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ORIGINAL RESEARCH

Spironolactone Reduces Aortic Stiffness in Patients With Resistant Hypertension Independent of Blood Pressure Change

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BACKGROUND: Aortic stiffness is an independent predictor of cardiovascular events in patients with arterial hypertension. Resistant hypertension is often linked to hyperaldosteronism and associated with adverse outcomes. Spironolactone, a mineralocorticoid receptor antagonist, has been shown to reduce both the arterial blood pressure (BP) and aortic stiffness in resistant hypertension. However, the mechanism of aortic stiffness reduction by spironolactone is not well understood. We hypothesized that spironolactone reduces aortic stiffness in resistant hypertension independently of BP change.

METHODS AND RESULTS: Patients with uncontrolled BP (≥140/90 mm Hg) despite use of ≥3 antihypertensive medications (including diuretics) were prospectively recruited. Participants were started on spironolactone at 25 mg/d, and increased to 50 mg/d at 4 weeks while other antihypertensive medications were withdrawn to maintain constant mean BP. Phase-contrast cardiac magnetic resonance imaging of the ascending aorta was performed in 30 participants at baseline and after 6 months of spironolactone treatment to measure aortic pulsatility, distensibility, and pulse wave velocity. Pulse wave velocity decreased (6.3±2.3 m/s to 4.5±1.8 m/s, $P<0.001$) and pulsatility and distensibility increased (15.9%±5.3% to 22.1%±7.9%, $P<0.001$; and 0.28%±0.10%/mm Hg to 0.40%±0.14%/mm Hg, $P<0.001$, respectively) following 6 months of spironolactone.

CONCLUSIONS: Our results suggest that spironolactone improves aortic properties in resistant hypertension independently of BP, which may support the hypothesis of an effect of aldosterone on the arterial wall. A larger prospective study is needed to confirm our findings.

Key Words: aorta ■ hyperaldosteronism ■ resistant hypertension ■ spironolactone

Hypertension is the most prevalent risk factor for other cardiovascular disease, stroke, and renal disease, and one of the leading causes of death in the United States.[1] Using the new thresholds from the 2017 American College of Cardiology/American Heart Association guidelines, the prevalence of hypertension is 45.6% among US adults.[2] A substantial proportion of these patients do not achieve a target goal of <130/80 mm Hg.[3] Various factors account for poor blood pressure (BP) control, including lack of treatment, nonadherence to recommended treatment, and resistance to guideline-directed medical therapy.[4,5] Resistant hypertension (RHTN) is defined as BP that remains above goal despite concurrent use of 3 antihypertensive agents of different classes, of which one is ideally a diuretic, all prescribed at maximum recommended or maximally tolerated dosage.[6] Among adults with treated hypertension, apparent RHTN occurs in 12% to 15% of population-based and 15% to 18% of clinic-based reports.[7,8] Patients with hypertension have a high mortality rate, and aortic stiffness (AS) is an independent predictor of all-cause and

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CLINICAL PERSPECTIVE

What Is New?

- Data suggest that spironolactone improves aortic properties in patients with resistant hypertension independently of blood pressure change.

What Are the Clinical Implications?

- Spironolactone is recommended in patients with resistant hypertension and thus results from our study are directly relevant to clinical practice.
- Our finding that the improvement of arterial elastic properties in patients with hypertension undergoing aldosterone antagonist treatment can occur independently of its effect on blood pressure, if confirmed in a larger cohort, may lead to reconsideration of approaches for evaluation of the therapeutic efficacy of spironolactone in clinical practice.

Nonstandard Abbreviations and Acronyms

AD	aortic distensibility
AP	aortic pulsatility
AS	aortic stiffness
PAC	plasma aldosterone concentration
PWV	pulse wave velocity
RHTN	resistant hypertension

cardiovascular mortality.[9,10] Moreover, the presence of AS predicts cardiovascular events in the general population, even in the absence of hypertension or cardiovascular disease.[11,12]

Hyperaldosteronism (defined as plasma renin activity <1.0 ng/mL per hour and a urinary aldosterone level >12 µg/24 h during high urinary sodium excretion [>200 mEq/24 h]) is a common cause of RHTN.[13,14] Aldosterone is both a key hormone for volume homeostasis and a contributor to target organ damage.[15] Aldosterone excess increases aortic wall stiffness independent of mechanical stress.[15] Spironolactone is a mineralocorticoid receptor antagonist that has been shown to reduce mortality in patients with heart failure, and some of this benefit has been attributed to extrarenal effects on inhibiting fibrosis.[16–18] As spironolactone has been shown to reduce collagen synthesis and fibrosis,[16] we hypothesize that it has a beneficial effect on AS. However, any such effect cannot be easily demonstrated in the clinical setting because spironolactone is a potent antihypertensive agent often used to treat hypertension, including RHTN.[19,20] The antihypertensive effects of spironolactone may confound efforts to investigate vascular actions, which may be independent of BP lowering. In fact, BP reduction itself appears to improve aortic compliance in patients with hypertension.[21] Mahmud and Feely[22] have shown that, compared with thiazide diuretics, spironolactone leads to greater reduction in BP as well as improvement in arterial stiffness in patients with hypertension. As measures of AS are directly correlated with the BP profile, the study by Mahmud et al was not able to identify any BP-independent effect of spironolactone on AS.

To test our hypothesis that spironolactone reduces AS independent of its effect on BP, we analyzed data from a prospective study in patients with RHTN.[23] Spironolactone was introduced and force-titrated upward while other antihypertensive medications were withdrawn, to maintain the patient's original BP level. The AS indicators pulse wave velocity (PWV), aortic distensibility (AD), and aortic pulsatility (AP) were measured using cardiac magnetic resonance (CMR) imaging at baseline and after 6 months of spironolactone treatment in order to determine the spironolactone-dependent, BP-independent changes in aortic properties.

