SUBJECTS AND METHODS

Animals-1

The 6-week-old male C57BL/6J mice weighing 20 ± 1 g were housed in a temperature- and light-controlled room (22 ± 1 °C; 12-h light day cycle) with *ad libitum* access to tap water and a standard mouse chow. Animals were fed a low fat diet (LFD, $n = 8$; 10% lard; Research Diets Inc., New Brunswick, NJ, USA) or a high fat diet (HFD, $n = 8$; 60% lard; Research Diets Inc.), with the latter further randomized to either an MR inhibitor (eplerenone, 100 mg kg^{-1} per day, via gavage, $n = 8$; Pfizer Inc., Tokyo, Japan)-treated or a vehicle-treated group ($n = 8$) during the 12-week experimental protocol. Animals received daily gavage with either eplerenone or vehicle control until being killed on week 12. Daily food intake was measured in each group.

We implanted a telemetry transmitter probe (model PA-C20; Data Sciences Int., St Paul, MN, USA) into the 6-week-old male mice under sodium pentobarbital anesthesia (50 mg kg^{-1} intraperitoneally), and the flexible tip of the probe was positioned at the cervical arteries as described previously.[22] The mice were returned to their home cages and allowed to recover for 2 weeks before the start of the measurements. We monitored conscious blood pressure (BP), heart rate and the activity in unrestricted and untethered mice with the Dataquest IV system (Data Sciences Int.).[22]

After 12-week treatment with eplerenone, body weight was recorded. Mice were then anesthetized with ether and the abdomen was opened through a mid-line incision. Blood and urine were drawn for measuring biochemical assays. Kidneys were harvested and sliced sagitally. The half was snap frozen in liquid nitrogen and stored at −80 °C and the remaining was immersed in 10% neutral-buffered formalin and embedded in paraffin. The study protocol was performed in accordance with the animal experimentation guideline of Keio University School of Medicine.

Animals-2

To evaluate the renoprotective effects of the Rho-kinase inhibition, animals were fed an LFD ($n = 8$; 10% lard; Research Diets Inc.) or an HFD ($n = 8$; 60% lard; Research Diets Inc.) in the same way, with the latter further randomized to either a Rho-kinase inhibitor (fasudil, 30 mg kg^{-1} per day, via gavage, $n = 8$; Asahi Kasei Co., Tokyo, Japan)-treated or a vehicle-treated group ($n = 8$) during the experimental protocol. After 12-week treatment with fasudil, the mice were killed and the collection of blood, urine and tissues were performed in the same way as in Animal-1.

Morphological examination

Nuclei within a single glomerulus were counted in 50 hilar glomeruli in each animal. Diameters of glomeruli were measured by photographing at high magnification for image analysis.[23] These morphological evaluations were conducted by two independent observers in a blinded manner.

Immunohistochemistry

Kidney sections (4 μm) were stained with periodic acid-Schiff's, monoclonal mouse macrophage marker F4/80 (1:300 dilution) and Masson's modified trichrome to demonstrate collagen matrix.[23,24] The accumulation of matrix, and the extent of histochemical and immunohistochemical staining were quantified using computer-assisted image analysis. The number of macrophages was assessed by counting F4/80-positive nuclei (brown) per mm^2 with the use of image analysis software.[23] Sections incubated with normal goat serum instead of the primary antiserum served as the negative control.

Tissue aldosterone and immunoblotting

Tissue aldosterone was measured by liquid chromatography-electrospray ionization tandem mass spectrometry.[25] Western blotting was performed as described previously.[13] To examine the effects of aldosterone on Rho/Rho-kinase pathway, phosphorylated levels of myosin phosphatase target subunit-1 (MYPT1; Upstate Biochemistry, Lake Placid, NY, USA), one of the substrates of Rho kinase, were examined in the kidney and aldosterone-stimulated human mesangial cells (HMCs), with the use of phospho-MYPT1 levels at the inhibitory site (Thr696) as a marker for Rho-kinase activity. Immunoblotting was performed using specific antibodies against phospho-MYPT1 and mitogen-activated protein kinases, including extracellular signal-regulated kinases (ERK1/2) and p38.[26,27]

We prepared the nuclear extracts with commercially available kits (Panomics Inc., Fremont, CA, USA).[28] MR (Perseus Proteomics, Tokyo, Japan) protein levels in the nuclear fraction of the kidney were examined. Immunoreactive bands were detected using an ECL detection kit (Amersham Biosciences, Uppsala, Sweden).

Measurement of MCP-1 and TNF-α protein levels

Tissue monocyte chemoattractant protein-1 (MCP-1) and tumor necrosis factor-α (TNF-α) were measured using a commercially available enzyme-linked immunosorbent assay kit (Westang Bio-tech, Shanghai, China).[29] Briefly, kidney samples were homogenized separately in 0.1 M NaCl, 0.05 M Tris-Cl and 2% (v v^{-1}) Triton X-100 together with protease inhibitors (Roche Diagnostics, Melbourne, Australia). After centrifugation, the supernatants were collected and stored at −80 °C until assayed. The TNF-α and MCP-1 levels were measured using an enzyme-linked immunosorbent assay kit. All samples were assayed in duplicate. The results of TNF-α and MCP-1 were calculated as concentration per wet weight of the tissue (picograms per gram).

Cell culture and experimental protocols

Experiment-1. Aldosterone was obtained from Sigma (St Louis, MO, USA). An aldosterone receptor blocker, eplerenone, was kindly provided by Pfizer Inc.. HMCs in primary culture were purchased from Cambrex Bioproducts (Takara Bio Inc., Otsu, Shiga, Japan). The cells were cultured in SmBM medium (Clonetics Co., Walkersville, MD, USA) supplemented with 10% fetal bovine serum. For all experiments, early passaged (passages 3-6) HMCs were grown at 60-70% confluence and made quiescent by serum starvation for 48 h. Cells were incubated with various concentration of aldosterone (10^{-11} to 10^{-8} mol l^{-1}) for 1 h and were also treated with eplerenone for 30 min before the addition of aldosterone (1 nmol l^{-1}). HMCs were harvested in 100 μl lysis buffer containing 20 mmol l^{-1} of Tris-HCl, 250 mmol l^{-1} of sucrose and phenylmethylsulfonyl fluoride, as well as aprotinin and leupeptin (10 μg ml^{-1} each) 5 min after the aldosterone treatment. After three cycles of freeze and thaw, samples were centrifuged at 250 *g* at 4 °C for 5 min. The supernatant was then centrifuged at 100 000 *g* for 30 min at 4 °C. The supernatant was saved and the protein was subjected to immunoblotting.

Experiment-2. Cells were further supplemented with 100 nM human insulin (Sigma) in 2% fetal bovine serum, to mimic hyperinsulinemia, or incubated with standard 1 nM or 10 nM insulin in Dulbecco's modified Eagle's medium with 20% fetal bovine serum over 48-h period. Cells were cultured in 75-cm^2 flasks (90-95% confluency) for RNA isolation.

Quantitative real-time reverse transcription-polymerase chain reaction

Total RNA was extracted from the mouse kidneys using Trizol solution (Invitrogen, Carlsbad, CA, USA). Total RNA was subjected to reverse transcription in a 20 μl reaction mixture containing random primers and Superscript II enzyme (Invitrogen). Quantitative real-time polymerase chain reaction was performed with an ABI Prism 7700 Sequence Detection System using SYBR Green PCR Master Mix Reagent Kit (Applied Biosystems, Foster City, CA, USA).[30] Primers used were as follows: serum- and glucocorticoid-induced kinase 1 (SGK1): sense–5′-TGTCTTGGGGCTGTCC TGTATG-3′, antisense–5′-GCTTCTGCTGCTTCCTTCACAC-3′; platelet-derived growth factor subunit B (PDGF-B): sense–5′-CGAGTGCAAGACGCGTACA-3′, antisense–5′-GGCATTGGTGCGATCGA-3′; TNF-α: sense–5′-GGTGATCGGTCC CAACAAGGA-3′, antisense–5′-CACGCTGGCTCAGCCACTC-3′; MCP-1: sense–5′-TAGGCTGGAGAGCTACAAGAGGAT-3′, antisense–5′-AGACCTCTCTCTTGAG CTTGGTGA-3′; CYP11B1: sense–5′-ACTCCGTGGCCTGAGACG-3′, antisense–5′-CTCTGCCAGTTCGCGATA-3′; CYP11B2: sense–5′-ACTCCGTGGCCTGAGA CG-3′, antisense–5′-GAGAGCTGCCGAGTCTGA-3′; 3β-hydroxysteroid dehydrogenase (3β-HSD): sense–5′-GCAGACCATCCTAGATGTCAATCTG-3′, antisense–5′-CAAGTGGCTCATAGCCCAGATCTC-3′; and CYP21 hydroxylase: sense–5′-CA

AGAAACTCTCTCGCTCAGCCCT-3′, antisense-5′-CAACGTGCTGTCCTTGTCTCCA AA-3′. Polymerase chain reaction-amplified products were also electrophoresed on agarose gels to confirm that single bands were amplified. Levels of mRNA were normalized to those of β-actin (primers commercially available from Applied Biosystems).

Statistics

Results are expressed as mean ± s.e.m. Statistical significance was evaluated with the analysis of variance with a least significant difference *post-hoc* comparison using the SPSS software package (SPSS Inc., Chicago, IL, USA). Histological results were analyzed by Kruskal-Wallis non-parametric test. *P*-values <0.05 were considered statistically significant.

RESULTS

Effects of eplerenone on systemic blood pressure, renal function and metabolic parameters

Systolic BP, mean BP and heart rate were unaltered by an HFD. Systolic and mean BP tended to be reduced by eplerenone, but did not attain statistical significance ($P>0.1$; Figure 1a–c). Albuminuria was markedly increased in mice on HFD compared with those on an LFD ($P<0.01$; Figure 1d). The treatment with eplerenone reduced albuminuria nearly to the level of mice on LFD. Serum creatinine was not changed in mice on HFD nor was altered by eplerenone (LFD, $0.13 \pm 0.02\,mg\,dl^{-1}$; HFD, $0.18 \pm 0.06\,mg\,dl^{-1}$; HFD with eplerenone, $0.14 \pm 0.02\,mg\,dl^{-1}$; $P>0.1$). Body weights and kidney weights of mice on HFD were markedly greater than those of mice on LFD ($P<0.05$; Figure 1e and f). HFD-induced obesity was ameliorated by the treatment with eplerenone. Eplerenone did not affect the amount of food intake (LFD, 1.9 ± 0.1 g per day; HFD, 1.9 ± 0.1 g per day; HFD with eplerenone, 2.0 ± 0.1 g per day).

HFD had no significant effect on blood glucose levels (LFD, $175 \pm 5\,mg\,dl^{-1}$; HFD, $196 \pm 41\,mg\,dl^{-1}$; HFD with eplerenone, $206 \pm 14\,mg\,dl^{-1}$) or triglycerides (LFD, $69 \pm 10\,mg\,dl^{-1}$; HFD, $95 \pm 32\,mg\,dl^{-1}$; HFD with eplerenone, $76 \pm 17\,mg\,dl^{-1}$), but caused increases in serum insulin (LFD, $0.72 \pm 0.175\,ng\,ml^{-1}$; HFD, $7.56 \pm 2.35\,ng\,ml^{-1}$; $P<0.05$ vs LFD), as well as free fatty acid (FFA) (LFD, $0.65 \pm 0.09\,mg\,dl^{-1}$; HFD, $1.25 \pm 0.16\,mg\,dl^{-1}$; $P<0.05$ vs LFD) and total cholesterol (LFD, $107 \pm 6\,mg\,dl^{-1}$; HFD, $191 \pm 34\,mg\,dl^{-1}$; $P<0.05$ vs LFD). Elevated levels of insulin and FFA were ameliorated by the treatment with eplerenone (insulin: HFD + eplerenone, $2.04 \pm 0.34\,ng\,ml^{-1}$, $P<0.05$ vs HFD; FFA: HFD + eplerenone, $0.71 \pm 0.1\,ng\,ml^{-1}$, $P<0.05$ vs HFD).

