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Aldosterone Related Myocardial Extracellular Matrix Expansion in Hypertension in Humans: A Proof of Concept Study by Magnetic Resonance Imaging

Short Title: MRI assessment of aldosterone induced cardiac fibrosis

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ABSTRACT

**Background.** Experimentally, aldosterone promotes left ventricular (LV) hypertrophy and interstitial myocardial fibrosis in the presence of high salt intake.

**Objectives.** To assess the respective effects of aldosterone and blood pressure (BP) levels on myocardial fibrosis in humans.

**Methods.** We included 20 patients with primary aldosteronism (PA, high aldosterone-high BP), 20 patients with essential hypertension (HTN, average aldosterone-high BP), 20 patients with secondary aldosteronism due to Bartter/Gitelman syndrome (BG, high aldosterone-normal BP) and 20 healthy subjects (HS normal aldosterone-normal BP). Participants in each group were of similar age and sex distributions, and asymptomatic. Cardiac MRI including cine and T1 mapping was performed blind to the study group to quantify global LV mass index (g/m²), as well as intracellular mass index and extracellular mass index considered as a measure of myocardial fibrosis *in vivo*.

**Results.** Median [IQR] plasma aldosterone concentration (pmol/L) was as follows: PA: 709 [430, 918], HTN: 197 [121, 345], BG: 297 [180, 428] and HS: 105 [85, 227]. Systolic BP (mmHg) was as follows: PA: 147±15, HTN: 133±19, BG: 116±9 and HS: 117±12 mmHg. LV end-diastolic volume showed underloading in BG and overloading in PA patients (63±13 vs 82±15 ml/m², p<0.0001). Intracellular mass index increased with BP across groups (BG: 36[IQR 29-41]; HS: 40[36-46], HTN: 51[42-54], PA: 50[46-67], p<0.0001). Extracellular mass index was similar in BG, HS, and HTN (16[12-20], 15[11-18], and 14[12-17]) but 30% higher in PA (21[18-29], p<0.0001) remaining significant after adjustment for mean BP.

**Conclusions.** Only primary pathological aldosterone excess combined with high BP increased both extracellular myocardial matrix and intracellular mass. Secondary aldosterone excess with normal BP did not affect extracellular myocardial matrix.

**Keywords:** myocardial fibrosis, extracellular matrix, magnetic resonance imaging, primary hyperaldosteronism, myocardial remodeling

The study was registered at ClinicalTrials.gov (NCT02938910)

**Abbreviations:**
- A-wave: Atrial LV filling peak
- cMRI: Cardiovascular Magnetic Resonance Imaging
- E’: Early Diastolic Peak of Myocardial Velocity
- E-Wave: Early Diastolic Peak of Transmitral Flow
- ECM, ECMi, ECV: Extracellular Mass, Extracellular Mass Index, Extracellular Volume
- HTN: Hypertension
- ICM, ICMi, ICV: Intracellular Mass, Intracellular Mass Index, Intracellular Volume
- MRA: mineralocorticoid receptor antagonist
- MOLLI: Modified Look-Locker Inversion Recovery sequence
INTRODUCTION

The mineralocorticoid hormone, aldosterone, plays a key role in the homeostasis of sodium and potassium and blood pressure (BP) control in humans. However, the effects of aldosterone extend far beyond these physiological effects (1, 2). Indeed, aldosterone, together with high dietary sodium intake, stimulates inflammatory reactions, cellular hypertrophy, extracellular matrix formation and apoptosis in the vessels, the heart and the kidneys (3). Myocardial hypertrophy and fibrosis can be reversed with administration of non-antihypertensive doses of spironolactone, a mineralocorticoid receptor antagonist (MRA)(4). Aldosterone is thus directly involved in target organ damage in various cardiovascular and renal diseases (5). Primary aldosteronism (PA), due to excess aldosterone secretion by the adrenal glands, leads to hypokalemic hypertension and increased prevalence of maladaptive left ventricular hypertrophy (LVH) (6, 7). PA is further associated with higher risk of myocardial infarction, stroke and atrial fibrillation compared with essential hypertension alone (8, 9). Moreover, MRA were shown to decrease the risk of sudden death, progression towards heart failure and cardiovascular mortality independently of BP levels in patients with left ventricular (LV) systolic dysfunction (10). In patients with asymptomatic diastolic dysfunction, MRA therapy is associated with significant improvement in diastolic function and decreased circulating surrogate biomarkers of cardiac fibrosis (11). The relative contribution of the direct effects of aldosterone on target organ damage and fibrosis, including the heart, the kidneys and the vessels, and those indirectly triggered via aldosterone-induced BP increase is not well individualized in humans (1).

Currently, the diagnosis of cardiac involvement relies mainly on echocardiographic measures of LV morphology (i.e. hypertrophy: LV mass and thickness) and global ejection fraction.
(LVEF) as well as volume-dependent estimates of diastolic function. Cardiac magnetic resonance imaging (cMRI), a reference technique to investigate cardiac function and geometry, also offers dense replacement myocardial fibrosis assessment by using late gadolinium enhancement (LGE) which has demonstrated prognostic value in various ischemic and non-ischemic cardiac diseases (12, 13). T1 relaxation mapping of the myocardial tissue provides quantitative assessment of intracellular and extracellular compartments of the myocardium. The latter was shown to be highly correlated with interstitial fibrosis quantified from myocardial biopsies (14, 15).

