Adiponectin Replenishment Ameliorates Obesity-Related Hypertension


Abstract—Patients with obesity are susceptible to hypertension. We have reported that the plasma adiponectin levels are decreased in obesity and that adiponectin has many defensive properties against obesity-related diseases, such as type 2 diabetes and coronary artery disease. The aim of this study was to determine the relationship between adiponectin and hypertension in mice. We measured blood pressure and heart rate directly by a catheter in the carotid artery and indirectly by automatic sphygmomanometer at the tail artery. Obese KKAy mice had significantly lower plasma adiponectin levels and higher systolic blood pressure than control C57BL/6J mice at 21 weeks of age. Adenovirus-delivered adiponectin significantly decreased blood pressure in KKAy mice. The direct role of adiponectin on blood pressure regulation under insulin resistance–free state was investigated in adiponectin-knockout (KO) mice. Adiponectin KO mice developed hypertension when maintained on a high-salt diet (8% NaCl) without insulin resistance. The hypertension of salt-fed adiponectin KO mice was associated with reduced mRNA levels of endothelial NO synthase (eNOS) and prostaglandin I₂ synthase in aorta and low metabolite levels of endothelial NO synthase and prostaglandin I₂ synthase in plasma. Adiponectin therapy lowered the elevated blood pressure and corrected the above mRNA levels to those of the wild type. Our results suggest that hypoadiponectinemia contributes to the development of obesity-related hypertension, at least in part, directly, in addition to its effect via insulin resistance, and that adiponectin therapy can be potentially useful for hypertension in patients with the metabolic syndrome. (Hypertension. 2006;47:1108-1116.)

Key Words: hypertension, obesity ■ nitric oxide synthase ■ sodium, dietary ■ L-NAME

The cluster of hypertension, diabetes mellitus, and dyslipidemia in upper body obesity, collectively referred to as the metabolic syndrome, is a common cause of atherosclerotic cardiovascular diseases and one of the most serious threats to public health. Adipose tissue produces and secretes many bioactive substances,1–4 conceptualized as adipocytokines.5 Dysregulated production of adipocytokines, such as tumor necrosis factor-α, leptin, and plasminogen activator inhibitor type 1, is associated with the pathophysiology of obesity-related disorders.1–3

Adiponectin is an antiatherogenic4–8 and antidiabetic9–13 adipocytokine, identified by our group through the screening of adipose-specific genes in the human cDNA project.14 Other groups independently cloned the mouse homologue of adiponectin as ACRP30 and AdipoQ, respectively.15,16 Adiponectin is a plasma protein exclusively produced by adipose tissue,14 and the adiponectin gene is located on chromosome 3q27, which was reported to replicate linkage with the metabolic syndrome.21 Recently, we demonstrated that the I164T mutation of the adiponectin gene affects the prevalence of coronary artery disease and obesity-unrelated clustering of hypertension, diabetes mellitus, and dyslipidemia.22

Human studies of the vasodilator response to reactive hyperemia revealed that plasma adiponectin levels correlated significantly with endothelium-dependent vasodilation.23 Moreover, adiponectin treatment suppressed apoptosis by activating AMP-activated protein kinase, Akt kinase, and endothelial NO synthase (eNOS) signaling axis in cultured human endothelial cells.24,25 These data suggest that adiponectin is a protective factor against endothelial injury and that low production of adiponectin might relate to the pathophysiology of hypertension.

We reported previously that the adiponectin-knockout (KO) mice exhibited obesity, insulin resistance, and hypertension when fed a high-fat/high-sucrose/high-salt diet for 4 weeks.23 In clinical studies, obesity, hypoadiponectinemia, insulin resistance, and hypertension are closely associated with one another in the metabolic syndrome.11,19,20,26–28
Based on this background, it is important to define the direct relationship between hypoadiponectinemia and hypertension. The results of the present study showed that KKAy mice exhibited hypoadiponectinemia. KKAy mice develop maturity-onset obesity through the antagonism of the hypothalamic melanocortin system by ectopic expression of the agouti protein. The agouti and agouti-related protein compete with proopiomelanocortin-derived peptides for binding sites on melanocortin receptors to regulate food intake and energy expenditure. Furthermore, numerous studies have demonstrated that KKAy mice are good models of the metabolic syndrome, such as hypertension and diabetes mellitus. In the present study, we showed for the first time that adiponectin replenishment improved the hypertension of KKAy mice.