Effects of eplerenone on renal morphological changes and renal expression of inflammatory chemokines

In kidneys from mice on HFD, marked mesangial hypercellularity and enlarged glomerular size were noted (Figure 2a). As shown in Figure 2c, glomerular size was increased in mice on HFD compared with that in mice on LFD ($P<0.01$). Similarly, when assessed by the number of the nucleus, glomerular cellularity was increased in mice on HFD compared with that in mice on LFD (66.7 ± 3.0 vs 32.3 ± 2.5 nuclei per glomerular cross-section,

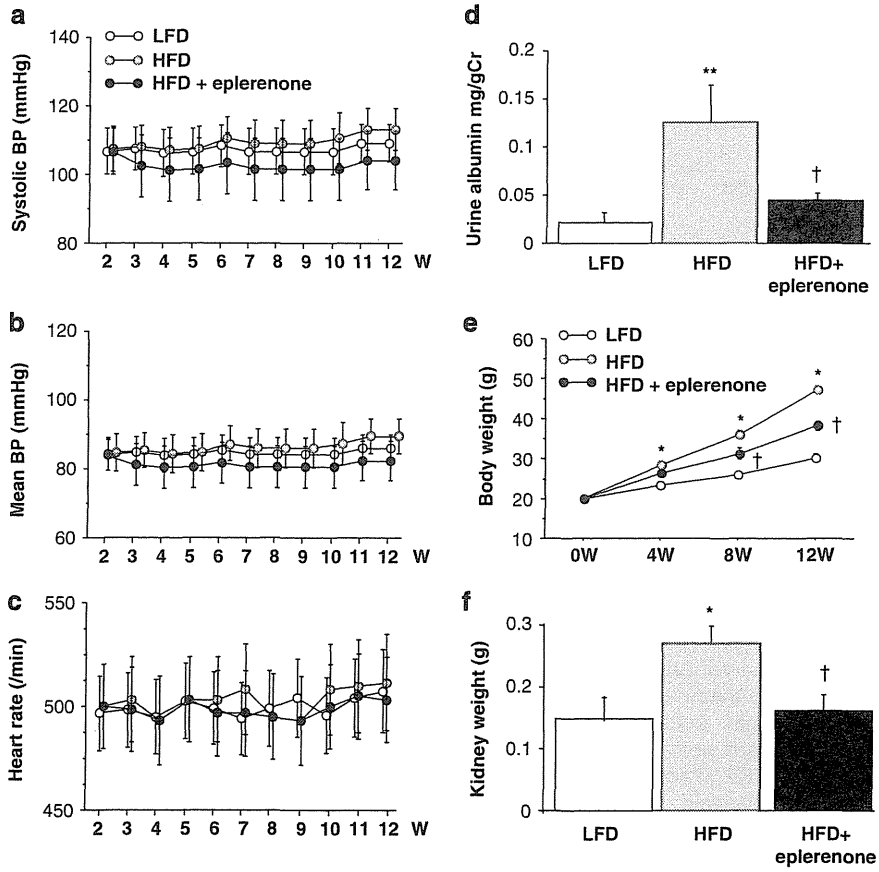

Figure 1. Effects of eplerenone on animal phenotype. (**a**–**c**) Systolic BP, mean BP and heart rate were neither unaltered by HFD nor was reduced by eplerenone. (**d**) Albuminuria was markedly increased in mice on HFD compared with those on LFD. Eplerenone reduced albuminuria. (**e**, **f**) Body weights and kidney weights of mice on HFD were markedly greater than those of mice on LFD. The diet-induced obesity was ameliorated by the treatment with eplerenone. Data were expressed as mean ± s.e.m. Cr, creatinine. *$P<0.05$, **$P<0.01$ vs mice on LFD; †$P<0.05$ vs untreated mice on HFD.

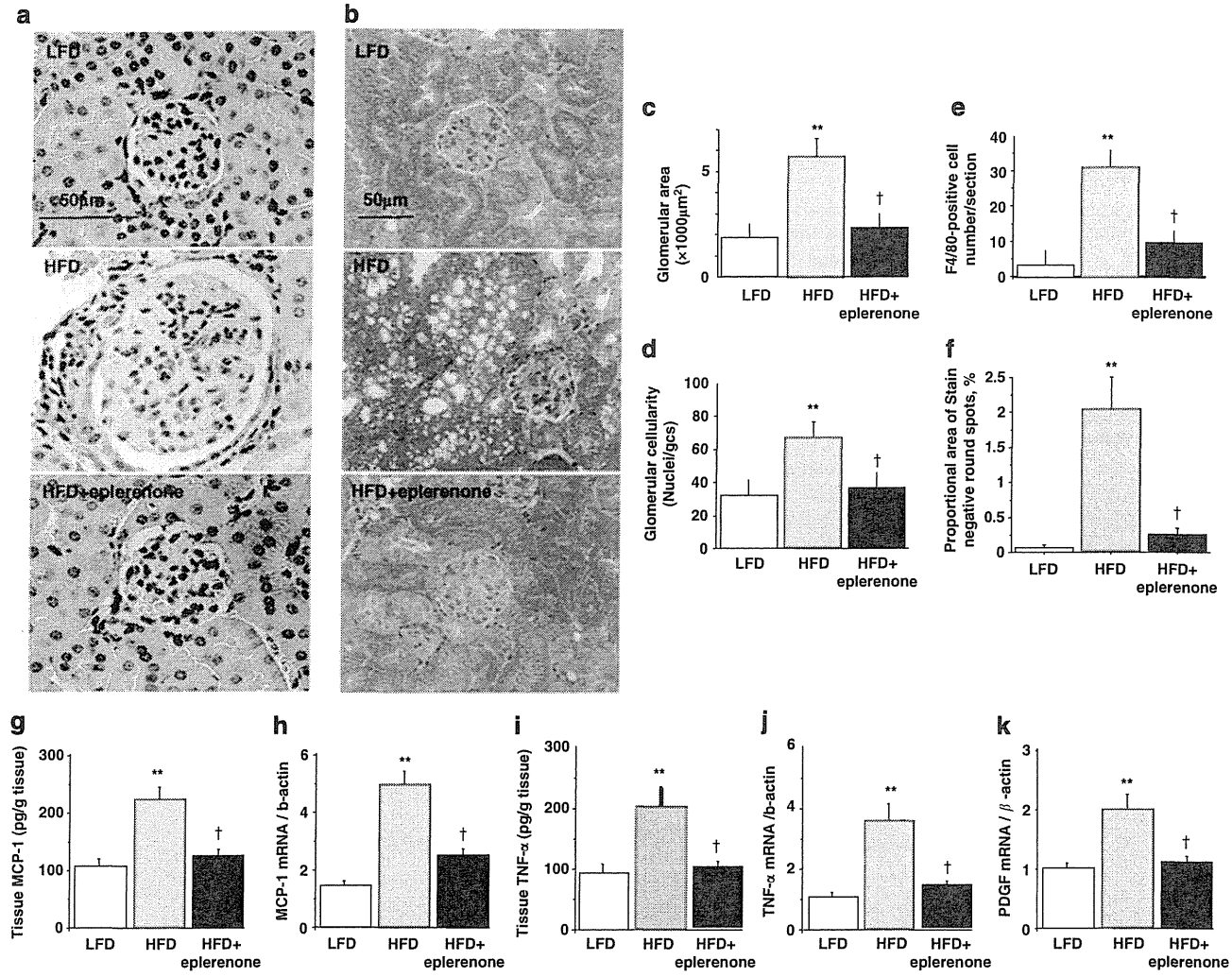

Figure 2. The effects of eplerenone on the HFD-induced renal damages, and renal expression of inflammatory chemokines. (**a**) Histology of F4/80-stained kidney section from mice on LFD, HFD and HFD with eplerenone. Magnification, × 400. Compared with mice on LFD, untreated mice showed marked glomerular hypercellularity and enlarged glomerular size. Eplerenone-treated mice showed near-normal glomerular histology. (**b**) Histology of Masson's modified trichrome-stained kidney section from mice on LFD, HFD and HFD with eplerenone. HFD showed no significant fibrotic changes. Alternatively, the stain-negative round spots are increased in tubules in obese mice and were reduced by eplerenone. (**c**) Glomeruli were markedly enlarged in mice on HFD, which change was reduced by eplerenone. (**d**) Glomerular cellularity was assessed by the number of nuclei per glomerular cross-section (GCS) in 50 hilar glomeruli per animal. (**e**) Macrophages were markedly infiltrated in the renal tissue of mice on HFD, which was improved by the treatment with eplerenone. (**f**) The stain-negative round spots are increased in obese mice and were reduced by eplerenone. HFD-fed mice showed increases in renal expressions of MCP-1 (**g, h**), TNF-α (**i, j**) and PDGF-B (**k**), all of which were attenuated by eplerenone. Data were expressed as the ratio of mRNA levels of MCP-1 (**h**), TNF-α (**j**) and PDGF-B (**k**) to that of β-actin in arbitrary units (a.u.), relative to controls assigned as a value of 1. Data were expressed as mean ± s.e.m. $^{**}P<0.01$ vs *C57BL mice on LFD*; $^{\dagger}P<0.05$ *vs untreated mice on HFD.*

$P<0.01$; Figure 2d). The HFD-induced changes in glomerular size and cellularity were nearly completely abolished by the treatment with eplerenone.

Marked infiltration of macrophages was observed in the renal tissue of mice on HFD (Figure 2e). The treatment with eplerenone pronouncedly abrogated the changes induced by HFD. We examined whether renal fibrotic changes were induced by obesity with Masson trichrome staining. HFD showed no significant fibrotic changes (Figure 2b). Alternatively, the stain-negative round spots were increased in obese mice and were reduced by eplerenone (Figure 2b and f). HFD-fed mice showed the upregulated renal expressions of MCP-1 (3.5-fold; Figure 2h), TNF-α (3.3-fold; Figure 2j) and PDGF-B (2.0-fold; Figure 2k). Protein levels of MCP-1 and TNF-α were similarly overexpressed in mice on HFD (Figure 2g and i). All of these changes were abolished by the treatment with eplerenone.

MR and SGK1 expression and aldosterone synthesis enzyme expression in kidneys from HFD-fed mice

Whether the aldosterone signaling pathway was augmented in kidneys from obese mice was examined. In kidneys of HFD-fed mice, MR protein levels in the nuclear fraction were increased (2.3-fold, $P<0.05$; Figure 3a). Similarly, SGK1, a transcriptionally regulated serine-threonine kinase and considered as one of the main effectors of MR-mediated signal transduction, was pronouncedly upregulated (5.5-fold, $P<0.01$; Figure 3b). The treatment with eplerenone suppressed the MR protein level in the nuclear fraction and downregulated the SGK1 expression.

