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Alterations in vascular function in primary aldosteronism – a cardiovascular magnetic resonance imaging study

Running title: Vascular function in primary aldosteronism

Patrick B MARK PhD¹

Stephen BOYLE MB, ChB¹

Lukas U ZIMMERLI MD²

Emily P MCQUARRIE MD¹

Christian DELLES MD¹

E Marie FREEL PhD¹

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1. BHF Cardiovascular Research Centre,

Institute of Cardiovascular and Medical Sciences,

University of Glasgow,

126 University Place,

Glasgow G12 8TA

2. UniversitätsSpital Zürich, Klinik und Poliklinik für Inner Medizin,

Rämistrasse 100, CH- 8091, Zürich, Switzerland

Correspondence: Dr Patrick B Mark

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BHF Cardiovascular Research Centre,
Institute of Cardiovascular and Medical Sciences,
University of Glasgow,
126 University Place, Glasgow G12 8TA, United Kingdom.

E-mail: patrick.mark@glasgow.ac.uk

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40 **Abstract**

Introduction

Excess aldosterone is associated with increased cardiovascular risk. Aldosterone has a permissive effect on vascular fibrosis. Cardiovascular magnetic resonance imaging (CMR) allows study of vascular function by measuring aortic distensibility. We compared aortic distensibility in primary aldosteronism (PA), essential hypertension (EH) and normal controls and explored the relationship between aortic distensibility and pulse wave velocity (PWV).

Methods We studied PA (n=14) and EH (n=33) subjects and age-matched healthy controls (n=17) with CMR, including measurement of aortic distensibility, and
50 measured PWV using applanation tonometry. At recruitment, PA and EH patients had similar blood pressure and left ventricular mass.

Results Subjects with PA had significantly lower aortic distensibility and higher PWV compared to EH and healthy controls. These changes were independent of other factors associated with reduced aortic distensibility, including aging. There was a significant relationship between increasing aortic stiffness and age in keeping with physical and vascular aging. As expected, aortic distensibility and PWV were closely correlated.

Conclusion These results demonstrate that PA patients display increased arterial stiffness compared to EH, independent of vascular aging. The implication is that
60 aldosterone invokes functional impairment of arterial function. The long-term

implications of arterial stiffening in aldosterone excess require further study.

Key words: Cardiac magnetic resonance imaging, aortic distensibility, primary aldosteronism, hypertension, pulse wave velocity, arterial stiffness

Introduction

The prevalence of Primary Aldosteronism (PA) is higher than previously thought. Aldosterone excess is found in approximately 10% of unselected hypertensives and up to 20% of subjects with resistant hypertension^{1, 2}. It is known that PA patients are at increased cardiovascular risk in comparison to subjects with essential hypertension (EH) and equivalent blood pressure, including dramatic increases in risk of cerebrovascular disease, myocardial infarction and prevalence of atrial fibrillation³. These observations and others suggest that aldosterone excess has significant adverse cardiovascular effects beyond its influence on blood pressure^{4, 5}. One potential mechanism linking aldosterone and cardiovascular disease relates to arterial stiffness; *in vitro* data suggest that aldosterone stimulates hyperplasia of vascular smooth muscle cells and additionally activates protein synthesis, increasing extracellular matrix and collagen deposition, leading to vascular fibrosis⁶⁻⁸. Patients with PA have been demonstrated to have increased carotid intima media thickness at ultrasound compare to EH, suggesting aldosterone excess contributes the fibrosis and thickening of the arterial wall⁹. In addition, aldosterone has also been shown to adversely affect endothelial function by enhancing oxidative stress and inflammation^{10, 11}. The potential sequelae of these effects include increasing vascular stiffness and atherosclerosis, which may impede conduit arterial function and worsen cardiovascular outcome.

Epidemiological studies have shown that increased arterial stiffness, most commonly

assessed by measurement of pulse wave velocity (PWV) is independently
90 associated with cardiovascular risk factors and morbidity^{12, 13}. PWV, measured
using applanation tonometry, is the 'gold-standard' technique of arterial stiffness
assessment in human subjects although alternative methods have emerged¹⁴. A
number of studies have demonstrated increased arterial stiffness or impaired arterial
conduit function in PA patients compared to EH, using PWV or augmentation index
(AIx), respectively, measured with applanation tonometry^{15, 16}. Cardiovascular
magnetic resonance imaging (CMR) permits visualisation of large arteries and
potentially offers an integrated non-invasive method of assessing vascular, as well
as ventricular function, during the same examination. Aortic distensibility (AD) is an
index of arterial stiffness and can be defined as the relative change in the cross-
100 sectional area of the aorta divided by central pulse pressure. AD measured with
CMR has emerged as a marker of vascular function in groups at elevated
cardiovascular risk^{17, 18}. CMR allows direct visualisation of blood vessels and is
independent of crude estimates of aortic length from tape measurements along the
body surface, which are required for PWV assessment with tonometry. CMR also
allows vascular assessment in subjects where tonometry may be challenging such
as obese subjects.

