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Quantitative morphology of the rat kidney during diabetes mellitus and insulin treatment

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Summary A morphometric study was performed on moderately hyperglycaemic streptozotocin diabetic rats after 10 and 50 days of diabetes, and on groups of rats that, after initial hyperglycaemia for 50 days, were insulin treated for 2 h or for 5, 15 or 38 days. A group of hyperglycaemic diabetic animals were fasted for 18 h. Another group of rats had acute hyperglycaemia induced by intravenous glucose injection. After 10 and 50 days of diabetes, kidney weight was increased by 55 and 93 %. Glomerular volume, tubule length, and tubular and interstitial volume increased in diabetic animals compared with controls. After 4 h insulin treatment, the kidney weight was 20 % decreased; after 5 days it was 31 % decreased. After 38 days the kidney weight was still 26 % greater than in controls. In diabetic animals, 18 h fasting induced a 30 % decrease in kidney weight. In normal animals, acute hyperglycaemia induced a 22 % increase in kidney weight. Volume fractions of most kidney structures remained similar in all groups. However, the glomerular volume fraction was smaller during kidney enlargement, and the tubular volume fraction was larger after induced hyperglycaemia compared with controls. In conclusion, high blood glucose levels in diabetic and normal animals are associated with increased kidney weight. In hyperglycaemic diabetic animals, normalization of blood glucose after insulin treatment or fasting was followed by a decrease in kidney weight. [Diabetologia (1997) 40: 802–809]

Keywords Diabetes mellitus, experimental, kidney, diabetic nephropathy, morphometry.

Kidney enlargement in streptozotocin (STZ) diabetic rats is well documented [1–4]. This growth is mainly due to hyperplasia and to a lesser extent to cellular hypertrophy [2, 5]. After insulin treatment and normalization of blood glucose concentration (BGS), kidney weight and glomerular volume decrease [6]. However, little is known about how fast the morphology changes following normalization of BG by insulin treatment and about the morphology of the diabetic kidney after insulin treatment or fasting.

In the present study, quantitative estimation of each tissue component was performed during experimental diabetes and during insulin treatment. The results were compared with the kidney weight response and morphology in fasted diabetic animals and with the kidney weight and morphology in normal animals with hyperglycaemia induced by i. v. glucose injection.

Information on kidney morphology in hyperglycaemia and normoglycaemia is a prerequisite to understanding better the physiological abnormalities found in diabetic patients.

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Abbreviations: CN, Controls; DH10, diabetic, hyperglycaemic for 10 days; DH50, diabetic, hyperglycaemic for 50 days; DH-N2 h, diabetic, hyperglycaemic for 50 days, insulin treated 4 h, normoglycaemic 2 h, DH-N5 d, normoglycaemic 5 days; DH-N15 d, normoglycaemic 15 days; DH-N38 d, normoglycaemic 38 days; DH-F, diabetic, hyperglycaemic for 50 days and fasted 18 h; CN-H10 m, controls, hyperglycaemic 10 min by i. v. glucose infusion, STZ, steptozotocin; BG, blast glucose concentration; PT, proximal tubule; DT, distal tubule; Taα, thick ascending loop of Henle; THP, Tamm-Horsfall glycoprotein.

Materials and methods

Animals. Female Wistar rats (Møllegaard, Eiby, Denmark) were housed at 21 °C at a 12-h light and 12-h dark cycles. The animals had free access to water and standard rat pellets (Altromin 1324). The mean body weight was 127 g ± 5 (mean ± SD) at the start of the experiment.

The study included normoglycaemic controls (CN) that lived from 50 to 88 days after the start of the experiment, and two groups of animals that were diabetic and hyperglycaemic for 10 (DH10) and 50 (DH50) days. The DH10 animals started the study later than the DH50 animals so that the two groups were the same age at the end of the study. The study also included five groups of diabetic animals that were initially diabetic and hyperglycaemic for 50 days (DH) and then treated with insulin aiming at normoglycaemia (DH-N) for various periods. Animals in all experimental groups were between 50 and 88 days of age like the controls. In addition, one group of DH50 animals were fasted (DH-F) and one group of controls was given i.v. glucose and was hyperglycaemic for 10 min (CN-H10m).

Experimental diabetes was induced by i.v. injection of 65 mg (STZ) (Upjohn Inc. Kalamazoo, Ill., USA). Diabetes and hyperglycaemia developed on day 2 after STZ administration. Thereafter DH animals were treated daily with small doses of long acting, heat treated ox insulin (Novo Ultralente; pH 5.5, not for human use, Novo, Copenhagen, Denmark) [23]. After 50 days of diabetes, the animals in group DH-N2 h received 10 IE insulin (Insulatard -X; Novo Nordisk) and BGc was measured every 30 min for 4 h. The other DH-N animals had their BGc measured in the morning and then received the above-mentioned Ultralente in insulin in doses designed to normalize the BGc for the following 24 h.