We further evaluated whether enhanced aldosterone signaling involved aldosterone production *per se* or the modification of MR function.[27] Plasma aldosterone levels were unaltered in mice on HFD (Figure 3c). In contrast, renal aldosterone contents were

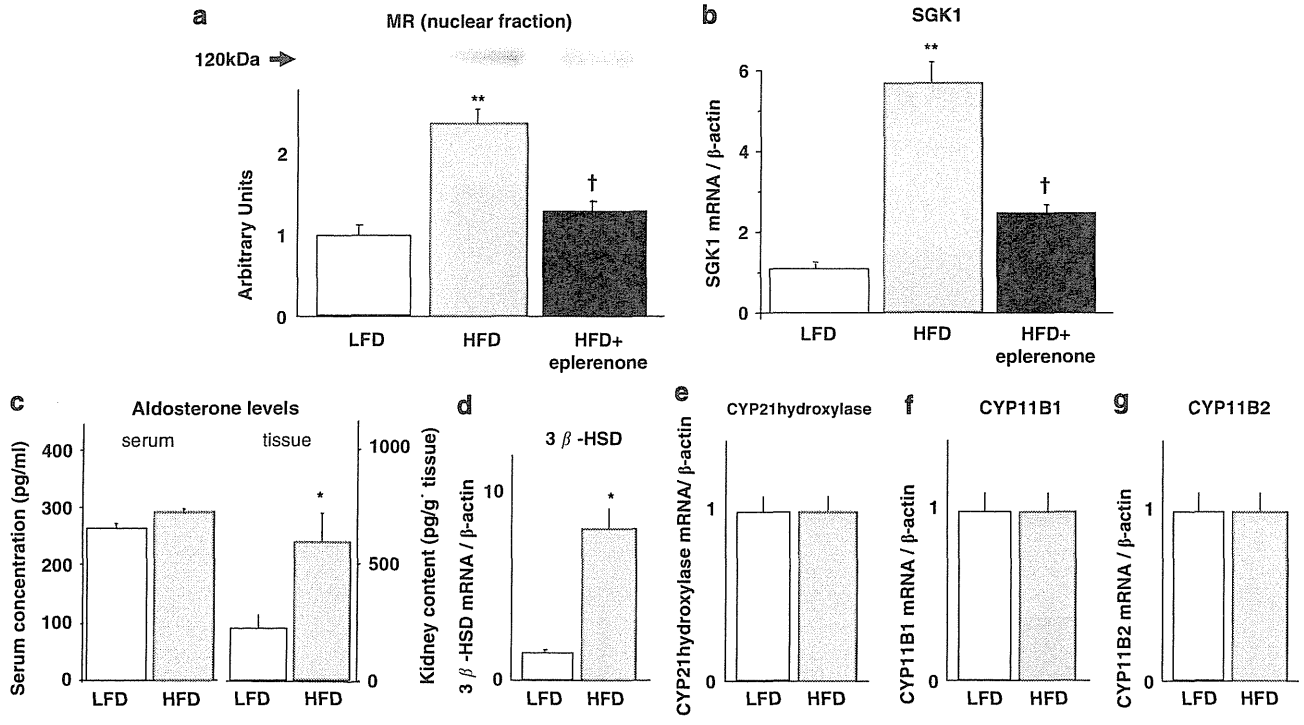

Figure 3. MR and SGK1 expression and aldosterone synthesis enzyme expression in kidneys from HFD-fed mice. (**a**) The protein in the nuclear fraction representing the MR was increased in HFD, suggesting that MR was enhanced with obesity. (**b**) HFD-fed mice showed increases in renal expressions of SGK1, which was attenuated by eplerenone. Data were expressed as the ratio of mRNA level of SGK1 to that of β-actin in arbitrary units (a.u.), relative to controls assigned as a value of 1. (**c**, left) Serum aldosterone levels did not differ between mice on LFD and those on HFD. (**c**, right) Tissue aldosterone in the kidneys was markedly increased. (**d**) HFD-fed mice showed increases in renal expressions of 3β-HSD, when compared with mice on LFD. Other aldosterone-synthesizing enzyme CYP21 hydroxylase (**e**), CYP11B1 (**f**) or B2 (**g**) was unchanged in mice on HFD. Data were expressed as the ratio of mRNA levels of 3β-HSD (**d**), CYP21 hydroxylase (**e**), CYP11B1 (**f**) and CYP11B2 (**g**) to that of β-actin in arbitrary units (a.u.), relative to controls assigned as a value of 1. Data were expressed as mean ± s.e.m. $^{*}P<0.05$, $^{**}P<0.01$ vs mice on LFD; $^{\dagger}P<0.05$ vs untreated mice on HFD.

increased by threefold in mice on HFD ($P<0.05$). The effects of HFD on the enzymes of aldosterone synthesis in renal tissues were evaluated. In HFD-fed mice, mRNA of 3β-HSD was upregulated (8.0-fold, $P<0.05$; Figure 3d). Other enzymes of aldosterone synthesis, including 21 hydroxylase, CYP11B1 and B2, were unaltered in mice on HFD (Figure 3e–g).

Rho-kinase activity in the kidney

Whether obesity enhanced the renal Rho/Rho-kinase pathway was examined. HFD significantly increased the level of Thr696-phosphorylated MYPT1 in renal tissues (2.0-fold induction, $P<0.05$ vs LFD; Figure 4a). The enhanced Rho-kinase activity was nearly completely abolished by the treatment with eplerenone ($P<0.05$ vs HFD). In contrast, neither p42/44 nor p38 was changed in mice on HFD (Figure 4b and c).

Activation of Rho/Rho-kinase pathway by aldosterone in an MR-dependent manner

Whether the MR activation enhanced Rho kinase was examined in cultured HMCs, using aldosterone as an MR agonist. Increasing concentrations of aldosterone elevated the phosphorylation levels of MYPT1, with a 2.2-fold elevation observed at 1 nmol l^{-1} ($P<0.05$ vs quiescent; Figure 4d). Stimulation with aldosterone (1 nmol l^{-1}) increased the phospho-MYPT1 levels at 90 min (1.9 ± 0.2-fold induction) and 3 h (1.4 ± 0.1-fold induction; Figure 4e). Preincubation with eplerenone (10 μmol l^{-1}) attenuated the aldosterone-induced increase in MYPT-1 phosphorylation in a dose-dependent manner (Figure 4f).

Effects of insulin on MR signaling pathway

SGK1 was upregulated with high concentration of insulin, but not with low concentration of insulin (2.5-fold, $P<0.05$; Figure 4g).

Effects of fasudil on systemic blood pressure, renal function and metabolic parameters

Systolic BP, mean BP and heart rate were unaltered by fasudil ($P>0.5$; Figure 5a–c). Serum creatinine levels in obese mice did not differ (LFD, 0.13 ± 0.02 mg dl^{-1}; HFD, 0.17 ± 0.05 mg dl^{-1}; HFD with fasudil, 0.13 ± 0.03 mg dl^{-1}; $P>0.1$). Albuminuria was markedly increased in mice on HFD, which was reduced by the treatment with fasudil ($P<0.01$; Figure 5d). HFD-induced obesity and enlarged kidneys were ameliorated by the treatment with fasudil ($P<0.05$; Figure 5e and f). Fasudil did not affect the amount of food intake (food intake, LFD, 2.0 ± 0.1 g per day; HFD, 2.0 ± 0.1 g per day, HFD with fasudil, 2.1 ± 0.1 g per day).

Effects of fasudil on renal morphological changes and renal expression of inflammatory chemokines

Marked infiltration of macrophages was observed in mice on HFD (Figure 6a and f). The HFD-induced changes in glomerular size and cellularity were nearly completely abolished by the treatment with fasudil (Figure 6b, d and e). The stain-negative round spots were increased in obese mice and were reduced by fasudil (Figure 6c and g).

Furthermore, the upregulated renal expressions, mRNAs (Figure 6i, k and l) and protein levels (Figure 6h and j) of MCP-1, TNF-α and PDGF-B in HFD-fed mice were abolished by the treatment with fasudil.

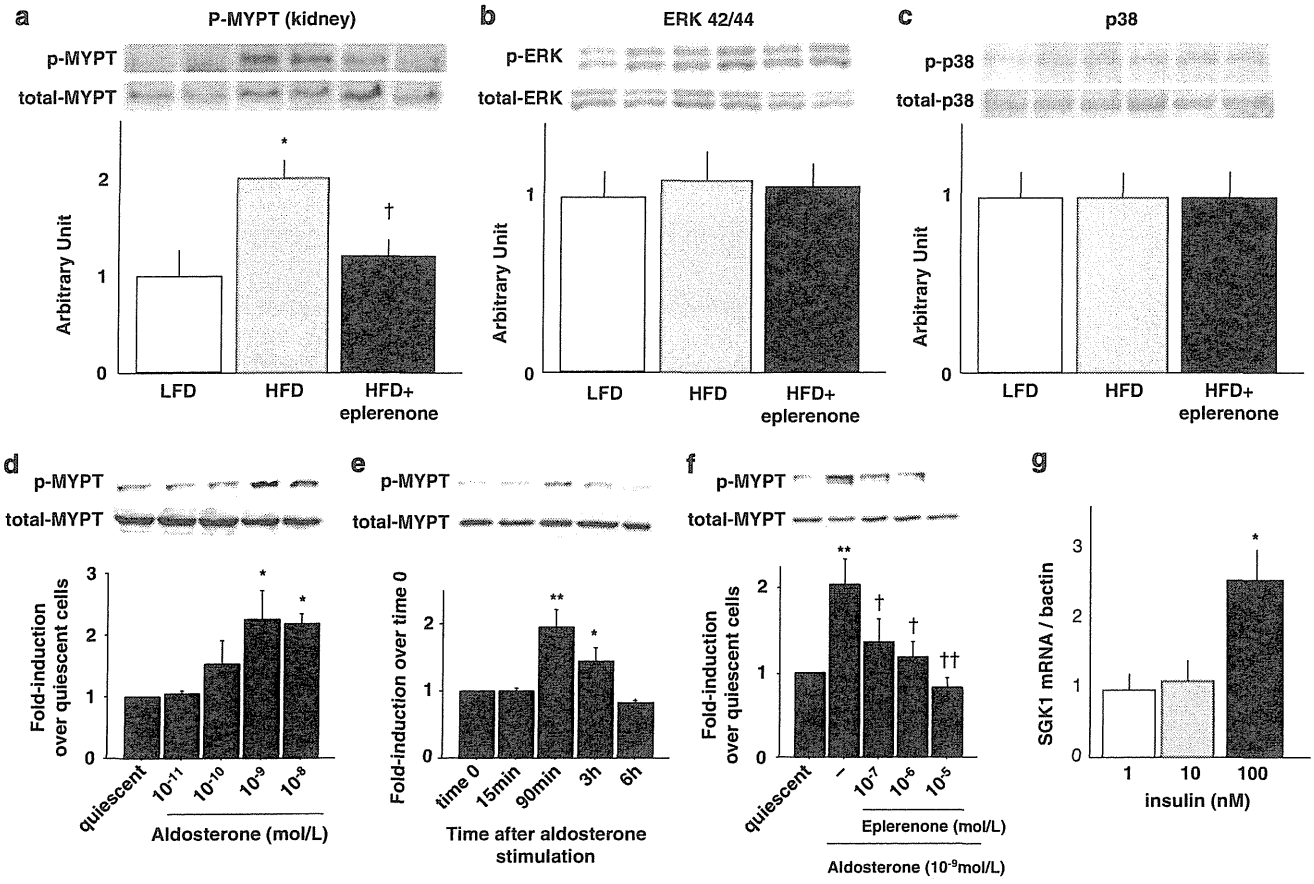

Figure 4. (Upper) Rho-kinase activity in the kidney of mice on HFD and the effects of eplerenone on the Rho-kinase activation. (**a**) Phosphorylation of MYPT was significantly increased in mice on HFD. The activation of Rho kinase was completely blocked by eplerenone treatment. (**b, c**) p42/44 or p38 in the kidney was unchanged in mice on HFD. Data were expressed as mean ± s.e.m. $^{*}P<0.05$ vs mice on LFD; $^{\dagger}P<0.05$ vs untreated mice on HFD. (Lower) Effects of aldosterone on Rho/Rho-kinase activity and effects of insulin on MR signaling pathway in primary mesangial cells. The stimulation with aldosterone increased Rho-kinase activity in a dose- (**d**, $n=4$) and a time-dependent manner (**e**, $n=4$). (**d**) Mesangial cells were incubated with various concentration of aldosterone for 1 h and the activation of Rho kinase was assayed by immunoblotting. Densitometric analysis of immunoblots is shown as values normalized by the expression levels of total MYPT1. $^{*}P<0.05$ vs quiescent cells. (**e**) Stimulation with aldosterone (1 nmol l^{-1}) significantly increased the level of phospho-MYPT1 at 90 min and 3 h. $^{**}P<0.01$, $^{*}P<0.05$ vs time 0. (**f**) Pre-incubation with eplerenone (10 μmol l^{-1}) attenuated the aldosterone-induced increase in MYPT-1 phosphorylation in a dose-dependent manner. $^{**}P<0.01$ vs quiescent; $^{\dagger\dagger}P<0.01$, $^{\dagger}P<0.05$ vs aldosterone stimulation without pre-treatment. (**g**) mRNA levels of SGK1 with high insulin treatment. $^{*}P<0.05$ vs 1 nM, 10 nM of insulin. Results are presented as mean ± s.e.m.