To date however, the relationship between AD, measured with CMR, and PWV has
not been assessed in PA patients or compared with EH subjects. Thus, we assessed

110 the relationship between CMR measures of vascular function, PWV, Alx and conventional CV risk factors in recently diagnosed PA with EH patients and healthy controls. To give insight into the mechanistic basis for any observed differences we also measured markers of inflammation and oxidative stress in PA and EH groups. We hypothesised that PA subjects would exhibit a more adverse vascular phenotype in comparison to EH subjects using CMR.

Subjects and Methods

Subjects

Fourteen patients with confirmed PA diagnosed in the Endocrine Department, Western Infirmary, Glasgow were studied. All were diagnosed with PA using Endocrine Society guidelines¹⁹. Briefly, screened subjects with an elevated aldosterone to renin ratio (ARR) (> 750 with aldosterone in pmol/l and renin measured as plasma renin activity in ng/ml/hr; >35 if renin measured as plasma renin concentration in mIU/mL) underwent repeat screening after withdrawal of all treatment affecting measurements of plasma renin and aldosterone (for 4-6 weeks). If the elevated ARR persisted, then autonomous aldosterone excess was confirmed using a saline suppression test. In this test, PA is 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 or magnetic resonance imaging) and adrenal vein sampling if surgery was to be considered in order to differentiate between unilateral and bilateral forms of PA.

Two control groups were studied. Thirty-three patients with EH comprised the first group. All patients with PH had a normal ARR and none were on mineralocorticoid receptor (MR) antagonists. No patient had pre-existing structural cardiac abnormalities (excluded by clinical examination and echocardiography where appropriate). All subjects were out-patients with no inter-current illness at the time of

study. A cohort of seventeen healthy volunteers with no past medical history and on no regular medication was also studied. This study was approved by the West of
140 Scotland Research Ethics Committee and all subjects gave informed consent.

CMR technique

All patients underwent CMR using a 1.5 Tesla MRI scanner (Sonata, Siemens, Erlangen, Germany). A fast-imaging with steady-state precession sequence was used to acquire cine images of the left ventricle (LV) in long axis followed by sequential short axis LV cine loops (8mm slice thickness, 2mm gap between slices) from the atrioventricular ring to the apex. Imaging parameters, which were standardized for all subjects, included: TR/TE/flip angle/voxel size/FoV = 3.14 ms/1.6 ms/60°/2.2 x 1.3 x 8.0 mm/340 mm.

150

AD was assessed from cine MR images in the transverse plane of the ascending aorta were obtained at the level of the main pulmonary artery (Figure 1) utilising a fast-imaging with steady-state precession sequence (TR=3.2 ms, TE=1.6 ms, flip angle =60°, FoV 276x340mm, pixel dimensions 2.3x1.3mm, slice thickness=7 mm). The approximately 10 second breath-hold CMR sequence resulted in prospective images with a temporal resolution of at least 22.5ms. During image acquisition, blood pressure was measured in the CMR scanner using a non-ferromagnetic brachial artery sphygmomanometer cuff (Schiller instruments, Baar, Switzerland).

LV mass was analysed short axis cine loops using tracing of epicardial and endocardial end-systolic and end-diastolic contours with LV mass calculated using analysis software (Argus, Siemens, Erlangen, Germany) and normalised to body surface area. Aortic distensibility was calculated as previously described from change in aortic volume and simultaneous brachial blood pressure acquired during the scan using the formula^{20, 21}:

$$\text{Aortic distensibility} = \frac{[(\text{Aortic volume})_{\text{max}} - (\text{Aortic volume})_{\text{min}}]}{[(\text{Aortic volume})_{\text{min}} * \text{pulse pressure}]}$$

where $(\text{Aortic volume})_{\text{max}}$ and $(\text{Aortic volume})_{\text{min}}$ are the maximal and minimal calculated aortic volumes obtained during the cardiac cycle. A schematic illustration of the contour tracing the cross sectional aortic volume is shown in Figure 1. All CMR scans were anonymised with a code number prior to analyses performed by two observers (P.B.M., S.B.) blinded to the clinical characteristics of the subjects.

Applanation tonometry measurement of pulse wave velocity

Measurements of PWV and Alx were carried out after 15 min of rest in the supine position using the SphygmoCor® Vx system (Atcor Medical, Sydney, Australia). PWV was measured from sequentially recorded electrocardiogram-gated carotid, femoral artery waveforms. Timing of the pulse wave was using the intersecting

tangent algorithm with carotid-femoral pathway measured using the distance obtained by subtracting the carotid measurement site to sternal notch distance from the sternal notch to femoral measurement site distance. PWV measurements were made in triplicate, and the mean values were used in the subsequent analysis. AIx, a measure of wave reflection, was determined from radial waveforms using the same device and corrected to heart rate of 75 beats per minute. A trained nurse (blinded to the clinical characteristics of the subjects) performed tonometry measurements.

Blood sampling

Venepuncture was performed in the PA and EH subjects for routine biochemistry on the day of scanning and analysed in the standard clinical laboratories at the Western infirmary, Glasgow. 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. Coefficient of variation of the QC was <5%. Plasma aldosterone was measured by direct radioimmunoassay (RIA) utilising the 'Coat-A-Count' system (Euro/DPC Ltd, Caernarfon, Wales). The radioisotope used was ¹²⁵I aldosterone. Coefficients of variation (% , within-batch and between-batch respectively) were: 2.3–5.4/3.8–15.7.

