Diabetologia (2002) 45: 253–261

Diabetologia

Kidney function and glomerulopathy over 8 years in young patients with Type I (insulin-dependent) diabetes mellitus and microalbuminuria

H-J Bangstad[1], R. Østerby[2], S. Rudberg[3], A. Hartmann[4], K. Brabrand[5], K. F. Hanssen[6]

[1] Department of Paediatrics, Ullevål University Hospital, Oslo, Norway
[2] Electron Microscopy Laboratory, Institute of Experimental Clinical Research and Institute of Pathology, Aarhus Kommunehospital, Denmark
[3] The Department of Woman and Child Health, Paediatric Unit, Karolinska Institute, Stockholm, Sweden
[4] Section of Nephrology, Department of Medicine, Rikshospitalet University Hospital, Oslo, Norway
[5] Department of Radiology, Rikshospitalet University Hospital, Oslo, Norway
[6] Department of Internal Medicine and The Diabetes Research Centre, Aker and Ullevål University Hospitals, Oslo, Norway

Abstract

Aims/hypothesis. We aimed to investigate prospectively the interrelation between kidney function and glomerular morphological changes over 8 years in young patients with Type I (insulin-dependent) diabetes mellitus and microalbuminuria.
Methods. Kidney biopsies were taken at baseline and after 8 years in 18 subjects who were 20 years of age (19–29 mean and range), had duration of diabetes for 11 years (7–18), and who had an albumin excretion rate of 45 μg/min (15–194). The glomerular ultrastructural parameters were analysed using stereological methods.
Results. At the end of the study three patients had an increased albumin excretion rate of more than 25 % a year, two of whom developed overt nephropathy. Glomerular filtration rate declined 2.3 ml/min · 1.73 $m^{-2} \cdot yr^{-1}$. Glomerular volume, volume fractions of matrix and mesangium, and basement membrane thickness showed an increase over the 8 years. Multiple regression analysis showed that mean 8-years HbA_{1c}, matrix volume $fraction_{baseline}$ and basement membrane thickness $BMT_{baseline}$ accounted for 70 % of the variation in AER at the end of the study. Mesangial volume $fraction_{baseline,}$ glomerular filtration $fraction_{baseline,}$ and mean 8-year HbA_{1c} accounted for 73 % of the change in glomerular filtration rate from baseline. Smoking was strongly associated with the glomerular filtration rate at baseline ($r = 0.65$). When glomerular filtration $rate_{baseline}$ was omitted from the equation, smoking was the only significant parameter linked to the change in glomerular filtration rate from the baseline.
Conclusion/interpretation. In patients who had diabetes for 20 years, long-term hyperglycaemia and glomerulopathy found 8 years prior to the study, and possibly smoking, affected renal function (i.e. albumin excretion rate and glomerular filtration rate). [Diabetologia (2002) 45: 253–261]

Keywords Diabetic glomerulopathy, basement membrane thickness, mesangial expansion, microalbuminuria, insulin-dependent, hyperglycaemia, stereology.

Since the term microalbuminuria was coined and was shown to predict clinical nephropathy in Type-I (insulin-dependent) diabetes mellitus in the early 1980s [1–3], the research has focused on the putative risk factors in the early stages of nephropathy and their relative importance for the decline in renal function. The present knowledge of these factors and the development of the ultrastructural changes in the glomeruli, i.e. basement membrane thickening and mesangial expansion, are scarce and primarily based on cross-sectional studies [4–8]. Long-term studies on homogeneous groups of Type I diabetic patients have not been carried out.

Hyperglycaemia has been shown to be associated with the degree of glomerulopathy in incipient nephr-

Received: 16 July 2001 and in revised form: 18 October 2001

Corresponding author: H-J Bangstad, M.D., Department of Paediatrics, Ullevål University Hospital, 0407 Oslo, Norway, e-mail: h.j.bangstad@ioks.uio.no
Abbreviations: BMT, Basement membrane thickness; Vv(mes/glom), mesangial volume fraction per glomerulus; Vv(mat/glom), matrix volume fraction per glomerulus; GV, glomerular volume; ERPF, effective renal plasma flow; FF, filtration fraction; CSII, continuous subcutaneous insulin infusion; CT, conventional treatment

opathy [5, 9, 10]. Improved metabolic control by either intensive treatment [11] or by pancreas transplantation [12] has been shown to arrest and even improve the glomerular changes in native kidneys.

Several additional factors like glomerular hyperfiltration [13], high blood pressure [14], smoking [15] and genetic predisposition [16–17] are probably coplayers in the development of diabetic nephropathy but their role in the more short-term development of the specific glomerulopathy is not clear.

The aim of our study was to investigate prospectively with sequential biopsies the relation between renal function and glomerular ultra-structural changes over 8 years in a well-characterized group of young patients with Type I diabetes and microalbuminuria.

Subjects and methods

Patients. A total of 18 patients were studied prospectively for 8 years (7.8–8.5). Patients were initially enrolled in an intervention study (continuous subcutaneous insulin infusion, CSII vs. conventional treatment, CT) for 2.5 years (2.2–2.8) in patients with microalbuminuria and poor blood glucose control. The details from this study have been presented [5, 11]. When the intervention study ended, two patients in the CSII-group did not adhere to the pump treatment modality, whereas one in the CT-group started with insulin pump. The patients were followed during the follow-up study by the same investigator (H-J Bangstad) at the outpatient clinic at 3 to 4 month intervals. Kidney biopsies and renal function tests were done at baseline [GFR and effective renal plasma flow (ERPF)], after 2.2 to 2.8 years (GFR and ERPF), and at 7.8 to 8.5 years (GFR).

All study patients accepted the final biopsy but two patients had to withdraw because of pregnancy. However, another two patients who dropped out of the intervention study before randomisation but after having kidney biopsies taken, were included in the 8-year follow-up study, giving a total of 18 patients. The two patients were followed in a clinical setting with the same regularity in the measurements of HbA_{1c} and urinary AER as the rest of the participants, blood pressures were obtained only in connection with the kidney biopsies. The Regional Ethics Committee approved the protocol and patients gave their written informed consent. No major complications were observed except for two incidences of macroscopic haematuria.