To decipher the respective roles of aldosterone and BP on myocardial hypertrophy and extracellular matrix remodeling on the human heart in vivo using cMRI, we designed a four-corner approach study including subjects with contrasting aldosterone and BP levels: 1) patients with primary aldosteronism (PA) with excess renin-independent aldosterone secretion by adrenal glands and high BP levels; 2) patients with Bartter/Gitelman (BG) syndrome, a salt-losing autosomal recessive tubulopathy, characterized by renin-dependent hyperaldosteronism and normal BP levels; 3) patients with essential hypertension (HTN) and average aldosterone levels; and 4) healthy subjects (HS) with both normal aldosterone and BP levels.

**METHODS**

The protocol was approved by the local ethics committee “Comité de Protection des Personnes Paris Ile de France IV” and registered at ClinicalTrials.gov (NCT02938910) and all participants gave written informed consent.

This was a cross-sectional pilot study which included a total of 85 consecutive subjects at the Hypertension Unit, the Center for Rare Renal diseases, and the Clinical Investigation Center of
Georges Pompidou hospital in Paris, France. Five patients were excluded because of incomplete cMRI exam due to claustrophobia or insufficient image quality (Supplemental figure 1).

**Hypertensive groups**

Twenty subjects with PA were included according to the following criteria: age 18 to 75 years; semi-recumbent plasma aldosterone/renin ratio >64pmol/mUI and plasma aldosterone concentration >500pmol/l in patients on treatment not interfering with the renin angiotensin system (e.g. alpha-blocker, calcium channel blocker or central antihypertensive drug) for at least 15 days, as previously described (16, 17).

Twenty subjects with similar age and gender distribution with HTN either on- or off-antihypertensive medications were included after a full investigation including biochemical tests, plasma hormone determinations, and adrenal and renal artery imaging according to the 2013 European guidelines (18) to exclude secondary hypertension.

**Normotensive groups**

Twenty subjects with BG syndrome were included in the study if they had a confirmed mutation (detailed in supplemental Table S1) in either the *SLC12A3* gene encoding for the basolateral chloride channel ClCKb or the *hTSC* gene encoding for the tubular thiazide sensitive sodium chloride co-transporter (19).

Twenty healthy normotensive HS (supine office blood pressure [BP] <140/90mmHg) with similar age and gender distribution were recruited at the Clinical Investigation Center.

Blood samples for standard biochemical parameters, blood count, and determination of hormonal and biological markers concentrations were collected at ≈08:00 am in fasting conditions after 1-hour rest in a semi-recumbent position. cMRI at 1.5 tesla and vascular
investigations were performed on the same day (extensive description of the method in Data Supplement).

**cMRI acquisitions**

cMRI examinations included: 1) cine short axis slices covering the left ventricle and atrium, 2) Late Gadolinium Enhancement images, performed 10 minutes after IV injection of 0.2mmol/kg of Gd-DTPA (Dotarem®, Guerbet, France), 3) T1 mapping before contrast and 15 minutes after contrast injection, acquired in a single mid-ventricular short-axis view using a Modified Look-Locker Inversion Recovery (MOLLI) sequence, 4) Phase contrast acquisitions of transmitral flow and basal myocardial longitudinal velocities, acquired as described previously for diastolic function assessment (20).

*Analysis of left ventricular morphology and function*

Cine SSFP data were analyzed by the same operator blind to study groups, for the semi-automatic tracing of endocardial and epicardial contours on all contiguous short axis slices during the end-diastolic and end-systolic phases, resulting in LV mass (LVM), end-diastolic (EDV) and end-systolic volumes. LV ejection fraction (LVEF), stroke volume (SV) and end-diastolic mass to volume ratio (M/V) were further calculated. All measures of ventricular volumes and mass were indexed to body surface area (BSA) and LV hypertrophy was defined by a LVM index (LVMi) >95 g/m$^2$ in men and >85 g/m$^2$ in women (upper 95th percentile of local reference values in 200 healthy subjects).

LV diastolic function assessment was performed from phase contrast acquisitions as previously described (20) resulting in early (E-wave) and late (A-wave) diastolic peaks of transmitral
mean flow velocities, total transmitral filling volume (ml) and early diastolic peak of myocardial longitudinal velocity (e’).

**Myocardial tissue characterization**

For myocardial tissue characterization, all analyses were done blinded to study groups. Visual analysis for the presence or absence of LGE was performed by an experienced operator. MOLLI images were analyzed using a custom software that enabled T1 map estimation (21). Quantitative T1 analysis included interventricular septum and blood pool native and post-contrast T1 values which were used for the estimation of the partition coefficient (\( \lambda \)), calculated using a previously described formula: 

\[
\lambda = \frac{(\text{post-contrast } R1_{\text{myo}} - \text{native } R1_{\text{myo}})}{(\text{post-contrast } R1_{\text{LV-Cavity}} - \text{native } R1_{\text{LV-cavity}})}
\]

with \( R1 = 1/T1 \). The extracellular volume (ECV, %) was calculated as follows: (1-hematocrit) * \( \lambda \). LV extracellular mass was defined as: 

\[
\text{ECM} = \text{LVM} \times \text{ECV} \quad (g)
\]

when intracellular mass was defined as: 

\[
\text{ICM} = \text{LVM} - \text{ECM} \quad (g)
\]

Intracellular volume (ICV, %) as ICV= 100 - ECV. Both ECM and ICM were indexed to BSA resulting in ECMi (g/m²) and ICMi (g/m²).

