Mineralocorticoid Receptor Blockade Reverses Obesity-Related Changes in Expression of Adiponectin, Peroxisome Proliferator-Activated Receptor-γ, and Proinflammatory Adipokines

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Background—In obesity, decreases in adiponectin and increases in proinflammatory adipokines are associated with heart disease. Because adipocytes express mineralocorticoid receptor (MR) and MR blockade reduces cardiovascular inflammation and injury, we tested the hypothesis that MR blockade reduces inflammation and expression of proinflammatory cytokines in adipose tissue and increases adiponectin expression in adipose tissue and hearts of obese mice.

Methods and Results—We determined the effect of MR blockade (eplerenone, 100 mg/kg per day for 16 weeks) on gene expression in retroperitoneal adipose and heart tissue from obese, diabetic db/db mice (n=8) compared with untreated obese, diabetic db/db mice (n=10) and lean, nondiabetic db/+ littermates (n=11). Expression of tumor necrosis factor-α, monocyte chemoattractant protein-1, plasminogen activator inhibitor type 1, and macrophage protein CD68 increased, and expression of adiponectin and peroxisome proliferator-activated receptor-γ decreased in retroperitoneal adipose tissue from obese versus lean mice. In addition, adiponectin expression in heart was reduced in obese versus lean mice. MR blockade prevented these obesity-related changes in gene expression. Furthermore, treatment of undifferentiated preadipocytes with aldosterone (10^-8 mol/L for 24 hours) increased mRNA levels of tumor necrosis factor-α and monocyte chemoattractant protein-1 and reduced mRNA and protein levels of peroxisome proliferator-activated receptor-γ and adiponectin, supporting a direct aldosterone effect on gene expression.

Conclusions—MR blockade reduced expression of proinflammatory and prothrombotic factors in adipose tissue and increased expression of adiponectin in heart and adipose tissue of obese, diabetic mice. These effects on adiponectin and adipokine gene expression may represent a novel mechanism for the cardioprotective effects of MR blockade. (Circulation. 2008;117:2253-2261.)

Key Words: adipose tissue • aldosterone • aldosterone antagonists • diabetes mellitus • inflammation • mineralocorticoid receptor • obesity

Obesity is a proinflammatory state characterized by adipose tissue inflammation; increased adipose tissue production of proinflammatory cytokines (eg, tumor necrosis factor-α [TNF-α] and monocyte chemoattractant protein-1 [MCP-1]) and prothrombotic factors (eg, plasminogen activator inhibitor type 1 [PAI-1]); and decreased adipose tissue production of adiponectin and peroxisome proliferator-activated receptor-γ (PPAR-γ).1,2 These obesity-related changes in adipose tissue are linked to the development of insulin resistance, type 2 diabetes mellitus, and cardiovascular injury.1–6

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Activation of the mineralocorticoid receptor (MR) has been implicated in mediating the inflammation observed in vessels, heart, and renal cortex of rodent models of diabetes mellitus and hypertension.7–10 Furthermore, large-scale clinical studies demonstrate beneficial effects of MR blockade on cardiovascular morbidity and mortality in patients with heart failure.11,12 MR blockade also decreases left ventricular mass in hypertensive patients with left ventricular hypertrophy with and without type 2 diabetes mellitus,13 improves coronary vascular function in patients with diabetes,14 reduces markers of inflammation in patients with diabetes,15,16 and decreases proteinuria in patients with diabetic and nondiabetic renal injury.17,18

In the present studies, we used in vivo and in vitro approaches to test the hypotheses that MR activation regulates expression of adipokines and that chronic MR blockade has beneficial effects on adipose tissue inflammation and

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adipose tissue expression of TNF-α, MCP-1, PAI-1, PPAR-γ, and adiponectin. In vivo studies were performed in obese, diabetic db/db mice and lean, nondiabetic db/+ heterozygous littermates. The effects of acute MR activation on expression of TNF-α, MCP-1, PPAR-γ, and adiponectin were assessed in 3T3-L1 cells.

