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Effect of Metabolic Regulation on Renal Leakiness to Dextran Molecules in Short-Term Insulin-Dependent Diabetics

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Summary. Renal clearance of dextran of two ranges of molecular size and glomerular filtration rate (GFR, ^{51}Cr-EDTA) were measured in seven short-term insulin-dependent diabetics (mean age 25 years). Measurements were carried out in the same patient during good and poor metabolic regulation (plasma glucose, mean ± SEM, 6.5 ± 0.9 and 14.8 ± 1.5 mmol/l, respectively). GFR was elevated in all patients during poor metabolic regulation (119 ± 6 ml/min/1.73 m^2, versus 99 ± 2 ml/min/1.73 m^2 during good control, $p < 0.01$). The average renal clearance of dextran with molecular weights ranging from 25,000 to 35,000 and 35,000 to 45,000 increased during poor metabolic regulation from 14.8 ± 0.8 to 19.8 ± 1.8 ml/min/1.73 m^2, and 5.2 ± 0.3 to 6.8 ± 0.6 ml/min/1.73 m^2, respectively ($p < 0.05$). The elevated GFR and renal dextran clearance found during poor metabolic regulation were normalized within one to three weeks of effective insulin treatment. This rapid reversibility can hardly be explained by the previously demonstrated enlargement in glomerular size and filtration surface area, since these alterations remain unchanged after more than one month of insulin treatment. The metabolic regulation did not influence the size-selective properties of the glomerular wall. Therefore, we suggest that the dominating mechanism involved in the GFR and renal dextran clearance alterations is functional, viz. increased filtration pressure.

Key words: Dextran, early diabetes, glomerular filtration rate, influence of metabolic regulation, kidney function.

It is well documented that glomerular filtration rate (GFR) and urinary albumin excretion rate are elevated in poorly controlled short-term juvenile diabetics [5, 15, 16, 17]. The mechanism of these alterations is not known.

Several investigators have, based on the characteristic finding of an increase in the calculated filtration fraction (GFR/RPF (renal plasma flow)), suggested an elevated intraglomerular filtration pressure as a possible mechanism [7, 15]. Recently, this suggestion has been challenged by some very elegant morphological studies by Østerby and her colleagues [11, 20]. These investigators demonstrated an enlargement of the glomeruli and an increase in the glomerular filtration area in newly diagnosed and short-term juvenile diabetics.

The aim of the present study was to investigate the influence of metabolic regulation in short-term insulin-dependent diabetics on renal dextran clearance and GFR, and to obtain information on the nature of the kidney function alterations (morphological versus functional origin), by using a short time period between the poor-control and good-control studies.

Material and Methods

Seven insulin-dependent diabetics (3 females and 4 males), aged 18–32 years (mean 25 years), all of whom had been fully informed of the nature of the study before giving their consent, were investigated. All patients were ketosis-prone. None of the patients were taking drugs. Their clinical and laboratory data are shown in Table 1. Microvascular permeability data has previously been presented in the same patients [17].

All patients were first studied during poor metabolic regulation. Two of the diabetics (no 4 and 6) were newly diagnosed. No 4 did not receive insulin, and no 6 was insufficiently treated with

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Table 1. Clinical data for seven short-term insulin-dependent diabetics

Subject no.	Age (years)	Sex	Duration of diabetes (months)	Insulin dose (U)	Plasma glucose[a] (mmol/l)		Urinary glucose excretion (grams/24 h)[b]		Legal's test of 24-hour urine[b]	
					G	P	G	P	G	P
1	24	M	17	18	4–12(8)	12–23(20)	11	161	0	0
2	18	F	10	34	3– 9(5)	16–39(25)	20	340	0	++
3	21	F	6	54	5–16(10)	20–36(23)	32	235	0	+
4	32	M	0.3	24	7–12(9)	10–20(16)	6	150	0	+++
5	29	F	7	12	5–12(8)	11–18(13)	2	29	0	0
6	24	M	0.3	22	4–15(10)	14–20(17)	35	220	0	+
7	18	M	40	30	7–16(10)	17–30(21)	38	186	0	0

G = Good metabolic regulation P = Poor metabolic regulation

[a] Plasma glucose measured 7 a. m., 10.30 a. m., 1.30 p. m., 4.30 p. m., 9.30 p. m., range with mean in bracket indicated for the last two days before the kidney function was determined

[b] Mean indicated for the last two days before the kidney function was determined

Table 2. GFR and renal dextran clearance in seven short-term insulin-dependent diabetics during good (I) and poor (II) metabolic regulation