METHODS

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Study Population and Study Design

Study participants (n=30, 54±7 years) were from a group of 45 consecutive patients referred for RHTN to the University of Alabama at Birmingham (UAB) Hypertension Clinic, who agreed to participate in 6-month follow-up research on spironolactone treatment (including CMR imaging) for assessment of the role of hyperaldosteronism in cardiac volume overload in RHTN.[23] Those with dedicated phase-contrast velocity mapping CMR imaging of the ascending aorta were included in the present analysis. RHTN was defined as BP >140/90 mm Hg at 2 clinic visits in spite of use of 3 antihypertensive medications, including a diuretic, at pharmacologically effective doses. Patients with a history of heart failure, primary hyperaldosteronism before enrollment, chronic kidney disease, or chronic steroid therapy were excluded. Patients with secondary causes of hypertension other than hyperaldosteronism, such as renovascular hypertension, pheochromocytoma, or Cushing syndrome were also excluded. At the time of enrollment, all patients had been taking a stable antihypertensive regimen

for ≥4 weeks. Clinical, biochemical and CMR imaging studies were performed at baseline and after 6 months of spironolactone treatment. In the present analysis, 5 participants were excluded as their baseline or 6-month CMR imaging studies lacked the phase-contrast images used for assessing AS (Figure 1). Another 10 patients did not complete the 6-month follow-up study after enrolling because of spironolactone intolerance (n=1), increased creatinine (n=1), hyperkalemia (n=1), uncontrolled BP (n=3), noncompliance to the study protocol (n=2), claustrophobia to CMR imaging study (n=1), and voluntary withdraw for adrenal venous sampling and adrenalectomy (n=1) as briefly previously described.[23] Demographic, baseline clinical characteristics, and measurements of the excluded and included participants were similar (Table S1). The study was approved by UAB's institutional review board and was conducted according to institutional guidelines. All participants provided written informed consent.

Spironolactone Treatment and Withdrawal of Other Antihypertensive Drugs

All participants were started on spironolactone 25 mg/d in addition to other antihypertensive medications and force-titrated to 50 mg/d at 4 weeks. After addition of spironolactone, other antihypertensive medications were withdrawn as needed to maintain constant BP. The sequence of withdrawal was as follows: centrally acting agents or vasodilators first, followed by β-blockers, calcium channel blockers, and renin-angiotensin system blockers.

BP Measurements

BP was noninvasively measured using a manual brachial mercury sphygmomanometer and an appropriately sized cuff after 5 minutes of rest. During each CMR imaging study, BP measurements were performed twice, before scanning and immediately after completion of scanning; these were performed in the scan room but outside the CMR imaging instrument. The average of 2 readings was recorded and used for analysis. The average pulse pressure (PP) was calculated as the difference in average systolic and average diastolic BP.

Biochemical Testing

Plasma aldosterone concentration (PAC), plasma renin activity (PRA), brain natriuretic peptide, serum potassium, and creatinine levels were measured in the morning between 8 AM and 9 AM with the patient in the upright sitting position. A 24-hour urine collection for aldosterone, cortisol, sodium, and creatinine was performed.

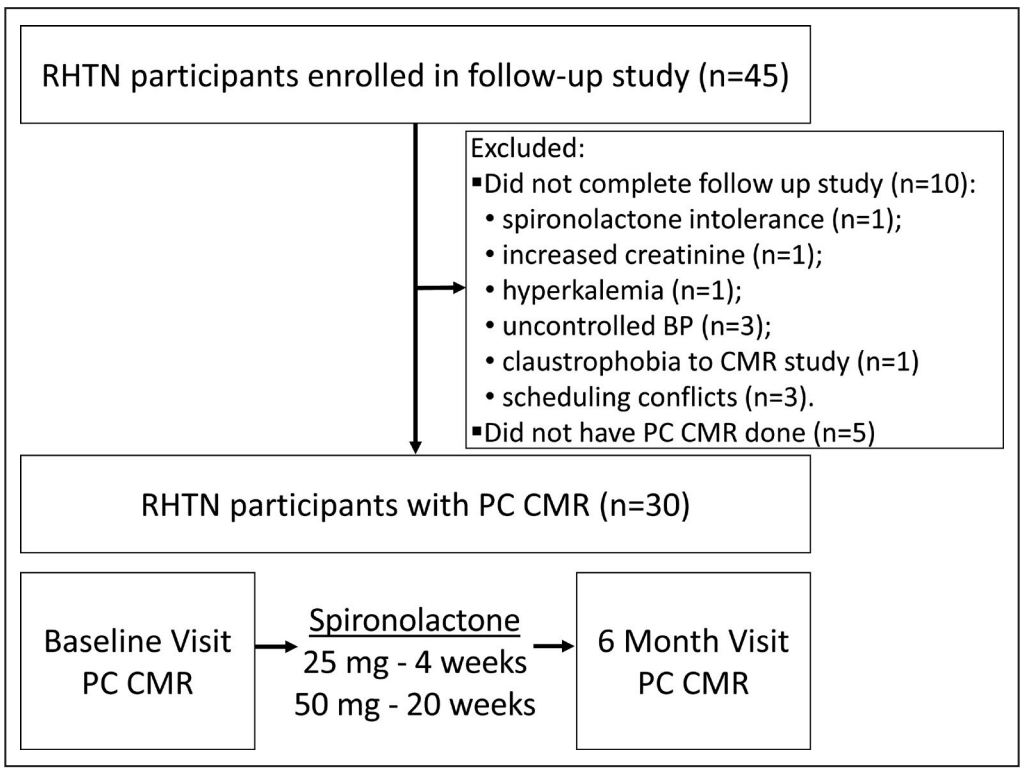

Figure 1. Flowchart of the study.
BP indicates blood pressure; CMR, cardiac magnetic resonance; PC, phase-contrast; and RHTN, resistant hypertension. Please see Methods for details.

PAC, PRA, and 24-hour urinary aldosterone was analyzed using liquid chromatography-tandem mass spectrometry (Mayo Medical Laboratories) with laboratory reference levels as follows: high PAC ≥16 ng/dL, high 24-hour urinary aldosterone ≥12 µg/24-hour, and suppressed PRA <1 ng/mL per hour.

CMR Imaging

CMR imaging was performed with a 1.5-T scanner optimized for cardiac imaging (Signa, GE Healthcare) using a 4-element phased-array surface coil and prospective ECG triggering. Cine imaging for left ventricular (LV) volume and function analysis was performed using a rapid steady-state free precession cine sequence (FIESTA; 10 lines per k-space segment). Standard 2- and 4-chamber, and short-axis views were obtained from appropriate scout images. The following typical parameters were used: matrix size, 256×128; field of view, 40×40 cm; slice thickness, 8 mm without gaps; repetition time, 3.9 ms; echo time, 1.6 ms; flip angle 45°; bandwidth 125 Hz/pixel; and typical acquired temporal resolution, 39 ms. Cine images were reconstructed into 20 cardiac phases. Mass Analysis Plus (version 5.1; Medis) software was used to evaluate LV volumes and function.

For analysis of AS, a single end-expiratory breathhold, ECG-gated phase contrast acquisition image plane oriented perpendicularly intersecting the ascending aorta was performed with 32 cardiac phases reconstructed. Contours of the ascending aorta were automatically created during all phases of the cardiac cycle using CAAS MR Flow 1.2 (Pie Medical Imaging). Contours were then manually corrected, if needed. The maximum and minimum cross-section areas of the ascending aorta were measured. Aortic flow-time curves and cross-section area-time curves were extracted for further analysis (Figure 2).

