Parallel Increase in Urinary Excretion Rates of Immunoglobulin G, Ceruloplasmin, Transferrin, and Orosomucoid in Normoalbuminuric Type 2 Diabetic Patients

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OBJECTIVE — Increased urinary excretions of several plasma proteins with different molecular radii <55 Å and different isoelectric points (pI), such as IgG, ceruloplasmin, transferrin, and orosomucoid, have been independently reported to precede the development of microalbuminuria in diabetic patients. We examined whether increases in urinary excretions of these proteins would be in parallel in the same patient.

RESEARCH DESIGN AND METHODS — Urinary excretion rates of proteins mentioned above in timed overnight urine samples were evaluated in 61 normoalbuminuric type 2 diabetic patients (group D) aged 40–60 years and in 17 age-matched control subjects (group C).

RESULTS — The excretion rates of these proteins were significantly higher in group D than in group C. These exhibited a strong linear correlation with each other and had a weak correlation with the excretion rate of *N*-acethylglucosaminidase. The excretion rate of α_2-macroglobulin with large molecular radii of 88 Å was not different between groups C and D, nor did they have any correlations with the excretion rates of the other proteins. Creatinine clearance and blood pressure levels in group D were significantly higher than those in group C.

CONCLUSIONS — In normoalbuminuric diabetic patients, excretion rates of plasma proteins with molecular radii <55 Å increased in parallel with each other. In view of our previous finding that urinary excretions of these plasma proteins selectively increased in parallel with enhanced glomerular filtration rate after acute protein loading, the present finding may be explained by renal hemodynamic changes, such as increased intraglomerular hydraulic pressure.

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Diabetic nephropathy is the most frequent cause of entering renal replacement therapy in industrialized countries such as the U.S., European countries, Australia, New Zealand, and Japan (1). Early recognition of renal changes increases the chance of preventing the development of diabetic nephropathy. Although microalbuminuria is generally recognized as the best available noninvasive predictor of diabetic nephropathy, several studies including ours have shown that increased urinary excretions of some kinds of plasma proteins with different molecular radii <55 Å and different isoelectric points (pIs) such as IgG (2–4), transferrin (5–8), ceruloplasmin (4,9), and orosomucoid (10) may precede the development of microalbuminuria in diabetic patients. Kazumi et al. (11) reported that increased urinary transferrin excretion could predict the development of microalbuminuria in type 2 diabetic patients.

Recently, we found that urinary excretions of plasma proteins with molecular radii <55 Å (IgG, IgG4, ceruloplasmin, transferrin, and orosomucoid, abbreviated to small-sized plasma proteins group [SPP]) selectively increased irrespective of their pI despite no increased urinary excretion of albumin (molecular radii = 36 Å, smaller than that of IgG, IgG4, and ceruloplasmin) when glomerular filtration rate (GFR) was increased by acute protein loading (APL) in healthy subjects (IgG, IgG4, and ceruloplasmin [12]; transferrin and orosomucoid [T.N., H.S., M.H., T.Mi., N.Y., T.Mo., T.S., J.K., H.F., M.K., S.I., unpublished data.]). In this study, increased urinary excretion of α_2-macroglobulin with molecular radii of 88 Å was not detected. These findings suggest that changes in renal hemodynamics, such as intraglomerular hydraulic pressure (GP), can be predicted by the measurement of urinary excretions of SPP. Subsequently, we found that renal clearances of SPP increased in subjects diagnosed with persistent impaired glucose tolerance for at least 2 years (13). Furthermore, we found that increased urinary excretions of IgG and ceruloplasmin were present in normoalbuminuric type 2 diabetic patients, especially with enhanced GFR or hypertension and were stabilized after strict glycemic control (4). Taken to-

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Abbreviations: APL, acute protein loading; GFR, glomerular filtration rate; GP, intraglomerular hydraulic pressure; NAG, *N*-acetylglucosaminidase; pI, isoelectric point; SPP, small-sized plasma proteins group; U-glu, levels of glucosuria.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

gether, we hypothesized that increased urinary excretions of SPP may reflect intraglomerular hypertension, which has been recognized as one of the main mechanisms of development and progression of diabetic nephropathy (14–16).

However, there is limited information on the mutual relationships among urinary excretions of SPP in normoalbuminuric diabetic patients. In other words, it is unclear whether one patient with increased urinary excretion of IgG would have increased urinary excretions of transferrin, ceruloplasmin, or orosomucoid. Therefore, in the present study, we examined correlations among urinary excretions of SPP in normoalbuminuric diabetic patients.

