Angiotensin type 1 receptor modulates macrophage polarization and renal injury in obesity

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Angiotensin type 1 receptor modulates macrophage polarization and renal injury in obesity. Am J Physiol Renal Physiol 300: F1203–F1213, 2011. First published March 2, 2011; doi:10.1152/ajprenal.00468.2010.—The mechanisms for increased risk of chronic kidney disease (CKD) in obesity remain unclear. The renin-angiotensin system is implicated in the pathogenesis of both adiposity and CKD. We investigated whether the angiotensin type 1 (AT1) receptor, composed of dominant AT1a and less expressed AT1b in wild-type (WT) mice, modulates development and progression of kidney injury in a high-fat diet (HFD)-induced obesity model. WT mice had increased body weight, body fat, and insulin levels and decreased adiponectin levels after 24 wk of a high-fat diet. Identically fed AT1a knockout (AT1aKO) mice gained weight similarly to WT mice, but had lower body fat and higher plasma cholesterol. Both obese AT1aKO and obese WT mice had increased visceral fat and kidney macrophage infiltration, with more proinflammatory M1 macrophage markers as well as increased mesangial expansion and tubular vacuolization, compared with lean mice. These abnormalities were heightened in the obese AT1aKO mice, with downregulated M2 macrophage markers and increased macrophage AT1b receptor. Treatment with an AT1 receptor blocker, which affects both AT1a and AT1b, abolished renal macrophage infiltration with inhibition of renal M1 and upregulation of M2 macrophage markers in obese WT mice. Our data suggest obesity accelerates kidney injury, linked to augmented inflammation in adipose and kidney tissues and a proinflammatory shift in macrophage and M1/M2 balance.

inflammation; chronic kidney disease

OBESITY IS INCREASING WORLDWIDE, with 66% of adults in the US overweight and 33% obese (12, 36). Obesity is associated with, and contributes to, development of type 2 diabetes mellitus, cardiovascular disease, and nondiabetic chronic kidney disease (CKD) (45). Adipose tissue is a complex endocrine organ that releases hormones and adipokines, including TNF-α, IL-1β, and monocyte chemotactic protein-1 (MCP-1), all components of the renin-angiotensin system (RAS), with diverse functions that affect glucose homeostasis, lipid metabolism, and inflammation (13, 41, 51). Macrophage infiltration is a common feature of obesity and many kidney diseases (5, 18). However, macrophages have significant heterogeneity in their functions (9). Macrophages have recently been classified into two polarization states, M1 and M2. M1 or “classically activated” macrophages are induced by classic immune pathways (such as LPS and interferon-γ), and enhance proinflammatory cytokine production, such as TNF-α, IL-1β, and IL-6. M2 or “alternatively activated” macrophages are generated by exposure to IL-4 and IL-13. M2 macrophages are important in resolution of inflammation and tissue repair through synthesis of anti-inflammatory cytokines IL-10 and IL-1 decoy receptor and endocytic clearance capacities (8). Inhibition of proinflammatory macrophages has beneficial effects in experimental chronic inflammatory renal injury (46). However, relevance to obesity-associated CKD is unknown.

RAS activation, via the AT1 receptor on macrophages, directly promotes macrophage infiltration and activation (10, 37, 40, 42, 43). The RAS activation is implicated in pathogenesis of both adiposity and CKD (6). An angiotensin receptor blocker, which decreases AT1 activity, attenuates obesity and metabolic abnormalities (20, 26).

Wild-type (WT) mice have two AT1 receptors, the dominant AT1a and less expressed AT1b. Recently, Crowley et al. (3) reported that AT1b mRNA is upregulated in podocytes in murine autoimmune nephritis when a systemic AT1a receptor is absent, suggesting that AT1b may have a compensating role in augmented kidney injury and inflammation. We hypothesize that the AT1 receptor is activated in macrophages in obesity and affects obesity-induced kidney injury by modulating macrophage polarization. We investigated this hypothesis using AT1a-deficient (AT1aKO) mice and studied the impact of AT1a vs. AT1b and an AT1 receptor blocker (ARB) on obesity-induced kidney injury.

