Structural and functional changes in the kidneys of high-fat diet-induced obese mice

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Deji N, Kume S, Araki S, Soumura M, Sugimoto T, Ishihara K, Chin-Kanasaki M, Sakaguchi M, Koya D, Haneda M, Kashiwagi A, Uzu T. Structural and functional changes in the kidneys of high-fat diet-induced obese mice. Am J Physiol Renal Physiol 296: F118–F126, 2009. First published October 29, 2008; doi:10.1152/ajprenal.00110.2008.—Metabolic syndrome has been reported to be associated with chronic kidney disease, but the mechanisms remain unclear. Although feeding of a high-fat diet (HFD) to C57BL/6 mice is reported to induce systemic metabolic abnormalities and subsequent renal injuries, such as albuminuria, similar to human metabolic syndrome, alterations in HFD-induced renal injuries have not been fully elucidated in detail. We therefore investigated the structural and functional changes in the kidneys of C57BL/6 mice on a HFD. Six-week-old mice were fed a low-fat diet (LFD; 10% of total calories from fat) or a HFD (60% fat) for 12 wk. Mice fed a HFD showed significant increases in body weight, systolic blood pressure, plasma insulin, glucose, and triglycerides compared with those on an LFD. Accompanying these systemic changes, mice on a HFD showed albuminuria, an increase in glomerular tuft area, and mesangial expansion. These systemic and renal alterations in mice on a HFD were prevented by body weight control with the dietary restriction of feeding a HFD. Furthermore, mice on a HFD showed renal pathophysiological alterations including renal lipid accumulation, an increased accumulation of type IV collagen in glomeruli, an increase in macrophage infiltration in the renal medulla, an increase in urinary 8-hydroxy-2′-deoxyguanosine excretion, and impaired sodium handling. In conclusion, this study suggests that local metabolic alterations in the kidney play important roles in the development of renal injury associated with metabolic syndrome in addition to systemic metabolic changes and an increase in body weight.

metabolic syndrome; dietary restriction; lipotoxicity; inflammation; salt-sensitive hypertension

MATERIALS AND METHODS

Animals. Male C57BL/6 mice were obtained from CLEA Japan (Tokyo, Japan). Six-week-old mice were housed in box cages, maintained on a 12:12-h light-dark cycle, and fed for 12 wk either a low-fat diet (LFD; 10% of total calories from fat, which consists of 5.5% of total calories from soybean oil and 4.5% kcal from lard) or HFD (60% of total calories from fat, which consists of 5.5% from soybean oil and 54.5% from lard) purchased from Research Diets (New Brunswick, NJ). Furthermore, a HFD restriction (HFDR) study was performed to prevent the CKD associated with metabolic syndrome.
explore whether systemic and renal alterations in the mice on a HFD were induced by feeding a HFD per se or an increase in body weight. Six-week-old mice were maintained under the dietary restriction on being fed a HFD for 12 wk to control an increase in their body weight at the same levels as those in the mice on a LFD. To adjust their body weight, the degree of the restriction of a HFD in each mouse on HFDR was determined by the measurement of body weight every 2 wk and comparison to the average reference value of body weight in the mice on a LFD. As a result, they were fed on a 60–80% restricted HFD of the average consumption in the mice on a HFD. Since each mouse was housed individually, one was assured that each mouse received the appropriate amount of HFD. At 4-wk intervals, body weight, blood pressure, and blood glucose were measured. For the measurement of blood pressure, conscious mice were placed on a heated pad (37°C) in temperature-controlled quiet room. After 5 min of rest, systolic blood pressure was measured by a programmable tail-cuff sphygmomanometer (BP98-A; Softron, Tokyo, Japan). The average of 10 consecutive measurements of each mouse was analyzed. Mice in each dietary group were placed in metabolic balance cages for 24-h urine collection to measure urinary albumin excretion (UAE). At week 12 of the experimental period, the mice were killed. In addition, to investigate serial renal alterations, some mice on a LFD and a HFD were killed at week 4 (n = 7 in LFD, n = 7 in HFD) and week 8 (n = 6 in LFD, n = 7 in HFD). Mice were anesthetized by intraperitoneal injection of pentobarbital sodium, and then the left kidneys were removed, their weight without the capsule was measured, and they were stored at −80°C for quantitative real-time PCR. After blood was drawn for biochemical assay, the right kidneys were fixed with 3.7% formaldehyde and 0.05% picric acid in a 6:4 mixture of cacodylate buffer (pH 7.4, adjusted to 300 mmol/kgH2O with sucrose). After 5 min of fixation, mice were perfused with PBS for 1 min, and the right kidneys were removed. One-half of each kidney was embedded in paraffin for periodic acid-Schiff (PAS) staining and immunohistochemistry, and the other half was frozen for immunofluorescent and oil-red O staining. The Research Center for Animal Life Science of Shiga University of Medical Science approved all experiments.

