Obesity-metabolic derangement preserves hemodynamics but promotes intrarenal adiposity and macrophage infiltration in swine renovascular disease

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Importantly, the constellation of metabolic derangements (ObM) that often accompanies obesity, including insulin resistance, elevated blood pressure, and atherogenic dyslipidemia (e.g., hypertriglyceridemia), contributes to the pathophysiological changes during obesity-related tissue injury, including the kidney (31). Although the underlying mechanism mediating kidney damage in ObM is incompletely understood, adaptations to increased body mass/excretory load or sodium retention, adverse effects of insulin resistance, excess of lipid products in the kidney, inflammation, and activated renin-angiotensin system have been all considered (2). Inflammatory cytokines implicated as important mediators of ObM-related kidney injury include TNF-α, IFN-γ, IL-6, leptin, and monocyte chemoattractant protein (MCP)-1, which might be derived from the cytokine-rich adipose tissue (31). In addition, the adaptation to increased body mass or excretory load also plays important role in the renal pathology by elevating the renal blood flow (RBF) and increasing the workload of the kidney (31).

Renal artery stenosis (RAS) often increases inflammation, microvascular remodeling, and tissue scarring in the kidney due to hypoperfusion and vasocostriction (21, 38). These deleterious processes might be exacerbated as a result of superimposed co-morbid conditions. However, whether concurrent ObM aggravates poststenotic kidney (STK) injury and the potential pathways have not been elucidated. We hypothesized that coexistence of ObM and RAS amplifies the STK inflammation and structural damage.

MATERIALS AND METHODS

Study Protocols

This study was approved by the Institutional Animal Care and Use Committee. Twenty-four 3-mo-old littermate Ossabaw pigs (Swine Resource, Indiana University) were randomized in four groups (n = 7 each) that included RAS and sham pigs fed with an ObM diet (ObM-RAS and ObM-sham; high-fat/high-fructose, SB4L, Purina Test Diet, Richmond, IN) (22) or standard chow [Lean-RAS and Lean-sham (normal control)]. Diets were fed for a total of 16 wk, with free access to water. Twelve weeks after initiation of diets, RAS induction or sham procedure was performed, followed 4 wk later by determination of renal function and oxygenation using multidetector computed tomography (MDCT) and blood oxygenation level-dependent (BOLD) MRI. Animals were then euthanized, and both kidneys were collected for assessments of the microvasculature, histology, and protein expression.

RAS Induction

Placement of a local irritant coil in the right main renal artery of the corresponding groups led to a gradual development of unilateral RAS, as previously described (5). Blood pressure was then measured continuously by a telemetry transducer (Data Science International, St. Paul, MN) implanted in the left femoral artery (9, 39).

The prevelance of obesity is on the rise world-wide (14, 27, 28). Obesity is associated with hypertension, diabetes, and atherosclerosis (37), and contributes greatly to cardiovascular morbidity and mortality. Obesity has also been identified to initiate chronic kidney disease, affect the progression of pre-existing renal diseases (3, 15), and is a strong independent risk factor for end-stage renal disease (16).
Table 1. Systemic characteristics in sham and renal artery stenosis (RAS) pigs with or without obesity metabolic derangement (ObM; n = 7 each)

<table>
<thead>
<tr>
<th></th>
<th>Lean</th>
<th>ObM</th>
<th>RAS</th>
<th>ObM-STK</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, kg</td>
<td>31.4</td>
<td>45.0</td>
<td>35.7</td>
<td>45.3</td>
</tr>
<tr>
<td>Degree of stenosis, %</td>
<td>87.9</td>
<td>85.6</td>
<td>85.6</td>
<td>86.6</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>98.9</td>
<td>112.2</td>
<td>122.2</td>
<td>136.2</td>
</tr>
<tr>
<td>Urine mAlb, µg/ml</td>
<td>6.6</td>
<td>21.5</td>
<td>153.5</td>
<td>221.1</td>
</tr>
<tr>
<td>Fasting glucose, mg/dl</td>
<td>163.1</td>
<td>179.3</td>
<td>186.4</td>
<td>184.6</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>3.1</td>
<td>6.6</td>
<td>1.9</td>
<td>9.3</td>
</tr>
<tr>
<td>TC, mg/dl</td>
<td>20.1</td>
<td>47.0</td>
<td>18.5</td>
<td>42.5</td>
</tr>
<tr>
<td>LDL, mg/dl</td>
<td>21.6</td>
<td>40.1</td>
<td>1.2</td>
<td>1.3</td>
</tr>
<tr>
<td>Plasma creatinine, mg/dl</td>
<td>1.1</td>
<td>1.1</td>
<td>0.07</td>
<td>0.16</td>
</tr>
<tr>
<td>PRA, ng·ml⁻¹·h⁻¹</td>
<td>0.02</td>
<td>0.10</td>
<td>30.2</td>
<td>41.5</td>
</tr>
<tr>
<td>Abdominal fat volume fraction, %</td>
<td>27.7</td>
<td>38.2</td>
<td>30.2</td>
<td>41.5</td>
</tr>
</tbody>
</table>