MATERIALS AND METHODS

Mice. Adult male (8-wk-old) AT1aKO mice on a C57BL/6J background and C57BL/6J WT mice were purchased from Jackson Laboratory (Bar Harbor, ME). Mice were housed in a pathogen-free barrier facility under normal conditions with a 12:12-h light-dark cycle at 21°C with 40% humidity and 12 air exchanges/h, with food and water ad libitum. All animal protocols were approved by the Vanderbilt University Institutional Animal Care and Use Committee according to National Institutes of Health guidelines.

Before the study, all mice were fed a standard normal chow diet (Purina Rodent “5001” meal, 23.4% protein, 4.5% fat, 6.0% fiber, 0.40% sodium, Tusculum Feed Center, Nashville, TN). Obesity was induced by placing mice on a high-fat diet (HFD; 58% of total calories from fat, F1850, Bioserv Industries, Frenchtown, NJ) as previously described (26). At age 10–12 wk, mice were divided into four groups and treated for 24 wk as follows: 1) WT on normal chow (lean WT; n = 10); 2) AT1aKO mice on normal chow (lean AT1aKO; n = 10); 3) WT mice on HFD (obese WT; n = 12); and 4) AT1aKO mice on HFD (obese AT1aKO; n = 10). To determine whether treatment with
ARB alters the phenotype of the WT macrophages, mice were treated
for 12 wk as follows: 1) WT mice on HFD (WT+HFD; n = 4) or
2) WT mice on HFD with ARB (losartan, 320 mg/l dry wt based on
effects in CKD; WT+HFD+ARB; n = 5). The dose of losartan (320
mg/l dry wt) is comparable to the dose of 25–32 mg/kg previously
used by our group and others to study CKD, suggesting that a higher
dose of ARB affords superior renoprotection in chronic kidney dis-
ease independently of hemodynamic effects (7, 11, 50). Normal adult
WT mice (age 10–12 wk) were studied as baseline control (n = 5).
Food intake was weighed daily, and body weight was measured every
4 wk. Fasting blood glucose, plasma, and urine were collected every
8 wk. Metabolic studies were performed as described below. Mice
were killed at 12 or 24 wk. White adipose tissue (epididymal fat pads),
skeletal muscle, and kidneys were harvested for morphological and
molecular analysis. To assess gene expression in macrophages, peri-
toneal macrophages were harvested from four mice in each group
above at the end of the study (see below).

Blood measurements. Blood glucose and plasma measurements
were made 6 h after daytime food withdrawal. Fasting blood glucose
levels were determined using an Accu-Check glucose monitor (Roche
Diagnostics Boehringer Mannheim, Indianapolis, IN). Plasma insulin,
adiponectin, and lipid were assessed at the Mouse Metabolic Phyto-
typing Center (MMPC) at Vanderbilt University (http://www.mc.vanderbilt.
edu/root/vumc.php?site=mmpc). Plasma insulin was measured by RIA
(Linco Research, St. Louis, MO). Plasma adiponectin was measured by
Luminex in a single-plex format (Millipore, formerly Linco Research, St.
Charles, MO). Plasma triglyceride, total cholesterol, and HDL cholesterol
levels were determined using kits (Raichem, San Diego, CA) at the
MMPC at Vanderbilt University.

Tail-cuff blood pressure measurement, urine albumin, and renal
function. Systolic blood pressure (SBP) was measured in trained,
conscious mice using tail-cuff impedance plethysmography (BP-2000
was collected from mice individually housed in polycarbonate meta-
bolic cages (Nalgene, Rochester, NY). Excretion of urinary albumin
was determined using albumin-to-creatinine ratio (ACR). Urinary
albumin and creatinine were determined using a mouse AlbueLL
ELISA kit and a Creatinine Companion kit (Exocell, Philadelphia,
PA). Plasma creatinine was measured with a Quantichrom Creatinine
Assay kit, using a modified Jaffé method that accurately measures
creatinine in mice, as previously described (Bioassay Systems) (16).

Analysis of body composition. Whole-animal body composition
was assessed using the minispec Live Mice Whole Body Composition
Analyzer (LF50) based on TD-NMR. This method provides a precise
method for measurement of lean tissue, fat, and fluid in live mice and
rats (Bruker Optik, Ettlingen, Germany). Animals were fasted for 16
h before scanning to reduce the variability attributed to food in the
digestive tract.