Methods

Animal Procedures
Male obese db/db mice (Jackson Laboratory, Bar Harbor, Me), which are homozygous for an inactivating mutation in the lepin receptor leading to hyperphagia, obesity, insulin resistance, and hyperglycemia, received either no drug treatment or the MR antagonist eplerenone via chow (0.6 mg eplerenone per gram chow) from age 8 to 25 weeks. The nondiabetic control group mice were male lean db/+ heterozygous littermates (Jackson Laboratory) studied from age 8 to 25 weeks. We reported previously that eplerenone reduces renal injury in these animals. Animals were kept in a room lighted 12 h/d at an ambient temperature of 22±1°C. Animals had free access to drinking water and Purina Rodent Chow (Purina, St Louis, Mo). Body weight was obtained at 8 and 25 weeks of age. At 25 weeks of age, systolic blood pressure was measured in conscious animals by tail-cuff plethysmography (Blood Pressure Analyzer, model 179, IITC Life Science), and animals were placed in individual metabolic cages for collection of urine for 24 hours. Mice were anesthetized with isoflurane. Blood, adipose tissue from the retroperitoneum, and hearts were harvested. Tissue samples were processed for immunohistochemistry studies as described previously. The Institutional Animal Care and Use Committee at Harvard University approved our experimental procedures.

Cell Culture
3T3-L1 cells (ATCC, Manassas, Va; No. CL-173) were maintained in Dulbecco’s modified Eagle’s medium (containing 4.5 g/L glucose) with 10% fetal calf serum. Twelve hours before treatment, cells were switched to Dulbecco’s modified Eagle’s medium without fetal calf serum. At the time of treatment, cells were washed twice with PBS and incubated with vehicle, aldosterone (10^-8 mol/L), or aldosterone (10^-7 mol/L) plus the water-soluble MR antagonist canrenone (10^-6 mol/L) in Dulbecco’s modified Eagle’s medium without fetal calf serum. The cells were harvested for RNA and protein analyses after 24-hour or 72-hour incubation, respectively. 3T3-L1 cells were differentiated into adipocytes in 6-well plates with the use of the 3T3-L1 adipocyte kit (Zen bio, Research Triangle Park, NC; No. KT-01) as described previously. Differentiated 3T3-L1 cells were cultured in Dulbecco’s modified Eagle’s medium, 0.5% fetal calf serum for 24 hours and then stimulated with aldosterone (10^-8 mol/L) for 24 hours.

Plasma and Urine Assays
Plasma insulin was measured with the LincoPlex mouse insulin assay (LINCO Research, St Charles, Mo). Plasma glucose and triglycerides were measured with the use of Roche Cobas Integra 400 (Roche Diagnostics, Indianapolis, Ind) via a hexokinase enzymatic reaction for determination of glucose and via an enzymatic and colorimetric method with glyceral phosphate oxidase and 4-aminophenazone for triglycerides. To estimate insulin resistance, the homeostatic model assessment (HOMA) index was calculated by the following formula: fasting plasma insulin (µIU/mL) x fasting plasma glucose (mmol/L)/22.5. The Cytometric Bead Array System was used according to the manufacturer’s instructions (mouse inflammation CBA kit; BD Biosciences, San Jose, Calif; catalog No. 552364) to measure protein concentrations of interleukin-6, interleukin-10, MCP-1, interferon-γ, TNF-α, and interleukin-12p70 in mouse plasma. Cytokine concentrations were determined by flow cytometry (BD Biosciences, San Jose, Calif; catalog No. 552364) to measure protein concentrations of interleukin-6, interleukin-10, MCP-1, interferon-γ, TNF-α, and interleukin-12p70 in mouse plasma. Cytokine concentrations were determined by flow cytometry (BD Biosciences, San Jose, Calif; catalog No. 552364) to measure protein concentrations of interleukin-6, interleukin-10, MCP-1, interferon-γ, TNF-α, and interleukin-12p70 in mouse plasma.

