Molecular and Cellular Biochemistry **284:** 175–182, 2006.
DOI: 10.1007/s11010-005-9047-9

Urinary liver-type fatty acid binding protein as a useful biomarker in chronic kidney disease

Atsuko Kamijo,[1] Takeshi Sugaya,[2] Akihisa Hikawa,[3] Masaya Yamanouchi,[4] Yasunobu Hirata,[5] Toshihiko Ishimitsu,[6] Atsushi Numabe,[6] Masao Takagi,[7] Hiroshi Hayakawa,[8] Fumiko Tabei,[8] Tokuichiro Sugimoto,[9] Naofumi Mise,[9] Masao Omata[5] and Kenjiro Kimura[1]

[1]*Internal Medicine, St. Marianna University School of Medicine, Kanagawa;* [2]*CMIC Co Ltd., Tokyo;* [3]*Eiken Chemical Co. Ltd., Tochigi;* [4]*Tanabe Seiyaku Co Ltd., Osaka;* [5]*Internal Medicine, the University of Tokyo, Tokyo;* [6]*Internal Medicine, Dokkyo University School of Medicine, Tochigi;* [7]*Internal Medicine, Tokyo Police Hospital, Tokyo;* [8]*Internal Medicine, Kanto Central Hospital, Tokyo;* [9]*Internal Medicine, Mitsui Memorial Hospital, Tokyo, Japan*

Abstract

Background: We reported that urinary *L*-FABP reflected the progression of chronic kidney disease (CKD). This study is aimed to evaluate the clinical significance of urinary liver type fatty acid binding protein (*L*-FABP) as a biomarker for monitoring CKD. *Methods:* Urinary *L*-FABP was measured using human *L*-FABP ELISA kit (CMIC.Co., Ltd., Tokyo, Japan). The relations between urinary *L*-FABP and clinical parameters were evaluated in non-diabetic CKD ($n = 48$) for a year. In order to evaluate the influence of serum *L*-FABP derived from liver upon urinary *L*-FABP, both serum and urinary *L*-FABP were simultaneously measured in patients with CKD ($n = 73$). *Results:* For monitoring CKD, the cut-off value in urinary *L*-FABP was determined as 17.4 μg/g.cr. by using a receiver operating characteristics (ROC) curve. Renal function deteriorated significantly more in patients with 'high' urinary *L*-FABP ($n = 36$) than in those with 'low' *L*-FABP ($n = 12$). The decrease in creatinine clearance was accompanied by an increase in urinary *L*-FABP, but not in urinary protein. Serum *L*-FABP in patients with CKD was not correlated with urinary *L*-FABP. *Conclusion:* Urinary excretion of *L*-FABP increases with the deterioration of renal function. Serum *L*-FABP did not influence on urinary *L*-FABP. Urinary *L*-FABP may be a useful clinical biomarker for monitoring CKD. (Mol Cell Biochem **284:** 175–182, 2006)

Key words: *L*-FABP, fatty acid binding protein, fatty acid, chronic kidney disease, tubulointerstitial damage

Introduction

A recent study of the American Heart Association suggests that CKD is an independent risk factor for developing cardiovascular disease, warns of the increasing number of patients with CKD, and emphasizes the importance of measuring clinical parameters in serum or urine [1]. However, there are few clinical markers to predict and monitor the progression of CKD.

Liver-type fatty acid binding protein (*L*-FABP) is expressed in the human proximal tubules. In the kidney disease, *L*-FABP expression in the proximal tubules was increased

Address for offprints: K. Kimura, Nephrology and Hypertension, St. Marianna University School of Medicine, 2-16-1 Sugao, Miyamae-Ku, Kawasaki 216-8511, Japan (E-mail: kimura@marianna-u.ac.jp)

and urinary excretion of *L*-FABP was accelerated [2]. In the clinical study, urinary *L*-FABP reflected the progression of CKD and might be a new clinical biomarker for predicting the progression of CKD [3].

In order to evaluate the clinical significance of urinary *L*-FABP as a biomarker for monitoring non-diabetic CKD, we examined the relations between the progression of CKD and urinary excretion of *L*-FABP in the outpatients with non-diabetic CKD.

