table 3 gives the correlations between the morphological parameters on the second biopsy or the morphological changes between the biopsies (Δ) and various clinical parameters. We found that the long-term metabolic control correlated strongly with BMT and also with V_V (matrix/glom), and V_V (mes/glom) on the second biopsy. The metabolic control between the biopsies correlated closely with the ΔBMT, ΔV_V (matrix/glom), and ΔV_V (mes/glom).

No correlations were noted between duration, gender, height, or any morphological parameters on the second biopsy, but age and the postpubertal duration correlated with the foot process width. Log UAE correlated most strongly

Table 2 | Comparisons between clinical and morphometric parameters in the groups of patients who, at the time of the second biopsy, were still normoalbuminuric and normotensive (NANT) or had received antihypertensive treatment during the follow-up because of MA or HT (AHT) or had developed complications, but had not yet been given AHT (MA/HT)

		NANT			AHT			MA and/or HT		
		n=19, 1st biopsy	*n*=19, 2nd biopsy	*P*-value	*n*=6, 1st biopsy	*n*=6, 2nd biopsy	*P*-value	*n*=4, 1st biopsy	*n*=4, 2nd biopsy	*P*-value
Duration	a	10.6	17.2		12.1	19.1		9.7	15.0	
HbA_{1c}	a	7.7	7.7		8.85	8.25	0.03	8.0	8.5	0.07
HbA_{1c} between biopsies	a		7.6			6.9			9.0	
% syst dipping	a	10.9	10.1		14.8	11.8		14	6.6	
BMT (nm)	a	480	471		585	510	0.046	497	524	
Matrix/glom	b	10.3	11.4	0.01	12.6	12.1		8.7	14	0.02
Mes/glom	b	19.2	22.3	<0.001	22.2	23.7		17.0	25.6	0.02

	Comparisons between the groups at 1st biopsy	Comparisons between the groups at 2nd biopsy	*P*-value between NANT and AHT groups at 1st biopsy	*P*-value between NANT and AHT groups at 2nd biopsy	*P*-value between NANT and MA/HTgroups at 1st biopsy	*P*-value between NANT and MA/HT groups at 2nd biopsy	*P*-value between AHT and MA/HT groups at 1st biopsy	*P*-value between AHT and MA/HT groups at 2nd biopsy
Duration								
HbA_{1c}	0.015	0.017	0.015			0.03		
HbA_{1c} between biopsies		0.009						0.0069
% syst dipping		0.054						0.048
BMT (nm)	0.007		0.005					
Matrix/glom	0.0045		0.002				0.0066	
Mes/glom	0.014						0.015	

Duration of diabetes is given in years. HbA_{1c} is the mean of all yearly HbA_{1c} until the first and second biopsy, respectively. HbA_{1c} between biopsies refers to the mean of yearly HbA_{1c} between the biopsies. The BMT have been corrected for 1.73 m^2. The matrix/glom and mes/glom refers to the volume fraction of mesangial matrix in the glomerulus, V_V (matrix/glom), and the volume fraction of the mesangium in the glomerulus, V_V (mes/glom), in %. Only significant changes are given.
a=median; b=mean.

Table 3 | Regression analyses of various morphological parameters on the second biopsy or changes between the biopsies (Δ morphology), as dependent variables

Univariate regression analyses

	Dependent variables						
Independent variables	ΔBMT	BMT	ΔV_V (matrix/glom)	V_V (matrix/glom)	ΔV_V (mes/glom)	V_V (mes/glom)	Foot process width
HbA_{1c} until 2nd biopsy		51%, $P<0.0001$, 78 nm		23%, $P=0.012$, 1%[b]		27%, $P=0.005$, 1.6%[a]	NS
HbA_{1c} between the biopsies	50%, $P<0.0001$, 59 nm		42%, $P=0.0002$, 1.4%		45%, $P=0.0001$, 2.05%[a]		
Age on 2nd biopsy		NS		NS		NS	34%, $P=0.0012$, 7.4 nm[a]
Postpubertal duration		NS		NS		NS	28%, $P=0.004$, 6.3 nm[a]
Log UAE ($n=20$)		NS		66%, $P<0.0001$, 2.9%[b]		41%, $P=0.002$, 3.9%	NS ($P=0.18$)
Daytime diastolic BP		29%, $P=0.003$, 5.8 nm[b]					14%, $P=0.049$, 3.3 nm
Night time diastolic BP		14%, $P=0.054$, 4 nm[b]					

