Renin-angiotensin system activation in renal adipogenesis

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1Department of Medicine and Therapeutics and 2Hong Kong Institute of Diabetes and Obesity and 3Li Ka Shing Institute of Health Sciences, The Prince of Wales Hospital, The Chinese University of Hong Kong, Hong Kong SAR, China

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Sui Y, Zhao H, Fan R, Guan J, He L, Lee HM, Chan JC, Tong PC. Renin-angiotensin system activation in renal adipogenesis. Am J Physiol Renal Physiol 298: F391–F400, 2010. First published November 25, 2009; doi:10.1152/ajpren.00445.2009.—The kidney is one of the major organs involved in whole-body homeostasis while chronic renal impairment usually leads to fat redistribution and hyperlipidemia. The aim of this study was to elucidate the role of tissue renal renin-angiotensin system (RAS) components, lipogenic peroxisome proliferator-activated receptor-γ (PPARγ), and cytokine TNF-α in the development of ectopic adipogenesis and lipid deposition. Adult male Sprague-Dawley rats were randomized into three groups: untreated uninephrectomized (UNX) rats, UNX rats treated with an angiotensin-converting enzyme inhibitor (ACEI), lisinopril, and sham-operated rats. All animals were euthanized at 10 mo postoperation. The untreated UNX rats showed increased protein expression of renin, angiotensinogen, PPARγ, and the angiotensin II type 2 receptor (AT2R) but reduced protein expression of AT1R and expression of renin, angiotensinogen, PPARγ postoperation. The untreated UNX rats also developed partial lipodystrophy with adipocyte dedifferentiation and adipogenesis. Treatment with the ACE inhibitor (ACEI) lisinopril largely prevented these nephrectomy-induced structural and functional changes. Here, we hypothesize that activation of RAS components and PPARγ in the remnant kidneys contribute to the ectopic adipogenesis and lipid accumulation. In this study, we used UNX rats and an ACE inhibitor to examine the renal expression of RAS components, PPARγ, and TNF-α that are involved in adipocyte differentiation and lipid metabolism.

DYSLIPEMIA AND ABNORMAL fat topography are associated with common noncommunicable diseases such as cardiovascular disorders, diabetes, and chronic kidney insufficiency. Recently, deviant fat distribution was found in African Americans with type 2 diabetes (8). Excessive fat accumulation in visceral organs and skeletal muscle combined with subcutaneous lipodystrophy is attributable to insulin resistance (8). Insulin resistance has been hypothesized to play a major role in the pathogenesis of metabolic syndrome, including abdominal obesity, type 2 diabetes, dyslipidemia, and hypertension (21). However, mechanisms underlying fat redistribution and etiological factors leading to ectopic adipogenesis and fat deposition are still unclear. Studies of human normal and overweight subjects demonstrated the presence and expression of renin-angiotensin system (RAS) components such as angiotensinogen (AGT), angiotensin II type 1 receptor (AT1R), and angiotensin-converting enzyme (ACE) in both visceral and subcutaneous adipose tissue (9). In addition, RAS components such as ANG II, AT1R, and the angiotensin II type 2 receptor (AT2R) have been implicated in adipocyte differentiation from precursor fibroblast to preadipocyte (19). ANG II stimulated adipocyte transformation and lipogenesis by increasing lipid storage via an AT2R-dependent pathway. On the other hand, AT1R activation was implied in the inhibition of adipogenesis. These effects of AT1R and AT2R on adipogenesis were related to peroxisome proliferator-activated receptor-γ (PPARγ) (18), one of the specific activators of adipogenic genes and the central engine of adipocyte differentiation (20). PPARγ serves as a master regulator in the formation of mature adipocytes (40). Lipodystrophy, insulin resistance, and RAS dysregulation are important features of human PPARγ deficiency states (14). Interestingly, suppression of endogenous production of the cytokine TNF-α stimulated PPARγ-induced adipogenic activity in human preadipocytes (44).

We have previously set up a unilateral nephrectomy (UNX) rat model characterized by chronic progressive renal impairment, glomerulosclerosis, hyperglycemia, hyperinsulinemia, and hyperlipidemia (51). The untreated UNX rats also developed partial lipodystrophy with adipocyte dedifferentiation and adipogenesis. Treatment with the ACE inhibitor (ACEI) lisinopril largely prevented these nephrectomy-induced structural and functional changes. Here, we hypothesize that activation of RAS components and PPARγ in combination with suppression of TNF-α in the remnant kidneys contribute to the ectopic adipogenesis and lipid accumulation. In this study, we used UNX rats and an ACE inhibitor to examine the renal expression of RAS components, PPARγ, and TNF-α that are involved in adipocyte differentiation and lipid metabolism.

