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Aldosterone and Myocardial Extracellular Matrix Expansion in Type 2 Diabetes Mellitus

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Abstract

Myocardial extracellular matrix expansion and reduced coronary flow reserve (CFR) occur in patients with type 2 diabetes mellitus without heart failure or coronary artery disease. Because aldosterone is implicated in the pathophysiology of cardiac fibrosis and vascular injury, the aim of this study was to test the hypothesis that aldosterone is associated with extracellular matrix expansion and reduced CFR in type 2 diabetes mellitus. Patients with type 2 diabetes mellitus without evidence of coronary artery disease were recruited. Blood pressure, lipid management, and glycemic control were optimized over 3 months. Cardiac magnetic resonance imaging with T1 mapping was used to measure myocardial extracellular volume (ECV). Cardiac positron emission tomography was used to assess CFR. On a liberal, 250 mEq/day sodium diet, 24-hour urinary aldosterone and change in serum aldosterone with angiotensin II stimulation were measured. Fifty-three participants with type 2 diabetes (68% men, mean age 53 ± 7 years, mean body mass index 32.2 ± 4.3 kg/m², mean glycosylated hemoglobin $6.8 \pm 0.7\%$, mean systolic blood pressure 126 ± 14 mm Hg) without infarction or ischemia by cardiac magnetic resonance and positron emission tomography were studied. Subjects had impaired CFR (2.51 ± 0.83) and elevated ECV (0.36 ± 0.05), despite normal echocardiographic diastolic function and normal left ventricular function. Myocardial ECV, but not CFR, was positively associated with 24-hour urinary aldosterone excretion ($r = 0.37$, $p = 0.01$) and angiotensin II–stimulated aldosterone increase ($r = 0.35$, $p = 0.02$). In a best-overall multivariate model (including age, gender, body mass index, glycosylated hemoglobin, and blood pressure), 24-hour urinary aldosterone was the strongest predictor of myocardial ECV ($p = 0.004$). In conclusion, in patients with type 2 diabetes mellitus without

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coronary artery disease, aldosterone is associated with myocardial extracellular matrix expansion. These results implicate aldosterone in early myocardial remodeling in type 2 diabetes mellitus.

Diabetic cardiomyopathy occurs even in the absence of coronary artery disease (CAD) and is a significant contributor to increased morbidity and mortality in patients with type 2 diabetes mellitus (T2DM).¹⁻³ Before the onset of symptomatic angina or myocardial infarction, patients with T2DM have subtle changes in cardiac structure and function, including impaired myocardial blood flow and expansion of the cardiac extracellular matrix.⁴⁻⁶ These abnormalities are independent of traditional cardiovascular risk factors and may contribute to cardiac dysfunction. Aldosterone and the mineralocorticoid receptor play a key role in the pathogenesis of vascular injury and cardiac fibrosis.⁷⁻⁹ T2DM is associated with elevated aldosterone levels in some studies, and mineralocorticoid receptor blockade reduces renal injury in patients with T2DM.^{10,11} Furthermore, there is evidence that aldosterone blockade reduces biomarkers of collagen turnover and cardiovascular mortality in patients with heart failure.¹² The impact of aldosterone on subclinical myocardial remodeling and microvascular dysfunction in patients with T2DM without CAD remains poorly characterized. Therefore, we tested the hypothesis that aldosterone levels correlate with myocardial matrix remodeling and microvascular dysfunction in men and women with T2DM.

Methods

This study is part of an ongoing clinical study, which enrolled men and women with T2DM, aged 18 to 70 years (ClinicalTrials.gov identifier NCT00865124). Analyses were performed with data obtained before randomization to drug therapy in the first 53 subjects who showed no evidence of previous myocardial infarction and/or reversible ischemia on cardiac positron emission tomography and cardiac magnetic resonance and who were in balance on a liberal sodium diet (urinary sodium >150 mmol/24 hours). Exclusion criteria were clinical evidence of coronary, cerebrovascular, or peripheral vascular disease; asthma; estimated glomerular filtration rate <60 ml/min/1.73m²; cigarette smoking; pregnancy; illicit drug use; contraindications to cardiac magnetic resonance or adenosine; uncontrolled hypertension (systolic blood pressure >160 mm Hg); and active major medical illness. The institutional review board at Brigham and Women's Hospital (Boston, Massachusetts) approved the study protocol, and all study participants provided written informed consent.