**Blood and urine analysis.** Triglycerides (TG), total cholesterol, and nonesterified fatty acid (NEFA) were measured using an L type TG H kit, Choleseterol E-test kit, and NEFA C-test kit (Wako Chemicals, Osaka, Japan). Plasma insulin was determined using an ELISA kit (Morinaga Institute of Biological Science, Tokyo, Japan). Plasma adiponectin was determined with a mouse-specific ELISA kit (Otsuka Pharmaceutical, Tokyo, Japan). Hemoglobin Alc (HbA1c) was measured using the DCA 2000 analyzer (Siemens Medical Solutions Diagnostics, Tokyo, Japan). Urinary albumin concentration was measured with an ELISA kit (Exocell, Philadelphia, PA), and UAE was expressed as total amount excreted in 24-h urine collection. To determine the oxidative DNA damage, the 24-h urinary level of 8-hydroxy-2′-deoxyguanosine (8-OH-dG) was determined using a competitive ELISA kit (8-OH-dG Check, Institute for the Control of Aging, Shizuoka, Japan).

**Abdominal computed tomography.** Mice in each dietary group were anesthetized by intraperitoneal injection of pentobarbital sodium, and then abdominal computed tomography (LCT100/A; AcroBio, Tokyo, Japan) was performed.

**Morphological analysis and immunohistochemistry.** Fixed kidneys were embedded in paraffin and were sectioned (3-μm thickness). To evaluate the renal structural changes, we performed PAS staining on these sections as described previously (26). From each mouse, 10 glomeruli cut at their vascular poles were supplied for morphometrical analysis. The extent of the mesangial matrix (defined as mesangial area) was determined by assessing the PAS-positive and nuclei-free area in the mesangium using a computer-assisted color image analyzer (Image-pro plus v. 6.1, Nippon Roper, Tokyo, Japan). To evaluate macrophage infiltration in the kidney, immunohistochemistry was performed with rat anti-F4/80 antibody (Serotec, Oxford, UK), which is a macrophage marker. The sections were deparaffinized and rehydrated. Endogenous peroxidase was quenched with 3% H2O2 for 15 min. Sections were incubated with the blocking reagent (Nichirei, Tokyo, Japan) to prevent nonspecific binding and were then incubated with rat anti-mouse F4/80 antibody (diluted 1:500) overnight at 4°C. After three rinses with PBS for 15 min, the sections were incubated with horseradish peroxidase (HRP)-conjugated anti-rat secondary antibody (diluted 1:100) for 1 h at room temperature. The sections were rinsed again with PBS and then examined using diaminobenzidine (DAB) substrate. For quantitative analysis, F4/80-labeled cells in the renal medulla were counted in 20 randomly selected high-power microscopic fields (HPF×400) per each animal and analyzed individually (28, 53).

**Electron microscopic study.** Mice on a LFD and a HFD at week 12 of the experimental period were anesthetized by intraperitoneal injection of pentobarbital sodium. Kidneys were perfused with 0.1 mol/l phosphate buffer, and then they were removed, cut into small tissue blocks (1 mm3), and fixed in 2.0% glutaraldehyde and 2.0% paraformaldehyde with 0.1 mol/l phosphate buffer at 4°C. After postfixation with 2% osmium tetroxide, tissues were dehydrated in a series of graded ethyl alcohol, substituting ethanol for propylene oxide, and critical-point dried. Ultrathin sections were double-stained with uranyl acetate and lead. Sections were examined with a JEM1200EX electron microscopy (JOEL, Tokyo, Japan) at 80 kV.