**Laboratory assays**

Plasma renin, aldosterone, BNP, biomarkers of collagen metabolism including inhibitor of type I metalloproteinase (TIMP1), C-terminal telopeptide of type I collagen (ICTP), C-terminal propeptide of type I procollagen (PICP), type 1 pro-matrix metalloproteinase (proMMP-1), N-terminal propeptide of type III collagen (PIIINP) were measured using commercially available kits (see Data Supplement).
**Statistical analysis**

This was a proof-of-concept pilot study designed to assess LV hypertrophy and myocardial tissue components *in vivo* using cMRI in contrasted conditions of exposure to variable aldosterone and BP levels. There was no pre-defined primary imaging endpoint and the number of subjects included in the study corresponded to a feasibility criterion.

Normality of the distribution of continuous variables was tested using Shapiro Wilk's test. Plasma hormones were log-transformed for statistical analyses. We compared the baseline characteristics between groups using one-way ANOVA (normally distributed variables) or Kruskall Wallis test (non-normally distributed variable) for continuous variables and with Chi-square test and Fisher test for categorical variables. Data were also analyzed by ANCOVA adjusted on mean BP values measured at the clinical examination. P-values less than 0.05 were considered as significant.

Paired comparisons were made with Tukey correction for multiple testing on continuous variables. For multiple testing on categorical variables, the paired comparisons were made with Bonferroni correction considering P<0.0083 as significant.

The correlation between variables was tested using the least square method or the Spearman's rank order test. We performed multivariate analysis using gender, age, mean BP and log-transformed plasma aldosterone concentration as independent variables and LVMi, ECMi, ICMi and ECMi/ICMi ratio as dependent variables.

Data were reported as percentages for categorical variables and means and standard deviations for normally distributed continuous variables and median and interquartile range (IQR) for non-normally distributed continuous variables. Statistical Analysis System (SAS) v. 9.4 (SAS Institute, Cary, NC) was used for the analyses. We deemed a P value <0.05 as significant.
RESULTS

Participant Characteristics

Age and gender were comparable across the 4 groups (Table 1). As expected, systolic and diastolic BP were higher in both HTN and PA groups compared to HS and BG groups. PA subjects had significantly higher BP levels than HTN subjects. The total number of antihypertensive drugs prescribed was similar between PA and HTN groups (p=0.72). The decreasing rank order of plasma aldosterone concentrations was PA, BG, HTN and HS. PA and BG patients had an opposite plasma renin profile.

Cardiac morphology and function

Table 2 summarizes LV, right ventricular (RV) and left atrial (LA) measurements. As expected, LVMi was much higher in both hypertensive groups (PA and HTN) than in both normotensive groups (BG and HS). The decreasing rank order of LVMi was PA, HTN, HS and BG. LVEDVi, LVESVi and LAVi, and LV systolic as well as diastolic function indices did not significantly differ between PA and HTN patients (Table 2, Figure 1). Only 4 over 20 patients with PA had LV hypertrophy and none in other groups. The LV mass-to-end diastolic volume ratio (M/V) did not differ between PA and HTN patients.

BG patients had lower LVEDVi, LVESVi, LAVi, RVEDVi, and lower transmitral filling volumes compared to HS. LV systolic as well as diastolic function indices were similar between BG and HS or PA patients. LV peak E-wave, peak A-wave and filling volumes were also lower in BG patients than in both HS and PA patients. Increased LVMi was associated to
male gender, MBP, and plasma aldosterone concentration in both univariate and multivariate analysis (Table S2).

**Cardiac Tissue Characterization**

One PA and one HTN patient had non-specific junctional late gadolinium enhancement by cMRI and no myocardial infarction was found. Native and post-contrast myocardial T1 relaxation times did not differ between groups (Table 2). Extra- and intracellular volume fractions differed significantly between groups in the ANOVA with no significant difference in pairwise analyses (Table 3). ECMi was markedly higher in PA patients than in the three other groups which had similar values (Table 3 and Figure 2). For the overall population, (n=80) in univariate analysis, both MBP and plasma aldosterone were associated to ICMi and ECMi. In multivariate analysis, the effect of aldosterone on ECMi was no more significant in the overall population after adjustment for MBP (Table S2). However, in multivariate analysis restricted to hypertensive groups PA and HTN, the effect of aldosterone on ECMi remained significant after adjustment for MBP (Table S3). ICMi was higher in PA than in HTN patients and higher in both PA and HTN patients than in BG patients and HS (Table 3). The ECMi/ICMi ratio was higher in PA than in the HTN patients (0.45±0.24 vs 0.31±0.10; p=0.023) but did not differ between BG patients and HS (0.47±0.22 vs 0.39±0.16, p=0.21). The 3-dimensional ellipsoid plots show the relative contribution of BP and log-transformed plasma aldosterone concentrations on LVMi, ECMi, and ICMi (Figure S2).

