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Glomerular volume and the glomerular vascular pole area in patients with insulin-dependent diabetes mellitus

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Abstract The vascular pole area (VPA) and glomerular volume were measured in renal biopsies from 9 insulin-dependent diabetes mellitus (IDDM) patients with normal albumin excretion rate (IDDM group 1), 38 IDDM patients with albumin excretion rate >15 μg/min (IDDM group 2) and 10 living kidney donors (ND). The volume of individual glomeruli was estimated as the sum of profile areas factored by the measured distance between levels, $t \sim 10$ μm, and VPA as the sum of chords multiplied by t. Mean glomerular volume was increased in IDDM patients but reached statistical significance only in IDDM group 2 ($P = 0.002$ vs ND). VPA was significantly different among the groups, mean (CV%) was 2036 (29) μm^2 in ND, 3555 (34) μm^2 in IDDM group 1, and 3528 (48) μm^2 in IDDM group 2, p = 0.004 and 0.001, IDDM versus ND. VPA calculated as a percentage of the surface area of the corresponding glomerulus was 2.4 (23)% in ND, 3.4 (27)% in IDDM group 1, and 3.3 (42)% in IDDM group 2; $P = 0.007$ and 0.01, IDDM versus ND. The intra-biopsy coefficient of variation was high (20–35%) and of the same order in all groups for all three measurements. Glomerular volume and absolute as well as relative size of VPA showed a positive correlation with estimates of mesangial expansion in IDDM group 2 and the VPA showed a negative correlation with GFR. Thus, part of the enlargement may represent a compensatory phenomenon triggered by the development of structural and functional abnormalities in the diabetic kidney.

Key words Diabetic glomerulopathy · Glomerular hypertrophy · Glomerular vascular pole · Insulin-dependent diabetes mellitus (IDDM) · Stereology

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Introduction

The vascular pole of the renal glomerulus is the critical point of regulation of glomerular flow and pressure. It is therefore of special interest in insulin-dependent diabetes mellitus (IDDM), which is usually associated with increased glomerular filtration rate and increased filtration fraction during the early phase of the disease [4, 14, 15].

The mechanism behind the early renal hyperfunction is not entirely clear. The hyperfunction phase is associated with renal enlargement [15], and in small biopsy series glomerular hypertrophy has been found [18]. The observation of correlation between the size of glomerular filtration surface and the level of glomerular filtration rate (GFR) indicated that structural modifications are part of the mechanism [11]. However, haemodynamic factors are also likely to be involved.

Detailed studies of glomerular size during the early years of diabetes are not available, but hypertrophy may regress to some extent, with stabilization of the metabolic state. In experimental diabetes it is possible to partially reverse the glomerular enlargement with metabolic normalization [7] when initiated after 1–3 months' duration of diabetes. In IDDM patients the glomerular hyperfunction usually remains at elevated levels for years and thus a persistent structural factor may be present. In pa-

tients developing the more advanced stages of diabetic glomerulopathy a further compensatory enlargement of the least affected glomeruli is seen [9, 20].

Increased nephron function, in particular a presumed elevated intracapillary pressure, as well as glomerular hypertrophy have been considered possible pathogenetic factors in ensuing development of glomerulosclerosis [3, 26]. Whether this mechanism is important in the long-term development of renal lesions in diabetes is not clear. At any rate it is of interest to obtain more data on glomerular volume, applying improved methodology. Kidney biopsy material from IDDM patients with either normal or elevated albumin excretion rates was available, obtained from a number of series over the years. Data on measures of glomerular size, and in particular the size of the vascular pole region, are presented in this paper.