The DH-F animals were diabetic for 50 days and insulin was omitted for 2 days before the 18-h fasting period. The animals had free access to water during the fasting period. BG was measured prior to fasting and every second hour. Normal, age matched animals were given 1 ml 50% glucose in the femoral vein and the kidneys were perfusion fixed after 10 min.

Fixation and preparation for light microscopy. The kidneys were fixed by retrograde perfusion through the abdominal aorta for 4 min with 1% glutaraldehyde in a modified Tyrode buffer with a final osmolality of 248 mosm and pH 7.4 [7].

The kidneys were cut horizontally into 2 mm slices with mounted razor blades [8]. From the slices, five randomly sampled $2 \times 2 \times 1$ mm blocks were cut from the cortex and from the outer stripe of the outer medulla (OSOM) and embedded in Epon 825. Semi-thin sections were stained with toluidine blue and periodic acid schiff (PAS) for light microscopy.

Stereology. The kidney volume was derived from the weight assuming a density for the kidney of one g/ml [4]. All volume densities were measured by point counting [9]. The stage of the microscope was set outside the section and the fields to be counted were selected by moving the stage in preselected steps marked on the handle of the microscope. Stereological measurements of the inner stripe of the outer medulla and the inner medulla were not done since satisfactory preservation of the medulla was not possible with the fixative of low osmolarity used for optimal fixation of the two outer zones [10]. The number of points falling on the glomerular tuft, proximal tubule wall, proximal tubule brush border, proximal tubule lumen, on the distal tubule wall, distal tubule lumen, and interstitium was counted. An average of 30 fields was counted per animal.

In the present study, the term distal tubule included the distal straight tubule, the distal convoluted tubule, the connecting tubule, and the collecting duct. The estimations were made this way because discriminating decisively between these distal nephron segments was not possible at the magnifications used. The descending and ascending thin limbs of the loop of Henle were not estimated separately, since they could not be distinguished from the vessels at the light microscope level. These structures were thus included in the interstitium. The volume of all glomeruli (V_G) was estimated by

$$V_G = V_v(c/k) \cdot V_v(g/c) \cdot kv$$

where $V_v(c/k)$ is the volume density of the cortex in the kidney, $V_v(g/c)$ is the volume density of glomeruli in the cortex, and kv is the kidney volume. Correspondingly, the total tubular volume (TWV) was estimated by

$$TWV = V_v(c/k) \cdot V_v(t/c) \cdot kv$$

where $V_v(t/c)$ is the volume density of tubuli in the cortex. The total length of a tubular segment per unit volume (L_v), was estimated by

$$L_v = 2 \cdot N_A$$

where N_A is the number of tubular profiles per unit area on the thin plastic sections [11]. The total length of tubules (L_T) in the cortex was estimated by

$$L_T = L_v(t/c) \cdot V_v(c/k) \cdot kv$$

where $L_v(t/c)$ is the length per unit volume of all tubules in the cortex. The mean cross-sectional area perpendicular to the longitudinal axis of a tubule (A) was estimated by

$$A = V/L$$

where V is the volume and L is the length of the tubule. Assuming a circular cross section, the average outer diameter (D_o) of tubules was calculated by

$$D_o = 2 \cdot \sqrt{A / \pi}$$

[32] and the inner tubular diameter (D_i) was estimated correspondingly by using the volume of the tubule lumen instead of the total tubule volume. The average height (H) of tubular cells was calculated as

$$H = (D_o - D_i)/2.$$

Statistical analysis. Differences between two experimental groups were tested with Student's *t*-test. When more than two groups were compared, one-way analysis of variance (ANOVA) was used. When the ANOVA reached significance, differences between groups were tested with Student's *t*-test with the Bonferroni adjustments made for multiple comparisons. *p* values below 0.05 were considered statistically significant. Values are presented as means ± SD.