In addition, we induced hypertension in adiponectin KO mice by providing a high-salt diet without affecting insulin resistance. Therefore, we advance the concept that obesity-related hypoadiponectinemia contributes to the development of hypertension both directly and indirectly via insulin resistance. Our results also suggest that adiponectin therapy is potentially useful for patients with the metabolic syndrome, especially those with hypertension and insulin resistance.

Methods

Animal and Animal Treatment

KKAy male mice were purchased from Japan CLEA (Tokyo, Japan). This strain is a cross between black KK female mice and obese yellow male Ay mice, features a deregulated overexpression of the agouti gene, and exhibits severe obesity, hyperlipidemia, and insulin resistance. Adiponectin-KO (APN-KO) mice were generated as described previously and backcrossed to wild-type (WT) C57BL/6J. In the present study, we showed for the first time that adiponectin replenishment improved the hypertension of KKAy mice.

In addition, we induced hypertension in adiponectin KO mice by providing a high-salt diet without affecting insulin resistance. Therefore, we advance the concept that obesity-related hypoadiponectinemia contributes to the development of hypertension both directly and indirectly via insulin resistance. Our results also suggest that adiponectin therapy is potentially useful for patients with the metabolic syndrome, especially those with hypertension and insulin resistance.

Blood Pressure Measurement

Systolic blood pressure (SBP) and heart rate (HR) were measured using either the tail-cuff technique with an automatic sphygmonanometer (BP98A; Softron) at the tail artery while the animals were restrained or by using indwelling arterial catheters into the carotid artery. Mice were trained to the tail-cuff apparatus at least twice. Ten readings were taken for each measurement, and a mean value was assigned to each individual mouse. The direct blood pressure measurements were achieved using a 1.4F catheter tip micromanometer (ARIA, Millar Instruments) inserted through the right carotid artery. Mice were anesthetized with isoflurane and placed on a temperature-controlled pad. Blood pressure was measured after a 30-minute stabilization period. The blood pressure was monitored for 15 minutes under restrained conditions, and then the average value of SBP was calculated and determined. The SBP levels measured by the tail-cuff method correlated well with those by the direct measurement through carotid artery catheter as reported previously.

Laboratory Methods

Blood samples were collected from mice in the fasting (12 hours) state. Serum total cholesterol, triglyceride, and glucose concentrations were measured with enzymatic kits (Wako Pure Chemicals), and insulin concentrations were assayed with an enzyme immunoassay kit (Glazyme, Wako Pure Chemicals). Adiponectin concentrations were determined with ACRP30 ELISA kits (Otsuka Pharmaceutical Co). Nitrate/nitrite concentrations were measured with a Nitrate/Nitrite Colorimetric Assay kit (Cayman Chemical Company) or with a Nitrate/Nitrite Fluorometric assay kit (Cayman Chemical Company). 6-Keto-PGF1α concentrations were measured with a 6-keto-PGF1α EIA kit (Cayman Chemical Company). Plasma levels of angiotensin II; aldosterone; and urinary concentrations of epinephrine, norepinephrine, and dopamine were measured by using appropriate biochemical methods in a commercial laboratory (SRL).

Gene Expression Analysis

Total RNA was extracted using an RNA-STAT kit (TEL-TEST) according to the protocol supplied by the manufacturer, and 0.5 μg RNA was reverse transcribed using a ThermoScript RT-PCR system (Invitrogen). Real-time PCR was performed on ABI-Prism 7700 using SYBR Green I as a double-stranded DNA–specific dye according to instructions provided by the manufacturer (Applied Biosystems). We used the primers listed in the online supplement (available at http://hyper.ahajournals.org). All of the results were normalized to 36B4.