RESEARCH DESIGN AND METHODS

— Sixty-one type 2 diabetic patients diagnosed with normoalbuminuria (38 men and 23 women age 52.7 ± 7.0 years, range 40–60) were recruited from outpatients of our hospital to participate in this study (group D). All patients met the criteria for type 2 diabetes proposed by the expert committee (17). No subjects had a history of disease other than diabetes, hypertension, or dyslipidemia. Absence of clinical renal disease was established from the clinical history, normal urinary sediment, and lack of detectable lesions such as unilateral or bilateral atrophy, urinary stones, hydronephrosis, or tumor in the kidneys by ultrasound examination. An electrocardiogram and a lack of symptoms of angina pectoris confirmed that all patients were also free from coronary artery disease. Careful physical examination and evaluation of medical histories showed that no patients had cerebrovascular disease or peripheral vascular disease.

Normoalbuminuria was defined as a urinary albumin excretion rate of <10 μg/min in all of serial timed overnight urine samples, according to the recommendation of the Japan Diabetes Association. Blood pressure was measured using a standard clinical sphygmomanometer with Korotkoff phase V as the diastolic values in a sitting position after 5 min at rest. Sixteen patients had been treated with antihypertensive agents and seven of whom had been treated with ACE inhibitors. Antidyslipidemic agents also had been administered in 14 patients (12 were statins and 2 were fibrates). Administration of antihypertensive agents or antidyslipidemic treatment was continued at equivalent dosage until this study was completed. Seventeen healthy age-matched volunteers (nine men and eight women age 51.9 ± 4.1 years, range 44–58) were recruited as a normal control population from members of the medical research staff at our hospital (group C). They had no history of diabetes, heart disease, hypertension, or known renal disease. They were normoglycemic, normotensive, and normoalbuminuric, and their urinary sediment was free from erythrocytes and other indications of renal diseases. All participants in this study gave their consent after being fully informed of the study protocol.

Study protocol

Timed overnight urine samples but not 24-h urine samples were collected based on the finding that fish and meat in diets induced transiently enhanced GFR, which caused increase in urinary excretions of some kinds of plasma proteins (12). Sixty-one patients were asked to collect exactly timed overnight urine samples on three different occasions according to the procedure previously reported (4). Concentrations of six different SPPs (albumin, IgG, ceruloplasmin, α_2-macroglobulin, transferrin, and orosomucoid) were measured to determine urinary excretion rates (albumin, IgG, ceruloplasmin, α_2-macroglobulin, transferrin, and orosomucoid excretion rates, respectively) of these substances. Geometric means of these urinary protein excretion rates of three serial urine samples of the patients were used for the evaluation. All of the patients visited our outpatients' clinic at least once a month. We could collect three serial samples from each patient within 2 or 3 months. Similarly, urinary concentration of *N*-acetylglucosaminidase (NAG), which is widely used as an indicator of proximal tubular damage, and levels of glucosuria were evaluated according to the same procedure. Blood samples were taken in the morning after the timed overnight urine samples were collected. Creatinine clearance was calculated using these overnight urine samples. The mean of three serial creatinine clearances was used for the evaluation. Additionally, levels of HbA_{1c}, total cholesterol, triglycerides, and HDL cholesterol were measured. In group C, timed overnight urine samples and blood were collected one occasion.

Laboratory procedures

Concentrations of IgG, ceruloplasmin, and α_2-macroglobulin of serum and urine samples were measured by immunoradiometric assay according to procedures previously reported (4,9,12,18). Intra- and interassay coefficient variances for the assays of these plasma proteins were almost 3 and 10%, respectively.

Levels of albumin, transferrin, and orosomucoid in urine were measured by radioimmunoassay using the double antibody technique (10,19). Intra- and interassay coefficients of variation for this method were 5 and 12%, respectively. Urinary NAG concentrations were measured by the *m*-cresol purple method (Shionogi, Osaka, Japan).

Urinary concentrations of creatinine and glucose and serum concentrations of creatinine, total cholesterol, triglycerides, and HDL cholesterol were measured by enzymatic methods using an automated multianalyzer (7600 Hitachi, Tokyo). HbA_{1c} was measured by high performance liquid chromatography using an automated analyzer (HLC-723GHb V A1c 2.2; Tosoh, Tokyo). The reference range of HbA_{1c} of our hospital laboratory was 4.3–5.8%. All urine and serum samples from the subjects were stored at −80°C until required. We used the ratio of renal clearance of a negatively charged ceruloplasmin (molecular radii of ceruloplasmin is 44 Å) to that of a neutrally charged IgG (molecular radii of IgG is 55 Å) as an indicator of the impairment of glomerular charge selectivity (4,20).

