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Urinary Excretion of Liver Type Fatty Acid Binding Protein Accurately Reflects the Degree of Tubulointerstitial Damage

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To investigate the relationship between liver-type fatty acid-binding protein (L-FABP), a biomarker of chronic kidney disease, in the kidney and the degree of tubulointerstitial damage, folic acid (FA)-induced nephropathy was studied in a mouse model system. As renal L-FABP is not expressed in wild-type mice, human L-FABP (hL-FABP) transgenic mice were used in this study. hL-FABP is expressed in the renal proximal tubules of the transgenic mice that were injected intraperitoneally with FA in NaHCO₃ (the FA group) or only NaHCO₃ (the control group) and oral saline solution daily during the experimental period. The FA group developed severe tubulointerstitial damage with the infiltration of macrophages and the deposition of type I collagen on days 3 and 7 and recovered to the control level on day 14. The gene and protein expression levels of hL-FABP in the kidney were significantly enhanced on days 3 and 7. Urinary hL-FABP in the FA group was elevated on days 3 and 7 and decreased to the control level on day 14. The protein expression levels of hL-FABP in both the kidney and urine significantly correlated with the degree of tubulointerstitial damage, the infiltration of macrophages, and the deposition of type I collagen. In conclusion, renal expression and urinary excretion of hL-FABP significantly reflected the severity of tubulointerstitial damage in FA-induced nephropathy. (Am J Pathol 2009, 174:2096–2106; DOI: 10.2353/ajpath.2009.080780)

function and the amelioration of tubulointerstitial damage retards the progression of kidney disease.^{1–3} Therefore, a biomarker that accurately reflects the degree of the tubulointerstitial damage should be a useful clinical tool to monitor kidney disease and its treatments.

Recently, urinary excretion of liver-type fatty acid binding protein (L-FABP) has been clinically recognized as a useful biomarker for monitoring chronic kidney disease and early detection of acute kidney injury.^{4,5} L-FABP is expressed in the human proximal tubules of the kidney.^{6,7} human L-FABP (hL-FABP) binds fatty acids and transports them to mitochondria or peroxisomes, where fatty acids are β -oxidized, and participates in intracellular fatty acid homeostasis.^{8,9} Some experimental studies of animal models of kidney disease with tubulointerstitial damage revealed that renal hL-FABP was up-regulated and urinary excretion of hL-FABP was increased.^{4,10–12} From our studies, we considered that the increase in urinary hL-FABP is caused by the increase in urinary excretion of hL-FABP from the proximal tubules.¹³ Furthermore, the excretion of urinary hL-FABP was lowered in response to various treatments eg, angiotensin II receptor blocker, statins, etc.^{14–16} Therefore, urinary hL-FABP may be an excellent biomarker in monitoring tubulointerstitial damage.

We reported previously that urinary hL-FABP reflected the severity of tubulointerstitial damage in renal biopsies of the patients with chronic kidney disease.¹² However, the dynamics of renal hL-FABP and the changes in urinary hL-FABP in the progression and regression of tubulointerstitial damage have not been investigated together in a single model system.

In a mouse model of folic acid (FA) induced nephropathy, acute kidney injury was provoked after intraperitoneal injection of FA. This resulted in focal or patchy

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It is well known in the variety of kidney diseases that tubulointerstitial damage leads to impairment of renal

progressive tubulointerstitial damage.^{17–20} In a preliminary experiment with this nephropathy model, we found that administration of one ml of saline daily to the mice by oral gavage after a single FA injection prevented the mice from dying of severe acute kidney injury. Severe tubulointerstitial fibrosis was induced on day 7 after injection of FA, but the tubulointerstitial damage had regressed on day 14. Therefore, the aim of this study was to evaluate the dynamics of renal hL-FABP and the changes of urinary hL-FABP excretion during both progression and regression of tubulointerstitial damage produced by injection of FA and administration of saline.

Materials and Methods

Animals

Studies were conducted in accordance with the St. Marianna University School of Medicine Institutional Guide for Animal Experiments.

As renal L-FABP is not expressed in the kidneys of the mice, *hL-FABP* chromosomal transgenic (Tg) mice were generated as described previously (patent no. WO0073791).¹² Mice were housed in the animal facilities of St. Marianna University School of Medicine with free access to food and water.

For this study, 10- to 12-week old female hL-FABP Tg mice ($n = 42$ body weight, 21.8 ± 0.2 g; mean $\pm$ SE) on a BALB/c background were used. The mice were divided into two groups: the FA group ($n = 27$) was given a single FA (Sigma-Aldrich Co., St. Louis, MO) intraperitoneal injection at a dose of 240 mg/kg in 0.3 M/L NaHCO₃. The control group ($n = 15$) received 0.3 M/L NaHCO₃ alone at a dose of 10 ml/kg. The doses of FA were critical for induction of severe tubulointerstitial damage and had been determined in earlier studies.^{21,22} All mice received a forced, daily oral administration of normal saline solution at 1 ml/mouse to prevent the death of mice by severe acute kidney injury. Following the measurement of body weight, all mice were housed overnight individually in metabolic cages with free access to tap water, and urine was collected on days 0, 3, 7, and 14. The sediment was removed from the urine samples by centrifugation (15,000 rpm for 5 minutes).

The mice of the FA group were sacrificed on day 3 ($n = 7$), day 7 ($n = 8$), and day 14 ($n = 12$). The mice of the control group were sacrificed on day 3 ($n = 5$), day 7 ($n = 5$), and day 14 ($n = 5$). In brief, under intraperitoneal anesthesia, blood was drawn from the inferior vena cava. Then, the left kidney was removed and fixed in 10% buffered formalin (WAKO Pure Chemical Industries, Ltd., Osaka, Japan) and methyl Carnoy's solution for staining. The right kidney was also removed, weighed, and snap-frozen in liquid nitrogen for analysis of protein and gene expression.

