Renin-Angiotensin System

Adipocyte Deficiency of Angiotensinogen Prevents Obesity-Induced Hypertension in Male Mice

Frederique Yiannikouris, Manisha Gupte, Kelly Putnam, Sean Thatcher, Richard Charmigno, Debra L. Rateri, Alan Daugherty, Lisa A. Cassis

See Editorial Commentary, pp 1389–1390

Abstract—Previous studies demonstrated that diet-induced obesity increased plasma angiotensin II concentrations and elevated systolic blood pressures in male mice. Adipocytes express angiotensinogen and secrete angiotensin peptides. We hypothesize that adipocyte-derived angiotensin II mediates obesity-induced increases in systolic blood pressure in male high fat-fed C57BL/6 mice. Systolic blood pressure was measured by radiotelemetry during week 16 of low-fat or high-fat feeding in Agtfl/fl and adipocyte angiotensinogen-deficient mice (AgtaP2). Adipocyte angiotensinogen deficiency had no effect on diet-induced obesity. Basal 24-hour systolic blood pressure was not different in low-fat-fed Agtfl/fl compared with AgtaP2 mice (124±3 versus 128±3 mm Hg, respectively). In Agtfl/fl mice, high-fat feeding significantly increased systolic blood pressure (24 hours; 134±2 mm Hg; \(P<0.05\)). In contrast, high-fat-fed AgtaP2 mice did not exhibit an increase in systolic blood pressure (126±2 mm Hg). Plasma angiotensin II concentrations were increased by high-fat feeding in Agtfl/fl mice (low fat, 32±14; high fat, 219±58 pg/mL; \(P<0.05\)). In contrast, high-fat-fed AgtaP2 mice did not exhibit elevated plasma angiotensin II concentrations (high fat, 18±7 pg/mL). Similarly, adipose tissue concentrations of angiotensin II were significantly decreased in low-fat and high-fat-fed AgtaP2 mice compared with controls. In conclusion, adipocyte angiotensinogen deficiency prevented high-fat-induced elevations in plasma angiotensin II concentrations and systolic blood pressure. These results suggest that adipose tissue serves as a major source of angiotensin II in the development of obesity hypertension. (Hypertension. 2012;60:1524–1530.) ● Online Data Supplement

Key Words: obesity ■ angiotensinogen ■ angiotensins ■ hypertension ■ adipose tissue

The prevalence of hypertension has surged over the last 10 years consistent with an increasing incidence of obesity. 1 In men, a rise in body mass index is the primary contributor to the increased prevalence of hypertension. 1 Factors that consistently link obesity to hypertension include increased sodium reabsorption and activity of the sympathetic nervous system and the renin-angiotensin system (RAS). 2–4

The RAS is activated in human and experimental obesity. 5–11 Previous studies demonstrated activation of the systemic RAS in experimental models of diet-induced obesity. 5,6,12,13 Obesity-prone rats fed a moderately high-fat (HF) diet exhibited increased plasma angiotensin II (Ang II) concentrations and elevated systolic blood pressures (SBPs). 5 Administration of an angiotensin type 1 receptor antagonist to obese rats normalized blood pressure. 12 In humans, administration of inhibitors of the RAS is an effective therapy for the treatment of obesity-related hypertension. 14 However, mechanisms for a stimulated RAS in the setting of obesity are not well defined.

Angiotensinogen (AGT) is the only known precursor to angiotensins 15,16 and the renin-angiotensin system (RAS). 2–4

The RAS is activated in human and experimental obesity. 5–11 Previous studies demonstrated activation of the systemic RAS in experimental models of diet-induced obesity. 5,6,12,13 Obesity-prone rats fed a moderately high-fat (HF) diet exhibited increased plasma angiotensin II (Ang II) concentrations and elevated systolic blood pressures (SBPs). 5 Administration of an angiotensin type 1 receptor antagonist to obese rats normalized blood pressure. 12 In humans, administration of inhibitors of the RAS is an effective therapy for the treatment of obesity-related hypertension. 14 However, mechanisms for a stimulated RAS in the setting of obesity are not well defined.