Values are means ± SE. MAP, mean arterial pressure; mAlb, microalbumin; HOMA-IR, homeostasis model assessment of insulin resistance; TC, total cholesterol; TG, triglycerides; PRA, plasma renin activity. *P < 0.05 vs. Lean-sham; †P < 0.05 vs. ObM-sham.

Fig. 1. Intrarenal oxygenation and medullary tubular function in stenotic (STK) and sham kidneys with or without obesity-metabolic derangement (ObM; n = 7 each). A: representative kidney blood oxygen level-dependent (BOLD)-MRI images obtained at baseline and after furosemide. Dashed lines (white) demarcate the zone encompassing the medullary regions. Cross-lines (black) differentiate vessels from hypoxic regions. B: oxygenation level (R2*) in the cortex. Renal artery stenosis (RAS) decreased cortical oxygenation level (increased R2*) in both Lean-STK and ObM-STK groups. C: oxygenation level in the medulla and its response to furosemide. Impaired tubular transport function was indicated in ObM-STK. #RAS: significant effect of RAS (2-way ANOVA). *P < 0.05 vs. Lean-sham; †P < 0.05 vs. pre-furosemide (paired t-test).
In Vivo Studies

MDCT. At 16 wk, MDCT scanning was performed to assess the STK and contralateral (CLK) kidney volume, RBF and glomerular filtration rate (GFR), as described previously (5, 9). Briefly, 160 consecutive scans were performed following a central venous injection of iopamidol (0.5 ml·kg⁻¹·2 s⁻¹). Then, MDCT images were reconstructed and displayed with the Analyze software package (Bio-medical Imaging Resource, Mayo Clinic, Rochester, MN). For data analysis, regions of interest (ROI) were drawn in the aorta, renal cortex, and medulla, and time-attenuation curves were used to obtain measures of RBF and GFR (9). Intra-abdominal adipose tissue volume was also measured from CT images and expressed as a fraction of the abdominal cavity volume, as described previously (22).

BOLD-MRI. Three days before MDCT, 3T BOLD-MRI (Signa Echo Speed; GE Medical Systems, Milwaukee, WI) was performed as described (11, 32) to assess intrarenal oxygenation, expressed as R2*, an index of deoxyhemoglobin concentration in the kidney cortex and medulla. To further examine tubular transport in the medullary thick ascending limb of Henle’s loop, BOLD measurements were repeated 15 min after a furosemide (20 mg) injection into an ear vein cannula. In viable medullary tubules, furosemide inhibits solute transport activity and thereby oxygen demand, and improves medullary oxy-

Table 2. Single-kidney hemodynamics and function in sham and stenotic kidneys (STK) from Lean or ObM pigs (n = 7 each)

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>ObM</th>
<th>Lean-STK</th>
<th>ObM-STK</th>
<th>Diet</th>
<th>RAS</th>
<th>DietxRAS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Renal volume, mm³</td>
<td>74.5 ± 5.6</td>
<td>90.1 ± 8.0</td>
<td>43.4 ± 11.9*</td>
<td>63.4 ± 9.8†</td>
<td>0.06</td>
<td>0.004</td>
<td>0.81</td>
</tr>
<tr>
<td>RBF, ml/min</td>
<td>427.5 ± 27.2</td>
<td>584.3 ± 95.4</td>
<td>238.6 ± 78.3</td>
<td>311.8 ± 62.7†</td>
<td>0.17</td>
<td>0.007</td>
<td>0.60</td>
</tr>
<tr>
<td>GFR, ml/min</td>
<td>50.5 ± 5.3</td>
<td>68.8 ± 7.7</td>
<td>27.8 ± 9.8*</td>
<td>36.3 ± 7.8*</td>
<td>0.11</td>
<td>0.003</td>
<td>0.54</td>
</tr>
</tbody>
</table>

Values are means ± SE. RBF, renal blood flow; GFR, glomerular filtration rate. *P < 0.05 vs. Lean-sham. †P < 0.05 vs. ObM-sham.

