Increased glomerular filtration rate in early metabolic syndrome is associated with renal adiposity and microvascular proliferation

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METABOLIC SYNDROME (MetS) is a constellation of interrelated metabolic risk factors that include obesity, dyslipidemia, hypertriglyceridemia, insulin resistance, and hypertension (2, 18). MetS affects ~25% of the adult population in the United States and 20–30% of adults in most other countries (22) and represents an increasingly important risk factor for the development and progression of chronic kidney disease (CKD) (41). MetS is associated with glomerular hyperfiltration (37), which precedes overt manifestation of cardiovascular disease. While elevated blood pressure (37) and/or glucose levels (39) that often coexist in MetS have been implicated in the pathogenesis of glomerular hyperfiltration, the mechanisms by which MetS induces renal injury are not completely understood.

Obesity is a major component of the current epidemic of MetS that leads to progressive lipid accumulation around and within key organs (ectopic fat storage), which may impair their function (30). In humans with type 2 diabetes, perirenal fat thickness is an independent predictor of kidney dysfunction (26). Although accumulating evidence links intrarenal lipid deposits in obese animals to kidney tissue injury (23, 25), the connection to renal hemodynamics and function is poorly understood. In obese rats, cerebrovascular remodeling (such as smaller luminal vessel diameter) correlates with development of hypertension and contributes to cerebral injury (31). However, the extent of renal microvascular remodeling in MetS and its temporal relationship to renal functional alteration remain unknown.

Therefore, our study was designed to test the hypothesis that increased glomerular filtration rate (GFR) in early MetS is associated with renal adiposity and microvascular remodeling. For this purpose, we studied both in vivo and ex vivo kidneys of Ossabaw pigs, which have a “thrifty genotype” to endure seasonal cycles of feasting and famine, thus constituting a unique large-animal model with a cluster of factors for MetS (15) closely resembling those observed in humans.

METHODS

Animals

The Institutional Animal Care and Use Committee approved this study. Twelve littermate Ossabaw pigs (Swine Resource, Indiana University) started a standard (lean; n = 6) or atherogenic (MetS; n = 6) diet. Kidney homodynamics and function, perirenal fat volume, and tubular dynamics were assessed in vivo by multidetector computed tomography (CT) and blood oxygen level-dependent (BOLD)-MRI. Microvascular architecture was assessed ex vivo with computed tomography (CT) and blood oxygen level-dependent volume, and tubular dynamics were assessed in vivo by multidetector (MDCT) for renal size, function, and changes after furosemide administration followed by multidetector computed tomography (MDCT; for renal size, function, and adiposity) 2 days later. Three days following the completion of in vivo studies, the pigs were euthanized with an overdose of pentobarbital sodium (100 mg/kg iv, Sleepaway, Fort Dodge Laboratories, Fort Dodge, IA) (27), kidneys were removed, immediately shock-frozen in liquid nitrogen and stored at −80°C, preserved in formalin, and prepared for micro-CT studies. Systemic and renal oxidative stress, inflammation, lipid accumulation, and microvascular architecture were then determined.

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**Systemic Measurements**

Blood pressure was measured with an intra-arterial catheter during the MDCT study. Plasma renin activity (PRA), endothelin (ET)-1, and TNF-α levels were evaluated as shown (19, 45). Plasma lipids [total cholesterol, triglyceride (TG), LDL, HDL], insulin, and glucose were measured by standard procedures. The glucose-to-insulin ratio and homeostasis model assessment index (HOMA) index (fasting plasma glucose × fasting plasma insulin/22.5) were used as indicators of insulin sensitivity (19). Systemic oxidative stress and nitric oxide (NO) metabolism were evaluated by plasma levels of oxidized LDL (Ox-LDL; AlpcO Diagnostics, Windham, NH), plasma 8-isoprostanes (EIA; Cayman Chemical, Ann Arbor, MI), and urine nitrate/nitrite (Cayman Chemical) following the manufacturers’ instructions.