At baseline the mean age (and range) of the patients was 20.1 years (18–29) and the duration of diabetes was 11.3 years (7–18). The clinical characteristics of the subjects are shown in Table 1. One had transient hypertension, i. e. normalized blood pressure after 6 months without antihypertensive treatment. However, from 6 years after baseline the patient was treated with ACE-inhibitor because of hypertension (> 140/90 mmHg). At baseline none of the subjects had proliferative retinopathy. Nine subjects smoked and the number of cigarettes smoked per day was noted yearly according to the interview.

Urine samples. The albumin concentration was measured by immunoturbidimetry in timed overnight samples. The interassay coefficient of variation was 4.7% in the range of 10 to 50 mg/l. The individual AER values presented are the mean of three measurements.

Table 1. Clinical data and structural parameters at baseline and end of an 8-year study in 18 young subjects with Type I diabetes and microalbuminuria at entry

	Baseline	End of study
HbA_{1c}(%)	10.1 (9.5–10.8)	9.4 (8.7–10.1)
AER (μg/min)[a]	32 (25–44)	18 (13–27)
Systolic BP (mm Hg)	126 (120–132)	131 (125–137)
Diastolic BP (mm Hg)	81 (76–85)	79 (75–82)
GFR (ml/min1 per 1.73 m^2)	143 (130–156)	125 (114–135)[b]
ERPF (ml/min per 1.73 m^2)	679 (609–748)	
FF	0.22 (0.19–0.26)	

[a] Median
[b] $p < 0.05$
Mean and 95% CI

Blood glucose control. During the first 3 years HbA_{1c} was analysed by high-performance liquid chromatography (HPLC) (Diamat analyser, Biorad, Richmond, Calif., USA). The normal range was 4.3 to 6.1%, with an interassay CV of 3%. For the rest of the study period an immunological method, DCA (Bayer, Leverkusen, Germany) was used. The normal range was 4.1 to 5.9% with an interassay CV of 3.4%. The DCA values were transformed to values corresponding to the HPLC method (multiplied by 1.04).

Blood pressure. Conventional mercury sphygmomanometer was used with patients sitting after a 10-min rest.

Renal function tests. Glomerular filtration rate was measured as inulin-clearance after oral water loading. High concentrations of interfering glucose were removed by glucose oxidase. At the end of the study I^{125} iothalamate clearance was applied. Effective renal plasma flow was calculated as renal clearance of para-amino-hippuran at a steady state concentration of 20 to 40 mg/ml. Filtration fraction was calculated as GFR/ERPF.

Renal biopsies. Percutaneous renal core biopsies at baseline were taken using an 18 gauge needle as described previously [5, 11]. The entire kidney biopsy was immediately fixed in a 2% glutaraldehyde solution in Tyrode buffer and mailed in the fixative to Aarhus. The core was subdivided into smaller blocks, which were dehydrated and embedded into vestopal.

At baseline each block of tissue was serially cut into 1μm thick sections, which were placed on slides and stained with toluidin blue. Glomeruli sampled for electron microscopy and for volume measurements were new appearing corpuscles in the block. Thin sections for electron microscopy were cut at 60 μm intervals and multiples thereof from the first baseline section in the block providing three sections through each glomerulus separated by 60 μm, with an independent random position within each glomerulus. Thin sections were obtained in three glomeruli, representing at least two different blocks in each biopsy, so that nine total cross sections were produced for measurements at low magnification. Glomerular volume was estimated applying Cavalieri's method using sections at 10 μm intervals [18].

At low electron microscopy magnification (~ 2300 ·) the total cross section was photographed with overlapping fields which were then pasted together to produce photomontages. The protocol for sampling and printing at higher magnification was repeated for all of the baseline biopsies with the new computer-based system as described below. Thus all of the measurements at higher magnification were done in baseline biopsies and the third biopsy at the same time and with exactly the same procedures.

The kidney biopsy at the end of the study was immediately fixed in a 2% glutaraldehyde solution and mailed in the fixative to Aarhus, cut into smaller blocks, dehydrated and embedded into epon (since vestopal was no longer available at this time).

At the end of the study each of the blocks were serially sectioned as described above. However, in this series the interval between the sections sampled for electron microscopy was 50 μm. As in the baseline biopsies three sections were obtained in each of three glomeruli sampled, representing at least two different blocks.

At the end of the study all images were digitised at the Philips CM10 microscope equipped with the SIS-system (Analy-SIS 3.0, Soft Imaging System, Hammer strasse 89, Münster, Germany). All recorded fields were saved at an optical disk, which was later taken to the office desk computer for the measurements. At low magnification (final magnification on the screen ~ 4900 ×), systematic sampling of the glomerular cross sections was done, covering about 83% of the total area. At higher magnification (corresponding to 12500 × on the computer screen) systematic independent sampling of the largest cross section in each glomerulus was done, providing a sampling fraction of about 64%. These recordings at higher magnification were repeated on the previously prepared thin sections from the baseline biopsies.

For measurements at low magnification volume fractions were obtained by standard point counting. On the photomontages a 1:8 grid was used counting coarse points hitting the glomerular polygon [19] and the glomerular tuft, i.e. all structures inside the epithelial covering. Fine points were counted hitting mesangial regions. Corresponding point counting using a 1:9 grid was done on the computer screen for the digital images.