DISCUSSION

Obesity and metabolic syndrome are important risk factors not only for cardiovascular complications, but also for the development of proteinuria and CKD. Multiple factors are assumed to contribute to the development of CKD in obesity, including systemic hypertension and dyslipidemia. In this study, we have demonstrated that HFD-induced obesity causes marked renal pathological changes, including glomerular hypercellularity, infiltration of macrophages and stain-negative round spots, which may represent increases in lipid droplets in kidneys from obesity (Figures 2 and 6). Furthermore, these alterations were prevented by the blockade of MR with eplerenone without alterations in systolic and mean BP. In the previous dog study, eplerenone actually lowered BP in obese animals.[31] In contrast to the dog studies, blood pressure tended to be reduced with eplerenone, but did not attain statistical significance in this study. Because systolic and mean BP were not changed by HFD, the effect of eplerenone might be small. It can be concluded that BP-independent effects of eplerenone are responsible for the reduction in renal injuries. Concomitantly, both nuclear MR protein levels and SGK1 expression in the kidney were elevated in HFD-fed mice, and these actions were abrogated by eplerenone (Figure 3). These observations suggest that the aldosterone/MR pathway constitutes a determinant of the development of CKD in obesity-related nephropathy. Alternatively, the intervention in the aldosterone/MR pathway would provide a clue to the novel therapeutic strategy in obesity-associated nephropathy.

Aldosterone is a potent mineralocorticoid that promotes renal sodium retention and induces hypertension. Several lines of studies have shown that increased serum aldosterone levels are linked to the development of obesity-associated hypertension.[32] Furthermore, accumulating evidence suggests that the excess aldosterone/MR activity provokes proteinuria and podocyte injury.[33] In rats with remnant kidney models, aldosterone administration increases proteinuria during the blockade of angiotensin II action with an angiotensin receptor blocker.[33] Increases in multiple factors, including mitogen-activated protein kinase,[15] plasminogen activator inhibitor-1,[34,35] transforming growth factor-β1,[20] MCP-1[refs 14,20] and reactive oxygen species,[15,36] have also been observed in renal tissues of aldosterone-infused animal models. Furthermore, SGK1 is considered as one of the main effectors of aldosterone.[37] Conversely, the blockade of MR with eplerenone substantially suppresses these parameters, and

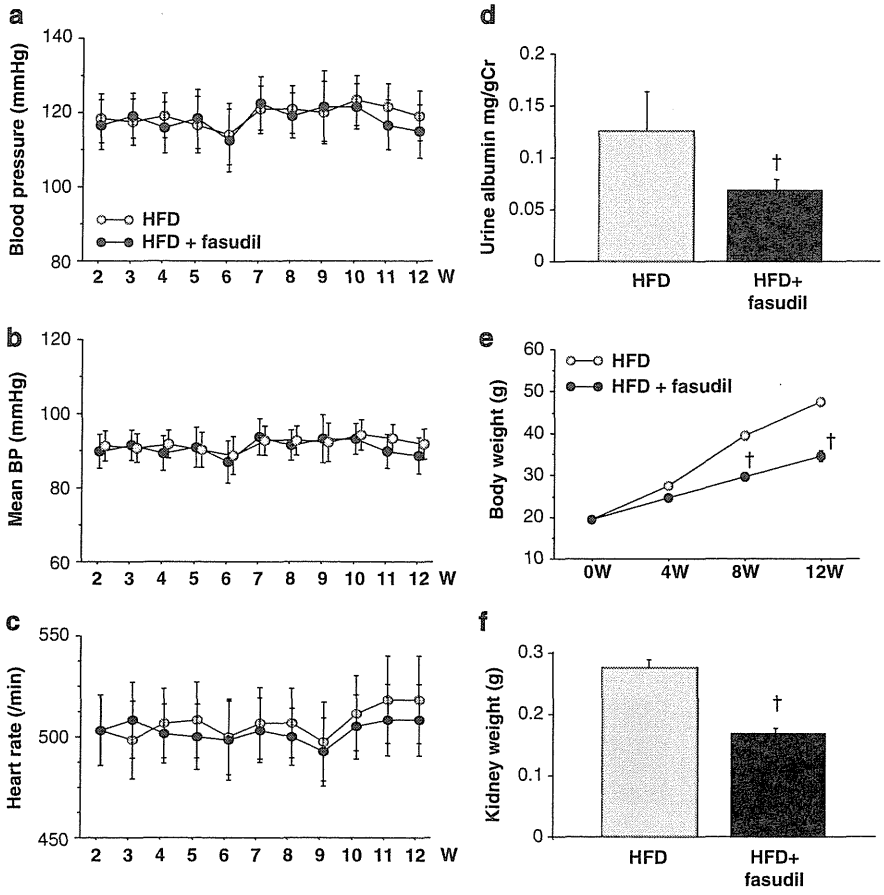

Figure 5. Effects of fasudil on animal phenotype. (**a**–**c**) Systolic BP, mean BP and heart rate were unaltered by fasudil. (**d**) Increased albuminuria in mice on HFD was reduced by fasudil. (**e**, **f**) The diet-induced obesity and enlarged kidneys were ameliorated by the treatment with fasudil. Data were expressed as mean ± s.e.m. Cr, creatinine. $^{\dagger}P<0.05$ vs untreated mice on HFD.

alleviates the renal injury induced by aldosterone.[38,39] In this study, we have demonstrated that the blockade of MR with eplerenone ameliorates the obesity-induced renal injury and abrogates the upregulated expression of MCP-1, TNF-α and PDGF-B (Figure 2), as well as SGK1 (Figure 3) in the kidney. These results suggest that obesity-associated renal injury involves the aldosterone/MR-mediated signaling pathway and renal inflammatory process. Although BP and serum aldosterone levels are not changed in this study, the renal arterioles tone may be changed by the reduced afferent arteriolar tone[40] and the elevated renal tissue aldosterone.[41] The observed findings (that is, enlarged glomeruli and glomerular hypercellularity) may be induced not by only the overexpression of inflammatory cytokines, but by hyperfiltration of the glomeruli.

Of note, our current study shows the elevation in renal tissue aldosterone contents and the activation of the MR-mediated signaling pathway in obese mice, despite unaltered serum aldosterone levels (Figure 3). Several lines of studies demonstrate that the MR signaling pathway is activated by a variety of factors, including insulin,[42] renal sympathetic nerve activation[43] and Rac1.[28] Aldosterone biosynthesis is mediated by several enzymatic pathways, including 3β-HSD, CYP11B2, CYP11A1 and 21-hydroxylase in the adrenal cortex. A recent study has reported that mesangial cells express the mRNA of 3β-HSD, CYP11B2 and 21-hydroxylase.[44] Mesangial cells are an aldosterone-producing tissue, in which LDL plays a major regulatory role in the expression of 3β-HSD and aldosterone production.[44] In this study, elevated renal tissue aldosterone contents but not serum aldosterone are supposed to account, at least in part, for the activation of the MR pathway in obesity. Of note, in *in vitro* study, high concentration of insulin induced the overexpression of SGK1 (Figure 4). These results show the link between metabolic disorders and MR signaling pathway in obesity.

Our study raises the possibility that tissue aldosterone is locally produced through the upregulation of 3β-HSD in obesity and contribute to effects in the renal glomerulus independently of the systemic renin–angiotensin–aldosterone system. In this regard, the transcription of these genes is regulated through the activation of signaling cascades that could be affected by adipocytokines.[45] Whether tissue aldosterone could be produced under the condition that aldosterone synthases other than 3β-HSD were not changed is not clear. Alternative explanations include increased aldosterone tissue uptake or decreased degradation within tissues. The precise mechanisms for the activation of the MR pathway and the enhanced renal aldosterone production in kidneys from obesity warrant further investigations.

This study has demonstrated the crucial role of Rho/Rho-kinase pathway in the development of nephropathy of non-genetic and HFD-induced obesity in C57BL/6J mice, a mouse model of metabolic syndrome. Evidence has been accumulated that Rho kinase is activated by several stimuli[46] and is involved in the pathogenesis or aggravation of renal damage in several renal injury and hypertensive models, including subtotally nephrectomized SHR,[15] Dahl salt-sensitive rats[47] and aldosterone-infused rats.[20] In this study, we have demonstrated that HFD-induced obesity causes enhanced Rho-kinase activity in the kidney tissue (Figure 4). We also showed the renoprotective effects of the Rho-kinase inhibition (Figures 5 and 6). Furthermore, the activation of

