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# Mineralocorticoid Receptor Blockade Improves Coronary Microvascular Function in Individuals With Type 2 Diabetes

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**Reduced coronary flow reserve (CFR), an indicator of coronary microvascular dysfunction, is seen in type 2 diabetes mellitus (T2DM) and predicts cardiac mortality. Since aldosterone plays a key role in vascular injury, the aim of this study was to determine whether mineralocorticoid receptor (MR) blockade improves CFR in individuals with T2DM. Sixty-four men and women with well-controlled diabetes on chronic ACE inhibition (enalapril 20 mg/day) were randomized to add-on therapy of spironolactone 25 mg, hydrochlorothiazide (HCTZ) 12.5 mg, or placebo for 6 months. CFR was assessed by cardiac positron emission tomography at baseline and at the end of treatment. There were significant and similar decreases in systolic blood pressure with spironolactone and HCTZ but not with placebo. CFR improved with treatment in the spironolactone group as compared with the HCTZ group and with the combined HCTZ and placebo groups. The increase in CFR with spironolactone remained significant after controlling for baseline CFR, change in BMI, race, and statin use. Treatment with spironolactone improved coronary microvascular function, raising the possibility that MR blockade could have beneficial effects in preventing cardiovascular disease in patients with T2DM.**

Individuals with type 2 diabetes mellitus (T2DM) have an increased risk of cardiovascular disease (CVD) (1). Diabetes accelerates coronary artery atherosclerosis and impairs coronary microvascular function (2,3). In the absence of significant epicardial coronary artery disease, patients with T2DM and impaired myocardial blood flow (MBF) (coronary flow reserve [CFR] below median) have a 3.2-fold increased rate of cardiac death in comparison with those with CFR above median (4). Thus, CFR is a good intermediate marker of CVD.

Aldosterone plays a critical role in the pathophysiology of CVD. In heart failure patients, mineralocorticoid receptor (MR) blockade improves cardiac morbidity and mortality (5). MR blockade reduces coronary microvascular damage in a rodent model of angiotensin II–dependent cardiovascular injury (6), suggesting that excess MR activation promotes injury to the coronary microvasculature. Further, preclinical studies demonstrate that excess MR activation contributes to vascular injury in obesity and diabetes (7–10).

We hypothesized that in humans with T2DM without clinical ischemic heart disease, addition of MR blockade to chronic ACE inhibitor (ACEI) therapy would improve coronary microvascular function, as assessed by quantitative positron emission tomography (PET) measures of CFR.

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## RESEARCH DESIGN AND METHODS

### Patient Population

Individuals with T2DM, aged 18–70 years, were enrolled in a double-blind, randomized, controlled study (clinicaltrials.gov NCT00865124). Exclusion criteria included the following: coronary, cerebrovascular, or peripheral vascular or renal disease (estimated glomerular filtration rate  $<60$  mL/min/1.73 m<sup>2</sup>); bronchospastic lung disease; gout if not on hydrochlorothiazide (HCTZ); serum potassium  $>5.0$  mmol/L; current smoker; pregnancy; use of potassium-sparing diuretics, oral contraceptives, hormone replacement therapy, or rosiglitazone; uncontrolled hypertension (systolic blood pressure [BP]  $>160$  mmHg or diastolic BP  $>100$  mmHg); ACEI intolerance; systolic BP  $<105$  mmHg off antihypertensive therapy; and other major medical illnesses. Partners HealthCare Institutional Review Board approved the protocol, and all participants provided written informed consent.

### Study Procedures

Participants completed a 3-month run-in phase followed by a baseline assessment, randomization to drug treatment, and posttreatment assessment. With initiation of the 3-month run-in, participants were placed on enalapril 20 mg daily and tapered off other antihypertensive medications except amlodipine 5–10 mg daily that was added for systolic BP  $\geq 140$  mmHg. Antidiabetic medications were adjusted to achieve a goal hemoglobin A1C (HbA<sub>1c</sub>)  $\leq 7\%$ . Simvastatin 20 mg daily was added for direct LDL  $>100$  mg/dL if participant was statin tolerant not on a statin. Participants measured BP and blood glucose daily and communicated readings to study staff weekly.