For measurements at high magnification basement membrane thickness was estimated with the orthogonal intercept method [20]. In the computer system intercepts were directly recorded, without subdivision into classes. From the total number of intercepts the harmonic mean thickness was calculated. Volume fractions were obtained by point counting using a 1:2:4 grid counting coarse points on glomerular tuft (defined similarly as by low magnification), medium points counted on mesangial space and fine points on mesangial matrix and peripheral basement membrane. The matrix star volume was estimated from random intercepts as described [6]

Mesangial volume fraction relative to the polygon space was measured from the set of nine photomontages in the baseline biopsies and in the last follow-up biopsy on the low magnification images from nine cross sections in each biopsy (measured area corresponding to 83% of the total). The volume fraction matrix/mesangium was estimated on the high magnification-images. Combined with the measurement of V_V(mes/glom) the estimate of V_V(mat/glom) was obtained. All volume fractions were estimated by the sum of points on the individual compartments divided by all points on the reference space for each biopsy. Likewise, basement membrane thickness and matrix star volume were estimated from the total number of intercepts measured in each biopsy.

Comparisons. All of the data at high magnification had been obtained previously on printed micrographs and a comparison could then be made between previous and recent data. Apart from using a different technique a different observer was involved in some of the measurements (BMT). A highly significant difference obtained in paired comparisons of old compared with new measurements on photographic prints versus on the computer screen, would indicate the need to redo all the baseline biopsies. However, a very strong correlation was obtained in the whole group between first and second measurements with p values of less than 10^{-4}.

Statistical analysis. Simple paired t-test was used when appropriate. Linear correlations were tested by least square regression to the mean. Stepwise multiple regression analyses were done to evaluate the independent influence of risk factors (mean 8-years HbA_{1c}, blood pressure, smoking during the study, baseline glomerular filtration rate, AER and morphological parameters) on renal function and morphological parameters respectively at the end of the study. Randomisation was resolved after the intervention study and the patients were treated as one group in the correlation analyses in this study. We found this acceptable since the idea of the intervention study was to test the effect of hyperglycaemia and not the efficacy of insulin pumps. However, we also analysed the data by Student's t test according to the intention to treat principle, i.e. those randomised to conventional treatment versus those on insulin pumps. Data are given as means (95% CI) with exceptions given in the text. AER values were logarithmically transformed before statistical analysis. Statistical significance was accepted with p values of less than 0.05. The statistical program of NCSS (Number Cruncher Statistical System, Kaysville, Utah, USA) version 2000 was used.

Results

Clinical data. HbA_{1c}, blood pressure and AER were not significantly changed after 8 years (Table 1). At the end of the study five of the patients had normal AER. Three patients increased their AER for more than 25% a year, two of whom developed macroalbuminuria (AER > 200 μg /min) (Fig. 1). No relation was found between mean 8-year HbA_{1c} and delta AER or blood pressure.

GFR decreased significantly during the study (Table 1) and the decline in GFR per year was 2.3 (4.5 – –0.1) ml/min per 1.73 m^2. Baseline GFR was highly correlated with smoking (number of cigarettes, $r = 0.65$ $p < 0.01$). GFR at the end of the study was negatively correlated with mean 8-year HbA_{1c} (Fig. 2).

Delta GFR was negatively correlated with HbA_{1c} ($r = –0.49$, $p < 0.05$, i.e. high HbA_{1c} = large decline in GFR), baseline GFR ($r = –0.82$, $p < 0.001$) (Fig 3), and FF at baseline ($r = –0.75$, $p < 0001$), but not with delta AER or delta BP.

Structure. All of the glomerulopathy parameters, the matrix/glomerular volume fraction [Vv(mat/glom)], the mesangial/glomerular volume fraction [Vv(mes/glom)], and the basement membrane thickness (BMT) showed a highly significant increment during the 8 years (Table 2) as did the glomerular volume (GV).

The associations between the change in matrix star volume and the change in Vv(mes/glom), $r = 0.52$ ($p = 0.03$), Vv(mat/glom), $r = 0.50$ ($p = 0.03$) and BMT, $r = 0.52$ ($p = 0.03$) were rather consistent. The associations between change in BMT and change in

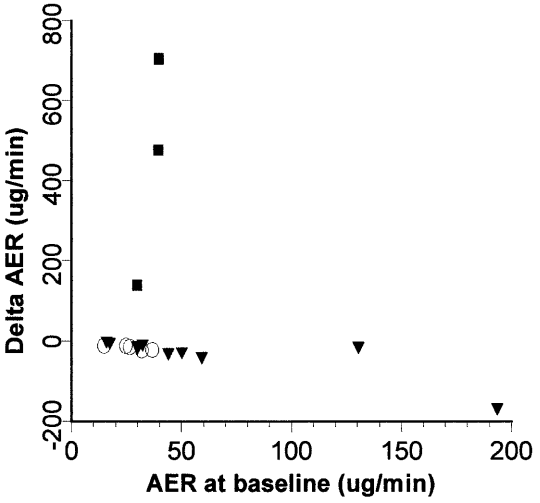

Fig. 1. Change in AER from baseline. Three subjects with AER-increment > 25 % per year (■), 10 subjects with no significant increment of AER during the study (▼), and five subjects with normoalbuminuria at the end of the study (○)

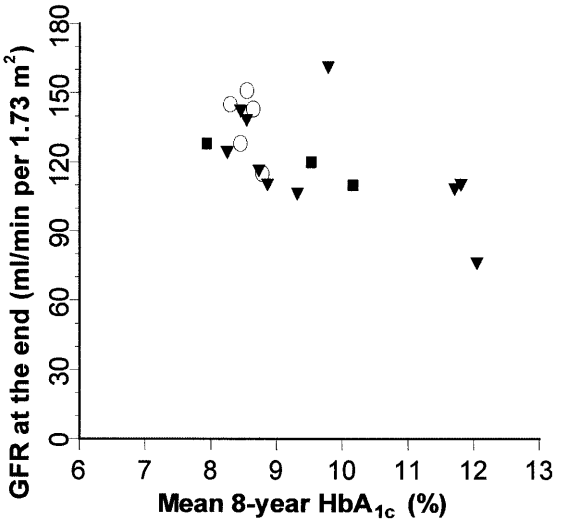

Fig. 2. GFR at the end of the study vs mean 8-year HbA_{1c}, $r = -0.61$, $p = 0.01$. Three subjects with AER-increment > 25 % per year (■), 10 subjects with no significant increment of AER during the study (▼), and five subjects with normoalbuminuria at the end of the study (○)

Vv(mat/glom), $r = 0.43$ ($p = 0.08$) and Vv(mat/glom), $r = 0.39$ ($p = 0.10$) were positive, but not significant.

