Role of Altered Renal Lipid Metabolism in the Development of Renal Injury Induced by a High-Fat Diet

Shinji Kume,* Takashi Uzu,* Shin-ichi Araki,* Toshiro Sugimoto,* Keiji Isshiki,* Masami Chin-Kanasaki,* Masayoshi Sakaguchi,* Naoto Kubota,† Yasuo Terauchi,‡ Takashi Kadowaki,† Masakazu Haneda,§ Atsunori Kashiwagi,* and Daisuke Koya*

*Department of Medicine, Shiga University of Medical Science, Otsu, Shiga, †Department of Metabolic Diseases, Graduate School of Medicine, University of Tokyo, Tokyo, ‡Department of Endocrinology and Metabolism, Yokohama City University Graduate School of Medicine, Yokohama, §Second Department of Medicine, Asahikawa Medical College, Asahikawa, Hokkaido, and Division of Endocrinology & Metabolism, Kanazawa Medical University, Kahoku-Gun, Ishikawa, Japan

ABSTRACT
Metabolic syndrome is associated with increased risk of chronic kidney disease, and the renal injury in patients with metabolic syndrome may be a result of altered renal lipid metabolism. We fed wild-type or insulin-sensitive heterozygous peroxisome proliferator–activated receptor γ-deficient (PPARγ+/−) mice a high-fat diet for 16 weeks. In wild-type mice, this diet induced core features of metabolic syndrome, subsequent renal lipid accumulation, and renal injury including glomerulosclerosis, interstitial fibrosis, and albuminuria. Renal lipogenesis accelerated, determined by increased renal mRNA expression of the lipogenic enzymes fatty acid synthase and acetyl-CoA carboxylase (ACC) and by increased ACC activity. In addition, renal lipolysis was suppressed, determined by reduced mRNA expression of the lipolytic enzyme carnitine palmitoyl acyl-CoA transferase 1 and by reduced activity of AMP-activated protein kinase. In PPARγ+/− mice, renal injury, systemic metabolic abnormalities, renal accumulation of lipids, and the changes in renal lipid metabolism were attenuated. Thus, a high-fat diet leads to an altered balance between renal lipogenesis and lipolysis, subsequent renal accumulation of lipid, and renal injury. We suggest that renal lipid metabolism could serve as a new therapeutic target to prevent chronic kidney disease in patients with metabolic syndrome.

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Metabolic syndrome, which is characterized by concurrent existence of obesity, dyslipidemia, hyperinsulinemia, hyperglycemia, and hypertension, is increasingly common because of increased prevalence of obesity. This syndrome is a growing health problem because of the associated increased risk for cardiovascular disease and premature death.1,2 Furthermore, a recent report suggested that individuals with metabolic syndrome are also at increased risk for developing chronic kidney diseases (CKD).3 Several pathomechanisms underlying the development of renal injury in metabolic syndrome have been proposed.4–8 Among them, renal lipid accumulation, lipotoxicity, has been reported to play an important role in the pathogenesis of renal injury in metabolic syndrome, although the precise mechanism of renal lipid accumulation has not been fully established.

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Correspondence: Dr. Daisuke Koya, Division of Endocrinology & Metabolism, Kanazawa Medical University, Kahoku-Gun, Ishikawa 920-0293, Japan. Phone: +81-76-286-2211; Fax: +81-76-286-6927; E-mail: koya0516@kanazawa-med.ac.jp

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elucidated. Excess energy intake, including high-fat diet (HFD), contributes to the development of metabolic syndrome. HFD also causes renal lipid accumulation and renal injury. Therefore, elucidation of precise mechanisms that are responsible for renal lipid accumulation under an HFD could suggest the possible mechanisms underlying the development of renal injury in metabolic syndrome and thus enhance the design of novel therapeutic strategies against this renal injury.

Various intracellular molecules regulate local lipid metabolism in several tissues, such as skeletal muscle and liver. An altered systemic glucose and lipid metabolism, the imbalance between lipogenesis and lipolysis in such tissues contributes to the local lipid accumulation and subsequent pathophysiologic changes. However, in the kidney, the role of local lipid metabolism in lipid accumulation and subsequent renal injury in metabolic syndrome has not been fully determined.

The purpose of this study was to clarify further the role of renal lipid metabolism in the development of renal injury in metabolic syndrome. We first examined how HFD could affect renal lipid metabolism and renal injury by using heterozygous peroxisome proliferator–activated receptor-γ-deficient (PPAR-γ−/−) mice, which were previously reported to be protected against HFD-induced obesity and insulin resistance.