Subject no.	Fasting plasma glucose (mmol/l)[a]		Standard bicarbonate (mmol/l)[b]		Plasma volume (l)		Plasma albumin (g/l)		GFR (ml/min × 1.73 m²)		Dextran clearance 25.000–35.000 daltons × 1.73 m²				Dextran clearance 35.000–45.000 daltons × 1.73 m²				Interval between I and II (days)
	I	II	I	II	I	II	I	II	I	II	I	CV	II	CV	I	CV	II	CV	
1	8.0	12.9	25.7	25.6	4.1	3.6	34.5	39.3	102	121	13.6	15.4	27.7	18.0	4.6	15.2	9.3	17.2	7
2	3.7	20.2	26.3	21.5	3.2	2.7	34.9	41.7	104	144	18.0	12.7	24.4	2.4	6.3	12.7	8.9	5.6	5
3	7.0	20.8	27.0	22.4	2.8	2.4	36.1	40.9	98	108	13.6	16.9	19.1	12.0	4.6	23.9	6.5	10.7	6
4	7.8	11.5	22.5	16.8	4.3	2.8	32.5	37.0	103	115	16.9	8.9	16.5	8.5	6.4	6.3	6.3	11.1	24
5	4.5	10.5	27.1	25.5	2.8	2.6	36.0	37.0	90	133	12.5	13.6	15.2	10.5	4.6	19.6	5.4	14.8	4
6	10.5	13.7	26.5	26.2	3.1	2.8	34.1	38.9	95	102	13.6	4.4	15.9	11.9	4.5	4.1	4.8	8.3	10
7	4.1	14.1	29.0	26.2	3.2	3.0	37.7	41.7	98	109	15.7	8.9	19.6	17.8	5.7	8.8	6.4	4.7	9
Mean	6.5	14.8	26.3	23.5	3.4	2.8	35.1	39.5	99	119	14.8	11.5	19.8	11.6	5.2	12.9	6.8	10.3	9
SEM	0.9	1.5	0.7	1.3	0.2	0.1	0.6	0.8	2	6	0.8	1.7	1.8	2.0	0.3	2.7	0.6	1.7	
p value	<0.01		<0.01		<0.01		<0.01		<0.01		<0.05				<0.05				

[a] Each value represents the mean of 4 determinations taken during the investigation 8 a. m., 9.30 a. m., 11 a. m., 1 p. m.

[b] Each value represents the mean of 2 determinations taken during the investigation 8 a. m., 1 p. m.

CV Intra-individual coefficient of variation (per cent)

crystalline insulin for 2 to 3 days before the first investigation. All the remaining patients were taken into hospital due to poor metabolic regulation at the last 2 to 3 visits in the out-patient clinic (interval 4–8 weeks). In patients no 1, 2, 5 and 7 insulin therapy was reduced or stopped one to two days prior to the first investigation. The second investigation in good metabolic control was carried out 4 to 24 days later, using identical experimental procedures. A subcutaneous injection of 8, 12 and 8 U of NPH insulin was given at 7 a. m. on the day of the second investigation to patients no 2, 3 and 7, respectively. None of the remaining four patients received insulin on the day of the second investigation.

The patients were studied in the morning after at least 12 hours fasting and half an hour rest in the supine position. All variables were measured on the same day between 8 a. m. and 1 p. m. They are listed below according to the time of the day on which they were carried out.

Plasma volume was determined with ^{125}I-labelled human serum albumin (code MIAK, Institute for Atomic Energy, Kjeller, Norway), by retropolation of the initial part of the disappearance curve (60 min) and from the injected dose.

GFR was measured after a single intravenous injection of ^{51}Cr-EDTA (9 a. m.) by studying the plasma disappearance for four hours, as described previously [4].

Renal dextran clearance was measured as previously described [13, 19]. To promote diuresis about one litre of tap water was given during the last hour before the start of the clearance periods (1000 hr). Urinary catheters were not employed. A single intravenous injection of 100 ml (10g/100ml) dextran (Rheomacrodex®, molecular weight range 10,000–80,000) was given over a 10 minute period. After an equilibration period of 30 minutes the bladder was emptied. Urine was collected quantitatively for 3 clearance periods of 20 minutes each. Three minutes before the

middle of each urine collection period a venous blood sample was drawn. The dextran was isolated by precipitation with picrinic acid before analysis, in order to remove interfering substances e. g. glucose. The total concentration of dextran in plasma and urine was determined by the anthrone method [6].

The molecular weight distribution of dextran in each sample was estimated by gel chromatography, adapted to automated routine and computer analysis by Arturson and Granath [1].

Plasma glucose was measured by a glucose-oxidase method on an autoanalyzer. Standard bicarbonate and pH were measured, using conventional laboratory techniques. Plasma albumin was measured by the method of Laurell [12]. Plasma immunoreactive growth hormone [8] was measured six times in each patient on samples taken at half hour intervals during the investigation.

Wilcoxon's non-parametric test for paired comparison was used for statistical analysis.

