Lipotoxic and inflammatory phenotypes in rats with uncontrolled metabolic syndrome and nephropathy

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THE RISK OF RENAL INJURY IN diabetes can be reduced by controlling the disordered metabolic and hypertensive syndromes (37). This basic notion implies that the renal disease in diabetes is engendered by the abnormal metabolic milieu. Accordingly, hyperglycemia (52), dyslipidemia (9, 24), hypertension (37, 3), and insulin resistance (34), which often times coexist as the metabolic syndrome (7, 18), have been considered as pathogenic. However, this knowledge has not been effectively translated into preventive or curative therapies (23). Recent work in animals generated a novel hypothesis: that anomalous immunological responses are triggered by the unregulated metabolic syndrome (31, 40, 49). This idea links metabolic derangements to inflammation, and it is appealing because it accounts for the insidious cell loss and fibrosis in diabetic nephropathy (1, 42).

Accordingly, experiments with mycophenolate mofetil (MMF) showed reduced injury from metabolic-hypertensive abnormalities (31, 40, 49). MMF is an immune suppressant (16) that limits inflammatory damage in models of diabetic (40, 49) and hypertensive nephropathy (40, 44). MMF inhibits inosine-5’-monophosphate dehydrogenase, preventing de novo synthesis of purines in T and B lymphocytes (16), although other hematological and renal cells are potential targets (54).

The early morbid renal phenotype of rats with the metabolic syndrome is characterized by accumulation of unutilized lipid and by severe capillary infiltration of polymorphonuclear neutrophils (PMN) and macrophages (14). Tissue lipid accumulation is thought to be in and of itself toxic (14, 30, 48, 50), in that it causes cell dysfunction, i.e., lipotoxicity (14, 30, 48, 50), and triggers inflammation, which further aggravates cell dysfunction (45). Accordingly, MMF might protect the kidney in diabetes because it attenuates both tissue lipid accumulation and systemic inflammation. We now show that in obese F1 hybrid Zucker fatty diabetic/spontaneous hypertensive heart failure (ZS) rats, MMF had very negligible effects on measured metabolic and renal lipotoxicity parameters. However, MMF lowered circulating cytokine-induced neutrophil chemoattractant-1 (CINC-1), a strong PMN chemotactic factor, as well as peripheral and renal PMN plus macrophages counts, and it dramatically reduced renal cytokine production and fibrosis. Hence, we propose that significant renal protection can be accomplished by directly interfering with systemic and renal inflammatory responses, independently of continued metabolic stress from hyperglycemia and lipotoxicity.

METHODS

Animals. The research involving animals adhered to The American Physiological Society “Guiding Principles in the Care and Use of Animals.” The investigative protocols were approved by the Institutional Animal Care and Use Committee at Indiana University. The pathogen-free rats used in these investigations were from Charles River (Wilmington, MA). We studied the ZSF1 rat, a first-generation (F1) hybrid rat derived from two well-characterized parental strains: the Zucker fatty diabetic (ZDF, fa/fa) and the spontaneous hypertensive heart failure rat (14). Hybrid rats were preferred over original strains to limit effects of potentially cosegregated genetic determinants beyond the selected leptin receptor mutations. There were four groups of five rats each maintained in our animal facility from 6 wk of age until termination at 28 wk of age. Group 1 consisted of lean male litter mates injected once daily with MMF (10 mg·kg⁻¹·day⁻¹), CellCept, Roche Pharmaceuticals, Nutley, NJ). Group 2 consisted of obese male injected once daily with 150 µl intraperitoneally (ip) of normal saline vehicle. Group 3 consisted of obese male litter mates injected once daily with MMF (10 mg·kg⁻¹·day⁻¹ ip). Group 4 consisted of obese male injected once daily with 150 µl ip of normal saline vehicle. The rats were fed Purina diet 5008 ad libitum and were housed in steel cages and acclimatized to 12:12-h light-dark (7 AM–7 PM).

The body weights and blood metabolic profiles of the rats were monitored throughout the experiment. Blood samples were obtained by sequential tail vein drawings and at termination.