Calculation of the Indices of Arterial Stiffness

Ascending AP, the relative change in lumen area during the cardiac cycle, was calculated using the equation (Figure 2)[24]: AP (%)=(A_{max}–A_{min})/A_{min}×100%; where A_{max} and A_{min} are the maximal and minimal calculated ascending aorta cross-section areas obtained during the cardiac cycle.

Ascending AD, the relative change in lumen area per unit change in pressure, was calculated using the equation[24,25]: AD (%/mm Hg)=AP (%)/PP.

PWV, a rate at which the systolic bolus of blood, pumped from the heart, travels through the vasculature, was calculated using the flow-area (QA) method (Figure 2)[26,27]: PWV=ΔQ/ΔA; where ΔQ is the change of flow across a vessel and ΔA is the change in the cross-sectional ascending aorta area during the acceleration phase of the systole. PWV was calculated from the plot of the ascending aortic flow versus cross-section area as the slope (m/s) of the best-fit line to the early systolic portion of the plot (acceleration phase).[26,27]

Statistical Analysis

Baseline and follow-up CMR imaging measurements, including aorta contouring and calculation of AS parameters, were performed blind without knowledge of other clinical data or the time point. Descriptive analyses were performed to summarize the demographics, comorbidities, and clinical and biochemical characteristics of study participants. Paired *t* test was used to compare values for biochemistry, BP, medications and CMR imaging findings at baseline and at 6 months of spironolactone treatment. Multivariable linear regression models were used to assess the relationship between the CMR imaging–derived indicators of AS in patients with RHTN adjusted separately for demographic factors (age,[28] sex,[29] race,[30] and hyperaldosteronism[14]), for basic cardiac function/hemodynamic factors (LV ejection fraction,[31] LV stroke volume,[32] heart rate,[33] mean arterial pressure,[34] and PP[34]), and for biochemical factors (serum creatinine,[35] serum potassium,[36,37] PRA,[38] and brain natriuretic peptide[39]). All values are represented as mean±SD, and $P<0.05$ was considered statistically significant for 2-sided tests. All analyses were performed using GraphPad Prism version 5.01 (GraphPad Software) and SPSS version 25 (IBM).

RESULTS

Baseline Characteristics

Of 45 participants enrolled, only 30 completed phase-contrast CMR imaging at baseline and at 6-month follow-up (Figure 1). At baseline, the participants were aged 53.7±6.7 years, 20 of 30 (66.7%) were men, and 19 of 30 (63.3%) were of Black race. Eighteen of 30 (60.0%) were diagnosed with hyperaldosteronism, 20 (66.7%) had obstructive sleep apnea, and 9 (30.0%) had diabetes mellitus. The mean±SD values of the group were: PAC (14.1±6.4 ng/dL), PRA (1.0±0.8 ng/mL per hour), PAC/PRA ratio (21.7±19.5), 24-hour urine aldosterone (16.0±7.4 µg), and 24-hour urine sodium (194±75 mmol) (Tables 1 and 2).

Changes in Biochemistry

Serum creatinine, serum potassium, and PRA significantly increased after 6 months of spironolactone treatment, with a reduction of brain natriuretic peptide (Table 2).

Changes in Hemodynamic Parameters

There was no significant change in systolic or diastolic BP, PP, or heart rate from baseline to 6 months of spironolactone treatment (Table 2).

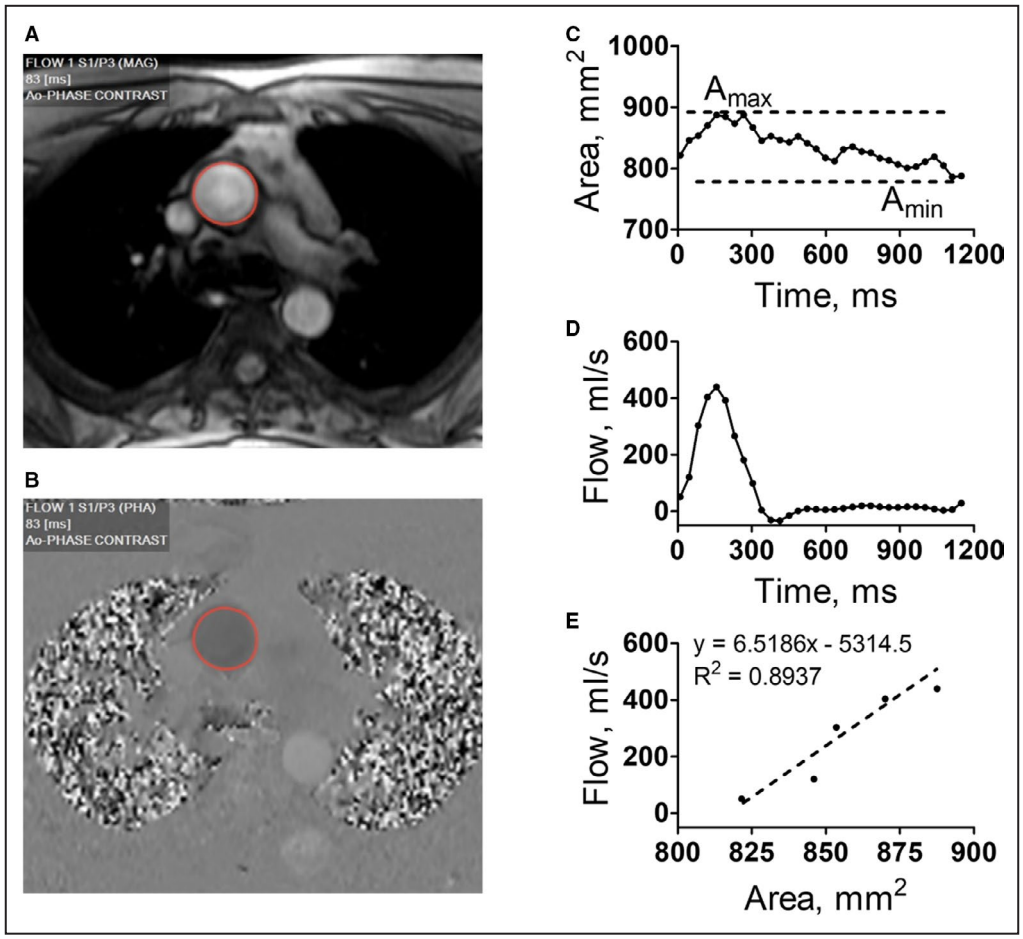

Figure 2. Representative example of phase-contrast cardiac magnetic resonance imaging of the ascending aorta cross-section and measurements of aortic stiffness estimates in a patient. **A** and **B**, Reconstructed magnitude (MAG) and velocity-sensitive phase (PHA) images with automatically detected contours of the ascending aorta (Ao). **C**, Plot depicting ascending aorta cross-section area change over cardiac cycle. Maximum and minimum areas (A_{max} and A_{min}) are used to calculate aortic pulsatility and distensibility (see Methods). **D**, Plot depicting ascending aorta flow over cardiac cycle. **E**, Scatterplot of early systolic (acceleration) phase of flow change vs area change in ascending aorta cross-section. The slope of best-fit linear regression was measured as aortic pulse wave velocity (see Methods).