Serum Biochemistry

Serum was isolated from blood by centrifugation (15,000 rpm for 15 minutes). Serum creatinine was measured by

the Jaffé method (Creatinine Test WAKO; WAKO Pure Chemical Industries, Ltd.). Serum urea nitrogen was measured by the urease-indophenol method (Urea N B; WAKO Pure Chemical Industries, Ltd.). Serum hL-FABP was quantified by a two-step sandwich enzyme-linked immunosorbent assay (ELISA) procedure (CMIC Co., Ltd., Tokyo, Japan).²³

Urinary Biochemistry

Urinary creatinine was quantified by the Jaffé method (the creatinine companion; Exocell Inc., Philadelphia, PA). Urinary albumin was quantified by an ELISA (Albwell; Exocell Inc.). Urinary *N*-acetyl- β -D-glucosaminidase (NAG) was quantified by using a commercially available chemical reagent (NAG; Shionogi & Co. Ltd., Osaka, Japan). Urinary hL-FABP was quantified by a two-step sandwich ELISA procedure (CMIC Co., Ltd.).²⁴

Renal Histological and Morphometric Analysis

For light microscopic analysis, the kidney was dehydrated and embedded in paraffin. Serial sections (1 μ m thick) were obtained for conventional histological assessment such as periodic acid-Schiff (PAS) staining, Azan-Mallory staining, and immunohistochemistry. The PAS-stained tissue sections were used to evaluate acute tubulointerstitial injury, which was defined as tubular dilatation with epithelial atrophy and without brush borders. Azan-Mallory stained tissue sections were used to evaluate chronic tubulointerstitial damage, which was defined as accumulation of extracellular matrix shown in blue and tubular atrophy. Under $\times 200$ magnification, 10 non-overlapping fields from the cortical region were selected, and the area of tubulointerstitial damage and the whole cortical area were measured with an image analyzer (WinRoof; Mitani Co., Tokyo, Japan). The degree of tubulointerstitial damage was evaluated as the ratio of the area of tubulointerstitial damage to the entire cortical area.^{12,23,25} These histological evaluations were performed in a blind manner by one of the researchers.

Immunohistological Analysis

Tissues fixed in methyl Carnoy's solution were embedded in paraffin. An indirect immunoperoxidase method was used to identify the antigens as described previously.^{11,26} Macrophages were identified with rat monoclonal antibody F4/80 (BMA Biomedicals, Augst, Switzerland), and type I collagen was identified with a rabbit polyclonal antibody (Cedarlane Laboratories Ltd., Ontario, Canada). The degree of macrophage infiltration in the cortical interstitium was measured as the ratio of the positive area of F4/80 to the entire cortical area under $\times 200$ magnification with an image analyzer (WinRoof). Similarly, the positive area of type I collagen was evaluated as the ratio of the positive area of type I collagen to the entire cortical area.

Tissues fixed in 10% buffered formalin and embedded in paraffin were used to perform the double immunohis-

tochemistry with monoclonal antibody against hL-FABP and rabbit polyclonal antibody against aquaporin (AQP)-1 (Chemicon Int., Temecula, CA) as a marker of the proximal tubule. hL-FABP immunostaining in the kidneys of the mice was performed with mouse monoclonal antibody against human L-FABP labeled as FABP-2, which was generated previously and reacted specifically with the endogenous mouse L-FABP expressed in the liver.^{11,12,26} In the first step, primary AQP-1 antibody was applied on the sections and the peroxidase-labeled anti-rabbit immunoglobulin of the DAKO EnVision System (DAKO, Tokyo, Japan) was used as a secondary antibody. Peroxidase activity was visualized using diaminobenzidine tetrahydrochloride (DAKO). In the second step, primary hL-FABP antibody was applied and the biotinylated anti-mouse secondary antibody (DAKO) was incubated followed by an avidin-biotin-alkaline phosphatase. The alkaline phosphatase activity was visualized using nitro blue tetrazolium chloride/5-brom-4-chloro-3-indolyl phosphate.

Measurement of Monocyte Chemoattractant Protein-1 and hL-FABP by ELISA

Frozen kidneys were homogenized in lysis buffer (0.1 mol/L phosphate buffer, 1 μ g/ml chymotrypsin, 1 μ g/ml leupeptin, 1% Triton X-100, and 0.05 mmol/L phenylmethyl sulfonyl fluoride) at 4°C. Supernatants were collected after centrifugation for 30 minutes at 15,000 rpm, and protein concentrations were measured by the Bradford method (Protein assay; Bio-Rad Laboratories, Inc., Hercules, CA). To determine the quantity of monocyte chemoattractant protein (MCP)-1 and hL-FABP proteins in the kidney, the proteins extracted by the method described above were measured with ELISA kits for MCP-1 (R&D Systems, Minneapolis, MN) and hL-FABP (CMIC Co., Ltd.). The concentrations of MCP-1 and hL-FABP were corrected for the total protein concentrations.

Western Blot Analysis

To determine hypoxia inducible factor-2 α (HIF-2 α)¹⁷ and the lipid peroxidation products produced by oxidative stress, ie, N ϵ -(Hexanoyl) Lysine (HEL),²⁷ protein extracts were prepared by homogenizing one-half of a kidney in lysis buffer (0.1 mol/L phosphate buffer, 1 μ g/ml chymotrypsin, 1 μ g/ml leupeptin, 1% Triton X-100, and 0.05 mmol/L phenylmethyl sulfonyl fluoride). A protein sample (40 μ g) was subjected to SDS-polyacrylamide gel electrophoresis, and the separated protein bands were analyzed by an enhanced chemiluminescence system (Amersham Int., Buckinghamshire, UK). Rabbit polyclonal antibody against HIF-2 α , (Santa Cruz Biotechnology Inc., Santa Cruz, CA) and monoclonal antibody against HEL (JalCA, Shizuoka, Japan) were used as primary antibody.

TaqMan Real-Time PCR Assay

Total RNA of the kidney was extracted using an RNeasy mini kit (Qiagen Inc., Valencia, CA) according to the

manufacturer's instructions. Total RNA (0.5 μ g) was reverse-transcribed using ExScript RT reagent kit (Takara Bio Co., Shiga, Japan). The TaqMan real-time PCR reactions were performed on a TaqMan ABI PRISM 7000 sequence detection system (Applied Biosystems, Foster City, CA) using TaqMan Universal PCR Master Mix (Applied Biosystems). hL-FABP, MCP-1, heme oxygenase (HO)-1, glutathione peroxidase (GPX)-1, receptor for advanced glycosylation end products (RAGE), matrix metalloproteinase 9 (MMP-9), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNAs were detected using TaqMan real-time PCR. Unlabeled specific primers and the TaqMan MGB probes (6-FAM dye-labeled) were purchased from Applied Biosystems. TaqMan conditions were as follows. After an initial hold of 2 minutes at 50°C and 10 minutes at 95°C, the samples were cycled 40 times at 95°C for 15 seconds and at 60°C for 1 minute. Expressions of hL-FABP, MCP-1, HO-1, GPX-1, and RAGE mRNAs in each sample were evaluated after normalization with GAPDH expression.

