Original Article

Urinary Excretions of Albumin and Type IV Collagen in Normotensive and Hypertensive Subjects

Toshihiko ISHIMITSU, Naoki MURAYAMA*, Teruo MEGURO*, Yo KAGEYAMA*, Kazuhisa OKADA*, Yasuhiro OKUBO*, Shigeyuki TAKEDA*, Kazuo HASEGAWA*, Yoshinobu KIKEGAWA*, Tsuneo OHWADA*, Hideo HOSOYA*, Junichi MINAMI, Hidehiko ONO, and Hiroaki MATSUOKA

Plasma albumin leaks into urine as a result of glomerular hypertension and basement membrane injury, while urinary type IV collagen derives from mesangial matrix and glomerular basement membrane. The purpose of this study was to elucidate the pathophysiological significance of these urinary microproteins as an indicator of cardiovascular organ injuries in hypertension. In health-checkup participants without diabetes, proteinuria, or microhematuria, and who were not being treated for hypertension or any other disease at the time of enrollment, urinary albumin and type IV collagen were measured and their relations to organ injuries and cardiovascular risk factors were evaluated. Of 1,079 subjects (40- to 65-year-old; 256 men and 823 women) enrolled in the study, 120 (11.1%) had untreated hypertension exceeding 140/90 mmHg. Urinary albumin was positively correlated with both age ($r=0.16$, $p<0.001$) and systolic blood pressure ($r=0.27$, $p<0.001$). Urinary type IV collagen was not only positively correlated with age ($r=0.12$, $p<0.001$) and diastolic blood pressure ($r=0.14$, $p<0.001$) but also negatively correlated with blood hemoglobin ($r=-0.12$, $p<0.001$). Urinary albumin, but not type IV collagen, had a significant relation to electrocardiographic signs of left ventricular hypertrophy ($p=0.012$) and retinal arteriosclerosis on fundoscopy ($p<0.001$). Thus both albumin and type IV collagen would seem to have increased in association with age and hypertension in this cohort. It is suggested that urinary albumin is an indicator not only of renal injury, but also possibly of development of cardiac hypertrophy and arteriosclerotic changes. Urinary type IV collagen, on the other hand, may be associated with renal tissue injuries that affect erythrokinetics.

(*Hypertens Res* 2000; 23: 459-466)

Key Words: hypertension, proteinuria, albumin, type IV collagen

Introduction

In protecting the kidneys from common diseases such as hypertension and diabetes mellitus, it is important to detect signs of renal injuries at an early stage and start intensive care in order to prevent further deterioration. To

this end, urinary albumin excretion can serve as an early indicator of renal injuries, since it has been shown to increase prior to the appearance of overt proteinuria (1-6). Urinary albumin has also been shown to correlate with the development of other cardiovascular organ injuries, such as cardiac hypertrophy and retinal arteriosclerosis (7-9). Furthermore, Damsgaard *et al.* (10) observed an

From the Division of Hypertension and Cardiorenal Disease, Department of Medicine, Dokkyo University School of Medicine, Tochigi, Japan, and *Utsunomiya Committee on Renal Disease Prevention, Utsunomiya, Japan.

This study was supported in part by a grant from the Research Foundation for Community Medicine of the Ministry of Home Affairs of Japan.

Address for Reprints: Toshihiko Ishimitsu, M.D., Division of Hypertension and Cardiorenal Disease, Department of Medicine, Dokkyo University School of Medicine, Mibu, Tochigi 321-0293, Japan.

Received March 18, 2000; Accepted in revised form April 10, 2000.

increase in mortality when subjects showing microalbuminuria were followed up for six years.

Other microproteins in urine, such as transferrin and type IV collagen, may also be useful in detecting the early stage of renal injuries. In particular, urinary excretion of type IV collagen possibly increases even before the increase in albumin excretion occurs in diabetic nephropathy (11-13). However, changes in urinary type IV collagen in the development of hypertensive nephrosclerosis have not yet been examined. Elevation of glomerular capillary pressure, glomerular hypertension, and basement membrane injury are known to induce leakage of plasma albumin. On the other hand, urinary type IV collagen derives from the mesangial matrix and glomerular basement membrane. Therefore, urinary excretions of albumin and type IV collagen may reflect different aspects of renal injuries. The purpose of this study was to elucidate the pathophysiological significance of urinary type IV collagen as an indicator of cardiovascular organ injuries in nondiabetic subjects.