Immunoblot

The protein was extracted from the thoracic aortas of adiponectin KO and WT mice and solubilized with solubilization buffer (1% Triton X-100, 50 mMol/L HEPES (pH 7.5), 150 mMol/L NaCl, 10% glycerol, 1.5 mMol/L MgCl2, 10 mMol/L NaF, 10 mMol/L sodium diphosphate decahydrate, 1% aprotinin, 5 μg/mL leupeptin, 1 mMol/L PMSF, and 1 mMol/L dithiothreitol). Whole cell lysates were resolved on 10% SDS-polyacrylamide gels, followed by electrophoretic transfer to nitrocellulose membranes (Amersham Life Science). The membranes were exposed to mouse monoclonal anti-eNOS antibodies (Transduction Laboratories, San Jose, CA) and then exposed to anti-mouse secondary antibodies conjugated with horseradish peroxidase. The bands were visualized by an enhanced chemiluminescence detection system (Amersham) and quantified by using National Institutes of Health Image analysis freeware. Band volume was determined as band intensity per area according to the instructions provided by the manufacturer.

Preparation and Delivery of Adenoviral Adiponectin

Adenovirus producing the full-length adiponectin was constructed with Adenovirus Expression Vector kit (TaKaRa). Plaque-forming units (2×109) of adenovirus-adiponectin (Ad-APN) or adenovirus β-galactosidase (Ad-β gal) were injected intravenously via the tail vein. Adenovirus-mediated adiponectin expression was detected exclusively in the liver using the RT-PCR method, indicating that the effect of adiponectin on other organs, including the arterial wall, was mediated by the blood stream.

Statistical Methods

Data are presented as mean±SEM. Differences between groups were evaluated by the Student t test or ANOVA with Fisher’s protected least significant difference test. A P<0.05 denoted the presence of a statistically significant difference. All of the calculations were performed by using a standard statistical package (StatView for Macintosh, version 5.0).

Results

Adiponectin Supplementation Decreases Blood Pressure in Obese Diabetic KKAy Mice

We studied genetically obese KKAy mice, which develop a maturity-onset obesity, type 2 diabetes, and hypertension. In the present study, the SBP of KKAy mice gradually increased after 13 weeks of age (13 weeks, 114±1.6; 17 weeks, 118±1.3; 21 weeks, 123±1.8; 23 weeks, 131±2.8 mm Hg). The
SBP of KKAy mice was significantly higher than that of WT C57BL/6J mice at 21 weeks (123±1.8 versus 106±1.8 mm Hg; P<0.01) under normal diet. Plasma adiponectin concentrations of KKAy mice (9.3±0.4 μg/mL) were approximately half of those of WT mice (17.8±1.3 μg/mL). Before Ad-APN treatment, plasma adiponectin concentrations were 9.1±0.8 μg/mL in the KKAy/Ad-APN group and 9.5±0.5 μg/mL in the KKAy/Ad-β gal group. On day 11 after injection of Ad-APN, plasma adiponectin concentrations were 56.8±5.0 μg/mL in KKAy/Ad-APN and 9.8±1.0 μg/mL in KKAy/Ad-β gal. Ad-APN treatment significantly reduced SBP compared with Ad-β gal control on days 7, 9, and 11 postinjection (119±2.5 versus 131±2.8 mm Hg; P<0.01; Figure 1a). Direct blood pressure measurement also showed that SBP was significantly lower in the KKAy/Ad-APN group than in the KKAy/Ad-β gal group on day 11 postinjection (106±1.6 versus 122±2.6 mm Hg; P<0.01; Figure 1b). The HR (670±15.9 bpm versus 687±17.8 bpm; P value not significant; Figure 1c), body weight (41.4±0.7 g versus 41.1±0.5 g; P value not significant; Figure 1d), food intake (6.0±0.8 versus 5.9±0.6 g per day; P value not significant), fasting plasma glucose (FPG), fasting immunoreactive insulin (IRI), total cholesterol, triglyceride, angiotensin II, aldosterone, and leptin concentrations were not different between KKAy/Ad-APN and KKAy/Ad-β gal during the observation period (Table 1). The plasma concentrations of nitrate/nitrite (NO metabolites) of Ad-APN–treated KKAy mice (17.4±1.8 μmol/L) were significantly higher than those of Ad-β gal–treated KKAy mice (11.1±1.5 μmol/L; P<0.05; Figure 1e). The eNOS mRNA levels in aorta of KKAy/Ad-β gal (●) and KKAy/Ad-APN (○). Results are mean±SEM.