Statistical Analysis

All values were expressed as mean $\pm$ SE. Differences among control and FA groups were analyzed by Dunnett's multiple comparison procedure. The correlations between control and FA groups (days 3, 7, and 14), (nonparametric distribution) was analyzed by the Mann-Whitney *U*-test using unpaired data. The correlation between each parameter was assessed by making scatter diagrams and calculating and analyzing correlation coefficients. These statistical analyses were performed with a computer software program for Microsoft Windows (SPSS; SPSS Japan Inc., Tokyo, Japan). Statistical significance was set at $P < 0.05$.

Results

Body and Kidney Weights

The body weights of the FA group were significantly lower than the control group on day 7 ($P < 0.05$) (Table 1).

The kidney weights of the FA group were significantly higher than the control group on days 3 ($P < 0.001$) and 7 ($P < 0.001$) and decreased on day 14 (Table 2).

Table 1. Body Weight of Control Group and FA Group

	Body weight	
	Body weight (g) control group	Body weight (g) FA group
Day 0	21.26 $\pm$ 0.29 ($n = 15$)	22.03 $\pm$ 0.25 ($n = 27$)
Day 3	21.45 $\pm$ 0.55 ($n = 5$)	21.09 $\pm$ 0.33 ($n = 7$)
Day 7	21.25 $\pm$ 0.54 ($n = 5$)	20.40 $\pm$ 0.54 ($n = 8$)*
Day 14	21.31 $\pm$ 0.22 ($n = 5$)	21.43 $\pm$ 0.44 ($n = 12$)

* $P < 0.05$ compared with the control group.

Table 2. Kidney Weight of Control Group and FA Group

	Kidney weight	
	Kidney weight (g) control group	Kidney weight (g) FA group
Day 3	0.134 ± 0.006 (n = 5)	0.196 ± 0.011 (n = 7)**
Day 7	0.140 ± 0.005 (n = 5)	0.169 ± 0.004 (n = 8)**
Day 14	0.144 ± 0.005 (n = 5)	0.155 ± 0.004 (n = 12)

**P < 0.001 compared with the control group.

Serum Biochemistry

The levels of serum urea nitrogen in the FA group were significantly higher than those in the control group on days 3 ($P < 0.005$) and 7 ($P < 0.0005$) (Table 3). On day 14, it had decreased to the level of the control group.

The levels of serum creatinine in the FA group tended to be higher than in the control group on days 3 and 7, but were not significant (Table 3).

The levels of serum hL-FABP in the FA group on each of the 3 days did not increase compared with the control group (Table 3).

Urinary Biochemistry

On days 3 and 7, the levels of urinary albumin (Figure 1A) in the FA group increased, but not significantly. On day 14, urinary albumin levels in the FA group were significantly higher than those in the control group (23.86 ± 13.60 mg/g cre vs 3.41 ± 0.34 mg/g cre $P < 0.05$).

The levels of urinary NAG (Figure 1B) in the FA group on days 3, 7, and 14 were significantly higher than the day 0 level of the same group (on day 3, 62.8 ± 6.3 U/g cre; on day 7, 67.3 ± 4.7 U/g cre; on day 14, 101.9 ± 14.5 U/g cre; vs FA group on day 0, 37.0 ± 3.8 U/g cre; $P < 0.05$, respectively). Urinary NAG levels between the FA and control groups differed significantly on days 3 ($P < 0.005$) and 14 ($P < 0.05$).

The levels of urinary hL-FABP (Figure 1C) in the FA and control groups differed significantly on days 3 (130.6 ± 28.4 ng/mg cre vs 58.6 ± 32.9 ng/mg cre, $P < 0.0001$) and 7 (240.9 ± 85.0 ng/mg cre vs 72.9 ± 28.6 ng/mg cre, $P < 0.01$), but not on days 0 and 14. The level of urinary hL-FABP in the FA group on day 7 was significantly higher than in the same group on day 0 ($P < 0.05$). On day 14, the urinary hL-FABP level of the FA group fell to nearly the day 0 value (77.7 ± 23.8 ng/mg cre).

Table 3. Serum Biochemistry

	Serum biochemistry					
	Serum urea nitrogen (mg/dl)		Serum creatinine (mg/dl)		Serum L-FABP (ng/ml)	
	control group	FA group	control group	FA group	control group	FA group
Day 3	40.0 ± 2.38 (n = 5)	97.9 ± 17.1 (n = 7) [†]	0.35 ± 0.03 (n = 5)	0.62 ± 0.12 (n = 7)	270.6 ± 63.6 (n = 5)	239.5 ± 14.0 (n = 7)
Day 7	37.3 ± 1.59 (n = 5)	145.9 ± 22.2 (n = 8) ^{††}	0.49 ± 0.07 (n = 5)	0.81 ± 0.10 (n = 8)	356.0 ± 36.1 (n = 5)	339.5 ± 36.4 (n = 8)
Day 14	49.9 ± 2.54 (n = 5)	49.2 ± 3.8 (n = 12)	0.46 ± 0.03 (n = 5)	0.49 ± 0.02 (n = 12)	378.6 ± 51.2 (n = 5)	313.6 ± 34.3 (n = 12)

[†]P < 0.005 and ^{††}P < 0.0005 compared with the control group of the same day.

Renal Histological and Morphometric Analysis

On day 3 of the acute phase, tubular dilation was widely observed (Figure 2A). On day 7 of the chronic phase, tubular fibrosis and tubular atrophy were noteworthy and, on day 14 of the regeneration phase, only segmental evidence of tubular fibrosis and tubular atrophy were found (Figure 2B). The degree of tubular dilation in a PAS-stained section is shown in Figure 2E, which shows quantitative data. The FA group on day 3 showed significantly greater differences from the control group ($14.4 \pm 2.7\%$ vs control group, $0.5 \pm 0.2\%$; $P < 0.001$). The degree of tubulointerstitial damage in an AZAN-Mallory stained section was shown in Figure 2F, which shows quantitative data. The FA groups on days 7 and 14 showed significantly greater differences from the control groups (on day 7, $20.7 \pm 5.9\%$ vs $0.4 \pm 0.1\%$, and on day 14, $8.8 \pm 3.9\%$ vs $0.4 \pm 0.1\%$; $P < 0.005$, for both). On day 14, the degree of tubulointerstitial damage in the FA group was relieved.

Immunohistological Analysis

The infiltration of macrophages (Figure 2, C and G) of the FA group on day 7 was significantly greater than in the control group ($5.3 \pm 1.2\%$ vs $0.6 \pm 0.2\%$; $P < 0.05$). On day 14, the infiltration of macrophages showed no difference in the FA and control groups.