**Circulating biomarkers of collagen metabolism**
PICP was significantly higher in PA patients than in other groups. None of the other measured circulating markers of collagen turnover differed between groups (Table 1).

DISCUSSION

To decipher the respective contribution of the direct or indirect effects of aldosterone on the human heart in vivo we studied four groups of subjects with contrasting aldosterone and BP levels. We used cMRI to noninvasively identify differential patterns of intracellular and extracellular myocardial mass components (25). This technique has been well validated in human with a good concordance between ECV derived from cMRI T1 mapping and histological ECV (26).

We found that the increase in cardiomyocyte mass was the prevailing response to increased afterload induced by hypertension, whereas significant extracellular matrix expansion per se, as assessed in cMRI by absolute extracellular mass index (ECMi) was observed only in PA but not in BG or in HTN patients. PA patients have both high BP and pathological aldosterone excess whereas BG patients have normal BP and physiological aldosterone excess and HTN patient’s high BP and average aldosterone levels. This effect remained significant between hypertensive subgroups after adjustment for MBP.

Aldosterone is a key determinant of cardiovascular disease leading to heart failure through indirect effects on water and salt homeostasis increasing volemia and BP as well as direct effects on the heart and vessels through activation of the MR pathway. Compared to HTN patients, PA patients are likely to have more severe left ventricular hypertrophy due to an increase in both myocyte size and extracellular matrix (fibrosis) as suggested by experimental animal studies (22). The main stimulus for cardiomyocyte hypertrophy is mechanical stress
secondary to increased preload or afterload, whereas myocardial fibroblast stimuli depend on a balance between collagen synthesis and collagen degradation enzymes, but may also be influenced by BP (23). In humans, alteration of myocardial texture has been reported in PA using ultrasounds suggesting increased cardiac fibrosis (24). However, the respective contribution of BP and high aldosterone has not been addressed.

In the present study, intra- and extracellular mass fractions measured in our healthy population were very close to values reported in histology studies for the normal myocardium (respectively ICM 73% vs 75%; ECM 27% vs 25%) (27). However, we did not find significant differences between native and post contrast T1 values across groups.

We observed a gradual increase in global LVMi and ICMi from BG patients, presenting with low preload and LV hypotrophy, to HS, with normal loading conditions, HTN with increased afterload and PA subjects who were the most hypertensive (Table 3, Figure 1-2). This is consistent with the fact that the main stimulus for cardiomyocyte hypertrophy is increased afterload or preload or both. The systolic function was found to be normal and unable to discriminate between groups and differences in diastolic function parameters were mainly due to differences in LV filling, hence preload conditions. By contrast, ECMi was similar in HS, BG and HTN but notably increased by 30% in PA patients (Table 3, Figure 2), remaining significant after adjustment for MBP. These results are in line with a previous study (28) identifying an increase in the frequency of myocardial fibrosis in PA as compared to HTN despite the absence of difference in severity or duration of hypertension between groups. The authors concluded that non-infarct myocardial fibrosis in primary aldosteronism, found independent of blood pressure, may be mediated partly through inflammation and oxidative stress. However, myocardial fibrosis in their study was defined as a diffuse pattern of late
gadolinium enhancement and compartmental analysis was not performed. The lack in ECV (%) expansion in hypertensive patients compared to normotensive controls has also been previously reported in the absence of LV hypertrophy (13, 14) as well as in the presence of hypertension without LV hypertrophy and without PA (15). Higher ECMi found in PA patients but not in BG patients with secondary aldosteronism in a sodium depleted state suggests a permissive effect of high BP on the profibrotic effect of aldosterone. Indeed, aldosterone remained a significant determinant of ECMi in hypertensive subjects (PA and HTN groups) independent of BP (Tables S2 and S3). As illustrated in supplemental Figure 2, both ICMi and ECMi as well as the ECMi/ICMi ratio varied significantly according to the presence of hypertension and/or hyperaldosteronism. Such compartmental changes could be observed despite a low prevalence of LV hypertrophy in our population. Our results confirmed that in hypertensive subjects LV hypertrophy can be due to increased ICMi alone (HTN) or increase in both ECMi and ICMi. By contrast, BG patients had markedly low cardiac volumes and LVMi related to low preload and low adaptative ICMi (Table 2, Figure 1).