Statistical analysis

Values are expressed as means ± SD or median with range. The Mann-Whitney test was used to calculate statistically significant differences of the values between groups C and D. The Pearson's correlation analysis was performed between two different urinary protein excretion rates. Due to skew-deviated distribution, logarithmically transformed urinary protein excretion rates were used for the correlation analysis. All calculations were made using the Stat View software package (Abacus Concept, Berkeley, CA).

RESULTS

— Table 1 shows clinical characteristics of 61 normoalbuminuric type 2 diabetic patients and 17 control subjects. HbA_{1c}, systolic blood pressure, and diastolic blood pressure were higher in group D than in group C, although the

Table 1—*Clinical characteristics of 17 control subjects and 61 normoalbuminuric type 2 diabetic patients*

Parameters	Control subjects	Diabetic patients
Age (years)	51.9 ± 4.1	52.7 ± 7.0
n (men/women)	17 (9/8)	61 (38/23)
BMI (kg/m^2)	22.8 ± 3.1	23.3 ± 2.9
Known duration (years)		9.3 ± 5.2
HbA_{1c} (%)	5.0 ± 0.23	6.9 ± 0.94*
Systolic blood pressure (mmHg)	113.4 ± 10.6	124.9 ± 11.6*
Diastolic blood pressure (mmHg)	70.3 ± 7.6	75.5 ± 8.6†
Total cholesterol (mmol/l)	5.3 ± 0.79	5.0 ± 0.70
Triglycerides (mmol/l)	1.2 ± 0.78	1.2 ± 0.89
HDL cholesterol (mmol/l)	1.6 ± 0.46	1.5 ± 0.48
Retinopathy (nil/simple/proliferative)		45/14/2
Hypertension treatment (%)		26.2
Hyperlipidemia treatment (%)		23.0
Diabetes treatment (diet/oral hypoglycemic agents/insulin)		20/29/12

Data are means ± SD unless otherwise indicated. *$P < 0.001$ and †$P < 0.01$ vs. control subjects revealed by Mann-Whitney test.

profiles of serum lipids concentrations did not differ between the groups.

Creatinine clearance, albumin excretion rates, IgG excretion rates, ceruloplasmin excretion rates, α_2-macroglobulin excretion rates, transferrin excretion rates, orosomucoid excretion rates, NAG excretion rates, and the levels of glucosuria (U-glu excretion rate) of timed overnight urine samples are listed in Table 2. Creatinine clearance and U-glu excretion rate were significantly higher in group D than in group C. Urinary leakage of IgG, ceruloplasmin, transferrin, and orosomucoid in group D significantly increased compared with those in group C, whereas that of α_2-macroglobulin did not differ between the two groups. The IgG excretion rates of 19 (31.1%) patients in group D exceeded the upper limit of those in group C. Similarly, the ceruloplasmin excretion rate of 18 (29.5%) patients, the transferrin excretion rate of 16 (26.2%) patients, and the orosomucoid excretion rate of 20 (32.8%) patients in group D exceeded the upper limits of those in group C, although the α_2-macroglobulin excretion rate of only 6 (9.8%) patients in group D exceeded the upper limit of those in group C. In these 19 patients with an elevated IgG excretion rate, 14 (73.7%) patients had elevated ceruloplasmin excretion rates, exceeding the upper limit of those in group C. Similarly, 13 (68.4%) and 11 (57.9%) of these 19 patients had elevated orosomucoid excretion rates and transferrin excretion rates, respectively. Contrastively, only 3 of these 19 patients had an elevated α_2-macroglobulin excretion rate.

Figure 1 illustrates correlations between IgG excretion rates and each of excretion rates of the other plasma proteins. Strong correlations existed between IgG and ceruloplasmin excretion rates (Fig. 1*B*, extremely strong linear correlations; $r = 0.890$ and 0.832 in groups C and D, respectively), between IgG and transferrin excretion rates (Fig. 1*D*), and between IgG and orosomucoid excretion rates (Fig. 1*E*) both in groups C and D. Compared with these results, the correlation between IgG and albumin excretion rates was significant but relatively weak (Fig. 1*A*). In contrast, α_2-macroglobulin excretion rates did not reveal significant correlations with IgG excretion rates in both groups C and D (Fig. 1*C*). In seven diabetic patients treated with ACE inhibitors, correlation coefficients of IgG excretion rates versus albumin excretion rates, ceruloplasmin excretion rates, transferrin excretion rates, and orosomucoid excretion rates were 0.575, 0.720, 0.623, and 0.558, respectively (indicated by solid circles throughout Fig. 1). These correlations were comparable with those in the remaining 54 patients without ACE inhibitors ($r = 0.640$, 0.837, 0.736, and 0.692 in IgG excretion rates versus albumin excretion rates, ceruloplasmin excretion rates, transferrin excretion rates, and orosomucoid excretion rates, respectively, indicated in open circles throughout Fig. 1). Levels of albumin excretion rates, IgG excretion rates, ceruloplasmin excretion rates, transferrin excretion rates, and orosomucoid excretion rates of patients treated with ACE inhibitors tended to be higher than those of patients without ACE inhibitors ($P = 0.0295$, 0.0655, 0.0412, 0.0501, and 0.0031, respectively). HbA_{1c} did not differ between patients with and without ACE inhibitors in group D (6.8 ± 0.65% with ACE inhibitors; 7.0 ± 0.97% without ACE inhibitors), but blood pressure levels in patients treated with ACE inhibitors was significantly higher ($P < 0.001$) than in patients without ACE inhibitors (134.7 ± 9.0/86.6 ± 6.1 mmHg; 123.6 ± 11.4/75.8 ± 8.1 mmHg, respectively).