As *L*-FABP is expressed not only in the kidney, but also in the liver, urinary *L*-FABP might also have been influenced by serum *L*-FABP. Furthermore, we evaluated the influence of serum *L*-FABP derived from the liver upon urinary *L*-FABP.

Methods

ELISA for measurement of L-FABP

The level of *L*-FABP was measured using human *L*-FABP ELISA kit (CMIC Co., Ltd. Tokyo Japan).

Study 1

Clinical significance of urinary L-FABP in CKD

Patient selection. Forty-eight adult patients from the outpatient clinics of the University of Tokyo Hospital (Tokyo, Japan), Dokkyo University School of Medicine (Tochigi, Japan), Tokyo Police Hospital (Tokyo, Japan), Kanto Central Hospital (Tokyo, Japan), and Mitsui Memorial Hospital (Tokyo, Japan) were enrolled in this study. Patients were followed from March 2001 to May 2002. The subjects were diagnosed as having CKD by renal biopsy ($n = 15$) or by clinical history ($n = 33$) without underlying systemic disease. The patients who had serum creatinine from 1.2 mg/dl (106.1 μmol/L) to 3 mg/dl (265.2 μmol/L) for men or from 0.9 mg/dl (79.6 μmol/L) to 2.3 mg/dl (203.3 μmol/L) for women were selected. Informed consent was obtained from all patients before beginning the study. All serum and urine samples were stored at $-70\,^{\circ}$C until analysis. Relevant clinical parameters were monitored every month or second month without a change in medication. Table 1 summarizes clinical and laboratory findings for the subjects.

Table 1. Patient characteristics (study 1)

Variable	$n = 48$
Mean ± SD (range) age (yr)	55 ± 11(29–77)
Male ($n = 36$)	54 ± 2 (29–77)
Female ($n = 12$)	57 ± 3 (39–69)
Mean blood pressure (mmHg)	101 ± 45
Serum creatinine (mg/dl)	1.8 ± 0.5
Creatinine clearance (mL/min)	45.9 ± 20.8
Total cholesterol (mg/dl)	208 ± 33
Urinary protein (g/g.cr)	1.3 ± 1.4
Urinary NAG (IU/g.cr)	9.3 ± 3.9
Urinary *L*-FABP (μg/g.cr)	89.3 ± 115.4
No of patients with renal biopsy (%)	15 (31%)
Mesangial proliferative glomerulonephritis	10
Membranous nephropathy	1
Focal segmental glomerulosclerosis	1
Membranoproliferative glomerulonephritis	1
Nephrosclerosis	2

Clinical parameters of serum and ambulatory spot urine

Serum levels of creatinine and total cholesterol, and urine levels of *L*-FABP, creatinine, protein and NAG (*N*-acetyl-β-D-glucosaminidase) were measured. Serum and urinary creatinine and serum cholesterol were measured by enzymatic methods, urinary protein by the pyrogallol red-molybdate complex method [4] and urinary NAG by using chlorophenol red-*N*-acetylglucosaminide (CPR-NAG) as a substrate. The levels of urinary parameters are expressed as a ratio of the level of urinary creatinine (cr.). Creatinine clearance (Ccr) was calculated by the Orita formula [5].

Clinical course and urinary L-FABP

To evaluate the progression of CKD, we analyzed Ccr in patients over the trial period (in months). For each patient, the progression rate of CKD was defined as the slope of the regression line of a line-graph plot of Ccr against trial length. The patients, whose renal function was deteriorated, had a disease progression rate, under -0.1 and the patients, which renal function was stable, had a disease progression rate above -0.1. For monitoring CKD, the cut-off points in urinary *L*-FABP and urinary protein were calculated by using a receiver operating characteristics (ROC) curve.

We divided the patients into two groups according to their levels of urinary *L*-FABP or urinary protein: the high group incorporated patients with higher levels of urinary *L*-FABP or urinary protein (equal to or more than the cut-off values for monitoring CKD), and the low group incorporated patients with lower levels (less than the cut-off value for monitoring CKD). Ccr, urinary *L*-FABP and urinary protein were assessed separately in the high and low groups.