Blood and urine analysis. The blood levels of triglyceride, cholesterol, creatinine, and blood urea nitrogen (BUN) were measured on a
Beckman CX4CE Clinical System in blood drawn from the tail vein.
C-reactive protein (CRP) was measured by the clinical laboratory at
the Veterans Affairs hospital. The following plasma cytokines were
measured using Multiplexed immunoassays with Lincoplex Instru-
mentation from Linco Research (St. Louis, MO): granulocyte-mac-
rophage colony-stimulating factor, IL-1α, MCP-1, IL-4, IL-1β, IL-2,
IL-6, IL-10, IL-12p70, IL-5, IFNγ, IL-18, CINC-1 (GRO/KC), and
TNF-α. Plasma adiponectin was measured by an ELISA kit specific
for rat adiponectin from B-Bridge International (Mountain View,
CA). Urine creatinine and protein were measured as previously
described (14).

Renal histology. Renal histology, immunohistochemistry, and mor-
phometry were conducted on paraffin embedded renal sections (14).
Each entire kidney section was analyzed; all glomeruli and tubules
were included in the calculations, and any degree of glomerular
sclerosis, ranging from focal to global lesions, was considered posi-
tive. Glomerular sclerosis and tubular atrophy data were expressed as
fractions of the total number of glomeruli and tubules. Interstitial
fibrosis was expressed as the percentage of the fibrosis area in
trichrome dye-stained sections. Lipid Oil red O stain was conducted
on 30-μm renal sections fixed with 4% paraformaldehyde as de-
scribed (14).

Renal polymorphonuclear leukocytes were labeled with naphtanol-
AS-D chloracetate esterase (Leder stain kit, Poly Scientific R&D, Bay
Shore, NY) (14). Macrophages were labeled with an anti-rat macro-
phage mouse monoclonal MAB1435 (Chemicon, Temecula, CA) (14).

Table 1. Metabolic syndrome phenotype

<table>
<thead>
<tr>
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<th>LMM</th>
<th>LMC</th>
<th>OMM</th>
<th>OMC</th>
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<tr>
<td>Body weight</td>
<td>485±15</td>
<td>487±11</td>
<td>578±15*</td>
<td>637±14*</td>
</tr>
<tr>
<td>Kidney weight</td>
<td>1.65±0.08</td>
<td>1.66±0.04</td>
<td>2.12±0.1†</td>
<td>2.89±0.07*</td>
</tr>
<tr>
<td>LW/BW</td>
<td>0.340±0.006</td>
<td>0.341±0.007</td>
<td>0.367±0.008†</td>
<td>0.454±0.006*</td>
</tr>
<tr>
<td>Liver weight</td>
<td>14.92±0.61</td>
<td>17.75±0.57</td>
<td>32.73±1.65†</td>
<td>46.47±1.47*</td>
</tr>
<tr>
<td>Heart weight</td>
<td>1.47±0.06</td>
<td>1.47±0.03</td>
<td>1.49±0.07</td>
<td>1.62±0.03</td>
</tr>
<tr>
<td>HW/BW</td>
<td>0.303±0.007</td>
<td>0.302±0.005</td>
<td>0.258±0.010*</td>
<td>0.254±0.003*</td>
</tr>
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</table>

Values are means ± SE. Body, kidney, heart, and liver weights are in grams.
KW/BW, HW/BW, and LW/BW: kidney, heart, and liver weights normalized
to body weight (g/g). LMM, lean males injected with mycophenolate; LMC,
lean male control; OMM, obese males injected with mycophenolate; OMC,
obese male control. *Significantly different from LMC and LMM, P < 0.05.
†OMM significantly different from OMC, P < 0.05, ANOVA.
followed by a rabbit anti-mouse second antibody (LSAB2 kit, Dako). The labeled cells were visualized at \( \times 400 \) magnification in 25 separate kidney fields/animal and expressed as cell number/\( \times 400 \) field.

The renal morphometric analysis was conducted with the programs Sigmascan and Sigmascan Pro, v. 4.0 (SPSS, Chicago, IL).

