Mineralocorticoid Receptor Antagonism Treats Obesity-Associated Cardiac Diastolic Dysfunction


Abstract—Patients with obesity and diabetes mellitus exhibit a high prevalence of cardiac diastolic dysfunction (DD), an independent predictor of cardiovascular events for which no evidence-based treatment exists. In light of renin-angiotensin-aldosterone system activation in obesity and the cardioprotective action of mineralocorticoid receptor (MR) antagonists in systolic heart failure, we examined the hypothesis that MR blockade with a blood pressure–independent low-dose spironolactone (LSp) would treat obesity-associated DD in the Zucker obese (ZO) rat. Treatment of ZO rats exhibiting established DD with LSp normalized cardiac diastolic function, assessed by echocardiography. This was associated with reduced cardiac fibrosis, but not reduced hypertrophy, and restoration of endothelium-dependent vasodilation of isolated coronary arterioles via a nitric oxide–independent mechanism. Further mechanistic studies revealed that LSp reduced cardiac oxidative stress and improved endothelial insulin signaling, with no change in arteriolar stiffness. Infusion of Sprague-Dawley rats with the MR agonist aldosterone reproduced the DD noted in ZO rats. In addition, improved cardiac function in ZO-LSp rats was associated with attenuated systemic and adipose inflammation and an anti-inflammatory shift in cardiac immune cell mRNAs. Specifically, LSp increased cardiac markers of alternatively activated macrophages and regulatory T cells. ZO-LSp rats had unchanged blood pressure, serum potassium, systemic insulin sensitivity, or obesity-associated kidney injury, assessed by proteinuria. Taken together, these data demonstrate that MR antagonism effectively treats established obesity-related DD via blood pressure–independent mechanisms. These findings help identify a particular population with DD that might benefit from MR antagonist therapy, specifically patients with obesity and insulin resistance. (Hypertension. 2015;65:1082-1088. DOI: 10.1161/HYPERTENSIONAHA.114.04912.)

Key Words: aldosterone ■ echocardiography ■ immune marker ■ metabolic syndrome X

Results of the recent Treatment of Preserved Cardiac Function Heart Failure with an Aldosterone Antagonist (TOPCAT) trial examining the clinical benefit of mineralocorticoid receptor (MR) antagonism in patients with heart failure with preserved ejection fraction (ie, diastolic heart failure) were negative. However, interpretation of these results is hampered by broad inclusion criteria resulting in a heterogeneous patient population that could mask specific patient subgroups that may preferentially benefit from MR antagonism. One of these potential populations is patients with diabetes mellitus, obesity, and the metabolic syndrome that exhibit >30% prevalence of cardiac diastolic dysfunction (DD), the major cardiac functional defect in heart failure with preserved ejection fraction. Indeed, smaller studies demonstrate improved diastolic function after MR antagonism in these patients. Thus, in conjunction with the renin-angiotensin-aldosterone system activation in obesity and metabolic syndrome, MR antagonism holds promise for the treatment of obesity-associated DD, but this and potential underlying mechanisms have not been studied and remain unclear.

DD contributes to the increase of cardiovascular risk in patients with obesity and diabetes mellitus; however, no clear evidence-based therapies for DD have been identified. DD is often accompanied by left ventricular (LV) hypertrophy, fibrosis, and impaired coronary flow reserve (ie, coronary microvascular dysfunction). It has recently been proposed that DD onset and progression occurs via systemic inflammation, contributing to cardiac/vascular oxidative stress and coronary microvascular endothelial dysfunction, ultimately leading to cardiac hypertrophy, fibrosis, and DD. Such a paradigm may explain recent epidemiological data demonstrating an association between...
coronary microvascular dysfunction and cardiac mortality in patients with diabetes mellitus. Importantly, the MR has been implicated in many components of this cascade. Specifically, MR activation promotes cardiovascular inflammation, oxidative stress, endothelial dysfunction, impaired coronary flow reserve, and cardiac fibrosis. In addition, recent reports reveal a role for the MR in enhancing proinflammatory immune cell phenotypes, including classically activated M1 macrophages and T-helper 17 lymphocytes. Based on these results, we hypothesized that MR signaling contributes significantly to obesity-associated DD and that MR blockade will interrupt disease progression, providing the first viable therapeutic avenue for DD in this specific patient population.

To test this hypothesis, we treated Zucker obese (ZO) rats with a blood pressure–independent low dose of the MR antagonist spironolactone (LSp). Previous work from our group and others demonstrates that ZO rats develop pronounced DD despite increased plasma renin activity. In addition to examining the effect of MR blockade in ZO rats, a role for the MR in the onset of and mechanisms underlying DD was examined in Sprague-Dawley (SD) rats treated with a subpressor dose of the MR agonist aldosterone.

Methods

For detailed description, see Methods in the online-only Data Supplement.

Results

Insulin Resistance and Kidney Injury Were Not Altered by LSp Treatment in ZO Rats

Compared with Zucker lean (ZL), ZO rats exhibited metabolic syndrome components including increased body weight, epididymal fat pad mass, insulin resistance (HOMA-IR [homeostatic model assessment of insulin resistance]), fasting plasma leptin, glucose, insulin, cholesterol, and triglyceride but not corticosterone (Table S2 in the online-only Data Supplement). LSp treatment (sc, 1 mg·kg⁻¹·day⁻¹) beginning at 29 weeks of age for 3 weeks had no effect on these parameters or serum potassium in ZO-LSp rats. Plasma aldosterone was reduced in ZO, but not ZO-LSp, rats versus ZL (Table S2) involving reduced plasma angiotensin II, despite increased plasma renin activity (Figure S1A and S1B). ZO rats exhibited proteinuria that was unchanged by LSp, and urinary sodium excretion was similar across all groups (Table S2).

MR Inhibition Normalized Obesity-Related Cardiac DD Independent of Blood Pressure

Systolic blood pressure was similar between all groups (Table S2). Compared with ZL, cardiac MR mRNA expression was unchanged in ZO but reduced in ZO-LSp, whereas mRNA expression of the MR target gene lipocalin-2 was increased in ZO rats regardless of treatment (Figure S1C). Consistent with our previous report, ZO rats exhibited abnormal diastolic function versus ZL (Figure 1A–1C; Table S3) and increased myocardial performance index, a heart rate–independent measure of diastolic and systolic function (Figure 1D). Myocardial performance index is increased because of abnormal diastolic function indicated by abnormal diastolic septal wall motion (decreased E/A′; Figure 1A), decreased propagation velocity of mitral inflow (Figure 1B), increased isovolumic relaxation time (Figure 1C), and increased LV filling pressure (increased E/E′; Table S3). Fractional shortening and ejection fraction were unchanged, indicating normal systolic function in ZO rats (Table S3). The abnormal diastolic parameters were largely ameliorated in ZO-LSp rats, indicating that MR antagonism treated established obesity-associated cardiac DD via blood pressure–independent mechanisms.

MR Inhibition With LSp Reduces Cardiac Fibrosis, But Not Hypertrophy, in ZO Rats

Given the well-described profibrotic action of MR signaling, we examined cardiac hypertrophy and fibrosis after MR inhibition. Compared with ZL, ZO rats exhibited LV hypertrophy, by LV-to-tibia length ratio (Figure 2A) and septal/posterior wall thicknesses (Table S3) that was unchanged by LSp treatment. Cardiomyocyte area tended to be increased in ZO (P=0.07) and was increased in ZO-LSp versus ZL (Figure 2A). However, elevated cardiac interstitial and perivascular fibrosis in ZO rats was reduced by LSp (Figure 2B). Conversely, cardiac mRNA expression of collagen I, collagen III, and transforming growth factor–β1 tended to be or was reduced in ZO rats regardless of treatment (Figure S1D). Thus, the normalization of diastolic function in obesity by MR inhibition involves reduced cardiac fibrosis without reduced hypertrophy.