Structure and clinical data. The increment in the glomerulopathy parameters showed a positive correlation with mean 8-year HbA_{1c} during the study (Table 3), but the relation was significant only for the Vv(mes/glom), (Fig. 3). In addition the increment in BMT was negatively correlated with GFR at the end, $r = -0.52$, $p < 0.05$.

The change in AER during the study was positively correlated with $Vv(mat/glom)_{baseline}$ ($r = 0.52$, $p < 0.05$), $Vv(mes/glom)_{baseline}$ ($r = 0.50$, $p < 0.05$), $BMT_{baseline}$ ($r = 0.49$, $p < 0.05$) and delta glomerular volume ($r = 0.64$, $p < 0.01$) but these results were primarily the consequence of the patients who increased their AER > 25 % per year (Fig. 4). AER at the end of the study was positively correlated with glomerular $volume_{end}$ ($r = 0.72$, $p < 0.001$), BMT_{end} ($r = 0.62$, $p < 0.01$), $Vv(mat/glom)_{end}$ ($r = 0.54$, $p < 0.05$) and delta glomerular volume ($r = 0.80$, $p < 0.0001$).

The influence of mean 8-years HbA_{1c}, baseline GFR, smoking and mean systolic and diastolic blood pressure on changes in morphological parameters and on current morphological parameters at the end of the study were analysed by stepwise multiple regression. It was found that only HbA_{1c} contributed significantly, i.e. to delta Vv(mes/glom), $T = 2.8$, $p < 0.01$.

When AER at the end of the study was applied as the dependent variable, mean 8-year HbA_{1c}, $Vv(mat/glom_{baseline})$ and $BMT_{baseline}$ contributed significantly to the model (Table 4), whereas smoking, duration, mean 8-years systolic and diastolic BP, $AER_{baseline}$ and $GFR_{baseline}$ did not. When the change in AER was used as the dependent variable, only

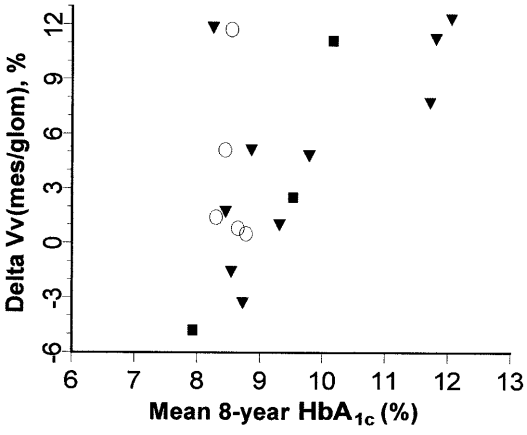

Fig. 3. Absolute change in Vv(mes/glom) vs mean 8-year HbA_{1c}, $r = 0.57$, $p = 0.01$. Three subjects with AER-increment > 25 % per year (■), 10 subjects with no significant increment of AER during the study (▼), and five subjects with normoalbuminuria at the end of the study (○)

$BMT_{baseline}$ contributed significantly ($T = 2$, 4, $p = 0.02$).

With GFR at the end of the study as the dependent variable, only mean 8-year HbA_{1c} contributed (negatively) significantly ($T = -3.3$, $p < 0.01$), whereas smoking, duration, $BMT_{baseline}$, $Vv(mat/glom)_{baseline}$, $Vv(mes/glom)_{baseline}$, AER, mean systolic BP and filtration fraction did not. For decline in GFR mean HbA_{1c} ($T = -2.8$, $p = 0.01$) and $GFR_{baseline}$ ($T = -6.2$, $p = 0.0001$), were significant. When $GFR_{baseline}$ was excluded from the analysis mean 8-year HbA_{1c} ($T = -2.6$, $p < 0.05$) and filtration fraction ($T = -3.4$, $p < 0.01$) added to the model ($r^2 = 0.68$). However

Table 2. Ultrastructural kidney parameters in 18 patients with Type I diabetes. Baseline and end of study (93–102 months)

Subjects *n*	Basement membrane thickness (nm)		Matrix/glomerular volume fraction (%)		Mesangial/glomerular volume fraction (%)		Matrix star volume (μm^3)		Glomerular volume ($10^6 \mu m^3$)	
	Baseline	End	Baseline	End	Baseline	End	Baseline	End	Baseline	End
1	324	389	12.7	16.8	19.8	27.5	24.0	35.1	4.87	4.91
2	536	594	11.4	8.9	18.3	15.1	18.3	3 3.9	2.13	2.36
3	449	402	9.3	12.5	18.4	23.5	24.2	28.2	2.21	2.28
4	476	597	11.4	16.0	16.6	28.5	21.0	81.2	2.91	3.45
5	548	577	10.1	13.1	19.3	24.2	22.7	59.5	2.37	3.42
6	647	777	13.8	21.8	20.6	31.7	24.5	50.3	3.19	4.30
7	471	672	10.0	16.1	20.3	31.6	29.9	86.4	3.03	3.61
8	522	695	10.5	16.0	17.1	29.4	22.3	67.5	1.96	2.01
9	505	564	8.4	12.2	16.4	17.8	18.5	27.9	2.44	2.81
10	551	494	10.1	12.6	21.0	19.5	22.9	20.3	2.55	2.60
11	467	606	10.0	12.6	22.9	23.7	10.8	28.6	2.56	3.12
12	444	553	10.5	12.4	20.0	20.5	35.0	62.9	3.41	3.40
13	606	747	14.3	17.6	31.4	26.6	36.9	33.8	3.75	
14	468	567	9.9	9.7	19.3	21.8	20.0	31.6	2.92	4.46
15	408	572	12.3	12.1	21.6	22.7	19.1	25.9	2.21	2.51
16	573	796	13.1	19.3	25.5	30.7	41.7	131.0	1.75	2.89
17	461	701	7.3	13.2	15.1	27.5	13.1	24.1	2.89	3.50
18	505	519	10.9	10.2	21.6	23.3	24.4	27.3	1.97	3.00
Mean	49 8	601	10.9	14.5	20.2	24.8	23.9	47.5	2.69	3.35
95%-	461-	544-	10.0-	12.9-	18.4-	22.4-	19.9-	33.0-	2.29-	2.87-
CI	535	658	11.8	16.2	22.1	27.1	27.8	62.0	3.05	3.83
Baseline vs end, p =	0.003		0.0002		0.004		0.01		0.02	

