Periaortic adipose tissue-specific activation of the renin-angiotensin system contributes to atherosclerosis development in uninephrectomized apoE<sup>−/−</sup> mice

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CHRONIC KIDNEY DISEASE (CKD) is now recognized to be an independent risk factor for cardiovascular mortality and morbidity, and underlying mechanisms of CKD-associated proatherogenic actions have been extensively investigated in both animal experiments and clinical studies (12, 24, 31, 32). Numerous previous studies have focused on the impaired bioavailability of nitric oxide (NO) accompanied by the increased level of asymmetric dimethylarginine (ADMA), endogenous NO synthesis inhibitor (41), activation of renin-angiotensin system (RAS) (4), and increased sympathetic nervous system activity (13, 33), all of which synergistically exaggerate oxidative stress and systemic inflammation, thereby contributing to the development of atherosclerosis. However, it is difficult to identify the causal relationship of each deleterious factor in the advanced stage of atherosclerosis, and the primary mechanism of minor renal insufficiency-induced atherosclerosis remains to be fully elucidated.

Recently, the perivascular adipose tissue has been shown to play a pivotal role in the pathogenesis of vascular remodeling and atherosclerosis through paracrine effects on the vasculature (8, 17, 18, 23a, 27, 38). Proinflammatory adipokines were markedly increased in the perivascular adipose tissue (PAT) in wild-type mice fed a high-fat diet (8). The epicardial adipose tissue in patients with acute coronary syndrome showed significantly higher gene expression and protein secretion of proinflammatory adipokines, unlike that observed in control subjects (18). Renal insufficiency has been reported to modulate visceral adipose tissue properties for exerting deleterious effects on lipid metabolism and energy homeostasis (37); however, its effects on perivascular adipose tissue and the causal relationship with CKD-associated atherosclerosis have not yet been investigated.

To elucidate the primary mechanisms of CKD-associated atherosclerosis, we focused on the PAT of apoE<sup>−/−</sup> mice and examined the effect of unilateral nephrectomy on the gene expression of adipokotines, including proinflammatory cytokines/chemokines and RAS components. To the best of our knowledge, we have demonstrated for the first time that PAT-specific increases in angiotensinogen (AGT) mRNA expression and angiotensin II (ANG II) secretion are significantly exerted in uninephrectomized apoE<sup>−/−</sup> mice at the early stage of atherosclerosis development. These findings suggest that PAT-specific activation of RAS, elicited by minor renal insufficiency, is likely a primary mechanism underlying the initiation of CKD-associated atherosclerosis and could be a therapeutic target to prevent cardiovascular diseases in patients with CKD.

METHODS

Animal preparation. ApoE deficient (apoE<sup>−/−</sup>) mice (C57BL/6) were obtained from Taconic (Germantown, NY). Eight-week-old...
male apoE−/− mice underwent uninephrectomy and were fed a high-cholesterol diet (36% fat, 1.25% cholesterol; Oriental Yeast, Tokyo, Japan) starting at 12 wk of age. The animals were housed in a room maintained at 22°C in a room with a 12-h light/dark cycles and drinking water ad libitum. Unilateral nephrectomy was performed as previously described (6). Anesthesia with a single intraperitoneal injection of ketamine (50 mg/kg) and xylazine (8 mg/kg) was maintained throughout the surgery. The depth of anesthesia was confirmed by lack of a tail pinch response. We made a dorsal midline incision of the skin, and dissected the left kidney free through a dorsovenal incision of the muscles and fascia near to the costal margin. The renal vessels and the ureter were carefully isolated, and a single ligature was placed around them and tied tightly. The distal portions were then cut, and the kidney was removed. Sham operations of control animals were performed in the same manner, but without removal of the kidney and the ureter. Finally, the musculofascial incisions were sutured, and the skin incision was closed by metal clips. The atherosclerotic lesion area in the thoracic aorta was evaluated at 16 or 20 wk of age. Before isolation of tissues (aortas), mice were killed by trans-cardiac perfusion under terminal anesthesia by intraperitoneal injection of pentobarbital (200 mg/kg). All investigations conformed to the Guidelines for Animal Experiments at the Kyoto Prefectural University of Medicine. Experiments were also approved by a local university ethics review board.

