Demonstration of Blood Pressure-Independent Non Infarct Myocardial Fibrosis in Primary Aldosteronism: A Cardiac MRI Study

Freel et al: Non-Infarct Myocardial Fibrosis in PA

E. Marie Freel, BSc, MBChB, PhD 1; Patrick B. Mark, MBChB, PhD 1; Robin A.P. Weir, MBChB, MD 2; Emily P. McQuarrie, MBChB 1; Karen Allan, BN 1; Henry J. Dargie, MBChB, MD 1; John D. McClure, PhD 1; Alan G. Jardine, MBChB MD 1; Eleanor Davies, BSc PhD 1; John M.C. Connell, MB ChB MD 3

1Institute of Cardiovascular and Medical Sciences
University of Glasgow
Glasgow G12 8TA

2Department of Cardiology
Hairmyres Hospital, East Kilbride
Glasgow G75 8RG

3Medical Research Institute
Ninewells Hospital and Medical School, University of Dundee
DD1 9SY

Correspondence to
Dr E Marie Freel
BHF Glasgow Cardiovascular Research Centre
126 University Place, Glasgow, G12 8TA
Telephone: +44 141 330 3412
Fax: +44 141 330 1689
Email: Marie.Freel@glasgow.ac.uk

DOI: 10.1161/CIRCIMAGING.112.974576

Abstract

**Background**—Primary Aldosteronism (PA) is common and associates with excess cardiovascular morbidity independent of blood pressure. Exposure to aldosterone and sodium leads to cardiac fibrosis and hypertrophy in humans and animals possibly mediated by inflammation and oxidative stress. We aimed to clarify the effects of aldosterone excess on myocardial structure and composition in human subjects with PA and essential hypertension (EH) using contrast-enhanced cardiac MRI (CMR) as well as explore the mechanistic basis for any observed differences.

**Methods and Results**—Twenty seven subjects with recently diagnosed PA and 54 EH controls were recruited. Subjects underwent gadolinium-enhanced CMR; non-infarct related myocardial fibrosis was identified by a diffuse pattern of late gadolinium enhancement (LGE). Patients also underwent assessment of pulse wave velocity (PWV), measurement of circulating superoxide anion and C-reactive protein as well as blood pressure and biochemical assessment. Subjects were well matched with no difference in severity nor duration of hypertension. There was a significant increase in the frequency of non-infarct LGE in PA (70%) when compared to EH subjects (13%; p<0.0001) with no difference in LV mass. PWV, superoxide and CRP were significantly higher in PA subjects.

**Conclusions**—These data illustrate that PA patients exhibit more frequent myocardial fibrosis as demonstrated by LGE using CMR imaging; this finding is independent of blood pressure. This may be mediated partly through inflammation and oxidative stress. This study highlights the importance of specific targeting of aldosterone excess as well as blood pressure reduction to minimise cardiac morbidity in Primary Aldosteronism.

**Key Words:** cardiac MRI, hypertension, aldosterone
Primary Aldosteronism (PA) is a common cause of hypertension; aldosterone excess is found in approximately 10% of unselected hypertensives and up to 20% of subjects with resistant hypertension (1;2). Moreover, patients with PA demonstrate increased cardiovascular morbidity in comparison to subjects with equivalent essential hypertension. In one study, where patients with PA were compared to matched hypertensives, PA patients demonstrated a 4-fold increase in stroke rate, 6.5-fold increase in risk of myocardial infarction (MI) and 12-fold increase in the prevalence of atrial fibrillation(3). Subsequent similar studies have confirmed these findings implying that aldosterone excess has significant adverse cardiovascular effects beyond its influence on blood pressure (BP) (4-6).

In animal models of PA, cardiac fibrosis and hypertrophy occur independent of BP, as demonstrated by amelioration of these effects by non-antihypertensive doses of the mineralocorticoid receptor (MR) antagonist, spironolactone(7). However, the exact mechanism behind the deleterious cardiovascular effects of aldosterone remains unclear.