Enrolled study participants were started on enalapril 20 mg/day and tapered off other antihypertensive medications. Amlodipine (5 to 10 mg/day) was added for systolic blood pressure >140 mm Hg. Simvastatin was added if low-density lipoprotein was >100 mg/dl at the screening visit. Diabetic medications were adjusted to target a glycosylated hemoglobin (HbA_{1c}) level <7%.

Study participants were admitted to an inpatient unit after 3 months of angiotensin-converting enzyme inhibitor or angiotensin receptor blockade therapy with at least the final month being enalapril and 3 months of statin therapy (if applicable; Figure 1). For 4 days before admission and continuing through admission, participants consumed an isocaloric, caffeine-free, liberal sodium (250 Na⁺ mmol/day) diet containing 100 mmol/day potassium, 1,000 mg/day calcium, 300 mg/day magnesium, and 30% carbohydrate by calories. Amlodipine was held 36 hours before admission. Oral hypoglycemic agents and insulin were held during fasting.

Urine was collected for 24 hours for aldosterone, creatinine, and sodium, after which morning blood samples were collected for serum aldosterone and plasma renin activity in subjects who had been supine and fasting since midnight. To assess the aldosterone response

to a controlled stimulus, supine subjects were next infused with angiotensin II at 3 ng/kg/min with reassessment of serum aldosterone after 60 minutes. The infusion rate was reduced to 1 ng/kg/min if systolic blood pressure increased to >180 mm Hg or diastolic blood pressure increased to >100 mm Hg.

Focused transthoracic echocardiography (Vivid i, 3.6-MHz bandwidth, phased-array transducer; GE Healthcare, Schenectady, New York) was performed for analysis of left ventricular diastolic function. Pulse-wave Doppler interrogation of mitral valve inflow was used to determine early (E) and atrial (A) left ventricular filling velocities. Tissue Doppler myocardial velocities at the lateral mitral annulus were measured (e' and a').

Study participants were studied with a whole-body positron emission tomographic/computed tomographic scanner (Discovery RX or STE LightSpeed 64; GE Healthcare, Milwaukee, Wisconsin). Regional myocardial blood flow was measured at rest and during an intravenous infusion of adenosine (0.14 mg/kg/min) using nitrogen-13 ammonia as a flow tracer. A nongated, low-dose transmission computed tomographic scan was acquired for correction of photon attenuation. Beginning with the intravenous bolus administration of nitrogen-13 ammonia (20 mCi), serial images were acquired for 20 minutes, as previously described.¹³ After 30 minutes, intravenous adenosine was infused for 4 minutes. Two minutes into the adenosine infusion, a second dose of nitrogen-13 ammonia was injected, and images were recorded again in the same sequence. Heart rate, blood pressure, and a 12-lead electrocardiogram were recorded at baseline and every minute during and after the infusion of adenosine. Coronary flow reserve (CFR) was determined as the ratio of adenosine-stimulated myocardial blood flow to baseline myocardial blood flow corrected for the baseline pulse-pressure product. Study participants with abnormal findings on cardiac positron emission tomography consistent with CAD were excluded.

Cardiac magnetic resonance imaging was performed using a 3-T magnet (Magnetom Tim Trio or Verio; Siemens Healthcare, Erlangen, Germany) with an 8-element phasedarray coil. The cardiac magnetic resonance protocol included localizers, cine function, late gadolinium enhancement imaging, and sequences for the assessment of myocardial extracellular volume (ECV). Electrocardiographically gated cine images were acquired in short-axis (contiguous 8-mm slices) and long-axis views of the left ventricle with a balanced steady-state free precession sequence (repetition time 3.4 ms, echo time 1.2 ms, matrix size 256×256 , field of view 32 to 34 cm). After an injection of 0.15 mmol/kg intravenous gadolinium diethylenetriamine penta-acetic acid (Magnevist; Berlex Pharmaceuticals, Wayne, New Jersey), late gadolinium enhancement was performed using standard techniques. Left atrial volumes were measured when technically feasible using the biplane area-length method and indexed to body surface area using commercially available software (cmr42; Circle Cardiovascular Imaging Inc., Calgary, Alberta, Canada).^{14,15} Quantitation of left and right ventricular volumes, function, and mass were performed using QMASS version 9.3 (Medis Medical Imaging Systems, Leiden, The Netherlands).