**Hemodynamic analysis.** The mean blood pressure and heart rate were measured under conscious and unrestrained conditions using a programmable sphygmomanometer (BP-98A; Softron, Tokyo, Japan), as described previously (23).

**Quantitative measurement of atherosclerotic lesions:** The mice were euthanized at the age of 16 or 20 wk, and the atherosclerotic lesions were analyzed. Image analysis was performed on oil red O-stained aortas using the Scion Imaging software. The thoracic aortic lesion area of each animal was measured as the percentage of the lesion area to the total thoracic aortic area.

**Real-time PCR.** Total RNA was extracted from adipose tissue or adipocytes and reverse transcribed to cDNA. Real-time PCR was performed using a Light Cycler ST300 (Roche Applied Science, Indianapolis, IN), with the SYBR Green PCR Master Mix and RT-PCR (Applied Biosystems) as described previously (23). Dissociation curves were monitored to check for the aberrant formation of primer dimers. PCR-amplified products were electrophoresed on 2% agarose gels to confirm the presence of a single band. Data were expressed as levels relative to the control.

**Enzyme-linked immunosorbent assay (ELISA).** Blood was collected from cut tails into tubes containing citrate at a final concentration of 0.01 M. Plasma was separated by centrifugation at 10,000 g for 10 min and stored at −20°C. A volume of 10 μl was used for mouse insulin and C-peptide assays using a commercial kit (Mouse insulin ELISA KIT, MS303, Morinaga Institute of Biological Science, Yokohama, Japan; Mouse C-peptide U-type ELISA KIT, AKRCP-031, Shibayagi, Gunma, Japan).

**Intraperitoneal glucose tolerance test (IPGTT).** Mice were fasted for 6 h and then injected intraperitoneally with glucose (2 g/kg). Blood glucose measurements were performed with a glucometer using blood samples from cut tails at baseline, 30, and 60 min after the injection of glucose. Plasma concentrations of insulin and C-peptide were measured 60 min after the injection.

**Culture and differentiation of primary adipose cells.** The PAT surrounding the descending thoracic aorta was isolated from 8-wk-old wild-type mice (C57BL/6), minced, and subjected to collagenase digestion [2 mg of collagenase in 2 ml of isolation buffer containing 0.123 M NaCl, 5 mM KCl, 1.0 mM CaCl₂, 4.5 mM glucose, 100 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), and 1.5% bovine serum albumin (BSA)] for 30 min, as described previously (26). The digested tissue was filtered through a 100-mm nylon screen. Collected cells were centrifuged (200 g) for 5 min. Adipogenic differentiation was induced as previously described (29). Freshly isolated cells were plated onto laminin-coated plates (BD Biosciences) in Dulbecco’s modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS) (GIBCO) and 10 μg/ml basic fibroblast growth factor (bFGF) (R&D Systems) and maintained in a 5% CO₂ atmosphere. The cells were allowed to grow to confluence and were then maintained at confluence for 2 days without changing the medium, before exposure to the differentiation cocktail (1 μg/ml insulin, 0.25 μg/ml dexamethasone, and 0.5 mM IBMX) in fresh medium without the addition of bFGF. After 72 h of exposure to the differentiation cocktail, the cells were maintained in DMEM with 10% FBS until day 3 or day 6 for harvest. RNA was isolated with the Trizol reagent (Invitrogen), and quantitative RT-PCR was performed according to the protocol described above.