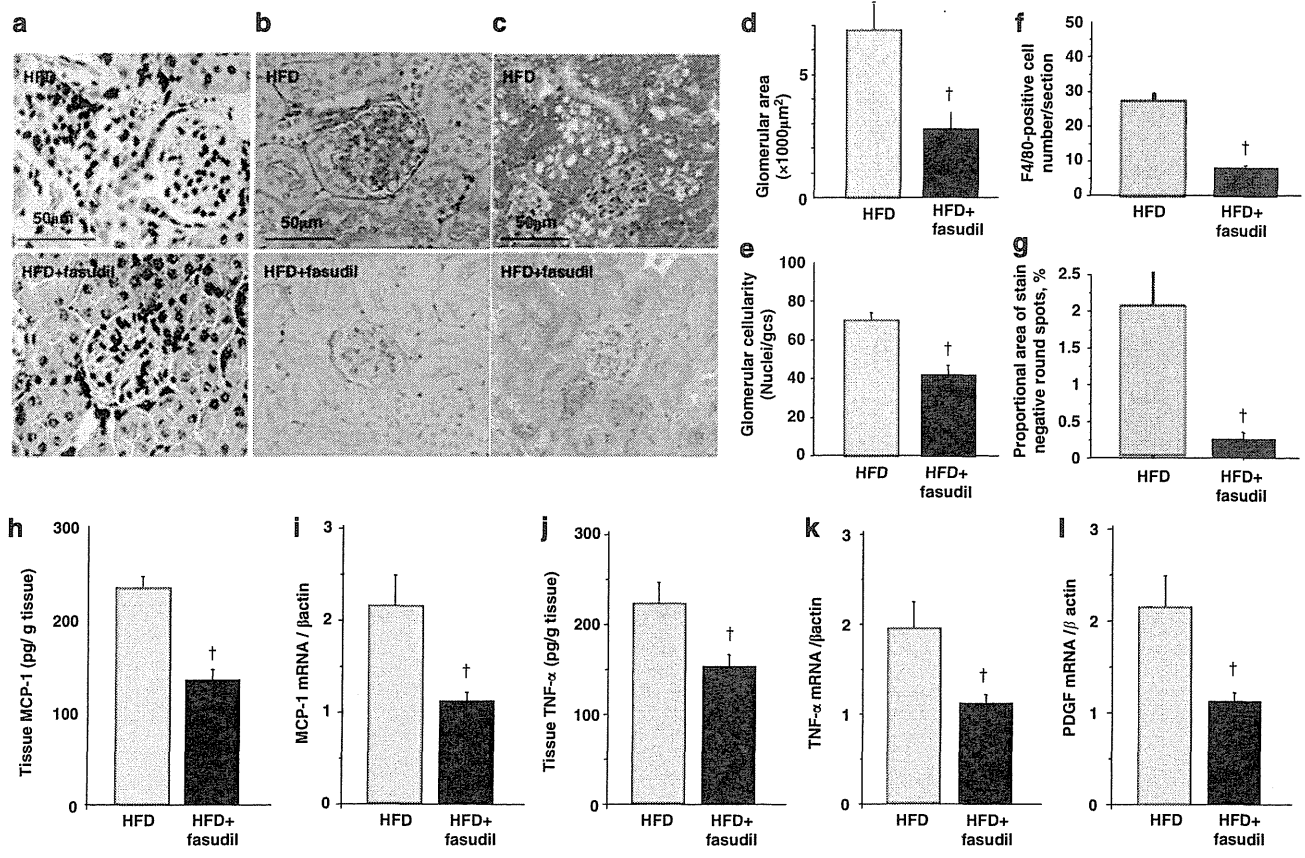

Figure 6. Effects of fasudil on renal morphological changes and renal expression of inflammatory chemokines. (**a**) Histology of F4/80-stained kidney section from mice on HFD and HFD with fasudil. (**b**) Periodic acid-Schiff's-stained kidney section. (**c**) Masson's modified trichrome-stained kidney section. (**d**, **e**) The HFD-induced changes in glomerular size and cellularity were nearly completely abolished by the treatment with fasudil. (**f**) Macrophages were markedly infiltrated in the renal tissue of mice on HFD, which was improved by the treatment with fasudil. (**g**) The stain-negative round spots are increased in obese mice and were reduced by fasudil. HFD-fed mice showed increases in renal expressions of MCP-1 (**h**, **i**), TNF-α (**j**, **k**) and PDGF-B (**l**), all of which were attenuated by fasudil. Data were expressed as the ratio of mRNA levels of MCP-1 (**i**), TNF-α (**k**) and PDGF-B (**l**) to that of β-actin in arbitrary units (a.u.), relative to controls assigned as a value of 1. Data were expressed as mean ± s.e.m. $^{\dagger}P<0.05$ vs untreated C57BL/6J mice with HFD.

Rho kinase is prevented by the treatment with eplerenone. *In vitro* study shows that MR stimulation activates Rho kinase in HMCs, which is inhibited by pre-treatment with eplerenone (Figure 4). Since Rho-kinase activation was observed with a peak at 90 min after aldosterone stimulation, non-genomic mechanisms appear to be involved in obesity-induced MR stimulation and the subsequent activation of Rho kinase. Of interest, a previous study shows that in rats treated chronically with aldosterone and salt, renal injury is associated with the activation of mitogen-activated protein kinase, including ERK1/2.[26] In our study, however, mitogen-activated protein kinase, ERK1/2 or p38, is not activated in obese mice (Figure 4). Thus, our findings suggest that the enhanced aldosterone/MR signaling pathway plays a key role in renal injury in obesity through the activation of Rho kinase.

Although this study demonstrates activation of MR and Rho kinase in kidneys from obese mice, the subsequent mechanisms for mesangial cell proliferation and macrophage infiltration remain undetermined. Rho kinase is reported to be linked to multiple factors that facilitate inflammation and tissue fibrosis.[48] Furthermore, our study shows that a Rho-kinase inhibitor (fasudil) abolishes the HFD-induced overexpression of MCP-1, TNF-α and PDGF-B (Figure 6). In this study, we have found that the overexpression of MCP-1, TNF-α and PDGF-B in the renal tissue of obese mice is abrogated by the blockade of the aldosterone/MR pathway (Figure 2). As eplerenone suppresses the upregulated Rho kinase in kidneys of obese mice (Figure 4a), these cytokines are anticipated to contribute importantly to the development of obesity-induced nephropathy as factors linking between aldosterone/Rho/Rho-kinase pathway and renal injury. The inhibition of Rho kinase by eplerenone may thus offer beneficial action on obesity-related nephropathy through the modification of these cytokines. We have demonstrated that HFD-induced obesity showed the round-shape stain-free spots in tubules, which may be a reminiscence of lipid accumulation areas. Of note, the degree of stain-free areas parallels the levels of several inflammatory markers, including MCP-1, TNF-α and macrophage infiltration, consistent with direct inflammatory changes by lipid accumulation in renal cells. In addition to MR/Rho-kinase pathway, this lipid accumulation may directly cause the overexpression of inflammatory cytokines, leading to the infiltration of inflammatory cells and renal injury.

Finally, the treatment with eplerenone partly prevented all the metabolic changes, including weight gain and hyperinsulinemia, in this study. Thus, it is difficult to discern which effects are due to normalization of obesity and which are due to direct action on the kidney. As we have shown in this study, direct action by the inhibition of MR/Rho/Rho-kinase pathway on the kidney accounts at least for the amelioration of the pathological changes and proteinuria in obesity-induced renal injuries. Moreover, the normalization of obesity itself could be due to the inhibition of MR/Rho/Rho-kinase pathway. This study has unveiled a novel observation showing a possible role of aldosterone in the

aggravation of obesity. Thus, we demonstrate that HFD-fed obese mice manifest a smaller body weight gain when treated with eplerenone (Figure 1e). In this regard, we previously reported that the inhibition of Rho kinase with fasudil alleviated the increase in body weight in Zucker obese rats, a genetic model of obese animals.[13] Of note, we have recently demonstrated that in cultured adipocytes, lipid accumulation after the differentiation elicits Rho-kinase activation. Furthermore, mechanical stretch of adipocytes elicits enhancement in Rho-kinase activity.[49] It is surmised therefore that hypertrophic process during lipid accumulation in adipocytes involves Rho-kinase activation through mechanical stretch as well as MR signaling pathway stimulation, and subsequently induces obesity. The intervention of Rho/Rho kinase and the MR pathway may constitute a novel strategy disrupting vicious circles aggravating obesity.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Ⅵ. 慢性腎臓病と各種疾患

6. 肥満・メタボリックシンドローム

脇野　修　伊藤　裕

要　旨

肥満・MetsはCKDのリスクにもなっている．肥満に伴う腎障害は巣状糸球体硬化症を主体とし，肥満関連腎症と呼ばれている．CKDでは腎性インスリン抵抗性症候群，腎性脂質異常症，腎性高血圧といったMetsと類似の病態を呈し両者は腎症を増悪させる可能性もある．治療の基本はMets・肥満の是正であり減量となるがCKDに存在するprotein energy wasting syndromeを考慮すると過度のカロリー制限は危険である．

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Key words　慢性腎臓病，肥満，メタボリックシンドローム，腎性インスリン抵抗性症候群

はじめに

メタボリックシンドローム（Mets）は心血管事故（CVD）リスクファクターとして注目されている．Metsは腹部肥満を基盤病態とし，糖代謝異常，脂質代謝異常，高血圧が合併した病態と定義されるが，肥満・Metsが慢性腎臓病（CKD）のリスクにもなっていることが注目されている．本稿では肥満・Metsに伴う腎障害とその治療戦略，管理上の問題点について述べるとともに，その逆の病態すなわち，CKDによるMetsの構成因子への影響についても解説する．

1. 疫　学

肥満と腎障害の関連を示す疫学的事実が報告されている．本邦における100,000人の沖縄のコホートを用いた前向き調査によれば，BMI（body mass index）の上昇に伴い高血圧や尿蛋白の存在とは独立に末期腎不全に至るリスクが上昇することが示されている．320,000人の米国のコホートを用いた検討でも同様の結果が報告されており，他の末期腎不全のリスクで調整したのちもなおBMIが末期腎不全のリスクであることが示されている．正常血圧のIgA腎症，片腎の患者においては血圧に関係なく肥満が腎障害の危険因子となっていること，肥満（BMI＞30）の健常者からの移植腎はやせ（BMI＜25）の健常者よ

慶應義塾大学腎臓内分泌代謝内科

Chronic Kidney Disease (CKD)—Recent Progress. Topics : VI. Chronic Kidney Disease (CKD) and Associated Disorders ; 6. CKD associated with obesity and metabolic syndrome.

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りの移植腎と比べて障害が多いことは肥満そのものと腎障害との関連を示唆するものである．またMetsと腎障害との関連についても同様に報告されている．NHANESIII研究では7,800人の集団が21年以上観察されており，Metsを有するとCKDとなるオッズ比が2.6倍であると報告されている．本邦の久山町研究ではオッズ比は2倍であった．その一方で肥満・Metsに合併する高血圧，耐糖能異常，脂質異常症の影響を完全に排除した臨床試験はない．さらにMetsの診断基準より明らかなようにBMIよりも内臓脂肪，腹部肥満のほうが腎機能障害とよく相関するというデータも存在する．肥満・Metsに伴う腎症のより正確な病態解明が必要と思われる[1]．

2. 肥満に伴う腎障害の病理所見

肥満に特徴的な腎病理所見として，肥満関連腎症obesity-related glomerulopathy（ORG）の概念が提唱されている．まず1974年に肥満症と蛋白尿との関連が初めて報告された．その後2001年にKambhamらは腎生検6,818症例中のBMI＞30の肥満71症例の解析を行った．その結果，ORGは腎生検施行例の2%の頻度であること，1986年から2000年の15年で10倍に頻度が増加していること，原発性のFSGS（focal segmental glomerulosclerosis）と比較してネフローゼの頻度が低く，血清アルブミンのレベルは高く，血清コレステロールのレベルは低く，浮腫の頻度が低いことなどが明らかとなりORGという疾患概念が確立した．現在，ORGは1．病的な肥満症（BMI＞40），2．浮腫を認めない蛋白尿，3．正常血清アルブミン値の3つをtriadとし，高血圧による腎硬化症および糖尿病腎症とを除外したものと定義される．予後については先述のKambhamらの報告によれば，8年間の観察期間で14%が血清クレアチニン値の倍加，3.6%で末期腎不全への進行が認められ，必ずしも良くないと考えられている[2]．

このORGで認められる腎病理所見は肥満に伴う糖代謝異常，高血圧，脂質代謝異常が関与し，病期としても尿蛋白がある程度認められる進行した腎障害を見ている可能性が高い．しかしこれらの合併症のない状態での腎生検所見は生検をする機会も少なく報告は少ない．この点についてReaらは腎障害のない肥満（BMI≧30，49名）および正常（BMI＜30，42名）の腎移植ドナーの腎生検所見を比較し検討している．その結果尿細管腔の拡張，尿細管の空胞の減少以外は病理組織に変化が認められなかったとしている．肥満に伴う腎障害の初期の変化が尿細管を中心とする障害であることは興味深い．

