The number of glomeruli in Type 1 (insulin-dependent) and Type 2 (non-insulin-dependent) diabetic patients*

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Summary. The number of glomeruli per kidney in Type 1 (insulin-dependent) and Type 2 (non-insulin-dependent) diabetic patients was estimated by an unbiased stereological method: the fractionator. No significant differences were observed between Type 1 and Type 2 diabetic patients without severe diabetic glomerulopathy and non-diabetic patients. Diabetic patients with proteinuria who were in the early stages of diabetic nephropathy also had a normal number of glomeruli. On the other hand, a subgroup classified as Type 1 diabetic patients with severe diabetic glomerulopathy had

significantly less glomeruli compared with Type 1 diabetic patients with mild or no glomerulopathy. A probable explanation is that Type 1 diabetic patients lose glomeruli in relation to the progression of diabetic glomerulopathy. A more theoretical alternative is, however, that development of diabetic glomerulopathy is facilitated by a low number of glomeruli.

Key words: Glomerular number, diabetic nephropathy, diabetic glomerulopathy, stereology.

Brenner et al. [1] have proposed that inborn differences in numbers of nephrons contribute to the susceptibility of patients with diabetes mellitus developing renal disease: patients endowed with a reduced filtration surface area might represent the subset of diabetic patients who exhibit persistent hyperfiltration early in the course of diabetes, and later progress to uraemia. According to this hypothesis, the initially increased whole kidney glomerular filtration rate (GFR) despite reduced numbers of nephrons may be explained by marked single nephron hyperfiltration induced by diabetic metabolic abnormalities. In fact, family studies have shown that diabetic nephropathy occurs in familial clusters in Type 1 (insulin-dependent) [2] and Type 2 (non-insulin-dependent) diabetic patients [3]. Genetically determined low numbers of glomeruli might predispose diabetic patients to diabetic nephropathy.

Another possibility is that glomeruli disappear during the course of diabetic glomerulopathy without leaving recognizable corpuscles, analogous to other chronic renal diseases [4]. If a difference in the number of visible glomeruli between diabetic patients, depending on the severity

of glomerulopathy, turned out to be significant, then comparisons of total filtration surface area and GFR would not make sense in studies where the number of glomeruli is assumed not to differ between the groups [5–8].

Kroustrup et al. [9], found a significantly increased glomerular filtration surface area per glomerulus in newly-diagnosed Type 1 diabetic patients. The result was based on observations made from a few glomeruli in one biopsy per patient. When the authors concluded that the total filtration surface area – in the whole kidney – was significantly increased in diabetic patients, they then assumed that diabetic patients do not have an abnormal number of glomeruli.

The number of glomeruli in diabetic patients has not previously been estimated. We wanted to make an unbiased and efficient estimation of the number of glomeruli in diabetic kidneys to determine whether the number of glomeruli is abnormal in diabetic kidneys [10, 11].

Subjects and methods

Kidneys were obtained from autopsy cases. Only one kidney was chosen per individual, the choice of left or right was arbitrary. During the years of kidney sampling most people in Denmark died in hospital [12], therefore, the sampled kidneys should constitute a representative sample of kidneys from deceased diabetic patients in Denmark.

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Table 1. The age, sex, height, surface area, kidney weight, glomerular number, diabetes duration, state of the renal function and histological classification of 25 Type 1 (insulin-dependent) diabetic patients