There is strong evidence that aldosterone is pro-inflammatory, can directly stimulate cardiac fibroblasts and can also increase vascular oxidative stress (by increasing availability of reactive oxygen species); these mechanisms can lead to scarring and fibrosis (8-10). In addition, aldosterone excess associates with increased arterial stiffness in both human and animal studies which is reversed by MR blockade; this may also contribute to cardiac structural changes. Finally, data have emerged supporting the role of intracellular calcium overload, in response to aldosterone-induced secondary hyperparathyroidism, in this process(11).

The effect of aldosterone excess on cardiac structure and function in humans is unclear; some studies show no influence on LV mass (LVM) while others suggest that PA associates with increased LVM compared to essential hypertension (12-15). However, most studies so far have assessed LV morphology using 2-dimensional echocardiography. Cardiac magnetic
resonance (CMR) imaging offers a validated, precise, 3-D measurement of LV mass and morphology. It is becoming widely used in clinical practice for assessment of LV function and dimensions and for demonstration of evidence of prior ischaemic heart disease (16;17). Furthermore, using gadolinium-based contrast agents, the presence of myocardial fibrosis can be implied by demonstrating the presence of ‘late’ gadolinium enhancement (LGE). Contrast-enhanced CMR imaging has provided additional insights into conditions classically associated with fibrosis such as MI and hypertrophic and dilated cardiomyopathy(17-19). The current study aimed to compare myocardial structure and composition in PA patients with matched essential hypertensives using contrast-enhanced CMR to explore the key hypothesis that subjects with aldosterone excess were more likely to exhibit LGE suggestive of cardiac fibrosis. In addition, we aimed to provide some insight into the mechanistic basis of any differences by measuring non-invasive parameters of arterial stiffness as well as comparing common markers of inflammation and oxidative stress between patient populations.

**Methods**

**Subjects**

Twenty seven subjects with confirmed PA diagnosed in the Western Infirmary, Glasgow were included. All were diagnosed with PA using Endocrine Society guidelines(20). Briefly, screened subjects with an elevated aldosterone to renin ratio (ARR) (> 750 with aldosterone in pmol/l and renin measured as plasma renin activity; >35 if renin measured as plasma renin concentration) underwent repeat screening after withdrawal of medications affecting measurements of plasma renin and aldosterone (for 4-6 weeks). If the elevated ARR persisted, then aldosterone excess was confirmed using a saline suppression test (PA confirmed if plasma aldosterone is >270pmol/L despite infusion of 2 litres of normal saline over 4 hours).
Subjects subsequently underwent adrenal imaging (computerised tomography) and adrenal vein sampling if appropriate in order to differentiate between unilateral and bilateral forms of PA. Importantly, all PA subjects included in the study were investigated either before or within one year of adrenalectomy or commencing specific medical therapy. The control group comprised 53 essential hypertensive (EH) subjects (2 per PA subject; except in one case where the control failed to complete the study) each matched to a PA subject for severity and duration of hypertension (established from casenote review), age and gender. All such patients had a normal ARR and none were on MR antagonists. No patient had pre-existing structural cardiac abnormalities (excluded by clinical examination and echocardiography where appropriate). All subjects were healthy out-patients with no inter-current illness at the time of study.

Study subjects were evaluated in the morning after fasting from midnight. They underwent measurement of weight, height and brachial BP (recorded as the average of triplicate measurements taken at intervals of 1 min using a validated oscillometric device (HEM-907; Omron Healthcare, Kyoto, Japan) after an initial 5 min of seated rest). All bloods were measured after 30 minutes of supine rest.

This study was approved by the West of Scotland Research Ethics Committee and all subjects gave informed consent.