**Gluomerular and Tubular Dynamics**

Single-kidney GFR and tubular dynamics were assessed in vivo using MRI and MDCT. Urine albumin concentration was assessed by EasyLyte Lithium Na/K/Li Analyzer (Medica). In vitro studies assessed the expression of angiogenic, oxidative, proinflammatory, and fibrogenic factors in the kidney. Angiogenic activity was evaluated by Western blotting of VEGF, its receptors (Cayman Chemical) following the manufacturers’ instructions. In vitro studies assessed the expression of angiogenic, oxidative, proinflammatory, and fibrogenic factors in the kidney. Angiogenic activity was evaluated by Western blotting of VEGF, its receptors.

**In Vivo Studies**

Each in vivo study included MRI followed by MDCT studies 2 days apart. For each study, animals were weighed, induced with IM Telazol (5 mg/kg) and xylazine (2 mg/kg), intubated, and ventilated with room air.

**MRI study.** Blood oxygen level-dependent (BOLD)-MRI was utilized to evaluate renal oxygenation and tubular dynamics, as we have previously shown (42, 43). Briefly, under 1%–2% isoflurane anesthesia, pigs were positioned in the MRI scanner (Sigma TwinSpeed EXCITE 3T system, GE Healthcare, Waukesha, WI), and BOLD images (5–6 axial-oblique) were acquired during suspended respiration through the upper, middle-, and lower pole during a 26– to 32-s acquisition. Imaging was repeated 15 min after a bolus injection of furosemide (0.05 mg/kg iv, Sigma, St. Louis, MO). Inhibition of O2 consumption by blocking sodium reabsorption in the thick ascending limb of Henle’s loop with this loop diuretic in healthy kidneys is followed by an ~10% decrease in intra-renal deoxyhemoglobin, and thus the BOLD signal $R_2^*$ (42).

MDCT study. The MDCT study was performed 2 days after MRI to evaluate renal structure and function. MDCT allows accurate and noninvasive evaluation of single-kidney volume, vascular volume fraction, regional perfusion, renal blood flow (RBF), GFR, and tubular function, as we have shown previously (14, 38). Briefly, catheters were placed under fluoroscopic guidance in the aorta and right atrium, and flow studies were initiated under baseline conditions. The flow studies were followed by a volume study in which kidneys were scanned from pole to pole for subsequent quantification of cortical and medullary volume.

MDCT images were then reconstructed and displayed with the Analyze software package (Biomedical Imaging Resource, Mayo Clinic, Rochester, MN). Regions of interest (ROI) were selected from the aorta, renal cortex, and medulla to generate time-attenuation curves and calculate cortical and medullary perfusion (ml/min−1·ml−1), normalized GFR (ml/min−1·ml−1), tubular mean transit time (MTT; s), and intratubular fluid concentration (ITC; arbitrary units) along the nephron (proximal tubule, Henle’s loop, and distal tubule) (10, 14). Single-kidney volume, vascular volume fraction (ml/ml), RBF (ml/min), and GFR (ml/min) were subsequently calculated (8, 11). Intra-abdominal adipose tissue was measured and expressed as volume and fraction, as described previously (19). For perirenal fat volume, a ROI was traced surrounding the right kidney on the MDCT-derived cross sections through the whole volume and was then expanded proportionally in three dimensions to the inner surface of the abdominal musculature. The perirenal fat was then measured based on the attenuation range for fat and expressed as a ratio to the volume of the corresponding kidney.

BOLD data were processed in MATLAB 7.10 (MathWorks, Natick, MA), as we have shown (42, 43). The BOLD index, $R_2^*$, was estimated in each voxel by fitting the MR signal intensity vs. echo time to a single exponential function and calculating the MR intensity decay rate. Medullary and cortical $R_2^*$ values were estimated from ROIs defined based on $T_2^*$-weighted MR images and calculating $R_2^*$ weighted-average of all ROIs for each compartment.