MATERIALS AND METHODS

Animals. Ethical approval for animal studies was provided according to the Animal Experimentation Ethics Committee of The Chinese University of Hong Kong and in accordance with the Animals (Control of Experiments) Ordinance of the Department of Health of the Hong Kong SAR Government.

Male Sprague-Dawley rats (300–350 g) were obtained from the Laboratory Animal Services Centre at The Chinese University of Hong Kong and maintained in the animal housing and care facility at the Prince of Wales Hospital. The animals were housed at 23 ± 1°C with a 12:12-h dark-light cycle, having free access to water and being fed a standard laboratory rat diet (5001 Rodent Diet; LabDiet, St. Louis, MO). The total duration of the study was 10 mo.

The animals were randomized into three groups: sham operation (n = 10), left unilateral nephrectomy (UNX; n = 10), and UNX plus treatment with the ACEI lisinopril (ACEI; n = 10). Lisinopril was dissolved in 3 ml sterile distilled water, with a once daily dosage of 4 mg/kg body wt. All the sham and UNX rats were also gavaged with distilled water (3 ml) as a placebo control.

Left UNX was performed according to the previously reported procedures (51). Briefly, the left kidney was removed via a flank incision, leaving the adrenal gland intact. Sham-operated rats under-
went surgical interventions of comparable durations without removal of the left kidney. At 10 mo postoperation, all rats were euthanized for biochemical and histopathological assessments.

**Biochemical studies.** Body weight and average 24-h intake of water and food of the experimental rats were monitored monthly. When rats were euthanized, fasting blood samples were taken for measurement of renal function, glucose, and blood lipid panels, including total cholesterol, triglyceride, LDL cholesterol, and HDL cholesterol according to the methods previously reported (51). Fasting serum insulin concentrations were measured using enzyme immunoassay with a rat insulin ELISA kit (Merodia, Uppsala, Sweden). Homeostasis model assessment-estimated insulin resistance (HOMA-IR) was calculated with the following formula: HOMA-IR = fasting insulin (mU/l) × fasting blood glucose (mmol/l) / 22.5.

**Histopathological examination.** Remnant kidneys in UNX rats and corresponding right kidneys in sham rats were removed at 10 mo postoperation when rats were euthanized. Specimens were fixed in 10% neutral formaldehdy for histological analysis or snap frozen in liquid nitrogen for protein assessment. After fixation for 24 h, tissues were dehydrated and embedded in paraffin for sectioning at 4-μm thickness. Sections were stained with hematoxylin and eosin (H&E). Stained slides were examined with a Zeiss Axiosplan 2 imaging microscope (Carl Zeiss, Hamburg, Germany), and representative images were captured using a SPOT digital camera.

**Immunofluorescence staining.** Kidney sections (4 μm) were stained for mouse anti-CD68 (dilution 1:100; DAKO, Carpinteria, CA); goat anti-renin (1:400; Santa Cruz Biotechnology, Santa Cruz, CA); goat anti-AGT and ANG II (angiostatin, dilution 1:200; Santa Cruz Biotechnology); rabbit anti-AT1R (1:50; Santa Cruz biotechnology); rabbit anti-AT2R (1:1,000; Abcam, Cambridge, MA), rabbit anti-PPARγ (1:100; Abcam); and rabbit anti-TNF-α (1:100; Abcam). First, tissue sections were blocked with 1% BSA for 30 min at room temperature after antigen retrieval with 10 mM citrate buffer. Then, sections were incubated with the primary antibodies overnight at 4°C. Immunofluorescence was detected with donkey anti-mouse, anti-rabbit, or anti-goat secondary antibodies (Molecular Probes, Eugene, OR) conjugated with Alexa 488 or 546 at a dilution of 1:400. Cell nuclei were counterstained with DAPI (1:25,000; Invitrogen, Carlsbad, CA). Slides were mounted with ProLong (Molecular Probes) antifading reagent, stored in the dark at 4°C, and examined within 1–3 days. Stained slides were examined with a Zeiss Axiosplan 2 imaging microscope (Carl Zeiss), and representative images were automatically taken using a SPOT digital camera.

