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ORIGINAL ARTICLE

**Ruth Østerby · Hans-Jacob Bangstad
Gudrun Nyberg · Susanne Rudberg**

On glomerular structural alterations in type-1 diabetes

Companions of early diabetic glomerulopathy

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Abstract Glomerular structural modifications were measured in kidney biopsies from two follow-up studies in type-1 diabetic patients with microalbuminuria and in kidney donors. Stereologic methods were used to obtain data on glomerular composition and absolute quantities per glomerulus to supplement data on diabetic glomerulopathy previously published. Diabetic patients at baseline (n=37) showed significant changes compared with controls (n=11). The volume fraction of tuft/glomerulus was increased, the proportion of capillary surface facing peripheral basement membrane was decreased (0.72± 0.04 vs 0.77±0.03, P=0.0008), the ratio of mesangial surfaces, urinary/capillary, was decreased (0.67±0.17 vs 1.11±0.28, $P<10^{-4}$), and the average capillary diameter was increased (8.9±0.9 µm vs 7.5±1.0 µm, P=0.0002). The total volume of mesangial extracellular material per glomerulus was increased (P=0.01), whereas glomerular volume was not significantly different from controls. Follow-up biopsies after antihypertensive treatment with ACE-inhibitor (n=7) or β-blocker (n=6; 36–48 months) and after intensive insulin treatment (n=7; 24–33 months) showed no change. In a conventionally treated group (n=9), the glomerular volume, the volume of extracellular material/glomerulus, and the capillary length increased. The mean capillary diameter did not correlate with the glomerular volume. In conclusion, the development of diabetic glomerulopathy entails structural modifications of the glomerular tuft. Antihypertensive and intensified insulin treatment seem to slow the progression of ultrastructural changes.

Keywords Antihypertensive treatment · Diabetic glomerulopathy · Glomerular volume · Microalbuminuria · Type-1 diabetes

Ruth Østerby (✉)
Electron Microscopy Laboratory, Building 03,
University Institute of Pathology, Aarhus Kommunehospital,
DK-8000 Aarhus, Denmark
e-mail: ro@em.au.dk
Tel.: +45-8949-2141, Fax: +45-8949-2150

Hans-Jacob Bangstad
Aker Diabetes Research Center, Oslo, Norway

Gudrun Nyberg
Transplant Unit, Sahlgrenska University Hospital,
Göteborg, Sweden

Susanne Rudberg
Department of Woman and Child Health,
Pediatric Unit Karolinska Institute, Stockholm, Sweden

Introduction

The appearance of microalbuminuria in type-1 diabetic patients indicates a high risk of further progression into diabetic nephropathy [12]. Glomerular ultrastructure has been investigated in several studies dealing with this phase of diabetic nephropathy. Groups of type-1 diabetic patients with microalbuminuria clearly demonstrate structural alterations indicating the presence of diabetic glomerulopathy [1, 5, 25]. Some controversies exist as to the very early stages in the development. We have found rather moderate degrees of glomerulopathy in patients with low-level microalbuminuria and almost normal structure in patients with normoalbuminuria [20]. However, other groups have found marked structural lesions in the normoalbuminuric and early microalbuminuric phase [4, 5, 10]. The patients presented here are type-1 diabetic patients with onset of disease at a young age. Most of the patients have a low range microalbuminuria and, correspondingly, a very moderate diabetic glomerulopathy, as reported previously [1, 25].

The diabetic glomerulopathy is first and foremost accumulation of the extracellular material, measured as an increase in basement membrane thickness and an increase in the volume fraction of the mesangial matrix. An increase in basement membrane thickness is the first detectable sign of glomerulopathy, closely followed by changes in mesangial matrix [20]. Most likely, the accumulation of extracellular material takes place in parallel in the mesangium and the peripheral basement membrane. However, since a large variation in measures of mesangial matrix exists within and among glomeruli, the

basement membrane thickness is the most sensitive parameter in these early stages. The present paper presents data on structural abnormalities that accompany the characteristic changes of diabetic glomerulopathy. As the extracellular matrix accumulation takes place, it seemingly involves many modifications of the glomerular structures.