Subjects and Methods

Subjects were 40- to 65-year-old participants in a health check program for adult diseases from December 1998 to April 1999 in Utsunomiya, Japan. The city of Utsunomiya lies 100 km north of Tokyo and has a population of approximately 440,000. The subjects came to the health center in the morning after an overnight fast. Urine and antecubital venous blood samples were collected for laboratory analyses. In addition to the qualitative analysis of urine using test paper for protein, sugar and occult blood, urinary concentrations of albumin and type VI collagen were determined by the immunoturbidimetric method (14) and the sandwich enzyme immunoassay (15), respectively. These values were expressed as a ratio to the urinary creatinine concentration measured by colorimetry. Blood cell counts and blood chemistry were examined using autoanalyzers. Serum high density lipoprotein (HDL) cholesterol was assayed in a supernatant of polyethylene glycol precipitation (16), and low density lipoprotein (LDL) cholesterol was calculated according to Friedewald's formula (17). Hypercholesterolemia was diagnosed when serum total cholesterol was 220 mg/dl or more according to the guidelines proposed by the Japan Atherosclerosis Society (18). Hemoglobin A_{1c}, an index of cumulative glycemic level, was determined by high pressure liquid chromatography (19).

Blood pressure was measured by a sphygmomanometer with the subject in a sitting position after a 10-min rest. Hypertension was defined as systolic blood pressure exceeding 140 mmHg and/or diastolic blood pressure exceeding 90 mmHg (20, 21). Body mass index was calculated as weight (kg) divided by height squared (m²), and values of 26.4 kg/m² or more were regarded as indicating

obesity, according to the criterion of the Japan Society for the Study of Obesity (22). Fundoscopic findings were used to grade subjects from 0 to 4 for each of hypertension and arteriosclerosis according to the classification of Scheie (23). A 12-lead resting electrocardiogram was recorded and the signs of left ventricular hypertrophy were scored on a scale of 0 to 2 according to the revised Minnesota code (24), with 0 indicating absence of the signs. High QRS voltage (code 3.1 or 3.3) was scored as 1. When this was accompanied by ST-T changes (code 4.1-4.3 or 5.1-5.3), a score of 2 was given (25).

The study protocol was in accordance with the recommendations of the World Medical Association for biomedical research involving human subjects (Somerset West version, 1996), and informed consent was obtained from each subject.

Data are presented as means $\pm$ SD or SE as indicated. Statistical analyses were performed using Stat View (version 5.0; SAS Institute Inc., North Carolina, USA), and Stat Flex (version 5.0; Artec Inc., Osaka, Japan) software. Differences between the two groups were analyzed by unpaired Student's *t*-test for parametric data, by Mann-Whitney *U*-test for nonparametric data, and by chi-square test for categorical data. Linear regression analysis was used to evaluate correlations between the two variables. Stepwise multiple regression analysis was performed to evaluate dependency between variables. In regression analyses, skewness of the data distribution was normalized by transformation employing the modified Box-Cox formula (26). A *p* value less than 0.05 was considered to indicate statistical significance.

Results

The following criteria were used to select subjects from among the 1,626 individuals who participated in the health check program described above. In order to avoid the effects of primary renal diseases, diabetic nephropathy and menstruation, subjects who were positive in test paper urinalysis for protein, sugar or occult blood were not included, and diabetic subjects presenting fasting blood glucose of 126 mg/dl or more or hemoglobin A_{1c} of 6.5% or more were excluded (27, 28). Subjects with serum creatinine of 1.5 mg/dl or greater were not included. Finally, patients who had been or who were being treated for hypertension, diabetes, or any other disease at the time of enrollment were excluded. This result in a final subject group of 1,079 individuals, or 256 men and 823 women.

Table 1 lists the physical characteristics and laboratory data of the study subjects. Naturally, men had higher body length, body weight, and serum creatinine. Body mass index and blood pressure were also higher in men than in women. White and red blood cell counts and liver enzymes were significantly greater in men than in women.