Quantification of ECV was performed using standard techniques described by our laboratory.^{16,17} T1 measurements were performed with a breath-hold Look-Locker sequence, which is based on a spoiled gradient-echo cine sequence (without steady-state free precession), with temporal resolution of 117 ms for precontrast T1 measurements and 55 ms for postcontrast T1 measurements. Briefly, a non-slice-selective BIR-4 adiabatic inversion pulse is applied after the detection of an R wave, followed by a segmented gradient-echo acquisition for 17 cardiac phases (repetition time 5 ms, echo time 2.2 ms, flip angle 10° , slice thickness 8 mm, matrix size 192×100 , field of view approximately 34×30 –34 cm, number of signals acquired 1, iPAT factor 2). T1 measurements were made at 1 mid left ventricular slice. One precontrast acquisition was obtained, and 2 or 3 postcontrast

acquisitions were acquired, with 5 minutes after contrast injection for the first postcontrast acquisition. Endocardial and epicardial contours were segmented, taking care to avoid blood pool. A region of interest was placed in the blood pool. The left ventricle was segmented into 6 sectors for analysis. Segmental T1 was derived by exponential fit of the inversion recovery. Segmental myocardial R1 (1/T1) was plotted against blood R1, and the slope of the linear fit defined the partition coefficient for gadolinium, λ_{Gd} , for each segment. Myocardial ECV for each segment was calculated by multiplying λ_{Gd} by (1 – hematocrit), and then the 6 myocardial segments were averaged for a final myocardial ECV.

Serum and urinary aldosterone were analyzed using the Siemens assay (Siemens, Los Angeles, California; intraassay variation 2.3% to 5.4%, interassay variation 3.8% to 15.7%), and plasma renin activity was analyzed using the Diasorin assay (Diasorin, Inc., Stillwater, Minnesota; intraassay variation 4.6% to 10%, inter-assay variation 5.6% to 7.6%). Blood levels of electrolytes, HbA_{1c}, and lipids and urine levels of sodium, creatinine, and microalbumin were assessed using routine clinical assays from the Laboratory Corporation of America (Raritan, New Jersey).

Baseline demographic, echocardiographic, cardiac magnetic resonance, and positron emission tomographic data are expressed as mean $\pm$ SD, except that medians and interquartile ranges are given for data that were not normally distributed. The primary end points for this analysis were the relation between 24-hour urinary aldosterone excretion and ECV as well as the relation between 24-hour urinary aldosterone excretion and CFR. Because CFR and ECV data were normally distributed, Pearson's correlation coefficients were used to express the association between continuous covariates and ECV and CFR. We constructed a best-overall multivariate model ($p < 0.10$ for model entry, $p < 0.05$ for model retention) for predictors of myocardial ECV, including covariates reflecting cardiometabolic risk (age, body mass index, gender, systolic and diastolic blood pressure, and HbA_{1c}), CFR, 24-hour urinary aldosterone excretion, and change in aldosterone with angiotensin II stimulation. All statistical analysis was performed with SAS version 9.3 (SAS Institute Inc., Cary, North Carolina) or JMP version 10.0 (SAS Institute Inc.).

Results

The study participants (68% men) included in the analysis had a mean age of 53 ± 7 years and a mean body mass index of 32.2 ± 4.34 kg/m² (see Table 1 for characteristics of the study population on the day of imaging procedures). The median duration of T2DM was 5 years, with 17% of study participants receiving insulin for glycemic control. Per the study protocol, all study participants were taking the angiotensin-converting enzyme inhibitor enalapril and had excellent glycemic, blood pressure, and lipid management per American Diabetes Association guidelines.¹⁸

Twenty-four hour urinary sodium excretion was 279 ± 67 mmol/day, and median 24-hour urinary aldosterone excretion was 6.28 μ g (Table 2). Study participants with above-median compared with below-median 24-hour urinary aldosterone excretion had lower high-density lipoprotein cholesterol (38 ± 9 vs 48 ± 11 mg/dl, $p = 0.0006$) despite similar statin use, had higher creatinine clearance (115 ± 19 vs 98 ± 20 ml/min/1.73 m², $p = 0.003$) and were more likely to require amlodipine for blood pressure control (41% vs 7%, $p = 0.009$). All other subject characteristics in Table 1 were similar in patients with above- versus below-median 24-hour urinary aldosterone excretion.