Cardiac MRI

Contrast-enhanced CMR was performed within 24 hours of the first study visit using a 1.5-Tesla Siemens Sonata (Siemens, Erlangen) with a phased-array chest coil, during breath-hold, and gated to the electrocardiogram. A steady-state free-precession sequence was used to acquire a short-axis cine stack of the LV from base to apex, consisting of 8-mm-thick slices with a 2-mm interslice gap. Ten minutes after the intravenous injection of a contrast agent
(gadoterate meglumine, ®Dotarem 0.1 mmol/kg; Guerbet, Roissy France) LGE images were acquired in the same views as for cine images, using a contrast-sensitive segmented inversion recovery sequence. The time to inversion was varied to obtain optimal nulling of the myocardium for the delayed enhancement sequences.

Post-processing was performed using commercially available Argus software (Siemens, Erlangen). Manual planimetry, performed by one observer blinded to underlying diagnosis, was used to trace the epicardial and endocardial contours of each short-axis slice acquired in the cine stack, allowing calculation of LV volumes, LVEF, and LV mass (myocardial density taken as 1.05 g/cm³). The most basal LV slice at both end-systole and end-diastole was defined as that in which the blood pool was surrounded by 50% or more of ventricular myocardium; papillary muscles were excluded from the LV volumes and included in the LV mass.

Left ventricular systolic dysfunction (LVSD) was defined as LV ejection fraction (LVEF) <55%, with LVH defined as left ventricular mass index (LV mass/body surface area; LVMI) >84.1 g/m² (male) or >76.4 g/m² (female) and LV dilation defined as end diastolic volume/body surface area >111.7 ml/m² (male) or 99.3 ml/m² (female) or end systolic volume >92.8 ml (male) or 70.3 ml (female) based on based on mean normal LV dimensions for healthy volunteers plus 2 standard deviations(21) .

Myocardial fibrosis was indicated by the presence of LGE as previously described with each image reviewed by two blinded independent observers. Images were assessed for the presence and pattern of gadolinium enhancement. Patients were classed as having positive LGE if LGE was seen on at least two (of three) views: short axis view, long axis view, and reverse phase sequences, to exclude artefact. The pattern of LGE was defined as infarct (subendocardial) or non-infarct (diffuse).
Assessment of arterial haemodynamics

Carotid-femoral and carotid-radial pulse wave velocity (PWV) was carried out using the SphygmoCor® Vx system (Atcor Medical, Sydney, Australia) by a single operator. PWV was measured from sequentially recorded electrocardiogram-gated carotid, radial and femoral artery waveforms. The aortic augmentation index (AIx), a measure of wave reflection, was determined from radial waveforms using the same device. The PWV and AIx measurements were made in triplicate, and the mean values were used in the subsequent analysis. Detailed descriptions of PWV and AIx measurements and their reproducibility have been reported previously(22).

Plasma measurements

Plasma renin activity (PRA) was measured by the Biodata Renin MAIA (Serono Diagnostics Ltd., Woking, Surrey, UK) with an intra-assay coefficient of variation (CV) of less than 10% between 0.3 and 18 ng/ml·h and a least detectable concentration of 0.3 ng/ml·h. The interassay batch variation over 1 year was 11% (QC mean values, 2.3 and 6.1 ng/ml·h).

Plasma aldosterone was measured by direct radioimmunoassay (RIA) utilising the ‘Coat-A-Count’ system (Euro/DPC Ltd, Caernarfon,Wales). The radioisotope used was $^{125}\text{I}$-aldosterone. CVs (%, within-batch and between-batch respectively) were: 2.3–5.4/3.8–15.7.

In 2009, the local method of renin measurement changed to plasma renin concentration (PRC)(23). This was measured using the Diasorin analyser (Stillwater, MN, USA) (normal range 5 – 500μIU/l). The intra-assay CV was <3.4% and inter-assay CV was <6.2%.

Oxidative stress status was assessed by analysing superoxide release from whole blood using an established method (24;25). In brief, venous blood was collected in lithium heparinate containing tubes and processed immediately. Superoxide levels were detected by electron paramagnetic resonance (e-scan R; Bruker BioSpin GmbH, Rheinstetten, Germany) with the
spin probe 1-hydroxy-3-carboxy-2,2,5,5-tetramethylpyrrolidine (CPH; Noxygen, Elzach, Germany) to a final concentration of 500 \( \mu \)M. Instrument settings were: centre field of 3375 G, modulation amplitude of 2.27 G, sweep time of 5.24 s, sweep width of 60 G and 10 scans. Superoxide levels were recorded as counts per minute for 10 minutes and a best fit regression line through these data points was constructed; the calculated slope of this line was used to measure the rate of superoxide anion production.