Structural analyses. Epididymal fat pads and the kidney cortex
were dissected and immersion-fixed in 4% paraformaldehyde/PBS
solution overnight at 4°C and embedded in paraffin using standard
techniques. Four-micrometer sections were dewaxed, rehydrated,
stained with periodic acid-Schiff, and immunohistochemistry was
performed as described below. A semiquantitative score (0–4) was
used to evaluate the degree of mesangial expansion and/or glomeru-
llosclerosis: 0 represents no lesion, 1 represents mesangial expansion
and/or glomerulosclerosis of <25% of the glomerulus, while 2, 3, and
4+ represents mesangial expansion and/or glomerulosclerosis of
25–50, >50–75, and >75% of the glomerulus (24). All glomeruli in
one section were evaluated and scored. Tubulointerstitial fibrosis was
assessed qualitatively. All sections were examined without the exam-
iner’s knowledge of the treatment protocol.

Immunohistochemistry. Immunostaining was performed in para-
formaldehyde-fixed, paraffin-embedded sections using the following
specific antibodies: 1) rat anti-mouse F4/80 for macrophages (1:20,
Serotec, Raleigh, NC) (25); 2) rabbit anti-phospho-c-Jun (Ser73, 1:100,
MMPC at Vanderbilt University (http://www.mc.vanderbilt.edu/root/vumc.php?site=mmpc). Plasma insulin was measured by RIA
(Linco Research, St. Louis, MO). Plasma adiponectin was measured by
Luminex in a single-plex format (Millipore, formerly Linco Research, St.
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levels were determined using kits (Raichem, San Diego, CA) at the
Table 1. Metabolic parameters in lean and obese WT and
AT1aKO mice

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<tr>
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<tr>
<td>Blood glucose, mg/dl</td>
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<tr>
<td>Lean WT</td>
<td>131.8 ± 4.9</td>
<td>133.0 ± 4.8</td>
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<tr>
<td>Lean AT1aKO</td>
<td>132.6 ± 2.7</td>
<td>132.6 ± 2.7</td>
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<tr>
<td>Obese WT</td>
<td>135.4 ± 6.0</td>
<td>188.6 ± 11.7*</td>
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<tr>
<td>Obese AT1aKO</td>
<td>132.9 ± 4.0</td>
<td>189.2 ± 9.3*</td>
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<tr>
<td>Plasma insulin, ng/ml</td>
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<tr>
<td>Obese WT</td>
<td>0.6 ± 0.0</td>
<td>4.9 ± 3.9†</td>
</tr>
<tr>
<td>Obese AT1aKO</td>
<td>0.5 ± 0.1</td>
<td>3.9 ± 0.7†</td>
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<td>Plasma adiponectin, µg/ml</td>
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<tr>
<td>Obese WT</td>
<td>12.8 ± 0.6</td>
<td>9.6 ± 0.7*</td>
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<tr>
<td>Obese AT1aKO</td>
<td>11.3 ± 0.7</td>
<td>11.5 ± 0.3‡</td>
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Values are mean ± SE. WT, wild-type; AT1aKO, ANG II type 1a
deficient. *P < 0.01, 24 wk vs. 0 wk, †P < 0.001, 24 wk vs. 0 wk, ‡P < 0.05
WT vs. AT1aKO at 24 wk.
Table 2. Plasma lipids in lean and obese WT and AT1aKO mice

<table>
<thead>
<tr>
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<th>Triglyceride, mg/dl</th>
<th>Total Cholesterol, mg/dl</th>
<th>HDL, mg/dl</th>
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<tr>
<td>Lean WT</td>
<td>79.3 ± 6.8</td>
<td>80.8 ± 0.8</td>
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<td>Lean AT1aKO</td>
<td>79.0 ± 5.3</td>
<td>116.6 ± 3.1*</td>
<td>58.6 ± 1.7*</td>
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<tr>
<td>Obese WT</td>
<td>76.9 ± 1.7</td>
<td>185.1 ± 7.3†</td>
<td>70.4 ± 1.6†</td>
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<tr>
<td>Obese AT1aKO</td>
<td>78.3 ± 3.5</td>
<td>213.3 ± 5.8§</td>
<td>71.7 ± 1.8§</td>
</tr>
</tbody>
</table>

Values are means ± SE. *P < 0.01 lean WT vs. lean AT1aKO, †P < 0.01 obese WT vs. lean WT, §P < 0.01 obese WT vs. obese AT1aKO. *P < 0.01 obese AT1aKO vs. lean WT.