The myocardial ECV in our diabetic population was 0.36 ± 0.05 (Table 3), which is elevated compared with published values of ECV in healthy subjects¹⁹ and with the mean ECV of 0.27 ± 0.03 ($p < 0.0001$) obtained in our laboratory in 10 healthy, normotensive volunteers

(mean age 54.8 ± 12.6 years, mean body mass index 28 ± 6 kg/m², mean systolic blood pressure 123 ± 8.3 mm Hg). In our diabetic study population, higher 24-hour urinary aldosterone excretion was associated with higher myocardial ECV, when treated continuously ($r = 0.37$, $p = 0.01$) and when stratified by median 24-hour urinary aldosterone excretion ($p = 0.03$) (Figure 2). This correlation persisted even after the removal of 2 study participants with 24-hour urinary aldosterone excretion 2.5 SDs above the mean ($r = 0.33$, $p = 0.02$). In addition, the change in aldosterone with angiotensin II infusion was associated with myocardial ECV ($r = 0.35$, $p = 0.02$; Figure 2). Myocardial ECV did not show a significant correlation with age, gender, race, body mass index, duration of T2DM, HbA_{1c}, lipids, blood pressure, amlodipine use, the left ventricular ejection fraction, indexed left ventricular mass, or diastolic function (transmitral E/A wave or E/e'). Myocardial ECV was associated with a higher left atrial volume index ($r = 0.37$, $p = 0.02$) and a higher left ventricular end-diastolic volume ($r = 0.29$, $p = 0.04$). In a best-overall multivariate model including age, gender, body mass index, HbA_{1c}, systolic and diastolic blood pressure, 24-hour urinary aldosterone excretion was a significant predictor of myocardial ECV ($p = 0.004$).

Peak adenosine-stimulated myocardial blood flow in our cohort was about 30% lower than that reported among age-matched healthy controls using the same method.⁴ Mean CFR in our population was 2.51 ± 0.83 , which is consistent with previous studies in patients with T2DM (Table 3).⁴ At the time of cardiac positron emission tomography, the mean blood glucose level was 110 ± 21 mg/dl. There was no correlation between CFR and either urinary aldosterone or change in aldosterone with angiotensin II ($p = 0.48$ and $p = 0.54$, respectively). Stress myocardial blood flow and CFR were similar in study participants with below- or above-median urinary aldosterone excretion. There was no linear association between CFR and myocardial ECV ($p = 0.25$) or between CFR and age, gender, race, body mass index, duration of T2DM, HbA_{1c}, lipids, and blood pressure. Twenty-four hour urinary aldosterone excretion was a significant predictor of ECV ($p = 0.005$) in a model adjusted for CFR treated as a continuous variable ($p = 0.13$).

Discussion

In this study, we used cardiac magnetic resonance and positron emission tomographic techniques to phenotype cardiac function, structure, and physiology in patients with T2DM. We established that in patients with T2DM without coronary disease, measures of aldosterone production are associated with an expanded myocardial extracellular matrix. Furthermore, the expansion in myocardial extracellular matrix was not correlated with CFR and was present despite a lack of significant diastolic dysfunction or ventricular hypertrophy. These results support the role of extracellular matrix expansion as an early disease marker in patients with T2DM and provide the first evidence to implicate aldosterone in this early phase of diabetic cardiac remodeling.

Our results are consistent with preclinical and clinical studies that establish aldosterone as an important profibrotic factor in the heart. For instance, in a deoxycorticosterone acetate salt rodent model, mineralocorticoid receptor activation leads to cardiac fibrosis and adverse remodeling of the intramural coronary circulation, independent of changes in blood pressure.^{8,9} Mineralocorticoid receptor blockade reduces angiotensin II-induced cardiac inflammation and fibrosis in rodents,⁷ and ameliorates left ventricular remodeling and dysfunction in dogs with chronic heart failure.²⁰ In humans with heart failure, mineralocorticoid receptor blockade diminishes cardiovascular morbidity and mortality and reduces biomarkers of collagen synthesis and degradation.^{12,21} Mineralocorticoid receptor blockade also decreases left ventricular hypertrophy in patients with hypertension²² and improves circulating markers of collagen turnover and integrated backscatter (a putative

echocardiographic marker of cardiac interstitial fibrosis) in patients with the metabolic syndrome.²³ In light of these well-known profibrotic effects of aldosterone, our finding of an association between aldosterone and myocardial ECV raises the possibility of a role for aldosterone in early myocardial remodeling in T2DM.