No.	Age (years)	Sex	Height (cm)	Surface area (m ²)	Kidney weight (g)	Glom. number (10 ³)	Duration of diabetes (years)	State of renal function	Histological classification
1	16	f	156	1.59	130	492	15	- P	I
2	33	m	170	1.73	180	759	29	- P	I
3	33	m	181	1.98	178	560	9	- P	0
4	33	f	165	1.64	149	548	29	+ P	I
5	40	m	164	1.53	74	365	35	D	II
6	41	m	170	1.69	124	531	27	D	II
7	43	m	161	1.55	172	1052	41	+ P	I
8	45	f	156	1.40	82	419	20	D	II
9	45	m	174	1.93	167	936	34	+ P	0
10	49	m	184	1.84	84	353	30	T + D	II
11	51	m	163	1.73	158	649	48	+ P	I
12	51	m	170	1.86	218	652	14	+ P	I
13	53	m	182	1.93	72	143	39	T	II
14	53	m	181	1.95	152	645	41	+ P	I
15	59	f	160	1.86	102	510	43	- P	0
16	63	f	170	1.79	157	640	34	- P	0
17	64	m	167	1.55	154	636	35	- P	0
18	64	f	164	1.63	117	482	29	D	II
19	65	f	162	1.63	59	318	56	D	II
20	65	m	170	1.73	164	511	25	+ P	I
21	68	m	179	1.92	174	626	35	+ P	0
22	70	m	169	1.64	127	379	59	- P	I
23	73	f	154	1.41	116	680	58	- P	0
24	77	f	170	1.71	77	389	53	- P	0
25	82	f	154	1.34	103	543	60	+ P	I
$\bar{X}$	54		167	1.72	149	623	37	Histological classification 0 + I	
CV	0.33		0.05	0.12	0.23	0.27	0.42		
$\bar{X}$	51		169	1.66	87	373	34	Histological classification II	
CV	0.20		0.06	0.11	0.28	0.34	0.34		
$\bar{X}$	58		166	1.70	131	617		Control subjects n = 36	
CV	0.34		0.05	0.13	0.26	0.25			

The values of control subjects are stated at the bottom. State of renal function: - P, no proteinuria; + P, proteinuria; D, dialysed; T, transplanted. Histological classification: 0, no diabetic changes; I, solitary glomeruli with diffuse and nodular lesions; II, many glomeruli with severe diffuse and nodular lesions. Type 1 diabetic patients with severe glomerulopathy, 5, 6, 8, 10, 13, 18, and 19 are exclusively classi-

fied in the categories "D" and "T" and "II". Patient no. 8 died in uraemia without dialysis or transplantation because treatment was determined futile. Patient no. 7 had a double crus amputation: height correction + 32 cm; weight correction + 4 kg. Patient no. 24 was femur and crus amputated: height correction + 32 cm; weight correction + 8 kg

Exclusion criteria

Macro- or microscopic kidney disease other than diabetic nephropathy, disseminated malignancies, cachexia, nephrectomy, removal of renal tissue (except for small biopsies), inborn kidney malformations, or treatment with cytostatics or regional X-rays. In the case of kidney transplantation in diabetic patients, the native kidney was taken. Patients were excluded if data about blood pressure and urine-protein were absent.

Control patients were also excluded if they had been treated for hypertension or diagnosed as hypertensive during the final hospitalization or had abnormal kidney function (proteinuria = reagent-strip testing for bromphenol blue reaction; positive: over 0.3 g protein per 1 litre urine), as were kidneys that later showed microscopic evidence of kidney disease. The control cases have been described in more detail in an earlier publication [13]. The results of the control cases are summarized in Table 1 and Table 2.

Inclusion criteria

Differentiation of Type 1 and Type 2 diabetes from other types was made in accordance with the World Health Organisation Study Group [14]. The differentiation between Type 1 and Type 2 diabetes

was based on the following criteria: (A) Patients treated without insulin for at least one year after diagnosis of diabetes irrespective of age, or aged above 45 years at diagnosis and survival on diet with or without oral hypoglycaemic drug therapy in a period of at least one month, were classified as Type 2 diabetic patients [15]. (B) Patients with diabetes not fulfilling the above criteria, with age at the time of diagnosis below 30 years and treated with insulin at onset of diabetes were classified as Type 1 diabetic patients, as were all insulin-treated patients with ketonuria at the time of diagnosis or later [16]. Diabetic subjects initially treated without insulin, but later with insulin, were excluded from this study.