Our results are consistent with experimental studies showing that elevated circulating aldosterone together with arterial hypertension are associated with cardiac macrophage and interstitial and perivascular fibroblast activation, resulting in heterogeneous myocardial structure alterations related to fibrosis (27). Cardiac fibrosis was observed in seminal murine renovascular hypertension models resulting from unilateral renal ischemia and high-salt diet except when hypertension was prevented by ACE-inhibitors or when aldosterone effects were prevented by low non-antihypertensive doses of MRA (27). Accordingly, in a rodent model of PA associated with elevated hypertension, cardiac injuries were more sensitive to MRA under
high salt intake than low salt intake (29). Furthermore, in hypertensive mice with LV hypertrophy, ECV expansion was prevented by spironolactone independently of BP (22). Increase in PICP (plasma carboxy-terminal propeptide of procollagen type I) in PA patients and post adrenalectomy decrease of this circulating fibrosis biomarker in such patients has been reported (30). We confirmed here the presence of increased PICP levels in our population of PA patients. But differences between groups were not significant after adjustment on BP levels. The other serum markers of collagen turnover that have been shown to be related to myocardial interstitial were not significantly different between groups in our study. Between groups, we further found no difference in BNP level that was reported to be related to the ECV with cMR in a large cohort of subjects included in the MESA study (31). This highlights that in a pathophysiological study even with a relatively small number of subjects, cMR can be more sensitive than such biological markers in detecting extracellular expansion. Finally, C-reactive protein levels were normal in each group, but we cannot rule out here the hypothesis that tissue inflammation can be induced by aldosterone and involved in extracellular expansion of the myocardium in PA subjects.

Monitoring myocardial ECM using cMRI may shed light on the mechanisms involved in the transition from hypertrophic remodeling to ventricular dilatation and dysfunction in patients subject to pressure overload at risk of developing heart failure. In the near future, novel cMRI myocardial tissue biomarkers such as intra and extracellular mass, in combination with structural and functional parameters may become new criteria to detect early disease and stratify cardiovascular risk and eventually become direct target endpoints to evaluate therapies (32) such as novel antialdosterone drugs, antifibrotic drugs or fibroblast-targeted therapy.
**Strengths of the study:** This proof of concept study benefits from a four corner approach with carefully matched groups and cMRI phenotyping tailored to study the respective effect of aldosterone and hypertension thanks to the inclusion of a group of BG subjects, a low prevalence condition (1/40000) and a healthy control group. The cross sectional study design does not allow drawing conclusions on causal determinants of extracellular matrix remodeling which will be the focus of future studies.

**Limitations** include the relatively small number of subjects when considering individual groups. The PA patients were more hypertensive than HTN, but we were able to demonstrate an independent effect of aldosterone on ECM, independent of hypertension. We acknowledge that normal myocardial T1 values obtained in our study were lower than published literature values. This was related to the readout-induced effect of the SSFP MOLLI method of our system at the time of the study and to the correction approximation after sampling of the longitudinal magnetization recovery when the inversion is imperfect (33). However, all subjects included in this work were studied on the same MRI system with exactly the same MOLLI sequence and acquisition settings. Furthermore, one might emphasize that no diagnostic T1 threshold value is provided in this work.

**Conclusions**

We demonstrated that the differential effects of aldosterone and hypertension on intra and extracellular myocardial components could be quantified noninvasively in humans using cMRI. While increase in intracellular mass is primarily a response to increased afterload, significant
extracellular expansion occurred only in patients with both hyperaldosteronism and hypertension demonstrating the inductive effect of increased blood pressure. Such quantitative knowledge of myocardial compartments offers the opportunity to develop personalized therapeutic strategies targeting cardiomyocytes and/or the extracellular matrix.

**CLINICAL PERSPECTIVES**

**What Is Known.** cMRI provides additional tissue-level information over generic measures of myocardial mass and thickness.

**What Is New.** By measuring myocardial intra and extracellular components by MRI we were able to demonstrate cardiac fibrosis in hypertensive patients with primary aldosteronism, and its absence in normotensive patients with secondary aldosteronism due to a congenital renal loss of sodium.

**What Is Next.** Monitoring myocardial ECM using cMRI shed light on the mechanisms involved in the transition from hypertrophic remodeling to ventricular dilatation and dysfunction in patients subject to pressure overload to identify patients at risk of developing heart failure. In the near future, novel cMRI myocardial tissue biomarkers such as intra and extracellular mass, in combination with structural and functional parameters may become new criteria to detect early disease and stratify cardiovascular risk and eventually become direct target endpoints to evaluate therapies such as novel antialdosterone drugs, antifibrotic drugs or fibroblast-targeted therapy. This approach will be also promising in the future development of personalized therapeutic strategies.

**Translational Outlook**
By measuring myocardial intra and extracellular components by MRI we were able to demonstrate cardiac fibrosis in hypertensive patients with primary aldosteronism. Such quantitative knowledge of myocardial compartments offers the opportunity to develop personalized therapeutic strategies targeting cardiomyocytes and/or the extracellular matrix.
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FIGURE LEGENDS:

Figure 1: Cardiac MRI images
One short axis view (upper row) and one four chamber view (lower row) of the LV obtained in SSFP cine MRI representative of each study group illustrate the progressive increase in LV mass between normotensive male subjects (BG, HS) and hypertensive male subjects (HTN and PA) and the smaller size of the heart including the left atrium in BG subjects.

Figure 2: Differences across groups in total LV mass (dotted bars), intracellular (white bars) and extracellular (grey bars) LV mass indexes measured by cardiac MRI.
As compared to HS, decrease in total LV mass index in BG is due to decrease in intracellular mass index, while increase in total LV mass index in HTN is due to a selective increase in intracellular mass index. By contrast, the greater increase in total LV mass index in PA was due to increase in both intracellular and extracellular mass indexes.