Table 1. Physical Characteristics and Laboratory Data of the Study Subjects

	Men (n=256)	Women (n=823)	p value
Physical findings			
Age (year)	52.8±8.8	50.6±8.0	<0.001
Body length (cm)	166.7±6.0	154.8±5.4	<0.001
Body weight (kg)	66.3±9.1	54.4±7.9	<0.001
Body mass index (kg/m ²)	23.8±2.8	22.7±3.0	<0.001
Systolic blood pressure (mmHg)	123±13	118±14	<0.001
Diastolic blood pressure (mmHg)	77±9	71±9	<0.001
Blood cell counts			
White blood cell (×10 ³ /mm ³)	6.09±1.69	5.55±1.47	<0.001
Red blood cell (×10 ⁴ /mm ³)	482±34	438±32	<0.001
Hemoglobin (g/dl)	15.5±0.9	13.2±1.3	<0.001
Hematocrit (%)	46.5±2.5	40.6±3.3	<0.001
Blood chemistry			
AST (U/l)	28±15	22±8	<0.001
ALT (U/l)	32±22	20±10	<0.001
γ-GTP (U/l)	57±56	23±19	<0.001
Creatinine (mg/dl)	0.91±0.13	0.68±0.09	<0.001
Total cholesterol (mg/dl)	210±31	213±37	
HDL cholesterol (mg/dl)	57±13	68±15	<0.001
LDL cholesterol (mg/dl)	126±29	127±35	
Triglycerides (mg/dl)	131±63	88±48	<0.001
Glucose (mg/dl)	95±9	92±8	<0.001
Hemoglobin A _{1c} (%)	5.03±0.34	4.97±0.35	0.009
Urinalysis			
Albumin (mg/g Cr)	12.1±12.5	14.4±27.2	0.014
Type IV collagen (μg/g Cr)	4.2±1.9	4.3±2.2	

Data are means±SD. AST, aspartate aminotransferase; ALT, alanine aminotransferase; γ-GTP, γ-glutamyl transpeptidase; HDL, high density lipoprotein; LDL, low density lipoprotein; Cr, creatinine.

Table 2. Stepwise Multiple Regression Analysis of Relations of Various Parameters to Systolic and Diastolic Pressure

Variable	Correlation coefficient	p value
Systolic blood pressure		
Age	0.185	<0.001
Body mass index	0.215	<0.001
Fasting blood glucose	0.238	<0.001
Hematocrit	0.113	<0.001
Diastolic blood pressure		
Age	0.140	<0.001
Body mass index	0.223	<0.001
Fasting blood glucose	0.189	<0.001
Hematocrit	0.204	<0.001

With regard to the serum lipids, HDL cholesterol was higher in women, while triglycerides were higher in men. Not only the fasting blood glucose, but also the hemoglo-

bin A_{1c} was slightly but significantly higher in men than in women. Urinary albumin excretion was slightly greater in women than in men, although there was no gender difference in urinary excretion of type IV collagen. Of 1,079 study subjects, 120 (11.1%), or 44 men (17.2%) and 76 women (9.2%), had hypertension, and 133 (12.3%), or 44 men (17.2%) and 89 women (10.8%), showed obesity. Hypercholesterolemia was observed in 482 subjects (44.7%), or 129 men (50.4%) and 353 women (42.9%).

In simple regression analyses, almost every parameters examined showed a significantly positive or negative correlation with blood pressure. However, some of these parameters were dependent on each other, and only four of the parameters, age, body mass index, fasting blood glucose and hematocrit, continued to show significant correlations to both systolic and diastolic blood pressure after the stepwise multiple regression analyses (Table 2).