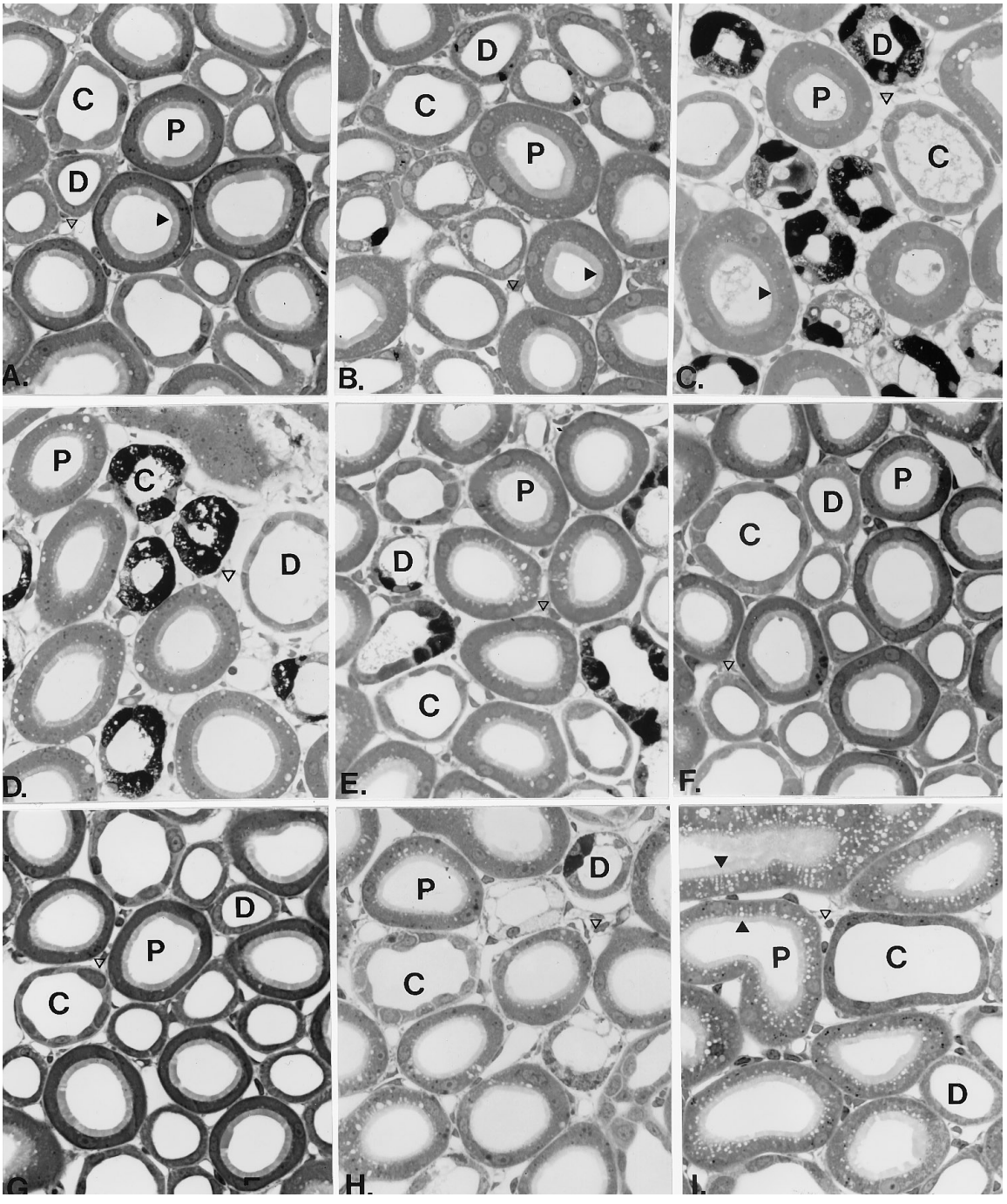

Fig. 1 A–I. Light micrographs of perfusion fixed kidney tissue from all experimental groups. Magnification × 275. P: proximal tubular profiles and ▶: brush border, **D**: distal tubular profiles, **C**: collecting duct profiles and ▽ interstitium. **A**: Control group (CN). Proximal and distal tubular profiles are open and round. In the proximal tubules, the brush border is clearly delineated and smooth as an indication of optimal fixation. **B**: Tissue from diabetic animal, hyperglycaemic for 10 days (DH10). In the proximal tubules the cells are higher than in controls and the brush border well preserved. In the distal tubules, discrete accumulation of glycogen is seen in some distal tubule profiles. **C**: Tissue from diabetic animal, hyperglycaemic for 50 days (DH50). The interstitium and the wall of the proximal tubule appear enlarged. Glycogen accumulation is seen in several distal tubular profiles. The accumulations either appear as solid black (PAS positive) areas or as more or less empty cells. The amount of interstitial tissue is increased. **D**: Tissue from diabetic animal, hyperglycaemic for 50 days, then insulin treated. The animal became normoglycaemic after 2 h and was perfusion fixed after 4 h (DH-N2 h). In the proximal tubules the cell height has decreased, but glycogen is still seen in the distal tubular profiles. **E**: Tissue from diabetic animal, hyperglycaemic for 50 days, then insulin treated for 5 days (DH-N5 d).Glycogen is still present in the distal tubular profiles but less than that of diabetic animals after 50 days duration of diabetes. **F**: Tissue from a diabetic animal, hyperglycaemic for 50 days, then insulin treated for 15 days (DH-N15 d). The tissue appears normal. G: Tissue from a diabetic animal, hyperglycaemic for 50 days, then insulin treated for 38 days (DH-N38 d). The tissue does not appear different from controls in A. **H**: Kidney tissue from a diabetic animal, hyperglycaemic for 50 days, then fasted for 18 h (DH-F). Glycogen is present in distal tubular profiles. **I**: Control animal that has been hyperglycaemic 10 min after i. v. glucose infusion. In the proximal tubules numerous vesicles are seen (▶).