To evaluate the changes in the clinical parameters, we examined the level of urinary *L*-FABP or urinary protein over the trial period (in months). For each patient, the change in urinary *L*-FABP or in urinary protein over the trial period was defined as the slope of the regression line of a line-graph plot of urinary *L*-FABP or urinary protein, respectively,

against trial length. These changes were compared with the progression rate of kidney disease.

Study 2

Patient selection. It has been reported that serum *L*-FABP was elevated in liver disease such as acute or chronic hepatitis [6]. Under such situations, urinary *L*-FABP might also have been influenced by serum *L*-FABP. In order to evaluate the influence of serum *L*-FABP derived from the liver upon urinary *L*-FABP, serum and urinary *L*-FABP were simultaneously measured in patients with CKD ($n = 73$), liver disease ($n = 71$) and healthy volunteers ($n = 71$) from May, 2000 to July, 2000. The patients from the outpatient clinic of the University of Tokyo Hospital, Tokyo, Japan, were enrolled. The patients with CKD were diagnosed as having primary chronic glomerular disease by renal biopsy ($n = 54$) or by clinical history, and they had no clinical or laboratory evidence of an underlying systemic disease. In liver disease, the patients were diagnosed chronic viral hepatitis without liver cirrhosis and malignant tumor and they had no abnormal urinalysis. Table 2 summarizes the clinical and laboratory findings of CKD and Table 3 summarizes those of liver disease. Reference value of urinary excretion of *L*-FABP was analyzed using urine samples of healthy volunteers in Eiken Chemical Co Ltd. ($n = 97$). Criteria for the healthy volunteers included (1) absence of a history of CKD and (2) absence of an abnormal finding in urinalysis.

Table 2. Patients evaluated for the influence of serum *L*-FABP derived from the liver upon urinary *L*-FABP in CKD (study 2)

Variable	$n = 73$
Mean (±SD) age-yr	49 ± 15
Sex-no. of patients (%)	
Male	39 (53%)
Female	34 (47%)
Serum creatinine (mg/dl)	1.1 ± 0.8
Total cholesterol (mg/dl)	204 ± 61
Serum *L*-FABP (ng/ml)	5.4 ± 4.4
Urinary protein (g/g.cr)	1.3 ± 2.4
Urinary NAG (IU/g.cr)	9.0 ± 6.0
Urinary *L*-FABP (μg/g.cr)	39.5 ± 73.1
Renal biopsy-no.of patients (%)	54 (74%)
Minor glomerular abnormalities	6
Mesangial proliferative glomerulonephritis	29
Membranous nephropathy	6
Focal segmental glomerulosclerosis	12
Membranoproliferative glomerulonephritis	1

Table 3. Patients evaluated for the influence of serum *L*-FABP derived from the liver upon urinary *L*-FABP in liver disease (study 2)

Variable	$n = 71$
Mean (±SD) age-yr	60 ± 12
Sex-no. of patients (%)	
Male	35 (49%)
Female	36 (51%)
Serum creatinine (mg/dl)	0.7 ± 0.2
Total cholesterol (mg/dl)	162 ± 27
AST (units/L)	58 ± 36
ALT (units/L)	62 ± 47
Serum *L*-FABP (ng/ml)	8.4 ± 6.7
Urinary protein (g/g.cr)	0.1 ± 0.1
Urinary NAG (IU/g.cr)	1.0 ± 0.6
Urinary *L*-FABP (μg/g.cr)	13.0 ± 9.3

The measurements of urinary or serum L-FABP

Urinary and serum *L*-FABP were measured by the method mentioned above.

Clinical parameters of serum and ambulatory spot urine

Serum creatinine, total cholesterol, urinary creatinine, protein and NAG were measured by the methods mentioned above. In the patients with liver disease, AST and ALT were measured by the standard method.

These researches were carried out according to the principles of the Declaration of Helsinki, that informed consent was obtained, and that our institutional review board has approved this study.

