Glomerular Charge and Size Selectivity Assessed by Changes in Salt Intake in Type 2 Diabetic Patients

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OBJECTIVE — To evaluate glomerular charge selectivity in patients with type 2 diabetes, we studied changes in fractional clearance of proteins with different sizes and charges when patients were placed on two diets with different salt contents.

RESEARCH DESIGN AND METHODS — Nineteen patients with type 2 diabetes and normoalbuminuria (<20 μg/min, n = 8), microalbuminuria (20–100 μg/min, n = 7), or advanced albuminuria (>100 μg/min, n = 4) were placed on a low-salt diet (85 mEq of sodium daily) or a high-salt diet (255 mEq of sodium daily) for 1 week, and then on the other diet, in random order. Fractional clearances of albumin and immunoglobulin G (IgG) were calculated on the last 3 days of each diet.

RESULTS — In patients with normoalbuminuria, the high-salt diet increased the fractional clearance of IgG, which is electrically neutral, but the fractional clearance of albumin, which is anionic, was unaltered, suggesting that the pore charge of the glomerular barrier was unaffected. However, in patients with microalbuminuria, the high-salt diet increased the fractional clearances of IgG and albumin equally, indicating some neutralization of the pore charge. Fractional clearance of IgG in these first two groups was similar when salt intake was low, so pore size was the same in these groups. In patients with advanced albuminuria, fractional clearance of IgG was higher than in the other groups, indicating that size selectivity had worsened.

CONCLUSIONS — In type 2 diabetic patients, charge selectivity is lost before size selectivity as diabetic nephropathy progresses.

Proteinuria is the first clinical sign of diabetic nephropathy. It is important to clarify the mechanism of proteinuria in diabetic patients. Impaired size selectivity (1,2) and charge selectivity (3–5) of the glomerular basement membrane seem to be involved. Decreased charge selectivity has been one explanation for microalbuminuria in diabetic patients (5). However, patients with advanced microalbuminuria (100–300 mg/day) have increased urinary excretion of immunoglobulin G (IgG) (3), which suggests that these patients lose some size selectivity at the same time at which they lose some charge selectivity. Decreased size selectivity is a major cause of macroalbuminuria (>300 mg/day) (1). Impairment of charge selectivity before a decrease in size selectivity may account for the development of microalbuminuria (6), but proof of this theory is lacking, especially for patients with type 2 diabetes; clinical evaluation of these two selectivities is difficult.

Other possible factors in proteinuria include hemodynamic forces (7). It is likely that a high-salt diet affects intrarenal hemodynamics such as glomerular capillary pressure. If the anionic charge of the basement membrane is preserved, urinary excretion of IgG, which is electrically neutral, and that of albumin, which is anionic, might change differently when a high-salt diet causes glomerular pressure to increase.

In this pilot study, patients with type 2 diabetes were placed on two diets with different salt contents. To evaluate glomerular charge selectivity, we studied the differences with the two diets in fractional clearance of two proteins, IgG and albumin, with their different sizes and charges.

RESEARCH DESIGN AND METHODS

Subjects

The subjects studied were 19 inpatients with type 2 diabetes in our hospital: 11 men and 8 women aged 40 to 74 years (mean ± SE, 60 ± 2 years). All patients met the criteria for type 2 diabetes suggested by an expert committee (8). Patients with a history of heart disease, nondiabetic renal disease, urinary tract infections, or serum creatinine >1.0 mg/dl were excluded. Patients treated with antihypertensive agents, currently or in the past, and patients with blood pressure >160/95 mmHg (measured with a standard clinical sphygmomanometer with Korotkoff phase V as the diastolic value) were also excluded. All patients were fully informed before giving their consent, and the study was approved by an institutional ethical committee. The patients entered the hospital, where they were placed on the standard diet, which contained ordinary levels of salt (170 mEq of sodium daily). When the plasma glucose level had been controlled for at least 2 weeks in the hospital, 24-h urine collection was performed on three consecutive days, and urine was assayed for albumin. The geometric mean of urinary albumin excretion for the 3 days was calculated. The patients were divided into three groups according to the level of albuminuria. Normoalbuminuria was defined as urinary albumin excretion <28.8 mg/24 h (<20 μg/min), microalbuminuria was defined as urinary albumin excretion of 28.8–144 mg/24 h (20–100 μg/min), and advanced albuminuria was defined as urinary albumin excretion >144 mg/24 h (>100 μg/min).