RESULTS

Systemic Metabolic Abnormalities

The characteristics of the four groups at 16 wk of experimental period are presented in Table 1. PPAR-γ+/+ mice on an HFD were significantly heavier than PPAR-γ+/+ mice on a low-fat diet (LFD). These obese mice showed significantly high plasma triglycerides, cholesterol, TNF-α and monocyte chemoattractant protein-1 (MCP-1) levels, compared with their counterparts on an LFD. Plasma adiponectin levels in PPAR-γ−/− mice were significantly lower than in PPAR-γ+/+ mice on an LFD. Moreover, PPAR-γ+/+ mice on an HFD showed hyperinsulinemia during 4 wk of HFD (Figure 1A) and hyperglycemia during 8 wk of HFD (Figure 1B). In contrast, PPAR-γ−/− mice were significantly protected against obesity, insulin resistance, and the altered adipokine secretions during the 16-wk HFD, although no differences in food intake were observed between PPAR-γ+/+ and PPAR-γ−/− mice (Table 1, Figure 1, A and B). Glucose intolerance (determined by intraperitoneal glucose tolerance test) and insulin resistance (determined by intraperitoneal insulin tolerance test) at 16 wk of HFD in PPAR-γ−/− mice were attenuated in PPAR-γ+/+ mice (Figure 1, C and D). PPAR-γ+/+ mice showed features of metabolic syndrome from the early stage of HFD, whereas these alterations under an HFD were attenuated in insulin-sensitive PPAR-γ+/+ mice, as previously reported.

Renal Injuries

We confirmed the significant downregulation of mRNA expression of PPAR-γ in the kidneys of PPAR-γ+/+ mice on both diets, compared with PPAR-γ+/+ mice (Table 2). Under an HFD, PPAR-γ+/+ mice exhibited a significant rise in urinary albumin excretion at 16 wk, although no significant differences were observed among the four groups at 4 and 8 wk (Figure 2). The increase in urinary albumin excretion at 16 wk was significantly inhibited in PPAR-γ−/− mice on an HFD (Figure 2). Examination of renal histopathologic changes with periodic acid-Schiff (PAS) in four groups revealed that HFD induced mesangial expansion in PPAR-γ−/− mice (Figure 3, B and M).

The expression of fibronectin was significantly increased in both the glomeruli and interstitium of PPAR-γ+/+ mice on an HFD (Figure 3, F and N, and J and O). In contrast, these HFD-induced glomerular and interstitial lesions were not observed (Figure 3, A, C, E, G, I, and K). Furthermore, under an HFD, the mRNA expression levels of fibronectin, type IV collagen, plasminogen activator-1, and MCP-1 were significantly increased in the renal cortex of PPAR-γ+/+ mice, and these changes were significantly attenuated in PPAR-γ−/− mice (Table 2).

Table 1. Characteristics of the four groups of mice at the end of the 16-wk experimental perioda

| Characteristic | PPAR-γ+/+ Mice | PPAR-γ−/− Mice |
|---------------|----------------|----------------|
| LFD           | HFD            | LFD            | HFD            |
| Body weight (g) | 32.6 ± 2.9    | 45.2 ± 3.1b,c  | 31.2 ± 3.4     | 40.5 ± 2.8b   |
| Food intake (g/d) | 2.71 ± 0.17   | 2.57 ± 0.19   | 2.62 ± 0.22    | 2.58 ± 0.16   |
| BP (mmHg) | 91.0 ± 11.3    | 95.2 ± 8.7    | 92.1 ± 9.0     | 92.2 ± 14.7   |
| Plasma triglyceride (mg/dl) | 60.5 ± 14.5   | 156.0 ± 33.1b  | 86.2 ± 21      | 152.4 ± 21.3b |
| Plasma cholesterol (mg/dl) | 122.3 ± 13.8  | 221.7 ± 18.9b  | 104.8 ± 10.9   | 201.3 ± 15.5b |
| Leptin (ng/ml) | 4.56 ± 1.11   | 10.1 ± 3.11bc  | 5.21 ± 1.22    | 16.5 ± 3.12b  |
| Adiponectin (μg/ml) | 8.21 ± 0.78   | 6.1 ± 0.31b,c  | 8.19 ± 0.21    | 7.89 ± 0.55   |
| MCP-1 (ng/ml) | 45.2 ± 11.1    | 154.0 ± 21.2bc | 47.0 ± 23.2    | 94.0 ± 13.9b  |
| TNF-α (ng/ml) | 9.50 ± 4.0     | 24.6 ± 4.6b    | 9.20 ± 3.1     | 19.9 ± 5.9b   |

aData are means ± SEM; n = 11 in each group.

bP < 0.05 versus PPAR-γ−/− mice fed LFD.

cP < 0.05 versus PPAR-γ+/+ mice fed LFD.
Renal Lipid Accumulation

Increased renal triglyceride content was observed in \( \text{PPAR}\gamma^{+/-} \) mice at 8 and 16 wk of HFD, although no significant increase was observed at 4 wk of HFD (Figure 4A). Furthermore, HFD-induced increases in renal triglyceride content at 8 and 16 wk of HFD were significantly reduced in \( \text{PPAR}\gamma^{+/-} \) mice (Figure 4A). During the experimental period, no significant differences in renal cholesterol content were observed among the four groups (Figure 4B). Oil-Red O staining of kidney sections in the four groups revealed that HFD caused marked neutral lipid accumulations in both the glomerular and tubulointerstitial lesion (Figure 5, B and F). These accumulations were markedly decreased in \( \text{PPAR}\gamma^{+/-} \) mice (Figure 5, D and H). In both \( \text{PPAR}\gamma^{+/-} \) and \( \text{PPAR}\gamma^{+/+} \) on an