Table 3. The relation (linear regression) between long-term hyperglycaemia (mean 8-year HbA_{1c}) and changes (Δ) in the glomerulus over 8 years in 18 young patients with Type I diabetes

		ΔBMT	Δmatrix/glomerular volume fraction	Δmesangial/glomerular volume fraction	Δmatrix star volume
Mean 8-year HbA_{1c}	*r*-value	0.38	0.33	0.57	0.09
	p-value	0.12	0.18	0.01	0.71

when both GFR and filtration fraction at baseline were removed from the equation, smoking was the only parameter of significance (T = –2.8, $p = 0.01$).

Intention to treat. The group which started with intensive treatment, ended up with a mean 8-year HbA_{1c} of 8.7 (8.1–9–2)%. This was not statistically different from those patients initially on conventional treatment and who had a mean HbA_{1c} of 9.7 (8.6–10.9) %, $p = 0.07$. There were no statistically significant differences in the development of the morphological changes in the two groups although there was a trend of a more pronounced mesangial expansion in the conventionally treated group. No differences were observed concerning change in AER, BP or GFR (data not shown).

Smokers. Nine of the 18 patients were smokers and they did not change their habit during the study. The mean number of cigarettes was 20.4 a day. The smokers were compared with the non-smokers. At baseline there were differences in AER, BP and morphological parameters (data not shown) between smokers and non-smokers but smokers had higher GFR [156 (137–175) vs 130 (114–146) ml/min per 1.73 m^2, $p < 0.05$]. During the study the structural changes tended to be more pronounced in the smokers, although not statistically significant. The smokers showed an increment in AER during the study which was significantly higher than in non-smokers, 134 (–73 – 342) vs –31 (–70 – 8) μg/min, $p = 0.04$. No significant differences were seen with respect to change in BP and GFR [–4.1 (–6.8 – –1.4) ml/min per 1.73m^2/year] vs [0.61 (–4.0–2.9) ml/min per 1.73m^2/year, $p = 0.08$] and mean HbA_{1c} 9.5 % (8.3 – 10.7) vs 9.2 % (8.4 –9.9).

Progresssors. Three of the patients had an increment ('progressors') in AER during the 8-year study. Their baseline data were compared with the five subjects who were normoalbuminuric at the end of the study and the rest of the series ($n = 10$) who did not have an increase in AER. No significant differences were found between the three groups but the progressors

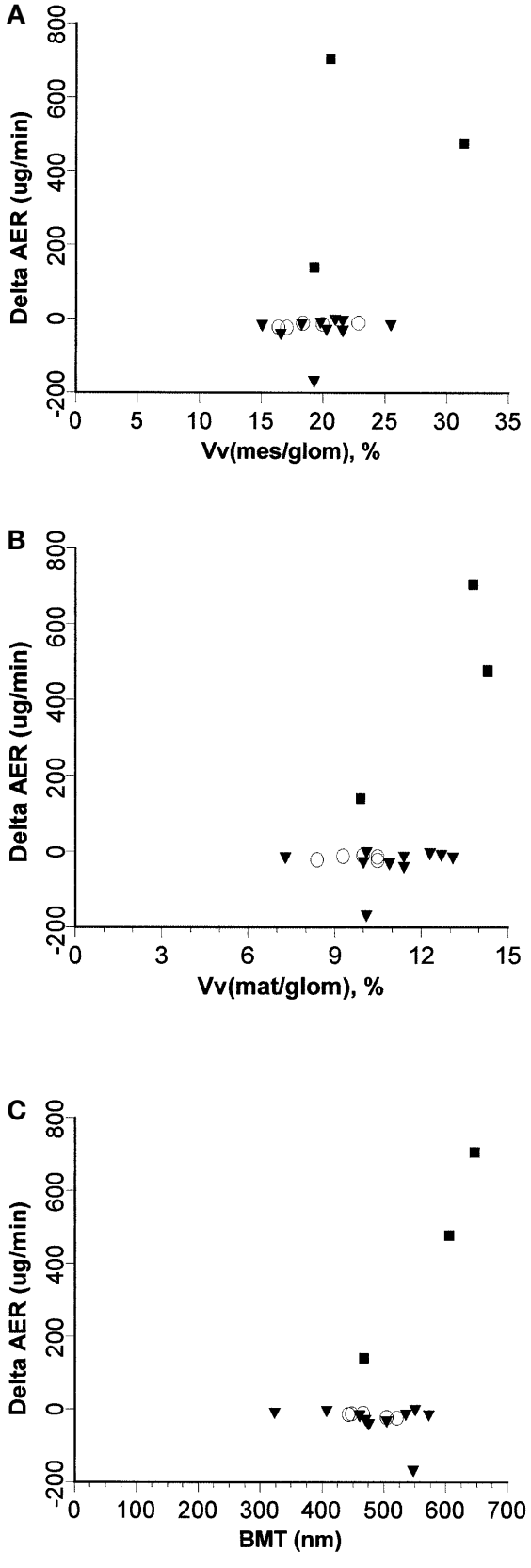

Fig. 4 A–C. Change in AER vs baseline values of (**A**) Vv(mes/glom), $r = 0.52$, $p < 0.05$, (**B**) Vv(mat/glom), $r = 0.50$, $p < 0.05$, (**C**) BMT, $r = 0.49$, $p < 0.05$. Three subjects with AER-increment > 25% per year (■), 10 subjects with no significant increment of AER during the study (▼), and five subjects with normoalbuminuria at the end of the study (○)

Table 4. Stepwise multiple regression analysis with AER at the end of the study as dependent variable in 18 young patients with Type I diabetes

	R^2-increment	T-value	*p* value
Baseline Vv(mat/glom)	0.26	3.5	0.004
Baseline BMT	0.23	3.3	0.005
Mean 8-year HbA_{1c}	0.12	2.4	0.03

$R^2 = 0.70$

showed a uniform trend towards having higher GFR, larger glomerular volumes, thicker basement membranes and larger matrix volume fractions (Table 5).