Fig. 2. Microcirculation and angiogenic activities in sham and STK from Lean and ObM pigs (n = 7 each). A: representative 3-dimensional tomographic images of the cortical microcirculation. B: quantifications of microvascular density in small, medium, and large vessels, and the average vessel diameter. Arrows (green) indicate arcuate arteries at the junction of the renal cortex and medulla. C: renal protein expressions of vascular endothelial growth factor (VEGF) and its receptor-2 FLK-1. Protein bands were quantified relative to GAPDH. Two representative bands from each group are shown. ▲RAS: significant effect of RAS; ▲DietxRAS: significant interaction of ObM diet and RAS (2-way ANOVA). *P < 0.05 vs. Lean-sham; §P < 0.05 vs. ObM-sham.
genation (decreases R2*) (11). Therefore, the change in R2* is taken as an index of the viability of the tubules. For data analysis, ROIs were manually traced in the cortex and medulla on the 7-ms echo time images that give the best anatomic details in each experimental period. For each echo time, the software automatically computed the average of MR signals within each ROI. The BOLD signal (relaxivity index, R2*) was measured both at baseline and after furosemide, and the change in R2* is presented as delta R2*.

Ex Vivo Studies

Blood and urine sample. Urine was collected before MDCT by bladder puncture and blood samples from the inferior vena cava (IVC). Urine microalbumin (mAlb; Arbor Assays, Ann Arbor, MI), creatinine, and a lipid profile (Roche) including total cholesterol, triglycerides, and LDL were assessed. Fasting blood glucose and insulin level were measured by standard procedures, and the homeostasis model assessment of insulin resistance (HOMA-IR) index (fasting plasma glucose / fasting plasma insulin / 2.25) was calculated to evaluate insulin sensitivity (4, 22). Plasma renin activity (RIA; DiaSorin) was measured to evaluate the renin-angiotensin system. Plasma 8-isoprostanes (EIA; Cayman Chemical, Ann Arbor, MI) and oxidized-LDL (Ox-LDL; Alpco Diagnostics, Windham, NH) levels served as systemic oxidative stress indices.

Assessments of systemic and kidney release of inflammatory cytokines. Levels of inflammatory cytokines were measured by ELISA in both

<p>| Table 3. Systemic levels of inflammatory markers in sham or RAS pigs with or without ObM (n = 7 each) |
|---------------------------------|------------------|------------------|------------------|--------------------|</p>
<table>
<thead>
<tr>
<th></th>
<th>Lean</th>
<th>ObM</th>
<th>Lean</th>
<th>RAS</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCP-1, ng/ml</td>
<td>0.09 ± 0.01</td>
<td>0.09 ± 0.02</td>
<td>0.2 ± 0.07</td>
<td>0.2 ± 0.04</td>
</tr>
<tr>
<td>TNF-α, ng/ml</td>
<td>0.03 ± 0.08</td>
<td>0.06 ± 0.00</td>
<td>0.16 ± 0.05</td>
<td>0.11 ± 0.03</td>
</tr>
<tr>
<td>sE-selectin, ng/ml</td>
<td>6.7 ± 3.7</td>
<td>4.8 ± 4.8</td>
<td>15.4 ± 7.1</td>
<td>36.8 ± 10.8</td>
</tr>
<tr>
<td>IFN-γ, ng/ml</td>
<td>0.14 ± 0.00</td>
<td>0.15 ± 0.02</td>
<td>0.55 ± 0.14</td>
<td>0.40 ± 0.11</td>
</tr>
<tr>
<td>IL-17, pg/ml</td>
<td>18.81 ± 1.80</td>
<td>26.82 ± 7.47</td>
<td>41.49 ± 27.76</td>
<td>66.50 ± 36.91</td>
</tr>
<tr>
<td>IL-10, pg/ml</td>
<td>8.12 ± 2.81</td>
<td>6.00 ± 1.34</td>
<td>4.85 ± 1.60</td>
<td>3.71 ± 0.92</td>
</tr>
</tbody>
</table>

Values are means ± SE. MCP-1, monocyte chemotactic protein-1; sE-selectin, soluble E-selectin. *P ≤ 0.05 vs. Lean-sham. †P ≤ 0.05 vs. ObM-sham.