Central Illustration. Respective and additive effects of aldosterone and hypertension in the overall remodeling of the left ventricle and each of its intra- and extracellular components.
Table 1: Clinical and biochemical data across groups

<table>
<thead>
<tr>
<th>Clinical characteristics</th>
<th>PA</th>
<th>HTN</th>
<th>BG</th>
<th>HS</th>
<th>P-value</th>
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<tr>
<td>Age, yrs</td>
<td>44 ± 12</td>
<td>46 ± 13</td>
<td>41 ± 11</td>
<td>39 ± 10</td>
<td>NS</td>
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<tr>
<td>Gender, M/F</td>
<td>10/10</td>
<td>9/11</td>
<td>7/13</td>
<td>7/13</td>
<td>NS</td>
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<tr>
<td>BMI, kg/m²</td>
<td>28 ± 3.8</td>
<td>25 ± 3.6</td>
<td>23 ± 3.3*</td>
<td>24 ± 2.8*</td>
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<td>Hemodynamic parameters</td>
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<td></td>
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<tr>
<td>Systolic BP, mmHg</td>
<td>147 ± 15</td>
<td>133 ± 19*</td>
<td>116 ± 9*;†</td>
<td>117 ± 12*;†</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Diastolic BP, mmHg</td>
<td>85 ± 10</td>
<td>75 ± 8*</td>
<td>65 ± 7*;†</td>
<td>65 ± 8*;†</td>
<td>&lt;0.0001</td>
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<tr>
<td>Heart rate, bpm</td>
<td>67 ± 9</td>
<td>64 ± 7</td>
<td>69 ± 11§</td>
<td>61 ± 8</td>
<td>0.0231</td>
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<td>Biology</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Kalemia, mmol/L</td>
<td>3.3 ± 0.4</td>
<td>3.8 ± 0.3*</td>
<td>2.6 ± 0.5*;†;§</td>
<td>4.0 ± 0.3*</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>C-reactive protein, mg/L</td>
<td>3.3 ± 4.3</td>
<td>1.8 ± 1.4</td>
<td>1.9 ± 1.6</td>
<td>1.7 ± 1.6</td>
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<tr>
<td>eGFR, ml/min/1.73m²</td>
<td>89.6 ± 24.8</td>
<td>86.4 ± 14.3</td>
<td>110.2 ± 24.2*;†</td>
<td>97.3 ± 13.3</td>
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<td>Hematocrit, %</td>
<td>39.3 ± 3.8</td>
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<td>40.6 ± 4.6</td>
<td>39.7 ± 2.7</td>
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</tr>
<tr>
<td>Plasma Renin, mUI/L</td>
<td>4.0 [2.0, 6.5]</td>
<td>52.5 [10.0, 119.0]*</td>
<td>63.5 [33.0, 94.0]</td>
<td>15.5 [6.5, 23.0]†</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Plasma Aldosterone, pmol/L</td>
<td>709 [430, 918]</td>
<td>197 [121, 345]*</td>
<td>297 [180, 428]†</td>
<td>105 [85, 227]†</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>B-Natriuretic Peptide, pmol/L</td>
<td>24.0 [18.0, 40.0]</td>
<td>15.5 [9.0, 23.0]</td>
<td>16.0 [12.0, 20.0]</td>
<td>18.0 [5.0, 24.0]</td>
<td>NS</td>
</tr>
<tr>
<td>ICTP, µg/L</td>
<td>4.3 [3.5, 4.7]</td>
<td>3.7 [3.4, 4.2]</td>
<td>3.5 [2.9, 4.0]</td>
<td>3.9 [3.2, 5.2]</td>
<td>NS</td>
</tr>
<tr>
<td>TIMP-1, ng/ml</td>
<td>133 ± 33</td>
<td>133 ± 34</td>
<td>144 ± 28</td>
<td>118 ± 26</td>
<td>NS</td>
</tr>
<tr>
<td>proMMP-1, ng/ml</td>
<td>3.6 [1.8, 5.9]</td>
<td>4.6 [2.4, 10.6]</td>
<td>4.2 [3.4, 5.4]</td>
<td>2.7 [2.0, 5.1]</td>
<td>NS</td>
</tr>
<tr>
<td>TIMP-1/proMMP-1,%</td>
<td>2.4 [1.5, 3.8]</td>
<td>2.8 [2.1, 8.1]</td>
<td>2.8 [2.3, 4.4]</td>
<td>1.9 [1.5, 5.0]</td>
<td>NS</td>
</tr>
<tr>
<td>PIIINP, µg/L</td>
<td>3.2 ± 0.8</td>
<td>3.0 ± 1.0</td>
<td>3.1 ± 0.8</td>
<td>2.9 ± 0.6</td>
<td>NS</td>
</tr>
</tbody>
</table>