To quantitate the presence of renin immunoreactivity in the juxtaglomerular apparatuses (JGA), blood vessels, and tubules, 10 cortex fields of ×200 magnification were counted in kidney sections from each rat and assessed by an observer who was blinded to the interventions. The number of JGA, blood vessels, and tubules with positive immunostaining for renin was then expressed as a mean of the total.

**Western bloting.** Total protein extracts were prepared from frozen renal cortex tissues. Briefly, tissue was homogenized in RIPA buffer. After centrifuging, the pellet was discarded and the supernatant was kept for further analysis. Protein concentrations in the supernatant were determined with a BCA Protein Assay Kit (Thermo Fisher Scientific, Waltham, MA). Tissue protein extracts (100 μg) and prestained molecular weight markers (Bio-Rad, Hercules, CA) were separated by SDS-PAGE using 10% gels. The resolved proteins were then transferred onto nitrocellulose membranes. The membranes were blocked for 1 h at room temperature with 5% skim milk, incubated with primary antibodies of goat anti-renin (1:1,000), goat anti-AGT (1:500), rabbit anti-AT1R (1:500), rabbit anti-AT2R (1:800), rabbit anti-PPARγ (1:500), and rabbit anti-TNF-α (1:100) in TBS containing 0.05% Tween 20 (TBS-T) with 5% skim milk overnight at 4°C. After washing with TBS-T, membranes were incubated with anti-rabbit or anti-goat secondary antibody conjugated to horseradish peroxidase (Upstate, Temecula, CA) with dilution of 1:2,000. Protein bands were detected by incubating the membrane with enhanced chemiluminescence reagent (Amersham, Piscataway, NJ) and then exposed to Hyperfilm. The major protein bands detected were ~40 kDa for renin, 60 kDa for AGT, 41 kDa for glycosylated AT1R, 53 kDa for nonglycosylated AT1R, 44 kDa for AT2R, 58 kDa for PPARγ, and 26 kDa for TNF-α. To ensure equal loading of proteins, membranes were probed with a rabbit anti-β-actin antibody (Abcam) at 1:10,000, which recognizes the β-actin protein at ~47 kDa. Signals were quantitated by densitometry and corrected for the β-actin signal, using the Kodak Digital Image Station 440CF and the ID Image Analysis software program.

**Statistical analysis.** Data are expressed as means ± SD. Statistics Package for the Social Sciences 10.0.7 for Windows 2000 (SPSS, Chicago, IL) was used to perform statistical analysis. One-way ANOVA was adopted to analyze the statistical differences of biochemical parameters among three groups, and the Bonferroni test was selected to conduct post hoc multiple comparisons between different groups. A two-tailed P value of <0.05 was taken as the criterion for a statistically significant difference.

**RESULTS**

**Body weight and renal impairment.** Over the period of 10-mo observation, body weight was similar among the three groups of animals (Table 1). Dramatic weight gain from an average of 331.6–550 g was found in the first 3 mo postoperation, followed by a slow weight gain period until 6 mo (9 mo of age). Eventually, the body weight was stable at ~600 g. These data indicate that UNX has little impact on the animal’s body weight, and UNX rats do not suffer from cachexia.

At 10 mo after uninephrectomy, the untreated UNX rats showed renal dysfunction (Table 1). Compared with sham rats, the untreated UNX rats had higher levels of serum urea and creatinine. Treatment with the ACEI lisinopril significantly normalized serum urea and creatinine levels.

**Hyperlipidemia and hyperinsulinemia.** Compared with sham rats, the untreated UNX rats showed significant elevations of serum lipid components: total cholesterol, triglyceride, LDL cholesterol, and HDL cholesterol (Table 1). The untreated UNX rats developed hypertriglyceridemia and hypercholesterolemia at 10 mo postoperation, whereas the ACEI treatment substantially normalized blood lipids levels.