**In Vitro Studies**

In vitro studies assessed the expression of angiogenic, oxidative, proinflammatory, and fibrogenic factors in the kidney. Angiogenic activity was evaluated by Western blotting of VEGF, its receptors.

### Table 1. Systemic characteristics in lean and metabolic syndrome pigs

<table>
<thead>
<tr>
<th></th>
<th>Lean</th>
<th>MetS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of pigs</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Body weight, kg</td>
<td>15.6 ± 1.6</td>
<td>24.2 ± 1.9*</td>
</tr>
<tr>
<td>Intra-abdominal fat, %</td>
<td>8.9 ± 1.8</td>
<td>20.0 ± 1.7*</td>
</tr>
<tr>
<td>Total cholesterol, mg/dl</td>
<td>76.3 ± 5.6</td>
<td>362.6 ± 76.6*</td>
</tr>
<tr>
<td>LDL cholesterol, mg/dl</td>
<td>34.8 ± 2.4</td>
<td>255.7 ± 63.5*</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dl</td>
<td>36.7 ± 3.8</td>
<td>101.6 ± 21.3*</td>
</tr>
<tr>
<td>LDL/HDL</td>
<td>1.0 ± 0.08</td>
<td>2.5 ± 0.5*</td>
</tr>
<tr>
<td>Plasma triglycerides, mg/dl</td>
<td>23.9 ± 4.3</td>
<td>26.3 ± 7.8</td>
</tr>
<tr>
<td>Mean arterial pressure, mmHg</td>
<td>112.2 ± 7.3</td>
<td>109.6 ± 9.6</td>
</tr>
<tr>
<td>Glucose, mg/dl</td>
<td>132.3 ± 54.9</td>
<td>132.4 ± 17.1</td>
</tr>
<tr>
<td>Insulin, μU/ml</td>
<td>0.11 ± 0.05</td>
<td>0.41 ± 0.11</td>
</tr>
<tr>
<td>Glucose-to-insulin ratio, mg/μU</td>
<td>14.3 ± 2.0</td>
<td>6.4 ± 2.3*</td>
</tr>
<tr>
<td>Homeostasis model assessment index</td>
<td>0.9 ± 0.6</td>
<td>2.6 ± 0.7</td>
</tr>
<tr>
<td>Plasma renin activity, ng·ml−1·h−1</td>
<td>0.11 ± 0.04</td>
<td>0.28 ± 0.20</td>
</tr>
<tr>
<td>Tumor necrosis factor-α, pg/ml</td>
<td>44.4 ± 15.5</td>
<td>47.8 ± 11.1</td>
</tr>
<tr>
<td>Endothelin-1, pg/ml</td>
<td>26.2 ± 3.2</td>
<td>31.8 ± 3.3</td>
</tr>
<tr>
<td>Oxidized LDL, mg/ml</td>
<td>328.1 ± 33.1</td>
<td>567.6 ± 201.3</td>
</tr>
<tr>
<td>8-epi-Isoprostane, pg/ml</td>
<td>329.3 ± 154.6</td>
<td>351.5 ± 60.6</td>
</tr>
<tr>
<td>Urinary nitrate/nitrite, μM</td>
<td>69.4 ± 10.8</td>
<td>128.1 ± 32.8</td>
</tr>
<tr>
<td>Urine protein, μg/ml</td>
<td>24.7 ± 20.0</td>
<td>62.8 ± 41.3</td>
</tr>
<tr>
<td>Urine sodium, mmol/l</td>
<td>61.4 ± 17.5</td>
<td>50.5 ± 13.2</td>
</tr>
<tr>
<td>Plasma sodium, mmol/l</td>
<td>134.7 ± 0.6</td>
<td>133.7 ± 0.6</td>
</tr>
</tbody>
</table>

Values are means ± SE. MetS, metabolic syndrome. *P ≤ 0.05 vs. lean.