Although NAG excretion rates were significantly higher in group D than in group C (Table 2), correlations between NAG excretion rates and each of excretion

Table 2—*Creatinine clearance and urinary excretion rates of six plasma proteins of timed overnight urine samples in control subjects and normoalbuminuric type 2 diabetic patients*

	Control subjects	Diabetic patients
Creatinine clearance (ml/min/1.73 m^2)	74.3 ± 17.2	122.8 ± 26.8*
U-glu excretion rate (mg/min)	0.032 (0.012–0.063)	0.30 (0–47)*
Albumin excretion rate (μg/min)	2.9 (1.1–8.2)	3.9 (0.5–9.6)
IgG excretion rate (μg/min)	1.2 (0.35–2.6)	1.7 (0.36–8.4)†
Ceruloplasmin excretion rate (ng/min)	21.6 (6.0–52)	38.4 (5.9–130)‡
α2-Macroglobulin excretion rate (ng/min)	11.0 (2.7–30)	7.9 (2.1–51)
Transferrin excretion rate (ng/min)	69 (20–230)	110 (21–620)‡
Orosomucoid excretion rate (ng/min)	170 (28–510)	370 (71–6400)†
NAG excretion rate (mU/min)	1.2 (0.094–2.5)	2.4 (0.037–12)*

Data are means ± SD or median (range). *$P < 0.001$, †$P < 0.01$, and ‡$P < 0.05$ versus control subjects revealed by Mann-Whitney test.