Materials and methods

The patients

Detailed studies of the glomerulopathy in the present series of type-1 diabetic patients have been published previously [2, 26]. The patients represent two separate follow-up studies. One study was conducted at Aker University Hospital with the purpose of studying the effect of metabolic control on the development of structural changes [2], and the other study was conducted at the Karolinska Institute in Stockholm, where the follow-up study concerned the effect of intervention with antihypertensive drugs [26]. All of the diabetic patients presented here had microalbuminuria at the time of the baseline biopsy, defined as an albumin excretion rate between 15–200 µg/min in overnight urine samples, present in at least two out of three consecutive urine samples. Patients from the separate studies had a similar degree of microalbuminuria, a similar age, and a similar duration of diabetes. Comparisons are made with kidney biopsies obtained from living related kidney donors, a biopsy being taken at the time of transplantation at Sahlgrenska University Hospital, Göteborg [19]. Clinical data for the patients and control group are presented in Table 1. The biopsy series were approved by the local ethics committees, and all patients had given written consent prior to the biopsies.

Upon entry into the study, a kidney biopsy was taken in diabetic patients under ultrasound guidance using an 18-gauge needle. The biopsies were sent in a 2% buffered glutaraldehyde fixative to the laboratory in Aarhus, where the processing for electron microscopy was carried out. The biopsies from Oslo were embedded in vestopal, whereas the remaining biopsies were embedded in epon. Follow-up biopsies were obtained in most of the diabetic patients after a treatment period. In the Stockholm series, the patients were allocated after the baseline biopsy into two treatment groups, one receiving an ACE-inhibitor (enalapril; 20 mg/day; group 1; *n*=7) and the other receiving a β-blocker (metoprolol; 100 mg/day; group 2; *n*=6) [26]. In the Oslo series, patients were likewise allocated into two groups. One received conventional insulin treatment with two or more injections daily, meaning that most of the patients received multiple insulin injections (group 3, CI, *n*=9). The other received intensified treatment using insulin pumps as described previously (group 4; CSII; *n*=7) [2].

The study on the effect of intensified treatment spanned 28 months (range 24–33 months), and the antihypertensive treated groups had an interval between baseline and follow-up biopsy of mean 38 months (range 36–48 months). In each group, the follow-up biopsy was processed similarly to the baseline biopsy. The two follow-up studies were not performed simultaneously; the vestopal series were earlier than the remaining part and thus, from the beginning, were not designed as controls for the antihypertensive-treated groups. In the non-diabetic control group, a wedge biopsy was taken after revascularization of the transplanted kidney. The specimens were mailed in buffered glutaraldehyde, and embedded into epon.

Light microscopy

The plastic embedded blocks were serially cut into 1-µm thick sections which were all consecutively picked up on slides and stained with toluidine blue. The serial sections were used for determination of glomerular volume, applying Cavalieri's method [8], as previously described in detail [23]. In each biopsy, an average of 12 glomeruli (range 6–26) were serially sectioned, and the volume of individual corpuscles was obtained as the sum of sectional areas multiplied with distance between measured levels. Measurement of profile areas was done through point counting at ~300× magnification. A sufficient number (minimum of six) of glomeruli cut exhaustively was not obtainable in four of the diabetic patients.

Table 1 Clinical data in the total series at baseline, median (range)

	Controls	Micro-albuminuric type-1 diabetic patients
n	11	37
Age (years)	31 (20–38)	19[a] (15–29)
Gender female/male	4/7	18/19
Duration of diabetes (years)		12 (6–18)
Age at onset (years)		7 (4–17)
Albumin excretion rate (µg/min)	Dipstick negative	27.5 (15–195)
Glomerular filtration rate (ml/min/1.73m^2)	107 (82–144)	128[b] (88–209)
Systolic blood pressure (mmHg)	120 (110–130)	125 (105–150)
Diastolic blood pressure (mmHg)	70 (60–85)	80[c] (65–98)