RESULTS

Systemic metabolic and hemodynamic parameters. Body weight was comparable between WT and AT1aKO mice at baseline [25.0 ± 0.4 vs. 25.8 ± 0.6 g, P not significant (pNS)]. WT and AT1aKO mice on HFD had similar food intake (2.28 ± 0.05 vs. 2.37 ± 0.05 g/day, pNS) and similar increases in body weight (60%) (Fig. 1A). However, obese AT1aKO mice had significantly less body fat (31%) than obese WT mice (39%) (Fig. 1B), confirmed by decreased epididymal fat pad mass (Fig. 1C). Interestingly, the whole body muscle percentage was significantly higher in AT1aKO mice on normal chow and HFD compared with WT mice (Fig. 1D). The liver-to-body weight ratio was similar in WT and AT1aKO mice (Fig. 1E).

Fasting blood glucose levels were similarly increased over baseline in obese WT and obese AT1aKO mice after 24 wk of a high-fat diet (Table 1). Plasma insulin levels were significantly increased over baseline in obese WT and numerically lower in obese AT1aKO mice. Plasma adiponectin levels were significantly decreased in obese WT mice but remained similar to baseline levels in obese AT1aKO mice (Table 1). Although circulating triglycerides did not differ among the groups, plasma total cholesterol levels were significantly higher in lean and obese AT1aKO mice compared with lean and obese WT mice (Table 2). Plasma HDL was also higher in lean AT1aKO mice, with similarly high levels in obese WT and obese AT1aKO mice (Table 2).

Obesity is associated with increased blood pressure (27). Our data confirmed mild but significantly higher SBP in obese WT vs. lean WT mice, but no hypertension in any AT1aKO mice (WT obese vs. lean 110 ± 2 vs. 96 ± 6 mmHg, P < 0.05; AT1aKO obese vs. lean 89 ± 5 vs. 90 ± 4 mmHg, pNS; P < 0.01 obese WT vs. obese AT1aKO).
Since rodents have two isoforms of the AT1 receptor, namely, AT1a and AT1b, we next studied AT1b receptor expression. AT1b mRNA was ~2.3-fold higher in visceral adipose tissue of obese AT1aKO vs. obese WT mice (fold change vs. lean WT: lean WT 1.00 ± 0.08, obese AT1aKO 1.21 ± 0.11, obese WT 0.90 ± 0.12, obese AT1aKO 2.26 ± 0.37, P < 0.01 obese AT1aKO vs. obese WT). In WT mice, obesity increased macrophage AT1a, but not AT1b, mRNA (Fig. 2A). In contrast, macrophage AT1b mRNA was markedly increased in obese AT1aKO vs. obese WT mice (Fig. 2B). Thus deletion of AT1a resulted in marked compensatory upregulation of AT1b.