### Table 2. Single-kidney hemodynamics and function in lean and MetS pigs

<table>
<thead>
<tr>
<th></th>
<th>Lean</th>
<th>MetS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Renal volume, ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cortext</td>
<td>39.5 ± 2.5</td>
<td>69.9 ± 4.9*</td>
</tr>
<tr>
<td>Medulla</td>
<td>12.5 ± 1.2</td>
<td>12.0 ± 1.0</td>
</tr>
<tr>
<td>Vascular volume fraction, ml/ml</td>
<td>0.36 ± 0.01</td>
<td>0.41 ± 0.02*</td>
</tr>
<tr>
<td>Cortext</td>
<td>0.43 ± 0.05</td>
<td>0.48 ± 0.03</td>
</tr>
<tr>
<td>Renal perfusion, ml/min−1·ml−1</td>
<td>4.4 ± 0.4</td>
<td>5.4 ± 0.5*</td>
</tr>
<tr>
<td>Cortext</td>
<td>4.0 ± 0.5</td>
<td>4.9 ± 0.7</td>
</tr>
<tr>
<td>Renal blood flow, ml/min</td>
<td>216.8 ± 16.2</td>
<td>411.0 ± 48.5*</td>
</tr>
<tr>
<td>Glomerular filtration rate, ml/min</td>
<td>31.7 ± 2.2</td>
<td>58.5 ± 7.1*</td>
</tr>
<tr>
<td>Filtration fraction, %</td>
<td>15.2 ± 1.1</td>
<td>14.5 ± 1.4</td>
</tr>
<tr>
<td>Mean transit time, s</td>
<td>41.0 ± 1.7</td>
<td>45.3 ± 1.7*</td>
</tr>
<tr>
<td>Proximal tubule</td>
<td>76.9 ± 3.1</td>
<td>86.0 ± 2.5*</td>
</tr>
<tr>
<td>Distal tubule</td>
<td>125.6 ± 5.0</td>
<td>127.8 ± 4.7</td>
</tr>
</tbody>
</table>

Values are means ± SE. *P ≤ 0.05 vs. lean.
Flt-1 and Flk-1 (1:200, Santa Cruz Biotechnology), the angiogenesis inhibitor thrombospondin-1 (TSP-1; 1:500, Abcam), matrix metalloproteinase (MMP)-1, MMP-10 (1:1,000, Abcam), and MMP-2 (1:200, Santa Cruz Biotechnology) expression. Renal redox status was evaluated by the in situ production of superoxide anion, detected by fluorescence microscopy using dihydroethidium (DHE; 20 μM, Sigma), by immunohistochemistry staining of ox-LDL (1:150, Abcam), and by protein expression of the p47 (1:200, Santa Cruz Biotechnology) and gp91 (1:500, Millipore) subunits of NAD(P)H oxidase, xanthine oxidase (XO; 1:10,000, Chemicon), nitrotyrosine (NT; 1:200, Cayman Chemical), endothelial nitric oxide synthase (eNOS), uncoupled eNOS (1:200, Santa Cruz Biotechnology), and activated phospho-eNOS (1:125, Invitrogen). Inflammation was investigated by staining of macrophage marker CD-163 (1:500, AbD Serotec) and by protein expression of monocyte chemoattractant protein-1 (MCP-1; 1:7,500, MyBioSource) and its receptor C-C motif receptor-2 (CCR-2; 1:1,000, Thermo), TNF-α (1:200, Santa Cruz Biotechnology), IFN-γ (1:1,000, MBL), IL-6 (1:1,000, Abcam), and NF-κB (1:5,000, Upstate). Endogenous antioxidative and anti-inflammatory defenses were evaluated by the expression of SOD-1 (1:200, Santa Cruz Biotechnology) and IL-10 (1:500, Abcam), respectively. Fibrosis was studied by trichrome staining and by protein expression of plasminogen activator inhibitor-1 (PAI-1; 1:2,500, BD Biosciences) and transforming growth factor (TGF)-β1 (1:200, Santa Cruz Biotechnology).