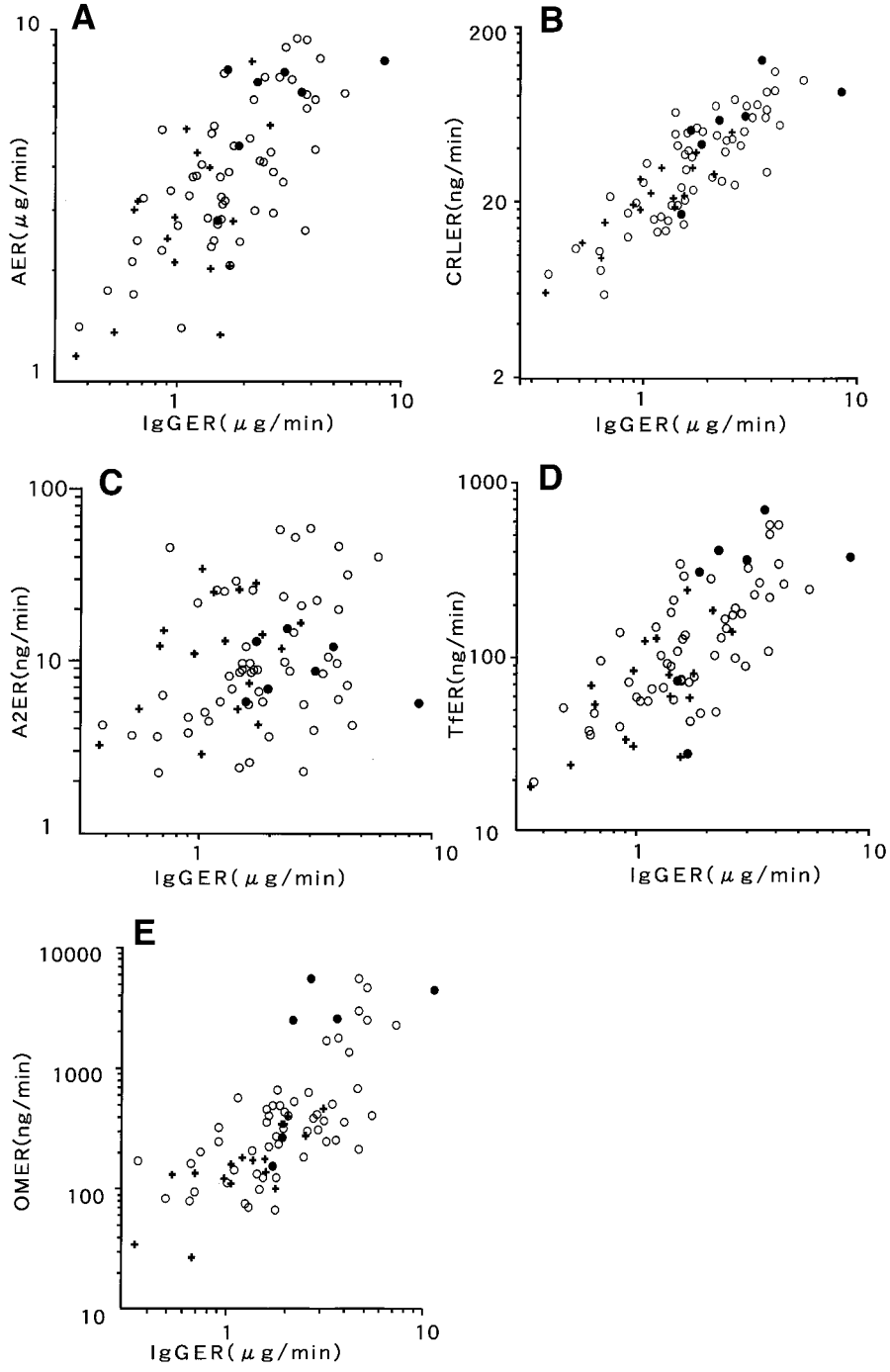

Figure 1—*Relationships between urinary IgG excretion rates and urinary albumin excretion rates* (A)*, urinary ceruloplasmin excretion rates* (B)*, urinary α_2-macroglobulin excretion rates* (C)*, urinary transferrin excretion rates* (D)*, and urinary orosomucoid excretion rates* (E) *of timed overnight urine samples in control subjects (cross symbols), normoalbuminuric type 2 diabetic patients treated with ACE inhibitors (●), and without ACE inhibitors (○). x- and y-axes are expressed on a logarithmic scale. A: Group C,* r = *0.573* (P = *0.0148*)*; group D,* r = *0.650* (P < *0.0001*)*. B: Group C,* r = *0.890* (P < *0.0001*)*; group D,* r = *0.832* (P < *0.0001*)*. C: Group C,* r = *0.334* (P = *0.194*)*; group D,* r = *0.287* (P = *0.0256*)*. D: Group C,* r = *0.664* (P = *0.0028*)*; group D,* r = *0.718* (P < *0.0001*)*. E: Group C,* r = *0.803* (P < *0.0001*)*; group D,* r = *0.692* (P < *0.0001*)*. In seven patients treated with ACE inhibitors of group D,* r *of IgG excretion rate versus AER, ceruloplasmin excretion rates, transferrin excretion rates, and orosomucoid excretion rates were 0.575, 0.720, 0.623, and 0.558, respectively. These correlations were comparable with those in the remaining 54 patients without ACE inhibitors* (r = *0.640, 0.837, 0.736, and 0.692 in IgG excretion rates versus AER, ceruloplasmin excretion rates, transferrin excretion rates, and orosomucoid excretion rates, respectively).*

rates of the other plasma proteins were weaker in group D than in group C (r = 0.278, 0.384, 0.426, 0.288, 0.355, and 0.461 in NAG excretion rates versus albumin excretion rates, IgG excretion rates, ceruloplasmin excretion rates, α_2-macroglobulin excretion rates, transferrin excretion rates, and orosomucoid, respectively, in group D; in the same order, r = 0.569, 0.580, 0.574, 0.567, 0.388, and 0.689, respectively, in group C). Additionally, in group D, both of these correlations related to NAG excretion rates, and correlations between U-glu excretion rates and each of the excretion rate of the other plasma proteins (r = 0.391, 0.437, 0.407, 0.157, 0.457 and 0.496 in U-glu excretion rates versus albumin, IgG excretion rates, ceruloplasmin excretion rates, α_2-macroglobulin excretion rates, transferrin excretion rates, and orosomucoid, respectively) were relatively weaker than those between IgG excretion rates and each of the excretion rates of the other proteins (indicated in the legend of Fig. 1). The glomerular charge selectivity index calculated as the ratio of renal clearance of ceruloplasmin to IgG was almost identical between groups C and D (1.5 ± 0.50 in group C; 1.4 ± 0.49 in group D).