**Measurement of ANG II concentrations.** After adequate perfusion with saline, the isolated PAT and epididymal white adipose tissue (WAT) were dissected and then stored at −80°C until measurement. Plasma was collected in tubes containing ethylenediaminetetraacetic acid (EDTA; final concentration, 1 mM). Each frozen adipose tissue

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Fig. 1. Atherosclerotic lesion development is significantly exaggerated in 20-wk-old uninephrectomized apoE−/− mice (UNX). A: representative photographs of en face oil red O-stained thoracic aortas in male apoE−/− mice fed a high-cholesterol diet for 4 or 8 wk, starting at 12 wk of age. B: quantitative analysis showing a significant atherosclerotic lesion development in 20-wk-old UNX mice compared with sham control mice. Values are means ± SE for at least 8 mice in each group. *P < 0.05 vs. 16-wk-old sham control mice. #P < 0.05 vs. 16-wk-old UNX mice. †P < 0.05 vs. 20-wk-old sham control mice.
was homogenized on ice in 0.9% saline/0.1 M HCl. The concentrations of ANG II were measured by a radioimmunoassay using a specific anti-ANG II antibody (SRL, Tokyo, Japan), as described previously (42).

Urinary catecholamine analyses. Mice were housed in individual metabolic cages for 24 h with free access to water and chow. Urine was collected in tubes containing 15 μl of 6 N HCl as a preservative. The concentrations of epinephrine and norepinephrine were determined by ELISA.

Statistical analysis. All data are expressed as means ± SE. Mean values were compared using ANOVA. If a statistically significant effect was found, Scheffe’s F-test was performed to detect the difference between the groups. P < 0.05 was considered statistically significant.

RESULTS

Atherosclerotic development is exaggerated in uninephrectomized apoE−/− mice. Atherosclerosis development of the thoracic aorta was analyzed in 16- and 20-wk-old uninephrectomized male apoE−/− (UNX) mice fed a high-cholesterol diet, starting at 12 wk of age. The atherosclerotic lesion area was comparable in the 16-wk-old UNX and sham control mice; however, the 20-wk-old UNX mice showed a significant ex-

Fig. 2. Periaortic adipose tissue activation of the renin-angiotensin system in 16-wk-old uninephrectomized apoE−/− mice (UNX). A: representative photographs of hematoxylin and eosin-stained sections of descending thoracic aortas in male apoE−/− mice fed a high-cholesterol diet for 4 wk, starting at 12 wk of age. Scale bar: 500 μm. B: pad weight and area of the periaortic adipose tissue surrounding the descending thoracic aortas in 16-wk-old mice, showing no significant difference between UNX and sham control mice. Values are means ± SE for at least 6 mice in each group. C: quantitative analysis of the mRNA expression levels of inflammatory adipokines by real-time PCR, showing no discernible difference between 16-wk-old UNX and sham control mice. Values are means ± SE relative to control mice for at least 6 mice in each group. *P < 0.05 vs. sham control mice. D: quantitative analysis of mRNA expression levels of renin-angiotensin system (RAS) components by real-time PCR, showing a significant increase in angiotensinogen (AGT) mRNA expression in periaortic adipose tissue from UNX mice compared with sham control mice at 16 wk of age. Values are means ± SE for at least 6 mice in each group. *P < 0.05 vs. sham control mice. E: MCP-1, monocyte chemoattractant protein-1. F: IL-6 did not differ between the two groups (Fig. 2, A and B). We next examined whether uninephrectomy modulated the gene expression levels of adipocytokines. The mRNA expression of inflammatory cytokines/chemokines such as TNF-α, monocyte chemoattractant peptide-1 (MCP-1), and IL-6 did not differ between the two groups (Fig. 2C). In contrast, the mRNA expression of angiotensinogen (AGT), but not ACE and angiotensin II type 1 receptor (AT1aR), was significantly elevated in the PAT of UNX mice compared with

aggregation of the oil red O-positive plaque area, by 54% compared with sham control mice (Fig. 1, A and B). The hemodynamic parameters and lipid profile did not differ between the two groups. The plasma creatinine concentration and the blood urea nitrogen level showed a moderate but significant increase in the UNX mice, suggesting that even minor renal insufficiency markedly accelerated the early stage of atherosclerosis development.