Stereological methods

To improve immersion fixation in formaldehyde, each kidney was split in half. The kidneys were weighed after a period of fixation of 1 to 6 months. A known fraction of the whole kidney was sampled systematically at random, and in that fraction the number of glomeruli was counted. Then, the total number of glomeruli was the number of counted glomeruli in that fraction multiplied by the reciprocal sampling fraction. The practical method (fractionator) has been explained in detail in a previous publication [11], and the theoretical basis has been explained by Gundersen [17].

Table 2. The 39 Type 2 (non-insulin-dependent) diabetic patients shown with their age, sex, height, surface area, kidney weight, glomerular number, diabetes duration, state of renal function and histological classification

No.	Age (years)	Sex	Height (cm)	Surface area (m ²)	Kidney weight (g)	Glom. number (10 ³)	Duration of diabetes (years)	State of renal function	Histological classification
1	35	f	163	1.90	107	650	6	-P	0
2	50	m	173	1.82	163	1124	2	-P	0
3	52	m	179	2.00	172	681	5	-P	0
4	53	m	182	2.16	228	1115	1	-P	0
5	53	m	165	1.68	132	442	9	-P	0
6	56	m	182	2.02	143	475	9	-P	0
7	57	f	170	1.86	228	793	13	-P	0
8	59	f	155	1.73	198	886	6	-P	0
9	60	f	161	1.52	109	530	1	-P	0
10	62	m	182	2.14	138	661	5	-P	0
11	64	f	155	1.58	155	658	6	-P	0
12	65	m	176	1.93	126	526	13	-P	0
13	65	m	162	1.69	153	540	5	-P	0
14	65	m	166	1.93	192	881	5	-P	0
15	65	f	160	1.46	48	251	6	-P	0
16	65	m	170	1.96	191	969	2	-P	0
17	65	m	174	2.06	139	504	10	+P	0
18	66	m	174	2.04	141	658	6	-P	0
19	66	m	160	1.51	134	632	2	-P	0
20	66	f	164	1.53	123	470	9	-P	0
21	66	f	161	1.82	105	532	3	-P	0
22	67	f	165	1.56	95	656	0.4	-P	0
23	68	m	165	1.77	164	663	0.2	-P	0
24	68	m	175	2.25	172	614	7	+P	II
25	69	f	160	1.62	161	548	15	-P	I
26	70	f	166	1.78	143	437	1	-P	0
27	71	f	158	1.58	129	458	10	-P	0
28	72	f	158	1.95	168	788	4	+P	I
29	73	m	166	1.82	137	833	10	-P	0
30	75	f	148	1.49	103	412	4	-P	0
31	76	m	176	1.73	197	502	20	+P	0
32	77	m	165	1.66	97	690	1	-P	0
33	78	m	170	1.81	146	640	8	+P	I
34	78	f	158	1.73	86	301	5	-P	0
35	79	f	158	1.81	148	524	14	-P	I
36	85	f	163	1.74	97	419	14	-P	0
37	85	f	168	1.87	82	379	8	-P	0
38	89	m	167	1.69	141	516	14	-P	0
39	89	m	170	1.81	120	348	8	+P	I
$\bar{X}$	67		166	1.80	141	608	7	Type 2 diabetic patients	
CV	0.17		0.05	0.11	0.28	0.34	0.69		
$\bar{X}$	69		165	1.67	128	594		Control subjects	
CV	0.19		0.05	0.13	0.29	0.28		n = 25	

The values of control subjects are stated at the bottom. State of renal function: -P, no proteinuria; +P, proteinuria. Histological classification: 0, no diabetic changes; I, solitary glomeruli with diffuse and nodular lesions; II, many glomeruli with severe diffuse and nodular lesions