Cardiac fibrosis is a common finding in diabetic heart disease, even in the absence of epicardial CAD.³ Expansion of the myocardial extracellular matrix and accumulation of interstitial collagen are characteristic of cardiac fibrosis.⁸ The cardiac magnetic resonance techniques used in our study quantify interstitial matrix expansion and have been validated against histologic assessment of interstitial matrix expansion in an animal model of diffuse myocardial fibrosis.²⁴ Furthermore, myocardial ECV by cardiac magnetic resonance is elevated in patients with cardiovascular diseases associated with extracellular matrix expansion, including aortic stenosis, dilated cardiomyopathy, and hypertrophic cardiomyopathy.^{19,25} ECV values in our diabetic population were elevated, suggesting that cardiac remodeling is present in patients with diabetes who are in good cardiometabolic control and who lack evidence of significant diastolic dysfunction.

Myocardial blood flow is impaired in patients with T2DM compared with healthy subjects.⁴ Our measurement of CFR was consistent with these previous reports in diabetic populations.^{4,13} We did not detect an association between aldosterone and CFR. However, given that mineralocorticoid receptor activity is modulated by multiple factors, it may still play a role in vascular dysfunction in T2DM.^{26,27} This remains an area for future investigation.

The absence of significant associations between standard cardiovascular risk factors and ECV or CFR may be related to the relatively mild diabetic heart disease in our population, to the rigorous control of cardiometabolic parameters, and/or to the homogeneity of our study population. Our inability to demonstrate a significant association between CFR and ECV may be related to a lack of power. Alternatively, these 2 common abnormalities in early diabetic heart disease may occur independently and through different mechanisms.

The lack of suppression of urinary aldosterone in our cohort despite long-term angiotensin converting enzyme inhibitor therapy and a controlled liberal dietary sodium intake demonstrates the difficulty in suppressing aldosterone chronically in a diabetic population. The aldosterone escape with angiotensin-converting enzyme inhibitor therapy may be related to the stimulation of aldosterone by adrenocorticotrophic hormone, potassium, and/or angiotensin II, which was not suppressed in our cohort, or potentially related to adipocyte production of aldosterone and/or stimulation of adrenal aldosterone production by adipocyte-derived factors.^{28,29}

The limitations of our study need to be viewed in the context of our study design. Despite a relatively small sample size, our population was rigorously phenotyped with regard to aldosterone, which was obtained under careful control of subjects' activity and dietary sodium and potassium intake. Although we did not perform coronary angiography to exclude CAD, combined stress positron emission tomography and late enhancement by cardiac magnetic resonance to exclude patients with known CAD is a comprehensive and diagnostically sensitive approach. Our study design did not allow us to determine whether the increase in extracellular matrix was related to T2DM, hypertension, or a combination of the 2 factors. However, the mean ECV in the 12 participants, who were not taking antihypertensive medications at enrollment and who were also not hypertensive per Joint National Committee 7 criteria (blood pressure $\leq 140/90$ mm Hg), was 0.34 ± 0.03 , which was similar to the mean ECV in the remaining participants (0.36 ± 0.05). We also lack histologic confirmation that the increased myocardial ECV represents diffuse collagen

deposition. Although studies have shown that diffuse fibrosis is an early phenotype of diabetic heart disease,³ it is possible that the myocardial interstitium may expand due to other pathologic processes, such as neovascularization and deposition of advanced glycation end products. This latter process can increase transforming growth factor β ,³⁰ thus promoting inflammation and fibrosis. In summary, our results implicate aldosterone in early myocardial remodeling in patients with type 2 diabetes mellitus.