C-reactive protein (CRP) was measured in a single run on all samples using a high-sensitivity method on a clinically validated automated platform (c311, Roche Diagnostics, Burgess Hill, UK). The analyser was calibrated and quality controlled using the manufacturers reagents, and according to their instructions (CV <5%).

Statistics

Data were analysed using SPSS (version 15 SPSS Inc, Chicago, Illinois) software. The matching of 2 EH subjects with each PA was taken account of using these triplets as the clustering variable. Specifically, continuous variables were compared using a linear mixed effects model; non-normally distributed variables underwent logarithmic transformation \((\log_{10})\) before analysis. The presence of myocardial fibrosis indicated by CMR was assessed using a General Estimating Equation model, with a logit link function.

Results

Clinical characteristics

Table 1 summarises the clinical details of PA subjects and their essential hypertensive (EH) controls. Eleven PA patients had adenomatous PA (APA) treated surgically; seven were studied prior to adrenalectomy. Sixteen subjects had bilateral aldosterone excess (13 with bilateral adrenal hyperplasia, 3 with Glucocorticoid Remediable Aldosteronism) treated
medically; three were on no specific treatment for aldosterone excess at the time of study. The remaining subjects had commenced specific treatment or undergone adrenalectomy less than a year before the study date (mean time between initiation of definitive treatment was 147 days). Medically treated subjects were on spironolactone (n=8; mean dose 69 mg), eplerenone (n=3; mean dose 108 mg) or amiloride (n=1, dose 20 mg). Groups were well matched in terms of severity and duration of hypertension as well as age. PA patients had a slightly higher BMI than EH subjects; this was unlikely to be of clinical significance. No subject in the EH group demonstrated an elevated ARR.

Cardiac dimensions

Twenty six PA patients underwent cardiac MRI scanning (one PA patient unable to tolerate MRI) along with 52 matched EH controls. Table 2 illustrates no significant difference in myocardial dimensions between PA and EH subjects. Two patients in the EH group had LVH compared with three patients in the PA group. No PA patients had LVSD with one EH patient demonstrating mild LVSD.

Pulse wave velocity

Carotid-femoral and carotid-radial PWV were significantly higher in the PA group (Table 3). There was no significant difference in AIx between the two groups.

Presence of late gadolinium-enhancement (LGE)

Gadolinium-enhanced CMR data were available for 24 PA patients (one not scanned, two refused gadolinium contrast). Of the 48 EH controls, seven could not be analysed for the presence of LGE because of breathing artefact meaning seven PA patients had only one matched EH control.
One PA patient demonstrated an infarct pattern on LGE; this patient had a previous anterior MI several years earlier. Two EH subjects demonstrated LGE pattern suggestive of previous MI. Once the analysis was unblinded, it was shown that one patient had pre-existing coronary artery disease. The other patient had no such personal history and was referred for cardiology follow up. These subjects were excluded from subsequent analysis.

Figure 1 gives typical long axis and basal/mid-cavity/apical short axis views of non-infarct LGE in PA subjects (Figure 1C and 1D) as well as an EH and PA subject with no LGE for comparison (Figure 1A and 1B respectively). The frequency of non-infarct LGE was significantly higher in the PA group (16/23; 70%) compared to EH subjects (5/39; 12.8%), p<0.0001 (Figure 2).

The presence of LGE was independent of BP and myocardial mass with no significant difference in these parameters in subjects (either PA or EH) with LGE when compared to those without LGE.