**Renal proteins.** The levels of unmodified and phosphorylated AMP-activated protein kinase (AMPK) isoforms, phosphorylated acetyl-CoA carboxylase (pACC), adipophilin, and adiponectin receptors 1 and 2 were measured on Western blots. The AMPK antibodies were all affinity-purified specific rabbit polyclonal antibodies to synthetic AMPK peptides (1:1,000 dilution, Cell Signaling Technology, Beverly, MA). The antibody to amino acids 73-85 of rat acetyl-CoA carboxylase was an affinity-purified rabbit polyclonal against pACC peptide (aa 73-85, phosphorylated at Ser79, Upstate, Lake Placid, NY). The mouse monoclonal antibody to adipophilin was raised to a synthetic adipophilin peptide (aa 5-27 from the NH\(_2\) terminus) from Research Diagnostics (Flanders, NJ). The rabbit antibodies to adiponectin receptors 1 and 2 were affinity purified to mouse synthetic peptides (AdipoR1 and AdipoR2) from Alpha Diagnostic International (San Antonio, TX). Anti-oxidized low-density lipoprotein receptor 1 (LOX-1) antibody was generated in rabbits against the LOX-1 peptide (aa 188 to 233, LOX-1 accession number NP_579840). The 46-amino acid LOX-1 peptide was synthesized by Dr. Suzanna Holgrath (California Institute of Technology, Pasadena, CA). The antibody was manufactured and affinity purified by Covance.

Table 2. **Albuminuria at 12 and 26 wk of age**

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<th>LMC</th>
<th>OMM</th>
<th>OMC</th>
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<tbody>
<tr>
<td>Pre: 12 wk of age</td>
<td>0.058±0.020</td>
<td>0.102±0.039</td>
<td>0.730±0.237*</td>
<td>0.527±0.078*</td>
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<tr>
<td>Post: 26 wk of age</td>
<td>0.010±0.001</td>
<td>0.012±0.002</td>
<td>1.423±0.112*</td>
<td>1.690±0.076*</td>
</tr>
</tbody>
</table>

Values are means ± SE expressed in mg/mg creatinine. *Significantly higher than respective lean rat group, \( P < 0.05 \).

Fig. 3. Oil red O stain of renal sections. Column A, lean rats treated with MMF; column B, lean rats injected with saline; column C, obese rats treated with MMF; column D, obese rats injected with saline. The 4 rows (top to bottom) are representative images taken from cortex to papilla. The red arrows point to renal tubules laden with lipid (red stain). The blue arrows point to dilated tubules in obese rats injected with saline vehicle.
Equal protein gel loading was verified with a primary rabbit antibody to conserved ribosomal protein L7a (RL7a) from the 60S subunit (41) (antibody R225, 1:1,000 dilution) from Cell Signaling Technology. Proteins were separated from renal cortex homogenates on 12% acrylamide SDS-PAGE gels, electrophoretically transferred to Immuno-Blot polyvinylidene difluoride membranes (Bio-Rad) at 15 mA, and labeled with specific primary antibodies followed by peroxidase-linked secondary anti-rabbit or anti-mouse IgG goat antibody (Pierce, Rockford, IL) (14). The relative intensities of the protein levels were measured on antibody-stained membranes using VitaScan software (ESA, Chelmsford, MA).

### Fatty acid oxidation

Fatty acid β-oxidation activity was measured as described elsewhere (17). Freshly isolated kidney cortices were homogenized, and homogenate aliquots (500 μg) were incubated in 0.2 ml of (in mM) 150 KCl, 10 HEPES (pH 7.2), 0.1 EDTA, 1 KPi buffer (pH 7.2), 5 Tris malonate, 10 MgCl2, 1 carnitine, and 5 ATP as well as 0.15% BSA and 50 μM [U-14C]palmitate (50,000 cpm of radioactive substrate). The reaction was carried out for 30 min at 25°C and stopped with 0.2 ml of 0.6 N perchloric acid. Nonreacted fatty acid was removed with 2 ml of n-hexane, and fatty acid radioactive degradation products in the water phase were counted.