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References

1. From AM, Scott CG, Chen HH. The development of heart failure in patients with diabetes mellitus and pre-clinical diastolic dysfunction: a population-based study. *J Am Coll Cardiol.* 2010; 55:300–305. [PubMed: 20117433]
2. Ho KKL, Pinsky JL, Kannel WB, Levy D. The epidemiology of heart failure—the Framingham Study. *J Am Coll Cardiol.* 1993; 22:A6–A13.
3. Regan TJ, Lyons MM, Ahmed SS, Levinson GE, Oldewurtel HA, Ahmad MR, Haider B. Evidence for cardiomyopathy in familial diabetes mellitus. *J Clin Invest.* 1977; 60:885–899.
4. Di Carli MF, Janisse J, Grunberger G, Ager J. Role of chronic hyperglycemia in the pathogenesis of coronary microvascular dysfunction in diabetes. *J Am Coll Cardiol.* 2003; 41:1387–1393. [PubMed: 12706936]
5. Ng ACT, Auger D, Delgado V, van Elderen SGC, Bertini M, Siebelink HM, van der Geest RJ, Bonetti C, van der Velde ET, de Roos A, Smit JWA, Leung DY, Bax JJ, Lamb HJ. Association between diffuse myocardial fibrosis by cardiac magnetic resonance contrast-enhanced T1 mapping and subclinical myocardial dysfunction in diabetic patients. *Circ Cardiovasc Imaging.* 2012; 5:51–59. [PubMed: 22135399]
6. Boyer JK, Thanigaraj S, Schechtman KB, Perez JE. Prevalence of ventricular diastolic dysfunction in asymptomatic, normotensive patients with diabetes mellitus. *Am J Cardiol.* 2004; 93:870–875. [PubMed: 15050491]
7. Rocha R, Stier CT, Kifor I, Ochoa-Maya MR, Rennke HG, Williams GH, Adler GK. Aldosterone: a mediator of myocardial necrosis and renal arteriopathy. *Endocrinol.* 2000; 141:3871–3878.
8. Brilla CG, Weber KT. Mineralocorticoid excess, dietary sodium, and myocardial fibrosis. *J Lab Clin Med.* 1992; 120:893–901. [PubMed: 1453111]
9. Young M, Head G, Funder J. Determinants of cardiac fibrosis in experimental hypermineralocorticoid states. *Am J Physiol Endocrinol Metab.* 1995; 269:E657–E662.
10. DeOliveira JM, Price DA, Fisher ND, Allan DR, McKnight JA, Williams GH, Hollenberg NK. Autonomy of the renin system in type II diabetes mellitus: dietary sodium and rectal hemodynamic responses to ACE inhibition. *Kidney Int.* 1997; 52:771–777. [PubMed: 9291199]
11. Epstein M, Williams GH, Weinberger M, Lewin A, Krause S, Mukherjee R, Patni R, Beckerman B. Selective aldosterone blockade with eplerenone reduces albuminuria in patients with type 2 diabetes. *Clin J Am Soc Nephrol.* 2006; 1:940–951. [PubMed: 17699311]
12. Zannad F, Alla F, Dousset B, Perez A, Pitt B. Limitation of excessive extracellular matrix turnover may contribute to survival benefit of spironolactone therapy in patients with congestive heart failure: insights from the Randomized Aldactone Evaluation Study (RALES). *Circulation.* 2000; 102:2700–2706. [PubMed: 11094035]
13. Di Carli MF, Bianco-Batlles D, Landa ME, Kazmers A, Groehn H, Muzik O, Grunberger G. Effects of autonomic neuropathy on coronary blood flow in patients with diabetes mellitus. *Circulation.* 1999; 100:813–819. [PubMed: 10458716]
14. Farzaneh-Far A, Ariyaratnam V, Shenoy C, Dorval JF, Kaminski M, Curillova Z, Wu H, Brown KB, Kwong RY. Left atrial passive emptying function during dobutamine stress MR imaging is a