Angiotensinogen (AGT) is the only known precursor to Ang II. Early studies demonstrated a high level of AGT gene expression in rat adipose tissue. 15,16 Moreover, AGT gene expression increased when stem cells were differentiated to mature white adipocytes. 17,18 In addition to AGT, adipocytes express multiple components of the RAS required for the synthesis of Ang II 19,20 and release angiotensin peptides. 19,21,22

In mice with transgenic overexpression of AGT in adipocytes, plasma AGT concentrations increased and were associated with increased SBP. 23 Conversely, adipocyte-specific AGT deficiency reduced plasma AGT concentrations and SBP in male and female mice fed a standard diet. 23 These results suggest that adipocyte-derived AGT modulates the systemic RAS and blood pressure control.

Expression of AGT in adipose tissue has been reported to increase, 5,6,24–26 decrease, 11,29 or not change 30 in human or experimental obesity. Differences in AGT gene expression in obese adipose tissue may arise from the source of tissue, species, or whether subjects exhibit obesity-related disorders. When corrected for adipose tissue mass, secretion of AGT from adipose tissue correlated positively to body mass index and SBP in obese humans and in HF-fed mice. 30 Moreover,
hypertrophied adipocytes, as well as other inflammatory cell types in obese adipose tissue, express components of the RAS required for the conversion of AGT to Ang II. Therefore, an expanded adipose tissue mass may serve as a source of systemic Ang II with obesity. We hypothesized that adipocyte-derived AGT or Ang II contributes to activation of the systemic RAS and blood pressure control in HF-fed male mice. In this study, we quantified effects of adipocyte-specific AGT deficiency on the development of obesity, activation of the adipose and systemic RAS, and on obesity-induced increases in blood pressure.

Methods

Animals
C57BL/6 mice with loxP sites flanking exon 2 of the AGT gene (1672bp including exon 2), as described previously, were bred to transgenic male C57BL/6 mice expressing aP2-Cre recombinase. For all of the studies, Agtfl/fl littermate controls were used for comparison with mice with adipocyte AGT deficiency (Agt aP2). Diets, radiotelemetry monitoring of blood pressure, measurement of plasma and adipose parameters, gene expression, and statistical analyses are described in online-only Data Supplement. Carotid artery catheters and radiotelemeters were implanted into anesthetized (isoflurane) mice during week 15 of diet feeding. At study end point, mice were anesthetized with ketamine/xylazine (100/10 mg/kg, IP) for exsanguination and tissue harvest. All of the procedures involving animals were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the institutional animal care and use committee at the University of Kentucky.

Results

Adipocyte AGT Deficiency Had No Significant Effect on the Development of Diet-Induced Obesity

Genotyping demonstrated the presence of a 182-bp band in Agt aP2 mice (Figure S1A). AGT mRNA abundance was not significantly influenced by diet or genotype in liver or kidneys from Agtfl/fl compared with AgtaP2 mice (Figure S1B and S1C). In white adipose tissue, AGT mRNA abundance was modestly but not significantly increased in HF-fed AgtaP2 compared with low-fat (LF)-fed Agtfl/fl mice (Figure S1D). AGT mRNA abundance was significantly decreased in adipose tissue from Agt aP2 compared with Agtfl/fl mice (Figure S1D; P<0.05).

In LF-fed mice, AgtaP2 mice had increased body weights compared with Agtfl/fl mice starting at week 5 (Figure 1A; P<0.05). With HF feeding, body weight increased in both genotypes compared with LF-fed mice (Figure 1A; P<0.05). In addition, fat mass increased significantly in HF-fed mice of each genotype compared with LF-fed mice (Figure 1B; P<0.05). However, body weight (Figure 1A), lean, and fat mass (Figure 1C; P<0.05) were not significantly different in HF-fed AgtaP2 and Agtfl/fl mice. HF-fed AgtaP2 mice exhibited impaired glucose tolerance (Figure S2; P<0.05) compared with LF-fed mice, with no differences between genotypes.