LSp Restores Coronary Arteriolar Endothelial Function in ZO Rats

We examined coronary microvascular function, an important regulator of cardiac perfusion, as this could underlie the normalization of diastolic function in ZO-LSp rats. Coronary dysfunction before LSp treatment was confirmed in a subset of rats at 14 to 16 weeks of age, in that ZO rats had impaired coronary dilation to insulin but not ACh (Figure S2). Similar to previous studies, ZO coronaries exhibited reduced ACh- and insulin-induced vasodilation at 32 weeks of age (Figure 3A). Constriction to the thromboxane analog U46619 was similar in all Zucker groups (Figure S3A). LSp treatment...
restored dilation to ACh (Figure 3A) but did not increase the abolished nitric oxide synthase–dependent component of ACh-induced dilation in ZO rats (Figure S3B). Insulin vasodilation (Figure 3A) and insulin-stimulated aortic endothelial Akt(Thr308) phosphorylation (Figure 3B) were improved by LSp. Last, arterioles from Zucker rats were not remodeled (similar wall:lumen ratios), LSp treatment did not reduce the obesity-associated increase in arteriolar elastic modulus (stiffness; Figure S3C), and dilation to sodium nitroprusside (NO donor) was unchanged, confirming normal smooth muscle NO sensitivity (Figure S3D) and indicating endothelial cell dysfunction. Together, these data indicate that MR inhibition in ZO rats improves coronary microvascular function in concert with reduced cardiac fibrosis and improved diastolic function.

MR Antagonism Abrogates Obesity-Associated Elevations in Cardiac Oxidative Stress

To further explore potential mechanisms underlying reduced cardiac fibrosis and improved diastolic function in ZO-LSp rats, we examined cardiac oxidative stress. Cardiac oxidative stress was elevated in ZO versus ZL rats (Figure 4A and 4B). Specifically, cardiac ROS production and cardiac 3-nitrotyrosine were increased in ZO rats (Figure 4A and 4B), whereas NADPH oxidase activity tended to be elevated (P=0.07; Figure 4A) versus ZL. Each of these, particularly NADPH oxidase activity, was reduced in ZO-LSp rats (Figure 4A and 4B). Thus, MR antagonism abrogates obesity-associated cardiac oxidative stress in conjunction with improved diastolic function and reduced fibrosis.

LSp Treatment Attenuates Obesity-Related Visceral Adipose Inflammation

Because of the role of adipose inflammation and adipose-derived cytokines in obesity-related disease processes, we considered whether the modulation of cardiac oxidative stress was associated with systemic alterations in adipose inflammation/cytokines. Circulating levels of the proinflammatory cytokines monocyte chemoattractant protein-1 (MCP-1), tumor

Figure 2. Increased cardiac fibrosis, not hypertrophy, in Zucker obese (ZO) rats is resolved by mineralocorticoid receptor antagonism. Cardiac weights, assessed by left ventricle-to-tibia length ratio, cardiomyocyte area (A), and cardiac interstitial/periarterial fibrosis (B) in Zucker rats. Interstitial collagen and periarterial fibrosis assessed by picrosirius red (PR) and Verhoeff-Van Gieson (VVG) staining, respectively. Representative images in lower panel, arrows indicate coronary arteries. Values are means±SE; n=5 to 8; *P<0.05 versus Zucker lean (ZL), **P<0.05 versus all other groups, †P<0.05 versus ZO, §P=0.07 versus ZL. GSI indicates gray scale intensity; and ZO-LSp, ZO treated with spironolactone.

Figure 3. Impaired coronary arteriolar endothelial function in Zucker obese (ZO) rats is restored by mineralocorticoid receptor antagonism. A, Vasodilator responses of coronary arterioles from Zucker rats to endothelium-dependent dilators acetylcholine and insulin and (B) insulin-stimulated Akt(Thr308) phosphorylation in endothelial lysates from Zucker aortas. Values are means±SE; n=5 to 8; *P<0.05 versus Zucker lean (ZL) or unstimulated, **P<0.05 versus all other groups. ZO-LSp indicates ZO treated with spironolactone.
necrosis factor-α, and the oxidative stress marker thiobarbituric acid reactive substances were increased in ZO rats and treatment with LSp reduced circulating MCP-1 (Figure 5A). Similarly, proinflammatory gene expression was increased in epididymal fat from ZO rats (Figure 5B). Specifically, expression of MCP-1, tumor necrosis factor-α, vascular cell adhesion molecule-1, intercellular adhesion molecule-1, and NADPH oxidase subunits (p22phox/p47phox) were increased in ZO versus ZL rats. LSp treatment normalized some, but not all, adipose inflammatory genes (Figure 5B). Along with increased inflammatory gene expression, ZO rats exhibit marked adipose immune cell infiltration indicated by increased immune marker mRNAs for M1 macrophages, assessed by the generalized macrophage marker F4/80 and M1 marker CD11c, as well as T cells, assessed by CD4 and the regulatory T (Treg) cell marker FoxP3 (Figure 5C). The presence of macrophage mRNAs was reduced in ZO-LSp rats (Figure 5C). Together, these data demonstrate that the MR plays a role in obesity-related adipose inflammation and immune cell recruitment.

**MR Antagonism Induces an Anti-Inflammatory Shift of Cardiac Immune Cell Markers in ZO Rats**

We next examined whether reduced cardiac fibrosis and oxidative stress in ZO-LSp rats was associated with an anti-inflammatory shift in the cardiac immune cell phenotype by examining cardiac immune cell mRNAs. Compared with ZL rats, ZO rats exhibited unchanged cardiac mRNAs for MCP-1 (gene and protein; Figure S4A) and vascular cell adhesion molecule-1 while tumor necrosis factor-α was reduced and intercellular adhesion molecule-1 was elevated, indicating cardiac inflammation (Figure S4B). Immune marker mRNAs for total macrophages (F4/80), proinflammatory M1 macrophages (CD11c), anti-inflammatory M2 macrophages (CD163), T cells (CD4), or Treg cells (FoxP3) were unchanged in ZO versus ZL (Figure 6). After LSp treatment, however, ZO-LSp rats exhibited increased mRNA expression for anti-inflammatory M2 macrophages (CD163) and Treg cells (FoxP3; Figure 6). These data suggest that the improvement in obesity-related cardiac DD, fibrosis, vascular function, and oxidative stress after MR antagonism is associated with an anti-inflammatory shift of cardiac immune cell marker genes.

**Aldosterone Infusion Recapitulates Some, But Not All, Consequences of Obesity**

Infusion of aldosterone (sc, 50 μg·kg⁻¹·day⁻¹) in SD rats for 3 weeks beginning at 8 weeks of age raised plasma aldosterone but not blood pressure (Table S4). SD rats infused with aldosterone (SD-Aldo) exhibited cardiac diastolic, but not systolic, dysfunction (Table S5; Figure S5A) without cardiac/cardiomyocyte hypertrophy (Figure S5B) but elevated cardiac

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**Figure 5.** Systemic and adipose inflammatory markers are elevated in Zucker obese (ZO) rats and attenuated by mineralocorticoid receptor antagonism. A, Plasma levels of monocyte chemoattractant protein-1 (MCP-1), tumor necrosis factor-α (TNF-α), and thiobarbituric acid reactive substances (TBARS) in Zucker rats. B, Gene expression of inflammatory (MCP-1, TNF-α, vascular cell adhesion molecule-1 [VCAM-1], intracellular adhesion molecule-1 [ICAM-1]) and oxidative stress (NADPH oxidase subunits p22phox/p47phox) genes in epididymal adipose from Zucker rats. C, Expression of macrophage (F4/80, CD11c) and T-cell (CD4, FoxP3) mRNAs in epididymal adipose of Zucker rats. Values are mean±SE; n=4 to 6; *P<0.05 versus Zucker lean (ZL), †P<0.05 versus ZO, §P=0.07 versus ZL, ¥P=0.08 versus ZL. ZO-LSp indicates ZO treated with spironolactone.
collagen accumulation/fibrosis (Figure S5C). SD-Aldo rats also exhibited impaired coronary arteriolar endothelium-dependent vasodilation to acetylcholine and insulin (Figure S5D) in conjunction with increased cardiac oxidative stress markers thiobarbituric acid reactive substances and 3-nitrotyrosine (Figure S5E). Similarly, SD-Aldo rats exhibited increased systemic inflammation (plasma MCP-1 and tumor necrosis factor-α; Figure S6A) and oxidative stress (plasma thiobarbituric acid reactive substances; Figure S6B); however, SD-Aldo rats did not exhibit elevated epididymal adipose inflammatory gene expression (Figure S6C). Thus, subpressor MR agonism by aldosterone recapitulated many of the cardiovascular, but not adipose, consequences of obesity, suggesting differential MR signaling in the presence of comorbidities.