### Baseline and 6-Month Assessment Protocol

Four days prior to and during the 2-day in-patient admission, participants consumed a caffeine-free, isocaloric diet (250 mmol/day Na<sup>+</sup>, 100 mmol/day K<sup>+</sup>, 1,000 mg/day Ca<sup>++</sup>, 300 mg/day Mg<sup>++</sup>, and at least 30% carbohydrate by calories). Participants stopped amlodipine 36 h prior to admission, and antidiabetic medications were adjusted to avoid hypoglycemia. Upon admission after an overnight fast, supine BP was measured every 5 min for 30 min, and the average was used for analysis. Blood samples were collected for HbA<sub>1c</sub>, glucose, and lipids, and 24-h urine collection for sodium, creatinine, and aldosterone was initiated. Participants underwent echocardiography for assessment of diastolic function, cardiac PET scan for determination of CFR (ratio of adenosine-stimulated to rest MBF), and cardiac MRI scan to determine left ventricular (LV) mass index and myocardial extracellular volume using techniques described previously (11). The following morning, after being supine and fasting from midnight onwards, blood was drawn for potassium, sodium, plasma renin activity, angiotensin II, and aldosterone. Assays were performed as previously described (11); angiotensin II was measured using ALPCO Immunoassay (Salem, NH).

### Drug Treatment

Participants without evidence of cardiac ischemia or prior myocardial infarction on baseline imaging were randomized 1:1:1 to 6 months of add-on daily therapy with one of three treatments: spironolactone 25 mg, HCTZ 12.5 mg with KCl 10 mEq, or matching placebo. To accommodate a funding reduction and considering the study rationale where the primary outcome was the effect of spironolactone versus HCTZ on CFR, the placebo arm was stopped after 80% of participants were randomized. All participants and study staff (except Investigational Drug Service, which was responsible for randomization) were blinded to treatment. Plasma potassium was measured at 1, 2, 4, 8, 16, and 24 weeks. A posttreatment assessment, which was identical to the baseline assessment, was completed at 6 months.

### Statistical Methods

Comparisons of treatment arms for demographic and other baseline variables were performed using Wilcoxon rank sum tests or  $\chi^2$  tests. The primary outcome was CFR, and the overall analysis framework was a repeated measures ANCOVA covering baseline and 6-month visit data. Spironolactone versus HCTZ was considered primary in the design, and HCTZ and placebo were expected to be similar. In addition to baseline CFR, covariates were selected from among those associated with vascular function (e.g., statin use, HbA<sub>1c</sub>, BMI, race, and age). All subsets were tested, retaining only those covariates contributing significantly to the multiple variable model. Diastolic function ( $E/e'$ ), as a measure of the impact of CFR on cardiac function, was a secondary outcome. Data are presented as means  $\pm$  SD. All statistical analyses were performed with SAS version 9.3 (SAS Institute, Cary, NC).

## RESULTS

Prior to randomization, 24 of 93 participants who entered the run-in period were excluded. Twenty-one met the following prespecified exclusion criteria: 1) evidence of ischemia or prior myocardial infarction on baseline cardiac PET and/or MRI imaging ( $n = 6$ ); 2) medical condition (lung mass, shortness of breath, seizures, uninephrectomy, atypical chest pain, kidney stones, or liver lesions) ( $n = 7$ ); 3) ACEI intolerance ( $n = 3$ ); 4) inability to meet blood glucose goals ( $n = 2$ ); 5) incarcerated ( $n = 1$ ); 6) enrolled in another study ( $n = 1$ ); and 7) illicit drug use ( $n = 1$ ). Two participants withdrew consent and one was lost to follow-up. Thus, 69 participants were randomized to drug treatment. Ninety-three percent (64 participants) completed both baseline and posttreatment assessments and are included in the analysis (Supplementary Fig. 1).