Periaortic adipose tissue-specific increase in angiotensinogen gene expression and ANG II concentration in uninephrectomized apoE−/− mice. We first examined the effect of uninephrectomy on the PAT weight, cross-sectional area, and morphological features of 16-wk-old UNX mice; however, there was no discernible difference between the two groups of mice (Fig. 2, A and B). We next examined whether uninephrectomy modulated the gene expression levels of adipocytokines. The mRNA expression of inflammatory cytokines/chemokines such as TNF-α, monocyte chemoattractant peptide-1 (MCP-1), and IL-6 did not differ between the two groups (Fig. 2C). In contrast, the mRNA expression of angiotensinogen (AGT), but not ACE and angiotensin II type 1 receptor (AT1aR), was significantly elevated in the PAT of UNX mice compared with
sham control mice (85%, $P < 0.05$) (Fig. 2D). Consistent with this finding, the PAT ANG II concentration in the UNX mice was significantly higher than that in the sham control mice ($P < 0.05$) (Fig. 2, E and F). We also examined the effect of uninephrectomy on the features of the epididymal WAT. The morphological features and pad weight were not different between the two groups (Fig. 3, A and B). The expression levels of inflammatory cytokines as well as the RAS components in UNX mice were equivalent to those measured in sham control mice (Fig. 3, C and D). Similarly, the ANG II concentration in the WAT and plasma did not differ between the two groups of mice (Fig. 3, E and F), suggesting that PAT-specific activation of RAS occurred in UNX mice.

Accumulation of inflammatory leukocytes in the PAT is not augmented in uninephrectomized apoE$^{-/-}$ mice. The adipose tissue is a complex mixture of various cell types (e.g., adipose cells, stromal vascular cells including preadipocytes, endothelial cells, macrophages, and T lymphocytes) (26). To investigate whether inflammatory leukocytes were the dominant source of ANG II production, we examined the effect of uninephrectomy on the accumulation of inflammatory leukocytes in the PAT from mice at 16 wk of age. The numbers of F4/80-positive cells assessed by immunohistochemical analysis were equivalent between the two groups (Fig. 4A). Consistent with this finding, the CD68 mRNA expression levels evaluated by real-time PCR did not differ between the two groups (Fig. 4B). We further examined the mRNA expression levels of NAD(P)H oxidase subunit because ANG II is known to induce reactive oxygen species via stimulated expression and activation of NAD(P)H oxidase. The expression level of Nox2, a phagocytic subunit of NADPH oxidase, was identical between the groups, which was consistent with the finding that accumulation of macrophages was equivalent between the two groups. In contrast, UNX mice showed a 2.3-fold increase in mRNA expression of Nox4, suggesting that oxidative stress was augmented in PAT of uninephrectomized mice. These findings support the notion that adipose cells, not accumulating leukocytes, are essential contributors to the PAT-specific activation of RAS and that PAT-specific activation of RAS con-

Fig. 3. Effect of uninephrectomy on the features of the epididymal adipose tissue in 16-wk-old apoE$^{-/-}$ mice. A: representative photographs of hematoxylin and eosin-stained sections of epididymal white adipose tissue in apoE$^{-/-}$ mice fed a high-cholesterol diet for 4 wk, starting at 12 wk of age. UNX: uninephrectomized apoE$^{-/-}$ mice. Scale bar: 200 µm. B: weight of the epididymal white adipose tissue (WAT), showing no significant difference between 16-wk-old UNX and sham control mice. Values are means ± SE for at least 6 mice in each group. C: quantitative analysis of the mRNA expression levels of inflammatory adipokines by real-time PCR, showing no discernible difference between 16-wk-old UNX and sham control mice. Values are means ± SE relative to control mice for at least 6 mice in each group. D: quantitative analysis of mRNA expression levels of RAS components by real-time PCR, showing no discernible difference between 16-wk-old UNX and sham control mice. Values are means ± SE relative to control mice for at least 6 mice in each group. E: ANG II concentration in the epididymal white adipose tissue from 16-wk-old UNX mice and sham control mice. Values are means ± SE for at least 6 mice in each group. F: plasma concentration of ANG II in 16-wk-old UNX mice and sham control mice. Values are means ± SE for at least 6 mice in each group.
tributes to the atherosclerosis development, at least in part, by augmentation of oxidative stress.