### Long-chain acyl-CoA dehydrogenase activity

Enzymatic activity of long-chain acyl-CoA dehydrogenase (LCAD)/medium-chain acyl-CoA dehydrogenase (MAD) was determined as previously described (13). Frozen renal cortices (20–50 mg) were homogenized as 20% (wt/vol) suspensions in ice-cold 100 mM HEPES (pH 7.6)/0.1 mM EDTA. The homogenates were then centrifuged at 7,000 g for 2 min, and LCAD activity was measured in the supernatant at 37°C by adding 5 μl of supernatant to 500 μl 100 mM HEPES (pH 7.6)/0.1 mM EDTA/200 μM ferricenium hexafluorophosphate/0.5 mM sodium tetradecylate/50 μM octanoyl-CoA. The results were calculated from the decrease observed over the initial 60-s period.

### RT-PCR

Total RNA was isolated from frozen kidney cortex using an RNAqueous 4PCR Kit (Ambion, Austin, TX). cDNA was synthesized from RNA with a StrataScript QPCR cDNA Synthesis Kit (Stratagene, La Jolla, CA) and then used as a template. Real-time PCR was performed with an i-Cycler (Bio-Rad) using the iQ SYBR Green Supermix reagents, according to the manufacturer’s instructions. The data were analyzed by the ΔΔCT method and normalized to the control group.
quantitative PCR was run on a MX4000 Multiples Quantitative PCR System (Stratagene) with Brilliant SYBR Green QPCR Master Mix. Standard curves for each target gene were obtained with the same system before the running of corresponding samples. The samples were always run in triplicate. The data were normalized to the mRNA content of β-actin in each sample and are reported as relative amount of mRNA compared with control samples.

Statistical analysis. The results are expressed as means ± SE. Any differences between groups were evaluated by one-way ANOVA and considered significant if P < 0.05.

RESULTS

Metabolic phenotype. MMF was initiated after the metabolic phenotype and the complicating nephropathy were well established at 12 wk of age and then continued until the rats were 28 wk old. Serum creatinine levels were comparable in all four groups of rats, but levels of glucose, cholesterol, and triglycerides, normal in lean rats, increased in all obese rats (Fig. 1).

Average body and organ weights at 28 wk of age (termination) are summarized in Table 1 (tables and figures list the MMF-treated groups before the respective control groups). MMF therapy significantly restricted renal and hepatic enlargement in obesity by 19 and 22%, respectively. Moreover, MMF limited the large increase in 24-h creatinine clearance that followed obesity and diabetes (Fig. 2). However, MMF did not alter proteinuria, already established before therapy was initiated (Table 2).

The larger kidneys of obese rats also contained larger amounts of lipid, which were preferentially localized in renal tubules from the cortex to the medulla, and these were not changed by MMF therapy (Fig. 3). The unutilized deposits of renal lipids were linked to higher levels of lipid droplet protein adipocyte differentiation-related protein (ADRP) (15, 38) and to higher levels of renal triglycerides plus lipid peroxides, and all of which were unaffected by MMF therapy. ADRP optical density (OD) on Western blots averaged: 0.97 ± 0.07 in lean male treated with mycophenolate (LMM); 1.00 ± 0.09 in lean male control (LMC); 1.49 ± 0.10 in obese males.

Table 3. Renal AMPK isoforms and pACC protein levels

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<thead>
<tr>
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<th>LMM</th>
<th>LMC</th>
<th>OMM</th>
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<tbody>
<tr>
<td>AMPKα</td>
<td>1.16 ± 0.12</td>
<td>1.00 ± 0.06</td>
<td>0.98 ± 0.02</td>
<td>1.31 ± 0.10</td>
</tr>
<tr>
<td>pAMPKα1</td>
<td>0.80 ± 0.09</td>
<td>1.00 ± 0.19</td>
<td>1.57 ± 0.09*</td>
<td>1.31 ± 0.05*</td>
</tr>
<tr>
<td>pAMPKα2</td>
<td>0.75 ± 0.13</td>
<td>1.00 ± 0.13</td>
<td>1.70 ± 0.10*</td>
<td>1.32 ± 0.14*</td>
</tr>
<tr>
<td>AMPKβ1</td>
<td>1.05 ± 0.07</td>
<td>1.00 ± 0.05</td>
<td>1.00 ± 0.08</td>
<td>0.90 ± 0.07</td>
</tr>
<tr>
<td>pAMPKβ1</td>
<td>1.29 ± 0.33</td>
<td>1.00 ± 0.01</td>
<td>3.44 ± 0.26*</td>
<td>3.74 ± 0.73*</td>
</tr>
<tr>
<td>pACC</td>
<td>0.82 ± 0.05</td>
<td>1.00 ± 0.14</td>
<td>1.44 ± 0.17*</td>
<td>1.79 ± 0.11*</td>
</tr>
</tbody>
</table>