Materials and methods

The present material represents a subset of cases in whom glomerular structure has previously been described in detail [1, 22, 25]. The studies were approved by the local ethics committees. This paper includes all those cases from the individual series in which at least seven vascular pole regions could be measured. The patients are thus subgroups of series designed to study the natural history of diabetic nephropathy [25], the effect of intensified insulin treatment [1] and the possible effects on renal structure of treatment with ACE inhibitors [22]. In the latter two series only biopsies taken at entry into the study (before the treatment trial) were used. In one further biopsy series (unpublished) including 16 albuminuric cases the patients had been receiving treatment with cilazapril or nifedipine for 18 months at the time of the biopsy. None of the other patients had been receiving antihypertensive treatment at the time of renal biopsy.

Nondiabetic control material was obtained from kidney donors for renal transplantation, in each of whom a biopsy was taken at the time of transplantation. In IDDM patients a percutaneous biopsy was obtained. It was immediately fixed in 2% glutaraldehyde and sent in the fixative from the different centres to the laboratory in Aarhus, where the embedding of small tissue blocs for electron microscopy was performed using either Vestopal or epon. In the control group an open wedge biopsy was obtained during the operation and the tissue was processed similarly for electron microscopy. The clinical data for the present series appear in Table 1. IDDM patients were grouped according to persistent albumin excretion rate (AER) above (elevated) or below (normal) 15 μg/min, recorded in timed overnight urine samples. The following group identifications are used: ND for nondiabetic control cases, IDDM group 1 for diabetic patients with normal AER, and IDDM group 2 for diabetic patients with elevated AER. In all series albumin was determined with immunoturbidimetry [24]. For the measurements of GFR different methods were applied in the individual series: either single intravenous injection of CrEDTA, single injection of inulin, continuous infusion of inulin, or single point plasma clearance of iohexol. The exact level of GFR is therefore not comparable in between the different series.

Each block of tissue was systematically sectioned with 1-μm-thick sections. The first section of the block was numbered 0, and thereafter all sections were picked up on slides and stained with toluidine blue. Levels separated by ~10 μm were used. For estimating the distance between levels the following procedure was used: starting the sectioning of the block a groove was cut at the right-hand side of the tissue block, parallel with the cut surface. The distance between cut surface and groove (about 300–400 μm), was measured by 1-μm steps of the microtome. Having completed sectioning of the block the new distance between the cut surface and groove was measured, and the average distance between levels, *t*, was calculated as the difference between first and last measurement divided by the number of levels in between (Fig. 2).

The volume of individual glomeruli *V*(G) was estimated by Cavalieri's method [10]:

$$\text{Glomerular volume, } V(G) = t \times \Sigma a$$

where *a* is the area of individual profiles, defined as the minimum circumscribed convex polygon [19]. Areas were measured by point counting using an Olympus microscope with a drawing tube attached and a point grid with a distance between points of about 34 μm. When the glomeruli could not be followed from top to bottom, the maximum profile area was used; assuming spherical shape of the glomerulus, then glomerular diameter

$$d = 2 \times \sqrt{a_{\max} / \pi} \quad \text{and} \quad V(G) = (\pi / 6) \times d^3$$

The number of glomeruli measured for determination of glomerular volume appears in Table 2. Cavalieri's method was applied for the majority of glomeruli, estimates from maximum area were used in only 18%. In 26 biopsies (5 ND and 21 IDDM) glomerular volume, measured by the Cavalieri method in at least ten glomeruli, was also calculated from the maximum area so that the two principles could be compared. The intra-biopsy coefficient of correlation was highly significant in all cases, with a median value of 0.80. However, maximum area systematically led to an overesti-

Table 1 Clinical data, median and (range) in nondiabetic controls (*ND*) and patients with insulin-dependent diabetes mellitus (*IDDM*) with different levels of albumin excretion in the urine (*AER*)

n	ND	IDDM patients group 1 AER <15 μg/min	IDDM patients group 2 AER >15 μg/min
Age (years)	10 31 (20–34)	9 33 (19–39)	38 24[a] (14–55)
Sex	4F, 6M	4F, 5M	20F, 18M
Systolic blood pressure (mmHg)	118 (110–130)	122 (106–142)	125 (105–153)
Diastolic blood pressure (mmHg)	70 (60–85)	73 (51–102)	80[b] (64–105)
Glomerular filtration rate[c], (ml/min/1.73 m^2)	107 (82–144)	123 (96–176)	113 (52–178)
Urinary albumin excretion rate, (μg/min)	Dipstick negative	5.0 (2–7)	40.3 (19–575)
Diabetes duration (years)		11 (5–34)	14[a] (6–44)