Renal injury. Although plasma creatinine levels were normal in all mice at 24 wk (lean WT 0.49 ± 0.10, lean AT1aKO 0.52 ± 0.08, obese WT 0.44 ± 0.06, obese AT1aKO 0.41 ± 0.02 mg/dl; pNS), HFD feeding induced structural kidney injury in WT mice that included mesangial expansion, tubular vacuolization, flattening, and dilation (P < 0.05 vs. lean WT, Fig. 3, A–C). AT1a receptor deficiency significantly potentiated these HFD-induced injuries (P < 0.05 vs. obese WT, Fig. 3, A–C). Electron microscopy revealed no or very limited foot process effacement, but segmental loss of glomerular endothelial fenestration in both obese WT and obese AT1aKO mice (data not shown). Glomerular basement membrane thickness was similar in obese WT and obese AT1aKO mice (206 ± 12 vs. 198 ± 10 nm, pNS). Compared with lean mice with normal kidneys, ACR rose comparably in both obese AT1aKO (4-fold increase) and obese WT mice (4.7-fold increase) (Fig. 3D). Renal nephrin mRNA expression, a corollary of podocyte differentiation, was variable but not different among groups (lean WT 1.3 ± 0.6 arbitrary units, lean AT1aKO 0.84 ± 0.3, obese WT 1.6 ± 0.4, obese AT1aKO 1.7 ± 0.3, pNS). Immunohistochemical analysis showed that obese AT1aKO mice had 47% increased tubular albumin expression vs. obese WT mice (Fig. 4A, B, P < 0.05), associated with 33% increased tubular megalin expression vs. obese WT mice (P < 0.05) (Fig. 4, E and F). To further quantitatively assess megalin expression, we performed Western blotting on whole kidney samples. Lean AT1aKO mice showed 1.5-fold higher megalin expression vs. lean WT mice (P = 0.06, Fig. 4, G and H), although immunohistochemical staining did not detect such change (Fig. 4, C and D). After HFD feeding, Western blotting confirmed that obese AT1aKO mice had 2.2-fold higher megalin expression vs. obese WT mice (P < 0.05, Fig. 4, G and H), suggesting that the ACR difference may be contributed to by alterations in tubular function.

Scattered and similar renal interstitial infiltration of macrophages was observed in both lean WT and AT1aKO mice (Fig. 5, A and B). Obesity increased renal F4/80+ macrophage infiltration in the interstitium, especially in obese AT1aKO mice (nearly 3-fold higher vs. obese WT, Fig. 5, A and B). We next examined expression of M1 macrophage markers (MCP-1, TNF-α) and the M2 macrophage marker (Ym-1). Kidney MCP-1 mRNA levels increased 3.5-fold in obese vs. lean WT mice (P < 0.05), but increased 21-fold in obese AT1aKO mice (P < 0.001 vs. lean WT and obese WT, Fig. 5C). Kidney TNF-α mRNA expression was similar in lean and obese WT but increased almost 2-fold in obese AT1aKO mice (P < 0.01, Fig. 5D). Kidney Ym-1 protein expression did not differ between lean WT and lean AT1aKO mice (Fig. 5, E and F). In contrast, kidney Ym-1 expression was decreased 48% in obese AT1aKO vs. obese WT mice (Fig. 5, G and H).

Systemic and adipose inflammatory parameters. Various stresses, including inflammation, activate c-Jun NH2-terminal kinases1 (JNK1) and transcription factor activator protein-1 (AP-1), which in turn upregulate proinflammatory and profibrogenic genes (29). We therefore assessed renal phosphorylated-c-Jun, a component of AP-1. Only rare immunostaining for phosphorylated-c-Jun was seen in tubular cells in lean WT and AT1aKO mice (Fig. 6A). Obesity significantly increased...
expression of phosphorylated-c-Jun, 3.9-fold in WT and 10-fold in AT1aKO mice, in both proximal and collecting tubules (P < 0.01 obese AT1aKO vs. obese WT) (Fig. 6, A and B).

Interestingly, obesity also induced higher renal c-Jun expression (data not shown) and phosphorylated JNK expression in tubules in obese AT1aKO vs. obese WT mice (P < 0.01, Fig. 6, C and D).