Values are means ± SE expressed as relative optical density (OD) of the 6 blots shown in Fig. 5. AMPK, AMP-activated protein kinase; pACC, phosphorylated acetyl-CoA carboxylase. *OMC and OMM significantly different from respective lean rat controls, either LMC or LMM, P < 0.05, ANOVA.

Table 4. Fatty acid oxidation

<table>
<thead>
<tr>
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<th>LMM</th>
<th>LMC</th>
<th>OMM</th>
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<tbody>
<tr>
<td>FAO</td>
<td>2.69 ± 0.29</td>
<td>2.50 ± 0.27</td>
<td>3.02 ± 0.61</td>
<td>2.55 ± 0.78</td>
</tr>
<tr>
<td>LCAD</td>
<td>48.4 ± 4.1</td>
<td>44.9 ± 6.0</td>
<td>46.6 ± 3.4</td>
<td>43.3 ± 4.0</td>
</tr>
</tbody>
</table>

Values are means ± SE. Fatty acid oxidation (FAO) activity is expressed as pmol·min⁻¹·g protein⁻¹. Long-chain acyl-CoA dehydrogenase (LCAD) activity is expressed as μmol·min⁻¹·g tissue⁻¹.
treated with mycophenolate (OMM); and 1.69 ± 0.13 in obese male control rats (OMC) (P < 0.05, ANOVA) when obese rats were compared with respective lean groups (Fig. 4). Furthermore, the higher levels of circulating and renal lipid were linked to higher levels of renal LOX-1, which facilitates uptake of oxidized lipid (14). The higher expression of renal LOX-1 protein in obese rats was also unaffected by MMF therapy. LOX-1 OD on Western blots averaged 0.94 ± 0.16 in LMM; 1.00 ± 0.10 in LMC; 1.98 ± 0.05 in OMM; and 2.02 ± 0.05 in OMC (P < 0.05, ANOVA) when lean rats were compared with obese groups (Fig. 5).

In obesity, excessive storage of unutilized lipid could result from inadequate activation of renal AMPK, a key enzyme that promotes fatty acid oxidation (FOA) (19, 21). Accordingly, levels of total and activated renal AMPK were measured using Western blotting. Figure 6 shows that while total AMPK levels were not altered by obesity, higher levels of all phosphorylated (i.e., activated) AMPK isoforms were consistently found (Table 3). This response was also not modified by MMF therapy. Moreover, levels of renal pACC (32), a downstream target for AMPK, were also increased in obesity, and were not altered by MMF. We projected that increased AMPK phosphorylation would stimulate FOA (53). However, renal FAO and renal LCAD activity, a key step in FOA (46), were not changed in obesity, regardless of MMF administration (Table 4). Thus AMPK signaling and subsequent FAO activity appeared to be uncoupled, revealing a significant disturbance of renal fatty acid metabolism in the leptin resistant obese ZS rats (14).

**Inflammation and renal fibrosis.** Systemic inflammation was estimated from blood cell counts and serum cytokines in lean and obese rats at termination (Table 5). In obese rats, higher counts of circulating PMN were normalized by MMF therapy. Lymphocyte blood counts were lower in obesity and seemed to be lowered even more by MMF, although the drug effect was not significant.

Adiponectin has important anti-inflammatory (28) and metabolic (47) activity, which in obesity might be limited by the very low levels of its renal AdipoR1 and AdipoR2 (Fig. 7). MMF therapy prevented the obesity-dependent fall in circulating adiponectin levels, which might have counteracted resistance from the depressed renal AdipoR1 and AdipoR2. AdipoR1 OD on Western blots averaged 0.94 ± 0.11 in LMM; 1.00 ± 0.02 in LMC; 0.70 ± 0.06 in OMM; and 0.77 ± 0.01 in OMC (P < 0.05) when lean groups were compared with obese groups. AdipoR2 OD on Western blots averaged 1.26 ± 0.12 in LMM; 1.00 ± 0.16 in LMC; 0.37 ± 0.04; in OMM; and 0.44 ± 0.11 in OMC (P < 0.05) when lean groups were compared with obese groups (ANOVA, Fig. 7).