CONCLUSIONS — The present study clarified that a substantial number of normoalbuminuric type 2 diabetic patients had increases in urinary excretions of SPP simultaneously without an increase in α_2-macroglobulin with large molecular radii (88 Å) when compared with the healthy control subjects. We also found that excretion rates of SPP exhibited a strong correlation with each other. In contrast, α_2-macroglobulin excretion rates did not have any correlation with any of the excretion rates of SPP. Previous reports on normoalbuminuric diabetic patients independently showed that one or two urinary excretions of SPP exceeded the upper limit in control subjects (2–10). The present study is the first report to demonstrate that excretion rates of all of SPP members increased in parallel in normoalbuminuric diabetic patients.

Kazumi et al. (11) reported that increased urinary transferrin excretion could predict a future development of microalbuminuria in normoalbuminuric type 2 diabetic patients. Together with the results of the present study, increased urinary excretions of SPP may equally have a predictive value of the future develop-

ment of microalbuminuria. This is clinically very important because additional methods for detection of a quite early stage in the development of diabetic nephropathy may be provided.

The mechanism behind these increases in urinary excretions of SPP in a substantial portion of normoalbuminuric diabetic patients is unclear. The etiology of proteinuria in diabetic nephropathy was reported to be due to three main factors: loss of glomerular charge selectivity (20–23), impairment of glomerular pore size selectivity (23–26), and increase in GP (14–16).

The impairment of glomerular charge selectivity is believed to be initiated from the stage of microalbuminuria both in type 1 and type 2 diabetes based on the relative increase in the ratio of the renal clearance of a negatively charged protein to that of a neutrally charged protein with similar molecular radii (20,22,23). In this study, we could not detect the impairment of glomerular charge selectivity in normoalbuminuric patients because parallel increases in urinary excretions of SPP with different pI (IgG, 7.4; ceruloplasmin, 4.4; transferrin, 5.7; orosomucoid, 2.7) were observed. Furthermore, the glomerular charge selectivity index calculated as the ratio of renal clearnace of a negatively charged ceruloplasmin to a neutrally charged IgG did not differ between groups C and D in the present study as reported previously (4,20). Thus, the increased excretion rates of SPP in group D cannot be explained by the impaired glomerular charge selectivity.

To explain transglomerular plasma protein passage, Deen et al. (27) proposed "the isoporous + shunt model," in which a large portion of the glomerular capillary wall is assumed to be perforated by restrictive pores of identical radii (29–31 Å) and a small portion by large nondiscriminatory pores (the shunt pathway, 110–115 Å) (28,29). When considering the normal pore size distribution quoted in these reports, α_2-macroglobulin might not pass through the shunt pathway easily because of its relatively large molecular radii, and SPPs may increase their flux through the shunt pathway if increased GP is present. Furthermore, if the glomerular pore size selectivity is impaired, urinary α_2-macroglobulin excretion may be assumed to increase when enhanced GP occurs. These ideas may be supported by our recent report that enhanced GFR caused by APL induced increases in urinary excretions of SPP but not of α_2-macroglobulin in healthy subjects (12). Based on these results, selectively increased excretion rates of SPP in the present study cannot be explained by the impairment of pore size selectivity but rather by enhanced GP. This notion appears to be partially supported by the finding that increased urinary IgG excretion was induced by exogenous norepinephrine administration, which may cause an increase in GP in control subjects and type 1 diabetic patients (30). In the present study, mutual relationships among excretion rates of SPP in patients treated with ACE inhibitors were similar to those in patients without ACE inhibitors. However, the levels of excretion rates of SPP tended to be higher in the former patients than in the latter. Considering that blood pressure was higher in the former patients than in the latter and that HbA_{1c} was not different, it seems likely that the difference in the levels of excretion rates of SPP between patients with and without ACE inhibitors may be due to blood pressure levels. Additionally, the levels of blood pressure were slightly but significantly higher in group D than in group C. The increase in excretion rates of SPP might be attributed simply to hypertension. Therefore, it will be necessary to examine whether the increase in urinary excretions of SPP would be detected in nondiabetic patients with essential hypertension.

Renal tubular reabsorption plays an important role on the urinary leakage of plasma proteins. Although the knowledge of renal tubular reabsorption of SPP is limited to albumin and transferrin at present, it was reported that a certain portion was reabsorbed through receptor-mediated endocytosis in the proximal tubule (31). In the present study, we evaluated proximal tubular function through measurements of NAG and effects of glucosuria on urinary SPP excretions. Although the NAG excretion rate was higher in group D than in group C, correlations between NAG and each of the SPP excretion rates and between U-glu and each of the SPP excretion rates were weak. Additionally, correlations between NAG and each of the SPP excretion rates were weaker in group D than in group C. These results indicated that disturbance of the proximal tubular reabsorption may not play a major role in high urinary excretion levels of SPP in our normoalbuminuric diabetic patients, although we cannot totally deny the possibility of disturbance of tubular reabsorption only by these relationships among NAG, glucosuria, and SPP. Further information of tubular handling mechanisms of SPP has to be elucidated.