We next assessed whether adipose tissue macrophage infiltration was also altered. Lean WT or AT1aKO mice had no detectable F4/80+ macrophages in epididymal fat tissue (Fig. 7, A and B). Obese WT mice had significantly increased adipose F4/80+ macrophage infiltration, with even greater infiltration in obese AT1aKO mice (Fig. 7, A and B). We next examined adipose tissue expression of M1 and M2 macrophage markers. Adipose tissue of obese WT mice had 6.7-fold higher mRNA expression of MCP-1 and 6.4-fold higher mRNA expression of IL-1β vs. lean WT mice (both P < 0.05 vs. lean WT, Fig. 8, A and B). Obese AT1aKO mice also had 5.9- and 6.4-fold higher adipose tissue MCP-1 and IL-1β vs. lean WT, respectively (both P < 0.05, Fig. 8, A and B). Adipose tissue TNF-α mRNA expression was not significantly different among groups (data not shown). In contrast, adipose expression of M2 markers, the mannose receptor and Ym-1, was significantly downregulated (31 and 35%, respectively) in obese AT1aKO vs. obese WT mice (Fig. 8, C, F, and G) (although no difference between lean mice, Fig. 8, C–E), leading to a higher adipose tissue M1/M2 ratio (expressed as IL-1β/mannose receptor, 8.6 ± 1.9 vs. 5.9 ± 1.0, Fig. 8H). Of note, despite an absence of macrophages in adipose tissues of lean AT1aKO mice, mRNA expression of MCP-1 and IL-1β was numerically (3.3- and 1.8-fold, respectively) higher in this tissue vs. lean WT (Fig. 8, A and B). These data suggest other cells, such as adipocytes or capillary endothelial cells, contribute to the adipose tissue inflammatory activation and that AT1a influences cytokine production in other cell types.

To further explore the impact of the AT1a receptor on macrophage polarization, we analyzed expression of M1 and M2 markers in isolated peritoneal macrophages. Macrophages from obese WT mice had increased MCP-1 and IL-1β mRNA vs. lean WT (2.0- and 3.5-fold, respectively). This M1 activation of macrophages was markedly enhanced in obese AT1aKO mice, with nearly 9-fold higher MCP-1 and 13-fold higher IL-1β mRNA levels vs. lean WT mice (P < 0.05 vs. lean and obese WT, Fig. 9, A and B). Macrophage M2 marker mannose receptor mRNA increased similarly in obese WT and obese AT1akO mice compared with lean levels (2.3- and
3.1-fold, respectively, Fig. 9C). Macrophage M2 marker Ym-1 mRNA was significantly decreased in obese WT but maintained in obese AT1aKO mice (Fig. 9D). Macrophage M2 marker TGF-β mRNA was increased in response to obesity in WT, but not in AT1aKO mice (Fig. 9E). Macrophage IL-10 and decoy IL-1 receptor mRNA expressions, M2 markers, were not significantly different among groups (Fig. 9, F and G). Thus the M1/M2 ratio was much higher in macrophages of obese AT1aKO vs. obese WT mice (Fig. 9H).

To determine whether pharmacological blockade of the AT1 receptor (ARB), which blocks both AT1a and AT1b, alters the macrophage phenotype response to obesity, we studied WT mice on HFD with or without ARB treatment for 12 wk. ARB treatment did not affect body weight but decreased blood glucose levels and epididymal fat mass (Table 3). ARB abolished the obesity-induced increase in renal macrophages (Fig. 10, A and B). ARB decreased obesity-induced upregulation of TNF-α, MCP-1, and IL-1β mRNA in the kidney and restored the obesity-induced decrease in mannose receptor and Ym-1 mRNA (Fig. 10, C–G).

**DISCUSSION**

In the present study, we investigated whether the AT1 receptor affects inflammation and kidney injury induced by obesity. Obesity accelerated kidney injury in WT mice. Unexpectedly, obesity-related kidney injury was even worse with genetic deletion of the AT1a receptor. Although obese WT mice had higher SBP than lean mice, SBP remained 110 mmHg in all groups. These data support the notion that the amplified structural kidney injury in obese AT1aKO mice is not primarily mediated by hypertension. Rather, our findings suggest that amplified structural kidney damage in obese AT1aKO mice was associated with augmented inflammation in kidneys and adipose tissue.
Our finding that obesity-related kidney injury is enhanced in the AT1aKO mice is consistent with a growing number of reports that AT1a deficiency can amplify tissue injury (2, 3, 33). We have previously found that unilateral ureteral obstruction (UUO)-induced renal injury was worsened by repletion with AT1aKO bone marrow (33). A recent study of chronic ANG II infusion in chimeric mice repleted with AT1aKO bone marrow showed greater hypertension, exaggerated renal expression of MCP-1, and persistent renal macrophage accumulation than in mice with AT1a intact bone marrow, suggesting that bone marrow-derived AT1 receptors may have a role in limiting mononuclear cell accumulation in the kidney (2). Furthermore, a lack of the AT1a receptor augmented kidney injury and inflammation in MRL-fasltpr/lpr (lpr) mice, a model of lupus (3).