### Table 2. Levels of mRNA expression in the renal cortex at the end of 16-wk experimental period

| Parameter               | \( \text{PPAR}\gamma^{+/-} \) Mice | \( \text{PPAR}\gamma^{+/+} \) Mice |
|-------------------------|-----------------------------------|-----------------------------------|
|                         | LFD      | HFD                  | LFD      | HFD                  |
| **PPAR-\( \gamma \)**   | 0.95 ± 0.29 | 1.21 ± 0.23\(^{b}\) | 0.33 ± 0.13\(^{b}\) | 0.51 ± 0.12           |
| Fibrosis and inflammation |         |                      |         |                      |
| fibronectin             | 0.85 ± 0.16 | 1.31 ± 0.22\(^{b}\) | 0.70 ± 0.29 | 0.97 ± 0.03           |
| type IV collagen        | 1.47 ± 0.18 | 2.10 ± 0.35\(^{b}\) | 1.43 ± 0.35 | 1.39 ± 0.21           |
| PAI-1                   | 0.81 ± 0.65 | 2.06 ± 0.37\(^{b}\) | 0.88 ± 1.22 | 0.98 ± 0.74           |
| MCP-1                   | 2.95 ± 1.22 | 5.73 ± 0.80\(^{b}\) | 2.89 ± 0.78 | 3.48 ± 0.33           |
| Fatty acid synthesis    |         |                      |         |                      |
| SREBP-1c                | 1.66 ± 0.80 | 2.77 ± 0.62\(^{b}\) | 1.55 ± 0.71 | 1.82 ± 0.25           |
| FAS                     | 1.56 ± 0.56 | 5.22 ± 2.13\(^{b}\) | 1.34 ± 1.45 | 2.45 ± 0.88\(^{c}\)   |
| ACC                     | 0.44 ± 0.19 | 3.70 ± 1.33\(^{b}\) | 0.54 ± 0.34 | 1.10 ± 0.34\(^{c}\)   |
| Fatty acid oxidation    |         |                      |         |                      |
| PPAR-\( \alpha \)       | 1.81 ± 0.99 | 3.04 ± 0.45          | 1.93 ± 0.69 | 2.11 ± 0.40           |
| CPT-1                   | 2.82 ± 0.98 | 2.11 ± 0.87\(^{b}\) | 2.88 ± 0.34 | 5.12 ± 0.67\(^{c}\)   |
| ACO                     | 0.84 ± 0.15 | 1.02 ± 0.15          | 1.03 ± 0.28 | 1.14 ± 0.25           |
| MCAD                    | 3.81 ± 0.79 | 3.01 ± 0.53          | 2.93 ± 1.49 | 4.11 ± 1.42           |

\(^{a}\)Data are means ± SEM; \( n = 11 \) in each group. PAI-1, plasminogen activator-1.

\(^{b}\)P < 0.05 versus \( \text{PPAR}\gamma^{+/-} \) mice fed HFD.

\(^{c}\)P < 0.05 versus \( \text{PPAR}\gamma^{+/-} \) mice fed LFD.
LFD, these renal neutral lipid accumulations were not observed (Figure 5, A, C, E, and G).

Renal Lipid Metabolism
Sterol regulatory element-binding protein-1c (SREBP-1c) is a transcriptional factor that regulates the transcriptional activity of the enzymes that are involved in lipogenesis, fatty acid synthase (FAS) and acetyl-CoA carboxylase (ACC).17,22 In the kidneys from all four groups, we measured the mRNA expressions of SREBP-1c, FAS, and ACC at 4 and 16 wk. The mRNA expression levels of these molecules were increased in the kidneys of PPAR-γ+/+ mice on an HFD at both time points (Tables 2 and 3). However, these changes were not observed in PPAR-γ−/− mice (Tables 2 and 3). Furthermore, under an HFD, ACC protein content was increased in the kidneys of PPAR-γ+/+ mice at 16 wk but not in PPAR-γ−/− mice (Figure 6, A and B).

We next measured the mRNA expression levels of the molecules that are involved in lipolysis. At both 4 and 16 wk, we did not observe any differences in mRNA expression levels of PPAR-α, acyl-CoA oxidase, (ACO), and acyl-CoA dehydrogenase (MCAD) in the kidneys among the four groups (Tables 2 and 3). However, at both 4 and 16 wk of HFD, a significant decrease in mRNA expression of carnitine palmitoyl transferase-1 (CPT-1) in the kidney of PPAR-γ+/+ mice was observed, although this was not found in PPAR-γ−/− mice (Tables 2 and 3).

The 5′ AMP-activated protein kinase (AMPK) phosphorylates and inactivates ACC, resulting in a decrease in intracellular level of malonyl-CoA, thereby relieving inhibition of CPT-1 activity and accelerating lipolysis.23 Phosphorylation of both AMPKα(Thr172) (Figure 6, A and C) and ACC(Ser79) (Figure 6, A and D) were significantly decreased in the kidneys of PPAR-γ+/+ mice on an HFD at 16 wk. In contrast, these HFD-induced decreases in phosphorylation of AMPKα(Thr172) (Figure 6, A and C) and ACC(Ser79) (Figure 6, A and D) were not observed in the PPAR-γ−/− mice.