In the previous study of APL in the healthy subjects, urinary excretion of albumin did not increase despite the increased urinary excretions of SPP when enhanced GFR occurred after APL (IgG, IgG4, and ceruloplasmin [12]; transferrin and orosomucoid [T.N., H.S., M.H., T.Mi., N.Y., T.Mo., T.S., J.K., H.F., M.K., S.I., unpublished data]). In the present study, the albumin excretion rate had a statistically significant but relatively weak correlation with the IgG excretion rate both in groups C and D. The exact reasons behind this discrepancy for changes in albumin excretion rates in both studies have yet to be elucidated. However, in view of the fact that the subjects in APL (12) were young healthy subjects (mean age 25.6 years), the difference in age of subjects may partially explain this discrepancy. That is, although completely intact renal handling of plasma protein leakage in young healthy subjects may not permit an increase in the albumin excretion rate following the hemodynamic changes after APL, the albumin excretion rate in the middle-aged subjects and the patients in the present study may have a tendency to increase depending on renal hemodynamic changes because renal handling of plasma protein leakage in them may not be completely intact. This idea may be supported by the following two findings. One is that in reanalysis of the previous APL study (12), the IgG excretion rate did not correlate with the albumin excretion rate ($r = 0.071$) but rather the ceruloplasmin excretion rate ($r = 0.702$) at baseline. The other is that in the present study, the correlation between the albumin excretion rate and the IgG excretion rate was statistically significant but weaker than those among the IgG excretion rate and each of the ceruloplasmin excretion rate, the transferrin excretion rate, and the orosomucoid excretion rate. Thus, the albumin excretion rate may be a less sensitive marker to reflect changes in renal hemodynamics than IgG excretion rates, ceruloplasmin excretion rates, transferrin excretion rates, and orosomucoid excretion rates.

Urinary excretion of IgG has been analyzed as a marker of impaired glomerular pore size selectivity (23,26,27). However, the present study and the previous study (12) suggest that the IgG excretion rate may increase under the following conditions: impaired glomerular pore size selectivity and enhanced GP. Therefore, it must be emphasized that increased urinary excretion of IgG should be evaluated as a marker of both increased GP and impaired glomerular pore size selectivity.