Fig. 3. Kidney release of inflammatory cytokines in sham and STK from Lean and ObM pigs (n = 7 each group). A: MCP-1. B: TNF-α. C: sE-selectin. D: IFN-γ. E: IL-17. F: IL-10. ObM-STK group showed magnified inflammation in the kidney. ▲RAS: significant effect of RAS; ▲Diet: significant effect of ObM diet (2-way ANOVA). *P ≤ 0.05 vs. Lean-sham.
the IVC and STK renal veins (RV), including MCP-1 (VS0081S-002, Kingfisher Biotech), TNF-α (KSC3011 Invitrogen), IFN-γ (VS0259S-002, Kingfisher), soluble E-selectin (sE-selectin, P4988, Biotang), IL-17 (VS0260S-002, Kingfisher), and the anti-inflammatory marker IL-10 (KSC0101, Invitrogen). The gradient of these cytokines between RV and IVC was assessed, and their net release from the STK was calculated ([RV-IVC] X RBF) (12).

Animals were euthanized after a 3-day recovery with a lethal intravenous dose of 100 mg/kg pentobarbital sodium (Sleepaway, Fort Dodge Laboratories, Fort Dodge, IA). Both kidneys were removed using a retroperitoneal incision and immediately dissected and preserved for micro-CT (renal microvasculature) and tissue studies (Western blotting and histology). Abdominal and perirenal fat were excised and prepared for ex vivo studies.

Micro CT. After the kidney was flushed, microfil MV122 (an intravascular contrast agent) was perfused into the STK under physiological pressure through a cannula ligated in the renal artery. Samples were prepared and scanned at 0.5° angular increments at 18-µm resolution, and images were analyzed as previously described (39). The spatial density of microvessels (defined as diameters <500 µm) in the inner and outer halves of the renal cortex were calculated using Analyze and classified according to diameter as small (20–40 µm), medium (40–200 µm), or large (200–500 µm) microvessels (39).

Histology and Western blotting. Intrarenal inflammation was assessed by macrophage (MØ) staining (CD163, 1:50, Abcam). Their subpopulations were evaluated by coexpression of either inducible nitric oxide synthase (iNOS) for proinflammatory M1-MØ or arginase-1 (1:50, both Abcam) for M2-MØ, which resolve inflammation and elicit tissue repair (19, 29). Intrarenal oxidative stress was assessed by Ox-LDL staining (1:50, Abcam) and Western blotting for p47 (1:200, Santa Cruz Biotechnology), and angiogenic activity by expression of vascular endothelial growth factor (VEGF) and its receptor-2 FLK-1 (1:200, both Santa Cruz Biotechnology). Kidney fibrosis was examined by trichrome staining and glomerulosclerosis score (% of sclerotic of 100 glomeruli) (5, 6), and oil-red-O staining for kidney fat deposition. Abdominal and perirenal fat tissues were also examined for inflammation by M1-MØ and TNF-α (1:50, Abcam) staining (30) and fibrosis by trichrome. Histochemical analysis utilized a computer-aided image-analysis program (AxioVision, Carl Zeiss MicroImaging, Thornwood, NY). GAPDH (1:5,000 Covance, Emeryville, CA) served as a loading control for Western blotting.

Fig. 4. Macrophage (MØ) phenotype in the kidney of sham and RAS with or without ObM (n = 7 each group). A: representative images (×40) of immunofluorescence staining for M1- and M2-MØ [CD163 red, M1 (inducible nitric oxide synthase; iNOS) or M2 (arginase-1) green, double staining yellow]. B: quantification of M1-MØ-positive cells. C: M1/M2 ratio phenotype subpopulations. ObM-STK exhibited amplified proinflammatory MØ infiltration. #RAS: significant effect of RAS; *Diet: significant effect of the ObM diet (2-way ANOVA). *P < 0.05 vs. Lean-sham; §P < 0.05 vs. ObM-sham; †P < 0.05 vs. Lean-STK.
**Statistical Analysis**

Statistical analysis was performed using JMP software package version 9.0 (SAS Institute, Cary, NC). Results are expressed as means ± SE. Two-way ANOVA was used to analyze the effects of RAS and diet as separate factors, and their interactions, followed by Tukey’s test as appropriate. A paired Student’s t-test was performed for comparisons within groups (BOLD medullary R2* before and after furosemide; function of STK vs. CLK). Results were considered significant for \( P \leq 0.05 \).