Macrophage infiltration in the kidney has an important role in the development and progression of renal diseases. Macrophage phenotypes and function are critical determinants of the balance of promoting tissue injury vs. resolution of injury (17). Activation of the AT1 receptor has a direct influence on monocyte/macrophage infiltration (28). Our previous data demonstrate that systemic ANG II amplifies macrophage-driven atherosclerosis (34). However, the specific role of the AT1 receptor on macrophages has been controversial. The absence of AT1 receptors on macrophages has deleterious effects in pathological conditions including atherosclerosis or kidney fibrosis (15, 33). We therefore considered the possibility that macrophage functions may be modulated by systemic AT1a deficiency in this obesity model. We found that obese WT mice had enhanced renal macrophage infiltration and that this response was even greater in obese AT1aKO mice.
perhaps related to increased AT1b. To characterize the impact of the AT1 receptor on macrophage polarization in obesity-related kidney injury, we analyzed expression of a panel of M1 and M2 macrophage markers in the kidney, adipose tissue, and isolated peritoneal macrophages. Obesity increased renal M1 markers in WT mice, a response that was further amplified in kidneys of AT1aKO mice, suggesting that abnormally activated inflammation may contribute to the more severe kidney injury in obese mice. Interestingly, kidney Ym-1 expression, a marker specific for the M2 macrophage phenotype, was significantly suppressed in obese AT1aKO compared with obese WT mice. Thus lack of the AT1 receptor, with an increase in AT1b, shifted the M1/M2 balance in the kidney, promoting renal inflammation.

Our finding of augmented AT1b in macrophages in AT1aKO mice in response to obesity supports a role for AT1b in this obesity-related kidney injury when AT1a is deleted. The improvement of renal injury and inflammation in HFD with ARB, which blocks both receptors, further emphasizes the importance of AT1 in mediating injury in response to obesity. Recently, Aki et al. (1) demonstrated that, in a model of antiglomerular basement membrane, renal infiltration of M1 and M2 macrophages in the kidney, adipose tissue, and isolated peritoneal macrophages. Obesity increased renal M1 markers in WT mice, a response that was further amplified in kidneys of AT1aKO mice, suggesting that abnormally activated inflammation may contribute to the more severe kidney injury in obese mice. Interestingly, kidney Ym-1 expression, a marker specific for the M2 macrophage phenotype, was significantly suppressed in obese AT1aKO compared with obese WT mice. Thus lack of the AT1 receptor, with an increase in AT1b, shifted the M1/M2 balance in the kidney, promoting renal inflammation.

To further explore the downstream mechanisms activated with this enhanced kidney inflammation induced by obesity, we assessed phosphorylated JNK and phosphorylated c-Jun, a component of the transcription factor AP-1. AP-1 regulates many genes involved in the progression of kidney disease. The transcriptional activity of AP-1 is regulated by phosphorylation of Ser63 and 73 through SAPK/JNK (4). Macrophages can be activated through JNK to induce TNF-α production. MCP-1 induces inflammatory responses of tubular epithelial cells via AP-1 activation (29, 44). Our data suggest that AP-1 is activated in the renal tubular epithelial cells of obese AT1aKO mice and thus could contribute to the more severe kidney injury in these mice. Compared with obese WT, obese AT1aKO mice had less proteinuria that was associated with preserved nephrin mRNA and increased tubular albumin and megalin expression. Interestingly, we previously showed that alterations in albumin and magalin correlate with tubule function (31). Thus double transgenic mice generated by crossing megalin knockout mosaic mice (lacking megalin expression in 60% of proximal tubule cells) with NEP25 mice (a transgenic line expressing human CD25 in the podocyte), and injuring these mice with immunotoxin caused massive nonselective proteinuria and mild glomerular and tubular injury. Comparing megalin-containing to megalin-deficient proximal tubule cells showed that albumin, immunoglobulin light chain IgA and IgG preferentially accumulated in proximal tubule cells expressing megalin. On the other hand, increased megalin has been reported to be associated with reduced proteinuria in a diabetic nephropathy model (39). These results show that megalin plays a pivotal role in reabsorption of small to large molecular size proteins.
proteins and provides direct in vivo evidence that reabsorption of filtered proteins triggers events leading to tubule injury (31). Our observations also support the possibility that AT1a deficiency, with AT1b upregulation, regulated megalin expression and increased tubular protein reabsorption in obese AT1aKO mice. Further studies will be needed to explore the mechanisms by which the AT1 receptor regulates megalin expression.