The deposition of type I collagen (Figure 2, D and H) of the FA group on days 7 and 14 were significantly greater than those of the control group (on day 7, $35.0 \pm 4.3\%$ vs $0.4 \pm 0.1\%$ and on day 14, $14.7 \pm 2.0\%$ vs $0.3 \pm 0.1\%$; $P < 0.005$, for both). On day 14, the deposition of type I collagen in the FA group was significantly diminished compared with on day 7 of the FA group.

The photomicrographs of Figure 2, A–D concurred with the morphometric analyses in Figure 2, E–H.

Expression of MCP-1 in the Kidney

The level of gene expression of MCP-1 (Figure 3A) in the FA kidneys on days 3 and 7 increased significantly over their corresponding control groups (on day 3, 1.32 ± 0.47 vs 0.16 ± 0.04 , $P < 0.05$; on day 7, 1.83 ± 0.57 vs 0.09 ± 0.03 ; $P < 0.005$, values in arbitrary units). On day 14, although gene expression level of MCP-1 was higher

Figure 1. Time course of urinary albumin (A), NAG (B), and hL-FABP (C) levels in the hL-FABP Tg mice. Filled circles indicate the FA group and open circles indicate the control group. FA group, on day 0 ($n = 27$), day 3 ($n = 27$), day 7 ($n = 20$), day 14 ($n = 12$); control group, on day 0 ($n = 15$), day 3 ($n = 15$), day 7 ($n = 10$), day 14 ($n = 5$). * $P < 0.05$, ** $P < 0.01$, § $P < 0.005$, and ¶ $P < 0.0001$ compared with the control group of the same day; * $P < 0.05$ compared with 0 day of the same group.

in the FA group than in the control, the difference was not significant.

The level of protein expression of MCP-1 (Figure 3B) in the FA kidneys on days 3 and 7 increased significantly over their corresponding control groups (on day 3, 3.86 ± 0.95 vs. 0.50 ± 0.07 ng/mg protein, $P < 0.01$; on day 7, 6.79 ± 2.07 vs. 0.39 ± 0.06 ng/mg protein; $P < 0.005$). On day 14, protein expression level of MCP-1 tended to be higher in the FA group than in the control, but was not significant.

Expression of hL-FABP in the Kidney

The levels of gene expression of hL-FABP (Figure 4A) in the FA kidneys on days 3 and 7 increased significantly over their corresponding controls (on day 3, 1.00 ± 0.14 vs. 0.48 ± 0.08 , $P < 0.05$; on day 7, 0.97 ± 0.18 vs. 0.43 ± 0.01 , $P < 0.01$, values in arbitrary units). Thereafter, on day 14, gene expression levels of hL-FABP in FA and control groups were nearly the same.

Figure 2. Histological findings of PAS staining (A), Azan-Mallory staining (B), immunohistochemistry using antibody to F4/80 (C), and antibody to type I collagen (D) in the control group and the FA group on days 3, 7, and 14. Magnification = original $\times 100$. The three areas were also assessed quantitatively (Figures E, F, G, and H) as described in *Materials and Methods*. Filled bars indicate the FA group and open bars indicate the control group; values are mean $\pm$ SE. FA group, day 3 ($n = 7$), day 7 ($n = 12$); control group, on day 0 ($n = 5$), day 3 ($n = 5$), day 7 ($n = 5$), day 14 ($n = 5$). * $P < 0.05$, ** $P < 0.01$, and § $P < 0.005$ compared with the control group of the same day. & $P < 0.05$ compared with 7 day of the FA group.

Figure 3. Expression of MCP-1 in the kidney. **A:** Expression of MCP-1 mRNA transcripts was determined by TaqMan real-time PCR and normalized to that of GAPDH mRNA transcripts in the same sample. **B:** The expression of MCP-1 protein was determined by ELISA and corrected for the total amount of protein. Filled bars indicate the FA group and open bars indicate the control group. Values are the mean $\pm$ SE; FA group, day 3 ($n = 7$), day 7 ($n = 8$), day 14 ($n = 12$); control group, on day 0 ($n = 5$), day 3 ($n = 5$), day 7 ($n = 5$), day 14 ($n = 5$). * $P < 0.05$, ** $P < 0.01$, and $^{\S}P < 0.005$ compared with the control group of the same day.

The levels of protein expression of L-FABP (Figure 4B) in the FA kidneys on days 3 and 7 increased significantly over their corresponding controls (on day 3, 4.29 ± 0.47 vs. 2.14 ± 0.47 $\mu\text{g/mg}$ protein, $P < 0.01$; on day 7, 6.27 ± 1.09 vs. 1.46 ± 0.39 $\mu\text{g/mg}$ protein, $P < 0.0001$). On day 14, the protein expression level of hL-FABP in the FA kidney decreased to nearly the same level as the control.

Double Immunohistochemical Staining of hL-FABP and AQP-1

To investigate localization of expression of hL-FABP, a double immunohistochemistry procedure was performed with monoclonal antibody against hL-FABP and rabbit polyclonal antibody against AQP-1, a marker of the proximal tubule (Figure 5, A–C). In the control group, many double positive tubules of hL-FABP/AQP-1 were observed in the cortical area, whereas in the FA group on day 3, the double positive tubules of hL-FABP/AQP-1 were widely found in both cortex and medulla. On day 7, the double positive tubules of hL-FABP/AQP-1 were not expressed in the extremely damaged tubular structures of the cortical lesion and consequently its number decreased in the cortical area. The double positive tubules of hL-FABP/AQP-1 showed remarkable expression in the medullary lesions. On day 14, in accordance with the regression of the tubulointerstitial damage, the double positive tubules of hL-FABP/AQP-1 were found in the cortical area and hL-FABP expression decreased in the medulla.

Western Blot Analysis of HIF-2 α and HEL

HIF-2 α was detected as a 120 kDa band in Western blot analysis of the FA group on day 7, whereas it was not observed in the control and FA groups on days 3 and 14 (Figure 6).

One of the lipid peroxidation products, HEL-modified protein band was observed as two bands in the FA group on day 7, whereas it was not observed in the control group and FA groups on days 3 and 14 (Figure 7).

Gene Expressions of HO-1, GPX-1, RAGE, and MMP-9 in the Kidney

The levels of gene expression of HO-1 (Figure 8A) in the FA kidneys on days 3 and 7 increased significantly over their corresponding controls (on day 3, 1.20 ± 0.36 vs. 0.39 ± 0.03 , $P < 0.05$; on day 7, 0.68 ± 0.15 vs. 0.37 ± 0.03 , $P < 0.05$, values in arbitrary units). On day 14, while the gene expression level of HO-1 in the FA kidney decreased compared with day 7, it was still significantly higher than the control.