**RESULTS**

**Animal Characteristics**

At 16 wk, ObM groups had higher body weight and visceral obesity, reflected by an increased abdominal fat volume fraction (Table 1). RAS groups had increased mean arterial pressure (MAP) in both Lean and ObM compared with their relative controls. The ObM diet had a stimulating effect on MAP but did not significantly increase MAP in either RAS or sham (both \( P > 0.10 \)). ObM but not Lean groups also developed hyperinsulinemia and insulin resistance (HOMA-IR), as well as elevated triglyceride and LDL levels. Collectively, this cluster of abnormalities suggested the development of obesity-metabolic syndrome in ObM groups. In addition, RAS increased urine concentration of mAlb in both Lean and ObM (\( P > 0.50 \) ObM-RAS vs. Lean-RAS) and had an effect on increasing plasma creatinine regardless of diet, although it was not elevated in either group. The ObM diet had no effect on mAlb or plasma creatinine. The ObM diet and RAS elevated PRA only in the ObM-RAS group (\( P = 0.01 \) vs. Lean-sham). No interaction between ObM and RAS was found for these parameters.

**Renal Hemodynamics, Function and Oxygenation**

RAS reduced volume and GFR in Lean-STK, suggesting decreased blood supply and renal function (Table 2). RAS also decreased RBF and GFR in ObM-STK compared with its sham control but not compared with Lean-sham (all \( P > 0.20 \)), suggesting the blood supply and function in ObM-STK were

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**Fig. 5.** Systemic and local oxidative stress in sham and RAS pigs with or without ObM (\( n = 7 \) each group). A: systemic 8-isoprostanes and oxidized-low density lipoprotein (Ox-LDL) levels. B and C: representative images (×20) and quantification of kidney Ox-LDL expression. D: renal protein expression of p47 quantified relative to GAPDH. Two representative bands from each group are shown. ObM-RAS exhibited enhanced oxidative stress in both circulation and the STK. ▲ Diet: significant effect of the ObM diet; ▲ RAS: significant effect of RAS; ▲ DietxRAS: significant interaction of the ObM diet and RAS (2-way ANOVA). * \( P < 0.05 \) vs. Lean-sham; § \( P < 0.05 \) vs. ObM-sham; † \( P < 0.05 \) vs. Lean-RAS.
relatively preserved compared with normal pigs. RAS elevated BOLD-MRI cortical R2* in both Lean-STK and ObM-STK compared with Lean-sham, indicating lower cortical oxygenation (Fig. 1). In the medulla, although the basal R2* was unaffected by either RAS or diet, only ObM-STK revealed a diminished R2* response to furosemide (P = 0.10 for Post-furosemide vs. Pre-furosemide, Fig. 1C). In addition, RAS decreased delta R2* only in ObM-STK (P = 0.03 vs. Lean-sham) but not Lean-STK (Fig. 1D). These data indicated impaired medullary tubular oxygen-dependent transport activity in ObM-STK.

**Intrarenal Microcirculation**

While there was no significant effect of RAS on the vessel density in Lean-STK, there was a decrease in ObM-STK in the density of both small (20 – 40 μm) and large vessels (200 – 500 μm) in both the outer and inner cortex (vs. sham control). Of note, the ObM diet interacted with RAS to aggravate its effect on small vessels (Fig. 2, A and B). In contrast, ObM-sham enhanced neovascularization in these vessels. The average vessel diameter was unchanged.

Consistent with these findings, the ObM diet and RAS also interacted to downregulate the expression of the angiogenic factor VEGF and further decreased its receptor FLK-1 in ObM-STK compared with sham control (P = 0.002). In contrast, Lean-STK increased VEGF, and ObM-sham elevated FLK-1 (Fig. 2C). Hence, the coexistence and interaction of the ObM diet and RAS mediate intrarenal microvascular remodeling in ObM-STK by suppressing neovascularization and blunting angiogenic activity.

**Inflammation**

The levels of systemic inflammatory markers are presented in Table 3. RAS elevated in both Lean and ObM the levels of MCP-1, TNF-α, and IFN-γ. In addition, RAS also elevated sE-selectin in ObM but not Lean. The ObM diet had no effect on systemic levels of these cytokines. IL-17 and anti-inflammatory IL-10 levels were unchanged.