Table. Characteristics of Lean db/+ and Obese db/db Mice at 25 Weeks

<table>
<thead>
<tr>
<th></th>
<th>db/+ (n=11)</th>
<th>db/db (n=10)</th>
<th>db/db Eplerenone (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight gain from 8 to 25 weeks, g</td>
<td>8.5±0.6</td>
<td>19.9±1.8*</td>
<td>18.3±2.1*</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>32±0.6</td>
<td>57±2.2*</td>
<td>55±2.0*</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>112±5</td>
<td>121±5</td>
<td>126±2</td>
</tr>
<tr>
<td>Urinary aldosterone/creatinine, ng/mg</td>
<td>0.09±0.02</td>
<td>0.24±0.02†</td>
<td>1.18±0.07‡</td>
</tr>
</tbody>
</table>

Blood measurements

| Glucose, mg/dL | 159±21 | 769±41* | 680±70* |
| MCP-1, pg/mL | 190±4 | 286±7* | 224±5¶ |
| HOMA index | 35±11 | 334±9¶ | 132±27§ |
| HOMA adiponectin, relative units | 1.00±0.09 | 0.68±0.06** | 0.93±0.16 |
| HOMA adiponectin/total adiponectin | 0.51±0.01 | 0.47±0.02 | 0.48±0.03 |

Data are mean±SE. *P<0.001 vs db/+; †P<0.05 vs db/+; ‡P<0.001 vs db/db; §P<0.05 vs db/db; |n|=5 to 7 per group; ¶P<0.01 vs db/+; #n=4 to 6 per group; **P<0.01 vs db/+ by Fisher exact test.

Quantitative Real-Time Polymerase Chain Reaction
Total mRNA was extracted from adipose tissue with the RNeasy Lipid Tissue Mini Kit (Qiagen Sciences, Germantown, Md) and from heart or 3T3-L1 cells with the RNeasy Mini Kit (Qiagen Sciences). cDNA was synthesized from 5 μg RNA with the First Strand CDNA Synthesis kit (Amersham, Buckinghamshire, UK). Polymerase chain reaction amplification reactions were performed with TaqMan gene expression assays in duplicate with the use of the ABI Prism 7000 Sequence Detection System (Applied Biosystems, Foster City, Calif). The ΔΔ cycle threshold method was used to determine mRNA levels. Target gene expression was normalized to 18S RNA levels.

Western Blot Analysis
Protein in 3T3-L1 cells was analyzed by quantitative Western blotting as previously described. Briefly, 3T3-L1 cells were sonicated in lysis RIPPA buffer (Santa Cruz Biotechnology Inc, Santa Cruz, Calif), followed by centrifugation. Equal amounts (30 μg) of the protein were subjected to electrophoresis on 10% SDS-polyacrylamide gels. In addition, detection of circulating levels of high-molecular-weight (HMW) adiponectin was conducted as described. Briefly, 1 μL of plasma was loaded to 3% to 15% PAGE.
SDS-PAGE gel under nonreducing and nonheat denaturing conditions. The gels were transferred onto a nitrocellulose membrane by electroblotting. Membranes were immunoblotted with 1:5000 anti-adiponectin antibody (Chemicon, Temecula, Calif) or 1:5000 anti-PPAR-γ antibody (Santa Cruz Biotechnology). The blots were scanned with the Epson Perfection 1650 scanner, and densitometric analysis was performed with Imagequant 5.2 software (Molecular Dynamics).