Table 1. End body weight, kidney weight, and blood glucose

	n	Body weight (BW) (g)	Kidney weight (KW) (g)	KW/BW (%)	Blood glucose (mmol/l)
CN	7	230.0 ± 20.2	0.85 ± 0.08	0.37 ± 0.04	5.0 ± 0.4
DH10	5	207.8 ± 3.3	1.32 ± 0.22[b]	0.64 ± 0.11[b]	22.3 ± 3.0[b]
DH50	9	182.9 ± 19.2[a]	1.64 ± 0.19[b]	0.90 ± 0.09[b]	20.6 ± 2.9[b]
DH-N4	6	169.8 ± 6.9	1.31 ± 0.13[c]	0.77 ± 0.07[c]	4.4 ± 1.9[e]
DH-5d	6	206.4 ± 4.7[c]	1.13 ± 0.04[e]	0.55 ± 0.01[e]	3.3 ± 1.2[e]
DH-15d	5	213.0 ± 17.3[d]	1.10 ± 0.05[e]	0.52 ± 0.02[e]	3.3 ± 1.1[e]
DH-38d	5	223.4 ± 14.0[e]	1.06 ± 0.09[e]	0.47 ± 0.04[e]	7.3 ± 5.0[e]
DH-F18h	5	183.0 ± 7.9[e]	1.19 ± 0.14[e]	0.65 ± 0.06[e]	4.5 ± 0.9[e]
CH10	5	227.8 ± 10.4	1.23 ± 0.05[b]	0.54 ± 0.02[b]	25.7 ± 3.8[b]

Values are means ± SD.
[a] $p < 0.01$; [b] $p < 0.001$ for comparison with controls
[c] $p < 0.05$; [d] $p < 0.01$; [e] $p < 0.001$ for comparison with the diabetes 50 days group

Table 2. Volumes of kidney structures

Group	*n*	Glomerulus	Proximal tubule		Distal tubule		Interstitium
		Volume (mm^3)	Wall (mm^3)	Lumen (mm^3)	Wall (mm^3)	Lumen (mm^3)	(mm^3)
CN	7	32.2 ± 2.4	211 ± 20	101 ± 39	70 ± 13	45 ± 16	96 ± 12
DH10	5	43.3 ± 8.5[a]	343 ± 40[c]	136 ± 38	122 ± 25[c]	101 ± 34[b]	139 ± 22[b]
DH50	9	60.0 ± 5.8[c]	410 ± 60[c]	203 ± 62[b]	157 ± 33[c]	113 ± 24[c]	164 ± 21[c]
DH-N4h	6	50.6 ± 3.1	330 ± 41	162 ± 26	120 ± 19	94 ± 10	114 ± 31[e]
DH-N5d	6	44.8 ± 4.2[e]	277 ± 24[e]	121 ± 21[d]	102 ± 6[e]	76 ± 10[d]	128 ± 22[e]
DH-N15d	5	45.3 ± 3.1[e]	290 ± 20[e]	104 ± 21[d]	106 ± 5[e]	69 ± 5[e]	108 ± 30[e]
DH-N38d	5	40.4 ± 2.7[f]	266 ± 26[f]	121 ± 19[d]	92 ± 17[e]	61 ± 10[f]	116 ± 7[f]
DH-F18h	5	53.2 ± 10.6	290 ± 47[e]	140 ± 24	107 ± 12[d]	108 ± 10	107 ± 8.4[f]
CH10	5	59.6 ± 4.5[c]	340 ± 14[c]	95 ± 4	116 ± 8[c]	121 ± 8[c]	107 ± 5

Values are means ± SD.
[a] $p < 0.05$; [b] $p < 0.001$; [c] $p < 0.001$ for comparison with controls
[d] $p < 0.05$; [e] $p < 0.01$; [f] $p < 0.001$ for comparison with the diabetes 50 days group

Results

Light micrographs of sections from all experimental groups are shown in Fig. 1.