**Micro-CT.** Shortly after euthanasia, a kidney lobe was flushed and perfused with a radio-opaque polymer (Microfil MV122, Flow Tech) under physiological pressure through a cannula ligated in a branch of the renal artery, and scanned with micro-CT at 0.5° angular increments at 18-μm resolution, as previously described (16, 44, 46). The spatial density, average diameter, and tortuosity of microvessels (diameters 20–500 μm) in the renal cortex and outer and inner medulla were measured using Analyze and classified according to diameter as small (20–40 μm), medium (40–100 μm), or large (100–500 μm) microvessels (11, 44).

**Quantification of triglyceride in kidney tissue.** Kidney tissue (100 mg) was homogenized in 1 ml of 5% Triton X-100 solution. The samples were heated slowly to 80–100°C for 5 min or until the Triton X-100 solution turned cloudy and then was cooled down to room temperature. The heating process was then repeated once. The samples were centrifuged at 14,000 rpm for 5 min. Fifty microliters of 10-fold diluted supernatant was used in the assay with a triglyceride quantification kit (BioVision).

**Histology.** Staining was performed in 5 μm of either frozen or paraffin-preserved midhilar renal cross sections following standard protocols, then semiautomatically quantified by a computer-aided image-analysis program (MetaMorph, Meta Imaging, Molecular Devices, Sunnyvale, CA) (38). DHE staining was quantified in 10 random fields, expressed as the fraction of surface area, and the results from all fields were averaged. A glomerular score (% of sclerotic glomeruli) was also calculated (8–11).

**Statistical Analysis**

Results are expressed as means ± SE. Comparisons within and between groups were performed with paired and unpaired Student’s t-tests with the Bonferroni correction, respectively. Least-square regression was used to explore the relationship between GFR and tissue measures. Statistical significance was accepted at $P \leq 0.05$.

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**Fig. 1.** Representative coronal images of blood oxygen level-dependent MRI (A–D) and quantification of their basal $R_2^*$ (E) and response to furosemide (F). The arrow indicates hypoxic medulla. MetS, metabolic syndrome. *$P \leq 0.05$ vs. baseline.
RESULTS

Systemic Characteristics

As summarized in Table 1, the MetS pigs were ~50% heavier compared with the lean group, accompanied by an accumulation of visceral fat. The MetS pigs expressed changes in lipids characterized by increased total cholesterol, LDL, and LDL/HDL ratio. Blood pressure, PRA, ET-1, and TNF-α remained unchanged between the two groups. Glucose and insulin levels were not different between the groups, but mild insulin resistance in MetS was reflected in lower glucose-to-insulin ratio ($P = 0.038$) and a trend for insulin level ($P = 0.06$) and HOMA index ($P = 0.09$) to increase. Nitrate/nitrite tended to increase in MetS pigs ($P = 0.07$), while the systemic oxidative stress markers ox-LDL and 8-iso-prostane were unchanged. Urine protein and plasma and urine sodium levels were not different between the two groups.

Renal Hemodynamics and Function

RBF and GFR were almost twofold higher in the MetS compared with the lean group (Table 2), mostly due to an increase in renal cortical volume, vascular volume fraction, and perfusion, whereas filtration fraction, medullary size, and perfusion showed no difference between groups. Longer proximal and Henle’s MTT in MetS pigs indicated slower proximal and Henle’s tubular flow. Henle’s ITC was significantly elevated in the MetS group, suggesting augmented fluid reabsorption that likely started at a proximal segment. BOLD-MRI demonstrated similar basal cortical and medullary oxygenation and responses to furosemide in the two groups (Fig. 1). Although furosemide primarily inhibits the Na-K-Cl cotransporter in the medullary thick ascending limb, we have previously shown that cortical oxygenation also slightly increases after furosemide injection (42).