[a] P=0.0001; [b] P=0.009; [c] P=0.001

Electron microscopy

From the first three new appearing glomeruli in the blocks, thin sections were cut for electron microscopy at three levels separated by 50 µm. The protocol ensured unbiased sampling of glomeruli and levels within the glomerulus. From each of the nine levels, photomontages of the whole cross section were produced at a magnification of 2300× for the determination of volume fractions through point counting using double grids and, using as reference space, "glomerulus", defined as the circumscribed minimal convex polygon [16]. Estimated volume fractions were tuft per glomerulus, V_V (tuft/glom), mesangium per glomerulus, V_V (mes/glom), and capillary space per glomerulus, V_V (cap/glom). Surface densities of interfaces were estimated through counting intersections between grid lines and trace of the interface: the filtration surface, defined as the interface between the peripheral basement membrane and the urinary space, and mesangial surfaces, towards capillary and urinary space, respectively (Fig. 1). The ratio of intercepts with two identified interfaces is a direct estimate of the ratio of the two areas. Finally, capillary length density L_V (cap/glom), was estimated from the number of external capillary profiles per glomerular area [8]. The estimates were thus obtained through standard stereologic methods. The average capillary diameter was obtained from the ratio of capillary volume fraction and length density. This provides average cross sectional area, and the capillary diameter is then estimated assuming circular cross sectional area [21].

At a higher magnification (~9800×), a subsample of the area (~24%) was obtained in a systematic, independent way. This set of micrographs, obtained only from the largest of three profiles in each of the three glomeruli, was used for estimation of the basement membrane thickness, volume fraction of the matrix, V_V (matrix/tuft), and volume fraction of the peripheral basement membrane, V_V (PBM/tuft). Combination with the low magnification measurements [V_V (tuft/glom)] provides volume fractions relative to glomerular space. In each of the two series the same observer

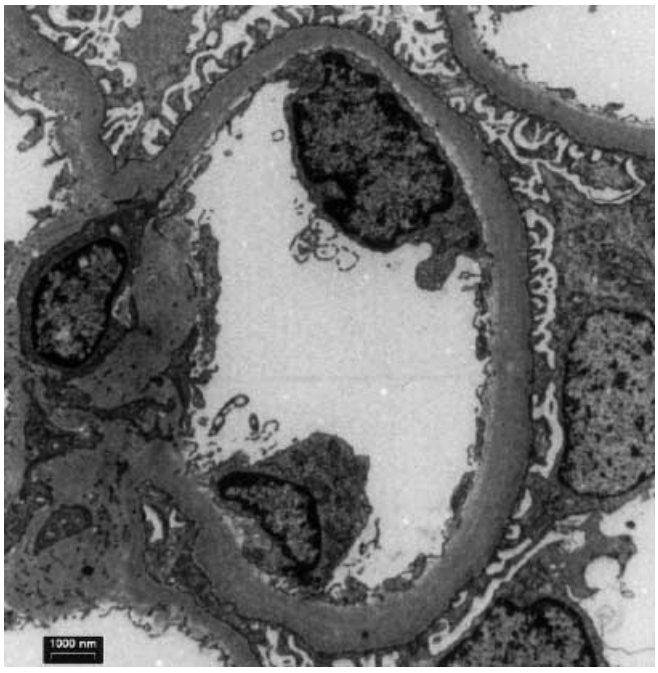

Fig. 1 The electron micrograph illustrates the interfaces of interest. The filtration surface is the interface between the capillary and urinary space. Mesangial surfaces face the capillary lumen and the urinary space. The stereologic estimates of surfaces were obtained at a lower magnification covering entire glomerular profiles

performed the corresponding measurements in baseline and follow-up biopsies, whereas there was not identity in observers between group 1 and group 2 versus group 3 and group 4.

Statistical methods

Comparisons between the diabetic patients and controls at baseline were done with the Student's *t*-test. The change over time in the follow-up biopsies was tested by means of paired, double-sided *t*-test and comparison of changes among the groups using analysis of variance (ANOVA). Correlation among different variables was estimated using least square methods.

Results

Controls versus diabetic patients at baseline

In Table 2 the results of a number of data expressing the glomerular composition are presented. All diabetic patients entering the study are included, i.e., from both follow-up series. The glomerular tuft (capillaries + mesangium) occupies a larger fraction of the glomerular space in diabetic patients, i.e., the glomerular space is more densely occupied by the capillary network at the expense of the urinary space.

In non-diabetic glomeruli, the distribution of extracellular material is about half in the mesangial matrix and half in the peripheral basement membrane. This latter proportion decreases in diabetic patients, although not significantly so in the present series.