Table 3 shows the results of the stepwise multiple regression analyses examining the relations of various parameters to left ventricular hypertrophy, retinal arteriosclerosis, and urinary excretions of albumin and type IV col-

lagen, respectively. Electrocardiographic signs of left ventricular hypertrophy were positively correlated with age, systolic blood pressure, serum creatinine and serum γ -glutamyl transpeptidase (γ -GTP). Sclerosis of the retinal arteries on fundoscopy was also correlated with age, systolic blood pressure and serum γ -GTP. With regard to the urinary microproteins, both albumin and type IV collagen

Table 3. Stepwise Multiple Regression Analysis of Relations of Various Parameters to Electrocardiographic Signs of Left Ventricular Hypertrophy, Retinal Arteriosclerosis on Fundoscopy, and Urinary Excretions of Albumin and Type IV Collagen

Variable	Standardized coefficient of correlation	<i>p</i> value
Left ventricular hypertrophy		
Age	0.072	0.023
Systolic blood pressure	0.066	0.035
Serum creatinine	0.091	0.004
Serum γ -GTP	0.083	0.008
Retinal arteriosclerosis		
Age	0.202	<0.001
Systolic blood pressure	0.186	<0.001
Serum γ -GTP	0.143	<0.001
Serum ATP	-0.091	0.007
Urinary Albumin		
Age	0.158	<0.001
Systolic blood pressure	0.267	<0.001
Retinal arteriosclerosis	0.069	0.019
Serum creatinine	-0.227	<0.001
Serum γ -GTP	0.090	0.002
Urinary type IV collagen		
Age	0.122	<0.001
Diastolic blood pressure	0.142	<0.001
Blood hemoglobin	-0.123	<0.001
Serum creatinine	-0.130	<0.001
Serum triglycerides	0.118	<0.001

ALT, alanine aminotransferase; γ -GTP, γ -glutamyl transpeptidase.

were correlated positively with age and negatively with serum creatinine. Urinary albumin was correlated with systolic blood pressure, and urinary type IV collagen was correlated with diastolic blood pressure. In addition, urinary albumin showed a positive correlation with serum γ -GTP, while urinary type IV collagen had a positive correlation with serum triglycerides.

Table 4 shows the interrelationship among the indices of cardiovascular organ injuries, *i.e.*, electrocardiographic signs of left ventricular hypertrophy, retinal arteriosclerosis on fundoscopy, and urinary excretions of albumin and type IV collagen. There were positive correlations among each of left ventricular hypertrophy, retinal arteriosclerosis and urinary albumin. However, urinary type IV collagen was not significantly correlated with left ventricular hypertrophy or retinal arteriosclerosis, although it was significantly correlated with urinary albumin.

When 54 subjects, corresponding to 5% of the total number, who had the highest urinary albumin values (>37 mg/g creatinine) were compared with 54 subjects who had the highest urinary type IV collagen values (>7.9 μ g/g creatinine), only seven subjects overlapped. In Table 5, the data of these seven subjects have been removed, and the parameters on the remaining 47 subjects with urinary albumin greater than 37 mg/g creatinine and 47 subjects with urinary type IV collagen greater than 7.9 μ g/g creatinine are compared. The high urinary albumin group showed higher blood pressure, higher liver enzymes, higher LDL cholesterol and more frequent retinal arteriosclerosis than the high urinary type IV collagen group. On the other hand, red blood cell counts, blood hemoglobin and hematocrit were lower in the high urinary type IV collagen group than in the high urinary albumin group.

Discussion

Among the various parameters examined in this study, age, body mass index, fasting blood glucose and hematocrit were independently correlated with blood pressure. Aging and obesity are well-known contributing factors to blood pressure elevation. Although patients with diabetes

Table 4. Univariate Correlations between Electrocardiographic Signs of Left Ventricular Hypertrophy, Retinal Arteriosclerosis on Fundoscopy, and Urinary Excretions of Albumin and Type IV Collagen

Dependent variable	Independent variable	Correlation coefficient	<i>p</i> value
Left ventricular hypertrophy	Retinal arteriosclerosis	0.140	<0.001
	Urinary albumin	0.076	0.012
	Urinary type IV collagen	-0.033	N.S.
Retinal arteriosclerosis	Urinary albumin	0.173	<0.001
	Urinary type IV collagen	0.044	N.S.
Urinary albumin	Urinary type IV collagen	0.331	<0.001

N.S., not significant.

Table 5. Comparison of 47 Subjects with Urinary Albumin Greater than 37 mg/g Cr to 47 Subjects with Urinary Type IV Collagen Greater than 7.9 μ g/g Cr. (Seven Subjects Showing Both Urinary Albumin Greater than 37 mg/g Cr and Urinary Type IV Collagen Greater than 7.9 μ g/g Cr Are Excluded.)