Emerging data suggest that macrophages recruited into adipose tissue of obese individuals are proinflammatory and contribute to insulin resistance (32, 48). Obesity also induces a macrophage phenotypic switch toward proinflammatory M1 macrophages in adipose tissue (8, 21, 22, 35). Consistent with previous studies (47, 49), we found that obesity increased macrophage infiltration in adipose tissue and markedly increased M1 markers. In the absence of the AT1a receptor, with increased AT1b, this response in adipose tissue was augmented. Interestingly, although expression of M1 markers in visceral fat tissue was not different between obese WT vs. obese AT1aKO mice, expression of the potentially beneficial M2 markers was markedly downregulated by AT1 receptor deficiency with concurrently increased AT1b. A similar shift in the M1/M2 ratio in peritoneal macrophages was seen in obese AT1aKO mice due to a robust increase in M1 markers.

Obesity not only induced greater inflammation in kidney and adipose tissue in obese vs. lean AT1aKO mice, it also increased body weight and blood glucose levels, without affecting SBP. These results complement findings by Rong et al. (38), who showed that high-fat- vs. normal chow-fed AT1aKO mice developed increased body weight, epididymal adipose tissue mass, and blood glucose levels, also without an effect on SBP (38). The higher SBP, as well as body weight in our study, likely reflect the later start point (10–12 vs. 8 wk) and longer duration of the follow-up in our study (24 vs. 12 wk). Not surprisingly, administration of ARB, which blocks both AT1a and AT1b receptors, improved all the metabolic abnormalities in obese AT1aKO mice (38).

Table 3. Metabolic parameters in ARB-treated obese mice

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<th>0 wk</th>
<th>12 wk</th>
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<td>Blood glucose, mg/dl</td>
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<tr>
<td>WT+HFD</td>
<td>115.1 ± 3.1</td>
<td>216.6 ± 8.8*</td>
</tr>
<tr>
<td>WT+HFD+ARB</td>
<td>111.1 ± 5.6</td>
<td>142.4 ± 6.7*†</td>
</tr>
<tr>
<td>Body weight, g</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WT+HFD</td>
<td>24.0 ± 0.2</td>
<td>44.8 ± 1.5*</td>
</tr>
<tr>
<td>WT+HFD+ARB</td>
<td>25.9 ± 0.7</td>
<td>42.3 ± 0.9*</td>
</tr>
<tr>
<td>Epididymal fat mass, g</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WT+HFD</td>
<td>1.2 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>WT+HFD+ARB</td>
<td>0.9 ± 0.1</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE. ARB, angiotensin receptor blocker; HFD, high-fat diet. *P < 0.01, 12 wk vs. 0 wk. †P < 0.01, WT+HFD+ARB vs. WT+HFD at 12 wk. ‡P < 0.05, WT+HFD+ARB vs. WT+HFD at 12 wk.
In summary, obesity aggravates kidney injury, contributed to by augmented inflammation in adipose tissue and the kidney. Our data suggest that the AT$_1$ receptor modulates macrophage phenotypes and functions in obesity. Lack of AT$_{1a}$ receptors with AT$_{1b}$ upregulation on macrophages thus not only promotes macrophage infiltration into adipose tissue and kidney but also adversely shifts the M1/M2 balance in response to obesity. Pharmacological inhibition of the AT$_1$ receptor with ARB in obese WT mice abolished obesity-induced renal macrophage infiltration, further suggesting a role of the AT$_1$ receptor in obesity-induced kidney injury. We speculate that direct infiltration of inflammatory M1 macrophages into the kidney as well as macrophage infiltration into adipose tissue and adipocyte-specific metabolites, including free fatty acids, leptin, and adiponectin, contribute to obesity-related kidney injury (14).

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