In addition to hyperlipidemia, higher 2-h oral glucose tolerance test blood glucose and fasting insulin levels and higher

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**Table 1. Renal impairment and blood lipids at 10 mo postoperation**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sham</th>
<th>UNX</th>
<th>ACEI</th>
</tr>
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<tbody>
<tr>
<td>Body weight, g</td>
<td>593 ± 42</td>
<td>564 ± 49</td>
<td>606 ± 48</td>
</tr>
<tr>
<td>Food intake (24 h), g</td>
<td>33 ± 5</td>
<td>34 ± 6</td>
<td>32 ± 6</td>
</tr>
<tr>
<td>Water intake (24 h), ml</td>
<td>41 ± 7</td>
<td>73 ± 9*</td>
<td>44 ± 5</td>
</tr>
<tr>
<td>Serum creatinine, μmol/l</td>
<td>42.9 ± 9.8</td>
<td>98.5 ± 25.5; 45.6 ± 6.1*</td>
<td>7.0 ± 1.9</td>
</tr>
<tr>
<td>Serum urea, mmol/l</td>
<td>7.0 ± 1.9</td>
<td>19.6 ± 7.3; 7.3 ± 1.1*</td>
<td>6.7 ± 0.5</td>
</tr>
<tr>
<td>Oral glucose tolerance test blood glucose (2 h), mmol/l</td>
<td>6.7 ± 0.5</td>
<td>7.9 ± 0.8*</td>
<td>7.0 ± 0.3*</td>
</tr>
<tr>
<td>Fasting serum insulin, mU/l</td>
<td>0.77 ± 0.14</td>
<td>0.93 ± 0.24*</td>
<td>1.06 ± 0.10*</td>
</tr>
<tr>
<td>Total cholesterol, mmol/l</td>
<td>1.8 ± 0.9</td>
<td>4.8 ± 0.8*</td>
<td>1.5 ± 0.5*</td>
</tr>
<tr>
<td>Triglyceride cholesterol, mmol/l</td>
<td>0.69 ± 0.36</td>
<td>1.49 ± 0.82*</td>
<td>0.53 ± 0.23*</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/l</td>
<td>0.50 ± 0.83</td>
<td>1.15 ± 0.48*</td>
<td>0.36 ± 0.10*</td>
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</table>

Values are means ± SD. UNX, uninephrectomized; ACEI, angiotensin-converting enzyme inhibitor; HOMA-IR, homeostasis model assessment-estimated insulin resistance. ‡P < 0.05, §P < 0.01 vs. sham. *P < 0.05, §§P < 0.01 vs. UNX.
HOMA-IR were found in UNX rats (Table 1). The ACEI treatment showed little effect on the improvement of HOMA-IR because of the elevated fasting serum insulin concentration.

**Fat accumulation in remnant kidney cortex.** Fat accumulation in the remnant kidneys included adipogenesis and lipid deposition. Histological examination demonstrated focal preadipocytes and fat tissues in the remnant kidneys of untreated UNX rats (Fig. 1). Normally, fat deposits were confined within the renal sinus; neither the renal cortex nor the renal medulla contained any adipose tissue in sham rats. However, UNX rats showed patches of adipocytes in the inner cortex (Fig. 1A). Immunohistochemical staining of CD68 differentiated these adipocytes from the macrophage-derived foamy cells, which were often found at the peripheral region of the renal adipose tissue. Treatment with the ACEI lisinopril generally prevented the ectopic adipogenesis in the remnant kidney cortex.

In agreement with the development of hyperlipidemia in untreated UNX rats, lipid deposition appeared as foci and patches of CD68-immunoreactive foamy cells accumulated in glomeruli and tubulointerstitial compartments (Fig. 1B). Such lipid-laden macrophages were not easily identified by routine HE staining, but readily detected by using specific immunomarkers. Interestingly, foamy cell accumulation also often occurred adjacent to the ectopic adipose tissue (Fig. 1A). Such renal adipogenesis and lipid deposition were rarely seen in the ACEI-treated rats.

**Expression of RAS components, PPARγ, and TNF-α.** Since RAS components are involved in adipocyte differentiation from fibroblast-like precursor cells during adipogenesis, we thus detected protein expression of renin, AGT, ANG I/II, AT1R, and AT2R in the renal cortex using both immunofluorescence microscopy and immunoblotting assay. Renal tissue expression of renin in sham rats was confined to a few cells in

![Fig. 1. Adipose deposition in remnant kidney cortex.](http://ajprenal.physiology.org/)
JGA (Fig. 2A). In untreated UNX rats, renin immunoreactivity could be found in tubular cells, glomerular cells, and vascular cells in addition to the JGA cells (Fig. 2A). Immunolocalization of renin in epithelial cells of the proximal convoluted tubules was detected in untreated UNX rats but not in the other two groups of animals. The renal cortical adipocytes were consistently negative for renin. Table 2 shows the immunolocalization and quantification of renin in renal cortex. Western blotting showed a twofold increase in renin expression in the remnant kidneys of untreated UNX rats (Fig. 2B). Treatment with the ACEI lisinopril significantly reduced cellular renin expression in UNX rats to a level slightly higher than that in sham rats (Fig. 2, Table 2).