Patient characteristics and baseline laboratory data for each treatment group are displayed in Table 1. All participants had a normal LV ejection fraction ( $>50\%$ ), normal LV mass index ( $\leq 80$  g/m<sup>2</sup>), and normal diastolic function ( $E/e' \leq 15$ ). Investigational Drug Service halved the enalapril and spironolactone doses in one participant with

**Table 1—Characteristics of study population at baseline assessment**

	Spironolactone group	HCTZ group	Placebo group
<i>n</i>	23	24	17
Mean age (years)	56 ± 6	53 ± 7	55 ± 10
Male ( <i>n</i> [%])	17 (74)	13 (54)	10 (59)
Race ( <i>n</i> [%])			
Caucasian	17 (74)	17 (71)	8 (47)
African American	4 (17)	6 (25)	7 (41)
Other	2 (9)	1 (4)	2 (12)
BMI (kg/m <sup>2</sup> )	31.4 ± 4.5	32.5 ± 5.4	31.3 ± 4.2
BP (mmHg)			
Systolic	123 ± 11	124 ± 14	125 ± 13
Diastolic	75 ± 7	74 ± 9	77 ± 10
Duration of diabetes (years)	9 ± 7	7 ± 6	7 ± 6
Diabetes medications ( <i>n</i> [%])			
Metformin	16 (70)	20 (83)	16 (94)
Insulin	3 (13)	3 (13)	3 (18)
Sulfonylurea	7 (30)	7 (29)	7 (41)
Thiazolidinedione	1 (4)	1 (4)	0 (0)
GLP-1 analog	1 (4)	1 (4)	2 (12)
Dipeptidyl peptidase-4 inhibitor	1 (4)	0 (0)	0 (0)
Antihypertensive medications ( <i>n</i> [%])			
Enalapril	23 (100)	24 (100)	17 (100)
Amlodipine	7 (30)	6 (25)	4 (24)
Statin use ( <i>n</i> [%])	17 (74)	20 (83)	11 (65)
Fasting laboratory data			
Blood glucose (mg/dL)	105 ± 23	106 ± 25	105 ± 24
Total cholesterol (mg/dL)	150 ± 35	153 ± 24	139 ± 24
LDL cholesterol (mg/dL)	81 ± 27	82 ± 20	75 ± 21
HDL cholesterol (mg/dL)	47 ± 12	45 ± 12	41 ± 12
Triglycerides (mg/dL)	113 ± 39	130 ± 77	120 ± 72
HbA <sub>1c</sub> (%)	6.6 ± 0.4	7.0 ± 0.9	7.0 ± 0.7
Serum sodium (mmol/L)	139.5 ± 2.1	139.0 ± 2.1	139.2 ± 1.5
Serum potassium (mmol/L)	4.2 ± 0.3	4.3 ± 0.3	4.2 ± 0.2
Creatinine clearance rate (mL/min)	129 ± 30	126 ± 26	124 ± 38
Plasma renin activity (ng/mL/h)	1.5 ± 2.2	2.3 ± 3.3	2.4 ± 3.9
Serum angiotensin II (pg/mL)	18.01 ± 8.89	22.59 ± 6.17	19.15 ± 5.82
Serum aldosterone (ng/dL)	3.13 ± 1.46	3.21 ± 1.19	3.84 ± 2.14
Echocardiography			
Mitral inflow			
E (m/s)	0.76 ± 0.14	0.74 ± 0.14	0.68 ± 0.13
A (m/s)	0.68 ± 0.17	0.66 ± 0.16	0.67 ± 0.17
Deceleration time (ms)	220.38 ± 37.94	212.04 ± 37.36	216.88 ± 31.75
E/A ratio	1.15 ± 0.23	1.13 ± 0.29	1.05 ± 0.23
Tissue Doppler imaging			
e' (m/s)	0.11 ± 0.02	0.11 ± 0.03	0.11 ± 0.02
E/e' ratio	7.24 ± 2.00	6.92 ± 1.59	6.58 ± 1.68
Cardiac MRI			
LV mass index (g/m <sup>2</sup> )	46.4 ± 12.2	43.6 ± 10.9	46.7 ± 11.2
LV ejection fraction (%)	61.4 ± 4.5	60.2 ± 7.0	60.4 ± 5.0
Myocardial extracellular volume	0.36 ± 0.06	0.34 ± 0.04	0.38 ± 0.04
24-h Urine results			
Sodium (mmol/24 h)	291 ± 74	258 ± 72	256 ± 77
Creatinine (mg/24 h)	1,599.7 ± 407.5	1,510.4 ± 326.9	1,537.8 ± 466.1
Potassium (mmol/24 h)	97.8 ± 15.0	91.1 ± 19.1	88.8 ± 29.1
Aldosterone (μg/24 h)	6.49 ± 6.46	7.19 ± 5.16	6.17 ± 4.99