Adiponectin KO Mice Develop Salt-Induced Hypertension Without Insulin Resistance

To investigate the direct role of adiponectin on blood pressure regulation in the absence of insulin resistance, we also studied adiponectin KO mice. At the 3-week feeding of high-salt diet, SBP was significantly higher in KO mice than in WT mice.

TABLE 1. Characteristics of Ad-β Gal- and Ad-APN–Treated KKAy Mice

<table>
<thead>
<tr>
<th>Variables</th>
<th>Ad-β Gal (n=9)</th>
<th>Ad-APN (n=9)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adiponectin, μg/mL</td>
<td>9.5±0.5</td>
<td>9.1±0.8</td>
<td>NS</td>
</tr>
<tr>
<td>FPG, mmol/L</td>
<td>10.35±0.82</td>
<td>11.16±0.82</td>
<td>NS</td>
</tr>
<tr>
<td>IRI, μU/mL</td>
<td>392.8±88.9</td>
<td>352.3±38.8</td>
<td>NS</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>183.0±20.1</td>
<td>197.3±17.1</td>
<td>NS</td>
</tr>
<tr>
<td>T-chol, mmol/L</td>
<td>3.53±0.28</td>
<td>3.36±0.17</td>
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<td>Triglyceride, mmol/L</td>
<td>1.55±0.16</td>
<td>1.67±0.15</td>
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<tr>
<td>Angiotensin II, pg/mL</td>
<td>172.7±83.5</td>
<td>174.0±75.2</td>
<td>NS</td>
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<tr>
<td>Aldosterone, pg/mL</td>
<td>488.8±105.0</td>
<td>412.7±147.1</td>
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<tr>
<td>Leptin, ng/mL</td>
<td>34.88±2.81</td>
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HOMA-IR indicates homeostasis model assessment of insulin resistance; T-chol, total cholesterol; NS, not significant. Data are mean±SEM.
(126±3.1 versus 103±1.1 mm Hg; P<0.01; Figure 2a). The direct blood pressure measurement by indwelling catheters also showed that SBP was significantly higher in KO mice (118±1.2 mm Hg) than in WT mice (103.0±1.7 mm Hg) at the 3-week feeding of high-salt diet (P<0.01; Figure 2b). The HR (766±6.2 bpm versus 766±4.7 bpm; P value not significant) and body weight (30.4±0.5 g versus 29.5±0.7 g; P value not significant), FPG (5.74±0.17 mmol/L versus 5.48±0.11 mmol/L; P value not significant) and IRI (4.9±1.5 μU/mL versus 7.5±1.8 μU/mL; P value not significant) were not different between KO and WT mice during the observation period (Figure 2c and 2d).

Characterization of Adiponectin KO Mice and WT Mice
There were no significant differences in plasma Na, Cl, K, FPG, IRI, homeostasis model assessment of insulin resistance, total cholesterol, triglyceride, angiotensin II, aldosterone, and leptin concentrations, as well as in urinary volume, total urinary epinephrine, norepinephrine, and dopamine concentrations between KO and WT mice (Table 2). In addition, there were no significant differences in insulin-mediated suppression of plasma glucose between adiponectin KO and WT mice (Figure 2e).

To determine the mechanism of hypertension in KO mice, we examined the mRNA levels encoding proteins associated with hypertension. After salt overload, the mRNA levels of eNOS and prostaglandin (PG) I₂ synthase (PGIS) in aorta and eNOS in kidney were significantly lower in KO mice than in WT mice, although no significant differences were observed in the mRNA levels of inducible nitric oxide synthase, PG E synthase, endothelin-1, and adrenomedullin in aorta and renin and epithelial sodium channel in kidney between KO mice and WT mice (Figure 3a and 3b). The plasma concentrations of nitrate/nitrite as NO metabolites tended to be lower in KO mice (7.3±1.5 μmol/L) than in WT mice (10.9±1.6 μmol/L) after salt overload, but the difference was not statistically significant (P=0.09; Figure 3b). The plasma level of 6-keto-