BG: Bartter Gitelman Syndrome, HS: Healthy Subjects, HTN: Hypertension, PA: Primary aldosteronism. eGFR: estimated glomerular filtration rate (MDRD formula). ‡P values were defined by ANOVA and Kruskal-Wallis for continuous variables and with Chi-square test and Fisher test for categorical variables. Paired comparisons were made with Tukey correction for multiple testing on continuous variables considering *P < 0.05 vs PA, †P < 0.05 BG or HS vs HTN, §P < 0.05 BG vs HS.
Table 2: MRI parameters of Cardiac Geometry and Function: comparison across groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>PA</th>
<th>HTN</th>
<th>BG</th>
<th>HS</th>
<th>P-value</th>
<th>P-value adj</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Left Ventricular and Atrial Parameters</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LVEDVi, ml/m²</td>
<td>82.0 ± 14.9</td>
<td>75.1 ± 14.2</td>
<td>62.8 ± 12.8*†§</td>
<td>76.8 ± 12.9</td>
<td>0.0003</td>
<td>0.0002</td>
</tr>
<tr>
<td>LVESVi, ml/m²</td>
<td>33.3 ± 8.3</td>
<td>29.3 ± 7.4</td>
<td>25.7 ± 6.6*†§</td>
<td>32.1 ± 5.2</td>
<td>0.0046</td>
<td>0.0053</td>
</tr>
<tr>
<td>LV ejection fraction, %</td>
<td>59.4 ± 6.0</td>
<td>61.0 ± 5.7</td>
<td>59.1 ± 5.6</td>
<td>58.0 ± 4.6</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>LV cardiac index, ml/min/m²</td>
<td>3.3 ± 0.7</td>
<td>3.0 ± 0.6</td>
<td>2.6 ± 0.7*</td>
<td>3.0 ± 0.7</td>
<td>0.0068</td>
<td>0.0058</td>
</tr>
<tr>
<td>LVMI, g/m²</td>
<td>71 [65, 86]</td>
<td>64 [56, 68]*</td>
<td>50 [48, 58]*†</td>
<td>56 [53, 62]*</td>
<td>&lt;0.0001</td>
<td>0.0011</td>
</tr>
<tr>
<td>M/V, g/ml</td>
<td>0.85 [0.76, 1.24]</td>
<td>0.86 [0.71, 1.01]</td>
<td>0.87 [0.74, 0.92]</td>
<td>0.73 [0.64, 0.86]</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Diastolic LV thickness, mm</td>
<td>8.3 [7.5, 10.6]</td>
<td>8.2 [7.3, 8.8]</td>
<td>7.1 [6.3, 8.3]*</td>
<td>7.5 [6.6, 7.9]*</td>
<td>0.0048</td>
<td>NS</td>
</tr>
<tr>
<td>Left Atrial Volume index, ml/m²</td>
<td>39.5 ± 9.5</td>
<td>34.7 ± 5.9</td>
<td>26.6 ± 9.4*†§</td>
<td>35.9 ± 6.6</td>
<td>&lt;0.0001</td>
<td>0.0001</td>
</tr>
<tr>
<td><strong>Right Ventricular Parameters</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RVEDVi, ml/m²</td>
<td>81.0 ± 13.8</td>
<td>79.3 ± 13.6</td>
<td>66.1 ± 11.4*†§</td>
<td>81.1 ± 14.7</td>
<td>0.0014</td>
<td>0.0011</td>
</tr>
<tr>
<td>RVESVi, ml/m²</td>
<td>33.5 ± 7.2</td>
<td>35.3 ± 6.1</td>
<td>32.1 ± 5.7</td>
<td>37.8 ± 8.0</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>RV ejection fraction, %</td>
<td>58.6 ± 6.3</td>
<td>55.8 ± 6.1</td>
<td>51.3 ± 4.2*</td>
<td>53.2 ± 6.3*</td>
<td>0.0011</td>
<td>0.0057</td>
</tr>
<tr>
<td>RV mass index, g/m²</td>
<td>16.1 ± 3.0</td>
<td>16.5 ± 3.0</td>
<td>15.4 ± 2.9</td>
<td>17.5 ± 2.9</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Diastolic function Parameters</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak E-wave, ml/s</td>
<td>361 ± 133</td>
<td>314 ± 97</td>
<td>258 ± 75*§</td>
<td>362 ± 123</td>
<td>0.0117</td>
<td>0.0176</td>
</tr>
<tr>
<td>Peak A-wave, ml/s</td>
<td>343 ± 97</td>
<td>294 ± 91</td>
<td>213 ± 68*†</td>
<td>261 ± 80*</td>
<td>0.0001</td>
<td>0.0278</td>
</tr>
<tr>
<td>E/A</td>
<td>1.1 [0.7, 1.3]</td>
<td>0.9 [0.8, 1.3]</td>
<td>1.1 [0.8, 1.6]</td>
<td>1.3 [1.0, 1.9]</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Filling volume, ml</td>
<td>78.0 ± 21.2</td>
<td>64.8 ± 16.7</td>
<td>52.5 ± 13.5*§</td>
<td>74.5 ± 21.0</td>
<td>0.0002</td>
<td>0.0010</td>
</tr>
<tr>
<td>Peak S-wave, cm/s</td>
<td>13.5 [8.9, 17.6]</td>
<td>10.0 [7.0, 12.7]</td>
<td>12.3 [8.9, 14.1]</td>
<td>12.5 [10.5, 17.1]</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Peak E’-wave, cm/s</td>
<td>6.3 [4.4, 9.0]</td>
<td>10.2 [5.7, 16.3]</td>
<td>7.5 [5.3, 16.0]</td>
<td>9.0 [4.9, 18.0]</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Peak A’-wave, cm/s</td>
<td>7.6 [5.1, 9.4]</td>
<td>7.9 [5.1, 14.0]</td>
<td>5.6 [4.1, 11.2]</td>
<td>6.1 [3.9, 7.6]</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