**Immunofluorescence imaging and oil-red O staining.** To evaluate extracellular matrix (ECM) accumulation, immunofluorescence staining was performed with rabbit anti-mouse type IV collagen antibody (Chemicon, Temecula, CA). Frozen sections were fixed using 10% formalin in PBS for 10 min and preincubated with 2% BSA in PBS for 10 min at room temperature. Then, they were covered overnight at 4°C with type IV collagen antibody (diluted 1:200) in the preincubation solution. The sections were rinsed three times with PBS, incubated with 2% goat serum in 2% BSA in PBS at room temperature, and incubated for 1 h at 4°C with the secondary antibody, rhodamine-conjugated goat anti-rabbit IgG antibody (diluted 1:100, MP Biomedicals, Solon, OH). After a rinsing with PBS, the sections were mounted using mounting medium. The kidney sections were then imaged with a fluorescence microscope (BX61, Olympus, Tokyo, Japan). Frozen sections were also used for oil-red O staining by which the renal accumulation of neutral lipids was evaluated, as previously reported (42).

**RNA extraction and quantitative real-time PCR.** Total RNA was isolated from the whole kidney based on the TRIzol protocol (Invitrogen Life Technologies, Carlsbad, CA). cDNA was synthesized using reverse transcript reagents (Takara Bio, Otsu, Japan). iQSYBR Green Supermix (Bio-Rad Laboratories, Hercules, CA) was used for real-time PCR (ABI Prime TM 7500 Sequence Detection System, PerkinElmer Applied Biosystems, Foster City, CA). The levels of mRNA expression were quantified using the standard curve method. Standard curves were constructed using serially diluted standard template. Ct values were used to compute the levels of mRNA expression from the standard curve. Analytical data were adjusted with the levels of mRNA expression of β-actin as an internal control. The sequence of primers were the following: β-actin (forward) tggatgccaggattc, (reverse) ctgctgctgatcaataagaa; renin (forward) at-gaaggggtgcttggggc, (reverse) atggcggaggggggcactct; angiotensin-converting enzyme (ACE) (forward) tggagaaagcgaggaaggtc, (reverse) agagttttgaaagttgctcacatca and angiotensinogen (forward) tcaagacggagagagagaa, (reverse) cttagatgagacaagggagagagaa.

**Acute salt loading study.** To examine the urinary sodium excretion in response to acute salt loading in mice on either a HFD or LFD at week 12 of the experimental period, salt loading was performed as previously reported (13). After fasting for 12 h, mice were given 1.5 ml of 0.9% saline intraperitoneally and placed into metabolic cages. Subsequently, urine was collected hourly for the next 6 h, and urinary volume and urinary sodium excretion were measured.

**Long-term salt loading study.** To test whether long-term high salt intake affects the blood pressure in the mice on a HFD, we performed...
a 4-wk salt loading using the mice on either a HFD or a LFD. After the mice were maintained on either diet (equally containing 0.1% NaCl in each diet) with tap water for 8 wk as described above, they were maintained on the high salt loading with each diet by replacement of tap water with 1% saline for 4 wk, from week 9 to week 12 of the experimental period. Blood pressure was measured before and after the salt loading, and the change in systolic blood pressure was analyzed. A salt sensitivity index was defined as a reciprocal of the slope of the pressure-natriuresis curve. A pressure-natriuresis curve was constructed by plotting the 24-h urinary sodium excretion normalized to 24-h urinary creatinine excretion and systolic blood pressure (24, 46).

Statistical analysis. Results are expressed as means ± SE. One-way ANOVA followed by Scheffe’s test was used to determine the significance of differences among three independent groups. Comparisons between two groups were performed by using a Mann-Whitney U-test for two independent groups and a Wilcoxon signed-rank test for repeated measurements. A P value of < 0.05 was considered statistically significant.