3. 肥満に伴う腎障害の発症機序

さまざまな因子が肥満に伴う腎障害の機序として想定されている．

1）アディポサイトカイン（adipocytokine）

脂肪組織より分泌されるcytokineすなわちadipocytokineが腎障害に重要な役割を有する．肥満の血圧調節，腎機能に関与する主たるadipocytokineにはアンジオテンシノーゲン，レプチン（leptin），アディポネクチン（adiponectin）などがあげられる．leptinの血中レベルは肥満においては上昇し，leptinは摂食を減少させ，エネルギー消費を亢進させる作用を有する．leptin受容体が腎臓において強く発現していることより，leptinの腎臓における直接作用が想定されている．leptinは培養糸球体内皮細胞においてTGFβ（tumor growth factor β）の発現を亢進させ，leptinの持続注入モデルでは血圧に変化をきたさずにタンパク尿，糸球体硬化が認められた．以上よりleptinが肥満関連腎症の発症因子の一つと考えられている．さらに近年adiponectinの腎臓における意義が明らかとなった．adiponectinの作用はインスリン感受性の亢進，抗炎症，抗動脈硬化作用

などである．肥満，糖尿病，インスリン抵抗状態においてはその血中レベルが低下することが知られている．adiponectine欠損マウスでは腎臓のpodocyteの足突起の融合が認められ，アルブミン尿が認められた．そしてadiponectine欠損マウスにadiponectineを補充すると病理組織の正常化およびアルブミン尿の改善が認められた．adiponectineの低下が肥満における腎障害の機序を説明する新たな因子と注目されている．

2）腎細胞内の脂肪蓄積

脂肪そのものが腎細胞，尿細管細胞，メサンジウム細胞，内皮細胞に障害を引き起こすことが知られている．これを腎臓におけるlipotoxicityという．肥満における内臓脂肪の蓄積は遊離脂肪酸の血中レベルを上昇させる．この遊離脂肪酸は細胞内でミトコンドリアに取り込まれ，β-酸化に利用される．肥満においてはそのミトコンドリアにおける遊離脂肪酸の取り込みが低下する．そのため，細胞内に蓄積する脂肪酸およびその代謝産物（diacylglycerol, fatty acy CoA, ceramide）などがprotein kinase C，NF-κBを活性化し，炎症や細胞死を引き起こす．Leviらのグループは脂肪酸やコレステロール生合成のmaster geneであるSREBP（sterol-regulatory element binding protein）の発現が亢進していることが肥満の腎臓のlipotoxicityの原因であるとしている．SREBPは転写因子でSREBP-1a, SREBP-1c, SREBP-2 に分類されSREBP-1 は細胞内の脂肪酸合成に，SREBP-2 はコレステロール合成に関与する．肥満の腎臓ではこれら転写因子の発現が亢進し，細胞内にlipidが蓄積し，細胞障害に働くことを報告している．さらに脂質異常の腎機能に及ぼす影響についてTG(triglyceride)-rich lipoproteinの腎毒性が指摘されている．VLDL（very low density lipoprotein）およびLDL（low density lipoprotein）受容体がメサンジウム細胞に発現しておりメサンジウム細胞の増殖，TGF-βの発現亢進を引き起こすことが報告されている．またアルブミンに結合している遊離脂肪酸（albumin-saturated free fatty acid）の尿細管細胞障害も指摘されている．アルブミン尿中に存在するalbumin-saturated free fatty acidは脂溶性であり細胞膜を通過し，細胞障害や炎症を引き起こすことが報告されている[3]．

3）腎血行動態の異常

肥満特有の血行動態が腎症を引き起こす一つの因子となっている可能性が示唆されている．肥満の患者は交感神経活性の亢進，Na（ナトリウム）再吸収の亢進，RAS（renin angiotensin system）の亢進等により難治性の高血圧をきたしやすい．この高血圧が腎障害を引き起こす．さらに糸球体の過剰濾過も腎症進行の危険因子となっている．その原因としてRAS系の亢進，ネフロン数の低下のほかに内臓脂肪組織増加に伴う腎実質の圧迫が挙げられる[3]（図1）．内臓脂肪の増加は先述のadipocytokineの発現異常にも関連するため，高血圧，eGFR(estimated glomerular filtration rate）はBMIよりも内臓脂肪量すなわちwaist-hip ratio，腹囲径によく相関することが示されている．我々は内臓脂肪のなかでも特に腎周囲の脂肪組織の意義に注目している．以前より内臓脂肪の除去によりラットの寿命延長が認められることが報告されている．腎周囲脂肪織の除去が肥満関連腎症の改善につながるかを検討している．

4）インスリン抵抗性と高インスリン血症

肥満の主要な病態としてインスリン抵抗性，高インスリン血症が挙げられる．肥満では腎臓のインスリン感受性が保たれており，インスリンのNa再吸収作用が亢進し肥満高血圧の原因となることが知られている．またインスリン抵抗性は血管内皮細胞においては一酸化窒素の合成の低下を引き起こし動脈硬化の原因となる．これら間接的な原因でインスリン抵抗性，高インスリン血症が腎障害を引き起こす．一方インスリンの直接作用も重要である．ポドサイト特異

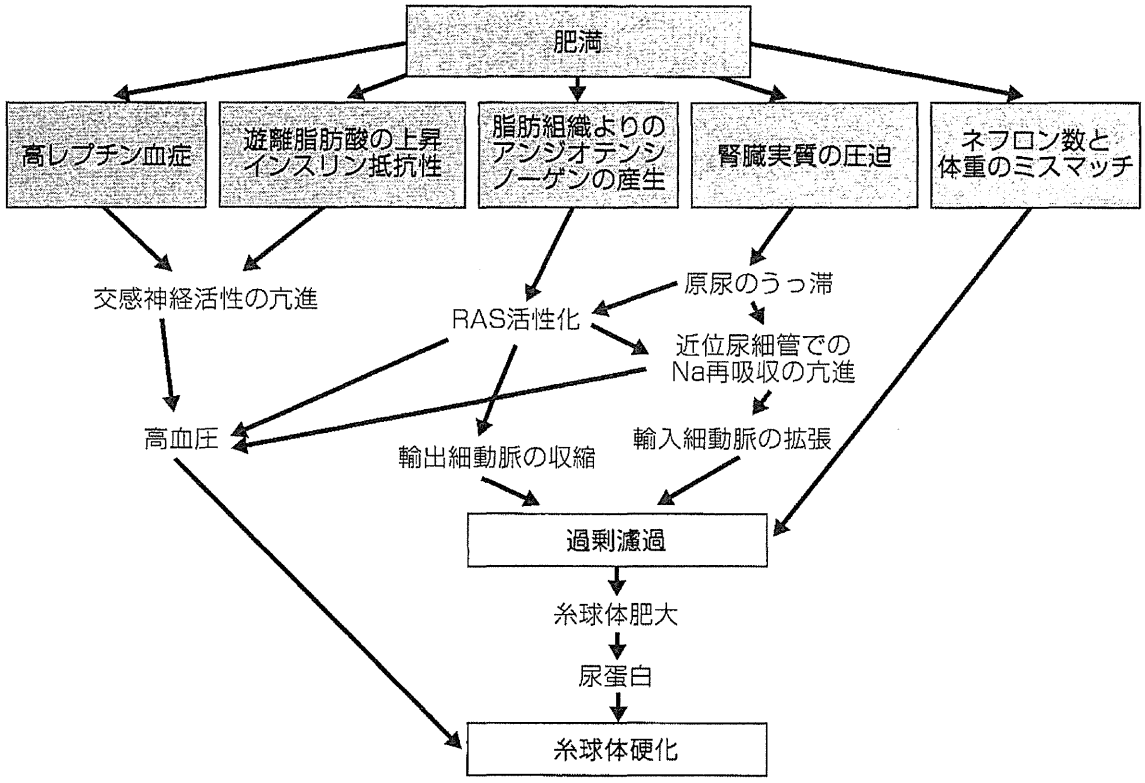

図 1. 肥満症における腎血行動態.
RAS, renin-angiotensin system

的なインスリン受容体欠損マウスでは生後5週で尿蛋白陽性となりメサンジウム領域の増生，基底膜の肥厚など糖尿病性腎症に似た組織像を呈することが報告されている．すなわちインスリン抵抗性が腎臓に生じ，ポドサイトにインスリンシグナルが入らなくなると腎症が発症することが考えられる．また細胞レベルで高インスリン血症がRASの活性を亢進させることが言われている[4]．尿細管におけるインスリン受容体の発現は高く尿細管のエネルギー代謝，糖の取り込みに重要である可能性が示唆される．したがってインスリン抵抗性，高インスリン血症が腎尿細管のエネルギー代謝に影響を及ぼす可能性も考えられる．

4. CKDにおける代謝異常

Metsおよびその各コンポーネントはCKDの発症進展因子となるが，その逆の関連も存在し，CKDがMetsの各コンポーネントを増悪させ，それが悪循環を形成するという病態が考えられる．この悪循環の遮断は治療戦略上重要となる（図2）．この項ではCKDのMetsのコンポーネントに対する影響について述べる．

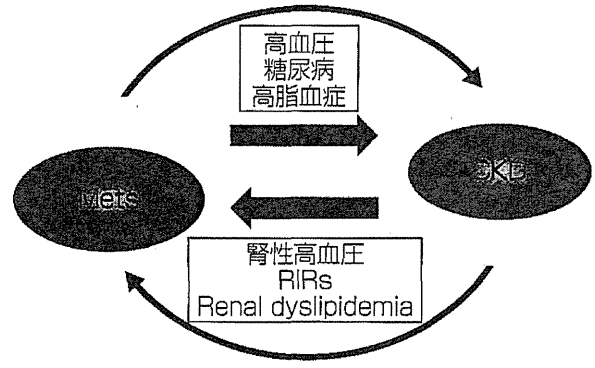

図 2. MetsとCKDとの間の悪循環

トピックス

1）腎性インスリン抵抗性症候群（renal insulin resistance syndrome：RIRs）

CKDの早期においてもすでにインスリン抵抗性が生じているということが報告されている．BeckerらはCKDのstage 1の段階でもHOMA指数で算出したインスリン抵抗状態が既に上昇していることを報告しており，RIRsと命名している[5]．また糖尿病でなくともeGFRが50 ml/分/1.73 m^2以下になればインスリン抵抗性が認められることがグルコースクランプ法で証明されている．このRIRsのメカニズムについては活性型ビタミンDの欠乏，腎性貧血，尿毒素物質がこれまで報告されている．またCKDの患者はTNFα（tumor necrosis factor α），IL-6（interleukin-6）といったインスリン抵抗性を引き起こすサイトカインが上昇していることが報告されている．これらサイトカインの血中レベルでの異常は腎臓でのクリアランスの低下とともに脂肪組織での発現異常が原因であることが指摘されている[4]．我々はRIRsの発症機序として1．内因性のNO（一酸化窒素）合成酵素阻害物質であるADMA（asymmetric dimethylarginine）の脂肪組織局所での濃度上昇，2．腎機能障害に伴う血中アルドステロン濃度の上昇が重要であることを明らかにしている．