**Discussion**

In summary, we have demonstrated that MR antagonism with a blood pressure–independent dose of spironolactone is able to reverse established obesity-related DD. Treatment with LSp largely normalized obesity-associated increases in cardiac fibrosis, oxidative stress, coronary endothelial dysfunction, and systemic and adipose inflammation/immune cell recruitment in ZO rats in vivo. In addition, LSp treatment promoted an anti-inflammatory shift in cardiac immune cell mRNAs involving increased Treg and M2 macrophage markers in the absence of changes in systemic metabolic parameters, serum potassium, or kidney injury. Importantly, the benefit of LSp occurred with reduced circulating aldosterone in this model of normotensive obesity, indicating a limitation of plasma aldosterone as a marker of MR activation. Similar improvement in cardiac phenotype with MR blockade was previously reported in a model of low-aldosterone salt-sensitive hypertension.23 Reduced circulating aldosterone in the ZO rat can be accounted for by a significant reduction in plasma angiotensin II, despite increased plasma renin activity indicating reduced hepatic angiotensinogen production or reduced angiotensin-converting enzyme activity in this model. It is likely that cardiac MR activation occurs mainly via corticosterone (unchanged in ZO rats) because of its equivalent affinity for the MR and the possibility of local cardiac and adipose aldosterone production cannot be ruled out. To our knowledge, this is the first study to demonstrate that MR antagonism effectively treats DD in a model of obesity and insulin resistance, and these results reveal novel deleterious roles for MR in the development of obesity-associated cardiovascular dysfunction.

It is established that the benefit of MR antagonists in the treatment of systolic heart failure involves suppression of cardiac collagen accumulation.26,27 Our data support a similar cardiac antifibrotic role of MR antagonism in the context of obesity-related DD in agreement with small human studies demonstrating reduced circulating markers of fibrosis with MR blockade in patients with obesity.5,5 In the ZO rat, this likely involves increased collagen degradation or decreased production after LSp because collagen gene expression was reduced in these rats. An important departure between this study and those in patients with obesity, however, relates to the lack of effect of LSp to reduce cardiac hypertrophy in ZO rats, whereas LV mass index/cardiac dimensions were reduced by spironolactone in patients with obesity.5,5 The lack of reduction in hypertrophy in ZO-LSp rats involves increased cardiomyocyte area, suggesting that reduced hypertrophy is not necessary for improved cardiac diastolic function in obesity and insulin resistance, further supporting the importance of reduced fibrosis underlying the clinical benefit of MR antagonists in DD.

The link between coronary perfusion (ie, coronary arteriolar function) and cardiac diastolic function is well established. For instance, in healthy dogs, reduced coronary perfusion increases cardiac isovolumic relaxation time (ie, impaired cardiac relaxation).28 In agreement with previous work,20,21 ZO rats demonstrated impaired coronary arteriolar vasodilation at 14 to 16 weeks of age that was normalized by LSp beginning at 29 weeks of age, and this may explain the improvement in coronary flow reserve in patients with type 2 diabetes mellitus after MR blockade.13 Our data suggest that improved endothelium-dependent vasodilation after LSp involves NO pathways (ie, prostacyclin/EDHF) and direct improvement in endothelial insulin signaling without improved arteriolar stiffness. To our knowledge, this is the first report of MR-dependent modulation of endothelial insulin signaling. Our data are consistent with a recent study demonstrating that knockout of the leukocyte/endothelial MR prevented aortic endothelial dysfunction in diet-induced obesity involving modulation of cyclooxygenase-1–dependent pathways.29 Future studies are necessary to fully delineate MR-dependent modulation of endothelium-dependent vasodilator pathways in obesity. Regardless, the improvement in coronary endothelium-dependent vasodilation by LSp is clinically relevant because coronary endothelial dysfunction and impaired coronary flow reserve are independently predictive of acute/long-term cardiovascular events.30

MR-dependent modulation of inflammatory pathways may be a central component of obesity-related cardiac dysfunction underlying the therapeutic effect of MR antagonists. Our data demonstrate that MR antagonism attenuated systemic and adipose inflammation and immune cell recruitment, particularly involving M1 macrophages, similar to previous mouse studies.29,31,32 In addition, we demonstrate that in the presence of cardiac inflammation (ie, increased intercellular adhesion molecule-1 mRNA) but not obesity-related alteration of cardiac immune cell marker mRNAs, LSp treatment increased cardiac mRNAs for anti-inflammatory M2 macrophages and Treg cells. Recent evidence demonstrates a role...
of MR signaling as a direct, physiologically relevant modulator of macrophage polarization and of the T-helper 17/Treg axis. Together with our data demonstrating abrogated cardiac, but not systemic, oxidative stress after LSp treatment, these data suggest an important pathogenic role of local MR signaling in obesity-related cardiovascular dysfunction. Thus, we speculate that the anti-inflammatory influence of these cell types may underlie the improved cardiac phenotype after LSp treatment in ZO rats; however, future mechanistic studies addressing this issue are necessary.

Interrogation of the systemic effects of MR activation in this study revealed several additional surprising findings that warrant further examination. First, LSp treatment did not improve kidney injury/proteinuria or increase serum potassium in the ZO rat, suggesting that MR activation either does not contribute to kidney injury in this model or that our dose of spironolactone is too low to sufficiently block renal MR. Indeed, the dose used in this study inhibits ≈35% of radio labeled aldosterone binding in vivo. In addition, our results suggest that MR signaling in various tissues is context-dependent because aldosterone infusion in healthy SD rats recapitulated some (cardiac/coronary dysfunction, cardiac/systemic inflammation). Furthermore, our data suggest a novel anti-inflammatory shift of cardiac immune cell markers after LSp in obesity that warrants further study. In DD caused by etiologies in which the inciting factors are different, MR antagonists may not be beneficial. This context-dependent role of MR signaling in DD may have contributed to the recent negative results of the TOPCAT trial. Because MR signaling plays an integral role in the development of DD in obesity, further study on MR antagonists to treat DD specifically in patients with insulin resistance and obesity is warranted.

Perspectives
We have demonstrated that LSp, too low to cause significant changes in serum potassium or blood pressure, treats established obesity-associated DD. This benefit of MR blockade involves reduced cardiac fibrosis, improved coronary microvascular function, and attenuated cardiac oxidative stress and systemic inflammation. Furthermore, our data suggest a novel anti-inflammatory shift of cardiac immune cell markers after LSp in obesity that warrants further study. In DD caused by etiologies in which the inciting factors are different, MR antagonists may not be beneficial. This context-dependent role of MR signaling in DD may have contributed to the recent negative results of the TOPCAT trial. Because MR signaling plays an integral role in the development of DD in obesity, further study on MR antagonists to treat DD specifically in patients with insulin resistance and obesity is warranted.

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Disclosures
None.

References


**Novelty and Significance**

**What Is New?**

- Treatment with the mineralocorticoid receptor (MR) antagonist spironolactone reverses established cardiac diastolic dysfunction in obese, insulin resistant rats and is associated with reduced cardiac oxidative stress and fibrosis, attenuated systemic and adipose inflammation, and improved cardiac immune cell markers.
- MR blockade improved coronary microvascular endothelial function in obesity via improved nitric oxide–independent vasodilation and restored insulin-dependent signaling to Akt with no change in arteriolar structure/stiffness.
- The benefit of MR antagonism occurred, despite reduced circulating aldosterone highlighting a limitation of using aldosterone as a surrogate for MR signaling.

**What Is Relevant?**

- Cardiac diastolic dysfunction, the major cardiac defect in diastolic heart failure, is common in patients with obesity and insulin resistance although no clear evidence-based therapies exist.