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Received for publication 8 August 1997 and accepted in revised form 26 November 1997.

Abbreviations: GFR, glomerular filtration rate; IgG, immunoglobulin G.

Table 1—*Clinical findings at beginning of study*

Stage of nephropathy	*n*	Age (years)	BMI (kg/m²)	Duration of diabetes (years)	HbA_{1c} (%)	Urinary albumin excretion (mg/24 h)	Systolic blood pressure (mmHg)	Diastolic blood pressure (mmHg)
Normoalbuminuria	8	65 ± 3	22.1 ± 0.8	11 ± 2	8.8 ± 0.4	16 ± 7 (8–27)	134 ± 5	72 ± 1
Microalbuminuria	7	55 ± 4	23.8 ± 1.8	11 ± 3	8.0 ± 0.5	65 ± 13 (31–122)*	133 ± 6	75 ± 4
Advanced albuminuria	4	61 ± 7	23.3 ± 1.9	12 ± 3	8.1 ± 0.5	479 ± 91 (309–635)*†	131 ± 4	75 ± 4

Data are means ± SE. Data for urinary albumin excretion include ranges in parentheses. *$P < 0.01$ vs. normoalbuminuria; †$P < 0.01$ vs. microalbuminuria.

Study protocol

After the plasma glucose level had been controlled, the patients were placed on a low-salt diet (85 mEq of sodium daily) or high-salt diet (255 mEq of sodium daily) for 1 week, after which they switched to the other diet, with no time intervening. The order of these diets was random. The amount of salt used as seasoning during cooking of the low-salt meals was less than usual. When on the high-salt diet, patients sprinkled salt (85 mEq of sodium daily) over the food. The patients' compliance with the diets was checked by a nurse after every meal. The completeness of 24-h urine collection was checked by analysis of urinary excretion of creatinine and sodium. Patients were asked to avoid strenuous exercise during the 2 weeks of the study. Protein constituted 20% of the calories in the diet, and each patient received the same number of calories each day, although there were differences between patients in the number of calories consumed daily. Medication other than insulin and antihyperglycemic agents was not administered.

On the last 3 days of each diet period, 24-h urine collection was performed, and the urine was assayed for calculation of the daily excretion rates of sodium, creatinine, albumin (molecular wt, 69,000; isoelectric point, 4.7), and IgG (molecular wt, 156,000; isoelectric point, 7.3). All samples were stored at −70°C until assayed. On the last day of each diet period, we measured plasma renin activity, concentrations of albumin, total IgG, and creatinine to calculate the glomerular filtration rate (GFR) and fractional protein clearances. The creatinine clearance for the 24-h urine collection was used as the GFR and corrected for body surface area (1.73 m²). The fractional protein clearance was calculated as urinary protein excretion/(serum protein concentration × GFR). The GFR and fractional protein clearances were expressed as the geometric means of 3 days. On the last day, 24-h blood pressure monitoring was done, with measurements made every hour, using a portable monitor and an oscillometric technique (Listmini BP-8800; Colin, Aichi, Japan). The mean arterial pressure was calculated by the addition of one-third of the pulse pressure to the diastolic pressure.