Adenomatous versus bilateral PA

PA subjects underwent further analysis to determine if cause of aldosterone excess affected cardiac outcomes. There was no significant difference in the frequency of LGE between subgroups- 73% (8/11) of APA demonstrated LGE compared to 69% (9/13) of patients with bilateral aldosterone excess (p =0.6). Similarly, there was no significant difference in mean BP, age, myocardial mass or LVMI between subgroups.

Plasma markers

Whole blood superoxide levels were significantly higher in PA patients compared to EH subjects (Figure 3). Plasma CRP levels were also significantly higher in PA patients (Figure 4).
Discussion

These data illustrate that subjects with Primary Aldosteronism are significantly more likely to exhibit myocardial fibrosis (as demonstrated by non-infarct pattern of LGE) when compared to subjects with equivalent essential hypertension. Additionally, this phenomenon may be partly mediated by oxidative stress and inflammation given the significantly higher levels of whole blood superoxide and CRP found in PA subjects in comparison to essential hypertensives. These data are the first, to our knowledge, to demonstrate the presence of BP-independent myocardial fibrosis using gadolinium-contrast CMR in humans with PA and support the concept that targeting of aldosterone excess in addition to lowering BP are equally important in this patient group.

The results from this study confirm previous animal studies which demonstrated that aldosterone can cause cardiac fibrosis independent of its effect on BP or on the development of ventricular hypertrophy(26;27). Crucially, these pro-fibrotic actions of aldosterone only develop in animals fed a high salt diet(28). Mean urinary sodium excretion did not differ significantly between the two patient groups we studied, but the mean excretion rate in both indicates that our patients were consuming a salt-rich diet characteristic of our local population. We speculate that this feature is likely to exacerbate the deleterious effects of aldosterone excess in our study.

Regional myocardial fibrosis is well described using delayed gadolinium contrast enhancement during cardiac MRI(29). Areas of myocardial damage and collagen deposition have a much slower washout rate of gadolinium-based contrast than healthy myocardium, leading to markedly increased signal intensity on T1-weighted imaging(30). This phenomenon of ‘late gadolinium enhancement’ (LGE) is well established in ischaemic heart disease which associates with discrete areas of LGE typically within the subendocardium(29).

More recently, we have described a more diffuse pattern of LGE in patients without coronary
artery disease which probably represents less severe myocardial fibrosis or milder degrees of excess collagen deposition(31). Whilst LGE cannot be used synonymously for fibrosis without cardiac biopsy data, there was no evidence of other possible causes (myocarditis, interstitial amyloid) within our patient groups supporting our conclusion that diffuse LGE in these subjects was as a consequence of aldosterone-driven myocardial fibrosis. These results confirm and extend earlier studies by Rossi et al who, using echocardiography and videodensitometry, demonstrated alterations in myocardial textures consistent with increased collagen deposition in PA and not essential hypertensive subjects(32).

Non-infarct LGE was found in only 13% of our EH subjects. This proportion may seem lower than expected; a recent study by Rudolph et al demonstrated LGE in 50% of subjects with essential hypertension(33). However, these subjects all had CMR evidence of global LVH and were pre-selected by the presence of LVH on echocardiography implying more significant underlying hypertension with the expectation of more significant cardiac structural abnormalities. In contrast, LVH was only demonstrated in 2 of our EH subjects. The presence of cardiac fibrosis without LVH is reminiscent of the original experiments of Karl Weber and colleagues. In rodents with renovascular or aldosterone/salt induced hypertension fibrosis was seen not only of the hypertrophied left ventricle but also of the normotensive, non-hypertrophied right ventricle implying that fibrosis can occur due to humoral (ie.aldosterone) rather than haemodynamic factors.

Pulse wave velocity was significantly higher in PA vs EH patients implying increased arterial stiffness in this group. These results confirm previous similar data as well as findings of Rizzoni et al who demonstrated increased total and type III vascular collagen in the extracellular matrix of small resistance arteries in PA and matched essential hypertensive patients (34-36). There was no difference in augmentation index (AIx) between groups. The reasons for this are speculative but may reflect the relatively ‘young’ age of our cohort; it has
been postulated that, since pulse pressure only increases significantly after the 5th decade, stiffening of the large arteries tends to occur in later life (37).