- Low-dose MR blockade with spironolactone normalized cardiac and coronary vascular function independent of changes in blood pressure, systemic insulin resistance, kidney injury, and serum potassium in a rat model.

**Summary**

Low-dose MR blockade is an effective treatment for cardiac diastolic and coronary microvascular dysfunction associated with obesity and insulin resistance. These results have implications for the use of MR antagonism in the treatment of obesity-related cardiovascular dysfunction.
Mineralocorticoid Receptor Antagonism Treats Obesity-Associated Cardiac Diastolic Dysfunction


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SUPPLEMENTAL MATERIAL

Mineralocorticoid Receptor Antagonism Treats Obesity-associated Cardiac Diastolic Dysfunction

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SUPPLEMENTAL MATERIAL

Methods
Animals. All animal protocols were approved by the Institutional Animal Care and Use Committees of the Harry S. Truman Memorial Veterans’ Hospital and the University of Missouri. Animals were housed in a temperature-controlled room (12:12-h light dark cycle) and provided ad libitum water and standard rodent chow (Formulab 5008, Purina Mills, St. Louis, MO). Male lean and obese Zucker fa/fa and Sprague-Dawley rats were purchased from Harlan (Indianapolis, IN) at 6-10 wk of age. Zucker obese rats were randomly assigned to placebo (ZO) or low dose spironolactone (ZO-LSp) treatment paradigms. Under isoflurane anesthesia (2-4% in 100% O2), rats were implanted with time-release, matrix-driven delivery pellets (Innovative Research of America, Sarasota, FL) containing either placebo or spironolactone (sc; 1 mg·kg⁻¹·day⁻¹) for 21 days beginning at 29 wk of age. This dose of spironolactone was chosen based on previous evidence that it does not reduce blood pressure in a RAAS-dependent model of hypertension1 and that anti-androgenic and progestogenic effects are absent while ~35% of in vivo aldosterone binding is inhibited in the rat2. Zucker lean (ZL) rats received placebo pellets. To ensure a treatment design, the age of pellet implantation was chosen based on published evidence that ZO rats exhibit established defects in cardiac diastolic function and coronary endothelium-dependent vasodilation by 28 wk of age3-5. Early impairment of coronary insulin-induced dilation was examined in a subset of untreated (no pellet implantation) ZL and ZO rats euthanized at 14-16 wk of age. Twenty-four hour urine collection was performed during the final week of treatment in all animals. SD rats were randomly assigned to vehicle (SD) or aldosterone (SD-Aldo) treatment. Under isoflurane anesthesia (2-4% in 100% O2), rats were implanted with osmotic mini-pumps (Alzet model 2004, Cupertino, CA) containing either vehicle (ethanol in saline) or aldosterone (50 µg·kg⁻¹·day⁻¹) for 21 days beginning at 8 wk of age. SD rats were maintained on a normal sodium diet (0.28% in chow with no additional salt in drinking water). This dose of aldosterone was chosen based on previous evidence demonstrating no change in blood pressure by radiotelemetry and aortic endothelial cell dysfunction6 and SD, rather than ZL, rats were chosen for the infusion studies as this is a common strain utilized in this type of experiment. Animals were fasted 5 hours prior to euthanization, anesthetized with isoflurane (2-4% in 100% O2), blood was subsequently collected from the abdominal aorta, processed to plasma and serum, and frozen at -80°C. Animals were euthanized by exsanguination.

Blood pressure measurement. Blood pressure was determined by tail-cuff (Coda 8, Kent Scientific, Torrington, CT) during the final week of treatment. Rats were acclimated to restraints and tail cuffs for 3 days. Blood pressure was determined on the fourth day with 10-25 (minimum of 5) cuff inflations averaged for each animal.

Echocardiography. Two-dimensional echocardiography (GE Vivid i, GE Healthcare, Wauwatosa, WI) was performed under isoflurane anesthesia (2-4% in 100% O2) in apical long and parasternal short axis views at the level of the left ventricular (LV) mid-cavity or the mitral inflow with M-mode and pulsed wave (PW) Doppler, according to Hoit7 and as previously described 5. Briefly, in M-mode, aortic (Ao) and left atrial (LA) diameters were obtained in long axis while LV septal and posterior wall thicknesses in diastole (SWTd and PWTd, respectively), LV internal diameter at end diastole and end systole (LVIDd and LVIDs, respectively), fractional shortening (FS), and ejection fraction (EF) were determined in short axis. Relative wall thickness (RWT) was calculated as: RWT = (SWTd+PWTd) / LVIDd. Tissue Doppler imaging (TDI) was performed in apical four chamber view with the sample volume at the septal annulus to assess early (E’) and late (A’) diastolic annular motion/velocity. PW Doppler was performed in the apical four-chamber view with the sample volume at the level of the LV mitral inflow tract for the determination of early (E) and late (A) transmitral peak diastolic flow velocity, isovolumic relaxation (IVRT) and contraction (IVCT) times, and ejection time (ET). Myocardial performance index (MPI), also known as the Tei index, was calculated as: (IVCT+IVRT) / ET. Finally, also in apical four chamber view, color M-mode recordings of mitral inflow during early diastole at the mitral leaflets were assessed to evaluate propagation velocity (Vp) of LV inflow, a correlate of the rate of chamber relaxation. All
Doppler spectra were recorded across 5-8 cardiac cycles at a sweep speed of 200 mm/s and parameters were assessed in 3 cardiac cycles each from 3 different spectra. All data were collected and analyzed offline by a single observer.

**Blood and urine chemistries.** Blood glucose (AlphaTrak, Abbott) was determined immediately prior to euthanasia. Plasma total cholesterol and triglycerides, serum potassium, and urine protein, creatinine, and sodium were measured using an Olympus AU680 automated chemistry analyser (Beckman-Coulter, Brea, CA) and plasma insulin was determined using a rat-specific ELISA by a commercial laboratory (Comparative Clinical Pathology Services, Columbia, MO). Plasma aldosterone and corticosterone were determined in duplicate by RIA at the Vanderbilt Hormone Assay & Analytical Services Core (Nashville, TN). In addition, serum samples were assayed for leptin, monocyte chemotactic protein-1 (MCP-1) and tumor necrosis factor-α (TNF-α) using multiplex cytokine assays (Millipore Milliplex, Billerica, MA & Bio-Rad Bio-Plex Pro, Hercules, CA) on a MAGPIX instrument (Luminex), according to the manufacturer’s instructions. Plasma renin activity was determined using a modification of the Sensolyte 520 Rat Renin Assay kit (Anaspec, Fremont, CA). Briefly, 90µl of plasma was combined with 10µl of diluted FRET-conjugated rat renin substrate (1:50 dilution). For kinetic measurement, fluorescence was measured with a Synergy H4 96-well plate reader (BioTek) at excitation/emission of 490/520 every 5 min for 60 min at 37°C. Plasma renin activity was assessed by the linear slope of the appearance of 5-carboxyfluorescein. Plasma angiotensin II concentration was determined by ELISA (Alpco Diagnostics, Salem, NH), according to manufacturer instructions.