- predictor of cardiac events in patients with suspected myocardial ischemia. *JACC Cardiovasc Imaging*. 2011; 4:378–388. [PubMed: 21492813]
15. Lang RM, Bierig M, Devereux RB, Flachskampf FA, Foster E, Pellikka PA, Picard MH, Roman MJ, Seward J, Shanewise JS, Solomon SD, Spencer KT, St John Sutton M, Stewart WJ. Recommendations for chamber quantification: a report from the American Society of Echocardiography's Guidelines and Standards Committee and the Chamber Quantification Writing Group, developed in conjunction with the European Association of Echocardiography, a branch of the European Society of Cardiology. *J Am Soc Echocardiogr*. 2005; 18:1440–1463. [PubMed: 16376782]
 16. Broberg CS, Chugh SS, Conklin C, Sahn DJ, Jerosch-Herold M. Quantification of diffuse myocardial fibrosis and its association with myocardial dysfunction in congenital heart disease. *Circulation-Cardiovasc Imaging*. 2010; 3:727–734.
 17. Jerosch-Herold M, Sheridan DC, Kushner JD, Nauman D, Burgess D, Dutton D, Alharethi R, Li D, Hershbeger RE. Cardiac magnetic resonance imaging of myocardial contrast uptake and blood flow in patients affected with idiopathic or familial dilated cardiomyopathy. *Am J Physiol Heart Circ Physiol*. 2008; 295:H1234–H1242. [PubMed: 18660445]
 18. American Diabetes Association. Standards of medical care in diabetes—2012. *Diabetes Care*. 2012; 35:S11–S63. [PubMed: 22187469]
 19. Ugander M, Oki AJ, Hsu LY, Kellman P, Greiser A, Aletras AH, Sibley CT, Chen MY, Bandettini WP, Arai AE. Extracellular volume imaging by magnetic resonance imaging provides insights into overt and subclinical myocardial pathology. *Eur Heart J*. 2012; 33:1268–1278. [PubMed: 22279111]
 20. Suzuki G, Morita H, Mishima T, Sharov VG, Todor A, Tanhehco EJ, Rudolph AE, McMahon EG, Goldstein S, Sabbah HN. Effects of long-term monotherapy with eplerenone, a novel aldosterone blocker, on progression of left ventricular dysfunction and remodeling in dogs with heart failure. *Circulation*. 2002; 106:2967–2972. [PubMed: 12460880]
 21. Pitt B, Zannad F, Remme WJ, Cody R, Castaigne A, Perez A, Palensky J, Wittes J. The Randomized Aldactone Evaluation Study I. The effect of spironolactone on morbidity and mortality in patients with severe heart failure. *N Engl J Med*. 1999; 341:709–717. [PubMed: 10471456]
 22. Pitt B, Reichel N, Willenbrock R, Zannad F, Phillips RA, Roniker B, Kleiman J, Krause S, Burns D, Williams GH. Effects of eplerenone, enalapril, and eplerenone/enalapril in patients with essential hypertension and left ventricular hypertrophy: the 4E-Left Ventricular Hypertrophy Study. *Circulation*. 2003; 108:1831–1838. [PubMed: 14517164]
 23. Kosmala W, Przewlocka-Kosmala M, Szczepanik-Osadnik H, Mysiak A, O'Moore-Sullivan T, Marwick TH. A randomized study of the beneficial effects of aldosterone antagonism on LV function, structure, and fibrosis markers in metabolic syndrome. *JACC Cardiovasc Imaging*. 2011; 4:1239–1249. [PubMed: 22172779]
 24. Messroghli DR, Nordmeyer S, Dietrich T, Dirsch O, Kaschira E, Savvatis K, H-Ici DO, Klein C, Berger F, Kuehne T. Assessment of diffuse myocardial fibrosis in rats using small-animal Look-Locker inversion recovery T1 mapping. *Circ Cardiovasc Imaging*. 2011; 4:636–640. [PubMed: 21917782]
 25. White SK, Sado DM, Flett AS, Moon JC. Characterising the myocardial interstitial space: the clinical relevance of non-invasive imaging. *Heart*. 2012; 98:773–779. [PubMed: 22422587]
 26. Krug AW, Pojoga LH, Williams GH, Adler GK. Cell membrane-associated mineralocorticoid receptors? *Hypertension*. 2011; 57:1019–1025. [PubMed: 21482958]
 27. Joffe HV, Kwong RY, Gerhard-Herman MD, Rice C, Feldman K, Adler GK. Beneficial effects of eplerenone versus hydrochlorothiazide on coronary circulatory function in patients with diabetes mellitus. *J Clin Endocrinol Metab*. 2007; 92:2552–2558. [PubMed: 17488800]
 28. Briones AM, Nguyen Dinh Cat A, Callera GE, Yogi A, Burger D, He Y, Corrêa JW, Gagnon AM, Gomez-Sanchez CE, Gomez-Sanchez EP, Sorisky A, Ooi TC, Ruzicka M, Burns KD, Touyz RM. Adipocytes produce aldosterone through calcineurin-dependent signaling pathways. *Hypertension*. 2012; 59:1069–1078. [PubMed: 22493070]

29. Goodfriend TL, Egan BM, Kelley DE. Plasma aldosterone, plasma lipoproteins, obesity and insulin resistance in humans. *Prostaglandins Leukot Essent Fatty Acids*. 1999; 60:401–405. [PubMed: 10471129]
30. Li JH, Huang XR, Zhu HJ, Oldfield M, Cooper M, Truong LD, Johnson RJ, Lan HY. Advanced glycation end products activate Smad signaling via TGF- β -dependent and -independent mechanisms: implications for diabetic renal and vascular disease. *FASEB J*. 2004; 18:176–178. [PubMed: 12709399]

3-Month Run-In

- Begin enalapril 20mg daily
- Taper off other anti-hypertensives
- Add/increase amlodipine 5-10mg daily for systolic blood pressure ≥ 140 mmHg
- Optimize glycemic control, Hgb A1c goal $\leq 7\%$
- Add simvastatin 20mg daily for dLDL > 100 mg/dL if not on a statin
- Start 250 mEq Na⁺ isocaloric diet 4 days prior to inpatient procedures

Inpatient Study Procedures

Figure 1.
Detailed study schema.