Data are expressed as mean ± SD unless stated otherwise. There were no significant differences between treatment groups prerandomization.

a 4-week plasma potassium >5.5 mmol/L; no further hyperkalemia was noted and study staff remained blinded as to treatment. One participant in the HCTZ group had enalapril increased to 40 mg daily for the final 4 months by his cardiologist.

Average treatment duration was 5.9 ± 0.5 months for spironolactone, 5.6 ± 0.9 months for HCTZ, and 5.7 ± 0.3 months for placebo (P = NS). Table 2 shows the changes in study parameters between visits. There were significant and similar decreases in systolic BP with spironolactone and HCTZ. Serum potassium increased significantly with spironolactone but not with other treatments. There were no significant changes from baseline in HbA<sub>1c</sub>, total cholesterol, HDL, calculated LDL (cLDL), triglycerides, and BMI with any treatment. Diastolic function, LV mass index, LV ejection fraction, and myocardial extracellular volume were unaffected by treatment.

**MBF and Flow Reserve**

Complete MBF and CFR data (Table 3) were available in 60 participants; four participants had technical difficulties

with the dynamic PET images (spironolactone, n = 1; HCTZ, n = 2; and placebo, n = 1). There was a significantly greater increase in CFR from baseline to posttreatment in the spironolactone group as compared with the HCTZ group (0.33 vs. -0.10, P = 0.04) and as compared with the combined HCTZ and placebo groups (0.33 vs. -0.05, P = 0.047).

An ANCOVA model predicting CFR posttreatment revealed a significant effect of treatment (P = 0.03), taking into account race (P = 0.07), statin use (P = 0.03), baseline CFR (P < 0.0001), and BMI change over the treatment period (P = 0.0002). Factors not contributing to the model included age, sex, insulin use, amlodipine use, duration of diabetes, baseline BMI, hypertensive status at screen, and either the baseline or change with treatment of HbA<sub>1c</sub>, BP, rest rate pressure product assessed during PET, potassium, TSH, total cholesterol, cLDL, and triglycerides. A priori treatment group contrasts demonstrated that CFR increased with spironolactone significantly more than with HCTZ (P = 0.02), placebo (P = 0.05), and the combined HCTZ/placebo