DISCUSSION
Here, we show that HFD induces the alteration of renal lipid metabolism by an imbalance between lipogenesis and lipolysis in the kidney per se, as well as systemic metabolic abnormalities and subsequent renal lipid accumulation and renal injury. Furthermore, these renal involvements under an HFD are ameliorated in insulin-sensitive PPAR-γ+/− mice.

Recently, HFD was reported to induce renal injury, although the exact mechanisms have not been fully clarified.13,24 Several reports have suggested that renal lipid accumulation, lipotoxicity, is associated with the development of such renal injury.13 It is interesting that our results show that HFD induces systemic metabolic abnormalities such as insulin resis-
tance during 4 wk of HFD and subsequent renal lipid accumu-
lation during 8 wk of HFD and finally renal injury at 16 wk of
HFD. Furthermore, these HFD-induced renal involvements
are ameliorated in insulin-sensitive PPAR-α mice. These
results suggest that lipotoxicity in the kidney could be one of
the important mechanisms for the development of renal injury
associated with metabolic syndrome.

To date, the precise mechanisms for renal lipid accumula-
tion have not been fully determined. However, there is growing
evidence that the increased renal lipogenesis plays a role in the
pathogenesis of renal injury.11,13,25,26 Therefore, we investi-
gated whether HFD increases renal mRNA expression levels of
SREBP-1c, FAS, and ACC, which are involved in lipogenesis.
Similar to previous reports,11,13,25,26 mRNA expression levels of
these molecules were increased in the kidneys of PPAR-γ+/-
mice during 4 wk of HFD, whereas these were not observed in
the kidneys of insulin-sensitive PPAR-γ+/- mice. Therefore,
we can show that the increase in renal lipogenesis is observed
from the early stage of HFD, before neutral lipid accumulation
in the kidney. These observations provide further evidence that
the accelerated renal lipogenesis contributes to the develop-
ment of renal lipid accumulation under insulin resistance.

In addition to renal lipogenesis, we examined the effects of
HFD on renal lipolysis to determine its role in the development
of renal lipid accumulation. Our results showed that mRNA
expression levels of CPT-1, which is one of the key enzymes
involved in lipolysis, were significantly decreased during the 4
wk of HFD but not in PPAR-γ+/- mice. These results suggest
that renal lipolysis decreases under insulin resistance, which
may contribute to renal lipid accumulation. PPAR-α also regu-
lates lipolysis in various tissues.27 However, we failed to find
significant differences of renal mRNA expression levels of
PPAR-α among the four groups. Furthermore, we could not
observe differences of renal mRNA expression of ACO and
MCAD, which are transcriptional target molecules of PPAR-α.
These results suggest that HFD might not affect mRNA expres-
sion of PPAR-α or activity of PPAR-α in this mouse model of
metabolic syndrome.

In this study, we found decreased renal mRNA expression
levels of CPT-1, although those of ACO, MCAD, and PPAR-α
were not changed, in PPAR-γ+/- mice on an HFD. We there-
fore focused on the activity of the AMPK pathway to explore
this discrepancy, because this pathway is a key regulator of
intracellular lipid metabolism in other tissues23 and because
activated AMPK inactivates ACC, resulting in a decrease in
malonyl-CoA, with subsequent release of inhibition of CPT-1
expression levels and acceleration of lipolysis.23 Under an
HFD, phosphorylation of AMPKα(Thr172) and ACC(Ser79)
was significantly decreased in the kidneys of PPAR-γ+/-
mice but not in the kidneys of PPAR-γ+/- mice. These results sug-
gest that decreased AMPKα activity in the kidney under an
HFD could increase the activity of ACC and intracellular ma-
lyonyl-CoA content, resulting in the decreases in renal mRNA
expression of CPT-1. These results could provide new evidence
that a decrease in lipolysis via inhibiting the AMPK–CPT-1
pathway but not PPAR-α could contribute to renal lipid accu-
umulation under an HFD. Furthermore, these results suggest
that posttranslational activation of ACC by inhibiting AMPK
activity under an HFD might contribute to the acceleration of
renal lipogenesis, as well as increased renal expression of ACC.

Figure 4. Triglyceride (A) and cholesterol (B) contents in the
kidneys of mice in each group. Data are means ± SEM for five to
11 mice in each group. *P < 0.05 versus PPAR-γ+/+ mice on an
LFD; †P < 0.05 versus PPAR-γ+/- mice on an HFD.