Immunofluorescent Microscopy
Retroperitoneal adipose tissue sections (4 μm) were fixed in ice-cold acetone for 10 minutes and preincubated with blocking solution containing 1% preimmune serum for 10 minutes. Tissue slices were incubated overnight at 4°C with mouse primary antibody to adiponectin (Chemicon, Temecula, Calif) and rabbit primary antibody to MR (Santa Cruz Biotechnology). After 3 washes with 0.5% blocking solution in PBS, tissue slices were incubated at 37°C for 30 minutes with secondary goat anti-mouse antibody tagged with Alexa Fluor 488 and secondary goat anti-rabbit antibody tagged with Alexa Fluor 594. After they were washed with PBS and deionized water, the slices were air-dried, mounted with Vectashield mounting medium containing 4',6-diamidino-2-phenylindole (DAPI) (Vector Laboratories Inc, Burlingame, Calif; catalog No. H1200), and stored under cool and dark conditions. Images were obtained with a Nikon Eclipse 90i microscope (Nikon Instruments Inc, Melville, NY) and processed with the use of NIS-Elements Advanced Research Imaging Software AR 2.30, SP3 (Nikon Instruments Inc) according to the manufacturer’s instructions. Alexa Fluor 488 yields a green color, Alexa Fluor 594 a red color, and DAPI a blue color. In the absence of the primary antibody, minimal fluorescence was noted.

Data Analysis
Data were analyzed by 1-way ANOVA followed by the Tukey or Newman-Keuls post hoc test for multiple comparisons. Differences in means with probability values ≤0.05 were considered statistically significant. Values are expressed as mean±SE.

The authors had full access and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results
Characteristics of Lean db/+ and Obese db/db Mice
We studied 3 groups of animals from age 8 to 25 weeks: (1) lean, nondiabetic db/+ mice; (2) obese, diabetic db/db littermates receiving no treatment; and (3) obese, diabetic db/db mice receiving eplerenone 100 mg/kg per day from age 8 to 25 weeks. At age 25 weeks, db/db mice were heavier than db/+ mice and had elevated blood glucose levels (Table). Treatment of db/db mice with eplerenone did not have a significant effect on body weight, weight gain from 8 to 25 weeks, blood glucose levels, or systolic blood pressure (Table). Urinary aldosterone to creatinine ratios in 24-hour urine samples collected at 25 weeks were higher in db/db mice than in db/+ mice and were further elevated in db/db mice receiving eplerenone (Table).

Effect of Obesity and MR Blockade on Adipose Tissue Expression of Adipokines
A 7- to 10-fold increase occurred in expression of proinflammatory cytokines TNF-α and MCP-1 and the macrophage marker CD68 in retroperitoneal adipose tissue from 25-week-old obese db/db mice compared with lean db/+ mice.
animals (Figure 1A to 1C). Adipose tissue from obese animals also showed elevated levels of PAI-1 mRNA compared with adipose tissue from lean animals (Figure 1D). Treatment with eplerenone markedly reduced mRNA levels of MCP-1, TNF-α, PAI-1, and CD68 in adipose tissue of db/db mice (Figure 1). Consistent with the gene expression studies in adipose tissue, plasma levels of MCP-1 were increased in db/db mice versus db/+ mice, and treatment of db/db mice with eplerenone significantly reduced plasma MCP-1 levels (Table). Adipose tissue mRNA levels of MR and interleukin-6 and plasma levels of interleukin-6, interleukin-12p70, TNF-α, and interferon-γ were similar in the 3 groups (data not shown).

**Effect of Obesity and MR Blockade on Cardiac and Adipose Tissue Expression of Adiponectin and PPAR-γ**

We determined adipose tissue expression of adiponectin, PPAR-γ, and leptin (Figure 2A to 2C). Leptin mRNA expression in fat was significantly increased in diabetic db/db mice versus db/+ mice, consistent with the loss of functional leptin receptors in db/db mice. In contrast, the levels of adiponectin and PPAR-γ mRNA were markedly decreased in adipose tissue of obese db/db mice compared with lean db/+ mice. MR blockade in db/db mice reduced adipose tissue expression of leptin and increased expression of both adiponectin and PPAR-γ to levels similar to those observed in lean animals (Figure 2A to 2C).

Obese db/db mice had reduced levels of adiponectin mRNA in heart compared with lean db/+ mice (Figure 2D). Treatment of db/db mice with eplerenone increased cardiac expression of adiponectin to levels observed in lean animals (Figure 2D). In contrast, PPAR-γ expression in heart was similar across the 3 groups (Figure 2E). Cardiac mRNA levels of MR, PAI-1, and TNF-α were similar in the 3 groups, and no leptin mRNA was detectable (data not shown).