RESULTS

Systemic alterations in mice in each dietary group. We first investigated the development of metabolic syndrome in C57BL/6 mice on a HFD and the effects of the dietary restriction of feeding a HFD on systemic metabolic alterations. The body weight and the levels of blood glucose were significantly heavier and higher in the mice on the HFD compared with those in mice on the LFD throughout the entire experimental period (Fig. 1, A and B). Similarly, the systolic blood pressure was significantly higher in this group at weeks 8 and 12 of the experimental period (Fig. 1C). On the other hand, mice on HFDR, in which the average of food intake was 10.5 kcal/day and a 72% consumption of the mice on a HFD, and were maintained on the high salt loading with each diet by replacement of tap water with 1% saline for 4 wk, from week 9 to week 12 of the experimental period (Fig. 1C). The kidney weight was heavier in the mice on HFDR (Fig. 3). In the renal morphological analysis, the glomerular tuft area in the mice on a HFD tended to be large rather than that in the mice on a LFD at week 4 and was significantly larger at weeks 8 and 12. Also, the mesangial matrix area was significantly larger in the mice on the HFD at week 4, and this phenomenon continued until week 12. Similarly, these renal morphological changes in the mice on a HFD at week 12 were not observed in the mice on HFDR (Fig. 4). These results suggested that the increase in body weight, rather than the feeding of a HFD per se, contributed to the development of systemic and renal abnormalities in the mice on a HFD. Thus, in the further study, we focused on the detailed investigation of renal pathogenetic changes in the mice on a HFD which developed these systemic alterations and renal injury.

Renal injury in each dietary group. We next investigated the development of renal injury in C57BL/6 mice on a HFD and the effects of the dietary restriction of feeding a HFD on renal alterations. UAE in the mice on a HFD were similar to those of the mice on a LFD at weeks 4 and 8. Compared with mice on a LFD, mice on a HFD showed significantly increased UAE at week 12 (Fig. 3A). The kidney weight was heavier in the mice on the HFD at week 4, and this phenomenon continued until week 12 (Fig. 3B). These renal abnormalities in the mice on a HFD at week 12 were not observed in the mice on HFDR (Fig. 3). In the renal morphological analysis, the glomerular basement membrane, and foot process effacement were not observed in the mice on HFDR (Fig. 4). These results suggested that the increase in body weight, rather than the feeding of a HFD per se, contributed to the development of systemic and renal abnormalities in the mice on a HFD. Thus, in the further study, we focused on the detailed investigation of renal pathogenetic changes in the mice on a HFD which developed these systemic alterations and renal injury.

Renal pathogenetic changes in mice on a HFD. In the mice on the HFD at week 12, electron microscopic examination showed the increased extracellular matrix, irregularly thickening glomerular basement membrane, and foot process effacement in some part of the glomeruli (Fig. 5). Immunofluorescence study revealed that the accumulation of collagen type IV, one of the major extracellular matrix proteins, was increased in the glomeruli of the mice on a HFD (Fig. 6, A and B). In the kidney sections from mice in each group examined by oil-red O staining, the accumulation of neutral lipid was detected in

![Fig. 1](http://ajprenal.physiology.org/)

Fig. 1. Changes in physiological parameters over the experimental period in mice on each diet. Plots are shown of body weight (A), blood glucose level (B), and systolic blood pressure (C) over time in mice on a low fat-diet (LFD), a high fat-diet (HFD), or high-fat diet restriction (HFDR). Values are means ± SE; n = 14–19, 14–19, and 10 in mice on a LFD, a HFD, or HFDR, respectively. Statistical analyses were performed by 1-way ANOVA followed by Scheffe’s test. *P < 0.05 vs. mice on a LFD. †P < 0.05 vs. mice on HFDR.
the glomeruli and proximal tubules of the mice on a HFD, but not in the mice on a LFD (Fig. 6, C and D). Also, the F4/80 (a macrophage marker)-positive cells were mainly observed in the renal medulla (Fig. 6, E and F). The number of the F4/80-positive cells in the renal medulla significantly increased in mice on a HFD than those on a LFD (4.64 ± 0.52/HPF vs. 2.74 ± 0.45/HPF, \( P < 0.05 \)) by a Mann-Whitney U-test, suggesting the presence of an inflammatory process in the kidney of the mice on a HFD. Furthermore, the levels of mRNA expression of renin, ACE, and angiotensinogen at week 12 of the experimental period significantly increased in the kidneys of the mice on a HFD compared with those in the mice on a LFD (Fig. 7).