NS=not significant.
Duration is the duration of diabetes since onset until examination, and age is given in years. HbA_{1c} until 2nd biopsy is the mean of all yearly HbA_{1c} until second biopsy. HbA_{1c} between the biopsies is the mean of yearly HbA_{1c} between the biopsies. Postpubertal duration is the duration of diabetes after puberty. ΔBMT and BMT are corrected for (in %) 1.73 m^2. Results are given in the univariate regression analyses as R^2, P-level and estimate. The estimate refers to the effect on the dependent variable if the independent variable changes one unit (e.g. if HbA_{1c} increases 1% the BMT will increase 78 nm).
[a]One patient excluded because of high Cook's distance.
[b]Two patients excluded because of high Cook's distance.

with V_V (matrix/glom) but also with V_V (mes/glom) and inversely with slit pore length density ($r^2=30\%$, $P=0.013$), but not with total slit pore length.

The daytime diastolic BP correlated directly with the BMT and foot process width.

Multiple regression analyses of morphology on the second biopsy

In the multiple regression analyses, the BMT and V_V (matrix/glom) on the second biopsy were ascribed to gender (males at risk) and long-term metabolic control (adjusted $r^2=64\%$, $P<0.0001$ and adjusted $r^2=24\%$, $P=0.01$, respectively). Furthermore, the long-term metabolic control and log UAE explained 63% of the V_V (matrix/glom) ($P<0.0001$).

DISCUSSION

Our aim in this study was to determine over time the changes in kidney morphology in a cohort of normoalbuminuric, NANT patients with type I diabetes.

One-third of these patients developed MA and/or HT after having diabetes for 17 years, and the DG changes became worse, although not all patients showed clinical signs of this. Those who developed a complication and have been given AHT and improved their metabolic control did not progress in their morphological changes.

We corrected the GV and BMT for body surface area (BSA) as we are studying growing adolescents and young adults. The large GV agrees with the findings of others,[5,17] as also does the increase in GV between the biopsies.[18,19] We found increases in both the NANT group and the entire group, although others report GV expansion only in MA patients or in relation to UAE.[3,5,7,20]

In the present study, no significant change occurred in BMT, when corrected for BSA in the whole group of patients, a finding also reported by Fioretto,[18] who studied NA and MA patients as well. In our AHT-treated group, however, we observed a decrease in BMT between the biopsies. In the untreated NANT and MA/HT group, the V_V (matrix/glom) and V_V (mes/glom) increased during the 6-year follow-up, which have been reported in follow-up studies of NA and MA patients.[18,19,21] Our AHT-treated patients showed stable V_V (matrix/glom) and V_V (mes/glom). In the ESPRIT study,[22] no changes were seen in the AHT-treated (enalapril or nifedipine) and placebo groups. In that study, all patients were micro- or macroalbuminuric and had advanced morphological changes, even at the start of treatment, which may indicate irreversible morphological changes or too short a treatment. Some believe that it may take as long for the morphological changes to resolve as they did to develop.[22] Fioretto showed a decrease in the morphological parameters only after 10 years of normoglycemia following a pancreas transplantation and, in the ESPRIT study, it was suggested that 36 months of treatment was too short to change the morphology.[22,23] However, we found a regression or stable morphological values after only 2 years of AHT treatment in combination with improved metabolic control. This would suggest that the very early DG changes are reversible. In an antihypertensive study of MA patients, no change occurred in BMT during 3 years, neither with ACE-I nor with metoprolol.[24] The authors suggest that a better metabolic control in the metoprolol-treated group was the reason for the lack of progression in that group.