Table 2 Glomerular compositional data at baseline; mean with inter-individual variation (CV%). *glom* glomerulus; *PBM* peripheral basement membrane; *ECM* extracellular material; *total ECM* peripheral basement membrane and mesangial matrix; *ND* kidney donors; *tuft* mesangial regions and capillaries; V_V *(tuft/glom)* volume fraction of tuft relative to the circumscribed polygon (glom); V_V *(PBM/total ECM)* volume fraction of PBM (in the capillary walls) relative to total ECM

	ND	Type-1 diabetic patients with micro-albuminuria	*P* value
n	11	37	
V_V (tuft/glom)	0.57 (6.1)	0.69 (5.4)	$<10^{-4}$
V_V (PBM/total ECM)	0.48 (6.8)	0.44 (12)	0.07
Filtration surface/ total capillary surface	0.77 (3.9)	0.72 (5.7)	0.0008
Ratio of mesangial surfaces, urinary/capillary	1.11 (25)	0.67 (25)	$<10^{-4}$
Capillary diameter (μm)	7.5 (14)	8.9 (10)	0.0002

Table 3 Total quantities per glomerulus; mean with inter-individual variation (CV%). *glom* glomerulus; *V (glom)* glomerular volume; *V (mes)* mesangial volume; *V (matrix)* volume of mesangial matrix. *ns* not significant; *ND* kidney donors

	ND	Type-1 diabetic patients with micro-albuminuria	*P* value
n	9	33	
V (glom) ($10^6\mu m^3$)	2.45 (29)	2.92 (30)	ns
V (mes) per glom, ($10^6\mu m^3$)	0.45 (33)	0.61 (37)	0.06
V (matrix) per glom ($10^6\mu m^3$)	0.23 (33)	0.34 (36)	0.01
Filtration surface per glom (mm^2)	0.33 (32)	0.37 (31)	ns
Capillary length per glom (mm)	22.3 (27)	23.1 (34)	ns

Statistically significant changes were observed in the glomerular interfaces; considering the capillary surfaces, the fraction made up of the filtration surface, i.e., towards the urinary space, is decreased in diabetic patients. Also, the mesangial surfaces show a shift, such that the fraction of the total surface facing the capillary is increased relative to the urinary surface. In the controls, the two subsets of interfaces are of the same order of magnitude. The estimated capillary diameter was significantly larger in diabetic patients than in the controls. This pertained whether the diameter was calculated from average cross sectional area or from average capillary circumference [21].

From the combination of glomerular volume and densities, total quantities per glomerulus were calculated and are shown in Table 3. Although the mean glomerular volume was larger in the group of diabetic patients than in controls, this difference did not reach statistical signif-

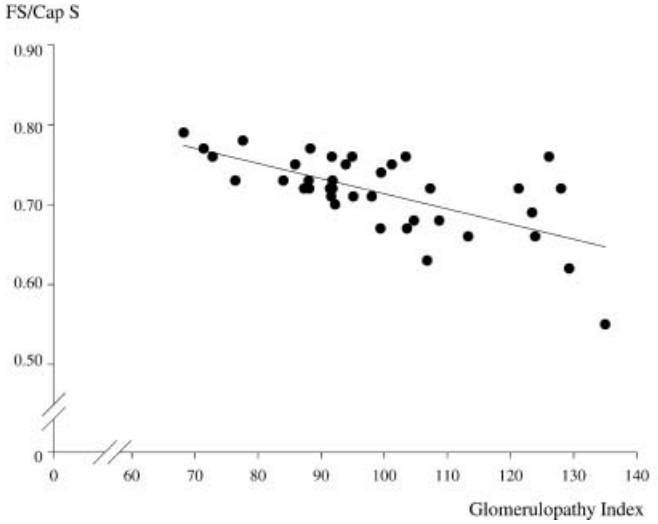

Fig. 2 The fraction of total capillary surface constituted by the peripheral capillary wall decreases with increasing diabetic glomerulopathy as estimated using the glomerulopathy index: BMT/10 + V_V (matrix/glomerulus)% + matrix star volume

icance, i.e., we did not find a marked glomerular hypertrophy at this stage of diabetic renal disease. The total volume of mesangial matrix is significantly increased in the diabetic group. As for the capillary dimensions, neither the total filtration surface per glomerulus nor the capillary length showed any differences between the two groups. It is noteworthy that the variation within groups of the fractional volumes and surface densities is at a somewhat lower level than that for the total quantities.