Example: The two kidney halves were embedded in agar and placed on their cut surfaces. Each half was cut perpendicular to its length into a series of slabs at intervals of 4 mm with a tissue slicer. The first kidney slab was sampled among slabs no. 1–4 by means of a random number (R) between 1 and 4. Every fourth slab in the series was sampled, i.e. $p_1 = \frac{1}{4}$. In the second series the first slab was sampled among no. 1–4 by means of $R \pm 2$. Medulla was excised from the sampled slabs except for a narrow rim to avoid excision of glomeruli. The sampled slabs were embedded upright in agar maintaining their original order, thus forming two “shortened” kidney halves. Agar slabs were cut parallel to the length of the “shortened” kidney halves perpendicular to the foundation at intervals of 5 mm producing agar slabs containing kidney strips. The kidney strips were sampled according to the sampling principle described in the first step with

$p_2 = \frac{1}{5}$. The precision was even further increased in this sampling step using the “Modulus System” [11]. The sampled strips were embedded in agar in their original order. They were cut perpendicular to their length at intervals of 1.5 mm producing agar slabs containing kidney blocks. The maximum dimensions of the kidney blocks were thus $4 \times 5 \times 1.5 = 30 \text{ mm}^3$. Every seventh kidney block was sampled as described above, i.e. $p_3 = \frac{1}{7}$. These kidney blocks were dehydrated in graded alcohol, embedded in plastic (JB-4, Polysciences, Inc., Warrington, Pa., USA) and serially sectioned on an LKB Histo-range microtome with a section thickness of 15 μm . Every eighth section (sampling section) was sampled systematically at random, i.e. $p_4 = \frac{1}{8}$. At this step the total fraction sampled of the renal cortex

Fig. 1. The number of glomeruli in the group of Type 1 (insulin-dependent) diabetic patients with mild or no glomerulopathy (○) is not correlated to age. Patients with severe glomerulopathy are indicated by (●)

Fig. 2. The number of glomeruli in the Type 1 (insulin-dependent) diabetic patients with mild or no glomerulopathy (○) regresses significantly with kidney weight. The slope is 3300 glomeruli per gram kidney weight. The Type 1 diabetic patients with severe glomerulopathy are indicated by (●)

was: $f_n = \frac{1}{4} \times \frac{1}{5} \times \frac{1}{7} \times \frac{1}{8} = \frac{1}{1120}$. The section succeeding a sampling section was also collected, and designated the look-up section. The sections were stained with periodic acid Schiff.

Two standard light monocular microscopes with mirrors were used to project the fields of vision side by side onto a table in a dark room. A counting grid was drawn on the table in the projected field of vision of the first microscope. The sampling section was placed in the first microscope and the look-up section in the second microscope. The stage of the first microscope was moved by a motor in predetermined, equidistant steps in two orthogonal directions making the counting grid cover a known fraction of the sampling section, f_a . The linear magnification of $115\times$ and total grid area of $59,731 \text{ mm}^2$ resulted in an area of the section covered by the grid of $\frac{59,731}{115 \times 115} = 4.52 \text{ mm}^2$. The motor was adjusted to step 3.27 mm along the length of the microslide and 3.05 mm orthogonal to the length. Therefore the fraction of the section area covered by the grid was: $f_a = \frac{4.52}{3.27 \times 3.05} = 0.45$. The fraction of the renal cortex sampled at this step was $\frac{1}{1120} \times 0.45 \approx \frac{1}{2500}$.