Statistical analysis

All values are expressed as the mean ± standard deviation (SD). The correlation between two parameters (nonparametric distributions) was analyzed by Spearman's rank coefficient of correlation and the correlation between two groups (nonparametric distributions) analyzed by the Mann-Whitney *U*-test using unpaired data. To clarify the relation between urinary or serum *L*-FABP with clinical parameters, the multiple regression analysis based on the stepwise method was applied. Differences in parameters among patients with CKD, patients with liver disease and healthy volunteers were analyzed by Scheffe's multiple comparison procedure after the Kruska*L*-Wallis test was performed. These statistical analyses were done by Stat View 5.0 software (SAS Institute Inc., NC, USA). StatFlex 5.0 software (Artech Co.,

Ltd., Osaka, Japan) was used to obtain the ROC curve. *P*-values of less than 0.05 were considered to be statistically significant.

Results

Study 1

Clinical course and urinary L-FABP

For monitoring CKD, the cut-off value in urinary *L*-FABP was determined as 17.4 μg/g.cr. and that in urinary protein was 1 g/g.cr. The regression slope for the disease progression rate of patients in the high urinary *L*-FABP group ($\geq$17.4 μg/g.cr., $n = 36$) was significantly lower than that of patients in the low urinary *L*-FABP group ($<$17.4 μg/g.cr, $n = 12$) [regression slope, -0.464 and 0.069 (ΔmL.min^{-1}.month^{-1}), respectively; $p < 0.001$]. Renal function in the high urinary *L*-FABP group deteriorated more than that in the low urinary *L*-FABP group (Table 4). The decrease in Ccr was accompanied by an increase in urinary *L*-FABP in the high urinary *L*-FABP group (Table 4). In the low urinary *L*-FABP group, however, Ccr was stable and urinary *L*-FABP increased slightly (Table 4). The change in urinary *L*-FABP was significantly higher in the high urinary *L*-FABP group than in the low urinary *L*-FABP group [4.31 and 0.88 ($\Delta\mu$g/g.cr./month), respectively; $p < 0.05$].

The regression slope for the disease progression rate of patients in the high urinary protein group ($\geq$1 g/g.cr., $n = 26$) was significantly lower than that of patients in the low urinary protein group ($<$1 g/g.cr., $n = 22$) [regression slope, -0.58 and -0.03 (ΔmL.min^{-1}.month^{-1}), respectively, $p < 0.0001$]. Renal function in the high urinary protein group deteriorated more than in the low urinary protein group (Table 5). In the high urinary protein group, however, the decrease in Ccr did not follow the increase in urinary protein (Table 5). In the low urinary protein group, Ccr was stable and urinary protein increased slightly (Table 5). The change in urinary protein in the high urinary protein group was similar to that in the low urinary protein group [0.01 and 0.01 (Δg/g.cr./month), respectively, NS].

The nature of the change in urinary protein was clearly different from that in urinary *L*-FABP, which increased with the decline in Ccr.

Study 2

Clinical parameters correlated with serum L-FABP in renal or liver disease

Serum *L*-FABP levels in patients with CKD, patients with liver disease and healthy volunteers were 5.4 ± 4.4 ng/mL, 8.4 ± 6.7 ng/mL and 1.2 ± 0.7 ng/mL, respectively. Serum *L*-FABP levels in patients with liver disease were significantly higher than those in healthy volunteers and those in patients with CKD (Fig. 1a).

The reference value of urinary *L*-FABP level was 6.5 ± 5.4 ng/mL or 5.2 ± 3.6 μg/g.cr in healthy volunteers ($n = 97$). Urinary *L*-FABP levels in patients with liver disease (13.0 ± 9.3 μg/g.cr) were similar to those in healthy volunteers. Urinary *L*-FABP levels in patients with CKD (39.5 ± 73.1 μg/g.cr) were significantly higher than those in healthy volunteers and those in patients with liver disease (Fig. 1b).