Figure 2.

Association between aldosterone and myocardial ECV. Myocardial ECV was positively associated with 24-hour urinary aldosterone when treated continuously ($r = 0.37, p = 0.01$) (A) and when dichotomized by median 24-hour urinary aldosterone ($p = 0.03$) (B). Myocardial ECV was also related to the change in aldosterone with angiotensin II infusion ($r = 0.35, p = 0.02$) (C).

Table 1
Characteristics of the study population (n = 53)

Characteristic	Value
Age (yrs)	53 ± 7
Men	36 (68%)
European American	34 (64%)
African American	15 (28%)
Other	4 (8%)
Body mass index (kg/m ²)	32.2 ± 4.34
Blood pressure (mm Hg)	
Systolic	126 ± 14
Diastolic	76 ± 9
Duration of T2DM (yrs)	5 (2–10)
Number of diabetic medications	
Diet controlled	8 (15%)
Monotherapy	19 (36%)
2	26 (49%)
Antihypertensive medications	
Enalapril	53 (100%)
Amlodipine	13 (24%)
Statin use	40 (75%)
Fasting laboratory data *	
Blood glucose (mg/dl)	110 ± 21
Total cholesterol (mg/dl)	148 ± 31
Low-density lipoprotein cholesterol (mg/dl)	81 ± 24
High-density lipoprotein cholesterol (mg/dl)	43 ± 11
Triglycerides (mg/dl)	124 ± 72
Other laboratory data	
HbA _{1c} (%)	6.8 ± 0.7
Corrected creatinine clearance (ml/min/1.73 m ²)	107 ± 21
Serum thyrotropin (mIU/ml)	1.50 ± 0.70

Data are expressed as mean ± SD, as number (percentage), or as median (interquartile range).

* Fasting laboratory data obtained on the day of imaging studies.

Table 2
Renin-angiotensin-aldosterone system and blood pressure response to angiotensin II

Variable	Value
Before angiotensin II infusion	
Urinary aldosterone ($\mu\text{g}/24\text{ h}$)	6.28 (3.32–9.48)
Urinary sodium ($\text{mmol}/24\text{ h}$)	279 ± 67
Urinary potassium ($\text{mmol}/24\text{ h}$)	94 ± 20
Urinary creatinine ($\text{mg}/24\text{ h}$)	$1,644 \pm 398$
Serum aldosterone (ng/dl)	2.49 (2.49–4.03)
Plasma renin activity ($\text{ng}/\text{ml}/\text{h}$)	0.6 (0.1–2.3)
Serum potassium (mmol/L)	4.2 ± 0.23
Mean arterial pressure (mm Hg)	95 ± 11
After angiotensin II infusion	
Change in serum aldosterone (ng/dl)	5.80 (3.35–8.83)
Change in mean arterial pressure (mm Hg)	16 ± 7

Data are expressed as mean $\pm$ SD or as median (interquartile range).

Table 3
Cardiovascular imaging

Variable	Value
Cardiac magnetic resonance imaging	
ECV	0.36 ± 0.05
Left ventricular mass (g)	100 ± 29
Left atrial volume index (ml/m ²)	28.6 ± 8.9
Left ventricular ejection fraction (%)	59.8 ± 5.9
Left ventricular end-systolic volume (ml)	60.5 ± 17.8
Left ventricular end-diastolic volume (ml)	147.0 ± 33.2
Right ventricular ejection fraction (%)	50.9 ± 5.8
Cardiac PET	
Myocardial blood flow at rest (ml/min/g)	0.74 ± 0.21
Myocardial blood flow at rest (corrected) (ml/min/g)	0.83 ± 0.21
Myocardial blood flow during stress (ml/min/g)	1.96 ± 0.43
CFR (corrected)	2.51 ± 0.83
Mitral inflow	
E (m/s)	0.71 ± 0.14
A (m/s)	0.66 ± 0.16
Deceleration time (ms)	221 ± 38
E/A ratio	1.13 ± 0.28
Tissue Doppler imaging	
e' (m/s)	0.11 ± 0.03
E/e' ratio	6.74 ± 1.74

Data are expressed as mean $\pm$ SD.