Figure 5. (A through H) Representative photomicrographs of
Oil-Red O–stained kidney sections in each group of mice. Mag-
nifications: ×200 in A through D; ×400 in E through H.
In this study, we show that the improvements of systemic metabolic abnormalities result in the attenuation of HFD-induced renal lipid accumulation and renal injury with the improvement of renal lipid metabolism in PPAR-γ+/− mice. We previously reported that moderate reduction of PPAR-γ activity could decrease local lipid accumulation in the liver and skeletal muscle in PPAR-γ−/− mice.20 These results raise the question of whether the reduction of PPAR-γ activity could directly affect the improvement of renal lipid metabolism in the kidneys of PPAR-γ+/− mice on an HFD. Liver-specific PPAR-γ disruption could attenuate steatohepatitis with the reduction of lipid accumulation in leptin-deficient mice.28 Also, deletion of PPAR-γ in adipose tissues of mice protects against HFD-induced adipocyte hypertrophy, which inhibits obesity and insulin resistance.29 These reports suggest that a reduction of PPAR-γ activity may inhibit various diseases that are associated with local lipid accumulation in various peripheral tissues, including kidney. However, our study does not provide enough evidence to clarify whether PPAR-γ deficiency in the kidney directly regulates renal lipid metabolism, as well as other peripheral tissues.28,29 Further studies are required to determine the direct effects of PPAR-γ activity on renal lipid metabolism.

Several investigators have reported that PPAR-γ agonists can protect against the various types of renal injury through their anti-inflammatory and antifibrotic effects.30–32 In contrast, our results showed that systemic reduction of PPAR-γ expression could improve HFD-induced renal injury. We therefore suggest that both PPAR-γ agonists and PPAR-γ insufficiency in the absence of ligands can protect against renal injury that is associated with glucose and lipid metabolism abnormalities, at least in part, through the attenuation of both systemic and renal lipid metabolism. Furthermore, several reports show that PPAR-γ recruits other transcriptional co-repressor complexes in the absence of ligand and that these co-repressors are capable of down-regulating PPAR-γ-mediated transcriptional activity.33,34 This might be another mechanism through which both ligand binding to PPAR-γ and ligand-free PPAR-γ deficiency could promote renal protection.

Here, we present evidence that HFD causes renal lipid accumulation and renal injury with increased renal lipogenesis and decreased renal lipolysis, whereas these abnormalities are attenuated in insulin-sensitive PPAR-γ+/− mice. These results suggest that the improvement of an imbalance between renal lipogenesis and lipolysis results in a reduction of renal lipid accumulation and subsequent attenuation of renal injury under insulin resistance. Therefore, we propose that attenuation of renal lipid metabolism could serve as a new therapeutic strategy to prevent the development of CKD in metabolic syndrome.

Table 3. Levels of mRNA expression in the renal cortex at 4 wk

| Parameter                      | PPAR-γ+/− Mice | PPAR-γ+/− Mice |
|--------------------------------|----------------|----------------|
|                                | LFD            | HFD            |
| Fatty acid synthesis           |                |                |
| SREBP-1c                       | 1.21 ± 0.10    | 2.93 ± 0.31b,c | 1.29 ± 0.10    | 1.69 ± 0.16    |
| FAS                            | 1.54 ± 0.15    | 3.38 ± 0.67b,c | 1.63 ± 0.17    | 1.72 ± 0.15    |
| ACC                            | 0.57 ± 0.07    | 1.06 ± 0.17b,c | 0.59 ± 0.04    | 0.69 ± 0.13    |
| Fatty acid oxidation           |                |                |
| PPAR-α                         | 2.13 ± 0.61    | 3.13 ± 0.60    | 2.18 ± 0.24    | 2.78 ± 0.34    |
| CPT-1                          | 3.20 ± 0.29    | 2.03 ± 0.47b,c | 3.04 ± 0.35    | 2.77 ± 0.20    |
| ACO                            | 0.91 ± 0.22    | 1.09 ± 0.22    | 1.02 ± 0.28    | 0.90 ± 0.13    |
| MCAD                           | 3.56 ± 1.89    | 3.89 ± 1.10    | 4.45 ± 2.14    | 3.95 ± 1.50    |

aData are means ± SEM; n = 5 to 6 in each group.
bP < 0.05 versus PPAR-γ+/− mice fed LFD.
cP < 0.05 versus PPAR-γ+/− mice fed HFD.

CONCISE METHODS

Animal Models

PPAR-γ+/− mice were generated as described previously.21 Six-week-old mice were housed in box cages, maintained on a 12-h light/12-h dark cycle, and fed an LFD (10% of kilocalories from fat) or HFD (45% of kilocalories from fat) obtained from Research Diets (New Brunswick, NJ) for 16 wk. At the end of 16-wk period, body weight, BP, and blood glucose were measured. BP of conscious mice was measured at a steady state with a programmable tail-cuff sphygmomanometer (BP98-A; Softron, Tokyo, Japan). Mice were placed in metabolic balance cages for 24-h urine collection to measure albumin concentration. Mice were anesthetized and perfused as described previously.11 The right kidney was embedded in paraffin for PAS staining and immunohistochemistry or was frozen for Oil-Red O staining. Total RNA and protein were extracted from the remaining renal cortex of the left kidney. The Research Center for Animal Life Science of Shiga University of Medical Science approved all experiments.