Adipocyte AGT Deficiency Prevented Obesity-Induced Increases in SBP

In LF-fed mice, adipocyte AGT deficiency had no significant effect on SBP (Figure 2A; 24-hour; Figure S3), diastolic blood pressures, pulse pressures, physical activity, or heart rate (Table 1). Moreover, both genotypes of LF-fed mice exhibited significantly lower SBP during the day compared with night cycle (Figure S3; P<0.05). In HF-fed mice of both genotypes, heart rate increased significantly, whereas physical activity was significantly decreased (Table 1; P<0.05). In HF-fed Agtfl/fl mice, SBP (24-hour) was increased significantly compared with LF-fed mice (Figure 2A; P<0.05). In contrast, HF-fed AgtaP2 mice did not exhibit increased SBP compared with LF-fed mice of either genotype (Figure 2A).

In addition, SBP was significantly decreased in HF-fed AgtaP2 compared with HF-fed Agtfl/fl mice (24 hours; Figure 2A; P<0.05) during both the night and day cycles (Figure 2B; P<0.05).

Adipocyte AGT Deficiency Prevented Obesity-Induced Elevations in Plasma Ang II Concentrations

In LF-fed AgtaP2 mice, plasma AGT concentrations were not significantly reduced compared with LF-fed Agtfl/fl (Figure

Figure 1. Adipocyte angiotensigen (AGT) deficiency had no significant effect on obesity development. A, Weekly body weight in 16-week low-fat (LF) and high-fat (HF)-fed Agtfl/fl and AgtaP2 mice. Data are mean±SEM from n=15 to 17 (LF) or n=21 to 32 (HF) mice per group. *P<0.05 vs LF-fed Agtfl/fl. Lean mass (grams; B) and fat mass (grams; C) in LF- and HF-fed Agtfl/fl and AgtaP2 mice. Data are mean±SEM from n=5 mice per group (LF) and n=12 to 18 mice per group (HF). *P<0.05 vs LF within genotype.
Both genotypes of HF-fed mice exhibited a significant increase in plasma AGT concentrations compared with LF-fed controls (Figure 3A; P<0.05). Plasma Ang II concentrations were markedly increased in HF-fed Agtfl/fl compared with LF-fed Agtfl/fl mice (Figure 3B; P<0.05). In contrast, plasma Ang II concentrations were decreased in HF-fed AgtaP2 compared with HF-fed Agtfl/fl mice (Figure 3B; P<0.05) and were not significantly different from LF-fed controls of either genotype.

Because plasma AGT concentrations were increased to a similar extent in HF-fed mice of each genotype (Figure 3A), but Ang II concentrations differed markedly between genotypes (Figure 3B), we quantified plasma renin activity (PRA; angiotensin I generated by endogenous renin and AGT) and plasma renin concentration (PRC; angiotensin I generated by endogenous renin in the presence of exogenous AGT). PRA was not significantly different in LF- or HF-fed AgtaP2 compared with HF-fed Agtfl/fl mice (Figure 3B; P<0.05) and were not significantly different from LF-fed controls of either genotype.

To define mechanisms for marked reductions in plasma Ang II concentrations in HF-fed AgtaP2 mice, we quantified renin-like activity and Ang II concentrations in adipose tissue. Renin-like activity, as reflected by levels of angiotensin I released from adipose explants, was increased in HF-fed Agtfl/fl compared with HF-fed AgtaP2 mice (Figure S5; P<0.05). Concentrations of Ang II in adipose tissue (picograms per milligram of tissue) were reduced markedly in both LF- and HF-fed AgtaP2 compared with Agtfl/fl mice (Figure 5A; P<0.05). When Ang II concentrations in adipose tissue were normalized for total fat mass of mice, HF-fed Agtfl/fl mice exhibited a tendency toward increased total adipose Ang II content compared with LF-fed Agtfl/fl, which was significantly decreased in AgtaP2 mice (Figure 5B). In contrast to adipose, liver Ang II concentrations were at the limits of detection (<0.1 pg/mg of tissue) and were not influenced by genotype or diet (data not shown). To define mechanisms for differences in Ang II concentrations between adipose and liver, we quantified mRNA abundance of the prorenin receptor and angiotensin-converting enzyme (ACE). Adipose tissue from HF-fed mice of each genotype expressed relatively greater mRNA abundance for the prorenin receptor and ACE compared with liver (Figure 6A; P<0.05). Abundance of the prorenin receptor and ACE mRNA was modestly increased in adipose tissue from HF-fed Agtfl/fl mice compared with LF-fed Agtfl/fl controls (Figure 6B). Although renin gene expression was below detectable limits in adipose tissue, cathepsin D, and tonin, enzymes capable of processing AGT to angiotensin peptides were also expressed in adipose tissue of mice in each group (Figure S6).