Discussion

The impact of long-term hyperglycaemia on the development of structural changes has been shown in several studies. This study is a follow-up after a randomised, prospective study where intensive insulin treatment using insulin pumps was compared with conventional treatment over a period of 2.5 years [11]. In the initial part of the study a significant difference between the groups concerning HbA_{1c} was observed. The main finding was that in the intensive treated group none of the matrix-parameters increased, whereas they all increased in the conventional group. BMT increased in both groups but most strikingly in the conventional group. A strong correlation was found between mean HbA_{1c} during the study and increase in BMT and matrix/glomerular volume fraction. We have confirmed the importance of long-term hyperglycaemia for the development of glomerulopathy by showing a positive association between HbA_{1c} and the increment of all of the investigated parameters though the relation was significant only for the mesangial volume fraction. In the multivariate analysis, mean 8-year HbA_{1c} was the only factor that had an independent influence on the glomerular structures (the mesangial volume fraction). Although the impact of the randomisation was lost or reduced because some of the participants changed treatment modality after 2.5 years, the data were analysed according 'to intention to treat'. The difference in HbA_{1c} was 1%-point between the groups. This was not statistically significant, but a difference in HbA_{1c} of this magnitude could be considered to be of clinical relevance. However, except for a trend toward more pronounced mesangial expansion in the group with the poorest blood glucose control, no differences were found with respect to AER, blood pressure and GFR between the groups. We cannot exclude the possibility that a statistical type 2-error is responsible for not showing a difference in the two groups of patients.

The relative importance of hyperglycaemia in the present study seems less evident than in this study.

Table 5. Clinical data and structural parameters at baseline in 18 young subjects with Type I diabetes

	Group A	Group B	Group C
Age (years)	22 (8–37)	19 (18–21)	20 (14–26)
Duration (years)	11 (3–20)	12 (9–14)	10 (8–13)
AER (μg/min)	37 (23–50)	60 (19–101)	27 (17–37)
Systolic BP (mm Hg)	128 (102–154)	125 (117–133)	126 (106–146)
Diastolic BP (mm Hg)	82 (76–88)	78 (71–84)	85 (75–95)
GFR ($ml \cdot min^{-1} \cdot 1.73m^{-2}$)	150 (123–177)	143 (122–165)	138 (103–174)
GV ($10^6 um^3$)	3.1 (1.3–4.8)	2.7 (2.0–3.3)	2.5 (1.8–3.2)
Vv(mes/glom) (%)	23.8 (7.3–40.8)	19.9 (17.8–22.0)	19.0 (15.7–22.2)
Vv/mat/glom) (%)	12.7 (6.7–18.7)	10.9 (9.7–12.1)	9.7 (8.6–10.9)
BMT (nm)	574 (340–806)	485 (431–540)	477 (431–540)
Matrix star volume (μm^3)	27 (5–49)	24 (18–29)	22 (11–33)

Group A = three subjects with > 25 % increment in AER per year, group B = ten subjects with no change in AER during the study and group C = five subjects who were normoalbuminuric at the end. Mean and 95 % CI

This could point to other mechanisms also important for the development of glomerulopathy after duration of diabetes of more than 10 to 15 years. It should be noted that the mean HbA_{1c} of the 8 years was rather high, at 9.4 % (normal range 4.3–6.1 %).

This study in young Type I diabetic subjects followed for 8 years, showed a significant increment in the two main aspects of diabetic glomerulopathy, namely the thickening of the basement membrane and the matrix expansion measured as matrix volume fraction, 21 % and 36 % respectively, during the study period. The observation that a relative increase after almost 20 years with diabetes was most pronounced in the matrix volume fraction is in accordance with previous studies [10, 21].

Albumin excretion rate is an intermediate endpoint in diabetic nephropathy. In this study only three of the participants experienced a significant increase in albumin excretion rate defined as more than 25 % per year [22] during the course of the study, and five patients ended up with a normal albumin excretion rate. This correlates with observations in other series that 1:3 of the patients with microalbuminuria become normoalbuminuric without treatment [22]. In this study the previously described strong association between AER and the structural parameters was confirmed [4, 5, 7, 11]. In variance with these observations are the reports of rather advanced glomerulopathy in patients with low grade microalbuminuria [23, 24] and even normoalbuminuria [8, 24]. We were able to show that the baseline values of matrix volume fraction and BMT independently, but together with mean 8-year HbA_{1c} predicted the degree of AER at the end of the study. However, baseline-BMT was the only predictor of the increase in AER during the 8 years, possibly because of the higher precision in the BMT-measurements than in the matrix estimates [25]. Our findings are more or less an extension and confirmation of an earlier intermediate report from the same study [26]. When we investigated if conditions at the beginning of the study could distinguish between those who progressed in AER and those who did not, we found no significant differences. However there was a uniform trend in the structural changes that seemed to be most pronounced among the progressors, who on the other hand did not have the higher AER at baseline.