	Urinary albumin >37 mg/g Cr	Urinary type IV collagen >7.9 μ g/g Cr	<i>p</i> value
Physical finding			
Men/Women	11/36	8/39	N.S.
Age (year)	53.3 $\pm$ 1.2	53.6 $\pm$ 1.1	N.S.
Body length (cm)	156.2 $\pm$ 1.1	156.2 $\pm$ 0.9	N.S.
Body weight (kg)	58.0 $\pm$ 1.5	57.5 $\pm$ 1.1	N.S.
Body mass index (kg/m ²)	23.7 $\pm$ 0.4	23.5 $\pm$ 0.4	N.S.
Systolic blood pressure (mmHg)	132 $\pm$ 2	123 $\pm$ 2	0.002
Diastolic blood pressure (mmHg)	80 $\pm$ 1	76 $\pm$ 1	0.038
Blood cell counts			
White blood cell ($\times 10^3$ /mm ³)	6.18 $\pm$ 0.28	5.85 $\pm$ 0.27	N.S.
Red blood cell ($\times 10^4$ /mm ³)	460 $\pm$ 5	445 $\pm$ 5	0.036
Hemoglobin (g/dl)	14.0 $\pm$ 0.2	13.0 $\pm$ 0.3	0.032
Hematocrit (%)	42.6 $\pm$ 0.6	40.7 $\pm$ 0.7	0.042
Blood chemistry			
AST (U/l)	25 $\pm$ 1	23 $\pm$ 1	N.S.
ALT (U/l)	26 $\pm$ 2	20 $\pm$ 1	0.036
γ -GTP (U/l)	37 $\pm$ 5	23 $\pm$ 2	0.026
Creatinine (mg/dl)	0.72 $\pm$ 0.02	0.72 $\pm$ 0.01	N.S.
Total cholesterol (mg/dl)	221 $\pm$ 5	208 $\pm$ 4	0.046
HDL cholesterol (mg/dl)	65 $\pm$ 2	65 $\pm$ 2	N.S.
LDL cholesterol (mg/dl)	132 $\pm$ 4	122 $\pm$ 3	0.048
Triglycerides (mg/dl)	121 $\pm$ 10	104 $\pm$ 6	N.S.
Glucose (mg/dl)	97 $\pm$ 1	95 $\pm$ 1	N.S.
Hemoglobin A _{1c} (%)	5.0 $\pm$ 0.1	5.1 $\pm$ 0.1	N.S.
Urinalysis			
Albumin (mg/g Cr)	89.2 $\pm$ 11.4	16.0 $\pm$ 1.1	<0.001
Type IV collagen (μ g/g Cr)	4.6 $\pm$ 0.2	9.7 $\pm$ 0.5	<0.001
Left ventricular hypertrophy (%)	4.2	0.0	N.S.
Retinal arteriosclerosis (%)	17.0	4.2	0.044

Data are means $\pm$ SD. AST, aspartate aminotransferase; ALT, alanine aminotransferase; γ -GTP, γ -glutamyl transpeptidase; HDL, high density lipoprotein; LDL, low density lipoprotein; Cr, creatinine; N.S., not significant.

mellitus were not included in this study, the positive correlation between fasting blood glucose and blood pressure may suggest that insulin resistance is involved in the pathogenesis of primary hypertension (29–31). Higher hematocrit is also known to be associated with higher blood pressure, and this association, in turn, is thought to be related to decreased plasma volume in hypertensives (32, 33). These previously established relationships in the epidemiology of hypertension were thus reconfirmed in the present study.

An increase of either albumin or type IV collagen in urine was associated with higher age and higher blood pressure. In the regression analyses, there were positive correlations among left ventricular hypertrophy, retinal arteriosclerosis and urinary albumin. In contrast, urinary type IV collagen did not show significant correlation with

left ventricular hypertrophy or retinal arteriosclerosis. Furthermore, when the subjects with high urinary albumin were compared with the subjects with high urinary type IV collagen, the former had higher blood pressure and higher serum LDL cholesterol than the latter. Therefore, it is speculated that cardiovascular changes induced by hypertension and hyperlipidemia, such as cardiac hypertrophy or arteriosclerosis, are reflected by an increase in urinary excretion of albumin rather than urinary type IV collagen excretion.