Table 1. Characteristics of LF- and HF-Fed Agtfl/fl and AgtaP2 Mice

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<th>Agtfl/fl</th>
<th>AgtaP2</th>
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<td>116.6±1.</td>
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<td>Diastolic blood pressure, mm Hg</td>
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<td>100.2±1.6</td>
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<td>Pulse pressure, mm Hg</td>
<td>28.6±3.7</td>
<td>32.5±1.9</td>
<td>32.3±1.3</td>
<td>28.4±2.0</td>
</tr>
<tr>
<td>Activity, counts/min</td>
<td>8.7±1.3</td>
<td>6.5±0.9*</td>
<td>7.9±1.5</td>
<td>5.3±0.4*</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>575±7</td>
<td>589±6*</td>
<td>569±9</td>
<td>599±10*</td>
</tr>
</tbody>
</table>

Data are mean±SEM for 24-hour measurements from n=6 to 7 mice per group in LF and 9 mice per group in HF. HF indicates high fat; LF, low fat.

*P<0.05 vs LF within genotype.
Discussion

In this study, we investigated the role of adipocyte-derived AGT and Ang II in the development of obesity hypertension. A major finding is that adipocyte-specific deficiency of AGT prevented obesity-induced increases in SBP. Notably, adipocyte AGT deficiency also prevented obesity-induced increases in plasma Ang II concentrations, suggesting that adipose tissue is a significant contributor to systemic Ang II and blood pressure control. In support, adipose tissue Ang II concentrations tended to increase when normalized to total fat mass of obese mice and were reduced in HF-fed adipocyte AGT-deficient mice. In addition, adipose tissue expressed components (eg, ACE, prorenin receptor, cathepsin D, and tonin) that convert AGT to Ang II, whereas liver had relatively lower abundance of these processing enzymes and undetectable levels of Ang II. These results indicate that adipose tissue becomes a primary source of systemic Ang II and contributes to obesity-induced increases in SBP.

It is well accepted that the liver is the primary source of systemic AGT in rodents and humans. Whole body deficiency of AGT markedly decreased plasma AGT concentrations and blood pressure in mice. By comparison, adipocyte AGT deficiency in mice fed a standard diet reduced plasma AGT concentrations and SBP by 25%. In this study, Agt ap2 mice fed an LF diet did not exhibit significant reductions in plasma AGT concentrations and SBP and had slightly higher body weights compared with Agt fl/fl mice. These results suggest that differences in diet composition (LF versus standard mouse diet) may have influenced the body weight and blood pressure phenotype of adipocyte AGT deficiency. Results from this study demonstrate that plasma AGT concentrations were increased by obesity and were not influenced in adipocyte AGT-deficient mice. Thus, elevated systemic AGT concentrations in obese mice were not adipocyte derived and presumably came from liver. Moreover, modest elevations in AGT mRNA abundance in adipose tissue from HF-fed control mice, as demonstrated in this and previous studies, most likely did not contribute to increased plasma AGT concentrations in obese mice.

A surprising finding was that, despite elevations in plasma AGT concentrations in obese adipocyte AGT-deficient mice, plasma Ang II concentrations were markedly reduced. These results demonstrate dissociation of plasma AGT concentrations from plasma Ang II levels, suggesting that elevated plasma Ang II of obese Agt fl/fl mice was not derived from systemic AGT. In humans, although changes in systemic AGT concentrations can influence the systemic RAS, the high plasma AGT concentration in relation to the Michaelis constant for renin supports renin as the rate-limiting step in Ang II production. In contrast, previous investigators demonstrated that mice exhibit low PRA.