Plasma insulin levels after glucose loading are markedly elevated in uninephrectomized apoE−/− mice. Of the various substances upregulating angiotensinogen gene expression in adipose cells (21–26), we focused on insulin signaling because renal insufficiency has been reported to increase plasma insulin levels to compensate for insulin resistance in both animal experiments and clinical studies (3, 19, 35). While the plasma insulin levels under fasting conditions did not differ between the two groups, the homeostasis model assessment-insulin resistance (HOMA-IR) index for 16-wk-old UNX mice was significantly higher than for sham control mice (Fig. 5A). We next performed an IPGTT to examine the plasma insulin levels after glucose loading. Although the plasma glucose levels after intraperitoneal injection of glucose (2 g/kg) were equivalent between the two groups (Fig. 5B), the plasma levels of insulin and C-peptide at 60 min after the injection were markedly higher in 16-wk-old UNX mice than in sham control mice (Fig. 5C), suggesting that augmented secretion of insulin promptly compensated for insulin resistance in UNX mice.

In vitro stimulation of preadipocytes with insulin increases angiotensinogen gene expression during adipocyte differentiation. We examined the effect of in vitro stimulation with insulin on AGT mRNA expression in mature adipocytes. According to preliminary experimental results, mature adipocytes were stimulated with 2.4 nM of insulin for 8 h; however, the AGT mRNA expression level was as the same as that observed in the control (Fig. 6A). We next examined the effect of insulin on the AGT mRNA expression level in differentiating adipocytes. Even in the absence of insulin stimulation, the AGT mRNA expression was increased up to 12-fold in a time-dependent manner, accompanied by the elevated expression of the differentiation-related gene PPARγ (Fig. 6B). Although the AGT and PPARγ mRNA expression levels were not affected by insulin treatment (100 ng/ml) at day 3, the mRNA expression levels were markedly increased at day 6 (23- and 17-fold vs. day 0, respectively; P < 0.01). To examine the effect of insulin on adipocyte differentiation, we analyzed oil red O-positive area at day 6; however, it was hardly observed in both groups. We thus examined at day 11 and found that oil red O-positive area was significantly increased by 24% in the insulin-treated group compared with the untreated group (Figs. 6C), indicating that insulin treatment accelerated adipocyte differentiation accompanied by the increased expression of PPARγ. These findings suggest that AGT mRNA expression in perivascular adipose tissue is upregulated along with the differentiation of adipocyte, which is aggravated by insulin treatment.

Fig. 4. Effect of uninephrectomy on the accumulation of F4/80-positive cells and CD68 mRNA expression in the periaortic adipose tissue. A: immunofluorescence image showing F4/80 (green) and DAPI (blue) in the descending thoracic aorta of 16-wk-old UNX mice. Arrows indicate F4/80-positive cells. Quantitative analysis of F4/80-positive cells showed no difference between uninephrectomized apoE−/− mice (UNX) and sham control mice. M: media; L: lumen. B: quantitative analysis of CD68 mRNA expression levels by real-time PCR showing no difference between UNX and sham control mice. Values are means ± SE for at least 6 mice in each group.
CKD-associated atherosclerosis is completely inhibited by treatment with ANG II receptor blocker. To investigate the relative role of local RAS activation in the development of CKD-associated atherosclerosis, we examined the effect of ANG II receptor blocker (ARB) treatment on atherosclerotic lesion development in uninephrectomized apoE⁻/⁻ mice. Eight weeks of ARB treatment completely inhibited lesion development in uninephrectomized apoE⁻/⁻ mice to the same extent as sham control mice. Considering that plasma ANG II concentration did not differ between uninephrectomized apoE⁻/⁻ and sham control mice at 16 wk of age (Fig. 3F), antiatherogenic effects of ARB were likely to be exerted through the inhibition of not only systemic RAS but also PAT-specific RAS.