Blood glucose concentrations. In diabetic, hyperglycaemic animals the BG averaged 21.2 ± 3.0 mmol/l and the animals had continuous glycosuria. The mean BG in insulin treated diabetic animals was close to that of control rats (4.6 ± 3.4 mmol/l) although the variation was greater (Table 1).

Body weight. The mean initial body weight for all animals was 127 ± 5 g. The end body weight, kidney weight and end blood glucose are given in Table 21. After 50 days of diabetes, the body weight was 21 % lower than controls ($p < 0.01$), and 38 days after the start of insulin treatment, the body weight (223 ± 4 g) was similar to the controls (230 ± 20 g). In the DH-F group the body weight after insulin withdrawal and before fasting was similar to the DH50 group, but was reduced by 18 % during fasting.

Kidney weight. The kidney weight was 55 % higher ($p < 0.001$) in the 10 day diabetic animals and 93 % higher ($p < 0.001$) in the 50 day diabetic animals as compared with controls (Table 1). Within 4 h after the commencement of insulin treatment, the kidney weight decreased 20 % ($p < 0.05$), and a further 14 % during the next 5 days. During treatment from day 15 to 38 no further significant decrease in kidney weight was found. At the end of the 38 day treatment period, the kidney weight (1.06 ± 0.09 g) was still higher ($p < 0.01$) than in controls (0.85 ± 0.08 g). In the fasted diabetic animals, BG was normalized after 12 h (5.7 ± 1.1 mmol/l) and remained normal during the last 6 h. The mean kidney weight (1.19 ± 0.14 g) in these animals was comparable to that after five days of insulin treatment (1.13 ± 0.04 g). In animals with induced hyperglycaemia the kidney weight was increased by 31 % ($p < 0.001$) after 10 min (Table 1). Figure 2 shows the correlation between kidney weight and BGc.

Glomerular volume. The glomerular volume in diabetic animals was increased by 34 % after 10 days and by 86 % after 50 days compared with controls. Hyperglycaemia through i. v. glucose infusion in normal animals also increased the glomerular volume. Insulin treatment significantly decreased glomerular volume after 5 days (Table 2).

Proximal tubules. Apart from the enlargement, the morphology of proximal tubule (PT) cells appeared

Table 3. Cell and tubule dimensions

	n	Proximal tubule					Distal tubule			
		Length (m)	Outer diameter (μm)	Inner diameter (μm)	Cell height (μm)	Brush border height (μm)	Length (m)	Outer diameter (μm)	Inner diameter (μm)	Cell height (μm)
CN	7	246 ± 38	46.6 ± 2.5	21.2 ± 2.9	7.03 ± 0.43	5.81 ± 0.52	131 ± 22	33.1 ± 2.1	19.0 ± 5.8	6.28 ± 0.64
DH10	5	311 ± 21[a]	53.1 ± 6.3[a]	25.5 ± 1.2	8.42 ± 0.98[a]	6.28 ± 1.30	137 ± 25	44.0 ± 6.7[b]	31.6 ± 1.9[c]	7.17 ± 0.94
DH50	9	339 ± 34[c]	55.4 ± 3.6[b]	27.4 ± 3.4[b]	8.08 ± 0.88	6.08 ± 0.99	196 ± 41[b]	42.2 ± 5.7[b]	27.4 ± 3.8[b]	7.35 ± 1.23
DH-N4h	6	301 ± 23	53.1 ± 2.9	27.3 ± 2.3	7.83 ± 0.73	5.22 ± 0.41	173 ± 14	39.0 ± 3.5	25.7 ± 1.4	6.62 ± 0.91
DH-N5d	6	306 ± 26	47.7 ± 2.1[f]	22.4 ± 2.6[d]	7.12 ± 0.58	5.56 ± 0.36	174 ± 15	35.1 ± 4.5	22.4 ± 2.6[d]	6.02 ± 0.98
DH-N15d	5	286 ± 25[d]	49.2 ± 1.9[e]	21.6 ± 1.8[e]	7.88 ± 0.26	6.14 ± 0.43	161 ± 14	37.6 ± 0.9	23.6 ± 0.8	6.99 ± 0.29
DH-N38d	5	260 ± 35[f]	51.3 ± 1.4	24.3 ± 1.2	7.30 ± 0.28	6.00 ± 0.47	133 ± 31[d]	39.3 ± 2.1	24.4 ± 1.2	7.08 ± 0.43
DH-F18h	5	309 ± 41	49.9 ± 2.6	23.8 ± 1.9[d]	6.94 ± 0.47[d]	6.24 ± 0.15	179 ± 37	34.7 ± 2.6[d]	22.3 ± 1.6[d]	6.36 ± 0.69
CH10	5	307 ± 12[b]	47.7 ± 0.7	18.6 ± 0.8	8.28 ± 0.08[c]	6.30 ± 0.20	221 ± 14[c]	37.3 ± 4.1	26.5 ± 1.9[a]	5.25 ± 0.38[b]