It is known that patients with microalbuminuria could be hyperfiltrating although a decline in GFR could be seen already in the early phase of diabetic nephropathy and could reflect a more advanced glomerulopathy than in patients with a stable GFR [27]. A GFR above the upper normal limit after a duration of diabetes of 12 years has been shown to be a prerequisite for the development of incipient microalbuminuria 8 years later [13]. In children it has been shown that in two comparable groups those with hyperfiltration (> 140 ml/min per $1.73m^2$) were those who developed microalbuminuria over a 10-year period [28]. Our data however, show that two of the five patients who had normal AER, did hyperfiltrate at baseline and after 2.5 years. Experimental studies in rats show that increased GFR plays an important role for the initiation and progression of glomerulopathy [29]. One cross-sectional study in diabetic patients found a tendency toward an inverse association between current GFR and glomerular structural changes indicating that those with declining GFR already had rather severe morphological changes [21]. Another report found that glomerular hyperfiltrating in fact added to the prediction of the degree of early basement membrane thickening [10]. We investigated two aspects of GFR, firstly the influence of baseline GFR for the later development of structural changes and secondly what factors were predictive for the decline in GFR and the GFR-value at the end of the study. We were not able to find support for a significant influence of baseline GFR with the development of glomerulopathy. In addition, none of the structural parameters contributed independently to the GFR at the end of the study. The increment of BMT during the study was, however, negatively associated with GFR at the end, indicating that patients with declining renal function have a pro-

gressive BM thickening. The mean decline in GFR per year was 2.3 ml/min per 1.73m^2 which is quite low compared to the 5.2 ml/min per 1.73m^2 which have been published previously in patients with overt nephropathy [30].

Smoking is an indisputable risk factor for microvascular and macrovascular disease. In Type I diabetes it has been shown to independently increase the degree of nephropathy [15]. In our study half of the participants smoked an average of 20 cigarettes a day. They had a more pronounced worsening for all of the structural parameters compared to the non-smokers but the difference did not reach statistical significance. The smoking group had a definitely higher rise in AER during the study. Smokers also had a higher baseline GFR and a tendency to larger decline in GFR than the non-smokers and smoking was an independent risk factor for decline in GFR in a multivariate analysis.

So far no specific genes have explained the observed familial clustering [16, 17] of diabetic nephropathy. One study showed that apart from concordance in glycaemia, a strong concordance was found for severity of glomerular structural lesions in siblings with diabetes [31]. Several candidate genes with relation to the renin-angiotensin system have been proposed, e.g. insertion or deletion polymorphism of the angiotensin converting enzyme [32] and the angiotensinogen (ATG) gene [33]. An association between the D-allele of the *ACE*-gene and progression of diabetic glomerulopathy has earlier been described in adolescents with microalbuminuria [34]. However, in this smaller study we did not find support for a connection between the ID-polymorphisms and the progression of glomerulopathy (results not shown).

Blood pressure is found to be in the normal or upper normal range in incipient nephropathy and rises in parallel with AER [14]. In two studies [5, 6] no association between blood pressure and structure could be detected. However, in our recent study on the effect of antihypertensive treatment on early renal morphological changes in microalbuminuric Type I diabetic patients, we found that low blood pressure seems to be an important protector against progression of diabetic glomerulopathy [35]. In the present study we could not demonstrate that either systolic or diastolic blood pressure influenced the development of glomerular structure. In a recent study 24-h ambulatory blood pressure measurements were carried out and an association between mean arterial blood pressure and basement membrane thickness was found in adolescents with 10 years duration of diabetes [36].

In conclusion, we found that the structural glomerular changes found after 11 years of diabetes duration continued to develop during the following 8 years. A strong association between function (GFR and AER) and morphology was found. Basement membrane thickening and matrix expansion predicted an increase in AER. The level of long-term hyperglycaemia was an important risk factor for morphological changes but in this study the relative contribution was modest. The results also point to a possible effect of smoking on the decline in GFR.

Acknowledgements. This study was supported by grants from the Norwegian Diabetes Association, the Diabetes Research Centre, Aker and Ullevål University Hospital, Aarhus University Foundation, the Danish Diabetes Association, the Danish Medical Research Council, the Juvenile Diabetes Foundation International No. 190592 and the Novo Nordic Foundation. We thank Ms. K. Gerlach, Ms. B. Saugbjerg, Ms B. Iversen and Ms L. Lysgaard for their skilful technical assistance.

References

1. Viberti GC, Hill RD, Jarrett RJ, Argyropoulos A, Mahmud U, Keen H (1982) Microalbuminuria as a predictor of clinical nephropathy in insulin-dependent diabetes mellitus. Lancet i: 1430–1432
2. Parving H-H, Oxenboll B, Svendsen PAA, Sandahl Christiansen J, Andersen AR (1982) Early detection of patients at risk of developing diabetic nephropathy. A longitudinal study of urinary albumin excretion. Acta Endocrinol 100: 550–555
3. Mogensen CE (1987) Microalbuminuria as a predictor of clinical diabetic nephropathy. Kidney Int 31: 673–689
4. Mauer SM, Steffes MW, Ellis EN, Sutherland DER, Brown DM, Goetz FC (1984) Structural-functional relationships in diabetic nephropathy. J Clin Invest 74: 1143–1155
5. Bangstad H-J, Østerby R, Dahl-Jørgensen et al. (1993) Early glomerulopathy is present in young Type I (insulin-dependent) diabetic patients with microalbuminuria. Diabetologia 36: 523–529
6. Walker JD, Close CF, Jones SL et al. (1992) Glomerular structure in type I (insulin-dependent) diabetic patients with normo- and microalbuminuria. Kidney Int 41: 741–748
7. Berg UB, Torbjörnsdotter TB, Jaremco G, Thalme B (1998) Kidney morphological changes in relation to long-term renal function and metabolic control in adolescents with IDDM. Diabetologia 41: 1047–1056
8. Ellis EN, Warady BA, Wood EG et al. (1997) Renal structural-functional relationships in early diabetes mellitus. Pediatr Nephrol 11: 584–591
9. Bilous RW, Mauer SM, Sutherland DER, Najarian JS, Goetz FC, Steffes MW (1989) The effect of pancreas transplantation on the glomerular structure of renal allografts in patients with insulin-dependent diabetes. N Engl J Med 321: 80–85
10. Rudberg S, Østerby R, Dahlquist G, Nyberg G, Persson B (1997) Predictors of Renal Morphological Changes in the Early Stage of Microalbuminuria in Adolescents With IDDM. Diabetes Care 20: 265–271
11. Bangstad H-J, Østerby R, Dahl-Jørgensen K, Berg KJ, Hartmann A, Hanssen KF (1994) Improvement of blood glucose control in IDDM patients retards the progression of morphological changes in early diabetic nephropathy. Diabetologia 37: 483–490
12. Fioretto P, Steffes MW, Sutherland DER, Goetz FC, Mauer M (1998) Reversal of lesions of diabetic nephropathy after pancreas transplantation. N Engl J Med 339: 69–75