Laboratory procedures

The urinary albumin concentration was measured by immunoturbidimetry (Wako Pure Chemical Industries, Osaka, Japan). Intra- and interassay coefficients of variation were <7.5 and <6.9%, respectively (9). The urinary IgG concentration was measured by an enzyme-linked immunosorbent assay. Urinary sodium was measured with a flame photometer. HbA_{1c} (reference range, 4.3–5.8%) was measured by high-pressure liquid chromatography (Kyoto Daiichi Kagaku, Kyoto, Japan). Serum and urinary concentrations of creatinine were measured with a kit purchased from Kainosu (Tokyo) that is based on an enzymatic technique (10). The serum albumin concentration was measured by a chemical method (Daiichi Pure Chemicals, Tokyo) (11). The serum IgG concentration was measured by a nepherometric assay. Plasma renin activity was measured by radioimmunoassay.

Statistical analysis

Values are expressed as means ± SE or as a range. The changes within each group were evaluated by the paired *t* test. When one-way analysis of variance showed a significant difference among groups, logarithmic transformation was done if necessary, and results were evaluated by multiple comparison (Scheffé's *F* test). Statistical analysis was done with StatView J. 4.11 (Abacus Concepts, Berkeley, CA). A difference with a *P* value <0.05 was considered statistically significant.

RESULTS — There were no significant differences among the three groups in mean age, BMI, duration of diabetes, HbA_{1c}, or systolic and diastolic blood pressures (Table 1). When salt intake was low,

Table 2—*Effects of salt intake on GFR, mean arterial pressure, urinary excretion of sodium, and plasma renin activity in type 2 diabetic patients*

Stage of nephropathy	Salt level	Creatinine clearance (GFR) (ml · min^{-1} · 1.73 m^{-2})	Mean arterial pressure (mmHg)	Urinary sodium excretion (mEq/24 h)	Plasma renin activity (ng · ml^{-1} · h^{-1})
Normoalbuminuria	Low	95 ± 7	92 ± 3	58 ± 6	2.14 ± 0.77
	High	109 ± 11*	94 ± 4	190 ± 27†	0.25 ± 0.07*
Microalbuminuria	Low	121 ± 9‡	92 ± 3	66 ± 6	1.00 ± 0.27
	High	135 ± 9*	95 ± 4	159 ± 10†	0.33 ± 0.05*
Advanced albuminuria	Low	88 ± 4§	95 ± 4	78 ± 16	0.83 ± 0.39
	High	99 ± 5*	101 ± 4	164 ± 21†	0.38 ± 0.14

Data are means ± SE. Creatinine clearance for the 24-h urine collection was used as the GFR. Low, low-salt diet (85 mEq of sodium); high, high-salt diet (255 mEq of sodium). *$P < 0.05$ vs. low-salt diet; †$P < 0.01$ vs. low-salt diet; ‡$P < 0.01$ vs. normoalbuminuria with low-salt diet; §$P < 0.05$ vs. microalbuminuria with low-salt diet.

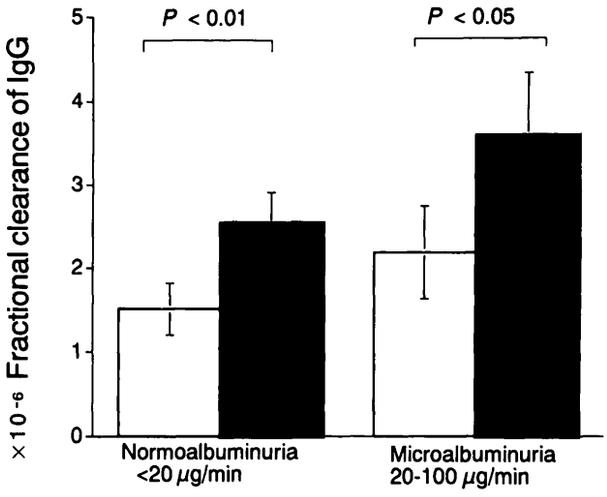

Figure 1—*Effects of high salt intake on fractional clearance of IgG in patients with normoalbuminuria or microalbuminuria. □, low-salt diet; ■, high-salt diet. Data are means ± SE.*