The levels of gene expression of GPX-1 (Figure 8B) in the FA kidneys on days 3 and 7 increased significantly over their corresponding controls (on day 3, 1.67 ± 0.15 vs. 1.28 ± 0.08 and on day 7, 1.76 ± 0.17 vs. 1.37 ± 0.08 , $P < 0.05$, for both). On day 14, the gene expression levels of GPX-1 in the FA kidneys decreased.

The level of gene expression of RAGE (Figure 8C) in the kidney on day 7 increased significantly compared

Figure 4. Expression of hL-FABP in the kidney. **A:** Expression of hL-FABP mRNA transcripts was determined by TaqMan real-time PCR and normalized to that of GAPDH mRNA transcripts in the same sample. **B:** The expression of hL-FABP protein was determined by ELISA and corrected for the total amount of protein. Filled bars indicate the FA group and open bars indicate the control group. Values are the mean $\pm$ SE; FA group, day 3 ($n = 7$), day 7 ($n = 8$), day 14 ($n = 12$); control group, on day 0 ($n = 5$), day 3 ($n = 5$), day 7 ($n = 5$), day 14 ($n = 5$). * $P < 0.05$ and ** $P < 0.01$ and $^{\S}P < 0.0001$ compared with the control group of the same day.

Figure 5. Double immunohistochemical staining of hL-FABP and AQP-1 in the kidney of the control group and the FA group on days 3, 7, and 14. **A:** Cortex and medulla; magnification = original $\times 40$. **B:** Cortex; magnification = original $\times 400$. **C:** Medulla; magnification = original $\times 400$.

with the control group (1.94 ± 0.38 vs. 0.72 ± 0.07 arbitrary units; $P < 0.05$, respectively).

The levels of gene expression of MMP-9 (Figure 8D) in the FA kidneys on days 3 and 7 increased significantly over their corresponding controls (on day 3, 0.19 ± 0.08 vs. 0.01 ± 0.00 , $P < 0.05$ and on day 7, 0.22 ± 0.07 vs. 0.02 ± 0.00 , $P < 0.01$). On day 14, while the gene expression levels of MMP-9 decreased, that in the FA kidney was still significantly higher than that in the control kidney (0.06 ± 0.02 vs. 0.01 ± 0.00 arbitrary units, $P < 0.05$).

Correlation between Each Parameter and Histological Changes

The FA model has two phases of acute tubular injury and subsequent fibrotic changes. Therefore, acute phase was defined as “on day 3” and acute tubular change (FA group, $n = 7$; control group, $n = 5$) was evaluated in PAS-stained tissue sections. Chronic phase was defined as “on days 7 and 14” and chronic tubulointerstitial damage (FA group, $n = 20$; control group, $n = 10$) was evaluated in Azan-Mallory stained tissue sections. Correlations between each of the parameters and histological changes were evaluated separately. The association between the clinical parameters and the histological changes consisting of tubulointerstitial damage, infiltration of macrophages (FA group, $n = 27$; control group, $n = 15$) and deposition of type I collagen (FA group, $n =$

Figure 6. Western blot analysis of HIF-2 α expression in FA induced mice. A representative image is shown using rabbit polyclonal antibody against HIF-2 α . In the FA group on day 7, a band of HIF-2 α was detected at 120 kDa.

Figure 7. Western blot analysis of HEL-modified protein expression in FA induced mice. A representative image is shown using mouse monoclonal antibody against HEL. Two bands of HEL were detected in the FA group on day 7.

27; control group, $n = 15$) were studied, and the findings are presented in Table 4.

Protein expression of hL-FABP was significantly and strongly correlated with all of the histological parameters ($r = 0.73$, vs tubulointerstitial damage of acute phase, $P < 0.01$; $r = 0.72$, vs tubulointerstitial damage of chronic phase, $P < 0.0001$; $r = 0.56$, vs infiltration of macrophages, $P < 0.001$; $r = 0.70$, vs deposition of type I collagen, $P < 0.0001$). Urinary hL-FABP was also significantly correlated with all of the histological parameters ($r = 0.70$, vs tubulointerstitial damage of acute phase, $P < 0.01$; $r = 0.56$, vs tubulointerstitial damage of chronic phase, $P < 0.01$; $r = 0.50$, vs infiltration of macrophages, $P < 0.005$; $r = 0.67$, vs deposition of type I collagen, $P < 0.0001$). Although urinary albumin levels were not correlated with tubulointerstitial damage of chronic phase and deposition of type I collagen, urinary albumin was significantly correlated with tubulointerstitial damage of the acute phase and the degree of infiltration of macrophages ($r = 0.70$, vs tubulointerstitial damage of acute phase, $P < 0.01$; $r = 0.40$, vs infiltration of macrophages, $P < 0.05$). Urinary NAG showed no correlation with any histological parameter.

Correlation between Gene Expression of hL-FABP and Oxidative Stress Marker

In acute phase (FA group, $n = 7$; control group, $n = 5$), gene expressions of hL-FABP were significantly correlated with HO-1 ($r = 0.76$, $P < 0.0001$) (Figure 9A) and GPX-1 ($r = 0.76$, $P < 0.001$) (Figure 9B), but not with RAGE ($r = -0.18$, NS) (Figure 9C).

In chronic phase (FA group, $n = 20$; control group, $n = 10$), gene expressions of hL-FABP were significantly correlated with HO-1 ($r = 0.72$, $P < 0.0001$) (Figure 9D), GPX-1 ($r = 0.70$, $P < 0.0001$) (Figure 9E), and RAGE ($r = 0.80$, $P < 0.0001$) (Figure 9F).