A glomerulus was counted, Q^- , only if it appeared in the sampling section and not in the look-up section: this event occurs only once per glomerulus in the section series. Thus all glomeruli were sampled with an equally known probability [18]. Glomeruli were only counted if they were surrounded by intact cortex. Glomeruli too close to either artificially cut edges or surfaces were excluded for counting by means of a specific counting rule [11]. An arbitrary point indicated on the grid was used to estimate the fraction of the sampled cortex sections used for glomerular counting, $\Sigma P/P_s$. P_s was the total number of times the arbitrary point hit renal cortex. P_t was the total number of times the point hit cortex used for glomerular counting. In our example $\Sigma Q^- = 141$, $P_t = 105$, $P_s = 195$

$$N(\text{glom}) = \frac{1}{f_n} \times \frac{1}{f_a} \times \frac{P_s}{P_t} \times \Sigma Q^- = 1120 \times \frac{1}{0.45} \times \frac{195}{105} \times 141 \approx 652 \times 10^3$$

$N(\text{glom})$ was the estimate of the total number of glomeruli in the kidney. All glomerular structures were counted – both open and occluded glomeruli.

The total variation ($CV_{\text{tot}} = \frac{SD}{\text{mean}}$) of the number of glomeruli in control subjects and diabetic patients was within the range of 25 to 34 % (Table 1 and 2). CV_{tot} resulted from biological variation (CV_{bio}), and variation of the estimates due to the stereological method (CE_{ste}): $CV_{\text{tot}}^2 = CV_{\text{bio}}^2 + CE_{\text{ste}}^2$ [19]. The coefficient of error ($CE_{\text{ste}} = \frac{SEM}{\text{mean}}$) of the method was estimated to 4.8 % [13]. Thus, the total variation of the number of glomeruli in a group of patients can almost be completely ascribed to true biological variation of the number of glomeruli among the individuals in the group of patients.

Histological classification

A very experienced pathologist made a semiquantitative light microscopical classification of all kidneys without knowledge of the patient category. Classifications were 0: no diabetic changes; I: solitary glomeruli with diffuse and nodular lesions; II: many glomeruli with diffuse and nodular lesions.

All control subjects were classified as 0. In "Results" we used only two categories for each type of diabetes: Patients from II with severe glomerulopathy (SGP) and patients from 0 and I with no or mild glomerulopathy (NMGP).

Clinical classification

All diabetic patients have been classified into four groups: – P: no proteinuria; + P: proteinuria; D: dialysed patients; T: kidney transplanted patients.

Body surface area was estimated using the method of DuBois and DuBois [20]. Patients with double sided crus or femur amputation were corrected for body length and weight, and patients with unilateral femur amputation were corrected for body weight, before estimating body surface area.

Statistical analysis

Student's unpaired t -test, standard multiple regression analysis and multiple hierarchical regression analysis [21], with a two-tailed significance level of 0.05 were used. Comparison of the slopes of the regression lines and their intersection with the ordinate was performed in accordance with Lentner [22].

Results

The data of the 37 control subjects have previously been tabulated [13]. Comparisons between control subjects and diabetic patients were performed after age- and sex-

Fig. 3. The individual and the mean values of the number of glomeruli for 36 control subjects (C) from Nyengaard and Bendtsen (13), for the whole group of Type 1 (insulin-dependent) diabetic patients (—), for the 18 Type 1 diabetic patients without severe glomerulopathy (---), for the seven Type 1 diabetic patients with severe glomerulopathy (-.-.-) and for the 38 Type 2 (non-insulin-dependent) diabetic patients without severe glomerulopathy. The mean value of number of glomeruli in the last group and the number of glomeruli in the single Type 2 diabetic patient with severe glomerulopathy do coincide. Patients with severe glomerulopathy are indicated by (●), whereas patients without severe glomerulopathy are indicated by (○).

matching: 36 of the control subjects were compared to the 25 Type 1 diabetic patients, and 25 of the control subjects were compared to the 39 Type 2 diabetic patients. The mean number and coefficients of variation in control subjects are listed at the bottom of Tables 1 and 2. In the control subjects the number of glomeruli shows significant negative regression with age ($r = 0.35$, $2p < 0.05$) and shows significant positive regression with kidney weight ($r = 0.68$, $2p < 0.05$).

Table 1 displays the data of the Type 1 diabetic patients, and the data of the Type 2 diabetic patients are listed in Table 2.