**Table 2—Change in study parameter with treatment†**

	Spironolactone group	HCTZ group	Placebo group	P value spiro vs. HCTZ	P value spiro vs. HCTZ + placebo
n	23	24	17		
Δ BMI (kg/m <sup>2</sup> )	0.07 ± 0.9	-0.06 ± 1.02	-0.11 ± 1.25	0.59	0.59
Δ BP (mmHg)					
Systolic	-7 ± 13*	-5 ± 10*	-1 ± 12	0.56	0.25
Diastolic	-5 ± 7‡	-2 ± 7	-2 ± 7	0.07	0.09
Δ Fasting laboratory data					
Glucose (mg/dL)	10.5 ± 23.9	8.3 ± 25.1	2.7 ± 11.8	0.99	0.52
Total cholesterol (mg/dL)	3.6 ± 32.1	2.4 ± 30.2	13.8 ± 32.5	0.24	0.12
LDL cholesterol (mg/dL)	2.9 ± 25.4	1.6 ± 25.2	9.7 ± 30.3	0.46	0.36
HDL cholesterol (mg/dL)	-2.0 ± 5.6	1.6 ± 5.0	2.8 ± 6.1	0.05	0.01
Triglycerides (mg/dL)	13.4 ± 37.7	1.9 ± 46.9	11.8 ± 48.3	0.74	0.65
HbA <sub>1c</sub> (%)	0.16 ± 0.39	0.08 ± 0.75	0.06 ± 0.45	0.94	0.64
Serum sodium (mmol/L)	-1.5 ± 2.6	-0.3 ± 2.1	0.0 ± 2.8	0.09	0.04
Serum potassium (mmol/L)	0.22 ± 0.3†	0.03 ± 0.3	0.04 ± 0.2	0.02	0.005
Δ 24-h Urine sodium (mmol/24 h)	-19.6 ± 76.9	3.9 ± 78.5	16.5 ± 71.3	0.31	0.15
Δ Creatinine clearance (mL/min)	-2.6 ± 21.4	-1.0 ± 20.4	-0.8 ± 13.0	0.96	0.98
Cardiac MRI					
Δ LV mass index (g/m <sup>2</sup> )	6.03 ± 22.50	4.81 ± 26.24	8.00 ± 24.05	1.00	0.91
Δ LV ejection fraction (%)	-0.87 ± 5.83	0.32 ± 8.25	1.08 ± 5.20	0.22	0.16
Δ Extracellular volume	0.00 ± 0.08	0.00 ± 0.04	0.00 ± 0.03	0.64	0.94
Echocardiography					
Mitral inflow					
Δ E (m/s)	-0.03 ± 0.15	-0.02 ± 0.09	0.01 ± 0.09	0.87	0.66
Δ A (m/s)	-0.02 ± 0.12	-0.02 ± 0.11	-0.01 ± 0.12	0.84	0.88
Δ Deceleration time (ms)	-17.93 ± 60.90	8.18 ± 61.24	7.56 ± 57.34	0.49	0.53
Δ E/A ratio	-0.02 ± 0.32	0.02 ± 0.18	0.04 ± 0.21	0.75	0.58
Tissue Doppler imaging					
Δ e' (m/s)	-0.01 ± 0.02	0.00 ± 0.02	0.00 ± 0.01	0.45	0.47
Secondary outcome					
Δ E/e' ratio	0.02 ± 1.61	0.06 ± 1.35	0.64 ± 1.95	0.65	0.85

†Posttreatment study parameter minus baseline study parameter. \*P < 0.05, indicates significant change from baseline within treatment group. ‡P < 0.01, indicates significant change from baseline within treatment group. spiro, spironolactone.

