Diabetologia 16, 125–128 (1979)

Diabetologia

Prevention of Diabetic Glomerulopathy in Streptozotocin Diabetic Rats by Insulin Treatment

Kidney Size and Glomerular Volume

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Summary. Kidney weight and glomerular volume have been studied in groups of insulin-treated streptozotocin diabetic rats maintained at high, or nearly normal plasma glucose levels. Kidney weight and glomerular volume in these groups were compared to a non-diabetic control group. – Rats with nearly normal plasma glucose levels (95 ± 35 to 182 ± 20 mg/100 ml) had the same kidney weight as the non-diabetic controls, 1.04 ± 0.14 and 1.07 ± 0.09 g, respectively. In the rats with constant high plasma glucose (338 ± 71 to 555 ± 86 mg/100 ml) kidney weight was significantly increased, 1.73 ± 0.20 g, compared to each of the two other groups. Glomerular volume was 0.599 $M\mu^3$ in the diabetic animals with nearly normal plasma glucose, a value very close to that of the non-diabetic controls, 0.587 $M\mu^3$. In animals with high plasma glucose concentrations glomerular volume was 0.775 $M\mu^3$, $2\ p < 0.03$ compared with both other groups. – The study indicates that good diabetic control for 6 months prevents the development of large kidneys and large glomeruli in dibatic rats.

Key words: Experimental diabetes, blood glucose control in rats, streptozotocin dibates in rats, kidney weight, glomerular volume, stereology.

Increased kidney size and glomerular filtration rate (GFR) have been demonstrated in diabetic patients both at the onset of diabetes [7] and after several years of disease [6, 3]. The increased kidney size and GFR found in patients with recent onset of diabetes can be normalised by strict insulin treatment for 3 weeks [7]. Glomerular volume is also enlarged in humans at the diagnosis of diabetes [19], and in long term diabetes the still functioning glomeruli are found to be enlarged [2]. The mechanism which leads to these changes is not known.

Streptozotocin diabetic rats show the same renal changes. A few days after the induction of diabetes the kidneys are enlarged and changes have been demonstrated up to 6 weeks after the induction of diabetes [15]. In one study it was found that the degree of kidney hypertrophy was correlated to the degree of diabetic derangement [16]. Glomerular volume was also enlarged in short term experiments in rats [17].

In the present study kidney weight and glomerular volume has been measured after a longer period (6 months) of streptozotocin diabetes in rats, to find out whether the degree of control of plasma glucose concentration has any influence on the magnitude of kidney growth and glomerular enlargement.

Materials and Methods

Female Wistar rats with a mean body weight of 188 ± 9 (SD) g and a mean age of 84 ± 12 days were given streptozotocin (90 mg/kg) IV. Insulin treatment was started 2 days later in the animals thät had developed gross glucosuria and ketonuria assessed with Clinistix® and Ketostix®.

The series of consecutive diabetic animals was separated systematically into two equal groups both treated with the same very long acting heat-treated non-commercial Ultralente insulin (NOVO) [10, 14] once a day in such a way that the rats of one group (8 animals) had plasma glucose values close to normal ("well-controlled") while in the rats of the other group (8 animals) plasma glucose levels were allowed to remain high on a small daily insulin dose ("poorly-controlled"). The degree of control was governed by plasma glucose determinations five days per week. Plasma glucose was measured between 8 and 9 a. m. and insulin was given shortly thereafter. The insulin dose was adjusted according to the results obtained [10]. Eight rats were kept as controls.

After 6 months, the 16 diabetic rats and the 8 control rats were anaesthetised with diazepam, 12 mg/kg, and pentobarbital, 50 mg/

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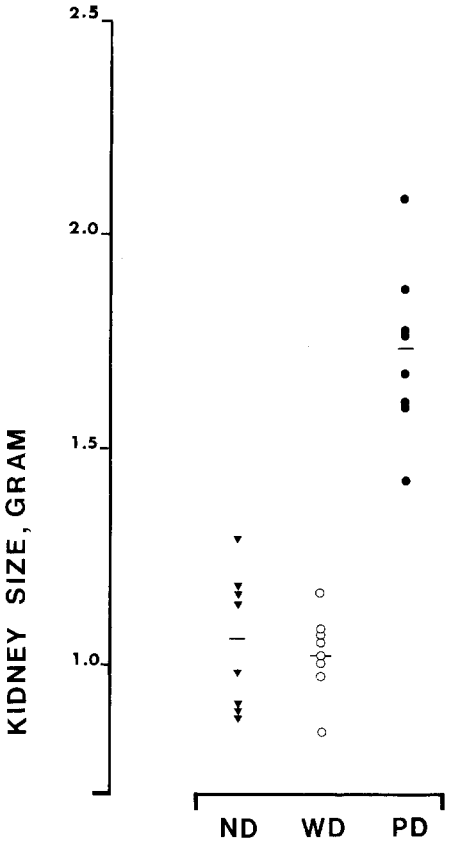