GFR was significantly higher in patients with microalbuminuria than in the other groups. In all groups, GFR was higher during the high-salt diet than during the low-salt diet (Table 2). With either diet, the mean arterial pressure was similar in all three groups. In all groups, mean arterial pressure was not significantly higher during the high-salt diet than during the low-salt diet. In all groups, urinary excretion of sodium was significantly higher during the high-salt diet than during the low-salt diet. In patients with normoalbuminuria or microalbuminuria, plasma renin activity was significantly higher during the low-salt diet than during the high-salt diet.

In patients with normoalbuminuria, the high-salt diet increased the fractional clearance of IgG from (1.51 ± 0.31) × 10^{-6} to (2.54 ± 0.37) × 10^{-6} ($P < 0.01$) (Fig. 1), but the fractional clearance of albumin was unchanged: (3.09 ± 0.58) × 10^{-6} with the low-salt diet, and (3.44 ± 0.74) × 10^{-6} with the high-salt diet (Fig. 2). In patients with microalbuminuria, the high-salt diet increased the fractional clearance of IgG from (2.19 ± 0.56) × 10^{-6} to (3.59 ± 0.75) × 10^{-6} ($P < 0.05$) (Fig. 1) and that of albumin from (7.32 ± 1.35) × 10^{-6} to (9.69 ± 1.64) × 10^{-6} ($P < 0.05$) (Fig. 2). In patients with advanced albuminuria, the high-salt diet caused no significant change in the fractional clearances of either IgG [from (11.3 ± 3.1) × 10^{-6} to (15.4 ± 3.3) × 10^{-6}] or albumin [from (89.6 ± 23.4) × 10^{-6} to (94.0 ± 15.4) × 10^{-6}].

The ratio of IgG clearance to albumin clearance in patients with normoalbuminuria was 0.488 ± 0.098 with the low-salt diet and 0.819 ± 0.082 with the high-salt diet ($P < 0.01$) (Fig. 3). However, the high-salt diet caused no significant change in this ratio in patients with microalbuminuria (0.303 ± 0.054 with the low-salt diet and 0.393 ± 0.079 with the high-salt diet) or advanced albuminuria (0.151 ± 0.010 and 0.169 ± 0.023, respectively). With the high-salt diet, the ratio was significantly lower in patients with microalbuminuria than in patients with normoalbuminuria (0.819 ± 0.082 and 0.393 ± 0.079, $P <$ 0.01). With the low-salt diet, the difference was not statistically significant.

When the three groups had the same salt intake (that is, with all three groups on either of the diets), fractional IgG clearance was significantly higher in patients with advanced albuminuria than in the other groups ($P < 0.01$). This clearance was not significantly higher in patients with microalbuminuria than in those with normoalbuminuria.

CONCLUSIONS — In this pilot study, a high-salt diet affected the fractional albumin clearance and the ratio of IgG clearance to albumin clearance differently in different stages of diabetic nephropathy. None of our patients dropped out of the study. For the different subjects, the amount of creatinine and sodium that should be found in 24-h collections of urine was not known, but there were no day-to-day variations in any patient. Furthermore, urinary excretion of sodium was significantly higher during the high-salt diet than during the low-salt diet. For these reasons, we believe that 24-h urine collection was complete. The high-salt diet caused no significant change in plasma renin activity in patients with advanced albuminuria, possibly because of the small sample size.