In the kidney, RAS elevated MCP-1 and IFN-γ only in ObM-STK but had no effect on Lean-STK. The ObM diet elevated sE-selectin in both sham and STK (Fig. 3). The release of IL-17 and IL-10 were again unchanged. Hence, RAS mainly activated...
inflammatory cytokines in the systemic circulation, but ObM-RAS magnified inflammation in both the systemic circulation and STK. Furthermore, the ObM diet amplified recruitment of M1-MØ in both RAS and sham compared with Lean-sham. Importantly, the ObM diet and RAS increased (albeit not synergistically) the M1/M2-MØ ratio in ObM-STK ($P = 0.0004$ vs. Lean-sham, $P = 0.018$ vs. ObM-sham, and $P = 0.0034$ vs. Lean-STK, Fig. 4A), an index of the proinflammatory phenotype shift of MØ, while there was no effect of RAS on the M1/M2-MØ ratio in Lean-STK. Therefore, in addition to elevating inflammatory cytokines in the kidney, ObM-STK also enhanced recruitment of proinflammatory M1-MØ, which is associated with the effect of the ObM diet.

**Oxidative Stress, Fat Deposition, and Renal Fibrosis**

The ObM diet and RAS increased the level of 8-isoprostane in ObM-RAS, and both ObM groups had elevated Ox-LDL (Fig. 5A), whereas Lean-STK was not affected. In the kidney, the RAS increased the expression of Ox-LDL in both STKs (both $P < 0.01$ vs. Lean, Fig. 5, B and C). However, ObM-STK also showed increased expression of p47, attributed to the effects of the ObM diet, RAS, and their interaction ($P = 0.002$ vs. Lean-sham, $P = 0.001$ vs. ObM-sham, and $P = 0.004$ vs. Lean-STK, Fig. 5D), whereas Lean-STK was not affected. Hence, the coexistence of the ObM diet and RAS also induced prominent oxidative stress in the ObM-RAS group in both systemic circulation and the STK.

The ObM diet and RAS induced distinct fat deposition only in ObM-STK, revealed by oil-red-O staining ($P = 0.002$ vs. Lean-sham, $P = 0.03$ vs. ObM-sham, and $P = 0.0007$ vs. Lean-STK, Fig. 6, A and C). The RAS increased tubulointerstitial fibrosis in both STKs (Fig. 6, B and D), but the ObM diet and RAS further synergistically increased the number of sclerotic glomeruli only in ObM-STK ($P = 0.01$ vs. Lean-sham and $P = 0.02$ vs. Lean-STK, Fig. 6).

**Fat Inflammation and Fibrosis**

Both ObM groups showed increased infiltration of M1-MØ, expression of TNF-α, and fibrosis in abdominal fat compared with Lean-sham (Fig. 7, A–D). Similarly, in the perirenal fat, ObM also elevated infiltration of M1-MØ, and in the ObM-STK, TNF-α expression as well (Fig. 7, E and F). The RAS also showed a stimulating effect on M1-MØ infiltration, although their number was not elevated in Lean-STK. These data...

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**Fig. 7.** MØ, inflammation, and fibrosis in the abdominal and perirenal adipose tissues of sham and RAS pigs with or without ObM ($n = 7$ each group). A and B: representative images ($\times40$) of immunofluorescence staining for M1-MØ (CD163 red, M1-iNOS green, double staining yellow) and TNF-α (green) and their quantifications. C and D: representative images ($\times20$) of trichrome staining in abdominal fat, and its quantification. E and F: representative images ($\times40$) of M1-MØ and TNF-α in perirenal fat. ObM diet enhanced M1-MØ and TNF-α expression in both abdominal and perirenal fat. ♦Diet: significant effect of ObM diet; ♦RAS: significant effect of RAS (2-way ANOVA). *$P \leq 0.05$ vs. Lean-sham; †$P \leq 0.05$ vs. Lean-STK.
suggest the ObM diet had enhanced inflammatory activity not only in abdominal adipose tissue but also in peripheral fat, particularly around the STK.

CLKs

Both ObM-CLK and Lean-CLK showed increased volume, RBF, and GFR compared with their corresponding STKs (all \( P < 0.01 \)). Furthermore, the ObM diet magnified these parameters in the CLK compared with Lean-sham (all \( P < 0.01 \)) and its GFR compared with Lean-CLK, indicating hyperfiltration in ObM-CLK (Table 4).

The RAS increased renal oxidative stress in the CLKs, reflected by elevated Ox-LDL in Lean-CLK and the expression of p47 in ObM-CLK (data not shown). Additionally, similar to the STK, the ObM diet also enhanced CLK fat deposition (both \( P < 0.01 \) vs. Lean-sham and vs. Lean-CLK, Fig. 8A), inflintra-

Table 4. Single-kidney hemodynamics and function in sham and contralateral kidneys (CLK) of RAS pigs with or without ObM (\( n = 7 \) each)

<table>
<thead>
<tr>
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<th>Sham</th>
<th>RAS</th>
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<tr>
<td></td>
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<td>68.8 ± 7.7</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SE. *\( P < 0.05 \) vs. Lean-sham. †\( P < 0.05 \) vs. Lean-CLK.