Serum levels of CINC-1 and GRO/KC, the rat CXCR2 receptor agonist (10), were elevated in obesity, and MMF normalized CINC-1 levels. In contrast, serum CRP and IL-1β, also elevated in obese rats, were not reduced by MMF therapy. On the other hand,
serum MCP-1 and IL-18 were unaffected by either obesity or MMF therapy. Ten additional cytokines were screened but were not detected in rat serum (see complete cytokine list in Table 5).

MMF also inhibited several critical components of obesity-induced renal inflammation (Table 6 and Figs. 8 and 9). For instance, large clusters of PMN in peritubular capillaries were virtually eliminated by MMF therapy (Table 6, Fig. 8). The numbers of interstitial, or peritubular, macrophages were even higher in obesity, and MMF significantly reduced their numbers (Table 6, Fig. 9). Glomerular PMN and macrophages were only slightly increased in obesity, and MMF reduced glomerular macrophage numbers (Table 6). It is noteworthy that inflammatory cells coexisted with severe fibrosis, which was widespread in glomerular and interstitial regions (Fig. 10). MMF therapy also significantly reduced the severity of glomerular and interstitial fibrosis by 19 and 23%, respectively (Table 7). We multiplied fractional areas of fibrosis per weight in each kidney and estimated that MMF reduced total renal fibrosis mass by ~44% (Table 7).

Renal mRNAs encoding IL-1β, IL-6, macrophage migration inhibitory factor (MIF), the CXCR2 receptor, and the transforming growth factor (TGF)-β1 receptor were all significantly increased in obesity, and all were corrected by MMF therapy. In contrast, mRNA encoding TGF-β1, TNF-α, and the TNF-α receptor superfamily was not affected by obesity or MMF therapy (Fig. 11).

**DISCUSSION**

We studied the roles of renal lipotoxicity and inflammation in ZS rats with uncontrolled metabolic syndrome and estab-
lished nephropathy. The rats were given daily MMF injections with the intention of reducing renal lipotoxicity and inflammation. MMF inhibited kidney enlargement and fibrosis, resulting in a remarkable overall 44% approximate reduction in renal scar formation. These effects were accompanied by reductions in creatinine clearance, but proteinuria was not changed significantly.

Renal lipotoxicity (14, 48) secondary to lipid accumulation complicates nephropathy of the metabolic syndrome (14, 48, 50), and it is thought to be an important element in its pathogenesis (14, 50). Kidneys of obese rats had higher levels of unutilized renal lipid, ADRP, the lipid storage protein (15, 38), lipid peroxidation products, and LOX-1, which is stimulated by oxidized lipid loads (14, 43). However, all these elevated indices of renal lipotoxicity were not affected by MMF administration. Higher levels of phosphorylated AMPK were found in obesity, induced perhaps by metabolic, nutritional, or oxidative stresses (14, 22, 29), and higher renal pACC levels were consistent with renal AMPK stimulation in obesity (51). However, AMPK phosphorylation did not result in greater renal FAO (55), pointing to either inadequate AMPK activation or to other unknown alterations of renal fatty acid metabolism in obese rats.

On the other hand, MMF improved key inflammatory parameters in obesity. Higher levels of circulating neutrophils and of CINC-1 (6, 10) were both corrected by MMF therapy. IL-1 stimulates CINC-1 production (2, 6), and it was also higher in obese rats, although IL-1 levels were not reduced by MMF, suggesting that MMF inhibited CINC-1 downstream of IL-1 activation. Obesity induced a remarkable 50-fold increase in serum CRP, which was not affected by MMF. In rodents, CRP is viewed as a sensitive marker of inflammation and not as a proinflammatory molecule (35, 36). Hence, CRP elevation might reflect features of inflammation refractory to MMF.