**Isolated coronary arterioles.** Following excision of the heart, the septum was removed and immediately placed in ice-cold physiological salt solution (PSS) containing (in mM): 145 NaCl, 4.7 KCl, 1.2 NaH2PO4, 1.17 MgSO4, 2 CaCl2, 5 glucose, 2 pyruvate, 0.02 EDTA, 3 MOPS, and 1% bovine serum albumin, pH 7.4 (bubbling not necessary since pH buffered by MOPS). The left ventricular free wall was immediately frozen and stored at -80°C. Septal coronary arterioles (<200µm internal diameter) were isolated, cleaned, and secured to glass micropipettes, as previously described. Arterioles were visualized with an inverted microscope equipped with a calibrated video micrometer (Colorado Video, Boulder, CO) for the determination of internal diameter which was recorded using Chart software and a PowerLab data acquisition system (ADInstruments, Colorado Springs, CO). Arterioles were pressurized to 60cmH2O and those free from leaks were equilibrated for ~1 h, during which time the bath temperature was raised to 37°C and the PSS changed every 10-15 min. Arterioles were preconstricted to 25-40% tone with the thromboxane A2 analog U46619 for examination of dilator responses. Dilation to acetylcholine (ACh; 1 nM – 10 mM), insulin (Novolin R, Novo Nordisk; 0.1 – 100 ng/ml), and the nitric oxide (NO) donor sodium nitroprusside (SNP; 1 nM – 10 mM) was assessed by cumulative addition of agonist to the vessel bath. The doses of insulin utilized represented physiological (fasting ~0.2 ng/ml; post-prandial ~2 ng/ml), pathophysiological (>5 ng/ml) and pharmacological (>10 ng/ml) levels. In a separate set of experiments, the NO-dependent component of ACh dilation was determined by assessment of dilation to ACh following 20 min pretreatment with the NO synthase (NOS) inhibitor Nω-nitro-L-arginine methyl ester (L-NAME; 300µM). The NOS contribution to ACh dilation was then determined by subtraction of the ACh response at each dose in the presence of L-NAME from the response to the corresponding dose in the absence of L-NAME in vessels from the same animal. Maximum passive diameter was determined at the end of the experiment for each vessel by replacement of the PSS with calcium-free PSS. Passive mechanics were examined only after 80mM KCl challenge yielded no vasoconstriction. Experiments were performed and data was analyzed as previously described. Young’s elastic modulus was calculated as coronary arteriolar stress (α) / strain (ε). Coronary dilator responses are presented as percent maximal dilation, calculated as [(Dd – Db)/(Dmax – Db)] × 100, and constrictor responses are presented as percent vasoconstriction, calculated as [(Db – Dd)/Db] × 100 where Dd is diameter after a drug intervention, Db is baseline diameter, and Dmax is maximal passive diameter.
Aortic insulin stimulation. The thoracic aorta was removed, immediately placed in ice-cold PSS, and cleaned of surrounding tissue. Two 1.5-2 cm aortic sections were opened longitudinally, transferred to individual wells containing phenol-free DMEM plus 0.1% BSA, and incubated at 37°C for 2 h. Subsequently, one section was stimulated with insulin (100nM) for 30 min while the other section received vehicle, similar to a previous report 12. Following stimulation, sections were pinned out lumen-side up, Laemmlli buffer containing protease and phosphatase inhibitors was applied and, using a sterile scalpel blade, the endothelial surface was mechanically scraped yielding an endothelium-enriched sample, as previously described 13. Samples were immediately frozen and stored at -80°C until processed for immunoblotting.

Oxidative stress assays. Total cardiac reactive oxygen species (ROS) was determined by lucigenin-enhanced chemiluminescence, as previously described 14. Briefly, tissue sections were homogenized in sucrose buffer (250 mM sucrose, 0.5 mM EDTA, 50 mM HEPES, and protease inhibitor tablet, pH 7.5) and centrifuged at 1,500 relative centrifugal force for 10 min at 4°C. Whole homogenate (100 μl) was added to 1.4 ml of 50 mM phosphate (KH2PO4) buffer (150 mM sucrose, 1 mM EGTA, 5 μM lucigenin, and 100 μM NADPH, pH 7.0) in vials after 1 h of dark adaptation, samples were counted every 30 s for 10 min on a scintillation counter, and counts over the last 5 min were averaged. Superoxide production was calculated as relative lumens per second per milligram of fresh tissue for each sample after subtraction of the background activity. Because baseline ROS accumulation in ZL rats varied between the several cohorts, all data are expressed relative to ZL within each cohort.

Cardiac NADPH oxidase activity was determined according to established methods 14. Briefly, aliquots of plasma membrane fractions containing 20 μg of protein were incubated with NADPH (100 mM) at 37°C. NADPH activity was determined by measurement of the conversion of Radical Detector (Cayman Chemical) in the absence and presence of the NADPH inhibitor diphenylene iodonium sulfate (500 μM) using spectrophotometric (450 nm) techniques. Data were calculated as milli-optical density units per minute and normalized to the protein content of the sample.

Cardiac and plasma lipid peroxide content was assessed in cardiac whole cell lysates using the thiobarbituric acid-reactive substance (TBARS) assay, according to manufacturer instructions (Zeptometrix Corp., Buffalo, NY).

RNA extraction and real-time PCR. Cardiac and epididymal adipose tissue samples were frozen immediately at sacrifice and subsequently homogenized in TRIzol solution using a tissue homogenizer (TissueLyser LT, Qiagen, Valencia, CA). Total RNA was isolated using the Qiagen RNeasy Lipid Tissue Kit and assayed using a Nanodrop spectrophotometer (Thermo Scientific, Wilmington, DE) to assess purity and concentration. First-strand cDNA was synthesized from total RNA using the High Capacity cDNA Reverse Transcription kit (Applied Biosystems, Carlsbad, CA). Quantitative real-time PCR was performed using the CFX Connect™ Real-Time PCR Detection System (BioRad, Hercules, CA). Primer sequences (Table S1) were designed using the NCBI Primer Design tool. All primers were purchased from IDT (Coralville, IA). A 20-μl reaction mixture containing 10 μl iTaq UniverSYBR Green SMX (BioRad, Hercules, CA) and the appropriate concentrations of gene-specific primers plus 4 μl of cDNA template were loaded in each well of a 96-well plate. All PCR reactions were performed in duplicate. PCR was performed with thermal conditions as follows: 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 45 s. A dissociation melt curve analysis was performed to verify the specificity of the PCR products. 18S and GAPDH primers were used to amplify the endogenous control product. In the present study, 18S and GAPDH CTs were not different among groups of animals within adipose and cardiac tissues. mRNA expression values were calculated as 2^ΔCT whereby ΔCT = 18S or GAPDH CT - gene of interest CT and are presented normalized to ZL or SD rats, which were set at 1.

Tissue staining, immunohistochemistry, and cardiomyocyte morphology. A portion of LV was immersion fixed in 3% paraformaldehyde, dehydrated in ethanol, paraffin embedded, and transversely sectioned in 5µm slices. Four sections each for 5-6 animals per group were examined. To evaluate cardiac fibrosis, as previously described 15, sections were stained with picrosirius red (PR) and Verhoeff-von Gieson.
(VVG) stain for the determination of cardiac collagen and periarterial fibrosis, respectively. Staining was quantified as the average percent staining in 5 images per animal with the aid of the thresholding function in MetaVue software. Periarterial fibrosis was quantified as the average percent VVG staining of the vascular wall of coronary vessels. Cardiac oxidative stress was assessed by immunostaining for 3-nitrotyrosine (AB5411, Millipore), as previously described. Briefly, 4 myocardial sections per animal were deparaffinized, rehydrated, and epitopes were retrieved in citrate buffer. Endogenous peroxidases and nonspecific binding were blocked with 3% H₂O₂ and avidin, biotin, and protein block, respectively. Sections were stained with primary antibody (1:200 dilution) overnight after which they were washed, incubated with secondary antibody, linked, labeled with streptavidin, and incubated with dianinobenzidine prior to staining with hematoxylin, rehydration, and mounting. Cardiomyocyte cross-sectional area was determined from VVG stained sections captured in cross-section and cross-sectional area was determined using Image J on cardiomyocytes with well-defined cellular membranes and visible nucleus, as previously described. Approximately 15 cells from 2 separate sites were measured and averaged per group and area is expressed relative to the control group in each strain.