Antibodies

Anti-phospho-acetyl CoA carboxylase(Ser79) was obtained from Upstate Cell Signaling (Lake Placid, NY). Anti-phospho-AMPKα(Thr172), anti-AMPKα(23A3), and anti-ACC were from Cell Signaling Technology (Beverly, MA).
Blood and Urine Analysis
Cholesterol or triglycerides were measured using the cholesterol CII kit or L type TG H kit (Wako Chemicals, Richmond, VA). Plasma insulin was determined using an ELISA (Exocell, Philadelphia, PA). Plasma leptin, MCP-1, and TNF-α were assayed with the immunoassay kit (R&D Systems, Minneapolis, MN). Plasma adiponectin was determined with a mouse-specific ELISA kit (Linco Research, St. Charles, MO). Urinary albumin excretion was measured with a mouse-specific sandwich ELISA system (Albuwell; Exocell) and was expressed as total amount excreted in 24 h.

Protein Extraction and Western Blot Analysis
The renal cortex was homogenized in an ice-cold lysis buffer containing 150 mmol/L NaCl, 50 mmol/L Tris-HCl (pH 8.0), 0.1% SDS, 1% Nonidet P-40, and protease inhibitor cocktail (Boehringer Mannheim, Lewes, UK). These samples were resolved by 10% SDS-PAGE and transferred to polyvinylidene fluoride membranes (Immobilon, Bedford, MA). The membranes were incubated with the appropriate antibodies, washed, and incubated with horseradish peroxidase-coupled secondary antibodies (Amersham, Buckinghamshire, UK). The blots were visualized by using an enhanced chemiluminescence detection system (Perkin Elmer Life Science, Boston, MA).

RNA Extraction and Quantitative Real-Time PCR
Total RNA was isolated from the renal cortex based on the TRIzol protocol (Invitrogen Life Technologies, Carlsbad, CA). cDNA was synthesized using reverse transcript reagents (Takara, Otsu, Japan). iQSYBR Green Supermix (Bio-Rad Laboratories, Hercules, CA) was used for real-time PCR (ABI Prism TM 7500 Sequence Detection System; Perkin-Elmer Applied Biosystems). The levels of mRNA expression of these molecules were quantified using standard curve method. Standard curves were constructed using serially diluted standard template. C_t value was 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. Primers used are described in Table 4.

Lipid Extraction and Analysis
Total lipid was extracted from the renal cortex by the method of Bligh and Dyer. Triglyceride and cholesterol contents were determined as described previously.

Morphologic Analysis
Fixed kidneys were embedded in paraffin, sectioned (3-μm thick), and then stained with PAS reagent as described previously. From each mouse, 20 glomeruli cut at their vascular poles were used for morphometric analysis. The extent of the mesangial matrix (defined as mesangial area) was determined by assessment of the PAS-positive and nucleus-free area in the mesangium using a computer-assisted color image analyzer (LUZEX F; Nikon, Tokyo, Japan). Immunohistochemical staining was performed with fibronectin-specific polyclonal anti-mouse antibody (A852/R5H; Biogenesis, Poole, UK). For evaluation of immunostaining for fibronectin, the percentages of area stained for fibronectin were graded as follows: 0, staining absent to 5%; 1, 5 to 25%; 2, 25 to 50%; 3, 50 to 75%; and 4, >75%. An investigator who was masked to sample identity performed the image analysis. Frozen sections were used for Oil-Red O staining, as previously reported.

Blood and Urine Analysis
Cholesterol or triglycerides were measured using the cholesterol CII kit or L type TG H kit (Wako Chemicals, Richmond, VA). Plasma insulin was determined using an ELISA (Exocell, Philadelphia, PA). Plasma leptin, MCP-1, and TNF-α were assayed with the immunoassay kit (R&D Systems, Minneapolis, MN). Plasma adiponectin was determined with a mouse-specific ELISA kit (Linco Research, St. Charles, MO). Urinary albumin excretion was measured with a mouse-specific sandwich ELISA system (Albuwell; Exocell) and was expressed as total amount excreted in 24 h.

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Table 4. Primer sequences for real-time PCR

| Primer | Forward | Reverse |
|--------|---------|---------|
| β-actin | CTTGCCTGACATCAAAGAGA | TGGATCCCAAGAGGTCCAT |
| Fibronectin | GCAAGCCAGTCTCCATCAT | CATTCTTGAGGAGCGTGTC |
| Type IV collagen | TACCTGCGCATCTTCCATAC | CCGATGTCGGTCTGCAAG |
| PAI-1 | GGACACCTCCATGCACTTTCA | TCTCATGAGTATCAGCAAGAT |
| MCP-1 | GCCCCCACCTACCTGCTGCTACT | CTCGCTGCGGACGAGCCT |
| SREBP-1c | GCCAACATGAGTCATTCCGAACCT | AGGAAACCTTCAGAGACAT |
| FAS | CACAACCATCACCATCAGAGT | GACGATGCAAACTGCAAGAT |
| ACC | CCAGCAGGATAATTACTTCTTGG | TCCCATTTGGAACACTAGGAG |
| CPT-1 | ACCACTCTGCGGACCTAGTCAAG | AGGCGATGCAAACTGCAAGAT |
| PPARα | CTTCAGAGCACCACCATCAGAT | GCGGAAGGGCTCCACCATTT |
| PPARγ | CAAATGCCATACGTTTGG | GCCATGGTGTGCTCTGGAAG |
| ACO | GACCACTATGGTGACATCCA | ACCAAACTTCGCGCTGGACAG |
| MCAD | TAATCGGAGGAGGAGGAGTTT |