Local expression of angiotensin was also increased in infiltrated renal cortical adipocytes (UNX-1 in Fig. 3A), renal tubular cells (UNX-2 in Fig. 3A), glomeruli, and blood vessels.

![Renin protein in renal cortex](image-url)
of untreated UNX rats. Positive staining was rarely found in sham rats (Fig. 3A). In parallel to the improvement of chronic renal impairment, the ACEI-treated rats showed largely normalized immunoreactivity of angiotensin (Fig. 3A). Western blotting confirmed the enhanced protein expression of AGT in the untreated UNX rats (Fig. 3B). Taken together, overexpression of renin and angiotensin in the remnant kidneys of untreated UNX rats was associated with uninephrectomy-induced renal fat accumulation.

Immunofluorescence microscopy revealed the differential immunolocalization of renal ANG II receptors, lipogenic PPARγ, and the antiadipogenic factor TNF-α (Fig. 4). Immunoreactivity of AT1R and TNF-α was found in normal tubular cells and arterial smooth muscle cells. The untreated UNX rats showed reduced renal immunoreactivity of AT1R and TNF-α due to the negative staining in the degenerative tubules and some arteries. In contrast, renal immunoreactivity of AT2R was rare and predominantly found in degenerative tubules. Therefore, only a few tubular cells were positive to the immunoreactivity of AT2R in the sham and ACEI-treated rats (Fig. 4), whereas ~10% of the tubules in the untreated UNX rats exhibited AT2R immunoreactivity. Similarly, renal immunoreactivity of PPARγ in tubular cells was found more frequently in the untreated UNX rats compared with the sham or ACEI-treated rats (Fig. 4). Taken together, collective actions of RAS components, PPARγ, and TNF-α may induce adipogenesis and lipid deposition in the remnant kidney cortex by paracrine or autocrine mechanisms.

Protein expression levels of AT1R, AT2R, PPARγ, and TNF-α in the renal cortex were also measured by Western blotting. The detected protein level was adjusted by the corresponding quantities in sham rats and expressed as ratios. In the remnant kidneys of untreated UNX rats, protein expression of AT1R was reduced (Fig. 5A). In contrast, protein expression of the lipogenic AT2R in the remnant kidneys of the untreated UNX rats was significantly increased (Fig. 5A). Consequently, the ratio of AT2R to AT1R was dramatically higher in untreated UNX rats (Fig. 5B). Consistently, the ACEI treatment generally normalized the protein expression of AT1R and AT2R as well as the AT2R-to-AT1R ratio (Fig. 5A and B). Protein expression of PPARγ was significantly increased in the untreated UNX rats compared with sham rats (Fig. 5A and C). Conversely, untreated UNX rats showed significantly reduced protein expression levels of TNF-α (Fig. 5A and D). The ACEI treatment substantially normalized the protein expression levels of PPARγ and TNF-α in the remnant renal cortex.

**DISCUSSION**

In this study, we reported dysregulated RAS components, PPARγ, and TNF-α associated with kidney cortex adipogenesis and lipid accumulation in UNX rats. These findings for the first time demonstrate the relationship between ectopic adipogenesis and localized tissue RAS activation in vivo. Previous studies of the angiotensin system and PPARγ in adipose regulation mainly focused on the adipocytes in fat deposits (1) and on the adipose cell lines in vitro (46). The novel findings in this study support the notion that the tissue RAS participates in the pathogenesis of ectopic intraorgan adipogenesis, global fat distribution, and lipid metabolism.