**Immunoblotting.** Left ventricular cardiac tissue was homogenized in lysis buffer containing protease and phosphatase inhibitors in a TissueLyser (Qiagen). Protein content of cardiac and aortic endothelial cell lysates were determined by BCA (Pierce) and Quant-iT (Invitrogen) protein assays, respectively. Cardiac samples were diluted in Laemmli buffer, 40µg protein was separated by 4-15% or 18% SDS-PAGE, transferred to nitrocellulose membranes, blocked with 5% non-fat milk in Tris-buffered saline-Tween 20 (TBST) solution and incubated overnight with gentle agitation at 4°C with anti-MCP-1 (1:1000, BD Pharmingen 551217) primary antibody. Three micrograms of aortic endothelium-enriched lysate was separated by 4-15% SDS-PAGE, transferred, blocked, and exposed to anti-Akt (1:500, Cell Signaling 9272), anti-phospho Akt (Thr³⁰⁸, 1:500, Cell Signaling 4056), and anti-pan-actin (1:1000, Cell Signaling 4968) antibodies as described for cardiac lysates. Membranes were subsequently incubated for 1 hr with appropriate secondary antibodies conjugated to horseradish peroxidase (1:20,000 or 1:30,000) and protein expression was detected by enhanced chemiluminescence (Pierce) on a Bio-Rad phosphoimager and quantified in Image Lab (Bio-Rad). Cardiac protein expression was normalized to total protein determined by Ponceau staining since preliminary experiments demonstrated group differences in cardiac actin expression. Actin expression was similar in aortic endothelial samples and aortic protein expression was normalized to actin. All antibodies were prepared in TBST containing 5% BSA.

**Statistics.** Data presented as means ± SE. Statistical analysis performed using Student’s t test for planned comparisons, one- or two-way ANOVA with Fishers LSD post hoc analysis, as appropriate. A p value ≤0.05 was considered significant.

**Supplemental References**


### Table S1. Primer sequences for real-time quantitative PCR

<table>
<thead>
<tr>
<th>Gene Name</th>
<th>Forward</th>
<th>Reverse</th>
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<tr>
<td>18s</td>
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<td>CATTCTTGCCAAATGCTTTCG</td>
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<td>CD4</td>
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<td>CD11c</td>
<td>CTGTCACTAGCAGGCCACGA</td>
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<td>CD163</td>
<td>TGTAGGTTCATCATCTCTCGGTCCT</td>
<td>CACCTACCAAAGCGGAGTTGAC</td>
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<td>Collagen I</td>
<td>CCTGGTGCTCAAGGTTTCC</td>
<td>CACACGAGACCCAGCAG</td>
</tr>
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<td>Collagen III</td>
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<td>AGTGACGGCATCTAGAAACTGT</td>
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<td>F4/80</td>
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<td>GAPDH</td>
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<td>VCAM-1</td>
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<td>Parameter</td>
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<td>Zucker Obese</td>
</tr>
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<td>---------------------------------</td>
<td>-------------</td>
<td>--------------</td>
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<tr>
<td>Body Weight (g)</td>
<td>498 ± 12</td>
<td>797 ± 12*</td>
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<td>Systolic Blood Pressure (mmHg)</td>
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<td>Adrenal Weight (mg)</td>
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<td>Epididymal Fat Pad Weight (g)</td>
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<tr>
<td>Epididymal Fat:Tibia Length (g·cm⁻¹)</td>
<td>2.5 ± 0.1</td>
<td>5.7 ± 0.7*</td>
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</table>

**Blood Parameters**

- Plasma Leptin (ng·ml⁻¹): 25 ± 9, 156 ± 34*, 167 ± 34*
- Plasma Glucose (mg·dl⁻¹): 117 ± 3, 135 ± 7§, 138 ± 7*
- Plasma Insulin (ng·ml⁻¹): 3.7 ± 1.0, 11.0 ± 1.9*, 9.6 ± 2.5*
- HOMA-IR: 12 ± 2, 58 ± 6*, 45 ± 10*
- Plasma Cholesterol (mg·dl⁻¹): 120 ± 20, 363 ± 46*, 352 ± 61*
- Plasma Triglycerides (mg·dl⁻¹): 196 ± 35, 1018 ± 132*, 1024 ± 185*
- Serum Potassium (mEq·L⁻¹): 3.8 ± 0.2, 4.4 ± 0.3, 4.5 ± 0.2
- Plasma Aldosterone (pg·ml⁻¹): 159 ± 20, 101 ± 11*, 120 ± 15
- Plasma Corticosterone (ng·ml⁻¹): 766 ± 89, 762 ± 50, 776 ± 98

**Urine Parameters**

- Proteinuria (mg·mg Cr⁻¹): 6.4 ± 3.2, 36.6 ± 5.8*, 36.8 ± 5.2*
- Sodium Excretion (µmol·d⁻¹·g BW⁻¹): 2.3 ± 0.3, 1.8 ± 0.2, 2.3 ± 0.3

**Coronary Arteriole Parameters**

- Passive Diameter (µm): 151 ± 11, 155 ± 8, 151 ± 13
- Preconstriction (%): 36 ± 3, 31 ± 2, 32 ± 2

Values are mean ± SE, n = 4-10, *p<0.05 versus Zucker Lean, §p=0.08 versus Zucker Lean.
### Table S3. Cardiac function outcomes by echocardiography in Zucker rats

<table>
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<tr>
<th>Parameter</th>
<th>Zucker Lean</th>
<th>Zucker Obese</th>
<th>Zucker Obese + Spironolactone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart Rate (bpm)</td>
<td>361 ± 9</td>
<td>340 ± 7</td>
<td>341 ± 9</td>
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**Morphological parameters**

<table>
<thead>
<tr>
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<th>Zucker Lean</th>
<th>Zucker Obese</th>
<th>Zucker Obese + Spironolactone</th>
</tr>
</thead>
<tbody>
<tr>
<td>SWTd (cm)</td>
<td>0.139 ± 0.004</td>
<td>0.189 ± 0.005*</td>
<td>0.176 ± 0.006*</td>
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<tr>
<td>PWTd (cm)</td>
<td>0.158 ± 0.004</td>
<td>0.187 ± 0.006*</td>
<td>0.180 ± 0.007*</td>
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<tr>
<td>LVIDd (cm)</td>
<td>0.76 ± 0.02</td>
<td>0.79 ± 0.02</td>
<td>0.81 ± 0.01</td>
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<tr>
<td>LVIDs (cm)</td>
<td>0.33 ± 0.02</td>
<td>0.33 ± 0.02</td>
<td>0.33 ± 0.02</td>
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<tr>
<td>RWT</td>
<td>0.62 ± 0.01</td>
<td>0.66 ± 0.02</td>
<td>0.62 ± 0.01</td>
</tr>
<tr>
<td>LA (cm)</td>
<td>0.31 ± 0.01</td>
<td>0.39 ± 0.02*</td>
<td>0.36 ± 0.02</td>
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<tr>
<td>Ao (cm)</td>
<td>0.30 ± 0.01</td>
<td>0.32 ± 0.01</td>
<td>0.29 ± 0.01</td>
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<tr>
<td>LA/Ao</td>
<td>1.04 ± 0.04</td>
<td>1.22 ± 0.04</td>
<td>1.23 ± 0.08</td>
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**Diastolic parameters**

<table>
<thead>
<tr>
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<th>Zucker Obese</th>
<th>Zucker Obese + Spironolactone</th>
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<tbody>
<tr>
<td>E (m·s⁻¹)</td>
<td>0.95 ± 0.04</td>
<td>1.13 ± 0.05*</td>
<td>1.16 ± 0.03*</td>
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<tr>
<td>E' (m·s⁻¹)</td>
<td>0.092 ± 0.006</td>
<td>0.071 ± 0.005*</td>
<td>0.080 ± 0.004</td>
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<td>A' (m·s⁻¹)</td>
<td>0.034 ± 0.002</td>
<td>0.048 ± 0.004*</td>
<td>0.036 ± 0.002†</td>
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<td>E/E'</td>
<td>10.7 ± 1.1</td>
<td>16.3 ± 0.9*</td>
<td>14.9 ± 0.9*</td>
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<tr>
<td>E'/A'</td>
<td>2.8 ± 0.3</td>
<td>1.5 ± 0.1*</td>
<td>2.3 ± 0.1†</td>
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<tr>
<td>Vp (m·s⁻¹)</td>
<td>65 ± 3</td>
<td>42 ± 1*</td>
<td>60 ± 2†§</td>
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<tr>
<td>E/Vp</td>
<td>1.5 ± 0.1</td>
<td>2.7 ± 0.1*</td>
<td>2.0 ± 0.1**†</td>
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<tr>
<td>IVRT (ms)</td>
<td>18 ± 1</td>
<td>22 ± 1*</td>
<td>17 ± 1†</td>
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**Systolic parameters**