Renal Adiposity

The ratios of intra-abdominal fat-to-cavity and perirenal fat-to-kidney volumes were both markedly higher in the MetS group (Fig. 2). Higher levels of tissue-neutral fats in MetS were shown by renal oil-red-O staining (Fig. 3, A–E). Renal tissue triglyceride levels were also higher in MetS pigs and significantly positively correlated with GFR (Fig. 3F).

Inflammation and Oxidative Stress

Renal expression of IL-6 tended to increase ($P = 0.057$) in MetS, whereas the expressions of MCP-1, CCR2, TNF-α, INF-γ, and NF-κB were similar in both groups, and IL-10 expression was markedly elevated ($P = 0.003$) (Fig. 4, A and B). Elevated superoxide anion production and renal expression...

Fig. 2. Representative cross-sectional tomographic images of intra-abdominal fat (A and B), 3-dimensional images of perirenal fat (C–F), and their respective fractions (means ± SE; G). The yellow area (arrow) represents fat tissue. *$P \leq 0.05$ vs. lean.
of NT in MetS indicated an increase in oxidative stress and was associated with elevated antioxidant defense demonstrated by increased expression of SOD1 ($P < 0.046$) (Fig. 4, A–E). The expression of gp91, uncoupled eNOS, and XO was not different between the groups (data not shown). Upregulated renal phospho-eNOS expression (Fig. 5F) and a strong tendency of systemic nitrate/nitrite to increase ($P = 0.07$) (Table 1) may suggest increased NO bioavailability.

Microvascular Proliferation and Angiogenic Activity Were Increased in Smaller Cortical Microvessels

The density of cortical microvessels 20–40 μm in diameter was increased in the MetS kidney, while the density of larger microvessels was unchanged (Fig. 5, A–D). Cortical microvessels in obese pigs were also characterized by greater tortuosity (Fig. 5E). There was no change in the medullary microcirculation. Taken together, these suggested microvascular proliferation in the MetS group, which was supported by enhanced angiogenic activity and remodeling reflected by increased protein expression of VEGF, MMP-1, -2, and -10 and decreased protein expression of the angiogenesis inhibitor TSP-1 (Fig. 5, F and G).

Renal Structure Reflected Tubular Vacuolization

Renal fibrosis was not significantly greater ($P = 0.15$ vs. lean) in MetS (Fig. 6, A–C), and neither was the expression of TGF-β or PAI-1 (Fig. 6, D and E), or glomerulosclerosis (data not shown). In contrast, marked proximal tubular vacuolization was found in MetS kidneys (Fig. 6, F and G), suggesting tubular degenerative changes and atrophy.

DISCUSSION

The present study shows that elevated GFR in vivo in early MetS in Ossabaw swine is associated with significant intrarenal and perirenal adiposity, as well as microvascular proliferation and proximal tubular vacuolization, which precede pronounced systemic metabolic derangement. The relatively mild oxidative stress and inflammation in the kidney tissue argue against a major role for these mechanisms in triggering the observed angiogenesis.

Currently, there are more than 20 million CKD patients in the United States (13), which is paralleled by and associated with an epidemic increase in MetS (41). Several mechanisms by which advanced MetS may initiate and exacerbate CKD have been proposed (41), including hyperglycemia, hyperinsulinemia, and hypertension. However, the precipitating renal pathophysiological changes that occur early in MetS and their underlying trigger mechanisms remain poorly understood.