Various approaches have been used to evaluate charge and size selectivities. For evaluation of size selectivity, the fractional clearances of neutral dextrans of graded size have been measured (2), but the method is complicated and difficult to use in a clinical study. The fractional clearance of IgG, which has a high molecular weight, has been used in evaluations of size selectivity (12,13). However, this method should be used when the salt intake is con-

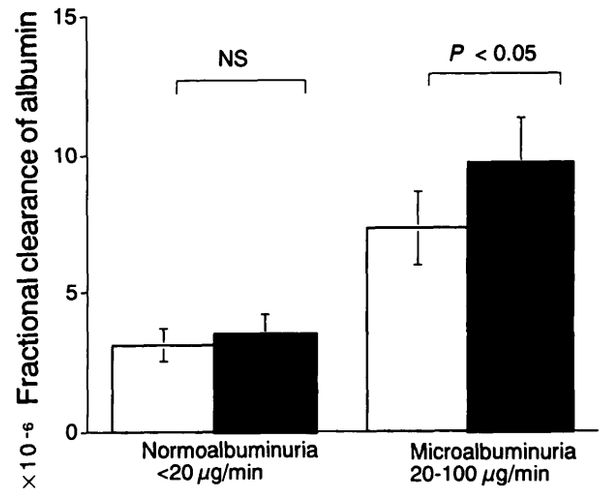

Figure 2—*Effects of high salt intake on fractional clearance of albumin in patients with normoalbuminuria or microalbuminuria. □, low-salt diet; ■, high-salt diet.*

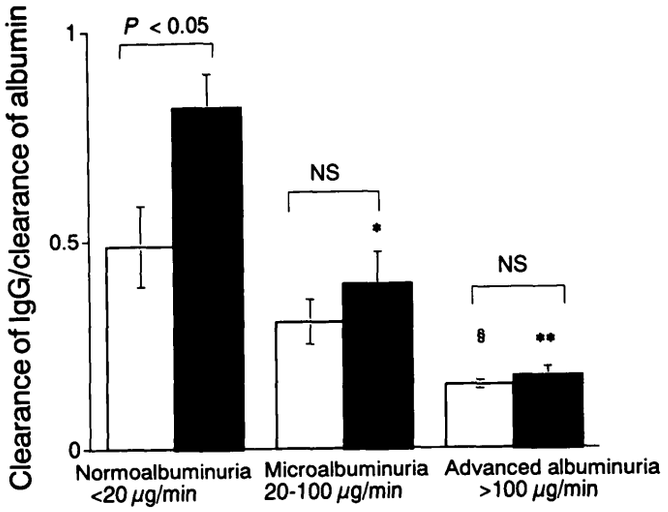

Figure 3—*Effects of high salt intake on the ratio of IgG clearance to albumin clearance in patients at different stages of diabetic nephropathy.* §P < *0.01,* *P < *0.05, and* **P < *0.01, all vs. normoalbuminuria.* □, *low-salt diet;* ■, *high-salt diet.*

stant, because the fractional IgG clearance is affected by salt intake, as we have shown here. Because IgG has a neutral charge, the increase in the fractional IgG clearance during the high-salt diet cannot be explained by changes in the number of glomerular polyanions, but it can be explained in terms of enlargement of pore size. If our results are applied to a lognormal pore-size distribution model (12), we see that an increase in salt intake shifts the pore size toward a larger diameter. Deckert et al. (3) found that patients with slight albuminuria (30–100 mg/day) have unchanged fractional IgG clearance compared with patients with normoalbuminuria, as we found here. However, in that study, urine was collected at home and the salt intake was not constant, so the results for fractional IgG clearance might have been inaccurate. For comparison of size selectivity on the basis of fractional IgG clearance among our three groups, we controlled salt intake.

One approach to the evaluation of charge selectivity is comparison of the fractional clearances of IgG and IgG4, which have the same molecular weights but different isoelectric points (7.3 for IgG and 5.5 for IgG4). Unfortunately, this method (1,4,6,14) has the drawback of giving results with a large coefficient of variation. The amount of IgG4 excreted in the urine is small, so accurate measurement is difficult, especially in patients in an early stage of diabetic nephropathy (1,6). In a study of impaired charge selectivity in patients with slight albuminuria (30–100 mg/day), the IgG/IgG4 selectivity index has been used (6), but urinary IgG4 could not be measured in some patients, even before water diuresis.