[a] Not significant IDDM group 1
[b] P=0.003 vs ND; P=0.03 vs IDDM group 1
[c] Measured with different methods in individual biopsy series

Table 2 Number of glomeruli for estimates of glomerular volume [(*V*(*G*)], vascular pole area (*VPA*) and VPA relative to glomerular surface (*VPA/GS*) with intra-biopsy variation and precision. Data are median and (range)

	V (G)	*VPA*	*VPA/GS*
No of glomeruli per biopsy			
ND	15 (8–32)	14 (8–26)	12 (8–23)
IDDM 1	11 (10–20)	8 (7–15)	8 (7–15)
IDDM 2	14 (10–24)	12 (7–19)	12 (7–18)
Intra-biopsy variation, CV%[a]			
ND	21 (16–28)	33 (26–44)	33 (21–41)
IDDM 1	24 (11–36)	32 (18–38)	25 (17–37)
IDDM 2	22 (11–48)	36 (18–108)	34 (22–87)
Intra-biopsy precision of estimates, CE%[b]			
ND	5.5 (3.8–8.4)	8.7 (6.3–12.5)	8.8 (6.4–14.6)
IDDM 1	7.2 (3.2–9.9)	9.8 (6.9–11.9)	8.0 (6.2–11.6)
IDDM 2	5.6 (2.7–15.3)	10.7 (5.6–26.3)	10.2 (5.9–21.2)

[a] CV=SD/mean
[b] CE=SEM/mean

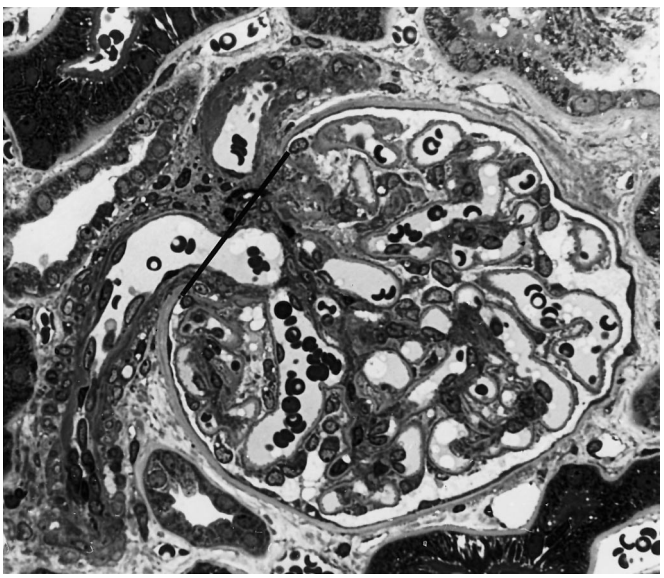

Fig. 1 A 1-μm-thick epon section traversing the vascular pole. The linear distance between the two points of reflection Bowman's capsule into the tuft is indicated by the *line*

mation of volume, with a median value of 10%. Taking the average of the four largest cross sections rather than just the largest section provided a closer correlation between the two methods, and such estimates from maximum profiles were therefore used.

The levels used for measurements were at distance of 10 μm and multiples of 10 μm thereof from the baseline section, thus providing a set of profiles with an independent position within newly appearing glomeruli.