References

1. Excerpts from the USRDS 2000 Annual Data Report: International comparison. *Am J Kidney Dis* 30:S177–S182, 2000
2. Deckert T, Feldt-Rasmussen B, Djurup R, Deckert M: Glomerular size and charge selectivity in insulin-dependent diabetes mellitus. *Kidney Int* 33:100–106, 1988
3. Jerums G, Allen TJ, Cooper ME: Triphasic changes in selectivity with increasing proteinuria in type 1 and type 2 diabetes. *Diabet Med* 6:772–779, 1989
4. Narita T, Fujita H, Koshimura J, Meguro H, Kiatazto H, Shimotomai T, Kagaya E, Suzuki K, Murata M, Usami A, Ito S: Glycemic control reverses increases in urinary excretions of immunoglobulin G and ceruloplasmin in type 2 diabetic patients with normoalbuminuria. *Horm Metab Res* 33:370–378, 2001
5. O'Donnel MJ, Martin P, Florkowski CM, Toop MJ, Chapman C, Holder R: Urinary excretion of transferrin excretion in type I (insulin-dependent) diabetes mellitus. *Diabet Med* 8:657–661, 1991
6. Cheung CK, Cockram CS, Yeung VFT, Schwaminathan R: Urinary excretion of transferrin in non-insulin-dependent diabetics: a marker for early complications. *Clin Chem* 35:1672–1674, 1989
7. McCormick CP, Konen JC, Shihabi ZK: Microtransferrinuria and microalbuminuria: I. In the diabetic human. *Clin Physiol Biochem* 8:53–58, 1990
8. Bernard AM, Ouled Amor AA, Goemaere-Vanneste J, Antonie JL, Lauwerys RR, Lambert A, Vandeleene B: Microtransferrinuria is a more sensitive indicator of early glomerular damage in diabetes than microalbuminuria. *Clin Chem* 34:1920–1921, 1988
9. Yamazaki M, Ito S, Usami A, Tani N, Hanyu O, Nakagawa O, Nakamura H, Shibata A: Urinary excretion rate of ceruloplasmin in non-insulin dependent diabetic patients with different stage of nephropathy. *Eur J Endocrinol* 132:681–687, 1995
10. Ito S, Tsuda A, Momotsu T, Igarashi K, Kasahara S, Satoh K, Shibata A: Urinary orosomucoid excretion rate in patients with non-insulin-dependent diabetes mellitus. *Acta Endocrinol (Copenh)* 120:584–590, 1989
11. Kazumi T, Hozumi T, Ishida Y, Ikeda Y, Kishi K, Hayakawa M, Yoshino G: Increased urinary transferrin excretion predicts microalbuminuria in patients with type 2 diabetes. *Diabetes Care* 22:1176–1180, 1999
12. Narita T, Kitazato H, Koshimura J, Suzuki K, Murata M, Ito S: Effects of protein meals on the urinary excretion of various plasma proteins in healthy subjects. *Nephron* 81:398–405, 1999
13. Fujita H, Narita T, Ito S: Abnormality in urinary protein excretion in Japanese men with impaired glucose tolerance. *Diabetes Care* 22:823–826, 1999
14. Hostetter TH, Rennke HG, Brenner BM: The case for intrarenal hypertension in the initiation and progression of diabetic and other glomerulopathies. *Am J Med* 72: 375–380, 1982
15. Zatz R, Meyer TW, Rennke HG, Brenner BM: Predominance of hemodynamic rather than metabolic factors in the pathogenesis of diabetic glomerulopathy. *Proc Natl Acad Sci U S A* 82:5963–5967, 1985
16. Anderson S, Brenner BM: Pathogenesis of diabetic nephropathy: hemodynamic considerations. *Diabetes Metab Rev* 4: 163–177, 1988
17. Expert Committee on the Diagnosis and Classification of Diabetes Mellitus: Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care* 20:1183–1195, 1997
18. Ito S, Usami A, Yamazaki M, Shibata A: A radioimmuno-metric assay for urinary α_2-macroglobulin. *Tohoku J Exp Med* 176: 137–147, 1995
19. Brodows RG, Nichols D, Shaker G, Kubasik NP: Evaluation of a new radioimmunoassay for urinary albumin. *Diabetes Care* 9:189–193, 1986
20. Narita T, Koshimura J, Suzuki K, Murata M, Meguro H, Fujita H, Kitazato H, Ito S: Effects of short-term glycemic control, low protein diet and administration of enalapril on renal hemodynamics and protein permselectivity in type 2 diabetic patients with microalbuminuria. *Tohoku J Exp Med* 189:117–133, 1999
21. Nakamura Y, Myers BD: Charge selectivity of proteinuria in diabetic glomerulopathy. *Diabetes* 37:1202–1211, 1988
22. Pietravalle P, Morano S, Cristina G, De Rossi MG, Mariani G, Cotroneo P, Ghirlanda G, Clementi A, Andreani D, Di Mario U: Charge selectivity of proteinuria in type I diabetes explored by Ig subclass clearance. *Diabetes* 40:1685–1690, 1991
23. Deckert T, Kofoed-Enevoldsen A, Vidal P, Nφrgaard K, Andreasen HB, Feldt-Rasmussen B: Size- and charge selectivity of glomerular filtration in type 1 (insulin-dependent) diabetic patients with and without albuminuria. *Diabetologia* 36: 244–251, 1993
24. Myers BD, Winetz JA, ChuiF, Michaels AS: Mechanisms of proteinuria in diabetic nephropathy: a study of glomerular barrier function. *Kidney Int* 21:633–641, 1982
25. Tomlanovich S, Deen WM, Jones HW III, Schwartz HC, Myers BD: Functional nature of the glomerular injury in progressive diabetic glomerulopathy. *Diabetes* 36:556–565, 1987
26. Scandling JD, Myers BD: Glomerular size-selectivity and microalbuminuria in early diabetic glomerular disease. *Kidney Int* 41: 840–846, 1992
27. Deen WM, Bridges CR, Brenner BM, Myers BD: Heteroporous model of glomerular size selectivity: application to normal and nephrotic humans. *Am J Physiol* 249: F374–F389, 1985
28. Myers BD: Pathophysiology of proteinuria in immune glomerular injury. *Am J Nephrol* 10:19–23, 1990
29. Tencer J, Frick IM, Öqvist B, Alm P, Rippe B: Size selectivity of the glomerular barrier to high molecular weight proteins: the upper size limitation of the shunt pathways. *Kidney Int* 53:709–715, 1998
30. Hoogenberg K, Sluiter WJ, Navis G, Van Haeften TW, Smit AJ, Reitsma WD, Dullaart RPF: Exogenous norepinephrine induces an enhanced microproteinuric response in microalbuminuric insulin-dependent diabetes mellitus. *J Am Soc Nephrol* 9:643–654, 1998
31. Verroust PJ, Kozyraki R: The roles of cubilin and megalin, two multiligand receptors, in proximal tubule function: possible implication in the progression of renal disease. *Curr Opin Nephrol Hypertens* 10:33–38, 2001