In this study, despite modest elevations in plasma AGT concentrations in obese mice, generation of angiotensin I from endogenous renin in plasma was not different between obese and lean mice. However, on addition of exogenous AGT, angiotensin I generation increased markedly, demonstrating that plasma AGT concentrations were rate limiting. However, obese mice could not effectively convert exogenous AGT to angiotensin I in plasma, suggesting obesity-induced alterations in renin. Mechanisms for obesity-induced reductions in active PRC are unclear. However, previous studies demonstrated that PRA was reduced in obese hypertensive but not normotensive women. Moreover, regional adiposity may influence renin, because central obesity was associated with increased PRA, whereas patients with peripheral obesity did not exhibit changes in renin activity. Interestingly, in HF-fed adipocyte AGT-deficient mice exhibiting low plasma Ang II concentrations, the total renin pool (including prorenin) increased in plasma. These results suggest that obese

Figure 4. Plasma renin activity (PRA; left y-axis) and plasma renin concentration (PRC; right y-axis) in low-fat (LF) and high-fat (HF)–fed mice of each genotype. Data are mean±SEM from n=8 to 20 mice per group. *P<0.05 vs LF within genotype.
adipocyte AGT-deficient mice respond to low systemic Ang II concentrations to increase prorenin production. Further studies are required to investigate changes in plasma renin with obesity. However, the rate-limiting nature of AGT in plasma of lean and obese mice, coupled with obesity-induced reductions in PRC, suggests that circulating concentrations of Ang II were not derived from blood-borne AGT.

In this study, reduced adipose tissue renin-like activity and Ang II concentrations paralleled reductions in plasma Ang II concentrations and SBP in HF-fed adipocyte AGT-deficient mice. These results suggest that adipose tissue converts AGT to Ang II, which is then released into the systemic circulation. In support, similar to previous findings of the prorenin receptor and ACE were modestly increased in adipose tissue. In addition, expression of several of these synthetic RAS components, as well as Ang II concentrations, were more abundant in adipose compared with liver. Conversion of AGT to angiotensin peptides through tonin or cathepsin may have contributed to increased conversion of AGT to Ang II in adipose tissue. In addition, expression of several of these synthetic RAS components may have contributed to increased Ang II–mediated regulation of sodium reabsorption and sympathetic nervous system activity contributed to the pronounced effects of adipocyte AGT deficiency to prevent obesity-associated hypertension.

In conclusion, results demonstrate that adipocyte-derived Ang II is a primary contributor to obesity-induced elevations in plasma Ang II concentrations and SBP. Reductions in plasma Ang II concentrations were paralleled by decreased adipose tissue Ang II concentrations in HF-fed adipocyte AGT-deficient mice. Adipose tissue expressed relatively greater abundance of Ang II synthetic processing enzymes and Ang II concentrations than liver. These results demonstrate that adipose tissue is a significant contributor to an activated systemic RAS in the development of obesity-associated hypertension.

Perspectives
Evidence supports activation of the RAS in obesity-related hypertension. Adipose tissue expresses several RAS components that may link obesity to hypertension. We demonstrate that adipocyte AGT deficiency prevents obesity-induced increases in plasma Ang II concentrations and SBP. Adipose tissue Ang II concentrations were markedly reduced in adipocyte AGT-deficient obese mice. Our results suggest that adipose-derived Ang II contributes to the systemic RAS and blood pressure control in obese mice.
mice. These results support the use of inhibitors of the RAS in the treatment of obesity hypertension. Moreover, interventions that could mitigate activation of the adipose RAS may provide novel areas of treatment for obesity-related hypertension.

Acknowledgments

We thank Eboni Lewis for skilled surgical implantation of radiotelemeters in mice, Michael Karounos for assistance with the development and maintenance of the breeding colony, and Victoria English for quantification of the RAS.

Sources of Funding

These studies were supported by the National Heart, Lung, and Blood Institute (HL73085, to Dr Cassis; HL62846, to Dr Daugherty; T32HL091812, to Dr Yiannikouris) and by the National Center for Research Resources (P20RR021954; to Dr Cassis).

Disclosures

None.