The sections for measurements of vascular pole area were sampled in the same way as those for glomerular volume. On sections traversing the vascular pole the chords, *c*, were measured as the linear distance between the two points of reflection of Bowman's capsule (Fig. 1). An approximate measure of the vascular pole area (VPA) is then (Fig. 2):

$VPA = t \times \Sigma c$

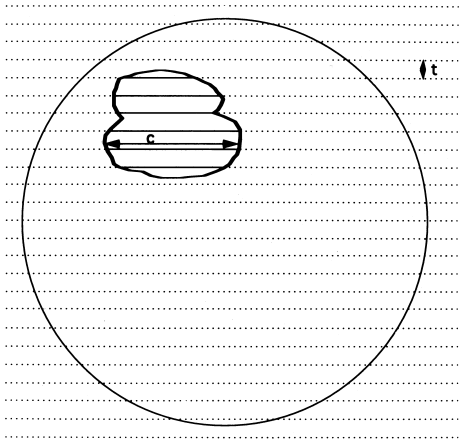

Fig. 2 Schematic representation of a spherical glomerulus with the vascular pole area indicated. The sections, separated by ~10 μm, traversing the vascular pole area are used for measurements of chords (*c*)

The size of VPA relative to the glomerular size was calculated by relating VPA to surface area of the corresponding glomerulus (VPA/GS): from the glomerular volume an estimate of the surface was calculated, assuming spherical shape of glomeruli: $d = [6/\pi \times V(G)]^{1/3}$, the glomerular surface, $GS = \pi \times d^2$. The minimum number of glomeruli per biopsy accepted for these calculations was seven. The actual number of glomeruli estimated in the three groups appears in Table 2.

The method used to measure the vascular pole area leads only to an approximation, which would be correct only if all sections were perpendicular to the area measured. However, this is not the case and the underestimation is larger when the vascular pole area is met where the glomerulus appears or disappears in the sequence of sections. The calculations were therefore repeated excluding all corpuscles in which the vascular pole was met at the first or the second level of the glomerulus. The results remained similar, but some biopsies had to be excluded owing to the lower number of glomeruli included. The bias caused by the angle between area and sectioning plane has not been evaluated in the present study. However, it is small compared with the size of the vascular pole area ($t << \Sigma c$) and, more importantly, is likely to be expressed equally in all three groups.

Another bias in the estimates is caused by any structural modification, such as shrinkage or swelling resulting from the tissue processing. These variables cannot be evaluated. In the present combined series two different types of plastic embedding were used. However, there were no indications of a systematic difference between the two types of embedding medium. Further, when the vascular pole area is estimated as a fraction of the tuft surface area, any structural modifications casued by tissue preparation should have the same effect on the two variables in the ratio.

The method used in the quantitative electron microscopy study to obtain basement membrane thickness and mesangial and matrix volume fractions and average capillary diameter in diabetic glomerulopathy has been described elsewhere [1, 17, 22, 25].

Differences among the three groups were evaluated by the nonparametric Kruskal-Wallis' test. When this test showed significant differences, results in groups were compared with the aid of the Mann-Whitney two-sample test. Correlations were studied by simple least-squares regression.

Results

The structural parameters characterizing diabetic glomerulopathy appear in Table 3. The increase in all three

Table 3 Glomerular structural data, mean and (CV)

	ND	IDDM patients group 1	IDDM patients group 2
Basement membrane thickness (nm)	365 (0.11)	442 (0.25)	606[a] (0.17)
Mesangial volume fraction V_V (mesangium glomerulus)	0.19 (0.10)	0.22[b] (0.14)	0.24[c] (0.25)
Matrix volume fraction V_V (matrix/glomerulus)	0.092 (0.17)	0.11 (0.23)	0.14[d] (0.28)

[a] $P<10^{-4}$ vs ND; P=0.0009 vs IDDM group 1
[b] P=0.03 vs ND
[c] P=0.002 vs ND
[d] P=0.0002 vs ND; P=0.02 vs IDDM group 1