Correlations among structural variables at the baseline

The shift in glomerular interfaces correlated with the degree of diabetic glomerulopathy; the fraction of capillary surface constituted by peripheral capillary wall correlated inversely with mesangial volume fraction (r=–0.66, $P<10^{-4}$) and the overall index of diabetic glomerulopathy (BMT/10 + V_V (matrix/glom)% + matrix star volume) [26], r=–0.56, $P<10^{-4}$ (Fig. 2). Also, the ratio of mesangial surfaces correlated inversely with the glomerulopathy index, r=–0.37, P=0.02, whereas it did not correlate with the mesangial volume fraction. The average capillary diameter did not show a correlation with the mean glomerular volume, neither in the control group nor in the combined diabetic groups at the baseline. Thus, individuals with the larger glomeruli did not tend to have a larger capillary diameter.

Correlations with clinical variables at the baseline

None of the correlations between the present structural parameters and the clinical variables that might be relevant in this context were statistically significant. The relationships studied were hemoglobin (Hb)A_{1C} versus glomerular volume, systolic and diastolic blood pressure versus V_V (tuft/glom) and capillary diameter, glomerular filtration rate (GFR) versus total filtration surface per glomerulus, and the ratio filtration surface (FS)/total capillary surface.

Follow-up biopsies

A significant increase in the mean glomerular volume was observed in the conventionally treated group (CI), and no increase was observed in the other groups (Table 4). The total structural quantities, to some extent, follow the changes in glomerular volume. Figure 3 shows the change in total volume of the extracellular material

Table 4 Total quantities per mean size glomerulus in follow-up biopsies in groups of diabetic patients treated with antihypertensive drugs (ACE-inhibitor, n=7; β-blocker, n=6), the conventionally treated group (CI, n=9), and the group treated with intensive diabetes treatment (CSII, n=7). *ACE-I* angiotensin converting enzyme inhibitor; *glom* glomerulus; *V(glom)* glomerular volume; *ns* not significant

	Group	Baseline		Follow-up		P[a]
		Mean	CV%	Mean	CV%	
V(glom) (10^6µm^3)	ACE-I	2.86	22	2.63	17	ns
	β-blocker	3.75	30	3.23	18	ns
	CI	2.78	35	3.03	35	0.04
	CSII	2.44	17	2.73	19	ns
V(mes) (10^6µm^3)	ACE-I	0.58	43	0.54	29	ns
	β-blocker	0.80	34	0.78	25	ns
	CI	0.60	41	0.65	36	ns
	CSII	0.51	15	0.58	19	ns
V(matrix) (10^6µm^3)	ACE-I	0.32	40	0.31	37	ns
	β-blocker	0.42	30	0.42	29	ns
	CI	0.34	44	0.41	41	0.02
	CSII	0.31	18	0.33	20	ns
Filtration surface (mm^2 per glom)	ACE-I	0.32	27	0.31	21	ns
	β-blocker	0.42	34	0.37	16	ns
	CI	0.40	37	0.42	32	ns
	CSII	0.35	16	0.37	18	ns
Capillary length (mm per glom)	ACE-I	19.2	32	17.4	19	ns
	β-blocker	25.9	36	22.9	15	ns
	CI	24.5	46	26.7	40	0.04
	CSII	22.4	14	22.6	18	ns

[a] Paired two-sided Student's *t*-test; comparison between baseline and follow-up

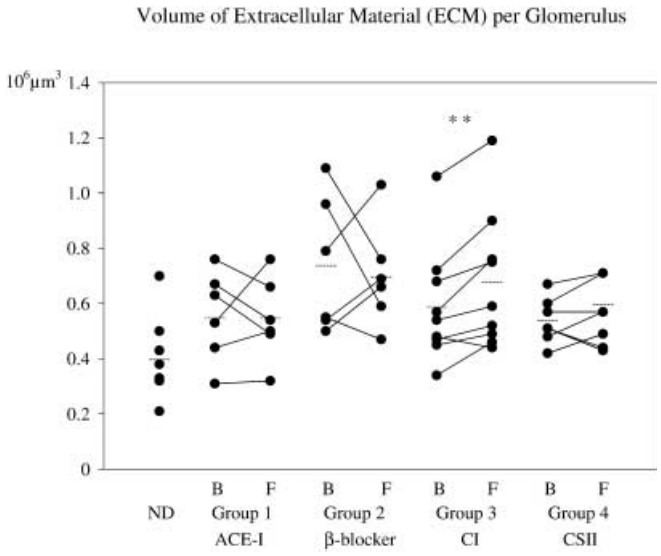

Fig. 3 Estimates of total extracellular material in kidney donors (*ND*) and the diabetic groups at entry into the study (*B*), and after the treatment period (*F*). The increase from baseline to follow-up in the conventionally treated group is statistically significant *(P*=0.009)

in the groups. This measure shows a significant increase in the CI group (*P*=0.009), whereas no significant change over time is seen in the other groups. Total mesangial volume did not change in any of the groups (Table 4).