Fig. 8. Renal fat deposition, MØ phenotype, and fibrosis in sham and the contralateral kidneys (CLK) of Lean and ObM pigs with renal artery stenosis (\( n = 7 \) each). A: representative kidney oil-red-O staining images (\( \times 20 \)) and its quantification showed increased fat deposition in ObM-CLK. B: representative images (\( \times 40 \)) of immunofluorescence staining for M1- and M2-MØ [CD163 red, M1-iNOS] or M2 [arginase-1] green, double staining yellow] and quantifications for M1-MØ-positive cells and the M1/M2-MØ ratio. As opposed to Fig. 4, the increase in the M1/M2 ratio in ObM-sham reached statistical significance because of the different groups involved in analysis. C: representative trichrome images (\( \times 20 \)) and quantifications for tubulointerstitial fibrosis and glomerulosclerosis. ♦Diet: significant effect of the ObM diet; ♦RAS: significant effect of RAS (2-way ANOVA). * \( P \leq 0.05 \) vs. Lean-sham; §\( P < 0.05 \) vs. ObM-sham; †\( P < 0.05 \) vs. Lean-CLK.
tion of M1-MØ, and the M1/M2 ratio (Fig. 8B). Both CLKs had mild but detectable tubulointerstitial fibrosis, but neither developed significant glomerulosclerosis (Fig. 8C). Therefore, the RAS induced mild oxidative stress and fibrosis in the CLK, while the ObM diet induced hyperfiltration and increased fat deposition and M1-MØ infiltration. Therefore, the coexistence of ObM resulted in greater injury in RAS-CLK.

DISCUSSION

This study shows that obesity associated with metabolic derangements, in addition to amplifying inflammation, interacts with renal artery stenosis to increase oxidative stress, impairs tubular function, blunts microvascular angiogenesis, and accelerates glomerulosclerosis in the STK, although at this early stage its GFR and RBF were relatively preserved. In the contralateral kidney, ObM also induces hyperfiltration and mediates fibrosis. Thus the constellation of the metabolic syndrome constitutes a risk factor for bilateral kidney structural damage in unilateral renovascular disease.

The proinflammatory effects of obesity on the kidney have been proposed previously (17, 26), yet its impact on the kidney subjected to chronic ischemia remained unidentified. In the present study, the coexistence of ObM and RAS magnified not only endothelial activation in the systemic circulation compared with RAS alone (sE-selectin), but also kidney release of inflammatory cytokines (MCP-1, sE-selectin, and IFN-γ), and in the bilateral kidneys induced infiltration of macrophages and MØ phenotype shift, which Lean-RAS did not. This observation extends findings in other forms of CKD on the contribution of a proinflammatory microenvironment to kidney injury (31), and underscores a key association of MØ infiltration and inflammatory phenotype with renal pathology in the ObM-complicated RAS. However, it is important to note that although the activated cytokines in ObM-STK were mainly attributed to the effect of the RAS, the RAS did not increase their release from Lean-STK. In addition, M1-MØ infiltration in the kidney was attributed only to the ObM diet. It is thus likely that during coexistence of ObM-RAS, the ObM diet plays a major role in kidney inflammation, both by directly amplifying M1-MØ and by facilitating the effect of RAS. Furthermore, adipose tissue contains adipocytes which are recognized as cytokine-secretory cells (36), including MØ that prevail in obesity (23, 35), and abundant inflammatory cytokines like TNF-α (34), as observed in the present study (abdominal and perirenal fat). Interestingly, our study also found that the ObM diet elevated M1-MØ in the STK, in parallel to its effect in the fat tissue, whereas the RAS had no effect on M1-MØ. Speculatively, increased M1-MØ and TNF-α activity in adjacent perirenal fat may potentially contribute to inflammation and MØ recruitment in the STK. On the other hand, elevated PRA in ObM-RAS may also reflect the contribution of angiotensin II to MØ activation and amplified inflammation via binding to its receptor on MØ (24). Further studies will be needed to elucidate the cross talk between the STK and its perirenal fat.