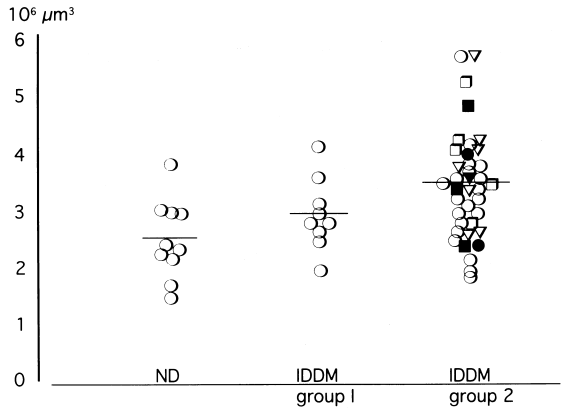

Fig. 3 For each biopsy the median value of 8–32 measurements of glomerular volume is plotted. In IDDM group 2 cases with AER > 200 mg/min are indicated as *filled symbols*. *Triangles* (∇) represent patients in treatment with ACE-inhibitor, and *squares* (❑), patients being treated with Ca-channel blocker

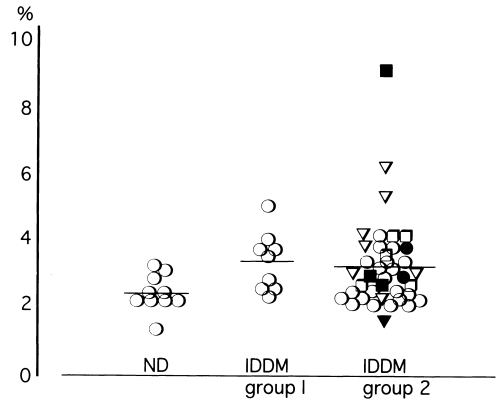

Fig. 5 VPA calculated as percent of the surface area of the corresponding glomerulus in individual glomeruli. The median value for each biopsy is shown in the plot. Signatures as in Fig. 3

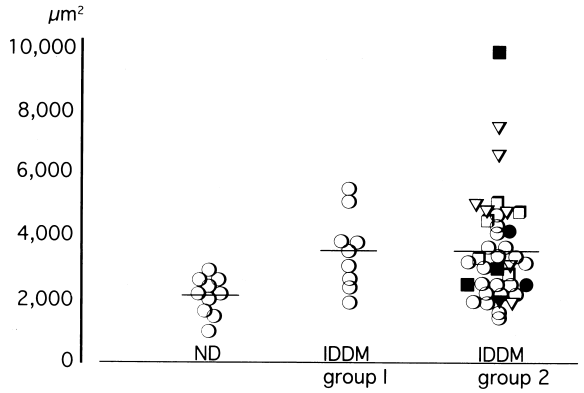

Fig. 4 Median vascular pole area in individual biopsies. Signatures as in Fig. 3

parameters in IDDM group 2 is highly significant; in IDDM group 1 only mesangial volume fraction is significantly increased.

The estimates of glomerular volume are shown in Fig. 3. The data are the median values for the individual biopsies. Only the IDDM patients with albuminuria showed a significantly increased volume compared with the controls (P = 0.002). Individual biopsies varied widely, as shown in Table 2. There were no significant differences among the groups in terms of the coefficient of variation. The precision of the estimate in individual cases, the coefficient of error, also appears in Table 2.

The results of the measurements of vascular pole area in the three groups are shown in Fig. 4. Both diabetic groups show significantly larger areas than the controls (P = 0.004 and 0.001, respectively for groups 1 and 2). The intra-biopsy variation in this structure was even wider than that for glomerular volume (Table 2).

The calculated estimate of vascular pole area as fraction of the surface area of the corresponding glomeruli is shown in Fig. 5. The relative size of the vascular pole area was also significantly increased in both diabetic groups (P = 0.007 and 0.01 versus controls), although the majority of diabetic patients had values within the normal range.

The size of the vascular pole area correlated with glomerular volume among biopsies in each of the groups. The relative size, VPA/GS, also showed a positive correlation with glomerular volume in group 2 of the IDDM patients.