As for the capillary data, no change was seen in the total filtration surface (Table 4) or capillary diameter (Table 5) from baseline to follow-up; only capillary length increased significantly in the CI group. The relationship between subsets of interfaces, filtration surface/capillary surface, and mesangial urinary versus capillary surface showed very similar mean values in baseline and follow-up biopsies. Still, the very moderate decrease in filtration surface/cap surface in the CSII group just reached statistical significance (Table 5). The stability within individual patients in several of these estimates is remarkable.

In the whole series, there was no correlation between the change in glomerular volume versus the change in capillary diameter. The relationship in the individual small groups showed an inverse correlation in group 1, i.e., with an increase in glomerular volume, there was a tendency to decrease in capillary diameter (data not shown). The results in Table 4 and Table 5 again show the low inter-individual variation (CV%) in compositional data and a much larger variation in total quantities.

Discussion

The characteristics of diabetic glomerulopathy are increases in the extracellular material. We have previously published from the same biopsy series that the intensively treated patients and the groups in antihypertensive treatment failed to show progression in glomerulopathy parameters from baseline to follow-up – contrary to observations in the group given only standard diabetes treatment [2, 26]. An increase in the matrix accumulation in afferent arterioles was demonstrated in the patients in antihypertensive treatment [7]. Accompanying the hallmark of glomerulopathy, extracellular matrix accumulation, we have observed various structural modifications as presented in this paper.

A number of shortcomings should be pointed to regarding the patient series. The two diabetic follow-up studies were in fact two separate studies not carried out contemporarily. However, the patients at baseline were very similar in the two subsets in terms of a large number of clinical variables, as discussed earlier [27]. Due to the variables, which were not similar in the two follow-up studies (point of time, embedding medium, and observers), main emphasis should be placed on the comparisons within groups of changes over time rather than comparisons among all four groups. The use of two dif-

Table 5 Compositional structural data in follow-up biopsies in groups of diabetic patients treated with antihypertensive drugs (ACE-inhibitor, *n*=7; β-blocker, *n*=6), the conventionally treated group (CI, *n*=9), and the group treated with intensive diabetes treatment (CSII, *n*=7). *ACE-I* angiotensin converting enzyme inhibitor; *glom* glomerulus; *tuft* mesangial regions and capillaries; *PBM* peripheral basement membrane; *ECM* extracellular material; *total ECM* PBM and mesangial matrix; V_V (tuft/glom) volume fraction of tuft relative to the circumscribed polygon (glom); *FS* filtration surface; *PBM/total ECM* volume fraction of PBM (in the capillary walls) relative to total ECM; *ns* not significant

	Group	Baseline		Follow-up		*P*
		Mean	CV%	Mean	CV%	
V_V (tuft/glom)	ACE-I	0.67	7	0.67	10	ns
	β-blocker	0.69	6	0.70	7	ns
	CI	0.69	5	0.68	6	ns
	CSII	0.70	5	0.68	4	ns
PBM/total ECM	ACE-I	0.46	16	0.46	16	ns
	β-blocker	0.44	6	0.40	9	ns
	CI	0.44	14	0.42	12	ns
	CSII	0.43	9	0.41	17	ns
FS/total capillary surface	ACE-I	0.72	9	0.74	8	ns
	β-blocker	0.71	7	0.72	5	ns
	CI	0.73	3	0.73	4	ns
	CSII	0.73	4	0.71	4	0.05
Capillary diameter (µm)	ACE-I	9.25	10	9.79	14	ns
	β-blocker	9.52	7	9.03	12	ns
	CI	8.40	9	8.28	9	ns
	CSII	8.36	11	8.44	6	ns