In this study, we took advantage of a unique swine model and cutting-edge imaging modalities to explore the underlying pathomechanisms of renal injury in an early stage of MetS. After 10 wk of an atherogenic diet, the Ossabaw swine developed early MetS, reflected by considerably elevated body weight and abdominal fat volume, hyperlipidemia, and insulin resistance. The early stage of MetS, implied by unchanged systemic triglyceride, glucose, and blood pressure levels, enabled the dissection of precipitating factors for renal functional disturbances. Our findings of increased in vivo GFR, RBF, and renal perfusion in the MetS pigs are analogous to the association of MetS in young people with glomerular hyperfiltration (24, 37). Because the filtration fraction was not different between the lean and normotensive MetS pigs, rather than...
increased filtration pressure, we interpret the rise in GFR to be likely secondary to increased blood flow, an early hemodynamic change and a pathogenic event in the development of CKD (36). Glomerular hyperfiltration may result from several mechanisms, including elevated blood pressure (37), increased proximal tubular sodium reabsorption with systemic volume expansion (3), or an attenuated tubuloglomerular feedback mechanism (39, 40). Importantly, while blood pressure was not elevated in our model, MDCT measurements demonstrated increased proximal tubular reabsorption (prolonged transit times and increased fluid concentration), while BOLD-MRI confirmed that medullary R2* responses to inhibiting solute reabsorption with furosemide remained intact. Although we did not directly pursue the underlying cause for the increased reabsorption, our findings indicate that increased glucose, insulin, or blood pressure levels were not required for this effect, since none of these were elevated in our MetS model. Furthermore, impaired insulin signaling in the kidney appears to affect the glomeruli and spare the tubules (29). Therefore, our results suggest that enhanced renal hemodynamics can precede many systemic metabolic abnormalities observed in advanced MetS.

In our study, MetS pigs were characterized by greater renal cortical, but not medullary, volume, vascular volume fraction, and perfusion, suggesting selective involvement of the renal cortex. It is plausible that marked chronic increases in cortical perfusion would be accompanied by increased numbers of vessels to support it. Indeed, we found significant cortical microvascular proliferation and remodeling in MetS pigs. Interestingly, only smaller microvessels (20–40 μm) proliferated, accompanied by increased expression of VEGF, raising the possibility that these new vessels are needed to sustain renal perfusion, which at least partially contributes to the elevated RBF and GFR observed in MetS. Pertinently, we have previously observed that inhibition of vascular proliferation using thalidomide, an anti-inflammatory and antiangiogenic drug, decreased basal renal hemodynamics and function in hypercholesterolemia (5).

Renal angiogenic activity in MetS pigs is reflected in increased expression of MMP-1 and MMP-2, which promote endothelial cell migration into the perivascular space, capillary sprouting, and microvascular development. However, newly generated vessels may not function properly because of their disorganized architecture, endothelial dysfunction, or increased permeability, or blind endings. Thus an increase in the number of fragile and permeable vessels may not necessarily benefit the kidney but may in fact exacerbate tissue injury through increasing extravasation of inflammatory factors. This assertion is supported by a recent finding that vasohibin-1, a
negative feedback regulator of angiogenesis, ameliorates glomerular hypertrophy and hyperfiltration in obese type 2 diabetic mice (35). On the other hand, maturation of the endothelial network involves remodeling and “pruning” of irregularly organized vessels into a structured network of branching vessels, which may also improve vascular function, and was suggested by increased expression of MMP-10 in MetS (20). Nevertheless, subsequent microvascular rarefaction and consequently renal injury through a positive-feedback mechanism may occur ultimately as MetS worsens.

Furthermore, elevated RBF and GFR in our study may also be associated with increased shear stress and NO bioavailability, as evidenced by elevated expression of phospho-eNOS and NT in renal tissue, and the tendency of urine nitrate/nitrite to increase in MetS pigs. Increased shear stress can stimulate angiogenesis and increase blood flow. NO levels are elevated in diabetic young adults (12) and obese children (21) and linked to glomerular hyperfiltration, oxidative stress, and inflammation, although NO synthesis might later decline with disease progression.