In the stepwise multiple regression analyses, left ventricular hypertrophy, retinal arteriosclerosis and urinary albumin each showed significant positive correlation with serum γ -GTP. This is possibly related to the liver injuries caused by alcohol consumption or obesity, either of which promotes blood pressure elevation and thereby facilitates

cardiovascular hypertrophy and sclerosis. Indeed, serum γ -GTP was positively correlated with body mass index ($r = 0.166$, $p < 0.001$) in the current study subjects. Moreover, earlier studies have shown dose-dependent increases in serum γ -GTP and blood pressure in subjects whose daily ethanol consumption was 30 ml or more (34, 35). We have previously reported that higher alcohol intake was associated with higher incidence of left ventricular hypertrophy (34), and we also have observed that fundoscopic lesions and urinary albumin excretion were increased in obese subjects (36). In the present study left ventricular hypertrophy was also correlated with serum creatinine. An increase in body size is supposedly associated with increases in left ventricular mass and serum creatinine level (37).

Both albumin and type IV collagen in urine had significantly negative correlation with serum creatinine concentration. The serum creatinine concentration increases with increasing body size and muscle mass, because urinary creatinine derives mainly from the skeletal muscles. On the one hand, an increase in glomerular filtration rate would be expected to lower the serum creatinine level, but on the other hand, this increase could potentially be accompanied by elevation of intraglomerular pressure and leakages of microproteins into urine (38). In addition to this, reduced reabsorption by the proximal tubuli also contributes to urinary microprotein excretions. In the present study, the values of urinary albumin and type IV collagen were expressed as a ratio to urinary creatinine. The urinary creatinine excretion is proportional to the body muscle mass. If the increase of urinary microproteins is smaller than the increase of urinary creatinine excretion in subjects with large body mass, a spurious negative correlation might be detected between serum creatinine and ratios of microproteins to creatinine in urine.

In addition to showing positive correlations with age and blood pressure, urinary type IV collagen was negatively correlated with blood hemoglobin concentration in the present study. Therefore, in addition to the development of nephrosclerosis induced by aging and hypertension, a number of factors related to the erythrokinetics are thought to contribute to the increase of urinary type IV collagen excretion. It is well-known that the erythropoiesis is greatly dependent on the stimulation by erythropoietin produced by the kidney. Therefore, if the erythropoietin production is influenced, even in the slightest degree, by certain changes in the kidney which bring about an increase in urinary type IV collagen excretion, such an influence may be involved in the negative correlation between blood hemoglobin and urinary type IV collagen observed in this study. In this context, it has been reported that relatively insufficient production of erythropoietin can cause anemia in nephrotic patients without renal dysfunction (39, 40).

Numerous significant correlations were observed in sim-

ple regression analyses of the parameters examined in this study. It is supposed that some of these are indirect correlations. In the present study, stepwise multiple regression analyses were applied in order to correct dependency between the parameters. It should be remembered, however, that these analyses were based on statistical handling of data rather than on clinical evidence. Therefore, further studies are needed to elucidate the pathophysiological significance of urinary microproteins in the early stages of renal injuries. In particular, it seems important to investigate the relations between histological findings and various aspects of renal functions, such as vascular, glomerular and tubular functions.

In this study, we investigated the relations of urinary albumin and type IV collagen to various parameters of physical and laboratory examinations. The results suggested that the concentration of either microproteins in urine increases with increasing age and blood pressure. In addition, the urinary albumin excretion seems to increase with the development of hypertrophic and sclerotic changes in cardiovascular organs. Urinary type IV collagen, on the other hand, may reflect other aspects of renal injury that are associated with erythrokinetics.

Acknowledgements

The authors thank the members of the health department of the Utsunomiya city office for their assistance in executing the study. We are also grateful for the help and advice provided by Dr. Eiji Kusano, Dr. Yasuhiro Ando and Prof. Yasushi Asano of the Department of Nephrology, Jichi Medical School, Tochigi, Japan.