Values are means ± SD
[a] $p < 0.05$; [b] $p < 0.01$; [c] $p < 0.001$ for comparison with controls
[d] $p < 0.05$; [e] $p < 0.01$; [f] $p < 0.001$ for comparison with the diabetes 50 days group

normal in diabetic animals (Fig. 1A–C). In the 10 day diabetic animals, PT were 27% longer (311 ± 21 m) than in controls (246 ± 38 m, $p < 0.05$). In 50 day diabetic animals both the PT and the distal tubule (DT) were longer (Table 3). In the group analysed 4 h after the start of insulin treatment, the PT was again 11% shorter (NS) whereas after 15 days it was 15% shorter ($p < 0.05$) and after 38 days it was 23% shorter ($p < 0.001$) compared with the DH50 animals (Table 3). The total PT wall volume was significantly larger after 10 and 50 days, both in the PT and DT, compared with controls (Table 2).

Compared with the DH50 animals, the PT wall volume was significantly smaller after 5 days of insulin treatment (277 ± 24 *vs* 410 ± 60 mm^3, $p < 0.01$). After 38 days of insulin treatment, the total wall volume of the PT was still not normalized whereas the wall volume of the DT was almost normalized.

Inner and outer tubular diameters increased in diabetes (Table 3). After insulin treatment the diameters again decreased, but were not fully normalized.

In the PT, cell height increased in the diabetic animals whereas the brush border showed insignificant changes (Table. 3). After insulin treatment, the height of most tubule cells decreased, but did not fully reach control values. Glucose infusion increased PT cell height and induced pronounced vesicle formation in the cytoplasm (Fig. 1I).

Distal tubules. In the distal straight tubule, glycogen accumulated with increasing duration of diabetes (Fig. 1B and C). Glycogen appeared as small dots and blots in the tubule wall and occupied 2.9% of the cell volume after 10 days diabetes. After 50 days the DT, contained 15% glycogen. After 4 h of insulin treatment the amount of glycogen was 11%, and after 5 days treatment glycogen occupied only 3%. After 15 and 38 days it was absent (Fig. 1.D–G). After fasting of the diabetic animals glycogen was still present in the cells of the DT (Fig. 1.H).

Interstitium. The total interstitial volume was significantly increased in the 10-day diabetic animals and further increased up to 50 days (Fig. 1A–C, Table 2). The decrease in interstitial volume was very pronounced within 4 h of the start of insulin treatment (Fig. 1D, Table 2).

Volume fractions. Volume fractions of the analysed structures showed only small changes between the study groups (Table 4). Aberrations from this pattern were seen in glomeruli of the D10 and D50 animals where the volume density was smaller compared with the controls. Intravenous administration of glucose was associated with an increased fractional volume of PT cells combined with an increased cell height and a concomitant decrease in luminal diameter.

Discussion

Two models of hyperglycaemia (STZ diabetes and glucose infusion) and two methods for normalization of diabetic hyperglycaemia (insulin treatment and fasting) were studied. Whatever method used, we found a positive correlation between blood glucose and kidney size (Fig. 2). In diabetic patients, the size of the kidney is increased during insulin treatment and in most studies it remains 20–30% larger [12, 13, 14]. Complete normalization of kidney size is not achieved in patients even after intensive treatment [14–17]. In experimental diabetes, preserving kidney weight by insulin treatment from the beginning of the disease is possible [18] but if treatment is started later the kidney weight cannot be completely normalized [6, 19]. In the present study, the reduction in weight could be divided into a rapid phase starting a