The high correlations that we observed between morphology and long-term metabolic control and the correlation

between changes in morphology and metabolic control between the biopsies accord with those of other studies[19,21,25,26] and emphasize the importance of good metabolic control. The unchanged DG parameters found in the AHT group may have been either owing to the AHT or the effect of the improvement in metabolic control. We think that the information to the patients of the need of treatment (AHT) for a complication is scaring and increase the patients' awareness of the importance of a good metabolic control. This may explain the improvement in metabolic control in the AHT group.

The increase in foot process width that we found on repeated biopsies have not been reported before so far as we know. The total slit pore length, as a product of slit pore length density and GV, was unchanged in contrast to a decrease in the slit pore length density. The total slit pore length is probably a more interesting factor to analyze, reflecting the total filtration area. The glomerular expansion between the biopsies is probably caused by compensatory mechanisms or secondary to pubertal changes. Other authors have shown a decrease in filtration surface area with duration[7] or between biopsies.[18] Our first biopsy showed an increase in foot process width and a decrease in the slit pore length density with increasing UAE,[6] which have also been reported by others.[27–29] In the present study, on the second biopsy, we observed only an inverse correlation between UAE and the slit pore length density, but not with the total slit pore length.

The patients were still hyperfiltrating on the second biopsy, as compared to the controls. The GFR showed no change between the biopsies, as noted by others.[18] We found no correlation between the actual or long-term GFR and morphology on the second biopsy or the morphological changes between the biopsies. Other authors likewise have detected no correlation between GFR and morphology.[7,19,21,26,30] However, Mauer *et al.*,[31] who studied patients with severer morphological changes than our patients, reported a decrease in GFR in patients with mesangial expansion exceeding 30%. The ESPRIT study,[22] which included micro- and macroalbuminuric patients, also showed a decline in GFR with time, age, and the severity of albuminuria. Therefore, the change in GFR seems to occur only after DG has deteriorated to DN with marked mesangial expansion.[7,31] The total slit pore length and the GFR were unchanged. We found no correlation between GFR and the total slit pore length, which may be caused by the short duration of the disease, small changes in filtrations slits, and a compensatory mechanism of the glomeruli to expand. Another explanation may be that mesangial expansion in an enlarging glomerulus, as we have shown, causes less damage than if the glomerulus cannot dilate any more.[32] Therefore, the GFR of our patients may change in future.

During the follow-up, our entire cohort showed an increase in night time diastolic BP. On the first biopsy, our group has reported a correlation between the night time BP and BMT, V_V (matrix/glom), and the foot process width.[14] Moreover, the night time BP on the first biopsy predicted some morphological changes on the second biopsy in the NA group.[16] On the second biopsy, the daytime diastolic BP correlated both to BMT and foot process width. Our group has also shown that non-dippers (systolic dipping $<7\%$, diastolic dipping $<14\%$) had severer morphological changes,[15] which accords to this study where we found less dipping in the MA/HT group. In accordance with our results, Drummond *et al.*[7] noted a correlation between the daytime diastolic BP and morphology. Thus, AMBP seems more informative than casual BP to which other authors did not find any correlations to morphology.[3,5,19,21,25,28,33]

In conclusion, the DG changes increased in NA patients and MA/HT group. However, the small group with better metabolic control and on AHT treatment showed no further advance of the DG, which indicates the importance of good metabolic control and strict BP surveillance and perhaps AHT early in the diabetic course.

MATERIALS AND METHODS

Patients

All patients with type I diabetes over 12 years of age, who had had diabetes for more than 5 years and attended the Children's Hospital at Karolinska University Hospital, Huddinge, were asked at the beginning of 1992 to take part in a kidney biopsy study in connection with their regular kidney function investigation. Forty-six unselected normoalbuminuric and normotensive patients had the first kidney biopsy carried out between 1992 and 1994, at a median age of 18 years. Six years later, they were asked to undergo a second kidney biopsy, 29 patients agreed and 17 declined. There were no differences in the clinical findings, renal function, or morphological parameters on the first biopsy in the patients, who underwent a second biopsy, as compared with those who did not.