<table>
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<th>Variables</th>
<th>WT (n=6)</th>
<th>KO (n=6)</th>
<th>P</th>
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<td>Plasma</td>
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<tr>
<td>Adiponectin, μg/mL</td>
<td>15.4±0.8</td>
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<td>Na, mEq/L</td>
<td>151.3±1.3</td>
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<td>Cl, mEq/L</td>
<td>114.3±0.3</td>
<td>112.3±0.8</td>
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<td>K, mEq/L</td>
<td>5.5±0.3</td>
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<td>FPG, mmol/L</td>
<td>5.48±0.11</td>
<td>5.74±0.17</td>
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<tr>
<td>IRI, μU/mL</td>
<td>7.5±1.8</td>
<td>4.9±1.5</td>
<td>NS</td>
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<tr>
<td>HOMA-IR</td>
<td>1.8±0.5</td>
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<td>NS</td>
</tr>
<tr>
<td>T-chol, mmol/L</td>
<td>2.08±0.23</td>
<td>2.07±0.13</td>
<td>NS</td>
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<tr>
<td>Triglyceride, mmol/L</td>
<td>0.89±0.12</td>
<td>0.93±0.06</td>
<td>NS</td>
</tr>
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<td>Angiotensin II, pg/mL</td>
<td>46.0±8.4</td>
<td>52.3±8.5</td>
<td>NS</td>
</tr>
<tr>
<td>Aldosterone, pg/mL</td>
<td>99.2±10.0</td>
<td>108.7±4.5</td>
<td>NS</td>
</tr>
<tr>
<td>Leptin, ng/mL</td>
<td>0.87±0.17</td>
<td>0.81±0.12</td>
<td>NS</td>
</tr>
<tr>
<td>Urine</td>
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<tr>
<td>Urine volume, mL/d</td>
<td>8.3±0.9</td>
<td>8.4±1.0</td>
<td>NS</td>
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<tr>
<td>Epinephrine, ng/d</td>
<td>32.2±3.2</td>
<td>28.9±4.2</td>
<td>NS</td>
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<tr>
<td>Norepinephrine, ng/d</td>
<td>347±14.9</td>
<td>314±29.9</td>
<td>NS</td>
</tr>
<tr>
<td>Dopamine, ng/d</td>
<td>1272±128.4</td>
<td>968±132.3</td>
<td>NS</td>
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</table>

HOMA-IR indicates homeostasis model assessment of insulin resistance; T-chol, total cholesterol; ND, not detected; NS, not significant. Data are mean±SEM.
Figure 3. Impaired production of NO and PG I₂ in adiponectin KO mice in response to salt overload. (a) mRNA levels of eNOS, PGIS, inducible NO synthase (iNOS), PG E synthase (PGES), endothelin-1 (ET-1), and adrenomedullin (AM) in aorta of WT (□ n=6) and APN-KO ( ■ n=6) mice. (b) mRNA levels of eNOS, renin, and epithelial sodium channel (ENaC) in kidney of WT (□ n=6) and APN-KO ( ■ n=6) mice. (c) Plasma levels of nitrate/nitrite and 6-keto-PGF1α in WT (□ n=8) and APN-KO ( ■ n=8) mice. (d) Protein levels of eNOS in aorta of WT (□ n=6) and APN-KO ( ■ n=6) mice. Results are mean ± SEM.
PGF1α, representing a PG I2 metabolite, was significantly lower in KO mice (1.08 ± 0.09 ng/mL) than in WT mice (1.85 ± 0.31 ng/mL; P < 0.05; Figure 3c). The protein levels of eNOS in aortas were significantly lower in KO mice than in WT mice (n = 6 in each group; Figure 3d). The mRNA levels of angiotensinogen and leptin in white adipose tissue and angiotensinogen in liver were not different between WT and KO mice (data not shown).