Fig. 1. Weight of the right kidney after 6 months of insulin-treated diabetes. ND = non-diabetic controls – WD = "well-controlled" diabetic rats – PD = "poorly-controlled" diabetic rats

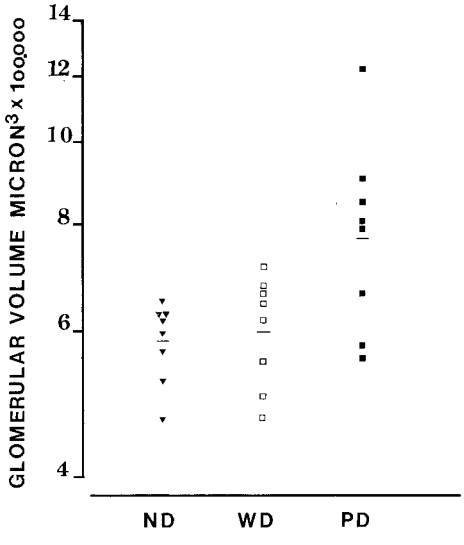

Fig. 2. Glomerular volume after 6 months of insulin-treated diabetes. About 100 glomerular cross sections were measured on the left kidney. ND = non-diabetic controls – WD = "well-controlled" diabetic rats – PD = "poorly-controlled" diabetic rats

kg. After opening of the abdomen, the right kidney was exposed and a ligature was placed around the kidney vessels. These were then cut very close to the kidney, the removed organ was quickly wiped and extrarenal fat removed. The kidney, still encapsulated, was then weighed. A retrograde perfusion of the left kidney through the aorta was then performed under standardised conditions with 12 ml of a special Tyrode solution (containing 3/4 of the ordinary NaCl content) [5] and continued for 10 min with the same solution with 0.5% Alcian Blue added [8]. The complete Tyrode solution had the following composition: NaCl 0.8, KCl 0.2, $CaCl_2$ 0.2, $MgCl_2 \cdot 6H_2O$ 0.1, $NaHCO_3$ 1.0, $NaH_2PO_4 \cdot H_2O$ 0.05, and glucose 1.0 g. The osmolality in the final solution was 370–376 mosm/kg and pH was 7.1 in all experiments. Four kidneys were discarded due to poor perfusion and 24 well perfused kidneys were used for further studies. The kidneys bleached immediately after the first perfusion had started and became evenly blue 30 seconds after the second perfusion had started. The left kidney was cut into halves vertically and mounted in paraffin. The quality of the perfusions was evaluated on sections without further staining. Only kidneys where all or nearly all glomeruli were heavily stained with Alcian Blue were used in the studies.

For measurements of glumerular volume paraffin sections were further stained with PAS. Mean glomerular volume was calculated in each animal from measurements of about 100 glomerular cross sections. Glomerulus is here equal to the glomerular tuft omitting Bowman's capsule and Bowman's space. All glomerular profiles appearing on one half of the kidney section were measured, the sections of the individual glomeruli being projected on to a grid with a series of concentric circles and thereby classified according to size. From the class distribution of these sectional areas the distribution of the true glomerular volume was calculated [13].

Since the distribution of the absolute areas is generally log normal the comparison between the groups has been carried out on logarithmically transformed values. The group mean calculated from the distributions equals the geometric mean of the original value. Student's t test for independent samples has been used to compare groups.

Results

The arithmetic mean weight of the right kidney was 1.07 ± 0.09 g in the group of non-diabetic control animals. In the "well-controlled" group of diabetic animals mean kidney weight was not different at 1.04 ± 0.14 g. The "poorly-controlled" diabetic animals had significantly larger kidneys, 1.73 ± 0.20 g, compared to the "well-controlled" diabetic animals (2 p = 0.00003) and to the non-diabetic control animals (2 p = 0.0003) (Fig. 1 and Table 1).

The geometric mean glomerular volume in the non-diabetic reference group was 0.587 ranging from 0.473 to 0.650 $M\mu^3$, in the "well-controlled" group it was 0.599 $M\mu^3$ (range 0.476 to 0.716). The "poorly-controlled" diabetic group had a geometric mean glomerular volume of 0.775 $M\mu^3$ (range 0.552 to 1.222). This value is statistically significantly different from the "well-controlled" group (2 p = 0.028) as well as from the non-diabetic control group (2 p = 0.014) (Fig. 2 and Table 1).