ferent embedding media (vestopal and epon) was an inevitable drawback since vestopal was no longer available after completing the first study. A comparison of structural parameters using the two different embedding media was initiated but could not be completed because vestopal was no longer available. The differences between the two media are probably minor since a long series of baseline data failed to show differences between the Norwegian (vestopal embedding) and the Swedish (epon embedding) patient series [24]. As to the control series, inevitable differences in terms of tissue preparation also pertain. It is probable that the biopsy procedure, taking wedge biopsies from the donor kidneys during the operation after revascularization, might affect the structures in a different way than in needle biopsies. It could be speculated that there would be some collapse of the glomerular tuft, which might lead to decrease in capillary diameter and a lower volume fraction of tuft/glomerulus. However, the differences observed here between controls and microalbuminuric diabetic patients are found to a larger extent in cases with advanced diabetic glomerulopathy [20, 22]. It is therefore suggested that the slight deviations from the controls observed here represent the initial stage in this development.

The reported total quantities, total volume, surface, and length are the result of the combined effect of glomerular composition and glomerular volume. The variation in the parameters, therefore, reflects the well-known large inter-individual variation in glomerular volume [13, 17]. In the early phase of diabetes, the acute glomerular hypertrophy has frequently been reported in diabetic patients and in experimental animals [6, 14]. In this acute hypertrophy, there is a remarkable retainment of structural composition, so that total volume, surfaces, and length are increased in parallel with the increase in volume [15]. These changes, e.g., increase in total mesangial volume and in volume of mesangial matrix, are not expressions of diabetic glomerulopathy. The early acute hypertrophy, probably to some extent, is reversible with the institution of insulin treatment [14]. This would fit with the fact that the patients with early micoalbuminuria do not show significantly increased glomerular volume. In the later phases, when the glomerulopathy has developed to some extent, a secondary phase of glomerular volume increase may be observed, probably as reflected in the changes seen in the conventionally treated group in this series: an enlargement of mean glomerular volume as a compensation for the compositional changes in the glomeruli [24].

In terms of glomerular function, the total size of filtration surface per kidney is the important structural parameter. Earlier studies have shown a correlation between estimates of total filtration surface and GFR [3, 9, 18]. We did not observe this positive correlation in the present series. However, it is probably only demonstrable when a wider range in GFR is considered. Within a more narrow range, it is likely that the other variables, i.e., the hemodynamic factors, are of dominating importance. The estimate of total filtration surface per glomerulus has no close relationship with the surface density, which is the measure immediately obtained from the electron micrographs. The surface density does decrease in diabetic glomeruli with the expansion of mesangial space as it has been reported several times [5, 11]. However, the surface density does not tell about the glomerular function as opposed to estimates of total filtration surface.

The functional implications of the shift in ratio of various interfaces are not very clear. It seems that the decrease in the proportion of the capillary surface facing the peripheral capillary wall with increasing diabetic glomerulopathy would be a contributing factor to the eventual loss of GFR. In these early stages, however, it is unlikely to have functional consequences. It should be noted that the estimates presented here are rather weak, being ratio estimators. However, the moderate changes observed here, compared with the controls, go in the same direction as those which are more readily demonstrable in more advanced stages of glomerulopathy [20, 22].

It is remarkable that we did not see a correlation between the capillary diameter and the glomerular volume in the baseline biopsies, indicating that the larger glomeruli are not characterized by a larger capillary diameter and, therefore, not necessarily by increased hydrostatic pressure on the capillary wall. Furthermore, there was no tendency to increase in capillary diameter, only capillary length, with the increase in glomerular volume.

The present data has shed some light on the stability of the various parameters in repeat biopsies. The observation time from baseline to follow-up was relatively short. However, during that period, it was possible to demonstrate progression of diabetic glomerulopathy parameters in the conventionally treated group relative to the other groups in this series [26]. Furthermore, in the two antihypertensive treated groups, a slight increase in matrix accumulation was demonstrated in the afferent arterioles [7].

The present results, demonstrating various structural modifications of the glomerular composition accompanying diabetic glomerulopathy, underline the fact that changes in certain structural subsets are likely to involve other structural modifications. In this respect, it is important to consider the whole glomerulus as a functioning unit. In this presentation, we have focused only on glomeruli, but just as important as it is to consider all glomerular compartments, the glomerular changes should be considered in conjunction with extraglomerular changes, which certainly take place along with the development of diabetic glomerulopathy. In particular, the accompanying changes in the ultrastructure of juxtaglomerular arterioles [7] may be detrimental for further progression.

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