In another method of evaluating charge selectivity, the ratio of IgG clearance to albumin clearance is used (3). However, the salt intake would probably affect this ratio as well as fractional IgG clearance, which is another reason salt intake must be controlled during the study. This ratio was different in patients with normoalbuminuria and microalbuminuria only when salt intake was high. This method has the drawback of giving different results depending on the salt intake. We decided for this reason not to study the ratio at one particular level of salt intake but rather to study the difference in the ratios at two levels of salt intake.

It is impossible to measure glomerular capillary pressure directly, but we believe that a high-salt diet increases glomerular pressure in diabetic patients for the following reasons. Normal kidneys autoregulate glomerular pressure over a wide range of blood pressures; tubuloglomerular feedback (15) and the myogenic response (16,17) participate in the regulation. However, both feedback (18,19) and the myogenic response of the afferent arterioles (20,21) are impaired in experimental animals with diabetes. Increased tubular sodium reabsorption is one explanation of impaired tubuloglomerular feedback. In the early phase of diabetes, such increased reabsorption may be the main cause of hypertension (22,23). In diabetic patients, impairment of these functions needed for autoregulation seem to increase glomerular capillary pressure when the salt intake is high, broadening the pores. If it were not for repulsive forces between the negatively charged albumin and the glomerular basement membrane, albumin could pass as easily as IgG through large pores. That is, if charge selectivity is not preserved, the ratio of IgG clearance to albumin clearance will not increase when salt intake is high.

In patients with normoalbuminuria, a high-salt diet increased fractional clearance of IgG. The increase in the fractional IgG clearance was not accompanied by an increase in the fractional albumin clearance, so the ratio of IgG clearance to albumin clearance rose. Our results indicate that in patients with normal albumin excretion, charge selectivity is preserved. Another explanation for increased fractional IgG clearance during the high-salt diet might be that tubular reabsorption of IgG is hindered more than reabsorption of albumin. This explanation is unlikely because IgG and albumin are handled in a similar way by the tubules (24).

In patients with microalbuminuria, the high-salt diet increased the fractional clearance of albumin as well as IgG; therefore, the ratio of IgG clearance to albumin clearance did not change significantly, indicating some neutralization of the pore charge. However, size selectivity was preserved, because when salt intake was the same, the fractional IgG clearance in patients with microalbuminuria was similar to that in patients with normoalbuminuria.

Fractional IgG clearance was higher in patients with advanced albuminuria than in the other groups, indicating that size selectivity as well as charge selectivity had worsened. The fact that fractional clearance of the two proteins that were tested during the high-salt diet was unchanged indicates that morphological changes were too severe for hemodynamic changes caused by different salt intakes to have any effect.

Charge selectivity was worse in patients with microalbuminuria than in patients with normoalbuminuria. In patients with advanced albuminuria, both size and charge selectivities were lost. These results in patients with type 2 diabetes are consistent with previous findings in patients with type 1 diabetes (3,6). The methods used here for the evaluation of charge and size selectivities are probably more reliable than those used previously.

Our results have clinical implications. Salt restriction reduced urinary albumin

excretion in patients with type 2 diabetes and microalbuminuria without significantly changing their blood pressure. Salt restriction might be shown in a larger study to be useful in the treatment of such patients.

In conclusion, the loss of charge selectivity occurs before the loss of size selectivity as diabetic nephropathy progresses in type 2 diabetic patients. Studies of larger numbers of patients, including obese ones, in various stages of diabetic nephropathy are needed to check these results and to clarify whether salt restriction is effective in the treatment of diabetic nephropathy.

Acknowledgments — We thank Professor R. Kikkawa, Third Department of Medicine, Shiga University of Medical Science, Otsu, Japan, for his helpful discussion.

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