Changes in Antihypertensive Medications

There was a significant reduction in number of other antihypertensive medications needed to maintain baseline BP from 4.4±1.2 at baseline to 2.7±1.1 at 6 months after addition of spironolactone (P<0.001, Table 2).

Changes in LV Function

No significant change in LV ejection fraction occurred after 6 months of spironolactone intervention (65.9±6.5% at baseline versus 66.9±6.7% at 6 months, P=0.360). There was a reduction of LV volumes from baseline to 6 months after spironolactone intervention (end-diastolic volume: 165±38 mL versus 153±39 mL, P=0.020; end-systolic volume: 55±17 mL versus 51±23 mL, P=0.331; stroke volume: 110±25 mL versus 101±21 mL, P=0.041). Cardiac output was not changed (7.4±1.6 L/min versus 7.1±1.3 L/min, P=0.290).

Changes in AS

Reference individual phase-contrast CMR imaging–derived measurements of ascending aorta maximum and minimum cross-sectional area, PWV, systolic and diastolic BP, and basic patient characteristics are presented in Table S2. Overall, there was a significant decrease in ascending aorta PWV (6.3±2.3 m/s to 4.5±1.8 m/s, P<0.001 [unadjusted]) and significant increases in ascending AP (15.9%±5.3% to 22.1%±7.9%, P<0.001 [unadjusted]) and distensibility (0.28%±0.10%/mm Hg to 0.40%±0.14%/mm Hg,

Table 1. Baseline Demographics, Comorbidities, and Biochemistry in Patients With Resistant Hypertension

Demographics	
Age, y	53.6±6.7
Men	20 (66.7)
Black race	19 (63.3)
Comorbidities	
Hypertension duration, y	20.9±10.7
Hyperaldosteronism	18 (60.0)
Obstructive sleep apnea	20 (66.7)
Diabetes mellitus	9 (30.0)
Coronary artery disease	1 (3.3)
Measurements	
Body mass index, kg/m^2	32.9±4.8
Fat percentage	33.9±8.2
Neck, cm	42.9±4.1
Waist, inch	42.8±5.0
Biochemistry	
Plasma aldosterone, ng/dL	14.1±6.4
Plasma aldosterone—PRA ratio	21.7±19.5
24-h Urine aldosterone, µg	16.0±7.4
24-h Urine protein, mg	346±769
24-h Urine cortisol, µg	151±76
24-h Urine sodium, mmol	194±75
24-h Urine potassium, mmol	73.2±26.6
24-h Urine calculated creatinine, mg	1622±464

Values are expressed as mean±SD or number (percentage). PRA indicates plasma renin activity.

P<0.001 [unadjusted]) following 6 months of spironolactone treatment (Figure 3A through 3C). These values of estimates of ascending aorta stiffness at baseline and after spironolactone treatment were similar to the corresponding values measured in patients who had either only baseline measurements or only follow-up phase-contrast CMR imaging measurements, and thus were excluded from the primary study analysis (Table S3).

Multivariable Regression

A multivariable linear regression model adjusted for effects of age, sex, race, and hyperaldosteronism shows that the difference in ascending aorta pulsatility and distensibility between baseline and 6 months was significantly associated with sex (Figure S1). The increase in ascending AP and AD in men was less than that in women (pulsatility: 3.5%±6.5% in men versus 11.5%±8.3% in women, *P*=0.008; distensibility: 0.08%±0.12%/mm Hg in men versus 0.20%±0.16%/mm Hg in women, *P*=0.027). The change in PWV was not sex dependent (Figure S1). In this model, race, hyperaldosteronism, and age (within the ranges studied) did not significantly affect spironolactone-related changes of the AS estimates (Figure S1). In separate multivariable linear models adjusted for effects of LV ejection fraction, stroke volume, heart rate, mean arterial pressure, and PP, and for effects of serum creatinine, serum potassium, PRA, and brain natriuretic peptide, none of these factors had significant effects on differences in the AS estimates (Figures S2 and S3, respectively).

DISCUSSION

This study is the first to show an effect of spironolactone on AS without a change in systemic BP in patients with RHTN, with increases in AP and AD, accompanied by a decrease in PWV. These changes in noninvasive estimates of AS suggest an improvement in elastic properties of the aorta with spironolactone administration.

Aldosterone, the primary endogenous ligand for the mineralocorticoid receptor, causes BP elevation as a result of changes in arteriolar vasoactive tone and sodium homeostasis, and has been shown to play an important role in the pathogenesis of RHTN.[40] Aldosterone excess leads to collagen accumulation and fibrosis in the left ventricle and aortic wall, and immunohistochemical evidence suggests that aldosterone receptors are present in the aorta.[38,41] Aldosterone also increases arterial stiffness and PP in salt-fed rats through alteration in the elastin and collagen densities, an effect that was prevented by treatment with a mineralocorticoid receptor antagonist.[42] However, data regarding the impact of aldosterone on vascular changes in humans are scant.[22,43] Aldosterone levels are elevated in 10% of patients with essential hypertension and up to 15% to 20% of patients with RHTN.[13] Aldosterone exacerbates oxidative stress and inflammation in vascular tissue, with adverse effects on endothelial function that lead to increased vascular stiffness, atherosclerosis, and ultimately to worsening of cardiovascular disease outcomes.[44]

AS is recognized as a major cardiovascular risk factor in individuals with hypertension.[9,10] Population studies, including the Rotterdam study, the Framingham Heart Study, and the Health ABC study all arrived at a common finding of increased AS (measured by Doppler flow–derived carotid-femoral PWV) associated with increased cardiovascular morbidity and mortality after adjusting for traditional risk factors.[11,45,46] The most extensively studied marker of AS, the carotid-femoral PWV, has proven to be a robust predictive marker for assessing future cardiovascular events and all-cause mortality beyond classical risk predictors such as the Framingham Risk Score and BP.[47] A recent post hoc analysis of SPRINT (Systolic Blood Pressure Intervention Trial) data by Vlachopoulos et al[48] that utilized estimated PWV, calculated based on

Table 2. Biochemistry, Clinic BPs, and Total Medications in Patients With Resistant Hypertension at Baseline and at 6 Months of Spironolactone Treatment