**Adiponectin Adenovirus Ameliorates High-Salt–Induced Hypertension and Modulates eNOS and PGIS mRNA Levels in Aorta of KO Mice**

To determine the effect of exogenous adiponectin replenishment, KO and WT mice were treated with Ad-APN or Ad-β gal. After 2 weeks on a high-salt diet, Ad-APN or Ad-β gal was injected intravenously via the tail vein. SBP was measured on days 2, 4, and 6 after injection. On day 7 after injection, adiponectin levels were 10.2 ± 0.7 μg/mL in KO/Ad-APN, not detectable in KO/Ad-β gal, 24.3 ± 0.8 μg/mL in WT/Ad-APN, and 15.8 ± 0.6 μg/mL in WT/Ad-β gal. Ad-APN treatment significantly decreased SBP compared with Ad-β gal control in KO mice on day 6 postinjection (108 ± 1.9 versus 120 ± 1.7 mm Hg; P < 0.01; Figure 4a), whereas no effects were observed in WT mice under high-salt diet (106 ± 3.3 versus 107 ± 1.9 mm Hg; P value not significant; Figure 4b). In addition, the hypotensive effect of Ad-APN for elevated blood pressure in KO mice was confirmed by direct SBP measurement using indwelling catheters on day 6 after injection (104 ± 1.5 versus 120 ± 2.5 mm Hg; P < 0.01; Figure 4c). Ad-APN–treated KO mice showed significantly higher eNOS and PGIS mRNA levels in aorta than Ad-β gal–treated KO mice (eNOS; 0.80 ± 0.15 versus 0.31 ± 0.04; P < 0.05; PGIS; 0.84 ± 0.27 versus 0.26 ± 0.10; P < 0.05). On the other hand, there were no differences in eNOS and PGIS mRNA levels between Ad-APN– and Ad-β gal–treated WT mice (eNOS; 1.03 ± 0.09 versus 1.00 ± 0.16; P value not significant; PGIS; 1.03 ± 0.14 versus 1.00 ± 0.16; P value not significant; Figure 4d and 4e).

**L-NAME Has No Effect on SBP in Adiponectin KO Mice Under High-Salt Diet**

To determine the in vivo effects of eNOS inhibition, we next studied the effects of L-NAME on SBP in KO and WT mice.
under high-salt diet. One-week administration of L-NAME resulted in a significant rise of SBP in WT mice (130±2.7 versus 103±1.1 mm Hg; \( P < 0.01 \)) but did not affect the SBP of adiponectin KO mice (131±3.3 versus 126±3.1 mm Hg; \( P \) value not significant; Figure 5a and 5b). To determine whether the salt-fed KO mice developed hypertension through impaired eNOS pathway, KO mice were treated with Ad-APN or Ad-\( \beta \)-gal under L-NAME or plain water administration after 2 weeks on a high-salt or normal-salt diet. Plasma adiponectin levels were 25.1±15.5 \( \mu \)g/mL in Ad-APN and not detectable in Ad-\( \beta \)-gal. On a normal-salt diet, the SBP of KO mice was similar to that of WT mice, and no difference was observed between Ad-APN and Ad-\( \beta \)-gal treatment (102±0.3 versus 102±0.3 mm Hg; \( P \) value not significant; Figure 5c). On high-salt diet, the SBP of KO mice was similar to that of L-NAME-treated WT mice (126±3.1 versus 128±1.7 mm Hg; \( P \) value not significant) and Ad-APN treatment significantly decreased SBP in KO mice compared with Ad-\( \beta \)-gal treatment (108±1.9 versus 122±1.5 mm Hg; \( P < 0.01 \)). The effect of Ad-APN in KO mice was abolished under L-NAME administration (126±2.7 versus 127±3.3 mm Hg; \( P \) value not significant Figure 5c).

Discussion

The major findings of the present study were the following: (1) adiponectin supplementation reduced the SBP of spontaneously hypertensive obese KKA\( y \) mice accompanied by increased levels of plasma NO metabolites; (2) salt-fed adiponectin KO mice developed hypertension, independent of obesity and insulin resistance, accompanied by reduced mRNA levels of eNOS and PGIS in aorta and eNOS in kidney and lower levels of eNOS and PGIS metabolites in plasma than salt-fed WT mice; (3) adenoviral delivery of adiponectin improved the hypertension and reversed the reduced mRNA levels of eNOS and PGIS in aorta of salt-fed KO mice; and (4) L-NAME–induced elevation of blood pressure was not observed in KO mice.