Glucose Tolerance Test and Insulin Tolerance Test
For glucose tolerance tests, mice were fasted overnight for 14 h followed by intraperitoneal glucose injection (1 g/kg body wt). Blood glucose was measured using tail blood collected at 0, 15, 30, 60, and 120 min after the injection.37 For insulin tolerance tests, mice were administered an injection of human regular insulin (Novolin R; Novo Nordisk, Clayton, NC) at 0.75 U/kg body wt intraperitoneally after a 6-h fast, and blood glucose was measured at 0, 15, 30, and 60 min.37

Statistical Analyses
Results are expressed as means ± SEM. ANOVA with subsequent Scheffe test was used to determine the significance of differences in multiple comparisons. P < 0.05 was considered statistically significant.

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DISCLOSURES

None.

REFERENCES

1. Lakka HM, Laaksonen DE, Lakka TA, Niskanen LK, Kumpusalo E, Tuomilehto J, Salonen JT: The metabolic syndrome and total cardiovascular disease mortality in middle-aged men. JAMA 288:2709–2716, 2002
2. Isomaa B, Almgren P, Tuomilehto J, Forsen B, Lahti K, Nissen M, Taskinen MR, Group L: Cardiovascular morbidity and mortality associated with the metabolic syndrome. Care 24:683–691, 2001
3. Chen J, Muntner P, Hamm LL, Jones DW, Batuman V, Fonseca V, Whelton PK, He J: The metabolic syndrome and chronic kidney disease in US adults. Ann Intern Med 140:167–174, 2004
4. Praga M: Obesity: A neglected culprit in renal disease. Nephrol Dial Transplant 17:1157–1159, 2002
5. Abrass CK: Overview: Obesity—What does it have to do with kidney disease? J Am Soc Nephrol 15:2768–2772, 2004
6. Bagby SP: Obesity-initiated metabolic syndrome and the kidney: A recipe for chronic kidney disease? J Am Soc Nephrol 15:2775–2791, 2004
7. Wisse BE: The inflammatory syndrome: The role of adipose tissue cytokines in metabolic disorders linked to obesity. J Am Soc Nephrol 15:2792–2800, 2004
8. El-Atat FA, Las SN, McFarlane SI, Sowers JR: The relationship between hyperinsulinemia, hypertension, and progressive renal disease. J Am Soc Nephrol 15:2816–2827, 2004
9. Gin H, Rigalleau V, Aparicio M: Lipids, protein intake, and diabetic nephropathy. Diabetics Metab 28(Suppl 4):45–53, 2000
10. Bonnet F, Cooper ME: Potential influence of lipids in diabetic nephropathy: Insights from experimental data and clinical studies. Diabet Metab 26:254–264, 2000
11. Sun L, Halaileh N, Zhang W, Rogers T, Levi M: Role of sterol regulatory element-binding protein-1 in regulation of renal lipid metabolism and glomerulosclerosis in diabetes mellitus. J Biol Chem 277:18919–18927, 2002
12. Spencer MW, Muhafeld A, Segerer S, Hudkins KL, Kirk E, LeBoeuf RC, Alpers CE: Hyperglycemia and hyperlipidemia act synergistically to induce renal disease in LDL receptor-deficient BALB mice. Am J Nephrol 24:20–31, 2004
13. Jiang T, Wang Z, Proctor G, Moskowitz S, Liebman SE, Rogers T, Li M, Seros T, Levi M: Diet-induced obesity in C57BL/6J mice causes increased renal lipolysis accumulation and glomerulosclerosis via a sterol regulatory element-binding protein-1c-dependent pathway. J Biol Chem 280:32317–32325, 2005
14. Jump DB, Botolin D, Wang Y, Xu J, Christian B, Demeure O: Fatty acid regulation of hepatic gene transcription. J Nutr 135:2503–2506, 2005
15. Jeukendrup AE: Regulation of fat metabolism in skeletal muscle. Ann N Y Acad Sci 967:217–235, 2002
16. Reddy JK, Rao MS: Lipid metabolism and liver inflammation. II. Fatty liver disease and fatty acid oxidation. Am J Physiol Gastrointest Liver Physiol 290:G852–G858, 2006
17. Horton JD, Goldstein JL, Brown MS: SREBP: Activators of the complete program of cholesterol and fatty acid synthesis in the liver. J Clin Invest 109:1125–1131, 2002
18. Kelley DE, Simoneau JA: Impaired free fatty acid utilization by skeletal muscle in non-insulin-dependent diabetes mellitus. J Clin Invest 94:2349–2356, 1994
19. Pan DA, Lilloja S, Kriketos AD, Milner MR, Baur LA, Bogardus C, Jenkins AB, Storlien LH: Skeletal muscle triglyceride levels are inversely related to insulin action. Diabetes 46:983–988, 1997
20. Yamauchi T, Kameon J, Waki H, Murakami K, Motojima K, Komeda K, Ide T, Kubota N, Terauchi Y, Tobe K, Miki H, Tsuchida A, Akanuma Y,
Nagai R, Kimura S, Kadowaki T: The mechanisms by which both heterozygous peroxisome proliferator-activated receptor gamma (PPARgamma) deficiency and PPARgamma agonist improve insulin resistance. *J Biol Chem* 276: 41245–41254, 2001