Previously, the effect of aldosterone excess on LV hypertrophy in humans has been controversial. In a separate study, Rossi et al showed, using echocardiography, that LV wall thickness and LVM were increased in PA subjects; this finding was also demonstrated by other investigators (12;13;38). However, other groups have shown no difference in LV dimensions between PA and EH subjects (15). Our data show no significant difference in LVM or LVMI between PA and EH patients. This may reflect relatively ‘mild’ hypertension demonstrated by the PA patients in this study. Additionally, most other studies of LV dimensions in PA and hypertensive patients rely on echocardiography data; cardiac MRI provides a more sensitive method of structural analysis and it is unclear how well MRI and echocardiographic findings correlate. In the previously quoted study by Rudolph et al, LVH using CMR was found in only 83 of 440 subjects (18%) with pre-identified LVH using echocardiography (33). It is pertinent that, in the only other study using relating aldosterone status with CMR findings, there was no significant difference in LVMI between essential hypertensive subjects with high versus low aldosterone production (39). PA patients did demonstrate an increased ejection fraction when compared to EH subjects. This may reflect the volume-expanded status of patients with mineralocorticoid excess. In an analogous situation, subjects with PA demonstrate increased eGFR in comparison with essential hypertension (suggestive of early glomerular hyperfiltration) and this resolves with treatment of aldosterone excess. (40)

Numerous human and animal studies demonstrate that aldosterone increases pro-inflammatory cells and cytokines leading to vascular inflammation and fibrosis (8;41). In addition, chronic aldosterone and salt treatment in rats increases the expression of NADPH oxidase in the myocardium which catalyses the formation of the superoxide anion which, in
tum, promotes inflammation and fibrosis (10;42). All of these effects are attenuated by the administration of MR antagonists and so suggest that aldosterone induces a pro-inflammatory phenotype, at least in part by increasing oxidative stress. This is supported by our finding of significantly elevated levels of the inflammatory hormone CRP as well as whole blood superoxide in PA subjects when compared to essential hypertensives. More recently, there has been evidence in rats with aldosterone excess that oxidative stress and cardiac fibrosis may occur as a consequence of intracellular cardiac calcium overload (11;43). Human and animal studies suggest that PA is accompanied by mild secondary hyperparathyroidism and resultant intracellular calcium overload in response to excess urinary and faecal loss of calcium and magnesium (11;44). Although we did not measure serum parathyroid hormone in our study, mean serum adjusted calcium was significantly lower in PA patients (2.37 mmol/L vs 2.47 mmol/L p<0.006) which supports this intriguing hypothesis.

Strengths of this study include the rigorous matching of newly diagnosed PA patients to subjects with essential hypertension as well as the use of the ‘gold standard’ technique of CMRI as a non invasive tool to accurately assess cardiac dimensions and gadolinium enhancement patterns characteristic of non-infarct related fibrosis.

Although PA subjects were studied as close to diagnosis as possible and always within a year of diagnosis/initiation of treatment, it could be that some subjects demonstrated rapid regression of LVH in response to medical or surgical management of aldosterone excess and this is a potential weakness of the study. In the studies of Catena et al, where a large cohort of PA patients were followed up for a mean of 7 years after medical or surgical therapy, there was a significant reduction in LVMI in PA within a year of surgical (although not medical) management despite similar reductions in BP (13). In our cohort, however, the mean time between initiation of specific treatment of aldosterone excess and study visit was less than 5 months and so it is unlikely that any substantial cardiac benefit would have been evident.
Moreover, a small number of PA patients (n=10) were studied prior to treatment of aldosterone excess. There was no significant difference in the frequency of LGE in this cohort when compared to the treated PA group (frequency of 56% and 67% respectively, p>0.05; data not shown). In addition, there was evidence of increased inflammation, oxidative stress and increased vascular stiffness in the PA group, supporting the theory that aldosterone excess was still exerting adverse effects. Indeed, the significantly positive findings of this study despite the inclusion of recently treated PA subjects may imply that the results would have been more striking if untreated patients had been studied and suggests that these results in fact represent a conservative summary of the adverse cardiovascular effects of aldosterone.