13. Rudberg S, Persson B, Dahlquist G (1992) Increased glomerular filtration rate as a predictor of diabetic nephropathy – an 8-year prospective study. Kidney Int 41: 822–828
14. Mogensen CE (1999) Microalbuminuria, blood pressure and diabetic renal disease: origin and development of ideas. Diabetologia 42: 263–285
15. Sawicki PT, Muhlhauser I, Bender R, Pethke W, Heinemann L, Berger M (1996) Effects of smoking on blood pressure and proteinuria in patients with diabetic nephropathy. J Internal Med 239: 345–352
16. Seaquist ER, Goetz FC, Rich S, Barbosa J (1989) Familial clustering of diabetic kidney disease. Evidence for genetic susceptibility to diabetic nephropathy. N Engl J Med 320: 1161–1165
17. Borch-Johnsen K, Norgaard K, Hommel E et al. (1992) Is diabetic nephropathy an inherited complication ? Kidney Int 41: 719–722
18. Gundersen HJ, Bendtsen TF, Korbo L et al. (1988) Some new, simple and efficient stereological methods and their use in pathological research and diagnosis. APMIS 96: 379–384
19. Østerby R, Parving HH, Nyberg G et al. (1988) A strong correlation between glomerular filtration rate and filtration surface in diabetic nephropathy. Diabetologia 31: 265–270
20. Østerby R (1973) A quantitative electron microscopic study of mesangial regions in glomeruli from patients with short term juvenile diabetes mellitus. Lab Invest 29: 99–110
21. Østerby R (1992) Glomerular structural changes in Type I (insulin-dependent) diabetes mellitus: causes, consequences and prevention. Diabetologia 35: 803–812
22. Mathiesen ER, Rønn B, Storm B, Foght H, Deckert T (1995) The Natural Course of Microalbuminuria in Insulin-dependent Diabetes: A 10-year Prospective Study. Diabet Med 12: 482–487
23. Fioretto P, Steffes MW, Mauer M (1994) Glomerular structure in nonproteinuric IDDM patients with various levels of albuminuria. Diabetes 43: 1358–1364
24. Østerby R, Schmitz A, Nyberg G, Asplund J (1998) Renal structural changes in insulin-dependent diabetic patients with albuminuria. APMIS 106: 361–360
25. Østerby R (1997) Research methodologies related to renal complications: structural changes. In: Mogensen CE, Handel E (eds) Research Methodologies in Human Diabetes. Walater de Gruyter, New York pp. 289–309
26. Bangstad H-J, Østerby R, Hartmann A, Berg TJ, Hanssen KF (1999) Severity of Glomerulopathy Predicts Long-Term Urinary Albumin Excretion Rate in Patients With Type 1 Diabetes and Microalbuminuria. Diabetes Care 22: 314–319
27. Rudberg S, Østerby R (1997) Decreasing glomerular filtration rate – an indicator of more advanced diabetic glomerulopathy in the early course of microalbuminuria in IDDM adolescents? Nephrol Dial Transplant 12: 1149–1154
28. Chiarelli F, Verrotti A, Morgese G (1995) Glomerular hyperfiltration increases the risk of developing microalbuminuria in diabetic children. Pediatr Nephrol 9: 154–158
29. Zatz R, Meyer TW, Rennke HG, Brenner BM (1985) Predominance of hemodynamic rather than metabolic factors in the pathogenesis of diabetic glomerulopathy. Proc Natl Acad Sci USA 82: 5963–5967
30. Parving HH, Smidt UM, Hommel E et al. (1993) Effective antihypertensive treatment postpones renal insufficiency in diabetic nephropathy. Am J Kidney Dis 22: 188–195
31. Fioretto P, Steffes MW, Barbosa J, Rich SS, Miller ME, Mauer M (1999) Is diabetic nephropathy inherited? Studies of glomerular structure in type 1 diabetic sibling pairs. Diabetes 48: 865–869
32. Marre M, Bernadet P, Gallois Y et al. (1994) Relationships between angiotensin I converting enzyme gene polymorphism, plasma levels, and diabetic retinal and renal complications. Diabetes 43: 384–388
33. Fogarty DG, Harron JC, Hughes AE, Nevin NC, Doherty CC, Maxwell AP (1996) A Molecular Variant of Angiotensinogen Is Associated With Diabetic Nephropathy in IDDM. Diabetes 45: 1204–1208
34. Rudberg S, Rasmussen LM, Bangstad HJ, Østerby R (2000) Influence of insertion/deletion polymorphism in the ACE-I gene on the progression of diabetic glomerulopathy in type 1 diabetic patients with microalbuminuria. Diabetes Care 23: 544–548
35. Rudberg S, Østerby R, Bangstad HJ, Dahlquist G, Persson B (1999) Effect of angiotensin converting enzyme inhibitor or beta blocker on glomerular structural changes in young microalbuminuric patients with Type I (insulin-dependent) diabetes mellitus Diabetologia 42: 589–595
36. Torbjörnsdotter T, Jaremko G, Berg U (2001) Glomerulopathy in relation to nocturnal hypertension in normoalbuminuric adolescents with Type I diabetes. Diabetologia 44: 865–873