Of the 29 patients, aged 24 (21–26) years, who had had a second biopsy 17 (14–19) years after the onset of diabetes, 19 were still normoalbuminuric and normotensive,[16] but 10 had developed HT (8 patients) and/or MA (4 patients). Seven patients had been treated during some part of this period, but one for less than a year and he is therefore regarded as an untreated patient with complications. Five of the treated patients received ACE-inhibitors because of MA (two patients) and HT (three patients) and one a β-blocker because of HT.

At the time of the second biopsy, they were classified as patients who (a) were normoalbuminuric and normotensive (called NANT), (b) developed complications and were on AHT during at least 2 years, or (c) developed MA and/or HT during the follow-up, but had not started treatment at the time of the second biopsy (MA/HT).

The investigations were performed over a 3-day period as described in the previous paper.[16]

The study was approved by the Ethics Committee of Karolinska Institutet and carried out after informed consent had been obtained from the patients.

Clinical follow-up

After the first biopsy, patients were examined clinically every third month in the Children's Hospital or, after the age of 18–20 years, in the Adult Diabetic Out-patient Clinic at Karolinska University Hospital, Huddinge. At every check-up, the HbA_{1c} and BP were

measured, and at least once or twice yearly, the UAE. The diagnosis of HT was made either by casual measurements or via AMBP measurements. The patients who had casual HT (>140/80–90 mmHg) on several occasions were classified as hypertensive. If they had a single measurement of casual HT, they underwent AMBP. If mean daytime or night time systolic and/or diastolic BP > 95th percentile for a given height and gender,[34] he was classified as hypertensive. Eight patients were classified as hypertensive.

Four patients developed persistent MA, defined as UAE $>20\,\mu g\,min^{-1}$ in two of three urine samples during a 6-month period.

Metabolic control

Metabolic control was evaluated every third month as HbA_1 or HbA_{1c}. The HbA_1 values were converted to HbA_{1c}.[16] The normal reference values of HbA_{1c} are 3.4–5.0%, which are about 0.7–1.0% lower than those in the Diabetes Control and Complication Trial study.[35,36] The long-term metabolic control was determined by the mean of all yearly HbA_{1c}.[16] The metabolic control between the biopsies was calculated as the mean of yearly HbA_{1c} between the first and second biopsy.

Ambulatory BP

The 24-h AMBP was recorded with portable automatic Space Labs 90 207 equipment (Space Labs Inc., Wokingham, UK), as described elsewhere.[14,15,34] AMBP readings were successful in a median of 90% at the first biopsy and 94% at the second biopsy.

No AMBP was carried out in two patients at the first biopsy. The percentage of the night time fall in BP ('dipping') was calculated as: (daytime BP−night time BP) × 100/daytime BP.[37,38] Soergel *et al.*[38] found 13 (6)% systolic and 23 (9)% diastolic dipping in controls.

Urine samples

UAE was determined in timed overnight urine collections with an automated immunonephelometric method (Behring Nephelometer Analyser; Behringwerken, Marburg, Germany) on the first biopsy, and an immunoturbidometric method (Roche Regencies and Hitachi equipment 917; Roche, Boston, MA) during the follow-up and on the second biopsy. Both methods were calibrated to show the same values. The detection limit for the albumin concentration was $4\,mg\,l^{-1}$.

On the first biopsy, no urine samples were available in four patients and six had urinary albumin concentrations below the detection level; on the second biopsy, 10 patients had values below the detection level.

Puberty status

The median age at the onset of diabetes was 7.2 (3.7–10.5) years and all our patients were regarded as having had their onset before puberty. As accurate data based on Tanner's values were not available in all cases, we arbitrarily chose the age of 11 years in female and of 12 years in male patients for the start of puberty.[39,40]

Renal function tests

Renal function was determined every second or third year from the onset of diabetes and mean 3.5 and 5.5 renal function tests were performed before first and second biopsy, respectively. The means of all previous renal hemodynamic data were calculated for each patient before the first and second biopsy.