Discussion

Although progressive tubulointerstitial damage was continually observed in the FA-induced nephropathy model, its regression was successfully induced by the administration of saline. In this animal model study, we evaluated the dynamics of renal hL-FABP and the change in urinary hL-FABP in the variety of tubulointerstitial damage. After

Figure 8. Gene expressions of HO-1, GPX-1, RAGE, and MMP-9 in the kidney. The expressions of HO-1 (A), GPX-1 (B), RAGE (C), and MMP-9 (D) mRNA transcripts were determined by TaqMan real-time PCR and were normalized to those of GAPDH mRNA transcripts in the same sample. Filled bars indicate the FA group and open bars indicate the control group. FA group, day 3 ($n = 7$), day 7 ($n = 8$), day 14 ($n = 12$); control group, on day 0 ($n = 5$), day 3 ($n = 5$), day 7 ($n = 5$), day 14 ($n = 5$). * $P < 0.05$ and ** $P < 0.01$ compared with the control group of the same day.

injection of FA and administration of saline, the onset of acute kidney injury was confirmed by the striking increase of serum urea nitrogen on day 3. At the same time, the expressions of HO-1 and GPX-1 were significantly higher in the FA group than in the control group. On day 7, severe tubulointerstitial inflammation and tubulointerstitial fibrosis were histologically observed and HEL-modified protein was produced. On day 14, these changes were attenuated. In the course of this kidney injury, the gene and protein expressions of renal hL-FABP were significantly up-regulated on days 3 and 7, only to fall on day 14. Urinary hL-FABP levels in the FA group significantly increased on days 3 and 7 over the control group but decreased on day 14. With respect to the associations revealed with tubulointerstitial inflammation and fibrosis:

- Urinary NAG showed no association with tubulointerstitial inflammation and fibrosis.
- Urinary albumin showed a significant correlation with tubulointerstitial inflammation and tubulointerstitial damage of acute phase.
- Protein expression of renal hL-FABP and level of urinary hL-FABP were significantly correlated with both tubulointerstitial inflammation and fibrosis.

From these results, urinary hL-FABP was considered to accurately reflect the degree of tubulointerstitial damage and to be useful as a real time indicator for tubulointer-

stitial damage. Furthermore, the change of urinary hL-FABP was shown to depend on the change in expression of hL-FABP in the proximal tubules.

In FA induced nephropathy, it is known that depletion of interstitial capillaries and tissue hypoxia occur,¹⁷ reactive oxygen species production is enhanced and consequently, lipid peroxidation products are generated.²⁸ HIF-2 α expression induced in the FA kidney on day 7 suggested the presence of tissue hypoxia. HO-1, an acute stress marker, is known to be a microsomal enzyme that catalyzes the oxidation of heme to the antioxidant molecules, biliverdin and carbon monoxide.²⁹ The expression of HO-1 is induced by oxidative stress. GPX-1, a member of the glutathione peroxidase family, plays an important role for the detoxification of hydrogen peroxide, and is one of the most important antioxidant enzymes.³⁰ Reactive oxygen species also produce advanced glycation end products, which are activated by binding to RAGE³¹ and accelerates the transdifferentiation of epithelial cells to form myofibroblasts and aggravate the tubulointerstitial damage. RAGE promotes renal disease through the activation of intracellular signaling pathways that promote oxidative stress. In the earlier stage of lipid peroxidation, HEL is found in the kidney.³² HEL is formed by oxidative modification by oxidized omega 6 fatty acids such as linoleic acid or arachidonic acid. In our model, HO-1, GPX-1, RAGE, and HEL ex-

Table 4. Correlation between Each Parameter and Histological Change

	Tubulointerstitial damage		Infiltration of macrophages	Deposition of type I collagen
	acute phase (day 3)	chronic phase (days 7 and 14)		
Protein expression of L-FABP	0.73 ($n = 12$) ($P < 0.01$)	0.72 ($n = 30$) ($P < 0.0001$)	0.56 ($n = 42$) ($P < 0.001$)	0.70 ($n = 42$) ($P < 0.0001$)
Urinary L-FABP	0.70 ($n = 12$) ($P < 0.01$)	0.56 ($n = 30$) ($P < 0.01$)	0.50 ($n = 42$) ($P < 0.005$)	0.67 ($n = 42$) ($P < 0.0001$)
Urinary albumin	0.70 ($n = 12$) ($P < 0.01$)	0.41 ($n = 30$) (NS)	0.40 ($n = 42$) ($P < 0.05$)	0.18 ($n = 42$) (NS)
NAG	0.25 ($n = 12$) (NS)	0.04 ($n = 30$) (NS)	-0.20 ($n = 42$) (NS)	0.01 ($n = 42$) (NS)

Figure 9. Correlation between gene expression of hL-FABP and gene expressions of HO-1, GPX-1, and RAGE. In acute phase (FA group, $n = 7$; control group, $n = 5$), gene expressions of hL-FABP were significantly correlated with HO-1 (A, $r = 0.76$, $P < 0.0001$) and GPX-1 (B, $r = 0.76$, $P < 0.001$), but not with RAGE (C, $r = -0.18$, NS). In chronic phase (FA group, $n = 20$; control group, $n = 10$), gene expressions of hL-FABP were significantly correlated with HO-1 (D, $r = 0.72$, $P < 0.0001$), GPX-1 (E, $r = 0.70$, $P < 0.0001$), and RAGE (F, $r = 0.80$, $P < 0.0001$).

pressions were increased in the FA kidneys. Oxidative stress therefore, was considered to play a significant role in the development of tubulointerstitial damage in the FA mice.

Renal hL-FABP was up-regulated in the proximal tubules of the FA mice. We previously reported that renal hL-FABP expression was up-regulated in the unilateral ureteral obstruction model, in which oxidative stress contributes to severe tubulointerstitial damage.¹¹ Therefore the onset of oxidative stress generated in the FA mice might promote the expression of renal hL-FABP. Moreover, the results of double immunohistochemistry revealed that the location of hL-FABP expression changed from the convoluted proximal tubules to the straight portion of the proximal tubules (S3 segment) during the time course of renal injury. Because the initially damaged tubules in the FA mice were the proximal convoluted tubules, the *de novo* expression of hL-FABP in the S3 segment of the undamaged proximal tubules might be up-regulated to prevent tubulointerstitial damage. However it is conceivable that there is a possibility that L-FABP secreted from S1 and S2 might be absorbed in S3, which may reduce renal injury. Further studies for the dynamics of hL-FABP in renal disease are needed.

Injection of FA provoked tubulointerstitial damage, and not glomerular injury. However, the level of urinary albumin was increased in the course of renal damage. The dysfunction of tubular reabsorption might contribute to increasing the excretion of urinary albumin. On day 14 of the FA mice, tubulointerstitial inflammation and fibrosis were attenuated. However the level of urinary albumin level remained high. It is possible that tubular function had not recovered despite the structural restoration of tubulointerstitial damage on day 14.

Urinary NAG was heretofore considered to be a marker of acute tubulointerstitial damage. However, in this study urinary NAG levels were increased not only in the FA mice, but also in the control mice. Thus, the measurements of urinary NAG in mice may not be a reliable marker of acute interstitial damage.

Because human L-FABP is expressed not only in the kidney, but also in the mouse liver in the human L-FABP Tg mouse, urinary L-FABP might have been influenced by serum L-FABP. However serum L-FABP did not increase in this model. Therefore the increased level of urinary L-FABP level was considered to be due to the L-FABP excreted from the proximal tubules into urine.