Table 4. Volume densities of kidney structures

Group	*n*	Glomerulus	Proximal tubule			Distal tubule		Interstitium
		(%)	Wall (%)	Lumen (%)	Brush border (%)	Wall (%)	Lumen (%)	(%)
CN	7	7.5 ± 0.4	31.3 ± 3.4	14.8 ± 5.2	18.0 ± 1.8	10.3 ± 1.1	6.6 ± 1.9	14.2 ± 1.6
DH10	5	6.3 ± 0.3[b]	32.9 ± 2.3	12.7 ± 2.4	15.1 ± 1.0[c]	11.6 ± 0.9	9.4 ± 1.7[a]	13.4 ± 1.5
DH50	9	7.6 ± 0.3	31.1 ± 2.2	15.2 ± 3.4	16.5 ± 2.5	11.9 ± 2.0	8.6 ± 1.2[a]	12.6 ± 1.6
DH-N4h	6	7.5 ± 0.7	31.3 ± 1.6	15.3 ± 1.2	15.6 ± 1.1	11.3 ± 1.1	8.9 ± 0.6	10.8 ± 2.4
DH-N5d	6	7.9 ± 0.5	30.8 ± 2.6	13.4 ± 2.4	16.9 ± 1.1	11.3 ± 1.0	8.4 ± 1.0	14.2 ± 2.2
DH-N15d	5	8.2 ± 0.3[d]	33.6 ± 0.6[d]	12.0 ± 1.9	17.3 ± 0.5	12.3 ± 1.1	8.0 ± 0.6	12.5 ± 3.3
DH-N38d	5	7.6 ± 0.4	31.6 ± 1.8	14.3 ± 1.4	17.4 ± 1.2	10.9 ± 1.7	7.3 ± 1.1	13.9 ± 1.2
DH-F18h	5	8.6 ± 0.9	30.4 ± 1.3	14.6 ± 1.2	18.8 ± 0.6	11.3 ± 0.7	11.4 ± 0.5[f]	11.3 ± 0.6
CH10	5	9.6 ± 0.4[c]	34.9 ± 0.5	9.7 ± 0.4	16.4 ± 0.1	11.9 ± 0.9[a]	12.5 ± 0.7[c]	11.0 ± 0.4[b]

Values are means ± SD.
[a] $p < 0.05$; [b] $p < 0.01$; [c] $p < 0.001$ for comparison with controls
[d] $p < 0.05$; [e] $p < 0.01$; [f] $p < 0.001$ for comparison with the diabetes 50 days group

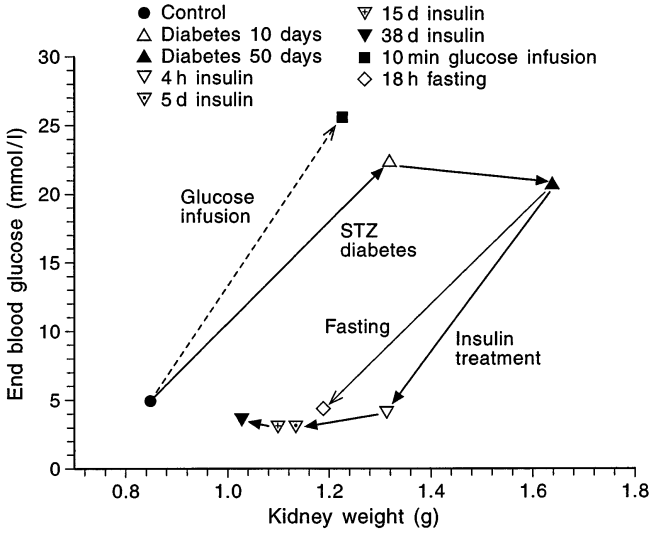

Fig. 2. Correlation between end blood glucose and kidney weight. Hyperglycaemia in diabetic animals and in normal animals after glucose infusion induced an increase in kidney weight. The kidney weight was further increased with longer duration of hyperglycaemia (DH-50). A fall in kidney weight was induced in DH animals by insulin treatment or by fasting. Insulin treatment of longer duration further decreased the kidney weight (DH-N5 d,15 d,38 d). However, complete normalization of the kidney weight was not achieved

few hours after normoglycaemia and a slower phase with a further reduction for the following days and weeks. Although insulin was given at doses aiming at normalizing BG, hyperglycaemic episodes during the days before perfusion fixation may influence the kidney weight at the end of the study and thus prevent the kidney size from returning to control values.

The glomerulus is a major site of functional impairment in diabetic nephropathy, and end stage renal disease is associated with an increase in mesangium and in basement membrane material and subsequent closure of the glomerulus [20, 21, 22, 23]. The present study shows a smaller relative volume growth of glomeruli as compared with the tubules in the early stages of STZ diabetes as has been shown previously [4]. Studies on 2–6 day diabetic rats using incorporation of radioactive thymidine into kidney cells and measurements of labelling indexes showed no increase of labelling in glomeruli [5]. Unbiased, morphometric measurements of the number of all nuclei in glomeruli in 10 day diabetic rats showed a 20 % increase and after 50 days a 47 % increase [25]; however, the type of cell that divides is not known. After 80 days diabetes, glomeruli contain 25 % more capillaries [26]. The development of new capillaries involves formation of new endothelial cells and basement membrane, whereas the podocytes probably do not participate as they rarely divide [5]. Consequently the podocytes with their complicated foot processes must reorganize to cover the new capillary surfaces. One mechanism to accomplish this, known both from human and experimental studies, is broadening of the epithelial foot processes [27], but other mechanisms are also possible. The two studies suggest a slow growth of glomeruli mainly by hyperplasia.