Results

Tables 1 and 2 show the metabolic variables studied in each patient before and during the day of the investigation. With the exception of patient no 4 (venous pH 7.27 and ketonuria), no patient was ketoacidotic during the day of the first investigation. The average venous pH was not significantly reduced during poor compared to good control, 7.33 versus 7.37, respectively. All patients were slightly dehydrated during poor metabolic control, particularly patient no 4, as indicated by the reduced plasma volume, 2.8 ± 0.1 versus 3.4 ± 0.2 l during good control ($p < 0.01$). Plasma albumin was elevated in all patients during poor compared to good metabolic control, 39.5 ± 0.8 versus 35.1 ± 0.6 g/l, respectively ($p < 0.01$). Plasma growth hormone concentration was not significantly different between poor and good control, 4.4 ± 1.0 versus 5.2 ± 1.3 ng/ml, respectively. Arterial blood pressure did not change significantly from poor to good control, 113/72 ± 6/3 and 108/69 ± 6/3, respectively.

Table 2 shows GFR and renal dextran clearance during poor and good metabolic control in the seven short-term insulin-dependent diabetics. The GFR was elevated in all patients during poor control, on average by 20%, and normal during good control ($p < 0.01$). The dextran values represent the average of the 3 clearance periods. Dextran clearance was elevated in 6 out of the seven patients during poor metabolic regulation, on average by 31% and 34% for the low and high molecular dextran, respectively ($p < 0.05$). Thus the ratio between the low and the high molecular dextran clearance did not change during poor and good metabolic regulation. The dextran clearance was unchanged in the untreated ketoacidotic, dehydrated patient no 4.

The 3 patients (no 2, 3 and 7) who had a subcutaneous injection of 8 to 12 U of NPH insulin on the day of the good control experiments did not differ from the remaining 4 as regards GFR and renal dextran clearance. The decrease in plasma glucose during the 5 hour investigation period was 2.8 mmol/l in the former group versus 2.0 mmol/l in the latter.

Discussion

Mogensen [14] has previously demonstrated increased renal dextran clearance in three newly diagnosed juvenile diabetics before insulin treatment and normalization after insulin treatment. Our findings in seven short-term insulin-dependent diabetics during poor and good metabolic regulation is in agreement with this. The findings in both studies of increased renal dextran clearance indicate an elevated transglomerular passage of neutral macromolecules, since dextrans are neither excreted nor reabsorbed to any measurable extent by the renal tubular cells [19].

The mechanisms involved in the elevated transglomerular passage of neutral dextrans can either be an increase in the permeability-surface area product (PS), and/or an increased hydrostatic pressure gradient across the glomerular membrane. If increased permeability in the form of enlargement of the pores in the glomerular membrane were present during poor metabolic control, then a pronounced increase in high molecular dextran clearance can be predicted. However, this was demonstrated neither in the present nor in the previous study [14], ruling out increased pore size as a mechanism. These findings suggest that the size-selective properties of the glomerular capillaries (P) is probably not altered in uncontrolled short-term juvenile diabetes, although an increased number of normal size pores per unit surface area was not excluded.

The glomerular wall does not discriminate only on the basis of molecular size and recent reviews [2, 18], have clearly stressed that the glomerular barrier also acts as a charge-selective filter. Unfortunately, it is not possible to elucidate this function in diabetes in man, since charged dextrans induce toxic side effects.

Recent quantitative morphometric studies of the kidney in newly diagnosed and short-term juvenile diabetics by Østerby and Gundersen [20] and Kroustrup, Gundersen and Østerby [11] have clearly demonstrated an increase in glomerular size, capillary volume, basement membrane material and glomerular filtration surface area (+ 80%). These findings might be taken to explain the elevated GFR and increased transglomerular passage of dextran. This explanation is of course only valid to explain our findings if the morphological alterations are quickly reversible, viz. within one to three weeks. However,

insulin treatment for five weeks in the above mentioned newly diagnosed diabetics did not affect the morphological alterations [11]. Thus we conclude that PS product variations can not account for the changes in GFR and transglomerular passage of uncharged macromolecules that are reversible in the course of few weeks.

We have no direct information in man on the hydrostatic pressure gradient across the glomerular membrane viz. the difference between intracapillary and proximal tubular pressure. We do know that the calculated filtration fraction (GFR/RPF) is greatly increased in poorly controlled juvenile diabetics (0.28) compared to 0.20 in non-diabetics [15]. The following variables can induce an increased filtration fraction (FF); increased permeability-surface area product of the glomerular membrane, reduced colloid oncotic pressure difference and increased hydrostatic pressure gradient [3]. As discussed above, PS product variations can hardly explain the rapid changes in FF. The plasma protein and albumin concentration is either normal or elevated, as in the present study, during poor metabolic control [15], ruling out oncotic pressure as a mechanism. Having excluded these two possibilities we are left with an increased hydrostatic pressure gradient as the most likely explanation of the present demonstrated kidney function alterations.

Recent studies of kidney function in diabetic (streptozotocin) rats supports this suggestion. Hostetter et al. [10] found that the insulin treated diabetic rats in poor metabolic control (blood glucose 375 mg%) had elevated GFR and renal plasma flow with increased transcapillary hydrostatic pressure (35.2 versus 44.4 mm Hg in control and insulin treated diabetic rats, respectively). Furthermore, the ultrafiltration coefficient (the product of water permeability of the glomerular wall and the surface area available for filtration) was not significantly different between the above mentioned two groups of rats.

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