Obesity confers a higher risk of hypertension.\textsuperscript{26–28} Recently, numerous reports have demonstrated that dysregulated production of adipocytokines is involved in the pathophysiology of obesity-related disorders.\textsuperscript{1–3} The adipocytokine adiponectin has antiatherosclerotic and antidiabetic properties, and the plasma adiponectin levels are significantly low in obese patients, especially those with visceral fat accumulation.\textsuperscript{19} Accumulating evidence suggests that visceral fat obesity
is precedent and causative of hypertension and cardiovascular disease in metabolic syndrome. In our recent analysis, hypoadiponectinemia was an independent risk factor of hypertension in human subjects, independent of obesity and insulin resistance. In addition, we reported recently that subjects with I164T mutation of adiponectin gene, who exhibited remarkable hypoadiponectinemia, had higher prevalence of coronary artery disease and hypertension unrelated to obesity. These findings suggest that hypoadiponectinemia contributes directly to the development of hypertension in humans. Next, we further investigated the role of adiponectin on blood pressure in the mouse model. Adiponectin KO mice did not display the phenotype of the metabolic syndrome under normal diet. On high-fat/high-sucrose diet, however, the KO mice developed severe insulin resistance. In addition, the KO mice showed delayed clearance of free fatty acid in plasma and low levels of fatty-acid transport protein 1 mRNA in muscle, although no differences were observed in total cholesterol levels and triglyceride levels in plasma.

On high-fat/high-sucrose/high-salt diet, the KO mice developed hypertension and diabetes mellitus with impaired acetylcholine-induced vasorelaxation of aortic rings. In the present study, we showed that obese KKAy mice had hypoadiponectinemia and that adiponectin supplementation ameliorated the hypertension in these mice. In addition, adiponectin KO mice developed hypertension without insulin resistance when maintained on a high-salt diet. These results suggest that hypoadiponectinemia, per se, is not sufficient for the development of hypertension but contributes to its development under insulin resistance and/or salt overload, although further studies are necessary to determine the blood pressure response to various doses of adiponectin.

In vascular endothelial cells, we have reported that adiponectin promoted the phosphorylation of AMP-activated protein kinase, protein kinase Akt/protein kinase B, and eNOS and that the adiponectin-AMP-activated protein kinase-Akt-eNOS signal was essential for the antiapoptotic and angiogenic effects. It has been reported that some polymorphism of the human PGIS gene was an independent risk for systolic hypertension and that PG I2-deficient mice developed hypertension with the thickening of arterial walls. Furthermore, an interaction between NO and PG pathways has been reported. In this study, we demonstrated that adiponectin KO mice exhibited salt-induced hypertension accompanied by reduced mRNA levels of eNOS and PGIS in aorta and eNOS in kidney. In addition, adenoviral delivery of adiponectin improved the salt-induced hypertension and reversed the reduced mRNA levels of eNOS and PGIS in aorta of KO mice. In addition, t-NAMe had no effect on SBP in adiponectin KO mice under a high-salt diet. On the other hand, there were no significant differences in plasma Na, Cl, K, angiotensin II, aldosterone, and leptin concentrations, in total urinary catecholamines, and in the mRNA levels of angiotensinogen and leptin in white adipose tissue, angiotensinogen in liver, and renin and epithelial sodium channel in kidney between salt-fed KO and WT mice, although it is possible that other mechanisms are involved in the development of hypertension. Thus, the present study suggests that the impaired adiponectin–eNOS–PGIS pathway in the systemic vasculature might be, at least in part, associated with the hypertension of salt-fed adiponectin KO mice, although further studies are necessary to elucidate the precise mechanism.