GFR and ERPF were determined by clearances of inulin and para-aminohippuric acid as described previously.[16] In the statistical analyses, various measurements of GFR have been used, for example, the GFR at the first and second biopsy, the difference between the GFR at the biopsies and the mean of previous GFR before the first and second biopsy. We compared the patients' renal function with that of 58 healthy children and young adults (presumptive kidney donors), who had been matched for age and gender, evaluated in our unit. The controls had GFR 116 (11) $ml\,min^{-1}$ per $1.73\,m^2$ and 115 (13) $ml\,min^{-1}$ per $1.73\,m^2$, ERPF 640 (99) $ml\,min^{-1}$ per $1.73\,m^2$ and 628 (113) $ml\,min^{-1}$ per $1.73\,m^2$, and FF 18.4 (2.5)% and 18.7 (2.8)% at ages corresponding to first and second biopsy (median 18.5 and 24.0 years of age, respectively). Hyperfiltration was defined as a GFR > 2 s.d. of the controls.

Renal biopsy

The biopsies were taken under local anesthesia with ultrasound guidance (Acuson XP 10 and Acuson Sequoia 512, Mountain View, CA), an automatic biopsy device (Bard Magnum Biopsy Instrument; Urological, Covington, CA) and a 16-G needle (Bard Magnum Core Tissue Biopsy Needle; Urological) as described in the previous paper.[16] Samples for light and electron microscopy were postfixed in 2% glutaraldehyde and 4% paraformaldehyde in phosphate buffer and embedded in Polybed 812.[16] Every biopsy was examined by a single nephropathologist (GAJ) without the knowledge of the patient's history.

The morphometric analyses in the light and electron microscopy (Philips 420, Eindhoven, The Netherlands) were carried out in the same way as described previously.[16] The sampling of the glomerular profiles taken for ultra-thin sectioning was carried out and corrected by a grating grid (EF Fullan Inc.; 28 800 lines $inch^{-1}$), as described elsewhere.[6] In the grid, the surface density of the peripheral capillary walls, S_V (pcap/glom), was calculated using the intersections (I) with the formula:

$$S_V\,(\text{pcap/glom}) = 2\times I/(P\times(2d/\text{mag}))\ (\mu m^{-1})$$

and the length density of filtration slits (L_V (slit pore/glom)) using the number of their related filtration slits (Q) was estimated with the formula:

$$L_V\,(\text{slit pore/glom}) = 2\times Q/(P\times(d^2/\text{mag}^2))\ (\mu m^{-2})$$

In the formulas above, P is the total points in the reference space, d is the distance between each point of the grid and mag is the final magnification.

The total slit pore length per mean glomerular volume (GV) (m) was estimated by multiplying mean GV ($M\mu m^3$) and the slit pore length density (L_V (slit pore/glom) (μm^{-2}).

We analyzed the absolute values of GV and BMT and the $1.73\,m^2$ BSA-corrected values, as we were studying growing adolescents. In Table 1 and in the first paragraph on changes in renal morphology, we report the corrected and uncorrected values, but in the following text and in Tables 2 and 3, the GV and BMT values have been corrected for BSA.

Statistical analyses

The mean and s.d. are given for normally distributed values and median and lower and upper quartiles for skewed data. The Student's paired t-test was used for normally distributed variables. The Mann–Whitney U-test and Wilcoxon's matched-pair test were

used for continuous data not normally distributed. When comparing the groups, one-way analysis of variance (ANOVA) was used, followed by *post hoc t*-tests with the Bonferroni adjustment. For variables having a skewed distribution, we used Kruskal–Wallis ANOVA by ranks, with Bonferroni adjustment.

Univariate and multiple regression analyses were employed to evaluate the correlations between the clinical and morphological findings. Values were excluded if the analyses of residuals and Cook's distance showed an outlier. Not more than two factors entered in the multiple regression analyses owing to the small sample size. $P < 0.05$ was considered significant, but in the multiple regression analyses, a P-value < 0.01 was considered significant in order to approximately maintain an overall 5% level. The statistical program of Statistica 6.1 was used.

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