Obese db/db mice, compared with lean db/+ control mice, had lower levels of circulating HMW adiponectin, a form of adiponectin that is decreased in humans with type 2 diabetes. Plasma levels of HMW adiponectin were similar in db/+ mice and db/db mice receiving eplerenone (Table). The ratio of HMW to total adiponectin was similar in the 3 groups (Table).

**Effect of Obesity and MR Blockade on HOMA Index and Triglyceride Levels**

Because our data demonstrate that MR blockade increases expression of insulin-sensitizing factors (adiponectin in heart and adipose tissue and PPAR-γ in adipose tissue) and
reduces expression of cytokines such as MCP-1 and TNF-α, which impair insulin sensitivity, we examined the effect of eplerenone on HOMA index and levels of triglycerides in blood samples obtained from anesthetized animals at the time of euthanasia. Triglyceride levels and HOMA index were elevated in obese diabetic db/db mice compared with lean mice, consistent with the insulin-resistant state of db/db mice, and eplerenone treatment reduced triglyceride levels and HOMA index (Table). Effect of Aldosterone on Expression of Adiponectin, PPAR-γ, and Adipokines in Cultured Adipocytes

Immunofluorescent staining of adipose tissue from db/db mice demonstrated expression of MR protein in adipocytes (Figure 3). To determine whether some of the effects of chronic MR blockade could be mediated through direct actions of MR on gene expression, we treated undifferentiated 3T3-L1 preadipocytes with aldosterone (10⁻⁸ mol/L) for 24 hours. Aldosterone increased mRNA levels of TNF-α 6-fold and mRNA levels of interleukin-6 and MCP-1 ~2-fold compared with control treatment (Figure 4A through 4C). In contrast, aldosterone treatment decreased mRNA levels of adiponectin and PPAR-γ to <50% of that observed with control treatment (Figure 4D and 4E). With 72 hours of exposure to aldosterone, a significant decrease occurred in protein levels of adiponectin and PPAR-γ, consistent with the aldosterone-mediated decrease in mRNA expression (Figure 4F and 4G). Furthermore, the MR antagonist canrenoate prevented these effects of aldosterone on gene expression (Figure 4). We also studied differentiated 3T3-L1 preadipocytes. Consistent with the observations in preadipocytes, incubation of differentiated adipocytes with 10⁻⁸ mol/L aldosterone for 24 hours caused a 33% decrease in adiponectin mRNA levels compared with cells incubated with vehicle (0.67±0.04 with aldosterone versus 1.00±0.03 with vehicle; P<0.01; n=3 per condition).

Discussion

These studies demonstrate reduced expression of adiponectin in heart and retroperitoneal adipose tissue and reduced expression of PPAR-γ in adipose tissue of obese, diabetic db/db mice compared with lean, nondiabetic db/+ mice. Furthermore, obese animals have increased adipose tissue inflammation and increased adipose tissue expression of PAI-1 and the proinflammatory cytokines TNF-α and MCP-1. Treatment of obese db/db mice with a MR antagonist reverses all of these obesity-related changes. Studies in 3T3-L1 preadipocytes demonstrate that aldosterone increases mRNA levels of TNF-α, MCP-1, and interleukin-6 and decreases mRNA and protein levels of adiponectin and PPAR-γ. Thus, some of the chronic effects of MR blockade in vivo may be secondary to blockade of the effects of MR on gene expression. These findings indicate that MR activation is a key factor mediating obesity-related changes in adipose tissue expression of proinflammatory and insulin-sensitizing factors and in regulating adiponectin expression in heart.