Measurements	Baseline	Spironolactone	*P* value
Biochemistry			
Serum creatinine, mg/dL	1.07±0.25	1.15±0.29	0.023
Serum potassium, mmol/L	3.77±0.36	4.24±0.40	<0.001
PRA, ng/mL per h	1.0±0.8	9.2±13.3	0.002
Brain natriuretic peptide, pg/mL	33.7±34.3	16.9±15.8	0.001
Blood pressure			
Systolic BP, mm Hg	142±17	138±21	0.342
Diastolic BP, mm Hg	83±12	81±14	0.564
Pulse pressure, mm Hg	59.5±12.6	57.2±15.2	0.320
Mean arterial pressure, mm Hg	103±13	100±15	0.450
Heart rate, beats per min	68.5±12.2	69.7±13.2	0.544
Total antihypertensive medications*	4.4±1.2	2.7±1.1	<0.001

BP indicates blood pressure; and PRA, plasma renin activity.
*Spironolactone not included.

patient's age and mean BP, found better survival in individuals whose estimated PWV responded to antihypertensive treatment independently of systolic BP reduction. This finding suggests a role for markers of AS as surrogate treatment targets in patients with hypertension.[48] Here, we utilized several CMR-derived measures of arterial stiffness, including aortic PWV, pulsatility, and distensibility. Since the first reported assessment of PWV by CMR imaging in 1989,[49] segmental and single-point methodology has been validated against tonometry[50–53] and used extensively in multiple clinical studies.[25–27] These indices of arterial stiffness measured by CMR imaging have emerged as reliable measures of vascular function with useful prognostic information.[28,54]

Increasing AS reduces AD, AP, and aortic compliance, and increases PWV. Aldosterone antagonism could ameliorate this increase in stiffness by either reducing sodium ion reabsorption,[55] increasing potassium levels,[56] or inhibiting fibrosis.[16] Mahmud and Feely[22] have shown that administration of spironolactone to untreated patients with essential hypertension leads to reduction in radial artery stiffness. However, because of a significant decrease in BP in those study participants, it was difficult to differentiate a possible effect of spironolactone on AS from its antihypertensive effect.[22] It has been shown that BP affects indices of AS.[21] Therefore, in the present study where BP was constrained to not change with therapy, we were able to assess the effect of spironolactone on AS independently of its antihypertensive effects.

The results of our study extend the information from previous studies in animals[57] and humans[58] showing that spironolactone has beneficial effects beyond BP lowering alone. Our data suggest that these unique properties of aldosterone, rather than its hypertensive effect, cause (or at least contribute) to physiology of the aortic vascular smooth muscle, leading to increased stiffness. The presence of aldosterone receptors in the aorta and other vessels also suggests a local action of mineralocorticoid receptor antagonism in the vasculature.[59] Potentially supporting this

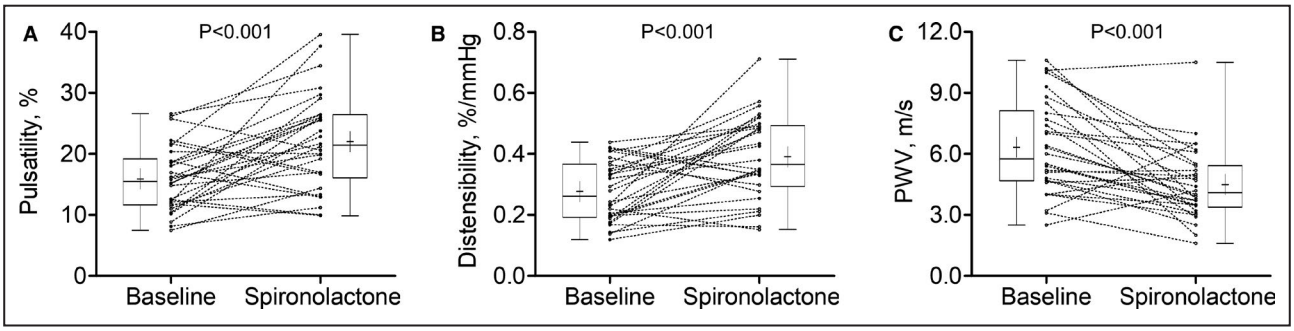

Figure 3. Effect of spironolactone on the ascending aorta pulsatility, distensibility, and pulse wave velocity (PWV).
Ascending aorta pulsatility (**A**), distensibility (**B**), and PWV (**C**) at baseline and after spironolactone intervention in individual patients (connected lines) and the group (box and whiskers; whiskers represent maximum and minimum; box edges represent 25th and 75th percentiles; center line the median, and cross the mean).

conjecture, in a study using female mice, low-dose spironolactone was shown to prevent the pathological aortic stiffening induced by a Western diet caused by blockade of vascular endothelial mineralocorticoid receptors.[60,61]

Increased AS has been reported to have different prognostic implications in men and women older than 55 years, with a 2-fold stronger association with mortality in women than in men.[62] Proximal AS is greater in women than men,[29] which may contribute to the greater risk of heart failure with preserved LV ejection fraction in women.[63] Recent CMR imaging and echocardiographic studies also reported a much faster decline in AD and arterial compliance in aging women than in men, despite no sex-related difference in PWV increase.[64–66] We undertook a multivariate analysis exploring the possible effects of multiple demographic and physiologic features on the stiffness parameters, and this suggested that the response to spironolactone treatment may be influenced by sex. However, the small study size, including a low number of women, limits our ability to infer an actual sex effect. In this respect, our potential finding of a greater effect of spironolactone in improving proximal AD and AP (although not PWV) in women warrants further research and confirmation. We are aware that a sex-specific relationship of aldosterone to cardiac structure and cardiovascular disease and a role of female steroid sex hormones in effects of aldosterone have been reported.[67,68] Furthermore, in the TOPCAT (Treatment of Preserved Cardiac Function Heart Failure With an Aldosterone Antagonist) trial, spironolactone therapy in patients with heart failure with preserved LV ejection fraction was associated with reduced all-cause mortality in women but not in men,[69] which also calls for more research in sex-specific clinical care in cardiovascular disease.