**Impaired sodium handling in mice on a HFD.** To explore one of the mechanisms of a HFD-induced elevation of systolic blood pressure, we finally examined the salt sensitivity in the mice on a HFD. The urinary sodium excretion in response to acute salt loading was examined first. During the first 4 h, urinary sodium excretion (Fig. 8A) and urinary volume (Fig. 8B) were significantly lower in the mice on the HFD than those in the mice on the LFD. At the end of 6 h, total urinary sodium excretion and urinary volume were slightly but not significantly lower, and smaller in this group. Next, the effect of a 4-wk salt loading on systolic blood pressure was examined. In the mice on the HFD, the increase in systolic blood pressure by a 4-wk salt loading was larger than that in the mice on the HFD without sodium loading at week 12 of the experimental period (13.6 ± 2.1 vs. 4.8 ± 2.5 mmHg, \( P < 0.05 \)) by a Wilcoxon signed-rank test, whereas the change in systolic blood pressure by a 4-wk salt loading was not observed in the mice on the LFD. The salt sensitivity index in the mice on a HFD significantly increased compared with those in the mice on a LFD (34.03 ± 6.3 in HFD vs. −1.17 ± 3.4 in LFD, \( P < 0.001 \), Fig. 9).

**DISCUSSION**

In this study, we confirmed that feeding a HFD to C57BL/6 mice induces major systemic alterations compatible to human metabolic syndrome, including obesity, hyperglycemia, hyperinsulinemia, hypertriglyceridemia, and hypertension. After the onset of metabolic syndrome, mice on a HFD developed increased UAE and glomerular lesions with the accumulation of extracellular matrix protein. Furthermore, we demonstrated renal pathophysiological alterations, including lipid accumulation, macrophage infiltration, increased oxidative stress, and impaired sodium handling in the mice on a HFD. These systemic alterations and renal injuries were prevented by the control of body weight with the dietary restriction of feeding a HFD, suggesting that the increase in body weight, rather than feeding of a HFD per se, contributes to the development of these abnormalities.

In this study, we observed several metabolism-associated pathophysiological alterations in the kidneys of mice on the HFD. Systemic lipid overload “lipotoxicity,” which can induce systemic inflammation and oxidative stress, is proposed as an important mechanism underlying metabolic syndrome (48, 51). In addition to these systemic metabolic alterations, the local alteration of lipid metabolism in the kidney is also considered to play an important role in the pathogenesis of the renal injury in metabolic syndrome (18, 27). We have previously reported that renal lipid accumulation and renal injuries, including glomerulosclerosis, interstitial fibrosis, and albuminuria, which may be caused by altered imbalance between renal lipogenesis and lipolysis, were induced by feeding a HFD to mice (27). In this study, in addition to renal lipid accumulation, we found macrophage infiltration and increased oxidative stress in the kidneys of mice on the HFD. Similarly, an increase in oxidative stress and the macrophage infiltration in the kidney have reportedly been observed in other obesity models [db/db mice (34, 44), KKAy mice (11, 15) and Otsuka Long-Evans Tokushima Fatty (OLETF) rats (23)]. In metabolic syndrome, the altered renal lipid metabolism may enhance lipotoxicity, oxi-

**Table 1. Characteristics at week 12 of experimental period**

<table>
<thead>
<tr>
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<th>Low-Fat Diet</th>
<th>High-Fat Diet</th>
<th>High-Fat Diet Restriction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma triglyceride, mg/ml</td>
<td>83.8±6.4</td>
<td>146.0±15.8*†</td>
<td>54.8±3.2</td>
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<tr>
<td>Plasma cholesterol, mg/ml</td>
<td>108.5±12.7</td>
<td>143.7±19.6</td>
<td>111.3±10.1</td>
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<tr>
<td>Nonesterified fatty acid, mg/ml</td>
<td>1.0±0.1</td>
<td>0.6±0.1*</td>
<td>0.7±0.1*</td>
</tr>
<tr>
<td>Hemoglobin A1c, %</td>
<td>3.2±0.1</td>
<td>3.9±0.1†</td>
<td>3.4±0.1</td>
</tr>
<tr>
<td>Plasma insulin, ng/ml</td>
<td>0.20±0.04</td>
<td>1.62±0.4†</td>
<td>0.04±0.01</td>
</tr>
<tr>
<td>Adiponectin, ng/ml</td>
<td>20.1±0.3</td>
<td>15.3±1.5†</td>
<td>20.1±0.1</td>
</tr>
<tr>
<td>Urinary 8-OH-dG, ng/day</td>
<td>7.4±1.6</td>
<td>38.6±8.3†</td>
<td>9.9±1.7</td>
</tr>
<tr>
<td>Food intake, g/day</td>
<td>3.2±0.3</td>
<td>2.8±0.4</td>
<td>2.0±0.2*</td>
</tr>
<tr>
<td>Food intake, kcal/day</td>
<td>12.3±1.1</td>
<td>14.6±1.9</td>
<td>10.5±0.9</td>
</tr>
</tbody>
</table>