21. Kubota N, Terauchi Y, Miki H, Tamemoto H, Yamauchi T, Komeda K, Satoh S, Nakano R, Ishii C, Sugiyama T, Eto K, Tsubamoto Y, Okuno A, Murakami K, Sekihara H, Hasegawa G, Naito M, Toyoshima Y, Tanaka S, Shiota K, Kitamura T, Fujita T, Ezaki O, Aizawa S, Kadowaki T, *et al.*: PPAR gamma mediates high-fat diet-induced adipocyte hypertrophy and insulin resistance. *Mol Cell* 4: 597–609, 1999

22. Rawson RB: The SREBP pathway: Insights from Insigs and insects. *Nat Rev Mol Cell Biol* 4: 631–640, 2003

23. Long YC, Zierath JR: AMP-activated protein kinase signaling in metabolic regulation. *J Clin Invest* 116: 1776–1783, 2006

24. Wei P, Lane PH, Lane JT, Padanilam BJ, Sansom SC: Glomerular structural and functional changes in a high-fat diet mouse model of early-stage type 2 diabetes. *Diabetologia* 47: 1541–1549, 2004

25. Wang Z, Jiang T, Li J, Proctor G, McManaman JL, Lucia S, Chua S, Levi M: Regulation of renal lipid metabolism, lipid accumulation, and glomerulosclerosis in FVBdb/db mice with type 2 diabetes. *Diabetes* 54: 2328–2335, 2005

26. Jiang T, Liebman SE, Lucia MS, Li J, Levi M: Role of altered renal lipid metabolism and the sterol regulatory element binding proteins in the pathogenesis of age-related renal disease. *Kidney Int* 68: 2608–2620, 2005

27. Lefebvre P, Chinetti G, Fruchart JC, Staels B: Sorting out the roles of PPAR alpha in energy metabolism and vascular homeostasis. *J Clin Invest* 116: 571–580, 2006

28. Matusue K, Haluzik M, Lambert G, Yim SH, Gavriloa O, Ward JM, Brewer B Jr, Reitman ML, Gonzalez FJ: Liver-specific disruption of PPARgamma in leptin-deficient mice improves fatty liver but aggravates diabetic phenotypes. *J Clin Invest* 111: 737–747, 2003

29. Jones JR, Barrick C, Kim KA, Lindner J, Blondeau B, Fujimoto Y, Shiota M, Kesterson RA, Kahn BB, Magnuson MA: Deletion of PPARgamma in adipose tissue of mice protects against high fat diet-induced obesity and insulin resistance. *Proc Natl Acad Sci U S A* 102: 6207–6212, 2005

30. Ishikii K, Haneda M, Koya D, Maeda S, Sugimoto T, Kikkawa R: Thiazolidinedione compounds ameliorate glomerular dysfunction independent of their insulin-sensitizing action in diabetic rats. *Diabetes* 49: 1022–1032, 2000

31. Guo B, Koya D, Isozono M, Sugimoto T, Kashiwagi A, Haneda M: Peroxisome proliferator-activated receptor-gamma ligands inhibit TGF-beta 1-induced fibronectin expression in glomerular mesangial cells. *Diabetes* 53: 200–208, 2004

32. Sarasidis PA, Bakris GL: Protection of the kidney by thiazolidinediones: An assessment from bench to bedside. *Kidney Int* 70: 1223–1233, 2006

33. Yu C, Markan K, Temple KA, Deplewski D, Brady MJ, Cohen RN: The nuclear receptor corepressors NCoR and SMRT decrease peroxisome proliferator-activated receptor gamma transcriptional activity and repress 3T3-L1 adipogenesis. *J Biol Chem* 280: 13600–13605, 2005

34. Cohen RN: Nuclear receptor corepressors and PPARgamma. *Nucl Recept Signal* 4: e003, 2006

35. Bligh EG, Dyer WJ: A rapid method of total lipid extraction and purification. *Can J Biochem Physiol* 37: 911–917, 1959

36. Koya D, Haneda M, Nakagawa H, Ishikii K, Sato H, Maeda S, Sugimoto T, Yasuda H, Kashiwagi A, Ways DK, King GL, Kikkawa R: Amelioration of accelerated diabetic mesangial expansion by treatment with a PKC beta inhibitor in diabetic db/db mice, a rodent model for type 2 diabetes. *FASEB J* 14: 439–447, 2000

37. Chin M, Isozono M, Ishikii K, Araki S, Sugimoto T, Guo B, Sato H, Haneda M, Kashiwagi A, Koya D: Estrogen and raloxifene, a selective estrogen receptor modulator, ameliorate renal damage in db/db mice. *Am J Pathol* 166: 1629–1636, 2005