**DISCUSSION**

In this study, we demonstrated for the first time that exaggerated atherosclerosis development elicited by uninephrectomy was closely associated with PAT-specific increases in AGT mRNA expression and ANG II concentration without enhanced accumulation of monocytes/macrophages, in which these features were not observed in the epididymal WAT. The HOMA-IR index and plasma insulin concentrations after glucose loading were markedly elevated in UNX mice, and in vitro stimulation of isolated PAT preadipocytes with insulin significantly increased AGT mRNA expression concomitant with the elevated expression of the adipocyte differentiation-related gene PPARγ. These findings suggest that augmented adipocyte differentiation through insulin resistance-associated hyperinsulinemia exerts PAT-specific activation of RAS, thereby promoting atherosclerosis in UNX mice.

Recently, the perivascular adipose tissue was implicated in the pathogenesis of atherosclerosis (8, 17, 18, 27, 38, 23a). The PAT anatomically contains the vasa vasorum, which forms a microvascular network in the adventitia of large arteries that supplies oxygen and nutrients to the outer layers of the vessel wall (16, 36). Because of the close interactions between perivascular fat and adventitial vasa vasorum, inflammatory adipocytokines and ANG II derived from the PAT likely contribute to the development of atherosclerosis. However, most studies have focused on infiltrating leukocytes, such as monocytes/macrophages and lymphocytes, which are crucial interaction partners with resident adipose cells and regulate the secretion of various adipocytokines from adipocytes and/or themselves. Considering that PAT-specific activation of RAS is not accompanied by the enhanced accumulation of leukocytes in this study, increases in AGT mRNA expression and secretion of ANG II is likely to be primarily attributed to adipose cells and not infiltrating leukocytes.

Cassis et al. reported that AGT mRNA was dramatically increased in periaortic fat as well as interscapular fat after bilateral nephrectomy (7); however, the precise mechanisms underlying PAT-specific activation of RAS remain undefined. A variety of mediators such as sympathetic nerve stimulation, several different fatty acids, and insulin have been reported to regulate AGT gene expression in adipose cells (1, 5, 10, 40). Moreover, ANG II itself has been shown to increase AGT expression by positive-feedback mechanism (21). It is therefore likely that PAT-specific activation of RAS contributes, at least in part, to the upregulation of AGT gene expression. To investigate an initial stimulus to increase AGT gene expression in UNX mice, we first examined the urinary catecholamine concentrations because it has been shown that the sympathetic nerve system is activated in CKD patients and that β-adrenergic stimulation increases AGT expression in adipose tissue (22, 34). However, the urinary catecholamine concentrations were equivalent between the two groups. Several different fatty acids, ligand activators of PPARγ, have also been reported to increase AGT mRNA expression in preadipose cells (30). We next examined the serum concentration of fatty acids at 16 wk.
of age; however, UNX mice showed the similar concentration levels of fatty acid as those observed in control mice. Clinical studies have demonstrated that CKD patients are susceptible to insulin resistance accompanied by postprandial hyperglycemia (3, 19, 35), and insulin stimulation has been shown to regulate AGT gene expression in adipose cells (2, 15). We thus examined the plasma insulin level and insulin resistance in UNX mice and found that UNX mice exhibited insulin resistance with a significantly higher plasma insulin level after glucose loading (Fig. 5). These findings strongly suggest that insulin is one of the primary mediators to regulate PAT-specific AGT gene expression in UNX mice.

The effect of insulin on adipose cell AGT gene expression has been widely investigated; however, conflicting results have been reported. Harte et al. demonstrated that insulin stimulation of isolated human abdominal subcutaneous adipocytes upregulated AGT mRNA expression through a TNFα-mediated mechanism (15), whereas insulin-induced downregulation has been observed in cultured Ob1771 and 3T3-F442A adipose cells (2). We first examined the direct effect of insulin on AGT expression in mature adipocyte isolated from wild-type PAT; however, the AGT mRNA expression level was not affected by insulin stimulation (Fig. 6A). Tamura et al. (39) reported that AGT mRNA was barely detectable in preadipocyte, and the level of AGT mRNA dramatically increased during adipocyte differentiation. We therefore examined the effect of insulin on AGT mRNA expression, using a previously described in vitro culture system (29). Along with the increased expression of the PPARγ gene, AGT mRNA expression increased time dependently in the absence of insulin stimulation, which was remarkably exaggerated by insulin treatment. Considering that insulin stimulation was performed during the first 3 days, augmented AGT mRNA expression induced by insulin was largely attributed to differentiating adipocytes rather than mature adipocytes.