Adipose tissues from obese db/db mice show obesity-related changes in adipose tissue inflammation and gene expression similar to those reported in humans. The current studies demonstrate that MR blockade prevents these obesity-related changes in adipose tissue, suggesting that obesity is associated with an activated MR. Multiple factors could contribute to increased MR activity in obesity, including increased levels of MR and aldosterone. Although cardiac and adipose tissue expressions of MR were similar in obese and lean mice, urinary aldosterone was elevated in the obese mice. Similarly, 24-hour urinary aldosterone levels are elevated in overweight obesity.
compared with lean individuals on a high-salt diet, without any differences in urinary free cortisol levels. These results suggest that increases in aldosterone contribute to increased MR activation in obesity. Adipose tissue is the main site of adiponectin production; however, adiponectin is also expressed in heart tissue. To our knowledge, this is the first report that adiponectin expression is decreased in hearts of obese versus lean mice and that MR blockade increases cardiac adiponectin expression. This effect is specific for adiponectin because cardiac expression of PPAR-γ is not regulated by obesity or eplerenone treatment. The decreased expression of adiponectin in heart and adipose tissue and reduced plasma levels of HMW adiponectin in the obese, diabetic mice are consistent with studies showing reduced adiponectin, and in particular HMW adiponectin, in individuals with insulin resistance and type 2 diabetes. Our observation that MR blockade increases adiponectin expression is consistent with a report showing an increase in circulating adiponectin levels in 9 diabetic individuals with poor glycemic control treated with spironolactone.

**Figure 4.** Expression of proinflammatory adipokines, adiponectin, and PPAR-γ in cultured 3T3-L1 preadipocytes. TNF-α (A), interleukin-6 (IL-6; B), MCP-1 (C), adiponectin (D), and PPAR-γ (E) mRNA levels and HMW adiponectin (F) and PPAR-γ (G) protein levels in 3T3-L1 preadipocytes treated with vehicle (control group), 10⁻⁸ mol/L aldosterone (ALDO), and 10⁻⁶ mol/L aldosterone with the MR antagonist 10⁻⁶ mol/L canrenoate (ALDO + MRA). Treatment was 24 hours for mRNA studies and 72 hours for protein studies. mRNA levels are expressed relative to 18S rRNA. Protein levels are expressed relative to β-actin. n=8 per condition for mRNA and n=6 to 8 for protein per group. Data are mean±SE.
In db/db mice, the improvements in adipokine expression with MR blockade did not appear to be mediated by changes in weight, glycemia, or blood pressure. However, we cannot rule out the possibility that our assays were not sufficiently sensitive to detect changes in glycemia and blood pressure. Our immunofluorescent studies demonstrate MR expression in retroperitoneal fat cells of db/db mice. To test whether MR activation has direct effects on adipocyte gene expression, we studied the effects of aldosterone in 3T3-L1 cells, which express a functional MR.30–32 Aldosterone increases expression of MCP-1, TNF-α, and interleukin-6 in cultured preadipocytes, consistent with the stimulatory effects of aldosterone on MCP-1 expression in brown adipocytes,33 on MCP-1 and PAI-1 expression in the vasculature,34,35 and on interleukin-6 levels in humans.36 Our studies do not determine whether these changes in gene expression are due to direct transcriptional effects of MR or are mediated through the rapid effects of MR on intracellular signaling pathways that could lead indirectly to changes in gene expression.

Two studies demonstrate a role for MR activation in the differentiation of 3T3-L1 preadipocytes into adipocytes, a process accompanied by increases in adiponectin expression.30,32 This aldosterone-mediated increase in adiponectin during adipocyte differentiation differs from the present studies showing decreases in adiponectin with aldosterone in both undifferentiated and differentiated 3T3-L1 cells and may indicate that the ultimate effects of MR activation depend on the underlying state of the cell. However, our observation that MR activation decreases adiponectin and PPAR-γ expression in 3T3-L1 cells is in agreement with our observation that MR blockade increases adiponectin and PPAR-γ expression in adipose tissue of obese diabetic mice.