In this study, the untreated UNX rats at 10 mo postnephrectomy demonstrated increased renin immunoreactivity in both JGA and the proximal tubules. Our findings are partially inconsistent with previous work reported by Gilbert et al. (10) demonstrating that in rats with subtotal nephrectomy, renin expression was reduced in the JGA but increased in renal tubular epithelial cells. The reason for variance in renin expression between the Gilbert study and that of the present study is not entirely clear. However, the animals were investigated 10 mo following left nephrectomy in this study as opposed to 12 wk after the drastic renal mass ablation performed by right subcapsular nephrectomy and infarction of approximately two-thirds of the left kidney in the work reported by Gilbert et al. The renal failure in rodents with subtotal nephrectomy was relatively acute and severe, and the follow-up periods were usually shorter (<4 mo) to avoid animal death before the conclusion of the observation. In the Gilbert report, the rats with subtotal nephrectomy had significant body weight loss, heavy proteinuria, and elevated serum creatinine level at 12 wk postsurgery. In the present study and in our previous work (51), body weight loss and renal dysfunction were not found in the untreated UNX rats within 3 mo following uninephrectomy. Therefore, the differences in the chronicity and severity of renal insufficiency may account for the difference in renin expression between the present observation and Gilbert’s study (10).

Experimental studies indicate that several humoral and tissue factors that may be responsible for the renal compensatory hyperplasia can initiate signaling mechanisms underlying the metabolic, functional, and structural alterations following UNX (2, 3). Activation of ANG II contributes to adipocyte proliferation and differentiation despite of the conflicting observation and interpretation from in vivo and in vitro experiments using different models. Some (6, 7, 41) but not all reports (17, 29) indicate that ANG II promotes adipocyte differentiation. In contrast to the controversial roles of ANG II in adipogenesis, the major ANG II receptor subtypes, AT1R and AT2R, are generally considered to regulate adipocyte differentiation in opposite directions. Published data suggested that RAS components modulate adipocyte transformation through their stimulating action via AT2R and inhibiting action via AT1R. On one hand, clinical studies of human subjects have shown that AT1R blockade by telmisartan, irbesartan and losartan induces adipogenesis in subcutaneous fat through the activation of the lipogenic PPARγ, and such stimulation is prevented by a PPARγ antagonist, GW9662 (18). On the other hand, AT2R stimulates adipocyte differentiation. Knockout mice with a genetic deletion of AT2R show a decrease in adiposity (49). Therefore, the actions of ANG II on adipogenesis may be dependent on the balanced expression of AT1R and

### Table 2. Immunolocalization and quantification of renal renin at 10 mo postnephrectomy

<table>
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<tr>
<th></th>
<th>Sham</th>
<th>UNX</th>
<th>ACEI</th>
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<tbody>
<tr>
<td>Juxtaglomerular</td>
<td>1.1 ± 0.2 (0–1)</td>
<td>1.1 ± 0.2 (0–1)</td>
<td>1 ± 0 (1–4)</td>
</tr>
<tr>
<td>apparatus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tubule</td>
<td>0.4 ± 0.2 (0–1)</td>
<td>1.8 ± 0.4 (0–4)</td>
<td>0 ± 0 (0–0)</td>
</tr>
<tr>
<td>Artery</td>
<td>0.6 ± 0.3 (0–1)</td>
<td>0.7 ± 0.2 (0–1)</td>
<td>0.1 ± 0.1 (0–1)</td>
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Values are means ± SE. *P < 0.001 vs. sham or ACEI.
AT2R. In the present study, local RAS activation coexisted with PPARγ overexpression and TNF-α downregulation in the remnant kidney cortex, where adipogenesis occurred by transformation of fibroblast-like cells to preadipocytes. These findings may at least partially explain the development of renal adipogenesis and adipose infiltration in untreated UNX rats.

The second novel finding in the present study is the upregulation of PPARγ, a key regulator of adipocyte differentiation (25), in remnant kidney cortex with adipose accumulation. Novel actions of AT1R antagonists on adipocytes rely on the upregulation of the lipogenic PPARγ (22, 23, 34, 42). PPARγ regulates the gene expression of proteins that are involved in the differentiation of adipocytes from precursor fibroblast-like cells and contributes to the storage of fatty acids (26). In normal adipose tissue, adipocyte differentiation from precursor cells occurs simultaneously with activation of lipogenic pathways (11), leading to accumulation of lipid in adipose tissue and redistribution of lipids from other sites to adipose tissue. Moreover, PPARγ triggers a typical adipose program for adipogenesis (40) and modulates the number of adipocytes and the amount of stored lipids, to adapt total adipose tissue storage.
capacity to an appropriate level (12). Clinically, such insulin-sensitizing drugs show remarkable antidiabetic action (24) by recruiting metabolically active adipocytes for the increment of lipid storage capacity (38). However, PPARγ expression in nonadipose tissues such as the kidney cortex promotes ectopic adipose accumulation, which may potentially reduce insulin sensitivity and induce insulin resistance. In fact, increased expression of PPARγ and reduced expression of TNF-α have been found parallel to increased circulating levels of free fatty acids and triglycerides (4), which could induce ectopic fat accumulation in nonadipose tissues and subsequently result in insulin resistance (43). In this study, upregulation of PPARγ in the remnant kidney cortex was a response to both local RAS activation and systemic hyperlipidemia, a sign showing an attempt to decrease circulating lipids by increasing lipid storage in ectopic sites (Fig. 6).