There was a positive correlation between estimates of mesangial and mesangial matrix volume fractions versus glomerular volume, VPA and VPA/GS in IDDM group 2 (Table 4, Fig. 6). There was no correlation between the average glomerular capillary diameter, estimated from capillary surface-to-length ratio on low-magnification electron microscopy [17] and either glomerular volume or vascular pole area.

No tendency to an association between the vascular pole dimension and sex or age was seen in the control group. The duration of diabetes showed a positive corre-

Table 4 Correlations between glomerular and vascular pole size versus measures of glomerulopathy and clinical variables in IDDM group 2

	Glomerular volume		Vascular pole area		Vascular pole area/ glomerular surface,	
	r	*p*	*r*	*p*	*r*	*p*
Mesangial volume fraction V_V (mesangium/glomerulus)	0.45	0.005	0.70	$<10^{-4}$	0.65	$<10^{-4}$
Matrix volume fraction V_V (matrix/glomerulus)	0.40	0.01	0.68	$<10^{-4}$	0.65	$<10^{-4}$
Diabetes duration	0.13	ns	0.53	0.0007	0.52	0.0009
Glomerular filtration rate	–0.27	0.10	–0.47	0.003	–0.39	0.02

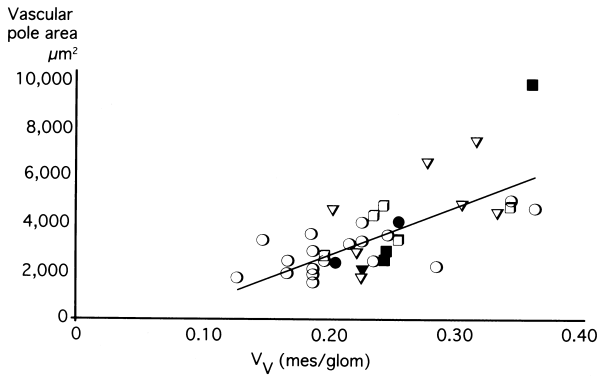

Fig. 6 The inter-biopsy correlation between vascular pole area and mesangial volume fraction in IDDM group 2 is statistically significant. r = 0.70, $P < 10^{-4}$. Signatures as in Fig. 3

lation with VPA in IDDM group 2 and with VPA/GS in the same group (Table 4), whereas no correlation was seen in IDDM group 1. An inverse correlation was obtained both between GFR and VPA and between CFR and VPA/GS in IDDM group 2 (Table 4). This relationship also persisted in a subgroup including only low range microalbuminuria, i.e. AER 15–50 μg/min (n = 23, r = –0.49, P = 0.02), but no correlation was seen in group 1. The systemic blood pressure did not correlate with structural dimensions in either group, and in IDDM group 2 no correlation was observed between the level of albumin excretion and structures.

Discussion

The glomerular hypertrophy that occurs in diabetes mellitus has attracted much attention. Early studies indicated glomerular hypertrophy observed at the time of diagnosis in a small group of patients [18]. The actual presence of acute glomerular hypertrophy at the onset of diabetes has been extensively supported by observations in experimental diabetes. In rats with induced diabetes, marked renal and glomerular hypertrophy is demonstrable within a few days [6, 23]. In the present series glomerular volume was not significantly increased in the IDDM group 1 patients. Earlier studies had also indicated that glomerular hypertrophy was less evident during the early years after the initial acute onset of diabetes [2, 18]. It may well be, therefore, that glomerular hypertrophy is not marked or is even absent in the great majority of diabetic patients for some years after the onset of diabetes. The observation of clearly increased glomerular volume in the patients with microalbuminuria may indicate the onset of compensatory hypertrophy even at this early stage of nephropathy. An alternative explanation could be that patients with larger glomeruli are more prone to the development of nephropathy. The present series included patients with long-standing diabetes, and it was found that these patients with a fairly slow development of nephropathy had more marked mesangial expansion at a relatively low level of albumin excretion than we have found in other series. The patients with long-term diabetes belonged preferentially to the biopsy series obtained after treatment with either a calcium channel blocker or an ACE (angiotensin-converting enzyme) inhibitor for 18 months. A drug effect on the structures measured in the present study cannot be excluded. However, there were no differences within the groups treated with the two drugs; neither was there any change in blood pressure or GFR over the 18 months, while AER showed a slight decrease. It seems more likely that the sluggish development of diabetic glomerulopathy during long-standing diabetes has favoured the development of compensatory changes and that it is compatible with more advanced glomerulopathy and hypertrophy before albuminuria appears. This seems to be supported by the results of Bilous et al. [2], who did not find a significant increase in glomerular volume in IDDM patients with or without nephropathy after a duration of 15 years, whereas patients with nephropathy and 25 years' duration of IDDM had enlarged glomeruli. Marked compensatory hypertrophy has previously been described in patients in more advanced stages of nephropathy [9].