In the stepwise regression analysis using serum *L*-FABP of patients with CKD with a dependent variable and seven clinical independent variables (serum creatinine, serum total cholesterol, urinary protein, urinary NAG and urinary *L*-FABP), serum *L*-FABP was found to be significantly correlated with serum creatinine ($F = 39.8$) and urinary NAG ($F = 10.5$) ($R = 0.69$, $p < 0.0001$) (Table 6). Serum *L*-FABP was not correlated with urinary *L*-FABP in patients with CKD. In the same analysis using serum *L*-FABP of liver disease patients with a dependent variable and nine clinical independent variables (serum creatinine, serum total cholesterol, serum AST, serum ALT, urinary protein, urinary NAG and urinary *L*-FABP), serum *L*-FABP was found to be significantly correlated with serum AST ($F = 51.7$), serum creatinine ($F = 6.9$) and urinary *L*-FABP ($F = 10.4$) ($R = 0.77$, $p < 0.0001$) (Table 7).

Discussion

CKD is a worldwide common disease and progresses to end stage renal disease. Therefore, the management of CKD is important. However, few clinical markers can predict and/or monitor the progression of CKD. In our previous study, we showed that urinary excretion of *L*-FABP may be a new marker for predicting the progression of CKD [3]. The present study indicated that urinary *L*-FABP might be a useful biomarker for monitoring CKD because the level of its excretion changes with the progression of CKD. Furthermore, serum *L*-FABP derived from the liver was not influenced by urinary *L*-FABP in CKD.

We investigated a correlation between the progression of kidney disease and the changes in urinary *L*-FABP or in urinary protein. Similar to a prospective analysis, the cut-off values for monitoring disease were measured by the ROC curve, and the patients were divided into high and low urinary *L*-FABP groups according to these cut-off values. Whereas renal function deteriorated in the high group, it was stable in the low group. However, urinary protein did not almost change as renal dysfunction progressed. The dynamics of urinary *L*-FABP differed from those of any other urinary

Table 4. Time-dependent change in urinary *L*-FABP and Ccr in high and low urinary *L*-FABP groups

Length of trial (months)	0	1	2	3	4	5	6	7	8	9	10	11	12
Urinary *L*-FABP $\geq 17.4\,\mu$g/g.cr. ($n = 36$)													
Ccr (mL/min)	41.2 ±16.0	34.8 ±18.3	39.3* ±14.8	36.3 ±15.7	37.1* ±16.3	34.5* ±14.7	34.8* ±15.0	34.5* ±14.4	35.6* ±17.1	36.3* ±15.1	37.0* ±15.3	33.3* ±13.7	33.9* ±13.7
Urinary *L*-FABP (μg/g.cr)	106.7 ± 129.6	133.3 ± 204.7	112.9 ± 110.8	83.0 ± 69.5	119.1 ± 152.8	155.5 ± 149.5	129.3* ± 142.8	159.0 ± 151.8	137.9 ± 104.6	168.3* ± 194.1	131.0* ± 93.2	114.2* ± 76.3	150.1* ± 164.7
Urinary *L*-FABP $< 17.4\,\mu$g/g.cr. ($n = 12$)													
Ccr (mL/min)	60.0 ± 27.5	69.6 ± 57.4	52.4 ± 21.7	67.8 ± 28.5	59.1 ± 30.2	56.3 ± 22.7	53.7 ± 26.2	67.0 ± 30.6	63.7 ± 33.6	62.5 ± 21.0	56.4 ± 34.8	66.6 ± 51.7	67.8 ± 29.5
Urinary *L*-FABP (μg/g.cr)	8.1 ± 4.6	12.5 ± 7.2	11.5 ± 7.7	17.8* ± 18.1	14.8 ± 12.1	22.1 ± 26.3	23.0 ± 24.7	16.5 ± 18.4	20.0 ± 18.5	17.2 ± 13.5	22.5 ± 14.7	23.2 ± 19.1	22.6 ± 24.0

Results are expressed as the mean ± SD.

*$p < 0.05$ compared to the value at the start of the follow-up period.