The plasma glucose values, urine glucose excre-

Table 1

		Non-diabetic control rats		"Well-controlled" diabetic rats		"Poorly-controlled" diabetic rats	
Kidney weight right kidney	(g)	1.07±0.09	NS	1.04±0.14	(2p = 0.00003)	1.73±0.20	(2p = 0.0003)
Glomerular volume left kidney	($M\mu^3$)	0.587 (0.473–0.650)	NS	0.599 (0.476–0.716)	(2p = 0.028)	0.775 (0.552–1.222)	(2p = 0.014)

Kidney weight is given as arithmetic mean ± SD. Glomerular volume is given as geometric mean and range. P. values shown between columns refer to comparisons between the two columns. P values shown after the last column refer to comparison between the first and the last column ($M\mu^3$ = million cubic micron). NS = $p > 0.05$

Table 2

	Non-diabetic control rats	"Well-controlled" diabetic rats	"Poorly-controlled" diabetic rats
n	8	8	8
Plasma glucose 8 a. m.	87 ± 9	182 ± 20	452 ± 41
11 a. m.	–	120 ± 47	338 ± 71
(mg/100 ml) 11 p. m.	–	95 ± 35	555 ± 86
Urine glucose (g)	–	0.19 ± 0.10	10 ± 2
Body weight after 6 months of diabetes (g)	310 ± 28	320 ± 27	222 ± 28
Insulin (U/kg) – mean dose	–	7.7 ± 2.2	1.5 ± 0.2

Results from the 6 monts' experiment. In the "well-controlled" group of diabetic animals plasma glucose measured at 8 a. m. is the mean value of 123 measurements in each animal. This individual mean value has been used to calculate the mean of the group. Plasma glucose at 11 a. m. and at 11 p. m. was only measured on two occasions after 3 and 5 months. Mean plasma glucose for the two other groups has been calculated similarly except that it was measured only 12 times during the period. In the "well-controlled" group the animals were without glucosuria on 62% of all days. Values are given as mean ± SD

tion, body weight, and amount of insulin administered are shown in Table 2.

Discussion

The present study was designed to answer the question if strict metabolic control prevents the development of kidney enlargement and increase of glomerular volume in long term streptozotocin diabetic rats. The results obtained indicate that carefully treated animals, kept nearly normoglycaemic for periods of 6 months, do not have large kidneys with large glomeruli.

Earlier studies in *human diabetics* have shown that shortly after the onset of diabetes, structural and functional kidney changes are demonstrable. The kidneys are enlarged [7], glomerular filtration rate (GFR) is increased [7] and so is glomerular volume [19] and the area of the glomerular filtration surface [4].

In studies dealing with short term *diabetes in animals*, similar functional and structural changes are found. Creatinine clearance is increased [18]. An increase is found in kidney weight, protein content and the RNA/DNA ratio in diabetic rats indicating hypertrophy [15]. These changes seem to be provoked by the degree of metabolic derangement and a correlation has been demonstrated between blood glucose level and kidney hypertrophy on the seventh day of streptozotocin diabetes in rats [16].

The early glomerular changes in man are *reversible*. In short term diabetic patients it has been shown that the GFR as well as the kidney size return to normal after insulin treatment [7]. In animal studies it is possible to *prevent* the development of kidney hypertrophy by insulin treatment in experiments of one week's duration [16].

All of the investigations mentioned above in short term diabetes mellitus and in short term experimental studies point to the metabolic condition as the causal factor leading to renal changes.

In diabetic patients undergoing ordinary insulin treatment it is known that kidney enlargement as well as increased GFR persist for many years after the onset of diabetes [6]. In agreement with these human studies creatinine clearance is reported to be increased after several months of diabetes in rats [18]. These authors, however, found lower kidney weight in the same diabetic rats and their findings are therefore not easily explicable.

In the *end stage* of diabetic glomerulopathy when many glomeruli are occluded and have ceased to function, a compensatory hypertrophy is seen of the

open glomeruli [2]. At this stage there is still enlargement of the whole kidney [3]. In the intermediate interval, i. e. between the early metabolically determined hypertrophy and the late compensatory hypertrophy, we do not have any information about the size of the glomeruli.

In the present study it has been shown that after several months of severe experimental diabetes, before the appearance of the classical morphological changes of diabetic glomerulopathy, the kidney and glomeruli are still enlarged as in the short term studies. Furthermore, the study has shown that the development of these changes is preventable by strict blood glucose control.

The study confirms the influence of the metabolic state on kidney and glomerular hypertrophy found in short term studies and also demonstrates that relatively good control over longer periods prevents hypertrophy. Similar results have also been obtained for glomerular basement membrane thickness [9, 11].

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Received: March 23, 1978
and in revised form: September 22, 1978

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