Regarding the role of renal hL-FABP in kidney disease, we previously reported that hL-FABP expression in the proximal tubules reduced inflammation in the interstitium and inhibited the development of tubulointerstitial damage in a protein overload model and in a unilateral ureteral obstruction model via an antioxidative function.^{11,12} Others had also reported on the antioxidative function of hL-FABP in *in vivo* and *in vitro* experiments.^{4,33–35} Therefore the FA model was generated with the wild-type mice similar to the Tg mice and the degree of tubulointerstitial damage in the wild-type mice compared with that in the Tg mice. Although the degree of macrophage infiltration was significantly higher in the FA wild-type mice than in the FA Tg mice (data not shown), there was no significant difference in tubulointerstitial damage between the FA wild-type mice and the FA Tg mice. Because many factors contribute to the pathogenesis of tubulointerstitial damage in the FA induced nephropathy model, it is likely that the presence of hL-FABP alone, as an endogenous antioxidative protein, had limited efficacy for prevention of tubulointerstitial damage.

Why did the administration of saline lead to the regression of tubulointerstitial damage? Because the gene expression of MMP-9, which is involved in the breakdown of extracellular matrix, was up-regulated on days 3, 7, and 14, this system contributed to attenuation of renal fibrosis. Although renal injury in the FA model is attributed to acute tubular obstruction by the rapid appearance of FA crystals, the direct nephrotoxicity of FA to the proximal tubules¹⁸ and dehydration, a detailed mechanism of renal injury has not been determined. Because the administration of saline might change the renal hemodynamics, prevent dehydration, wash out the early deposition of FA crystals from the tubules, and remove the toxicity of FA, absorption of fibrosis and proximal regeneration might be accelerated on day 14.

Tubular hypoxia provoked by peritubular capillary loss plays an important role for the development of renal injury in the FA model.¹⁷ To show the relationship of humans and the Tg mice in acute kidney injury, the kinetics of urinary hL-FABP in intravenous injections of radiocontrast was reported by Nakamura et al.³⁶ Radiocontrast also induced tissue hypoxia before induction of acute renal dysfunction,³⁷ which showed that there is a common pathophysiologic mechanism between renal injury by injection of FA and that of radiocontrast. They reported that urinary hL-FABP level increased significantly from 18.5 ± 12.8 ng/mg cre to 46.8 ± 30.5 ng/mg cre by the next day after injection of radiocontrast in the patients with radiocontrast nephropathy (which is defined as an increase in serum creatinine level of greater than 0.5 mg/dL, >44 micromol/L or a relative increase of more than 25% at 2 to 5 days after the procedure). At 2 and 14 days after the procedure, although urinary hL-FABP level remained high (at 2 days, 38.5 ± 28.5 ng/mg cre; at 14 days 34.5 ± 30.0 ng/mg cre), the values gradually decreased in proportion to regression of radiocontrast nephropathy. Because urinary hL-FABP was reported to reflect degree of tubular hypoxia, increase of urinary hL-FABP was considered to reflect a stress, ie, tubular hypoxia. Moreover, a decrease in urinary hL-FABP indicated a reduction of stress by wash out of radiocontrast. Urinary hL-FABP was considered to change in accordance with the degree of stress loaded in the proximal tubules. Because renal tissue was not obtained, the presence of tissue hypoxia by radiocontrast was not directly demonstrated in the study of Nakamura et al.³⁶ Further clinical studies are needed to clarify the kinetics of urinary hL-FABP in phases of acute kidney injury and repair.

In conclusion, the progression and regression aspects of tubulointerstitial damage in the FA model were demonstrated. Urinary hL-FABP accurately reflected the tubulointerstitial damage and changed in accordance with its various forms. Therefore, urinary hL-FABP is useful as a real time indicator for tubulointerstitial damage. Furthermore, the change in urinary hL-FABP was shown to depend on the change in renal hL-FABP expression.

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References

- Jacobson HR: Chronic renal failure: pathophysiology. *Lancet* 1991, 338:419–423
- Bohle A, Mackensen-Haen S, von Gise H, Grund KE, Wehrmann M, Batz C, Bogenschütz O, Schmitt H, Nagy J, Müller C, Müller G: The consequences of tubulo-interstitial changes for renal function in glomerulopathies. A morphometric and cytological analysis. *Pathol Res Pract* 1990, 186:135–144
- Mackensen-Haen S, Bader R, Grund KE, Bohle A: Correlations between renal cortical interstitial fibrosis, atrophy of the proximal tubules and impairment of the glomerular filtration rate. *Clin Nephrol* 1981, 15:167–171
- Yamamoto T, Noiri E, Ono Y, Doi K, Negishi K, Kamijo A, Kimura K, Fujita T, Kinukawa T, Taniguchi H, Nakamura K, Goto M, Shinozaki N, Ohshima S, Sugaya T: Renal L-type fatty acid-binding protein in acute ischemic injury. *J Am Soc Nephrol* 2007, 18:2894–2902
- Portilla D, Dent C, Sugaya T, Nagothu KK, Kundi I, Moore P, Noiri E, Devarajan P: Liver fatty acid-binding protein as a biomarker of acute kidney injury after cardiac surgery. *Kidney Int* 2008, 73:465–472
- Maatman RG, van de Westerlo EM, van Kuppevelt TH, Veerkamp JH: Molecular identification of the liver- and the heart-type fatty acid-binding proteins in human and rat kidney. Use of the reverse transcriptase polymerase chain reaction. *Biochem J* 1992, 288 (Pt 1):285–290
- Maatman RG, Van Kuppevelt TH, Veerkamp JH: Two types of fatty acid-binding protein in human kidney. Isolation, characterization and localization. *Biochem J* 1991, 273 (Pt 3):759–766
- Veerkamp JH, Peeters RA, Maatman RG: Structural and functional features of different types of cytoplasmic fatty acid-binding proteins. *Biochim Biophys Acta* 1991, 1081:1–24
- Sweetser DA, Heuckeroth RO, Gordon JL: The metabolic significance of mammalian fatty-acid-binding proteins: abundant proteins in search of a function. *Annu Rev Nutr* 1987, 7:337–359
- Negishi K, Noiri E, Maeda R, Portilla D, Sugaya T, Fujita T: Renal L-type fatty acid-binding protein mediates the bezafibrate reduction of cisplatin-induced acute kidney injury. *Kidney Int* 2008, 73:1374–1384
- Kamijo-Ikemori A, Sugaya T, Obama A, Hiroi J, Miura H, Watanabe M, Kumai T, Ohtani-Kaneko R, Hirata K, Kimura K: Liver-type fatty acid-binding protein attenuates renal injury induced by unilateral ureteral obstruction. *Am J Pathol* 2006, 169:1107–1117
- 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* 2004, 165:1243–1255
- Kamijo-Ikemori A, Sugaya T, Kimura K: Urinary fatty acid binding protein in renal disease. *Clin Chim Acta* 2006, 374:1–7
- Nakamura T, Sugaya T, Kawagoe Y, Ueda Y, Osada S, Koide H: Candesartan reduces urinary fatty acid-binding protein excretion in patients with autosomal dominant polycystic kidney disease. *Am J Med Sci* 2005, 330:161–165
- Nakamura T, Sugaya T, Kawagoe Y, Ueda Y, Osada S, Koide H: Effect of pitavastatin on urinary liver-type fatty acid-binding protein levels in patients with early diabetic nephropathy. *Diabetes Care* 2005, 28:2728–2732
- Nakamura T, Sugaya T, Kawagoe Y, Ueda Y, Koide H: Effect of pioglitazone on urinary liver-type fatty acid-binding protein concentrations in diabetes patients with microalbuminuria. *Diabetes Metab Res Rev* 2006, 22:385–389
- Yuan HT, Li XZ, Pitera JE, Long DA, Woolf AS: Peritubular capillary loss after mouse acute nephrotoxicity correlates with down-regulation