Table 5. Time-dependent changes in urinary protein and Ccr in the high and low urinary protein groups

Length of trial (months)	0	1	2	3	4	5	6	7	8	9	10	11	12
Urinary protein ≥1 g/g.cr. ($n = 26$)													
Ccr (mL/min)	40.9 ±16.1	34.8 ± 18.3	38.8* ± 14.9	36.1* ± 16.2	35.3* ± 15.5	31.7* ± 13.2	31.3* ± 13.3	33.1* ± 14.9	33.0* ± 16.6	34.5* ± 15.8	33.8* ± 14.3	27.8* ± 9.2	32.9* ± 13.2
Urinary protein (g/g.cr)	2.5 ± 1.8	1.9* ± 0.9	2.1 ± 1.4	1.9 ± 1.1	2.0 ± 1.3	2.4 ± 1.7	1.9 ± 1.2	2.4 ± 1.7	2.4 ± 1.6	2.6 ± 1.9	2.4 ± 1.3	2.0 ± 1.2	2.3 ± 1.6
Urinary protein <1 g/g.cr. ($n = 22$)													
Ccr (mL/min)	51.8 ±24.4	69.6 ± 57.4	47.0 ± 19.6	56.2 ± 28.4	52.9 ± 27.2	50.8 ± 21.0	49.6 ± 22.3	56.7 ± 28.7	53.2 ± 27.9	58.5 ± 17.7	49.4 ± 27.1	58.7 ± 27.1	63.1 ± 26.0
Urinary protein (g/g.cr)	0.3 ± 0.3	0.4 ± 0.3	0.4 ± 0.3	0.3 ± 0.2	0.3 ± 0.2	0.4 ± 0.3	0.6 ± 0.8	0.6* ± 0.7	0.5* ± 0.4	0.4 ± 0.4	0.6* ± 0.5	0.5 ± 0.6	0.5 ± 0.4

Results are expressed as the mean ± SD.

*$p < 0.05$ compared to the value at the start of the follow-up period.

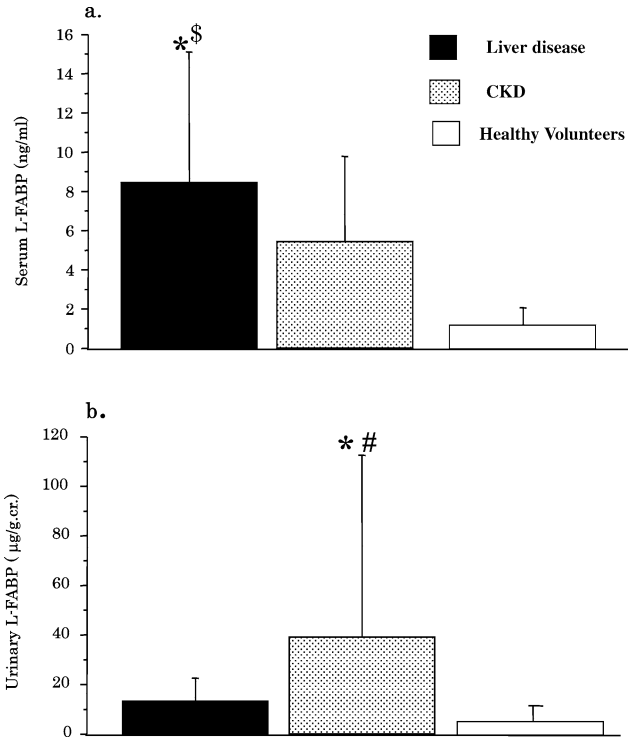

Fig. 1. (a) Bar graph of serum *L*-FABP levels in patients with CKD, patients with liver disease and healthy volunteers, mean ± SD. (b) Bar graph of urinary *L*-FABP levels in patients with CKD, patients with liver disease and healthy volunteers, mean ± SD. $^{*}p < 0.05$ compared to healthy volunteers, $^{\$}p < 0.05$ compared to patients with CKD, $^{\#}p < 0.05$ compared to patients with liver disease.

markers, which suggests that urinary *L*-FABP may be a more relevant clinical marker for monitoring the progression of CKD.

As *L*-FABP is not expressed in the proximal tubules of the wild type mice, we established human *L*-FABP gene transgenic mice and examined the dynamics of *L*-FABP in kidney disease [2]. In the experimental study, the stress overloaded on the proximal tubules, which induces tubulointerstitial damage, induced up-regulation of *L*-FABP gene expression and accelerated urinary excretion of *L*-FABP from the proximal tubules into urine [2]. From the results, we speculate that, in the proximal tubules of the patients whose renal function are deteriorated, *L*-FABP gene expression and urinary excretion of *L*-FABP have increased during the progression of renal dysfunction.