The glomerular volume decreases after insulin treatment. It is not known whether the number of capillaries decreases, but if this were the case, the excess basement membrane might accumulate in the glomerulus and in the mesangium. It can be speculated whether many periods of growth and regression may lead to repetitive accumulation of basement membrane.

Glomeruli also enlarge after intravenous glucose infusion in normal animals, showing that initial moderate enlargement of glomeruli can be seen, at least for a shorter period, without hyperplasia.

The proximal tubules increase both in length, internal and external diameters, and in cell height during diabetic growth. When studied by thymidine incorporation autoradiography [5], the net increase in number of cortical tubular cells after 6 days diabetes was approximately 32 %, and PT cell nuclei showed the largest uptake of thymidine of all cell types in the kidney [5]. Using stereological methods, the total number of PT cells was increased by 37 % and the

mean cell volume was increased by 12% after 3 months diabetes [28]. These studies clearly show that the PT has the potential to enlarge mainly by hyperplasia and that the growth starts within the first 6 days. In fact growth is most pronounced on the second day [5] and about the same increased number of cells is found also after 3 months with hyperglycaemia [28].

The reason for the growth of the PT is not fully understood, but several factors could be working in concert. Growth factors, including insulin like growth factor, which possibly stimulate growth in the PT, are increased in diabetes [29, 30]. The increased glomerular filtration rate imposes an extra workload on the tubules for reabsorption of filtered water and solutes, and the increased amount of Na,K-ATPase in the kidney in the STZ-diabetic kidney [31, 32] confirms an increase in sodium absorption.

During fasting the animals in the study had been normoglycaemic for 6 h, and since insulin was withdrawn 2 days before fasting was started, the normalization of BG was not due to an effect of insulin. We have previously shown that in control animals 18 h fasting does not significantly influence kidney weight (0.87 ± 0.03 in fasted vs 0.85 ± 0.08 in controls and BG is still normal) (R. Rasch, unpublished data) indicating that the reduction in kidney weight seen after fasting in this study is related to the diabetic condition.

The enlargement of the PT induced by glucose infusion in the present study was probably caused by swelling of the cells, the outer diameter being similar to controls but with a decreased inner diameter. Furthermore the cytoplasm contained numerous vacuoles, a phenomenon described as osmotic nephrosis, which also occurs after administration of mannitol, sucrose or dextran [33]. In the present study the rise in blood glucose was very rapid and pronounced after 10 min. In the study of Maunsbach et al. [33], infusion was given over longer periods with lower glucose concentrations, but with similar light microscopic changes with many cytoplasmic vacuoles. In animals with diabetic hyperglycaemia (groups DH10 and DH50) we found no apparent increase in the number of cytoplasmic vacuoles. Our findings suggest that acute hyperglycaemia in the daily life in diabetic patients may elicit episodes of osmotic nephrosis.

The distal tubule, like the proximal, also increases in length, diameter and cell size. Thymidine incorporation, indicating cell division [5], was increased in diabetic rats compared with controls but the change was not as striking as in the PT [28]. Glycogen accumulates in parts of the thick ascending limb of the loop of Henle (TAL) and the distribution has been described in detail [34]. In the cortex, most of the TAL cells contain glycogen to the extent that cell functions are probably impaired. TAL cells are the only cells in the body that form Tamm-Horsfall glycoprotein (THP), which is the major protein excreted in the urine [35]. The excretion rate is increased in diabetic patients with microalbuminuria and decreased in patients with macroalbuminuria [36]. In experimental diabetes of 80 days duration, the excretion rate is increased but the amount of THP in TAL cells and the amount of mRNA coding for THP is decreased [37]. These latter findings also suggest a decreased function of this segment of the kidney after long term diabetes. The THP binds to and acts as a regulator of circulating interleukins such as recombinant murine interleukin-1 and recombinant tumour necrosis factor [38] and thus its decreased production in diabetes may influence immunologic reactions and growth. The number of nuclei in PT and DT tubules was not significantly lower 3 weeks after normalization of BG [28]. Since the kidney weight was nearly normalized, the cell size was consequently smaller. The number of cells may not be normalized even after prolonged treatment and this may be one reason for why complete normalization of kidney weight is not seen.

If conclusions from the present study are applied to diabetic patients with poor regulation, the kidney can be expected to acutely fluctuate in size as BG levels change. Since kidney size is strongly associated with glomerular filtration rate [39] this should be taken into account when the glomerular filtration rate or the kidney size is measured for clinical or for research purposes.

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