Adipose tissue contains multiple cells including preadipocytes, adipocytes, small blood vessels, and macrophages. Cross talk between the different adipose tissue cell types, in particular between macrophages, preadipocytes, and adipocytes, is thought to be an important factor in promoting a proinflammatory state in obesity.25,57 It is possible that some of the eplerenone-mediated changes in adipose tissue gene expression may be related to changes in gene expression in these cells, as well as in adipocytes. Finally, leptin expression in adipose tissue is increased in db/db mice, which is attributed to the lack of a functional leptin receptor in these animals.19 Our observation that MR blockade reduces leptin expression in adipose tissue of db/db mice is consistent with a study showing aldosterone-stimulated increases in leptin gene expression in vitro33 and suggests that MR-modified factors are involved in leptin regulation. The leptin promoter contains multiple AP-1 sites,58 and MR has been shown to regulate transcription through inhibition of AP-1/nuclear factor κB protein–DNA complexes.39

Obesity-related changes in adipose tissue expression (eg, increases in cytokines such as TNF-α and MCP-1 and decreases in adiponectin and PPAR-γ) are thought to contribute to insulin resistance.4,22,28,40–43 Therefore, we examined indirect measures of insulin sensitivity (eg, levels of triglycerides, insulin, and glucose) in our studies. Obese, diabetic db/db mice are hyperglycemic and have elevated triglyceride levels and HOMA index compared with lean mice, consistent with an insulin-resistant state in db/db animals.19 Treatment with spironolactone leads to decreases in triglycerides and HOMA index, suggesting that MR blockade improves insulin sensitivity. Similarly, studies in healthy individuals on a high-salt diet demonstrate a positive correlation between aldosterone and insulin resistance that is independent of age and body weight index.26,44 Furthermore, in primary hyperaldosteronism, removal of an aldosterone-producing adenoma or treatment with a MR antagonist improves insulin sensitivity.45 Aldosterone also decreases insulin receptor substrate-1 in rat vascular smooth muscle cells, which reduces insulin sensitivity.46 These studies support the concept that aldosterone impairs insulin sensitivity.

Our studies suggest that MR activation plays an important role in obesity-related changes in cardiac expression of adiponectin and changes in adipose tissue function that lead to low-grade inflammation, insulin resistance, and ultimately cardiovascular injury. Although a number of treatments (diet and exercise47 and 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors48,49) reduce obesity-related changes in adipose tissue inflammation and improve insulin sensitivity, diabetes mellitus and cardiovascular injury remain major causes of morbidity and mortality in obese individuals. Large clinical trials demonstrate that MR blockade reduces cardiovascular morbidity and mortality in patients with heart failure on maximum therapy, including statins.11,12 The present findings suggest a novel mechanism for beneficial cardiovascular effects of MR blockade through reductions in adipose tissue inflammation and increases in adiponectin.

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Disclosures
None.

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CLINICAL PERSPECTIVE
Adipose tissue inflammation, increased expression of proinflammatory adipokines, and decreased expression of insulin-sensitizing adipokines are associated with an increased risk of insulin resistance and cardiovascular disease in obesity. These studies in the db/db mouse model of obesity and diabetes mellitus demonstrated increased aldosterone in obese versus lean mice, consistent with reports of increased aldosterone production in human obesity. Treatment of db/db mice with the mineralocorticoid receptor antagonist eplerenone reduced adipose tissue inflammation; reduced adipose tissue expression of monocyte chemoattractant protein-1, plasminogen activator inhibitor type 1, and tumor necrosis factor-α; and increased adipose tissue expression of insulin-sensitizing factors peroxisome proliferator-activated receptor-γ and adiponectin. In cultured preadipocytes, aldosterone increased expression of tumor necrosis factor-α, monocyte chemoattractant protein-1, and interleukin-6 and reduced expression of adiponectin and peroxisome proliferator-activated receptor-γ, lending further support to the concept that aldosterone has detrimental effects on adipokine expression. These studies suggest that excess mineralocorticoid receptor activation is present in obesity, contributing to changes in adipose tissue that promote low-grade inflammation, insulin resistance, and ultimately cardiovascular injury. Future studies are needed to determine whether mineralocorticoid receptor antagonists will have a beneficial effect on these outcomes in human obesity.