Study Limitations

Because of our relatively small sample size and other limitations, our results should be cautiously interpreted. Inclusion of an additional control group in which the medications are not changed or adjusted during spironolactone treatment could potentially shed additional light on the effect size and enable discernment of how much of an effect BP lowering could have on AS, in addition to any spironolactone effect on the vascular wall. We did not pursue this question, as these effects have already been partially explored.[22,70] However, we were able to explore the effects of possible demographic, hemodynamic, and biochemical interactions on our results, using multivariate regression analysis. Although none of the hemodynamic and biochemical factors we tested showed a significant role in the model, we cannot exclude potential additive contributing effects of some of the tested or other (untested here) factors affecting the changes in AS induced by spironolactone treatment. Nevertheless, our results are consistent with previously published effects of antihypertensive drugs in long-term trials.[71–73] The majority of our study cohort was composed of Black participants. Although the prevalence of RHTN in Black individuals is higher than in other races,[74,75] the generalizability of our findings to other patient populations with a different racial makeup might be limited. There are reports suggesting variances in some responses to spironolactone in different racial groups, especially in respect to patients with heart failure.[76–79] However, in the patients with RHTN, race was not significantly associated with BP response to spironolactone or electrolyte changes.[20,80] Also, the observed sex differences of spironolactone effects on aortic properties may be a chance finding, attributable to the lower female prevalence in the study cohort. In addition, the complex effect of aortic wave reflections, which are significant determinants of central aortic pressure and are typically different in men versus women (because of different height-related aortic arch length),[81] were not accounted for in this study. Accuracy of CMR imaging–measured AS estimates is subject to several systematic limitations, including relatively limited temporal resolution and effects of through-plane motion caused by ventricular contraction. In addition, BP was not simultaneously measured with CMR imaging, but instead was assessed at the beginning and end of the CMR imaging examination. These limitations are potential sources of random error effects. Also (although similar to other clinical studies), we used brachial rather than central BP for calculation of AD.[25,64] Potentially, this could be mitigated by employing validated, commercially available, noninvasive methods for assessment of aortic pressure waveforms.[82] These limitations exist, but because they are unbiased with respect to comparison of baseline and follow-up measurements, their impact is somewhat mitigated.

CONCLUSIONS

The results of our study suggest that the arterial stiffening in patients with RHTN may be, at least in part, caused by an effect of aldosterone on the vascular wall, independent of the elevation in BP, and is reversible with spironolactone treatment, independent of spironolactone's effects on BP reduction. Because of the exploratory study design and other limitations, our results should be considered hypothesis generating and cannot be generalized to a larger cohort without validation.

ARTICLE INFORMATION

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Disclosures

Dr Oparil reports research grants from Bayer, George Medicines Pty Limited, and Idorsia Pharmaceuticals, reports ad hoc lecture honoraria from Ascension, reports personal fees from Preventric Diagnostics, Inc as Chief Medical Officer and from Cincor Pharma Inc as Scientific Advisory Board Member, and reports annual stipend as Editor-in-Chief for Current Hypertension Reports (Springer Science Business Media LLC), all outside of the submitted work. The remaining authors have no disclosures to report.

Supplementary Material

Tables S1–S3
Figures S1–S3

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SUPPLEMENTAL MATERIAL

Table S1. Baseline characteristics of different groups of subjects enrolled in 6-month spironolactone treatment study.

Parameters	Included in analysis (n=30)	Not complete PC CMR (n=5)*	Study not completed (n=10)†	P-Value
Demographics				
Age (Years)	53.6±6.7	55.8±9.2	57.4±8.3	0.487
Male	20 (66.7%)	3 (80%)	6 (60%)	0.899
African Americans	19 (63.3%)	2 (40%)	5 (50%)	0.478
Co-Morbidities				
Hypertension Duration (Years)	20.9±10.7	23.0±7.7	14.5±11.4	0.210
Hyperaldosteronism	18 (60.0%)	2 (40%)	8 (80%)	0.358
Obstructive Sleep Apnea	20 (66.7%)	4 (80%)	5 (50%)	0.572
Diabetes	9 (30.0%)	2 (40%)	2 (20%)	0.694
Coronary Artery Disease	1 (3.3%)	0 (0%)	1 (10%)	0.561
Measurements				
Body Mass Index (kg/m^2)	32.9±4.8	36.0±4.6	38.5±7.7	0.219
Fat Percentage	33.9±8.2	34.7±11.2	33.9±8.4	0.351
Neck (cm)	42.9±4.1	42.4±2.5	42.9±3.2	0.958
Waist (inch)	42.8±5.0	46.7±4.7	43.7±3.6	0.238
Biochemistry				
Serum Creatinine (mg/dL)	1.07±0.25	1.14±0.44	1.13±0.26	0.743
Serum Potassium (mMol/L)	3.77±0.36	3.70±0.23	3.98±0.36	0.214
B-Type Natriuretic Peptide (pg/mL)	33.7±34.3	34.0±49.0	41.6±30.7	0.824
PAC (ng/dL)	14.1±6.4	15.6±7.5	15.6±8.9	0.783
PRA (ng/mL/h)	0.6 (0.6-1.0)	2.3 (0.6-4.8)	0.6 (0.6-0.6)	0.062
PAC/PRA Ratio	21.7±19.5	13.7±8.8	25.0±14.7	0.510
24-h Urine Aldosterone (µg)	16.0±7.4	12.4±6.5	18.3±9.7	0.396
24-h Urine Protein (mg)	346±769	447±517	287±141	0.922
24-h Urine Cortisol (µg)	151±76	138±120	163±85	0.867
24-h Urine Sodium (mmol)	194±75	165±135	157±55	0.409
24-h Urine Potassium (mmol)	73.2±26.6	63.6±32.7	85.8±76.1	0.602
24-h Urine Calculated Creatinine (mg)	1622±464	1606±525	1592±360	0.982
Total Antihypertensive Medications*	4.4±1.2	5.2±1.3	5.1±0.9	0.155

(*to be continued*)

Table S1 (Continued).

Baseline characteristics of different groups of subjects enrolled in 6-month spironolactone treatment study.

Parameters	Included in analysis (n=30)	Not complete PC CMR (n=5)*	Study not completed (n=10)†	P-Value
Blood Pressure				
Systolic Blood Pressure (mmHg)	142±17	137±9	153±13	0.109
Diastolic Blood Pressure (mmHg)	83±12	83±11	88±11	0.559
Pulse Pressure (mmHg)	59.5±12.6	53.8±12.3	65.6±14.8	0.236
Mean Arterial Pressure (mmHg)	103±13	101±9	109±9	0.259
Heart Rate (beats/minute)	68.5±12.2	65.8±9.4	64.5±9.6	0.605
CMR LV Function				
LVEF	65.4±6.4	70.2±14.1	64.7±8.5	0.411
LVEDVI	74.5±14.1	58.0±15.1	75.0±11.6	0.048
LVESVI	25.5±6.8	17.2±8.2	26.6±7.5	0.046
LVSVI	49.1±10.4	40.9±13.3	48.4±9.2	0.271
CO	7.1±1.6	5.8±1.7	6.8±0.8	0.210
Estimates of Ascending Aorta Stiffness				
Asc. Aorta Pulsatility, %	15.9±5.3	-	14.5±5.7	0.471
Asc. Aorta Distensibility, %/mmHg	0.28±0.10	-	0.23±0.12	0.255
Asc. Aorta PWV, m/s	6.3±2.3	-	5.5±1.7	0.298

*****This group includes 5 subjects that did not complete phase-contact CMR study of Ascending Aorta flow either at baseline (n=1) or after 6-month spironolactone treatment (n=4).