<table>
<thead>
<tr>
<th>Parameter</th>
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<th>Zucker Obese</th>
<th>Zucker Obese + Spironolactone</th>
</tr>
</thead>
<tbody>
<tr>
<td>EF (%)</td>
<td>90 ± 1</td>
<td>90 ± 1</td>
<td>92 ± 1</td>
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<tr>
<td>FS (%)</td>
<td>56 ± 1</td>
<td>57 ± 2</td>
<td>60 ± 2</td>
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<tr>
<td>MPI</td>
<td>0.39 ± 0.01</td>
<td>0.49 ± 0.02*</td>
<td>0.38 ± 0.01†</td>
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SWTd, septal wall thickness-diastole; PWTd, posterior wall thickness-diastole; RWT, relative wall thickness; LVIDd, LV inner dimension-diastole; LVIDs, LV inner dimension-systole; EF, ejection fraction; FS, fractional shortening; LA, left atrial diameter; Ao, aortic diameter; A', peak late septal annular velocity; E', early peak septal annular velocity; IVRT, isovolumic relaxation time; E, velocity of early mitral inflow; E/E' index of LA filling pressure; E/Vp, index of LV filling pressure; MPI, myocardial performance index. Values are mean ± SE, N=8-10. *p<0.05 versus ZL; †p<0.05 versus ZO; §p=0.09 versus ZL; ¥p=0.06 versus ZL.
Table S4. Phenotypic characteristics of Sprague-Dawley rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sprague-Dawley</th>
<th>Sprague-Dawley + Aldosterone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body Weight (g)</td>
<td>321 ± 5</td>
<td>331 ± 8</td>
</tr>
<tr>
<td>Systolic Blood Pressure (mmHg)</td>
<td>132 ± 12</td>
<td>153 ± 12</td>
</tr>
<tr>
<td>Epididymal Fat Pad Weight (mg)</td>
<td>2.7 ± 0.1</td>
<td>2.8 ± 0.1</td>
</tr>
</tbody>
</table>

**Blood parameters**

- Plasma Glucose (mg·dl⁻¹)  
  - Sprague-Dawley: 113 ± 8  
  - Sprague-Dawley + Aldosterone: 107 ± 5
- Plasma Insulin (ng·ml⁻¹)  
  - Sprague-Dawley: 0.38 ± 0.04  
  - Sprague-Dawley + Aldosterone: 0.40 ± 0.07
- HOMA-IR  
  - Sprague-Dawley: 0.10 ± 0.02  
  - Sprague-Dawley + Aldosterone: 0.12 ± 0.03
- Plasma Aldosterone (pg·ml⁻¹)  
  - Sprague-Dawley: 257 ± 37  
  - Sprague-Dawley + Aldosterone: 381 ± 29*
- Plasma Corticosterone (ng·ml⁻¹)  
  - Sprague-Dawley: 672 ± 46  
  - Sprague-Dawley + Aldosterone: 611 ± 47

**Coronary Arteriole Parameters**

- Passive Diameter (µm)  
  - Sprague-Dawley: 160 ± 18  
  - Sprague-Dawley + Aldosterone: 183 ± 8
- Preconstriction (%)  
  - Sprague-Dawley: 37 ± 2  
  - Sprague-Dawley + Aldosterone: 36 ± 3

Values are mean ± SE, n =5-10, *p<0.05 versus SD.
### Table S5. Cardiac function outcomes by echocardiography in Sprague-Dawley rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sprague-Dawley</th>
<th>Sprague-Dawley + Aldosterone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart Rate</td>
<td>348 ± 6</td>
<td>320 ± 7*</td>
</tr>
</tbody>
</table>

**Morphological parameters**

- SWTd (cm): 0.17 ± 0.007 vs. 0.19 ± 0.005*
- PWTd (cm): 0.18 ± 0.01 vs. 0.20 ± 0.01*
- LVIDd (cm): 0.64 ± 0.02 vs. 0.67 ± 0.02
- LVIDs (cm): 0.34 ± 0.02 vs. 0.35 ± 0.02
- RWT: 0.80 ± 0.03 vs. 0.77 ± 0.02
- LA (cm): 0.29 ± 0.02 vs. 0.33 ± 0.01*
- Ao (cm): 0.24 ± 0.01 vs. 0.24 ± 0.01
- LA/Ao: 1.17 ± 0.05 vs. 1.41 ± 0.06*

**Diastolic parameters**

- E (m·s⁻¹): 0.88 ± 0.03 vs. 0.87 ± 0.03
- E' (cm·s⁻¹): 0.07 ± 0.006 vs. 0.04 ± 0.006*
- A' (cm·s⁻¹): 0.05 ± 0.002 vs. 0.06 ± 0.003**
- E/E' : 12.5 ± 0.9 vs. 21.6 ± 107*
- E'/A' : 1.23 ± 0.14 vs. 0.71 ± 0.05*
- Vp (m·s⁻¹): 55 ± 2 vs. 47 ± 2†
- E/Vp: 1.6 ± 0.1 vs. 1.9 ± 0.2
- IVRT (ms): 16 ± 1 vs. 23 ± 1*

**Systolic parameters**

- EF (%): 82 ± 2 vs. 83 ± 2
- FS (%): 47 ± 3 vs. 48 ± 2
- MPI: 0.40 ± 0.01 vs. 0.54 ± 0.03*

* SWTd, septal wall thickness-diastole; PWTd, posterior wall thickness-diastole; RWT, relative wall thickness; LVIDd, LV inner dimension-diastole; LVIDs, LV inner dimension-systole; EF, ejection fraction; FS, fractional shortening; LA, left atrial diameter; Ao, aortic diameter; A’, peak late septal annular velocity; E’, early peak septal annular velocity; IVRT, isovolumic relaxation time; E, velocity of early mitral inflow; E/E’ index of LA filling pressure; E/Vp, index of LV filling pressure; MPI, myocardial performance index. Values are mean ± SE, N=8-10.

* p<0.05; †p=0.07; **p=0.05.
Figure S1. Plasma renin activity (PRA; A), plasma angiotensin II (Ang II) concentration (B) and cardiac mRNA expression of the mineralocorticoid receptor (MR), the MR target gene lipocalin-2 (Lcn-2; C) as well as (D) cardiac mRNA expression of collagen I (Col I), collagen III (Col III), and transforming growth factor-β1 (TGF-β1) in 32 week old Zucker rats. ZL, Zucker lean; ZO, Zucker obese; ZO-LSp, Zucker obese treated with low dose spironolactone. Values are mean ± SE; n=5-10; *p<0.05 versus ZL, **p<0.05 versus all other groups; ¥p=0.057 versus ZL; §p=0.08-0.09 versus ZL.
Figure S2. Vasodilator responses of isolated, pressurized coronary arterioles from 14-16 wk old ZL and ZO rats to the endothelium-dependent vasodilators insulin and acetylcholine. Phenotypic characteristics of these animals and the coronary arterioles studied are included in the Table. ZL, Zucker lean; ZO, Zucker obese. Values are mean ± SE; *p<0.05 versus ZL.