- of vascular endothelial growth factor-A and hypoxia-inducible factor-1 alpha. *Am J Pathol* 2003, 163:2289–2301
18. Fink M, Henry M, Tange JD: Experimental folic acid nephropathy. *Pathology* 1987, 19:143–149
19. Byrnes KA, Ghidoni JJ, Mayfield ED Jr: Response of the rat kidney to folic acid administration. I. Biochemical studies. *Lab Invest* 1972, 26:184–190
20. Byrnes KA, Ghidoni JJ, Suzuki M, Thomas H, Mayfield ED Jr: Response of the rat kidney to folic acid administration. II. Morphologic studies. *Lab Invest* 1972, 26:191–200
21. Klingler EL Jr, Evan AP, Anderson RE: Folic acid-induced renal injury and repair. Correlation of structural and functional abnormalities. *Arch Pathol Lab Med* 1980, 104:87–93
22. Parchure M, Ambaye RY, Lalitha VS, Gokhale SV: Acute toxicity of folic acid in mice. *Experientia* 1985, 41:72–73
23. Kamijo A, Sugaya T, Hikawa A, Yamanouchi M, Hirata Y, Ishimitsu T, Numabe A, Takagi M, Hayakawa H, Tabei F, Sugimoto T, Mise N, Omata M, Kimura K: Urinary liver-type fatty acid binding protein as a useful biomarker in chronic kidney disease. *Mol Cell Biochem* 2006, 284:175–182
24. 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* 2004, 143:23–30
25. Kamijo A, Kimura K, Sugaya T, Yamanouchi M, Hase H, Kaneko T, Hirata Y, Goto A, Fujita T, Omata M: Urinary free fatty acids bound to albumin aggravate tubulointerstitial damage. *Kidney Int* 2002, 62:1628–1637
26. Kamijo-Ikemori A, Sugaya T, Sekizuka A, Hirata K, Kimura K: Amelioration of diabetic tubulointerstitial damage in liver-type fatty acid binding protein transgenic mice. *Nephrol Dial Transplant* 2009, 24:788–800
27. Kato Y, Mori Y, Makino Y, Morimitsu Y, Hiroi S, Ishikawa T, Osawa T: Formation of Nepsilon-(hexanonyl)lysine in protein exposed to lipid hydroperoxide. A plausible marker for lipid hydroperoxide-derived protein modification. *J Biol Chem* 1999, 274:20406–20414
28. Doi K, Okamoto K, Negishi K, Suzuki Y, Nakao A, Fujita T, Toda A, Yokomizo T, Kita Y, Kihara Y, Ishii S, Shimizu T, Noiri E: Attenuation of folic acid-induced renal inflammatory injury in platelet-activating factor receptor-deficient mice. *Am J Pathol* 2006, 168:1413–1424
29. Jarmi T, Agarwal A: Heme oxygenase and renal disease. *Curr Hypertens Rep* 2009, 11:56–62
30. de Haan JB, Bladier C, Griffiths P, Kelner M, O'Shea RD, Cheung NS, Bronson RT, Silvestro MJ, Wild S, Zheng SS, Beart PM, Hertzog PJ, Kola I: Mice with a homozygous null mutation for the most abundant glutathione peroxidase. Gpx1, show increased susceptibility to the oxidative stress-inducing agents paraquat and hydrogen peroxide. *J Biol Chem* 1998, 273:22528–22536
31. Schmidt AM, Yan SD, Yan SF, Stern DM: The multiligand receptor RAGE as a progression factor amplifying immune and inflammatory responses. *J Clin Invest* 2001, 108:949–955
32. Ueno Y, Horio F, Uchida K, Naito M, Nomura H, Kato Y, Tsuda T, Toyokuni S, Osawa T: Increase in oxidative stress in kidneys of diabetic Akita mice. *Biosci Biotechnol Biochem* 2002, 66:869–872
33. Wang G, Gong Y, Anderson J, Sun D, Minuk G, Roberts MS, Burczynski FJ: Antioxidative function of L-FABP in L-FABP stably transfected Chang liver cells. *Hepatology* 2005, 42:871–879
34. Ek-Von Mentzer BA, Zhang F, Hamilton JA: Binding of 13-HODE and 15-HETE to phospholipid bilayers, albumin, and intracellular fatty acid binding proteins. implications for transmembrane and intracellular transport and for protection from lipid peroxidation. *J Biol Chem* 2001, 276:15575–15580
35. Raza H, Pongubala JR, Sorof S: Specific high affinity binding of lipoxygenase metabolites of arachidonic acid by liver fatty acid binding protein. *Biochem Biophys Res Commun* 1989, 161:448–455
36. Nakamura T, Sugaya T, Node K, Ueda Y, Koide H: Urinary excretion of liver-type fatty acid-binding protein in contrast medium-induced nephropathy. *Am J Kidney Dis* 2006, 47:439–444
37. Ries M, Basseau F, Tyndal B, Jones R, Deminiere C, Catargi B, Combe C, Moonen CW, Grenier N: Renal diffusion and BOLD MRI in experimental diabetic nephropathy. Blood oxygen level-dependent. *J Magn Reson Imaging* 2003, 17:104–113