Eighteen Type 1 diabetic patients had no or mild glomerulopathy (NMGP). Their mean number of glomeruli of $623 \pm 168 \times 10^3$ showed no significant difference compared to that of control subjects of $617 \pm 155 \times 10^3$ ($2p \gg 0.05$). The NMGP group showed no significant regression in number of glomeruli with age (Fig. 1; $r = 0.29$, $2p > 0.05$). Employing hierarchical regression analysis the NMGP group showed significant positive regression in the number of glomeruli with kidney weight (Fig. 2; $r = 0.52$, $2p < 0.05$) even after elimination of the influence of age and body surface area.

Comparison of the mean number of glomeruli of the 18 Type 1 diabetic patients classified as NMGP ($623 \pm 168 \times 10^3$), with the seven classified as severe glomerulopathy ($373 \pm 126 \times 10^3$), showed a significant difference of 40% (Fig. 3; $2p \ll 0.05$).

The mean number of glomeruli of the nine Type 1 diabetic patients with (+P) proteinuria ($685 \pm 185 \times 10^3$) did not differ significantly from the mean number of glomeruli of the nine Type 1 diabetic patients without (-P) proteinuria ($561 \pm 130 \times 10^3$) ($2p > 0.05$).

The NMGP group of Type 2 diabetic patients showed no significant difference in number of glomeruli ($608 \pm 205 \times 10^3$) when compared to control subjects ($594 \pm 164 \times 10^3$) ($2p \gg 0.05$), but they showed significant negative regression in the number of glomeruli with age (Fig. 4; $r = 0.42$, $p < 0.05$) and positive regression in the number of glomeruli with kidney weight (Fig. 5; $r = 0.70$, $2p \ll 0.05$), and also in the number of glomeruli with body surface area ($r = 0.42$, $2p < 0.05$) even after elimination of the influence of age. The NMGP group of Type 2 diabetic patients showed significant negative regression in the number of glomeruli with known diabetes duration ($r = 0.33$, $2p < 0.05$), without correction for age influence. The slope of the regression line of glomerular number with age, does not show a significant difference between Type 2 NMGP diabetic patients and the matched control subjects ($2p > 0.85$). The intersections of the respective regression lines with the ordinate also show no significant difference ($2p > 0.95$). The co-variation of age and diabetes duration has an r -value of 0.29. With the number of glomeruli being unknown, the two patients contributing the most to this co-variation were removed, which reduced the r -value to 0.20. At that point the regression of the number of glomeruli with age was no longer significant ($2p > 0.05$).

The mean number of glomeruli of the six Type 2 diabetic patients with (+P) proteinuria ($566 \pm 150 \times 10^3$) did not differ significantly from the mean number of glomeruli of the 33 Type 2 diabetic patients without (-P) proteinuria ($610 \pm 212 \times 10^3$) ($2p > 0.05$). Only one Type 2 diabetic patient was classified as having severe glomerulopathy.

Discussion

The number of glomeruli in kidneys from Type 1 and Type 2 diabetic patients with no or mild glomerulopathy did not differ significantly from non-diabetic control subjects. On the other hand, a subgroup of the Type 1 diabetic patients died in a severe state of renal function with severe glomerulopathy: this group had significantly fewer glomeruli compared to Type 1 diabetic patients with no or mild glomerulopathy. It is not possible to decide whether the group of diabetic patients who died with severe nephropathy had relatively few glomeruli at onset of diabetes, as proposed by Brenner et al. [1], or lost glomeruli during the course of diabetic renal disease. However, three important points contest the validity of the hypothesis of Brenner:

Fig. 4. In Type 2 (non-insulin-dependent) diabetic patients with mild or no glomerulopathy (○), the number of glomeruli declines significantly with age. The slope is -7400 glomeruli per year. Only one patient has severe glomerulopathy (●)