References


### Novelty and Significance

**What Is New?**
- Demonstration for the first time that adipocyte AGT deficiency reduces plasma Ang II concentrations and prevents the development of obesity-associated hypertension.
- Demonstration of dissociation of systemic concentrations of AGT from Ang II in the setting of obesity.

**What Is Relevant?**
- Because obesity accounts for almost the entire increased prevalence of hypertension in men in the United States, this is highly relevant to target novel therapies for hypertension.
- This study demonstrates that adipose tissue can be a major source of systemic Ang II in obesity-induced hypertension.

**Summary**
- Adipocyte AGT deficiency prevents obesity-induced increases in SBP.
- Adipocyte AGT deficiency markedly reduces plasma and adipose tissue Ang II concentrations in obese mice.
- The expanded fat mass from obesity becomes a significant source of systemic Ang II in the development of hypertension.
Adipocyte Deficiency of Angiotensinogen Prevents Obesity-Induced Hypertension in Male Mice

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Hypertension. 2012;60:1524-1530; originally published online October 29, 2012; doi: 10.1161/HYPERTENSIONAHA.112.192690

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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Adipocyte Deficiency of Angiotensinogen Prevents Obesity-Induced Hypertension in Male Mice

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University of Kentucky, Lexington, KY
Supplemental Materials and Methods.

**Experimental protocol.** Male mice (2 months of age) of each genotype were fed a LF (10% kcal as fat; D12450B; Research Diets Inc, New Brunswick, NJ) or HF diet (60% kcal as fat; D12492, Research Diets Inc, New Brunswick, NJ) for 16 weeks. Diets were matched in protein content (20% kcal) and provided energy at 3.85 or 5.25 kcal/gm (LF and HF, respectively). Diets were provided to mice *ad libitum*. Fat and lean mass were measured on conscious mice at study endpoint using NMR spectroscopy (Echo MRI®).

**Measurement of plasma and tissue AngII.** Plasma AngII concentrations were quantified in male mice fed a LF or HF diet as described previously. For tissue quantification of AngII, adipose tissue or liver (1:10 wt/vol) were homogenized on ice in buffer (0.1 N HCl, 80% ethanol, 50 mM EDTA, 0.1 mM o-phenanthroline, 0.1 mM pepstatin), centrifuged (20,000 g, 4°C for 20 min), and the supernatants stored at -20°C overnight. The next day, centrifugation was repeated, and supernatants were diluted with 0.1% orthophosphoric acid (0.1% wt/vol) and stored at 4°C for 6 hours. This process was repeated, and then samples were processed over C18 mini-columns. Eluted peptides were vacuum concentrated, and angiotensin peptides quantified by radioimmunoassay using an anti-rat AngII antibody as described previously. To quantify renin-like activity, explants of adipose tissue (10-15 mg) were incubated in buffer containing captopril (1 µM) and EDTA (0.05 M) for 4 hours. Angiotensin I released into the media was quantified by radioimmunoassay as described previously.

**Quantification of plasma renin, total renin, and AGT.** Plasma renin activity (PRA) was quantified indirectly by incubating mouse plasma (8 µl) at 37°C (30 min) in phosphate buffer containing EDTA (0.05 M) and enalapril (10 µM), followed by quantification of angiotensin I by radioimmunoassay as described previously. Plasma renin concentration (PRC) was quantified by incubating mouse plasma (8 µl) with exogenous AGT (25 nM) prepared from nephrectomized rats at 37°C (30 min) using the method described above. Plasma (10 µl) concentrations of AGT were quantified using a mouse total angiotensinogen assay kit (Immuno-Biological Laboratories Co, Gunma, Japan) as described previously. This kit does not distinguish intact AGT from des-asp AGT. Plasma total renin (renin+prorenin) was quantified using the rat Prorenin/Renin Total Antigen Assay (Molecular Innovation, MI, USA).

**Measurement of blood pressure.** Blood pressure was measured by radiotelemetry during week 16 according to methods described previously. Briefly, anesthetized (isoflurane) mice were implanted with carotid artery catheters and telemetry devices during week 15 of LF or HF feeding, allowed 1 week to recover, and then pressure was recorded continuously (5 minute sampling) for 3-5 days. Inclusion criteria were (1) pulse pressures > 20 mmHg, (2) standard deviation of the pulse pressure ≤ 9 mmHg and (3) the presence of diurnal differences in SBP between the night and light cycle.