Values are means ± SE; \( n = 10–13 \) mice on a low-fat diet (LFD), \( n = 11–13 \) mice on a high-fat diet (HFD), and \( n = 7–10 \) mice on high-fat diet restriction (HFDR). Food intake is the average throughout the experimental period. 8-OH-dG, 8-hydroxy-2′-deoxyguanosine. Statistical analyses were performed by 1-way ANOVA followed by Scheffe’s test. \(* P < 0.05 \) vs. mice on a LFD. †\( P < 0.05 \) vs. mice on HFDR.
dative stress, and inflammation in the kidney and subsequently cause renal abnormalities. We also performed the HFDR study to explore whether renal injuries observed in the mice on a HFD were induced by feeding a HFD per se or an increase in body weight. The mice on HFDR, of which body weight was controlled at the same levels as those in the mice on a LFD, did not develop systemic and renal alterations. In addition, these mice did not show the elevation of systolic blood pressure as well as those on a LFD. These results suggest that the control of body weight in the patients with metabolic syndrome is an important therapeutic target to prevent the development of CKD in addition to renal lipid metabolism.

We have previously reported that the patients with metabolic syndrome develop salt-sensitive hypertension (45), which is

Fig. 3. Renal changes over the experimental period in mice on each diet. A: 24-h urinary albumin excretion. B: kidney weight at week 12 in mice on a LFD, HFD, or HFDR. Values are means ± SE; n = 11, 11, 9 in the mice on a LFD, a HFD or HFDR for urinary albumin excretion and n = 10, 10, and 9 in the mice on a LFD, a HFD or HFDR for kidney weight, respectively. Statistical analyses were performed by 1-way ANOVA followed by Schefffé’s test. *P < 0.05 vs. mice on a LFD. †P < 0.05 vs. mice on HFDR.

Fig. 4. Histological features in the kidneys of mice on each diet. Representative light microscopic features of periodic acid-Schiff (PAS)-stained kidney sections from mice on a LFD (A), HFD (B), or HFDR (C) at week 12 of the experimental period. Original magnification ×400. Also shown is a quantitative analysis for glomerular tuft area (D) and mesangial matrix area (E) in the mice on a LFD, HFD or HFDR. Values are means ± SE; n = 10, 10, and 7 in the mice on a LFD, HFD, or HFDR, respectively. Statistical analyses were performed by 1-way ANOVA followed by Schefffé’s test. *P < 0.05 vs. mice on a LFD. †P < 0.05 vs. mice on a HFDR.
also one of the important mechanisms of renal dysfunction under metabolic syndrome (12, 45). In this study, the mice on the HFD exhibited a delay of urinary sodium excretion by acute salt loading and a salt-sensitive elevation of systolic blood pressure by a 4-wk salt loading. Also, the mRNA expression levels of renin, ACE, and angiotensinogen in the kidney of the mice on the HFD were increased compared with those in the mice on the LFD, suggesting renal RAS activation in the mice on the HFD. The activation of renal RAS is reported to be associated with impaired sodium handling in the kidney (5, 35, 38, 41, 47). Thus the abnormal salt sensitivity, which may be associated with the activation of renal RAS, is one of the mechanisms of renal injuries and hypertension in the mice on the HFD.

Fig. 5. Ultrastructural features in the kidney of mice on a HFD. Representative electron micrographs are shown from mice on a LFD (A and C) or a HFD (B and D) at week 12. Mesangial matrix expansion is indicated by an asterisk (B), irregular thickening of glomerular basement membrane by a black arrow (D), and foot process effacements by a white arrow (D). Original magnification ×5,000 (A and B) and ×20,000 (C and D). Mes, mesangial cell; CP, capillary lumen.