†This group includes 10 subjects that did not compete study protocol due to various reasons as follows: due to spironolactone intolerance (n=1), increased creatinine (n=1), hyperkalemia (n=1), uncontrolled BP (n=3), incompliance to the study protocol (n=2), claustrophobia to CMR study (n=1), and voluntary withdraw for adrenal venous sampling and adrenalectomy (n=1).

Based on distribution of continuous variables, parametric or non-parametric, one-Way ANOVA or Kruskal-Wallis test was used to compare means or medians of baseline variables of 3 groups of subjects enrolled in the study. Proportions of categorical variables in the groups were compared using Fisher's Exact test.

Table S2. Study subjects' demographic characteristics and CMR-related measurements of blood pressure, ascending aorta cross-section area, and pulse wave velocity.

Subjects	Age, yrs.	Race	Sex	Baseline					6-month of Spironolactone intake				
				SBP, mmHg	DBP, mmHg	$AscA_{max}$, mm^2	$AscA_{min}$, mm^2	PWV, m/s	SBP, mmHg	DBP, mmHg	$AscA_{max}$, mm^2	$AscA_{min}$, mm^2	PWV, m/s
1	55	AA	M	130	78.5	1292	1187	6	120.5	71.5	1032	833.8	4.4
2	45	AA	M	154.5	92	808.7	643.2	3.2	144	101.5	682.2	565.6	6.5
3	48	W	M	157	92	876.4	692.3	4.7	164	85	754	576.4	2.5
4	58	AA	M	135	81.5	1086	971.6	5.1	126	60.5	1056	960.8	5.2
5	61	AA	M	122	83.5	823.3	708.5	4.6	144	95.5	1050	836	4.8
6	48	W	F	123	55	496.1	408.5	4	146	75	586.6	420.2	3
7	52	AA	F	131	71	540.9	490.1	8	119.5	64	680.3	526.9	7
8	45	AA	M	153	103.5	978.1	803.8	8.8	112	79	1033	885.3	5.4
9	45	W	M	136	100.5	927.1	807.4	6.3	144.5	93	995.1	850.6	4
10	47	W	M	124	80	1444	1215	4	135.5	88.5	1328	1175	5
11	57	AA	M	138	77	727.4	595.2	4.8	132	75	995.1	850.6	3.7
12	64	AA	F	180	92	451	382	5.4	192.5	90.5	507.9	403.3	3.7
13	48	AA	M	128.5	67.5	886.4	736.4	7.7	125.5	78.5	756.4	630.4	4.7
14	57	AA	M	157	104	1083	926	7.1	97.5	63.5	887.5	786	6.5
15	47	AA	M	157.5	82.5	797.3	632	6.4	138	79	795	591.4	3.9
16	65	W	M	153	94	843.2	779.7	10.6	104	67	855.3	769.1	3.8
17	66	AA	F	164	89.5	549.9	488.8	7	136	74	694.6	631.6	6.1
18	57	W	M	120	76	678.2	589	5.5	135	68	897.6	709.9	3.45
19	49	AA	F	147	65	897.4	772.9	4.7	168.5	78	994.3	829.6	3.1
20	51	W	F	134	84	464.4	391.5	3.1	122	70	561	432.5	1.6
21	54	W	F	136	81	510.2	453	5.4	129	82	589.2	479.5	3.5
22	48	W	F	142	77	793.2	684.9	4	143	78	701.8	576.9	3.5
23	62	W	M	139	76	805.8	749.6	5.2	138	71	807.1	705.7	4.9
24	49	AA	M	137.5	85.5	1076	933.9	2.5	159.5	110.5	1100	876.4	4.2
25	57	AA	M	133	74	676.3	606.9	10.1	141	84.5	718.5	602.9	10.5
26	57	AA	F	130	74	960	871.3	10.2	126	77	1062.5	843.2	5.5
27	65	AA	M	143.5	77	662.1	558.4	9.3	162	97	650.3	514.2	3.2
28	56	AA	M	140	84.5	836.4	753.5	8.5	170	117	1068	881	2
29	51	W	F	127.5	76	505.2	450.8	7.4	118.5	65.5	614	445.9	2.9
30	45	AA	M	196	111	829.7	739.9	10	156.5	93.5	684.7	604.1	6.2
Average	*53.6*	-	-	*142*	*83*	*810*	*701*	*6.3*	*138*	*81*	*838*	*693*	*4.5*
SD	*6.7*	-	-	*17*	*12*	*240*	*212*	*2.3*	*21*	*14*	*203*	*188*	*1.8*

Age is at the time of study enrollment; AA= African American; W= Whites; M= male; F= female; SBP= systolic blood pressure; DBP= diastolic blood pressure; $AscA_{max}$= maximum (systolic) area of the Ascending Aorta; $AscA_{min}$= minimum (diastolic) area of the Ascending Aorta; PWV= pulse wave velocity.

Table S3. Estimates of ascending aorta stiffness after 6-month of spironolactone treatments in subjects included in vs. excluded from the primary study analysis.

Parameters	Included in analysis (n=30)		Excluded from analysis (n=15)		T-test P-value
	Baseline (n=30)	6-month SPL (n=30)	Baseline (n=11)*	6-month SPL (n=4)†	
Ascending Aorta Pulsatility, %	15.9±5.3	22.1±7.9	13.8±5.9	24.4±10.9	0.271 (unpaired) 0.584 (unpaired)
T-Test P-value	<0.001 (paired)		0.027 (unpaired)		
Ascending Aorta Distensibility, %/mmHg	0.28±0.10	0.40±0.14	0.23±0.11	0.49±0.24	0.199 (unpaired) 0.272 (unpaired)
T-Test P-value	<0.001 (paired)		0.012 (unpaired)		
Ascending Aorta PWV, m/s	6.3±2.3	4.5±1.8	5.4±1.7	3.8±2.2	0.213 (unpaired) 0.481 (unpaired)
T-Test P-value	<0.001 (paired)		0.166 (unpaired)		

SPL=spironolactone.

*****This group includes subjects that had only baseline phase-contrast CMR study of ascending aorta flow (10 subjects that did not compete 6-month treatment study protocol and were withdrawn and 1 subject that did not have phase-contact CMR study after 6-month spironolactone treatment).

†This group includes 4 subjects that had only follow-up phase-contrast CMR study of ascending aorta flow (they completed the treatment study but did not have phase-contact CMR study of ascending aorta flow at baseline).