References

1. Parving HH, Mogensen CE, Jensen HA, Evrin PE: Increased urinary albumin-excretion rate in benign essential hypertension. *Lancet* 1974; **1**: 1190-1192.
2. Viberti GC, Hill RD, Jarrett RJ, Argyropoulos A, Mahmud U, Keen H: Microalbuminuria as a predictor of clinical nephropathy in insulin-dependent diabetes mellitus. *Lancet* 1982; **1**: 1430-1432.
3. Mogensen CE: Microalbuminuria predicts clinical proteinuria and early mortality in maturity-onset diabetes. *N Engl J Med* 1984; **310**: 356-360.
4. Haffner SM, Stern MP, Gruber MK, Hazuda HP, Mitchell BD, Patterson JK: Microalbuminuria: potential marker for increased cardiovascular risk factors in non-diabetic subjects? *Arteriosclerosis* 1990; **10**: 727-731.
5. Agewall S, Persson B, Samuelsson O, Ljungman S, Herlitz H, Fagerberg B: Microalbuminuria in treated hypertensive men at high risk of coronary disease: The Risk Factor Intervention Study Group. *J Hypertens* 1993; **11**: 461-469.
6. Mimran A, Ribstein J, DuCailar G: Is microalbuminuria a marker of early intrarenal vascular dysfunction in