Fig. 6. Pathological alterations in the kidneys of mice on a HFD. Immunofluorescence study for collagen type IV on kidney sections from mice on a LFD (A) or HFD (B) at week 12 is shown. Original magnification ×400. C and D: oil-red O staining on kidney sections on a LFD (C) or a HFD (D) at week 12. Original magnification ×400. E and F: immunohistochemistry for F4/80 on kidney sections on a LFD (E) or a HFD (F) at week 12. Original magnification ×100.
Human metabolic syndrome is reported to be associated with CKD, defined as albuminuria and reduced glomerular filtration rate (7, 32). It is also known that the development of glomerulomegaly and focal segmental glomerulosclerosis has been linked to massive obesity (17, 20, 21). Most of the relevant histological examinations, however, have been limited to small autopsy series or operative cases because it is technically difficult to perform renal biopsies in patients with obesity. Therefore, it remains unclear whether there are specific pathological features of metabolic syndrome-associated kidney disease. In this study, we observed that C57BL/6 mice on a HFD developed renal functional and pathological abnormalities similar to those observed in the patients with obesity or metabolic syndrome. The evidence suggests that this model may be useful to elucidate the pathological features of CKD associated with metabolic syndrome.

Renal injuries associated with metabolic disorder have often been investigated by using genetically altered animals (4, 6, 39, 40), such as db/db mice (39), KKAy mice, obese Zucker rats, and OLETF rats. These animals are indeed useful for exploring how one or several specific genes contribute to the development of metabolic syndrome. However, there are several limitations in using these model animals for research on metabolic syndrome. These animals sometimes do not replicate the changes observed in humans (26, 37, 39), suggesting the artificial effects of gene modification. Furthermore, because of the congenital metabolic alteration due to a lack of a specific gene, they may show abnormal metabolic effects and renal injuries at an early age, even at birth (39). This is different from the long-term developed process of metabolic syndrome in humans. This also makes it difficult to explore the mechanisms of the initiation and the development of metabolic syndrome-associated kidney disease. In contrast, feeding a HFD to mice is a very simple method of examining lifestyle alteration and intervention. Feeding a HFD can induce complex metabolic dysfunctions in

![Graph](image-url)

**Fig. 7.** mRNA expression of renin, angiotensin converting-enzyme (ACE), and angiotensinogen in the kidney. Values are means ± SE; n = 7–11 in the mice on a LFD and n = 8–12 on a HFD. Statistical analyses were performed by a Mann-Whitney U-test. *P < 0.05 vs. mice on a LFD.

![Graph](image-url)

**Fig. 8.** Urinary sodium excretion after acute salt loading. Urinary sodium excretion (A) and urinary volume (B) in mice on a low fat-diet (LFD) or a high fat-diet (HFD) over 6 h following intraperitoneal injection of 0.9% saline are shown. Data are expressed as cumulative urinary sodium excretion. Values are means ± SE; n = 8 in the mice on a LFD and n = 9 on a HFD. Statistical analyses were performed by a Mann-Whitney U-test. *P < 0.05 vs. mice on a LFD.
normally developed mice at predictable times. They develop systemic and renal alterations without drug toxicity, genetic modification, or surgical invasion.

A limitation of this study is acknowledged. In this study, we investigated only HFD-induced renal abnormalities in male C57BL/6 mice. It has been reported that the degree of systemic metabolic alterations on feeding a HFD varies depending on the genetic background (36, 52). Differing impacts of genetic background on the development of metabolic syndrome and diabetes is also recognized in human populations with comparatively higher rates of diabetes and obesity, i.e., Hispanic, black, and Native-American groups (3, 25, 49). Several studies have suggested that C57BL/6 mice are “obese prone” as well as “susceptible to insulin resistance and glucose intolerance,” whereas other strains, such as C3H/He, 129Sv, and A/J mice, are resistant to obesity and diabetes. However, there are few reports concerning whether these genetic background factors contribute to the renal lipid metabolism and renal abnormalities as well as systemic metabolic changes. Further studies are required to explore any potential influence of the gene-environment interaction on HFD-induced renal abnormalities. In addition, we did not investigate the effect of different type of fat on renal injuries in the mice on a HFD. Unsaturated fatty acids have been reported to have beneficial effects on various renal diseases compared with saturated fatty acids (2, 8). Therefore, the difference in the fat source may influence the development of renal injuries in this model.

In summary, we characterized the structural and functional changes in the kidneys of C57BL/6 mice on a HFD as a mouse model of metabolic syndrome. We propose that further research using this mouse model would be helpful in elucidating the renal pathogenesis associated with metabolic syndrome and developing novel therapeutic strategies for the CKD associated with metabolic syndrome.

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