Depending on groups of comparison, paired or unpaired T-test was used as specified in the table.

FIGURE S1

Multivariable linear regression model (demographic variables).

Dependent variables:
A) difference in aortic pulsatility (d_AP), **B**) difference in aortic distensibility (d_AD), and **C**) difference in pulse wave velocity (d_PWV).

where difference = value after 6 months of spironolactone intake – value at baseline

Independent variables:
Age, Sex, Race, HyperAldosteronism.

Collinearity Statistics for model with dependent variables d_AP, d_AD, or d_PWV.

Independent Variables	Tolerance	Variance inflation factor (VIF)
Age	0.962	1.04
Sex	0.941	1.063
Race	0.936	1.069
HyperAldosteronism	0.966	1.035

A) Outcome = Difference in aortic pulsatility

Parameter	Beta Estimate	Standard Error	t Value	Pr > |t|
Intercept	-5.703	11.591	-0.492	0.627
Age	-0.123	0.206	-0.596	0.556
Sex	7.842	2.901	2.703	0.012
Race	-2.509	2.845	-0.882	0.386
HyperAldosteronism	3.019	2.754	1.096	0.283

B) Outcome = Difference in aortic distensibility

Parameter	Beta Estimate	Standard Error	t Value	Pr > |t|
Intercept	-0.238	0.225	-1.061	0.299
Age	0	0.004	0.051	0.96
Sex	0.129	0.056	2.298	0.03
Race	0.028	0.055	0.512	0.613
HyperAldosteronism	0.015	0.053	0.274	0.787

C) Outcome = difference in PWV

Parameter	Beta Estimate	Standard Error	t Value	Pr > |t|
Intercept	-3.144	3.702	-0.849	0.404
Age	0.073	0.066	1.111	0.277
Sex	0.166	0.927	0.179	0.859
Race	0.379	0.909	0.417	0.681
HyperAldosteronism	1.352	0.88	1.536	0.137

Units of change in the predictor variables: Age (1 year), Sex (1 (M)/0 (F)), Race (1 (African American)/0 (White)), Hyperaldosteronism: (1 (yes)/0 (no)).

FIGURE S2

Multivariable linear regression model (basic cardiac function and hemodynamic variables).

Dependent variables:
A) difference in aortic pulsatility (d_AP), **B**) difference in aortic distensibility (d_AD), and **C**) difference in pulse wave velocity (d_PWV).

Independent variables:
Difference in left ventricular ejection fraction (d_LVEF), stroke volume (d_SV), heart rate (d_HR), mean arterial pressure (d_MAP), and pulse pressure (d_PP).

where difference = value after 6 months of spironolactone intake – value at baseline

Collinearity Statistics for model with dependent variables d_AP, d_AD, or d_PWV.

Independent Variables	Tolerance	Variance inflation factor (VIF)
d_LVEF	0.49	2.04
d_SV	0.431	2.323
d_HR	0.708	1.413
d_MAP	0.771	1.298
d_PP	0.754	1.326

A) Outcome = Difference in aortic pulsatility

Parameter	Beta Estimate	Standard Error	t Value	Pr > |t|
Intercept	-6.778	1.443	-4.699	0.000
d_LVEF	0.147	0.218	0.677	0.505
d_SV	0.112	0.091	1.234	0.229
d_HR	0.179	0.153	1.168	0.254
d_MAP	0.095	0.086	1.096	0.284
d_PP	0.069	0.128	0.535	0.597

B) Outcome = Difference in aortic distensibility

Parameter	Beta Estimate	Standard Error	t Value	Pr > |t|
Intercept	-0.122	0.027	-4.442	0.000
d_LVEF	0.002	0.004	0.380	0.707
d_SV	0.002	0.002	1.233	0.229
d_HR	0.003	0.003	0.988	0.333
d_MAP	0.002	0.002	0.950	0.352
d_PP	-0.005	0.002	-1.973	0.060

C) Outcome = difference in PWV

Parameter	Beta Estimate	Standard Error	t Value	Pr > |t|
Intercept	1.416	0.419	3.378	0.002
d_LVEF	-0.129	0.063	-2.035	0.053
d_SV	0.023	0.026	0.887	0.384
d_HR	-0.066	0.044	-1.477	0.153
d_MAP	-0.001	0.025	-0.044	0.965
d_PP	0.039	0.037	1.040	0.309

Units of change in the predictor variables: LVEF (1 %), SV (1 ml), HR (1 bpm), MAP (1 mmHg), PP (1 mmHg).

FIGURE S3

Multivariable linear regression model (biochemical variables).

Dependent variables:
A) difference in aortic pulsatility (d_AP), **B**) difference in aortic distensibility (d_AD), and **C**) difference in pulse wave velocity (d_PWV).

Independent variables:
Difference in serum creatinine (d_SCr), serum potassium (d_SK+), plasma renin activity (d_PRA), brain natriuretic peptide (d_BNP).

where difference = value after 6 months of spironolactone intake – value at baseline

Collinearity Statistics for model with dependent variables d_AP, d_AD, or d_PWV.

Independent Variables	Tolerance	Variance inflation factor (VIF)
d_SCr	0.914	1.094
d_SK+	0.981	1.02
d_PRA	0.87	1.15
d_BNP	0.925	1.081

A) Outcome = Difference in aortic pulsatility

Parameter	Beta Estimate	Standard Error	t Value	Pr > |t|
Intercept	-8.546	2.735	-3.125	0.005
d_SCr	0.794	8.608	0.092	0.927
d_SK+	-1.334	3.528	-0.378	0.709
d_PRA	-0.241	0.123	-1.963	0.061
d_BNP	-0.009	0.106	-0.082	0.936

B) Outcome = Difference in aortic distensibility

Parameter	Beta Estimate	Standard Error	t Value	Pr > |t|
Intercept	-0.123	0.05	-2.457	0.022
d_SCr	-0.041	0.158	-0.259	0.798
d_SK+	0.003	0.065	0.051	0.960
d_PRA	-0.003	0.002	-1.489	0.150
d_BNP	-0.002	0.002	-1.181	0.249

C) Outcome = difference in PWV

Parameter	Beta Estimate	Standard Error	t Value	Pr > |t|
Intercept	1.349	0.784	1.719	0.098
d_SCr	-3.192	2.469	-1.293	0.208
d_SK+	0.503	1.012	0.497	0.624
d_PRA	-0.014	0.035	-0.404	0.690
d_BNP	0.031	0.03	1.023	0.317

Units of change in the predictor variables: Scr (1 mg/dL), SK+ (1 mMol/L), PRA (1 ng/mL/h), BNP: (1 pg/mL).