2）腎性脂質異常症（renal dyslipidemia）

CKDには早期より特有の脂質異常症が存在する[6]．その特徴は低HDL（high density lipoprotein）コレステロール（HDL-C）血症と高中性脂肪（TG）血症であり，Metsと同じであることは興味深い．低HDL-C血症の原因となっているのはApoA-IおよびApoA-IIレベルの低下である．その他TG-richなリポ蛋白であるVLDLの上昇，コレステロール逆転送系においてHDLにコレステロールを添加する酵素であるLCAT（lecithin cholesterol acyltransferase）の活性が低下していることなどが想定されている．一方高TG血症の原因としては1．ApoC-IIIレベルの上昇，2．腎臓での遊離脂肪酸のクリアランスの低下，3．lipolysisに重要な2つの酵素LPL（lipoprotein lipase）及びHL（hepatic lipase）の活性の低下，4．TG-richなリポ蛋白であるVLDLを肝臓に取り込むためのVLDL受容体のレベルの低下などが報告されている．先述のようにadipocytokineの発現異常がCKDの脂肪組織では存在していることを考えると，中性脂肪の貯蔵器官としての脂肪組織の機能異常が示唆される．我々は脂肪組織の成熟異常がこの機能異常を引き起こしrenal dyslipidemiaの原因となる可能性につき検討している．

3）危険因子の逆転現象（reverse epidemiology）とprotein-energy wasting syndrome（PEW）

Metsでは腹部肥満を基盤として高血圧，耐糖能異常，脂質異常症が合併することに主眼が置かれた診断基準となっている．したがって，減量，BMIの低下，降圧，脂質のコントロールがCVD発症の予防となる．ところがコレステロールの低下を含めたこの古典的なリスクファクターの管理がCVDの発症，死亡率に逆に作用する現象が報告されている．これが危険因子の逆転現象（reverse epidemiology：RE）であり，維持透析患者およびCKDにおいてはBMIの低下がCVD発症のリスクになり，BMIの上昇により死亡率が低下することが報告されている[8]（表1）．その他，維持血液透析患者では低血圧，低コレステロール血症，低ホモシステイン血症において有意に死亡率が高いことが報告されている[8]．Kovesdyらは透析前のCKDのコホートを血圧で4群に分け死亡率を調べたところ収縮期血圧133 mmHg未満の群をコントロールとして133～154 mmHg，155～170 mmHg，170 mmHgの各群の総死亡のハザード比は各々0.61, 0.62, 0.68であった．彼らは同様の解析を総コレステロール値でも施行し，総コレステロール値215 mg/dlを超えるCKD群をコントロールとすると153 mg/dl未満，153～182 mg/dl，183～215 mg/dlの各群の総死亡のハザード比は1.91, 1.36, 1.10であった．

表1. CKDにおけるRE（文献7より抜粋）

心血管リスク	一般人口	血液透析患者	CKD患者	留意点
BMI	BMIが高ければCVDのリスクが高くなる（*N Engl J Med* 341：1097-1105, 1999）.	BMIが高ければ生存率よい（*J Am Soc Nephrol* 14：2366-2372, 2003）.	BMIが高ければ生存率が高い（*Am J Kidney Dis* 46：863-870, 2005）. BMIが高ければ生存率が低い（*Ann Intern Med* 144：701-70a, 2006）.	COPD，慢性心不全，慢性関節リウマチ，高齢者では高いBMIは高い生存率.
血圧	高血圧ほどCVDのリスクが高くなる（*N Engl J Med* 345：1291-1297, 2001）.	低血圧ほど生存率が低い（*Kidney Int Suppl* 55：S173-S174, 1996）.	収縮期血圧と生存率についてはJ-shape現象が成立する（*Nephrol Dial Transplant* 21：1257-1262, 2006）. 拡張期血圧は低いほど生存率が高い（*J Am Soc Nephrol* 16：2170-2179, 2005）.	eGFR<30で血圧と生存率の関係が逆転する（*Nephrol Dial Transplant* 21：1257-1262, 2006）.
脂質	総コレステロール，LDLコレステロール高値およびHDL-コレステロール低値はCVDのリスクである（*N Engl J Med* 322：1700-1707, 1990）.	総コレステロール高値は生存率が高い（*Am J Kidney Dis* 15:458-482, 1990）.	コレステロール，中性脂肪が低値は生存率が低い（*J Am Soc Nephrol* 18：304-311, 2007）.	コレステロール値と生存率との関係はMICSやMIA症候群において逆転する（*J Am Soc Nephrol* 18：304-311, 2007）.

COPD：chronic obstructive pulmonary disease

EvansらはBMIについての検討を行いBMI 20.1～25の群をコントロールとすると20以下，25.1～30，30を超える各群の総死亡のハザード比はそれぞれ1.49，1.10，0.96であり，BMI>30が最も生存するという結果を得ている．本邦でも日本透析学会の疫学調査によれば，週3回の血液透析患者の2000年から2001年の1年間の生存に寄与する因子を解析したところ，透析前の血圧が高い患者ほど，またBMIが高いほど生存率が高いことが明らかにされている．REにおいて興味深い現象は腎臓移植した患者ではこの逆転現象が逆転し，肥満や高コレステロール血症，高血圧が死亡率上昇に寄与することである．これをreversal of REもしくはback to normal phenomenonと呼ばれている．肥満やMetsのCKDでは減量がCKDの進行やCVD発症に有効であることも考えると，CKDの経過のどこかで危険因子の逆転現象が生じていることとなる．このREの背景に存在するのがPEWである．

PEWとはタンパク質とエネルギーすなわち脂肪やグリコーゲンの蓄積が減少し，低栄養状態を引き起こす病態であり，protein-energy malnutrition（PEM）とも言われている．血液透析患者については毎回の透析操作で透析膜との反応で生じる白血球や補体の活性化が慢性炎症状態を引き起こし，PEWの原因となっている．これはmalnutrition-inflammation complex（cachexia）syndrome（MICS）やmalnutrition-inflammation atherosclerosis（MIA）syndromeとも言われている．しかし透析操作の変更ではこの現象は消失せず，腎不全そのものがPEW発症の背景に存在していると考えられている．例えば尿毒素やleptinの上昇が食欲を低下させ，低栄養を引き起こしている．以上よりCKDのステージのどこかでPEWが発症しREが生じていると考えられ，それに基づいた栄養面の評価が必要であると思われる．肥満・MetsによるCKDではこのREの時期を見定めリスクファクターをきめ細かく管理することが重要になってくると思われる．

表2. PEWの診断基準の一例（文献10より）

定義	
血液生化学	
	血清アルブミン<3.8 g/dl
	血清プレアルブミン（トランスサイレチン）<30 mg/dl（維持透析患者のみ）
	血清コレステロール<100 mg/dl
体格	
	BMI<23 kg/m^2
	体重減少（減量をせず）3か月で5%，6カ月で10%
	体総脂肪率<10%
筋肉量	
	筋肉量の減少　3か月で5%，6カ月で10%
	上腕筋周囲径の減少（50パーセンタイルより10%の低下）
	クレアチニン産生量
食餌量	
	食事療法をしない状況で蛋白摂取量が0.8 g/kg/日が2カ月以上（維持透析患者），0.6 g/kg/日（ステージ2-5のCKD）
	食事療法をしない状況でエネルギー摂取量が<25 kcal/kg/日が少なくとも2カ月以上

5. 治療戦略

肥満・MetsにともなうCKDの治療は運動，食事制限による減量が基本であり，adipocytokine，脂肪毒性lipotoxicityなどの発症因子の発現を制御することが重要と考えられる．より積極的な治療手段としてインスリン抵抗性を改善させるPPARγ（peroxisome proliferator-activated receptor γ）リガンド，脂質異常を改善させるPPARαリガンド，スタチンなども挙げられるが，CKDの進行に伴い体液貯留，横紋筋融解などのこれらの薬剤の副作用の発現に注意すべきである．bariatric surgeryによる体内脂肪除去は糖脂質代謝異常，adipocytokineの異常を改善することが報告されているが，長期の安全性や腎障害に対する影響については未知である．Mets患者を対象としたこれらの治療戦略に関する前向きの臨床試験も今後必要と思われる．

6. Metsに伴うCKDの食事療法

CKDにおいてはPEWが存在するため，Metsでの治療方針をCKDが進んだ状況においてもそのまま継続させてよいか議論の余地がある．reverse epidemiology現象を考慮した，独特な治療戦略が必要ではないかと思われる．Metsを伴うCKDの管理の一例として食事療法につき考察する．CKDの治療戦略の柱の一つとしてタンパク制限食が挙げられる．その一方で，Metsの治療戦略の柱の一つとしてカロリー制限がある．したがってMetsによるCKDの治療としてはタンパクおよびカロリーの制限が考えられるが，CKDのステージが進むにつれPEWが顕著になると，カロリー制限はタンパク制限下ではPEWを助長する．カロリー制限はむしろCKDのステージが進んだ症例では危険であり充分なカロリーを補充することが必要である．そして従来の食事療法では脂質20～25%[9]で糖質を中心にカロリーを摂取することが推奨されている．先述のようにCKDではPEWとともに全身のインスリン抵抗性

が存在する．我々はCKD stage 5の患者を中心に終日の血糖プロフィールを測定しており，CKD患者では食後高血糖が遷延することを明らかにしている．食後高血糖は糖尿病においては大血管障害のみならず，網膜症や神経障害などの様々な合併症との関連が報告されている．糖質の過剰摂取はこの食後高血糖を容易に引き起こすことを考慮すれば過剰な糖質によるカロリー補充は避けるべきと思われる．その一方で脂肪も重要なエネルギー源の一つである．FacchiniらはCR-LIPE食というフォーミュラー食を糖尿病患者に投与した．CR-LIPE食は炭水化物制限，高脂肪であり，さらにポリフェノールに富んだ食事である．その結果4年の経過観察で，死亡率が低く，腎機能の保持が可能であった．近年Ewersらは血液透析患者40名に不飽和脂肪酸のサプリメントを3カ月間投与した．その結果，一日の総カロリーが上昇したものの脂質代謝には異常が認められなかった．ドライウェイトで評価した栄養状態は上昇し，CRP（C-reactive protein）の低下が認められたと報告している．以上よりPEWおよびREを考慮した肥満，Metsに伴うCKDの食事療法は，低タンパク，低カロリーでよいがPEWの存在を考慮しエネルギー状態のモニターが必要と思われる．表2にPEWモニターの指標を示した[10]．このように肥満，MetsのCKDの食事療法は患者各自の状態にもより今後の検討課題であろう．

おわりに

以上肥満・MetsとCKDについて解説した．今後肥満人口の増加とともに透析人口の抑制のためにもこの病態は臨床上重要な位置を占めると思われる[11]．その一方でCKDの代謝面の問題点も無視できず，Mets，CKDの2大CVDリスクファクターを同時に見据えた管理が重要となってくると思われる．

著者のCOI（conflicts of interest）開示：本論文発表内容に関連して特に申告なし

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メタボリックシンドロームに伴う心腎連関の治療

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abstract

近年メタボリックシンドローム（MS）とともに心血管疾患（cardiovascular disease：CVD）のリスクとして慢性腎臓病（chronic kidney disease：CKD）が重視されており，CKDがCVDのリスクとなっていることを心腎連関という．また，MSはCKDを合併することにより，急速にCVDを引き起こす．MSとCKDの共通の基盤病態にインスリン抵抗性，レニン・アンジオテンシン系（RAS）の亢進がある．また，心腎連関のメディエーターであるasymmetrical dimethylarginine（ADMA）やアルドステロンも2つの病態をつなぐ物質と考えられる．MSおよびCKDの治療戦略においてはインスリン抵抗性の改善（チアゾリジン誘導体）やRASの抑制（アンジオテンシンⅡ受容体拮抗薬，アンジオテンシンⅡ変換酵素阻害薬），ミネラルコルチコイド受容体（MR）拮抗薬が重要である．