Fig. 5. In Type 2 (non-insulin-dependent) diabetic patients with mild or no glomerulopathy (○), there is a significant regression of the number of glomeruli with kidney weight. The slope is 3500 glomeruli per gram kidney weight. Only one patient has severe glomerulopathy (●)

Firstly, after many years of diabetes some diabetic patients develop clinically manifest diabetic nephropathy with persistent proteinuria and declining GFR [23]. Without antihypertensive treatment uraemia develops on average 4 years after the debut of clinically manifest diabetic nephropathy [24]. In our material there was no difference in the number of glomeruli when patients classified as “no proteinuria ($-P$)” were compared with patients classified as “proteinuria ($+P$)”, either for the group of Type 1 or Type 2 diabetic patients. These observations support the explanation that glomeruli disappear in the course of severe diabetic glomerulopathy. There is, however, also the possibility that the decrease in glomerular number in patients on hemodialysis or having been transplanted is related to renal replacement therapy.

Secondly, Moritz and Hayman [4] found that glomeruli disappear in the course of chronic renal disease such as chronic diffuse glomerular nephritis and arteriosclerotic

disease. They also produced unilateral renal damage in rabbits by means of X-rays and experimental hydronephrosis. They found that 70% of the glomeruli in the damaged kidney disappeared when compared to the normal kidney. This also suggests that fewer glomeruli in diabetic kidneys are a result of diabetic renal disease, contrary to the hypothesis of Brenner et al. [1].

Finally, Gundersen and Østerby [25] observed that long-term diabetic patients showed a considerably increased relative number of occluded glomeruli compared with control subjects, and they also observed that the relative number of occluded glomeruli correlated closely to the duration of diabetes.

Hirose et al. [6] observed a very close correlation between GFR and glomerular filtration surface area in Type 1 diabetic patients with less than 5 years duration of diabetes. Ellis et al. [5] and Østerby et al. [7, 8] observed the same correlation in a sample of diabetic patients with long duration of diabetes and clinically manifest diabetic nephropathy, though not uraemic. The authors assumed that no glomeruli disappeared in the course of the renal disease. Their studies contained no end-stage diabetic patients, whereas all our Type 1 diabetic patients with severe glomerulopathy were uraemic. Thus our results support the above-mentioned assumptions.

Kroustrup et al. [9] observed a significant increase in glomerular filtration surface area (80%) per nephron in newly-diagnosed Type 1 diabetic patients compared with control subjects. The authors assumed a significant increase in the total glomerular filtration surface area in diabetic patients from this observation, and thus required that the number of glomeruli did not differ between the groups. Our results are in apparent support of that requirement.

Supposedly, the regression of glomerular number with diabetes duration in Type 2 NMGP diabetic patients is the result of ageing. Occluded glomeruli have been observed in column formations perpendicular to the kidney surface in Type 1 diabetic patients with severe glomerulopathy [26]. This finding suggests that an extraglomerular factor (probably atherosclerosis) causes occlusion and eventually loss of glomeruli in diabetic glomerulopathy. It would seem that such an extraglomerular factor does not have much effect in as early a stage as Type 2 NMGP diabetic patients. The lack of an age effect in the number of glomeruli among the NMGP Type 1 diabetic patients is most reasonably explained by the smaller representation of older patients in this group of 18 patients.

The correlation of the number of glomeruli and age has an r^2 value as low as 10%, i.e. that age may “explain” about 10% of the observed variation in glomerular number. This means that either the effect of age on the number of glomeruli is not pronounced, or otherwise the impact of other variables tends to obscure the observable correlation of the number of glomeruli and age.

In conclusion, diabetic patients in general do not have an abnormal number of glomeruli at onset of diabetes. Glomeruli seem to disappear in the course of severe diabetic glomerulopathy, but the theoretical possibility remains that diabetic patients with fewer glomeruli are predisposed to develop diabetic renal disease.

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