The moderate structural changes in the kidney at the stage of microalbuminuria may be associated with functional consequences, since patients showing a slight decline in GFR during the years preceding the biopsy were found to have more advanced glomerulopathy than patients with a stable GFR [21].

The vascular pole area was clearly increased in both absolute and relative terms, and to the same extent in the normoalbuminuric IDDM patients as in patients with ele-

vated AER. It is particularly in the early and preclinical stages of nephropathy that the GFR is increased in diabetes mellitus. However, we found no correlation between GFR and VPA in IDDM group 1. In contrast, the inverse correlation in IDDM patients group 2 indicated that the enlargement of the vascular pole area together with glomerular volume increase in this group represents a compensation, which may be triggered by the developing glomerulopathy and the glomerular occlusion that starts to appear as a significant finding in patients with microalbuminuria. Obviously, the relationship between GFR and structural data must be regarded with some reservations, since different methods were used for GFR determination in the individual series.

The large interglomerular variation within biopsies in all the structural parameters reported here was inconvenient, since it necessitated sampling of a fairly large number of glomeruli. With the number of corpuscles measured per biopsy the coefficient of error was reasonably low, in most cases under 10%.

The localization of the glomeruli, whether superficial or juxta-medullary, cannot usually be established in biopsy tissue. There could well be systematic differences in the vascular pole area as has been reported for glomerular volume [5].

The Cavalieri principle for the estimation of glomerular volume is superior to previously used methods in that it is unbiased and provides estimates of intra-biopsy variation. The price to pay is that it is more time-consuming than working out estimates from average profile area [12]. The overestimation of glomerular volume from the maximum area, as seen in the present series, was previously reported by Lane et al. [13]. When the large intra-biopsy variation in glomerular volume was taken into account it was decided to include the (minority of) measurements from the maximum area as the average of the four largest areas, rather than having fewer determinations per biopsy.

The enlargement of the vascular pole area corresponds well with the recent demonstration of an enlargement of the juxtaglomerular apparatus in IDDM patients with micro-albuminuria [8], so that the whole region of importance for glomerular haemodynamic control is enlarged in a manner that is out of proportion to the glomerular enlargement. It is noteworthy, however, that these changes are not associated with an increase in glomerular capillary diameter. Similarly, it has been found that glomerular hypertrophy in experimental diabetes occurs by way of an increase in the number of capillaries and is not associated with an increase in capillary diameter [16]. The detailed measurements of the structures in the vascular pole region in the above mentioned study of IDDM patients showed that the luminal area of the afferent and efferent arterioles is increased to the same extent, so that the ratio between these two areas remains the same in the diabetic patients as it is in controls. This observation, and the lack of association between size of vascular pole area and capillary diameter, may be important when possible consequences for glomerular haemodynamics and, in particular, intracapillary pressure are considered. The interplay between these functional variables and the development of diabetic nephropathy needs further investigation.

The present study shows that the vascular pole area is increased in IDDM patients, both in terms of absolute size and relative to glomerular size. The enlargement of this region may partly represent a compensatory phenomenon triggered by the developing glomerulopathy.

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