Our previous study has shown that early obesity increased intrarenal microvascular proliferation and angiogenic activity (22). In the current study, we observed the similar effect in the ObM-sham group. The Lean-STK exhibited relatively preserved microvascular density, possibly due to a compensatory increase in VEGF in response to ischemia (13). However, the ObM diet interacted with the RAS and blunted in ObM-STK both neovascularization and angiogenic signaling. This finding suggests that microvessels spawned in ObM may be fragile and prone to loss (20). Additionally, prolonged and severe inflammation and oxidative stress interfere with upregulation of angiogenic factors (7, 8, 39). Indeed, suppressed microcirculation in ObM-STK was accompanied by marked inflammation (MCP-1, IFN-γ, and MØ) and magnified oxidative stress, which may explain the suppressed...
vessel density in ObM-STK that was not observed in Obm or the RAS alone. Therefore, the coexistence of ObM and the RAS may predispose the kidney to microvascular regression through amplified inflammation and oxidative stress.

Given the suppressed neovascularization ex vivo with no change in diameter, the relatively preserved RBF in the ObM-STK is unlikely due to microvascular remodeling. Rather, hemodynamic factors like increased cardiac output and blood volume induced by ObM may possibly lead to vasodilation and a rise in RBF in vivo. In contrast, Lean-STK had decreased RBF but relatively preserved microcirculation. Possibly as a result, ObM-STK and Lean-STK developed similar degrees of hypoxia (R2* by BOLD MRI), yet tubular injury (diminished response to furosemide) and renal scarring (glomerulosclerosis) were more pronounced in the ObM-STK. Reflected by oil-red-O staining, the ObM diet increased fat deposition in ObM-STK. Because free fatty acids bind to albumin (31), enhanced albumin excretion by the RAS may facilitate their excess glomerular filtration that is not apparent in Lean-STK and enhance their reabsorption by the renal proximal tubules, resulting in tubulointerstitial inflammation and fibrosis (18, 31). Through similar pathways (31, 33), increased renal fat deposition may also lead to glomerular injury, such as mesangial cell proliferation and extracellular matrix deposition (1). In line with this notion, the present study found that ObM synergized with the RAS to increase glomerulosclerosis.

The current study shows that obesity-metabolic derangement augments renal hemodynamics, reflected by preserved RBF and GFR in the STK and hyperfiltration in the CLK. Nevertheless, increased glomerulosclerosis and suppressed neovascularization in the ObM-STK may expose the remaining nephrons to greater risk of hyperfiltration, thereby accelerating their loss, following a similar pattern observed in the progression of chronic renal failure (25). This notion is supported by the observation of greater glomerulosclerosis in the ObM-STK than Lean-STK. In addition, increased glomerular filtration pressure may also facilitate fat deposition in the CLK and subsequent adverse effects. Therefore, the bolstered hemodynamics in Obm may not protect the kidney from the insult of renovascular disease but rather accelerate its structural damage. Indeed, STKs of patients with Obm are less likely to improve after revascularization (10), possibly due to greater irreversible damage compared with non-Obm patients.

Limitation

Our study is limited by the use of relatively young animals and the short duration of the disease, yet renal structure and function in our swine model are similar to humans. Furthermore, this model recapitulates many features of the metabolic syndrome in humans. Further studies will need to pursue individual pathways and longer durations of kidney injury to determine its reversibility in Obm-RAS. In addition, the possible effects of Obm on the progression of degree of RAS, the effectiveness of treatments like revascularization, and identification of specific factors influencing renal outcomes warrant further exploration.

Conclusion

As summarized in Fig. 9, our study suggests that obesity associated with metabolic derangements exerts complex roles in mediating kidney damage distal to renovascular disease, including aggravated inflammation that involves M1-MØ and related cytokines, blunted microcirculation, enhanced oxidative stress, and accelerated scarring in the kidney. These pathological alterations might be related to magnified fat deposition in the kidney and activated MØ in the perirenal fat tissue attributable to the Obm diet. Additionally, Obm not only directly induces injury in the kidney but also synergizes and facilitates the adverse effects of RAS on microvascular remodeling, glomerular damage, and tubular dysfunction. The preserved function in the ObM-STK may also permit disease progression in remaining nephrons. In conclusion, MØ-activated inflammatory renal pathways may constitute important mechanisms and potential therapeutic targets in addition to restoring blood flow in Obm-RAS kidneys. Prevention of obesity and its consequence likely will prove to be important strategies to combat the kidney injury subject to chronic ischemia.

GRANTS

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DISCLOSURES

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

AUTHOR CONTRIBUTIONS


REFERENCES