Ⅰ　はじめに

メタボリックシンドローム（MS）は内臓肥満を基盤とし，高血圧，耐糖能異常，脂質代謝異常を合併した病態である．日本においては平成15年に診断基準が示され，心血管事故のリスクとして社会的にも関心が深まった（図1）．われわれは，MSが内臓肥満より発症し，心筋梗塞，脳卒中といった心血管疾患（cardiovascular disease：CVD）へ至る過程を一連の流れとしてとらえたメタボリックドミノの概念を提唱し，MSを単なるリスクととらえるのみでは不十分であり，予後を見据えた治療戦略の構築と病因解明の重要性を強調している（図2）．

一方新たなCVDのリスクとして腎機能障害が指摘されている．腎機能の低下はそれが早期の段階であっても（例えば微量アルブミン尿の段階），すでにCVDのリスクとなっていることが多くの疫学データで証明されるようになり，慢性腎臓病（chronic kidney disease：CKD）の概念が米国腎臓財団（national kidney foundation：NKF）より提唱された[1]．また，糸球体濾過量（glomerular filtration rate：GFR）値および尿タンパクでCKDをステージ分類し，重症度を評価することが推奨されている．

以上を背景に，本稿ではまずCKDがCVDのリスクであることを示す心腎連関にMSがどうかかわるかを説明する．次にMSおよびCKDという二大症候群の共通の基盤病態について説明し，最後にこれらの病態を踏まえた治療戦略について述べる．

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1. メタボリックシンドロームの診断基準

腹腔内脂肪蓄積	ウエスト周囲径	男性≧85cm 女性≧90cm

内臓脂肪面積　男女とも≧100cm²に相当
上記に加え以下のいずれか2項目以上（男女とも）

高トリグリセリド血症 低HDL-コレステロール血症	かつ/または	TG≧150mg/dL HDL-C＜40mg/dL
収縮期血圧 拡張期血圧	かつ/または	≧130mmHg ≧85mmHg
空腹時高血糖		≧110mg/dL

2. 慢性腎臓病（CKD）の定義

＜1か2のどちらかを満足する場合＞
1. 腎障害（kidney damage）が3カ月以上継続する．
   ー腎障害とは腎臓の形態的または機能的な異常を指し，GFR低下の有無を問わない．
   ー腎障害の診断は，
   ・病理学的診断または
   ・腎障害マーカーによって行う．（このマーカーとしては血液または尿検査，または画像診断がある．）
2. GFR<60mL/分/1.73m²が3カ月以上継続する．この場合腎障害の有無を問わない．

図1　メタボリックシンドロームの診断基準

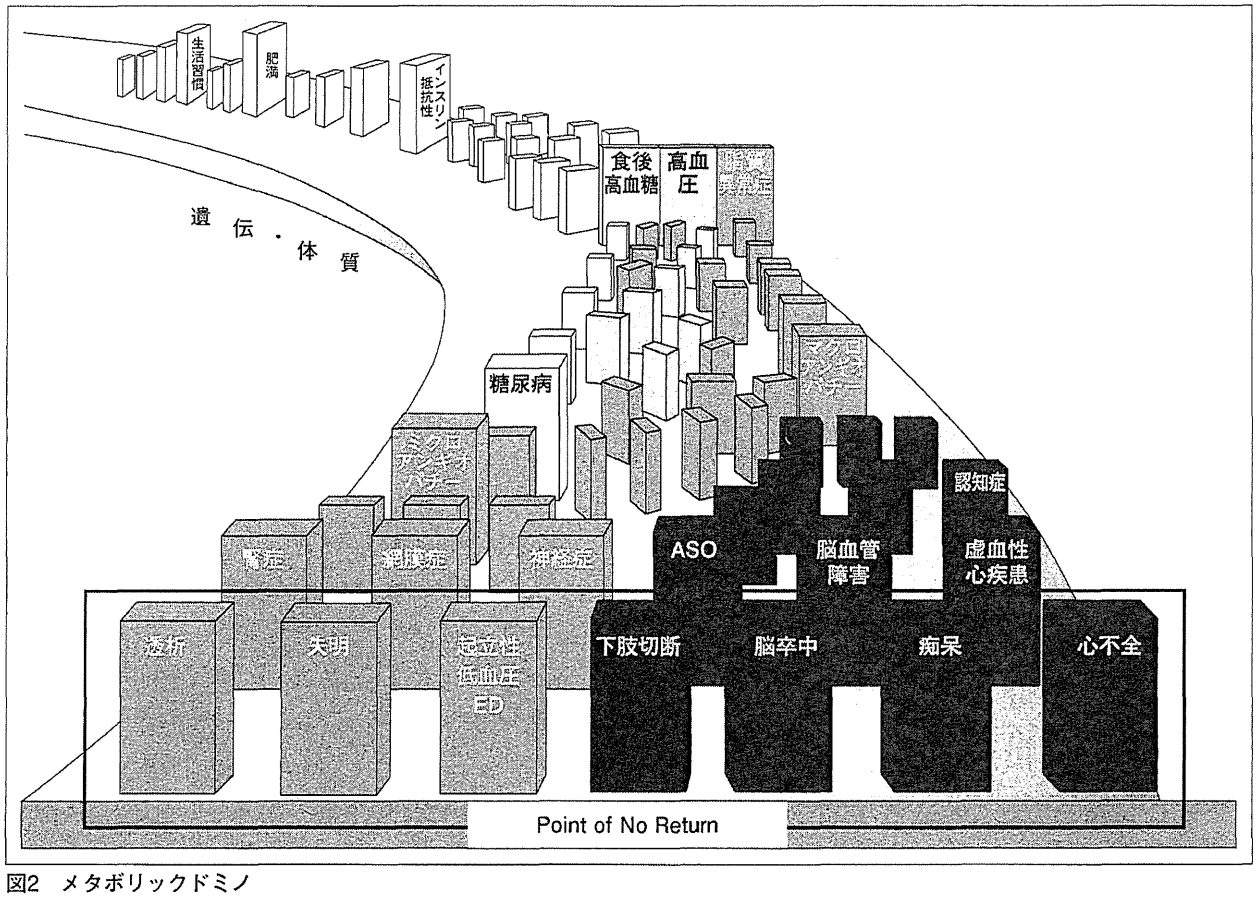

図2　メタボリックドミノ

II　慢性腎臓病のリスクとしてのMS

CKDがCVDのリスクであるという心腎連関が存在する一方で，MSがCKDのリスクであるというデータが報告されている．IsekiらはBMIと末期腎不全の発症につき100,000人の日本人の集団を対象に17年間観察を続けた．その結果BMIが増加するにつれて末期腎不全に移行するリスクが男性において高くなることが示された[2]．10,096名の非糖尿病患者からなるatherosclerosis risk in communities study（ARIC）においては，9年の観察期間でMSの存在（NCEP-ATPⅢの定義による）でCKD発症（推算GFRが60mL/分/1.73m²未満）のリスクが1.43倍

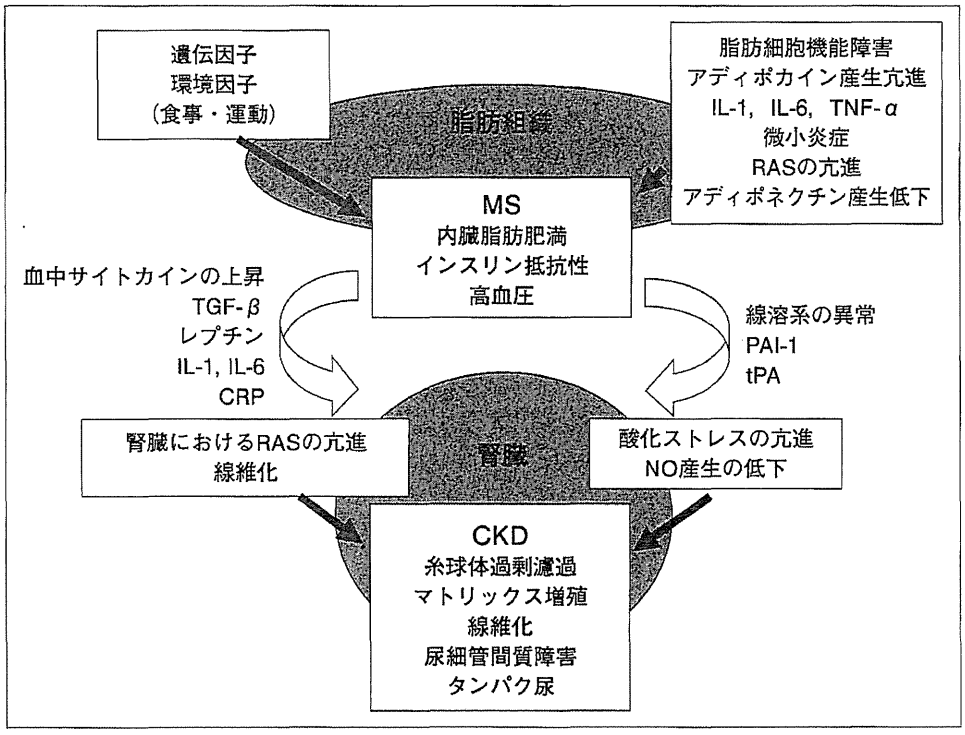

図3
CKDのリスクとしてのMS
〔参考文献5), 25)より引用改変〕

と報告されている．さらにMSの構成因子をまったく有さない患者に比べ，因子を1，2，3，4，5個有するものはCKD発症のリスクがそれぞれ1.13，1.53，1.75，1.84，2.45倍であった[3]．また，7,832名よりなる米国住民のコホートでの6年間の観察によるNHANEⅢ研究（the third National Health and Nutrition Examination Survey）ではMSを有する患者はMSを有さない患者に比べCKD発症（GFR＜60mL/分/1.73m^2）および微量アルブミン尿陽性の危険率がそれぞれ2.60倍，1.89倍であった[4]．このようなMSがCKDのリスクであるという疫学データを説明するメカニズムとして，さまざまな因子が想定されている（**図3**）[5]．また古くより肥満が腎機能障害の原因となることが指摘されていた（肥満関連腎症）．このように少なくとも一部のCKDにおいてはMSがその発症要因となるとともに，CKDは心腎連関を通じMSにおける心血管イベント発症のドミノ倒しの連鎖反応を加速させる因子となるものと思われる．

Ⅲ 慢性腎臓病とMSの共通の基盤病態

このようなMSとCKDとの相関は両者になんらかの共通の基盤病態が存在することが想定される．まず，インスリン抵抗性の存在である．MSの基盤病態にインスリン抵抗性があることは論は待たないが，CKDの早期においてもすでにインスリン抵抗性が生じているということが報告されている[6]．BeckerらはCKDのstage 1の段階でもHOMA指数で算出したインスリン抵抗状態が既に上昇していることを報告しており，腎性インスリン抵抗性症候群（Renal Insulin Resistance Syndrome：RIRs）と命名している[7]．またわれわれでもそれを示唆する報告を行っている[8]．このメカニズムについては活性型ビタミンDの欠乏，腎性貧血，尿毒素物質がこれまで報告されている．またCKDの患者はTNF-α，IL-6，レプチンといったサイトカインが上昇していることが報告されている[9]．さらに重要なことは，インスリン抵抗性はそれ自身がCKDのリスクになるということである．その機序は不明な点が多いが，高インスリン血症はレニン・アンジオテンシン系