<table>
<thead>
<tr>
<th>Phenytypic and coronary characteristics of 14-16 wk Zucker rats</th>
<th>Zucker Lean</th>
<th>Zucker Obese</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Phenotypic parameters</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>287 ± 9</td>
<td>558 ± 23*</td>
</tr>
<tr>
<td>Left ventricle : tibia length</td>
<td>193 ± 47</td>
<td>225 ± 10*</td>
</tr>
<tr>
<td><strong>Coronary arteriole parameters</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Passive diameter (μm)</td>
<td>167 ± 13</td>
<td>171 ± 11</td>
</tr>
<tr>
<td>Preconstriction (%)</td>
<td>27 ± 4</td>
<td>32 ± 3</td>
</tr>
</tbody>
</table>

Values are mean ± SE, n = 6-10, *p<0.05 versus Zucker Lean.
Figure S3. Functional and structural characterization of isolated, pressurized coronary arterioles from Zucker rats. (A) Vasoconstrictor responses to the thromboxane A_{2} analog U46619. (B) Nitric oxide synthase (NOS)-dependent component of acetylcholine-induced coronary vasodilation (difference with and without L-NAME). Right panel showing the percent relative NOS-dependent and non-NOS dependent contributions to 0.1mM acetylcholine dilation. (C) Coronary elastic modulus and wall:lumen ratio. (D) Vasodilator responses of arterioles to the NO donor sodium nitroprusside. ZL, Zucker lean; ZO, Zucker obese; ZO-LSp, Zucker obese treated with low dose spironolactone. Values are mean ± SE; n=3-7. *p<0.05 versus ZL, **p<0.05 versus all other groups, §p=0.06 versus ZL.
Figure S4. (A) Cardiac mRNA and protein expression of monocyte chemoattractant protein-1 (MCP-1) in Zucker rats. Representative immunoblot in inset. (B) Cardiac mRNA expression of tumor necrosis factor-α (TNF-α), vascular cell adhesion molecule-1 (VCAM-1), and intracellular adhesion molecule-1 (ICAM-1). Values are mean ± SE; n=5-6; *p<0.05 versus ZL, §p=0.08 versus ZL.
Figure S5. Infusion of aldosterone in Sprague-Dawley (SD) rats induces cardiac diastolic dysfunction, fibrosis, coronary microvascular endothelial dysfunction, and oxidative stress. (A) SD rats infused with aldosterone (SD-Aldo) exhibit cardiac diastolic dysfunction indicated by impaired indices of diastolic (E’/A’, Vp, and IVRT) and global (MPI) cardiac function. (B) SD-Aldo rats do not exhibit cardiac or cardiomyocyte hypertrophy, assessed by left ventricle-to-body weight (LV:BW) ratio and myocyte area, respectively. (C) Cardiac interstitial and periartrial fibrosis assessed by picrosirius red (PR) and VVG staining, respectively. Representative images on bottom right. (D) Vasodilator responses of isolated, pressurized coronary arterioles to the endothelium-dependent vasodilators acetylcholine and insulin and the nitric oxide donor sodium nitroprusside (SNP). (E) Cardiac oxidative stress assessed by presence of thiobarbituric acid reactive substances (TBARS), an index of lipid peroxidation, and 3-nitrotyrosine (3-NT) staining. Representative images on bottom right. E’/A’, ratio of early to late septal annular wall motion; GSI, gray scale intensity; IVRT, isovolumic relaxation time; MPI, myocardial performance index; Vp, propagation velocity of mitral inflow. Values are mean ± SE; n=4-10; *p<0.05 versus SD, ¥p=0.07 versus SD.
Figure S6. Aldosterone infusion in Sprague-Dawley (SD) rats increases markers of systemic, but not adipose, inflammation and oxidative stress. SD rats infused with aldosterone (SD-Aldo) exhibit elevated plasma levels of the inflammatory cytokines (A) monocyte chemoattractant protein-1 (MCP-1) and tumor necrosis factor-α (TNF-α) as well as (B) the lipid peroxidation marker thiobarbituric acid reactive substances (TBARS). (C) mRNA expression of MCP-1 and TNF-α in epididymal adipose tissue from SD rats. Values are mean ± SE; n=4-5; *p<0.05 versus SD.
动态血压监测（摘要）

高海拔对未经治疗和经过治疗的高血压患者动态血压的影响

高海拔心血管研究——安第斯研究

Ambulatory Blood Pressure in Untreated and Treated Hypertensive Patients at High Altitude

The High Altitude Cardiovascular Research—Andes Study

Grzegorz Bilo, Francisco C. Villafuerte, Andrea Faini, Cecilia Anza-Ramírez, Miriam Re Vera, Andrea Giuliano, Sergio Caravita, Francesca Gregorini, Carolina Lombardi, Elisabetta Salvioni, Jose Luis Macar lupu, Deborah Ossoli, Leah Landaveri, Morin Lang, Piergiuseppe Agostoni, José Manuel Sosa, Giuseppe Mancia, Gianfranco Parati

季晓平 译

健康人突然暴露于高海拔地区，血压会升高。然而，目前关于高海拔对高血压患者的影响及在这种情况下使用的降压疗效如何了解较少。高海拔心血管研究（High Altitude Cardiovascular Research, HIGHCARE）——安第斯山脉低地人研究的目的就是评估突然暴露于高海拔地区对高血压患者24小时动态血压的影响，以及对降压效果进行评估。在研究中，100例未经治疗的轻度高血压患者（收缩压144.1±9.8 mmHg，舒张压92.0±7.5 mmHg）随机分为双盲安慰剂组与替米沙坦80 mg+硝苯地平缓释片30 mg治疗组。我们分别监测停止治疗后，在海平面水平治疗6周后，突然升高到海海拔（3280 m）并立即返回海平面水平的患者24小时的动态血压。最终89例患者完成了研究（年龄56.4±17.6岁，52男性/37女性；体重指数，28.2±3.5 kg/ m²）。两组患者在高海拔地区的24小时收缩压均显著增加（安慰剂组增加11.0±9 mmHg；P<0.001；治疗组增加8.1±10.4 mm Hg；P<0.001）。积极的治疗使在海平面水平和高海拔地区的24小时收缩压均显著下降（安慰剂组 vs 治疗组：147.9±11.1 mmHg vs 132.6±12.4 mmHg；P<0.001；95%可信区间是10.9–19.9 mmHg），而且耐受性良好。同样，治疗组的舒张压、日间血压、夜间血压均显著降低，且在各种情况下对耐受性良好。本研究表明：（1）高血压患者突然暴露于高海拔地区，其24小时血压会显著上升；（2）在这种情况下的血管紧张素受体阻滞剂联合钙离子拮抗剂治疗是一种安全有效的选择。

（Hypertension, 2015;65:1266-1272.）

肾素-血管紧张素系统（摘要）

盐皮质激素受体拮抗剂治疗肥胖相关的心脏舒张功能障碍

Mineralocorticoid Receptor Antagonism Treats Obesity-Associated Cardiac Diastolic Dysfunction


曹新舟 陈志新 译 董波 审校

肥胖及糖尿病患者往往并发心脏舒张功能障碍——心血管事件的独立危险因素，针对这一危险因素并没有循证医学证据的支持。鉴于肥胖患者肾素-血管紧张素（renin-angiotensin-aldosterone system）系统的激活以及盐皮质激素受体拮抗剂在收缩性心力衰竭患者中的心脏保护作用，我们检验了这样的假说：在Zucker肥胖大鼠中，应用不引起血压变化的小剂量螺内酯治疗肥胖相关的的心脏舒张功能障碍。心脏超声证实：应用螺内酯治疗可使心脏舒张功能恢复正常，这可能与减轻心脏纤维化，经由非一氧化氮（NO）依赖性途径恢复小的冠状动脉分支内皮依赖性舒张功能有关，但是并没有减轻心肌肥厚。进一步的机制研究表明：在不改变小动脉管壁硬度的基础上，螺内酯可减轻心脏的氧化应激，增加内皮的胰岛素通路。在SD大鼠中，应用盐皮质激素受体激动剂醛固酮可再现类似Zucker肥胖大鼠心脏舒张功能障碍的模型。此外，在心脏免疫细胞mRNA水平显示：螺内酯治疗Zucker肥胖大鼠改善心功能与减轻全身性及脂肪组织的炎症及抗炎转化有关，特别是螺内酯选择性激活心脏巨噬细胞和调节性T细胞，螺内酯干预的Zucker肥胖大鼠并没有改变血压水平、血清钾水平、全身的胰岛素敏感性、改善肥胖相关的肾损伤及缓解蛋白尿。总体而言，这些数据说明盐皮质激素受体拮抗剂能够通过非血压依赖性途径有效治疗肥胖相关的心脏舒张功能障碍。这些研究可帮助我们确定心脏舒张功能障碍这一特殊人群，可能会获益于盐皮质激素受体拮抗剂的应用，尤其是肥胖及胰岛素抵抗患者。

（Hypertension, 2015;65:1082-1088.）