An adipose depot-specific increase in AGT gene expression has also been investigated in diet-induced obese animals, which often exhibit hyperinsulinemia due to insulin resistance (28). AGT mRNA expression level in liver was equivalent between sham control and UNX mice, suggesting that AGT gene expression in UNX mice is also regulated in a tissuespecific manner. Hainault et al. (14) examined the AGT gene expression levels in Zucker rats during the onset of obesity and found that AGT expression was significantly increased in adipose tissue, while the expression in liver was not changed. Lu et al. (21) also demonstrated that ANG II infusion into LDL receptor-deficient mice markedly increased AGT mRNA expression in adipose tissue but not in liver. Tamura et al. (39) reported that activation of the AGT promoter might be involved in the adipogenic differentiation-coupled gene expres-
tion. Although the precise mechanism of tissue-specific regulation of AGT gene expression has not been fully elucidated, AGT gene expression in adipose tissue is strongly implicated in nutritionally induced adipogenesis, which may be responsible for the adipose tissue-specific regulation of AGT gene expression.

Inflammatory response in visceral adipose tissue has been shown to contribute to the development of insulin resistance in obesity. However, UNX mice exhibited insulin resistance without any changes of inflammatory cytokines expression and/or macrophage infiltration in adipose tissue. Although the detailed mechanisms of insulin resistance in UNX mice remain undefined, perivascular adipose tissue-specific activation of the RAS is likely to exacerbate insulin resistance. Perivascular adipose tissue is found throughout the body and adipose tissue-derived hormones (adipokines) including ANG II could impair microvascular function in a paracrine manner (20), which affects insulin-mediated glucose disposal, thus contributing to the aggravation of insulin resistance (9). Although we did not examine the characteristics of perivascular adipose tissue around the microvessels in this study, it is likely that perivascular adipose tissue-specific activation of RAS induces microvascular dysfunction, thereby aggravating insulin resistance. Conversely, compensatory hyperinsulinemia could increase AGT gene expression in perivascular adipose tissue, leading to the activation of the RAS.

It is well known that ANG II is critically involved in the proinflammatory signaling pathway via AT1 receptor activation in a variety of cells. In particular, accumulation of proinflammatory leukocytes such as monocytes/macrophages plays a critical role in tissue inflammation in visceral adipose tissue. On the other hand, Fitzgibbons et al. (11) reported a depot-specific difference in response to proinflammatory stimuli. They compared the obesity-induced inflammatory response between thoracic perivascular adipose tissue and visceral white adipose tissue and found that thoracic perivascular adipose tissue had markedly lower expression of immune cell-enriched mRNAs such as F4/80 and CD68 compared with white adipose tissue. Furthermore, Padilla et al. (25) showed that mRNA expressions of inflammatory cytokines were markedly lower in thoracic perivascular adipose tissue than those in abdominal perivascular adipose tissue from male Wister rats. These findings strongly suggest that inflammatory response in adipose tissue is exerted in a depot-specific and region-specific manner, and thoracic perivascular adipose tissue is likely to be resistant to inflammatory stimuli.

In conclusion, this study demonstrated that the PAT-specific activation of RAS in uninephrectomized apoE−/− mice might be primarily involved in CKD-associated atherosclerosis. This depot-specific activation of RAS is likely induced by the increased expression of the AGT gene, at least in part through insulin-mediated stimulation of adipocyte differentiation. These findings provide insights into the role of the tissue RAS in the pathogenesis of CKD-associated atherosclerosis, and perivascular adipose tissue-specific RAS activation may represent a new therapeutic target to prevent cardiovascular diseases in patients with CKD.

**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

**AUTHOR CONTRIBUTIONS**


**REFERENCES**

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