In conclusion, we have demonstrated for the first time that subjects with PA are more likely to develop cardiac fibrosis, as evidenced by a non-infarct pattern of diffuse LGE on cardiac MRI, than matched subjects with essential hypertension. We have further shown that myocardial fibrosis in these subjects may be partly mediated by pro-inflammatory and oxidative stress effects of aldosterone excess. These data highlight that amelioration of aldosterone excess by specific medical or surgical measures in addition to BP reduction is crucial in this common patient cohort.

Acknowledgements

The authors acknowledge the work of Tracey Steedman and Kirsten Lanaghan with CMR imaging.

Sources of Funding

EMF is funded by a Clinician Scientist Fellowship awarded by the Medical Research Council (reference number G0802803).
Disclosures

None.

References


42. White PC. Aldosterone: direct effects on and production by the heart. J Clin Endocrinol Metab. 2003;88:2376-83.
<table>
<thead>
<tr>
<th></th>
<th>Primary Aldosterone (PA)</th>
<th>Essential hypertensives (EH)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex</strong></td>
<td>21 M/6F</td>
<td>42 M/11 F</td>
<td>-</td>
</tr>
<tr>
<td><strong>Age (y)</strong></td>
<td>53.8 (11.2)</td>
<td>55.1 (9.4)</td>
<td>0.06</td>
</tr>
<tr>
<td><strong>BMI (kg/m2)</strong></td>
<td>31.1 (5)</td>
<td>29.2 (4.6)</td>
<td>0.02</td>
</tr>
<tr>
<td><strong>Serum potassium (mmol/l)</strong></td>
<td>3.4 (0.5)</td>
<td>3.9 (0.4)</td>
<td>0.01</td>
</tr>
<tr>
<td><strong>Creatinine clearance (ml/min)</strong></td>
<td>110.1 (26.8)</td>
<td>116.1 (33.9)</td>
<td>0.5</td>
</tr>
<tr>
<td><strong>Plasma aldosterone (pmol/l)</strong></td>
<td>754 (299)</td>
<td>260 (208)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>ARR</strong></td>
<td>*1998 (975)</td>
<td><strong>153.6 (145.8)</strong></td>
<td><strong>13.78 (31.31)</strong></td>
</tr>
<tr>
<td><strong>Urine sodium (mmol/24h)</strong></td>
<td>173.8 (88.7)</td>
<td>176.4 (82.4)</td>
<td>0.71</td>
</tr>
<tr>
<td><strong>Urine potassium (mmol/24h)</strong></td>
<td>76.1 (29.3)</td>
<td>89.2 (29)</td>
<td>0.09</td>
</tr>
<tr>
<td><strong>Urine albumin (mg/24h)</strong></td>
<td>110 (131)</td>
<td>43.9 (71.4)</td>
<td>0.14</td>
</tr>
<tr>
<td><strong>Urine albumin:creatinine</strong></td>
<td>6.33 (7.9)</td>
<td>14.4 (11.7)</td>
<td>0.92</td>
</tr>
<tr>
<td><strong>SBP (mm/Hg)</strong></td>
<td>150.3 (26.6)</td>
<td>152.63 (19.3)</td>
<td>0.65</td>
</tr>
<tr>
<td><strong>DBP (mm/Hg)</strong></td>
<td>89.9 (11.9)</td>
<td>94.3 (11.2)</td>
<td>0.12</td>
</tr>
<tr>
<td><strong>Number of anti-hypertensive medications</strong></td>
<td>2.5 (1.6)</td>
<td>2.2 (1.4)</td>
<td>0.37</td>
</tr>
<tr>
<td><strong>Duration of hypertension (y)</strong></td>
<td>9.2 (8)</td>
<td>11.3 (8.3)</td>
<td>0.84</td>
</tr>
</tbody>
</table>

Data are means (+/- standard deviation). Data compared by linear mixed effects model.