- essential hypertension? *Hypertension* 1994; **23**: 1018-1021.
7. Cerasola G, Cottone S, D'Ignoto G, et al: Micro-albuminuria as a predictor of cardiovascular damage in essential hypertension. *J Hypertens Suppl* 1989; **7**: S332-S333.
 8. Redon J, Gomez-Sanchez MA, Baldo E, et al: Micro-albuminuria is correlated with left ventricular hypertrophy in male hypertensive patients. *J Hypertens Suppl* 1991; **9**: S148-S149.
 9. Pedrinelli R, Lindpaintner K, Dell'Omo G, et al: Urinary albumin excretion and atherosclerosis in essential hypertension. *Clin Sci* 1997; **92**: 45-50.
 10. Damsgaard EM, Froland A, Jorgensen OD, Mogensen CE: Microalbuminuria as predictor of increased mortality in elderly people. *Br Med J* 1990; **300**: 297-300.
 11. Yagame M, Suzuki D, Jinde K, et al: Significance of urinary type IV collagen in patients with diabetic nephropathy using a highly sensitive one-step sandwich enzyme immunoassay. *J Clin Lab Anal* 1997; **11**: 110-116.
 12. Ellis D, Forrest KY, Erbey J, Orchard TJ: Urinary measurement of transforming growth factor-beta and type IV collagen as new markers of renal injury: application in diabetic nephropathy. *Clin Chem* 1998; **44**: 950-956.
 13. Takizawa H, Satoh T, Kurusu A, et al: Increase of urinary type IV collagen in normoalbuminuric patients with impaired glucose tolerance. *Nephron* 1998; **79**: 474-475.
 14. Teppo AM: Immunoturbidimetry of albumin and immunoglobulin G in urine. *Clin Chem* 1982; **28**: 1359-1361.
 15. Obata K, Iwata K, Ichida T, et al: One step sandwich enzyme immunoassay for human type IV collagen using monoclonal antibodies. *Clin Chim Acta* 1989; **181**: 293-303.
 16. Izzo C, Grillo F, Murador E: Improved method for determination of high-density-lipoprotein cholesterol I: isolation of high-density lipoproteins by use of polyethylene glycol 6000. *Clin Chem* 1981; **27**: 371-374.
 17. Friedewald WT, Levy RI, Fredrickson DS: Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 1972; **18**: 499-502.
 18. Japan Atherosclerosis Society: Guideline for diagnosis and treatment of hyperlipidemias in adults. *J Jpn Atheroscler Soc* 1997; **25**: 1-34.
 19. John WG, Braconnier F, Miedema K, Aulesa C, Piras G: Evaluation of the Menarini-Arkray HA 8140 hemoglobin A_{1c} analyzer. *Clin Chem* 1997; **43**: 968-975.
 20. The Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure and the National High Blood Pressure Education Program Coordinating Committee: The sixth report of the Joint National Committee on prevention, detection, evaluation, and treatment of high blood pressure. *Arch Intern Med* 1997; **157**: 2413-2446.
 21. Guidelines Subcommittee of the WHO-ISH Mild Hypertension Liaison Committee: 1999 World Health Organization-International Society of Hypertension Guidelines for the Management of Hypertension. *J Hypertens* 1999; **17**: 151-183.
 22. Yoshiike N, Matsumura Y, Zaman MM, Yamaguchi M: Descriptive epidemiology of body mass index in Japanese adults in a representative sample from the National Nutrition Survey 1990-1994. *Int J Obes* 1998; **22**: 684-687.
 23. Walsh JB: Hypertensive retinopathy. Description, classification, and prognosis. *Ophthalmology* 1982; **89**: 1127-1131.
 24. Sokolow M, Lyon TP: The ventricular complex in left ventricular hypertrophy as obtained by unipolar precordial and limb leads. *Am Heart J* 1949; **37**: 161-186.
 25. Ostrander LD: Epidemiological study of the electrocardiographic diagnosis of left ventricular hypertrophy. *Circulation* 1966; **33**: 270-282.
 26. Ichihara K, Kawai T: Determination of reference intervals for 13 plasma proteins based on IFCC international reference preparation (CRM470) and NCCLS proposed guideline (C28-P, 1992): trial to select reference individuals by results of screening tests and application of maximal likelihood method. *J Clin Lab Anal* 1996; **10**: 110-117.
 27. The 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* 1997; **20**: 1183-1197.
 28. Alberti KG, Zimmet PZ: Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. *Diabet Med* 1998; **15**: 539-553.
 29. Ferrannini E, Natali A: Essential hypertension, metabolic disorders, and insulin resistance. *Am Heart J* 1991; **121**: 1274-1282.
 30. Reaven GM: Insulin resistance and compensatory hyperinsulinemia: role in hypertension, dyslipidemia, and coronary heart disease. *Am Heart J* 1991; **121**: 1283-1288.
 31. Ferrari P, Weidmann P, Shaw S, et al: Altered insulin sensitivity, hyperinsulinemia, and dyslipidemia in individuals with a hypertensive parent. *Am J Med* 1991; **91**: 589-596.
 32. Cirillo M, Laurenzi M, Trevisan M, Stamler J: Hematocrit, blood pressure, and hypertension. The Gubbio Population Study. *Hypertension* 1992; **20**: 319-326.
 33. Smith S, Julius S, Jamerson K, Amerena J, Schork N: Hematocrit levels and physiologic factors in relationship to cardiovascular risk in Tecumseh, Michigan. *J Hypertens* 1994; **12**: 455-462.
 34. Ishimitsu T, Yoshida K, Nakamura M, et al: Effects of alcohol intake on organ injuries in normotensive and hypertensive human subjects. *Clin Sci* 1997; **93**: 541-547.
 35. Choudhury SR, Ueshima H, Okayama A, Kita Y, Miyoshi Y: Relationship between blood pressure and alcohol consumption on the previous day in Japanese men. *Hypertens Res* 1998; **21**: 175-178.
 36. Nakamura M, Ishimitsu T, Matsuoka H, Ohnishi M, Hisauchi T: Implications of obesity for target organ injuries and cardiovascular risk factors in hypertensive subjects. *Jpn J Nephrol* 1997; **39**: 746-752.

37. Shigematsu Y, Hamada M, Hiasa G: Serum creatinine level underestimates hypertensive renal involvement in elderly patients with essential hypertension. *Hypertens Res* 1999; **22**: 297-301.
38. Okada N, Imanishi M, Yoshioka K, *et al*: Creatinine clearance as a substitute for the glomerular filtration rate in the assessment of glomerular hemodynamics. *Hypertens Res* 1999; **22**: 279-284.
39. Vaziri ND, Kaupke CJ, Barton CH, Gonzales E: Plasma concentration and urinary excretion of erythropoietin in adult nephrotic syndrome. *Am J Med* 1992; **92**: 35-40.
40. Ishimitsu T, Ono H, Sugiyama M, *et al*: Successful erythropoietin treatment for severe anemia in nephrotic syndrome without renal dysfunction. *Nephron* 1996; **74**: 607-610.