Table 6. Stepwise regression analysis for serum *L*-FABP in CKD

Independent Variables	*F* ratio
Serum Creatinine	**39.8**
Total cholesterol	1.0
Urinary Protein	1.2
NAG	**10.5**
L-FABP	0.6

Note. significant results in bold font.

Table 7. Stepwise regression analysis for serum *L*-FABP in liver disease

Independent Variables	*F* ratio
Serum Creatinine	**6.9**
Total cholesterol	0.3
AST	**51.7**
ALT	1.0
Urinary Protein	0.04
NAG	0.3
L-FABP	**10.4**

Note. significant results in bold font.

In addition to the kidney, *L*-FABP was expressed in the liver. Serum *L*-FABP derived from the liver was filtered through the glomeruli and might influence urinary *L*-FABP. Serum *L*-FABP levels were significantly higher in liver disease patients than in both CKD patients and the healthy volunteers; serum *L*-FABP levels were also correlated with AST, which is released from liver cells after injury. These results suggest that serum *L*-FABP derived from the liver is increased after liver injury. In CKD, because serum *L*-FABP was correlated with serum creatinine, it is considered to have accumulated in the circulatory system through a decrease in glomerular filtration rates. Urinary *L*-FABP levels in liver disease patients were significantly lower than those in kidney disease patients, and were similar to those in the healthy volunteers; serum *L*-FABP levels and urinary *L*-FABP levels were not correlated in kidney disease patients. These results suggest that serum *L*-FABP levels do not influence urinary *L*-FABP levels.

In conclusion, urinary excretion of *L*-FABP increases with the deterioration of renal function, but not urinary protein. Serum *L*-FABP did not influence on urinary *L*-FABP. Urinary *L*-FABP may be a useful clinical biomarker for monitoring CKD. We thus assume that urinary *L*-FABP may be utilized in the management of CKD.

Acknowledgments

We are indebted to Ms. Sanae Ogawa (Internal Medicine, the University of Tokyo) for assistance with the collection of urine and serum samples.

References

1. Sarnak MJ, Levey AS, Schoolwerth AC, Coresh J, Culleton B, Hamm LL, McCullough PA, Kasiske BL, Kelepouris E, Klag MJ, Parfrey P, Pfeffer

M, Raij L, Spinosa DJ, Wilson PW: Kidney disease as a risk factor for development of cardiovascular disease: a statement from the American Heart Association Councils on Kidney in Cardiovascular Disease, High Blood Pressure Research, Clinical Cardiology, and Epidemiology and Prevention. Circulation 108: 2154–2169, 2003

2. Kamijo A, Sugaya T, Hikawa A, Okada M, Okumura F, Yamanouchi M, Honda A, Okabe M, Fujino T, Hirata Y, Omata M, Kaneko R, Fujii H, Fukamizu A, Kimura K: urinary excretion of fatty acid-binding protein reflects stress overload on the proximal tubules. Am J Pathol 165: 1243–1255, 2004
3. Kamijo A, Kimura K, Sugaya T, Yamanouchi M, Hikawa A, Hirano N, Hirata Y, Goto A, Omata M: Urinary fatty acid binding protein as a new clinical marker for the progression of chronic renal disease. J Lab Clin Med 143: 23–30, 2004
4. Watanabe N, Kamei S, Ohkubo A, Yamanaka M, Ohsawa S, Makino K, Tokuda K: Urinary protein as measured with a pyrogallol red-molybdate complex, manually and in a Hitachi 726 automated analyzer. Clin Chem 32: 1551–1554, 1986
5. Horio M, Orita Y, Manabe S, Sakata M, Fukunaga M: Formula and nomogram for predicting creatinine clearance from serum creatinine. Clin Exper Nephrol 1: 110–114, 1997
6. Kamisaka, Maezawa H, Inagaki T, Okano K: A low molecular weight binding protein for organic anions (Z protein) from human hepatic cytosol: purification and quantitation. Hepatology 1: 221–227, 1981