Paired comparisons were made with Tukey correction for multiple testing on continuous variables considering *P <0.05 vs PA, †P <0.05 BG or HS vs HTN, ‡P <0.05 BG vs HS. P values were defined by ANOVA and Kruskal-Wallis and by ANCOVA adjusted on mean arterial pressure measured at the clinical examination.
### Table 3: Quantitative LV Myocardial Tissue Characterization by Cardiac MRI

<table>
<thead>
<tr>
<th></th>
<th>PA</th>
<th>HTN</th>
<th>BG</th>
<th>HS</th>
<th>P-value</th>
<th>P-value adj</th>
</tr>
</thead>
<tbody>
<tr>
<td>Native T1, msec</td>
<td>782 ± 184</td>
<td>773 ± 129</td>
<td>777 ± 158</td>
<td>759 ± 186</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Post contrast T1, msec</td>
<td>408 ± 58</td>
<td>439 ± 59</td>
<td>400 ± 45</td>
<td>417 ± 51</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Partition coefficient, lambda</td>
<td>0.49 ± 0.14</td>
<td>0.38 ± 0.09</td>
<td>0.51 ± 0.15†</td>
<td>0.45 ± 0.13</td>
<td>0.0188</td>
<td>0.0012</td>
</tr>
<tr>
<td>ECV, %</td>
<td>29.5 ± 9.4</td>
<td>23.3 ± 5.5</td>
<td>30.4 ± 10.2</td>
<td>27.0 ± 8.3</td>
<td>0.0480</td>
<td>0.0143</td>
</tr>
<tr>
<td>ECMi, g/m²</td>
<td>20.9 [17.7, 29.2]</td>
<td>14.0 [12.1, 16.5]†</td>
<td>16.4 [11.8, 20.1]†</td>
<td>15.4 [11.4, 18.0]†</td>
<td>&lt;0.0001</td>
<td>0.0208</td>
</tr>
<tr>
<td>ICV, %</td>
<td>70.5 ± 9.4</td>
<td>76.7 ± 5.5</td>
<td>69.6 ± 10.2</td>
<td>73.0 ± 8.3</td>
<td>0.0480</td>
<td>0.0143</td>
</tr>
<tr>
<td>ICMi, g/m²</td>
<td>49.8 [46.0, 67.3]</td>
<td>51.0 [42.3, 53.8]</td>
<td>35.6 [29.4, 40.7]†,†</td>
<td>40.3 [36.2, 46.2]†</td>
<td>&lt;0.0001</td>
<td>0.0007</td>
</tr>
<tr>
<td>ECMi/ICMi</td>
<td>0.45 ±0.24</td>
<td>0.31±0.10</td>
<td>0.47±0.22</td>
<td>0.39±0.16</td>
<td>0.0469</td>
<td>0.0153</td>
</tr>
</tbody>
</table>

Note: ECM, ECMi: extracellular mass and mass index; ICM, ICMi: intracellular mass and mass index; ECV: extracellular volume fraction; ICV: intracellular volume fraction. Paired comparisons were made with Tukey correction for multiple testing on continuous variables considering †P <0.05 vs PA, ‡P <0.05 BG or HS vs HTN, §P <0.05 BG vs HS. P values were defined by ANOVA and Kruskal-Wallis and by ANCOVA adjusted on mean arterial pressure measured at the clinical examination.
FIGURE LEGENDS:

**Figure 1: Cardiac MRI images**
One short axis view (upper row) and one four chamber view (lower row) of the LV obtained in SSFP cine MRI representative of each study group illustrate the progressive increase in LV mass between normotensive male subjects (BG, HS) and hypertensive male subjects (HTN and PA) and the smaller size of the heart including the left atrium in BG subjects.

**Figure 2: Differences across groups in total LV mass (dotted bars), intracellular (white bars) and extracellular (grey bars) LV mass indexes measured by cardiac MRI.**
As compared to HS, decrease in total LV mass index in BG is due to decrease in intracellular mass index, while increase in total LV mass index in HTN is due to a selective increase in intracellular mass index. By contrast, the greater increase in total LV mass index in PA was due to increase in both intracellular and extracellular mass indexes.

**Central Illustration.** Respective and additive effects of aldosterone and hypertension in the overall remodeling of the left ventricle and each of its intra- and extracellular components.
Hypertension

End Diastolic Volume

Concentric Myocardial Remodelling

Myocardial Hypertrophy

Established Generic Imaging Biomarkers

End Diastolic Volume / Total LV mass
Total LV Mass

Aldosterone

New Compartmental Imaging Biomarkers
Extra cellular mass (ECM)
Intra cellular mass (ICM)
Future Therapeutic Targets

Hypertension

Increased afterload is related to increased cardiomyocyte volume

Increased aldosterone is related to increased ECM in the presence of increased BP

Cardiovascular morbidity and mortality
Heart Failure, Arrhythmias, Sudden death