Table 3—Cardiac PET imaging parameters					
Characteristic	Spirolactone group	HCTZ group	Placebo group	<i>P</i> value spiro vs. HCTZ	<i>P</i> value spiro vs. HCTZ + placebo
<i>n</i>	22	22	16		
Primary outcome					
Change in global CFR (posttreatment minus baseline)*	0.33 ± 0.83	−0.10 ± 0.65	0.02 ± 1.03	0.04	0.05
Additional measures					
Change in rest global MBF (mL · g <sup>−1</sup> · min <sup>−1</sup> )*	−0.07 ± 0.16	0.01 ± 0.11	−0.07 ± 0.13	0.14	0.46
Change in stress global MBF (mL · g <sup>−1</sup> · min <sup>−1</sup> )*	0.06 ± 0.46	−0.02 ± 0.34	−0.08 ± 0.57	0.75	0.54
Prerandomization					
Global CFR	2.77 ± 0.82	2.92 ± 0.52	2.68 ± 0.93		
Rest global MBF (mL · g <sup>−1</sup> · min <sup>−1</sup> )	0.78 ± 0.23	0.70 ± 0.13	0.73 ± 0.20		
Stress global MBF (mL · g <sup>−1</sup> · min <sup>−1</sup> )	2.03 ± 0.38	2.00 ± 0.37	1.81 ± 0.40		
Posttreatment					
Global CFR	3.10 ± 1.04	2.83 ± 0.55	2.69 ± 0.96		
Rest global MBF (mL · g <sup>−1</sup> · min <sup>−1</sup> )	0.72 ± 0.20	0.71 ± 0.11	0.66 ± 0.17		
Stress global MBF (mL · g <sup>−1</sup> · min <sup>−1</sup> )	2.09 ± 0.50	1.98 ± 0.41	1.73 ± 0.61		

\*Posttreatment study parameter minus baseline study parameter. spiro, spironolactone.

groups ( $P = 0.01$ ). HCTZ and placebo had similar effects on CFR ( $P = 0.79$ ). The predicted change (95% CI) in CFR was +0.38 (0.11, 0.65) with spironolactone, −0.10 (−0.38, 0.18) with HCTZ, and −0.05 (−0.38, 0.28) with placebo after multivariable adjustment (Fig. 1).

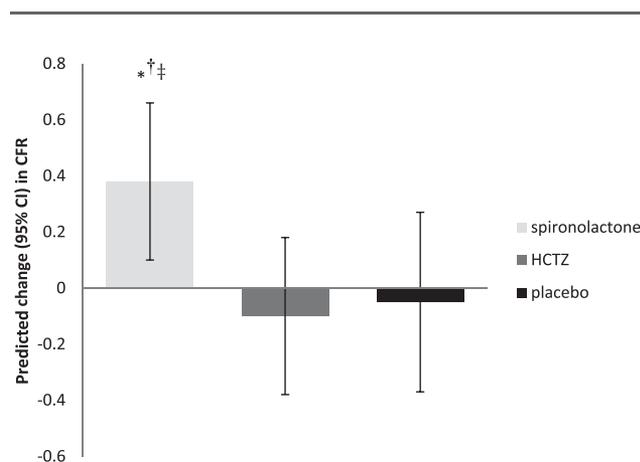
A secondary analysis to identify additional factors predicting posttreatment CFR found that both LV mass index ( $P = 0.03$ ) and baseline serum aldosterone ( $P = 0.02$ ), but not  $E/e'$  ( $P = 0.29$ ), contributed to the ANCOVA model, where the predicted change in CFR with spironolactone (+0.34 [0.06, 0.61]) remained significantly higher than with HCTZ ( $P = 0.006$ ) and combined HCTZ/placebo ( $P = 0.014$ ).

## DISCUSSION

Addition of spironolactone to standard therapy, including ACEI, improved CFR in patients with well-controlled T2DM without clinical ischemic heart disease, suggesting that excess MR activation contributes to coronary microvascular dysfunction in T2DM. Our observation that MR blockade improves CFR is consistent with the current understanding of MR biology. MR is expressed in endothelium, vascular smooth muscle cells (12,13), cardiomyocytes (14), and circulating leukocytes (15). MR activation causes vascular inflammation with increased ROS production and increased expression of PAI-1 and ICAM, vascular damage, vascular dysfunction, and perivascular fibrosis (6,13,15–17). In rodents, excess MR activity is associated with a proinflammatory phenotype involving the intramural coronary circulation and myocardium (18,19).