**Measurement of glucose tolerance.** Mice were fasted for 6 hours before quantification of glucose tolerance. Blood glucose was quantified at 0 min before injection of glucose solution (2 mg/kg body weight, ip) and at 15, 30, 60 and 120 min
after injection as described previously.²⁴

**Tissue RNA extraction and quantitative RT-PCR.** Tissue RNA was quantified as described previously.²⁴ Estimation of amplified gene products was normalized to 18S RNA for liver and kidney and analyzed using the 2^ΔΔCt method. For adipose tissue (HF versus LF diet), amplified gene products were normalized to the average of IPO8 and FBXL10 mRNA abundance. The primer sequences are illustrated in Table S1.

**Statistical analysis.** Data are expressed as mean ± SEM. All data were analyzed using SigmaPlot. Ordinary and repeated measures ANOVAs were employed to compare diet and genotype groups on quantitative outcome measures, followed by post-hoc tests with Holm-Sidak adjustments for multiple comparisons. When the assumptions underlying the ANOVA’s were not otherwise met, we nonlinearly transformed the observations before running the ANOVA’s to obtain valid p-values; however, for ease of interpretation, the Figures are based on the untransformed observations. Figure 6 data were analyzed by three-way ANOVA on log-transformed responses, with the modification that mouse-specific random effects were included. Statistical significance was declared at P<0.05.
References


Table S1

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Figure S1. A. Genotyping of tails from LF and HF-fed Agt^{fl/fl} and AgtP2 mice. Primers were used to detect Cre+ (5'-ACCTGAAGATGTTCGCGATT and 5'-CGGCATCAACGTTTTCTTTT), IL-2 gene (5'-CTAGGCCACAGAATTGAAAGATCT and 5'-GTAGGTGGAAATTCTAGCATCATCC). Cre+ mice were identified by a product of 182bp. B. AGT mRNA abundance in liver; C. AGT mRNA abundance in kidney; D. AGT mRNA abundance in white adipose tissue. Data are mean ± SEM from n = 6-10 mice/group. *, P<0.05 compared to Agt^{fl/fl} within diet group.
Figure S2. Glucose tolerance tests in LF and HF-fed Agt<sup>fl/fl</sup> and Agt<sup>ap2</sup> mice. A, Blood glucose concentrations at several time points after administration of glucose (2 mg/kg body weight). B, Area under the curve (AUC) for data in A, above. Data are mean ± SEM from n = 7-10 mice/group. *, P<0.05 compared to LF within genotype.
Figure S3. SBP (12 hour averages for each mouse) of LF-fed $Agt^{fl/fl}$ and $Agt^{AP2}$ mice during the night and light cycle. Carotid artery catheters and radiotelemeters were implanted at week 15 of LF, mice were allowed one week to recover, and SBP was recorded for 3 - 5 days. Data are mean ± SEM from n = 6-7 mice/group (LF) over the 3 - 5 days of recording. *, P<0.05 compared to night cycle within genotype.
Figure S4. Total renin (renin+prorenin) in plasma of LF and HF-fed Agt<sup>fl/fl</sup> and Agt<sup>AP2</sup> mice. Data are mean ± SEM from n = 5-6 mice/group. *, P<0.05 compared to LF within genotype.
**Figure S5.** Renin-like activity, as evidenced by generation of angiotensin I, in explants of adipose tissue from LF and HF-fed Agt^{fl/fl} and Agt^{sp2} mice. Data are mean ± SEM from n = 3-4 mice/group. *, P<0.05 compared to LF within genotype. **, P<0.05 compared to Agt^{fl/fl} within diet group.
Figure S6. Cathepsin D (A) and tonin (B) mRNA abundance in adipose tissue from LF and HF-fed Agt\textsuperscript{fl/fl} and Agt\textsuperscript{P2P2} mice. Data are mean ± SEM from n = 5-7 mice/group. P = 0.06 genotype by diet interaction.