In conclusion, we demonstrated in the present study that adiponectin supplementation reduced blood pressure both in obese KKAy mice and salt-fed adiponectin KO mice without affecting the insulin-resistant state. Both KKAy mice and salt-fed adiponectin KO mice developed hypertension accompanied by reduced mRNA levels of eNOS in aorta and kidney and low NO metabolite levels in plasma. These results suggest that hypoadiponectinemia contributes to the development of obesity-related hypertension via a direct effect on vasculature, in addition to its effect on insulin resistance, and that adiponectin supplementation is a potentially useful therapeutic modality for hypertension, as well as insulin resistance in the metabolic syndrome.

Perspectives

Obesity is closely associated with hypertension. The mechanisms underlying hypertension in obesity, however, have not been fully clarified. Adiponectin has many defensive properties against obesity-related diseases, such as type 2 diabetes and coronary artery disease. In the present study, we demonstrated for the first time that adiponectin treatment lowers obesity-related hypertension. Therefore, adiponectin treatment is potentially useful for hypertension and may help in establishing a unified approach for the treatment of the metabolic syndrome.

Acknowledgments

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References


Adiponectin Replenishment Ameliorates Obesity-Related Hypertension
Koji Ohashi, Shinji Kihara, Noriyuki Ouchi, Masahiro Kumada, Koichi Fujita, Aki Hiuge, Toshiyuki Hibuse, Miwa Ryo, Hitoshi Nishizawa, Norikazu Maeda, Kazuhisa Maeda, Rei Shibata, Kenneth Walsh, Tohru Funahashi and Iichiro Shimomura

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In a Hypertension article by K Ohashi et al (Ohashi K, Kihra S, Ouchi N, Kumada M, Fujita K, Hiuge A, Hibuse T, Ryo M, Nishizawa H, Maeda N, Shibata R, Walsh K, Funahashi T, Shimomura I. Adiponectin replenishment ameliorates obesity-related hypertension. Hypertension 2006;47:1108–1116), there was a mistake in describing plasma adiponectin levels before adenovirus injection in Table 1. On day 11 post-injection of Ad-APN, plasma adiponectin concentrations were 56.8±5.0 μg/mL in KKAy/Ad-APN and 9.8±1.0 μg/mL in KKAy/Ad-β gal, as mentioned in the text. As a matter of course, the plasma adiponectin levels were significantly higher in KKAy/Ad-APN than in KKAy/Ad-β gal. Therefore, the correct Table 1 is shown here. The authors regret the error.

### TABLE 1. Characterization of Ad-β gal- and Ad-APN–Treated KKAy Mice. Plasma Concentrations of Adiponectin, Fasting Plasma Glucose (FPG), Insulin RI (IRI), HOMA-IR, Total Cholesterol (T-chol), Triglyceride (TG), Angiotensin II, Aldosterone, and Leptin Concentrations in Ad-β gal- and Ad-APN-Treated KKAy Mice

<table>
<thead>
<tr>
<th>Variables</th>
<th>Ad-β gal (n=9)</th>
<th>Ad-APN (n=9)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adiponectin, μg/mL</td>
<td>9.8±1.0</td>
<td>56.8±5.0</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>FPG, mmol/L</td>
<td>10.35±0.82</td>
<td>11.16±0.82</td>
<td>NS</td>
</tr>
<tr>
<td>IRI, μU/mL</td>
<td>392.8±88.9</td>
<td>352.3±38.8</td>
<td>NS</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>183.0±20.1</td>
<td>197.3±17.1</td>
<td>NS</td>
</tr>
<tr>
<td>T-chol, mmol/L</td>
<td>3.53±0.28</td>
<td>3.36±0.17</td>
<td>NS</td>
</tr>
<tr>
<td>Triglyceride, mmol/L</td>
<td>1.55±0.16</td>
<td>1.67±0.15</td>
<td>NS</td>
</tr>
<tr>
<td>Angiotensin II, pg/mL</td>
<td>172.7±83.5</td>
<td>174.0±75.2</td>
<td>NS</td>
</tr>
<tr>
<td>Aldosterone, pg/mL</td>
<td>488.8±105.0</td>
<td>412.7±147.1</td>
<td>NS</td>
</tr>
<tr>
<td>Leptin, ng/mL</td>
<td>34.88±2.81</td>
<td>31.08±2.76</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS, not significant. Data are mean±SEM.