The improvement in CFR with MR blockade in the current study is consistent with the results of our pilot

study assessing effects of eplerenone in a crossover design on cardiac MRI determinations of CFR in 12 individuals with type 1 diabetes mellitus or T2DM and microalbuminuria (20). Additionally, we report herein that both statin use and weight loss were significant predictors of an improvement in CFR with treatment in our multivariable model; we believe the weight loss association is novel. The CFR benefits contrast with studies in diabetes showing



**Figure 1**—An ANCOVA model predicting the change with treatment in CFR. Spironolactone treatment improved CFR as compared with HCTZ ( $*P = 0.02$ ), placebo ( $†P = 0.05$ ), and combined HCTZ/placebo groups ( $‡P = 0.01$ ). HCTZ and placebo had similar effects on CFR ( $P = 0.79$ ). The predicted adjusted change (95% CI) in posttreatment CFR was 0.38 (0.11, 0.65) with spironolactone, −0.10 (−0.38, 0.18) with HCTZ, and −0.05 (−0.38, 0.28) with placebo. Model adjusts for race, statin use, baseline CFR, and the change in BMI over the treatment period.

no improvement (and in one study a detriment) with MR blockade in forearm vascular endothelial function (20–22), perhaps related to intrinsic differences in the regulation of the coronary versus peripheral vasculature.

The strengths of this physiological study include the well-controlled cardiometabolic phenotype, addition of MR blockade to standard medical therapy, comparison of MR blockade to another antihypertensive medication and to placebo, and the assessment of coronary microvascular function under highly controlled conditions that controlled for possible confounders such as dietary sodium, low or high glucose levels, lipid levels, and BP. We hypothesize that since this study excluded patients with ischemic heart disease, the improvements we saw in CFR with MR blockade reflect improvement in microvascular function. Furthermore, since 87% of our 69 participants had interpretable pre- and posttreatment CFR data, our results are likely applicable to patients with clinical characteristics similar to our study population.

Limitations include the lack of assessment of cardiovascular events, sample size, and duration of this physiological study. Further, although spironolactone improved CFR as compared with HCTZ and as compared with combined HCTZ and placebo treatments, we cannot rule out the possibility that HCTZ may have impaired CFR. We did not see an effect of MR blockade on diastolic function, possibly related to the lack of diastolic dysfunction at baseline, or on myocardial extracellular volume, possibly because cardiac remodeling takes longer than 6 months. Due to spironolactone's effects on potassium homeostasis, we restricted this study to individuals with good renal function. Novel MR antagonists, which preserve the cardiovascular benefits of spironolactone but lack the adverse potassium effects, are currently in development and could prove to be useful in patients with diabetes (23). Also, selective MR antagonists, like eplerenone, may prove to be beneficial in patients who cannot tolerate the antiandrogen or antiprogesterone effects of spironolactone. Finally, CFR is an intermediate marker for cardiovascular outcomes. It remains to be determined if there is a cause and effect relationship between CFR and cardiovascular health, and whether increasing CFR through administration of an MR antagonist will lead to reductions in cardiovascular events.

This proof-of-concept study demonstrating improvement in CFR with MR blockade may have important clinical implications. Impaired CFR is associated with increased mortality in patients with no evidence for CAD (4). Thus, it is possible that MR antagonists over and above ACEI/angiotensin receptor blocker therapy may lead to significant cardiovascular benefits in patients with diabetes. Future studies are needed to address this possibility.

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**Author Contributions.** R.G. recruited participants, conducted the study, interpreted data, and wrote the manuscript. A.D.R. recruited participants, helped in clinical management of study participants, conducted the study, and interpreted data. M.B.-G. helped in conducting the study and collected data. S.H. performed statistical analysis. C.F. helped with PET imaging analysis. R.V.S. performed and interpreted MRI scans. M.J.-H. analyzed MRI data. R.Y.K. directed MRI imaging. M.F.D.C. directed PET imaging and analysis. G.K.A. conceived the idea, procured funding, directed and conducted the study, interpreted data, and wrote the manuscript. All authors contributed to the manuscript and take full responsibility for its originality. G.K.A. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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