One of the major drivers of the association between metabolic factors and glomerular hyperfiltration is likely adipose tissue, a source of multiple adipocytokines, which are associated with GFR in patients with CKD and diabetes (37). Additionally, adipose tissue also produces and secretes IL-6 (1), which was slightly increased in MetS. These were conceivably produced by enhanced renal adiposity in the MetS kidney, as evidenced by significant perirenal and intrarenal fat accumulation. Importantly, inflammatory cytokines like IL-6 also support angiogenesis during adipose tissue growth by upregulating VEGF (33) and thus may have contributed to renal cortical angiogenesis in the MetS pigs. Indeed, adipose tissue is capable of stimulating angiogenesis (4, 17), and its mass can be regulated by its vasculature (34). Furthermore, GFR in this study was positively correlated with the amount of tissue triglycerides. Collectively, these data suggest that adipocytokines may mediate, at least in part, the observed association among increased adiposity, angiogenesis, and glomerular hyperfiltration, with a potential to foster the development of glomerular dysfunction and damage.
Ectopic perirenal fat has been proposed to physically compress the kidney, especially the inner medulla, which is not protected by the fibrous capsule, increasing renal interstitial fluid hydrostatic pressure and reducing tubular flow through the distensible loop of Henle. In the present study, the renal cortex seemed to be more vulnerable to early pathophysiological changes in MetS compared with the renal medulla. We observed in MetS pigs increased proximal reabsorption (which can extend to increase in ITC detectible in Henle’s loop). The unchanged perfusion, oxygen levels, and responses to furosemide in the medulla as detected by our imaging techniques do not support intense basal reabsorption activity in this region. In contrast, increased angiogenesis and perfusion in the cortex sustained adequate oxygen supply for proximal reabsorption, which does not involve substantial oxygen consumption. Lower distal NaCl delivery to the macular densa, however, may elicit an increase in GFR via the tubuloglomerular feedback mechanism. In the long term, these changes may generate a hemodynamic burden on the kidneys. Possibly, the marked proximal tubular vacuolization as recently observed in obese mice (28) may serve as a marker of tubular injury.

Interestingly, mild oxidative stress and inflammation were accompanied by activation of antioxidant and anti-inflammatory mechanisms in the MetS kidney, as demonstrated by increased expression of SOD-1 and IL-10. Furthermore, in addition to mediating angiogenesis, activation of MMP in the MetS kidney may also serve to curtail fibrosis, as we have previously suggested (7, 9). These observations imply that in early MetS endogenous defense mechanisms curtail oxidative stress and inflammation, yet might deteriorate as defense mechanisms are downregulated upon disease progression (6).

**Limitations**

The present study design and sample size did not enable establishing a causal relationship among increased GFR, adiposity, and angiogenesis in MetS, a rather complex biological system with multiple interactions. Future studies are needed to explore the role of specific mechanisms in MetS. This was unlikely due to the increases in serum cholesterol alone, because we have previously shown that high cholesterol pigs that are not heavier than pigs fed standard chow show no insulin resistance (19).

In summary, our study demonstrated early pathophysiological changes in the kidney of a swine model of experimental MetS both in vivo and in vitro. Importantly, it shows for the first time that increased GFR is associated with renal adiposity and microvascular proliferation in early MetS. Renal oxygen-

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Fig. 6. Representative images (A and B) and quantification (C) of renal fibrosis (trichrome blue). D and E: renal protein expression of transforming growth factor (TGF)-β1 and plasminogen activator inhibitor (PAI)-1 in MetS. The PAI-1 lanes located between the lean and MetS groups were spliced as indicated to remove lanes derived from an unrelated group on that blot. F and G: representative images showing marked proximal tubular vacuolization (black arrow) in the MetS kidney.
ation and medullary tubular dynamics were relatively preserved. As glomerular hyperfiltration may constitute an early, and possibly reversible, marker of CKD, renal adiposity and proliferative microvessels may represent novel therapeutic targets for preserving renal function in the early phase of MetS.

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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the authors.

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