This animal study demonstrated renal adipogenesis and fat deposition following unilateral nephrectomy. Unlike hyperlipidemia with a definitive impact on insulin resistance, the pathophysiological role of ectopic fat deposition has not been fully characterized. There are interests in the potential pathogenic effects of ectopic fat on homeostatic regulation. Some literature implied a crucial role of intraorgan fat in the development of insulin resistance and subsequently type 2 diabetes. In human and animal models, central obesity is often associated with fat deposition within and around nonadipose organs, predisposing the development of type 2 diabetes and cardiovascular disease. In obese animals fed a high-fat diet, fat deposited within and around the heart and eventually led to chronic heart failure (32). Therefore, ectopic fat accumulation in multiple organs may disturb the homeostasis of energy balance. Impressively, obese patients with type 2 diabetes undergoing a moderately hypocaloric diet intervention demonstrated a significant improvement in blood glucose and insulin sensitivity with only 8% loss of body weight and 81% reduction in hepatic lipid content (28, 35). Moreover, clinical treatment with thiazolidinediones improves insulin resistance by redistributing fat from liver cells to subcutaneous adipose tissue without altering the total amount of fat (27, 30, 36). Taken together, ectopic fat deposition and intracellular lipid content may be more important than the absolute fat quantity in the pathogenesis of insulin resistance (37).

In this study, the beneficial effects of ACEI treatment were not associated with significant changes in observed HOMA-IR or body weight. Similar observation was also found in a progressive renal failure rat model, Imai rats, demonstrating that long-term RAS blockage with olmesartan completely normalized hypertension, hyperlipidemia, proteinuria, and glomerulosclerosis without observed changes in body weight (39). The reason for the unchanged HOMA-IR after ACEI treatment may be related to the increased fasting serum insulin level in the ACEI-treated UNX rats. We have previously reported that
ACEI treatment completely normalized the reduced pancreatic β-cell area and diminished urine insulin excretion following uninephrectomy (45). Since the calculation of HOMA-IR is based on the product of fasting blood glucose and serum insulin levels, the elevated fasting serum insulin level in the ACEI-treated rats definitely raised the HOMA-IR score with compromised implication in insulin resistance. In our work, the HOMA-IR value should never mask the important beneficial effects of ACEI treatment on the normalization of renal function, insulin secretion, blood lipid profile, and ectopic fat deposition.

The association of RAS activation with renal adipogenesis and fat deposition in this report provides insight concerning the antidiabetic effects of RAS blockades. In the past decades, the contribution of the tissue RAS in disease pathogenesis has prompted the investigation of using RAS inhibition to prevent diabetes beyond lowering blood pressure. RAS blockades positively regulate insulin sensitivity and glucose metabolism (31). The development of type 2 diabetes and metabolic syndrome was delayed using RAS blockers in landmark clinical trials such as the CAPPP study (13, 33), HOPE study (15), ANBP-2 survey (47), and VALUE study (16). Meta-analyses affirm the antidiabetic effects of RAS blockades in “at risk” populations with hypertension (5), cardiovascular disease (13, 48), or with insulin resistance (50). The finding of the preventive effects of the ACEI lisinopril on ectopic fat deposition and hyperlipidemia described in this study is novel knowledge for persons with high risk of diabetes. Common molecular pathways modulating adiposity, blood pressure, and glucose and lipid metabolism warrant future studies for the better management of the global epidemic of diabetes and metabolic syndrome.

**ACKNOWLEDGMENTS**

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**DISCLOSURES**

No conflicts of interest are declared by the authors.
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