* ARR expressed using plasma renin activity (PRA; normal range < 750 pmol/litre/ng/ml/h)

** ARR expressed using plasma renin concentration (PRC; normal range < 35 pmol/litre/uIU/ml)

BMI: body mass index; ARR: aldosterone to renin ratio; SBP: systolic blood pressure; DBP: diastolic blood pressure
Table 2. Summary of cardiac dimensions of PA vs EH patients as analysed by cardiac MRI.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Primary Aldosteronism</th>
<th>Essential hypertension</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>EF (%)</td>
<td>77 (6)</td>
<td>70 (10)</td>
<td>0.002</td>
</tr>
<tr>
<td>LV EDV (ml)</td>
<td>123 (27)</td>
<td>132 (31)</td>
<td>0.2</td>
</tr>
<tr>
<td>SV (ml)</td>
<td>94 (20)</td>
<td>91 (22)</td>
<td>0.6</td>
</tr>
<tr>
<td>LVM (g)</td>
<td>135 (34)</td>
<td>128 (33)</td>
<td>0.2</td>
</tr>
<tr>
<td>LVMI (g/m²)</td>
<td>64 (13)</td>
<td>62 (13)</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Data are means (+/- standard deviation) compared by linear mixed effects model.

LVEDV: left ventricular end-diastolic volume, SV: stroke volume, LVM: left ventricular mass, LVMI: left ventricular mass index.
Table 3. Summary of peripheral applanation tonometry data of PA vs EH patients as analysed by Sphygmacor

<table>
<thead>
<tr>
<th></th>
<th>Primary Aldosteronism</th>
<th>Essential hypertension</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>PWV (m/s) (carotid-femoral)</td>
<td>0.99 (0.08)</td>
<td>0.90 (0.1)</td>
<td>0.006</td>
</tr>
<tr>
<td>PWV (m/s) (carotid-radial)</td>
<td>0.96 (0.06)</td>
<td>0.94 (0.06)</td>
<td>0.04</td>
</tr>
<tr>
<td>Augmentation index (%)</td>
<td>1.44 (0.17)</td>
<td>1.49 (0.15)</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Data are logged and expressed as means (+/- standard deviation) and compared by linear mixed effects model. PWV: pulse wave velocity.
**Figure Legends**

Figure 1. Horizontal long axis (i) and basal/mid cavity/apical short axis views (ii-iv) of contrast enhanced cardiac MRI images of:

A: EH subject (no LGE)
B: PA subject (no LGE)
C: PA subject (non-infarct LGE in images i and ii).
D: PA subject (localised non-infarct LGE in images i-iii)

EH: essential hypertension  PA: Primary Aldosteronism  LGE: late gadolinium enhancement

Figure 2. Frequency of non-infarct late gadolinium enhancement in PA vs EH patients.
Frequencies compared using logistic regression (general estimating equations). Subjects with LGE due to myocardial infarction were excluded

Figure 3. Whole blood superoxide levels in PA patients compared to matched EH.
Rate of production is derived from the gradient of the regression line drawn between 10 data points for each sample (full details in methods section)
Logarithmic transformed data (log_{10}) were compared using linear mixed effects model

Figure 4. C-reactive protein (CRP) levels in PA compared to EH subjects
Logarithmic transformed data (log_{10}) were compared using linear mixed effects model
Figure 1A (i)
Figure 1A (ii)
Figure 1A (iii)
Figure 1A (iv)
Figure 1B (i)
Figure 1B (ii)
Figure 1C (i)
Figure 1C (iii)
Figure 1C (iv)
Figure 1D (i)
Figure 1D (ii)
Figure 1D (iii)
Figure 1D (iv)
Figure 2
Figure 3

Rate of production (Au)

p<0.05
Figure 4

- Log CRP (mg/L)
- PA
- EH
- p<0.04