MMF therapy prevented the fall in adiponectin levels of obese rats. This effect is particularly significant in the context of renal adiponectin resistance, which likely resulted from the low numbers of renal AdipoR1 and AdipoR2 in obesity (26). Thus the potential benefits of MMF also included some degree of preservation of adiponectin anti-inflammatory (28, 33) and metabolic effects (27, 47).

Renal inflammation was investigated by measuring kidney mRNA encoding IL-1β (12), IL-6 (25), MIF (5), the CXCR2 cytokine receptor for CINC-1 (4), and also the TGF-β receptor 1 (11). Renal mRNAs encoding these proinflammatory cytokines and receptors were all increased in obesity and were normalized by MMF therapy. In contrast, renal mRNA encoding TGF-β1, TNF-α, and the TNF receptor were not altered by obesity or MMF therapy at age 28 wk.

Our data indicate that controlling lipotoxicity-dependent inflammation (8) drastically reduces renal fibrosis, a critical parameter in renal failure progression (20). Current views, based on work with targeted acyl CoA-synthase expression, posit inflammation as a consequence of tissue lipid accumulation (8). Our results are consistent with this view, and our data indicate that treatment of inflammation with MMF, presumably downstream of unaltered lipotoxic stress, protects the kidney from scar formation. It is noteworthy that renal protection by MMF occurred in conjunction with substantial reductions in numbers of neutrophils in the blood and kidney, and with inconsequential drug effects on hyperglycemia. Serum cholesterol and triglycerides were extremely high in all obese rats, and although MMF had a relatively minor depressing effect

### Table 7. Renal fibrosis

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<th>LMC</th>
<th>OMM</th>
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<tbody>
<tr>
<td>FGFA</td>
<td>0.156±0.013</td>
<td>0.212±0.015</td>
<td>0.334±0.014×†</td>
<td>0.408±0.018*</td>
</tr>
<tr>
<td>FIFA</td>
<td>0.110±0.01</td>
<td>0.118±0.015</td>
<td>0.424±0.019×†</td>
<td>0.552±0.027*</td>
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<tr>
<td>EKF M</td>
<td>0.183±0.021</td>
<td>0.195±0.025</td>
<td>0.90±0.045×†</td>
<td>1.60±0.10×†</td>
</tr>
</tbody>
</table>

Values are means ± SE. FGFA, fractional glomerular fibrosis area; FIFA, fractional interstitial fibrosis area; EKF M, estimated kidney fibrosis mass, or the product of individual kidney weights and individual FIFA determinations (organ weight × fractional fibrosis area). *Significantly different from LMC and LMM, P < 0.05. †OMM significantly different from OMC, P < 0.05, ANOVA.

![Fig. 11.](http://ajprenal.physiology.org/) mRNA encoding proinflammatory cytokines and receptors in renal cortex. mRNA levels encoding IL-1β, IL-6, macrophage migration inhibitory factor (MIF), the cytokine-induced neutrophil chemoattractant (CINC-1) receptor, and the transforming growth factor (TGF)-β receptor were all increased in control obese rats (filled bars), and MMF therapy prevented the rise in obesity (open bars) (P < 0.05 for all, ANOVA). In contrast, mRNA encoding TGF-β1, TNF-α, and the TNF-α receptor was not affected by obesity or MMF therapy.
late in the course, MMF did not alter the severe lipotoxic renal phenotype.

In conclusion, our data show that established nephropathy in the metabolic syndrome can be significantly improved by treating the inflammation derived from abnormal metabolism, even when the severely disturbed metabolic picture remains largely unchecked.

ACKNOWLEDGMENTS

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GRANTS

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REFERENCES

3. Cugini Azzollini N, Gagliardini E, Cassis P D, Bertini R, Colotta F, Dinarello CA.
6. Huang W, Dedousis N, Bandi A, Lopaschuk GD, O’Doherty RM.
9. Cooper ME, Jandeleit-Dahm KA.
12. Cugnì Azzollini N, Gagliardini E, Cassis P D, Bertini R, Colotta F, Dinarello CA.
14. Chen J, Muntner P, Hamm LL, Jones DW, Batuman Fonseca V, Muntner P, Hamm LL, Jones DW, Batuman Fonseca V, Jandeleit-Dahm KA.