Oxidative stress status was assessed by analysing superoxide release from whole

blood using an established method^{22, 23}. In brief, venous blood was collected in
200 lithium heparinate containing tubes, kept on ice and processed within half an hour.
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 μM . 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 min 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.

210 **Power calculation**

Based on our previous CMR studies of AD, if the true difference in the experimental
and control means is $3 \times 10^{-3} \text{ mmHg}^{-1}$, 12 PA subjects and 24 EH controls are
required to detect a significant difference between PA patients and EH patients with
power 0.8 and type I error of 0.05.

Statistical methods

Differences between groups were tested by Chi-squared or Fisher's exact test and
appropriate for categorical variables and by Student's t-test and Mann-Whitney-U
test for continuous variables. Correlations between cardiac dimensions, measures of

220 arterial function and continuous clinical variables were assessed with the Pearson and Spearman correlation co-efficients. Analysis of covariance was used to assess influence of covariates on differences between groups. All analyses were performed using the SPSS 19.0 software package (IBM, Armonk, NY, USA).

Results

Patient demographics, therapy and clinical variables

230 Patient demographics and the various measures of vascular function are shown in Table 1. There were no significant differences in age, gender or left ventricular mass index between the three groups. Blood pressure was numerically but not significantly higher in the PA groups compared to EH. Predictably, the healthy control group had a significantly lower body mass index (BMI), systolic, diastolic and pulse pressure compared to both the PA and EH subjects. As expected, PA subjects had significantly higher plasma aldosterone levels than PH but there was no difference between plasma CRP or whole blood superoxide production between these subject groups. As the lab changed assays from plasma renin activity to plasma renin concentration during the study, data on renin levels are not presented.

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There were no significant difference in use of angiotensin converting enzyme inhibitors, angiotensin receptor antagonists, beta-blockers or calcium channel antagonists at the time of study between the PA and EH subjects. No PA patients were receiving thiazide diuretics, which were used in 21 (63.6%) of EH patients. Of the 14 PA patients, four were on no specific therapy at the time of study, nine patients were receiving MR antagonists (seven on spironolactone, two on eplerenone) and one patient had undergone adrenalectomy seven months prior to study. In patients on MR antagonists the median duration of therapy was 180 days

(range 28-360 days). Therefore all PA subjects included in the study were
250 investigated either before, or within one year, of either adrenalectomy or
commencing specific medical therapy.

Left ventricular dimensions and function

There were no significant differences between LV mass between PA and EH
patients or controls, although LV mass was slightly higher in patients compared to
controls. However, PA patients did have smaller LV end diastolic volume compared
to both EH patients and controls. No group had evidence of LV systolic dysfunction.
In fact, PA patients had significantly higher LV ejection fraction than either EH
patients or controls (Table 2).

260

Measures of vascular function

AD was significantly lower in both PA and EH compared to healthy controls
($p < 0.001$), with AD additionally being significantly lower in PA compared to EH
subjects despite similar ages and blood pressures in the groups (Figure 2). There
was a significant negative correlation between age and AD in the whole cohort of
patients ($R = -0.454$, $p < 0.001$, Figure 3). Both markers of increased aortic stiffness
correlated with increasing BMI (AD $R = -0.277$, $p = 0.028$; PWV $R = 0.295$, $p = 0.022$).
There were no significant correlations between AD and plasma aldosterone levels,
any lipid parameter, C-reactive protein, IL-6, superoxide, calcium or phosphate.

270 There were no significant differences in AD between genders in PA or EH groups although AD was lower in male healthy controls compared to females (mean AD 3.9×10^{-3} vs. 5.2×10^{-3} , $p=0.055$) despite similar blood pressures. However, the number of females in the PA and EH groups was small.

Heart rate-corrected central Alx was significantly lower in the healthy controls compared to EH patients, with a trend towards being lower in PA patients. There was no significant difference in Alx between PA and EH subjects and there did not appear to be any significant relationship between Alx and any clinical variables other than blood pressure (see below).

280 Analysis of covariance performed to assess the differences in AD between the groups independent of age as a covariate, confirmed significantly lower AD in PA and EH patients compared to controls as a reference. Additionally this demonstrated that, for every increase in age by one year, AD reduces by $6.75 \times 10^{-5} \text{Hg}^{-1}$, irrespective of the effect of patient group (Table 3). By contrast PA subjects have a reduction of $1.0 \times 10^{-3} \text{Hg}^{-1}$ compared to PH patients; therefore essentially, PA conveys an excess equivalent of 14.8 years additional aging compared to EH with comparable blood pressure. Figure 4 illustrates that PWV was significantly higher in both PA and EH compared to controls ($p<0.001$), with PWV additionally being significantly higher in PA compared to EH subjects ($p=0.001$)

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Relationship between measures of vascular function

As would be expected for complementary methods of assessment of vascular function, there was a significant correlation between AD and PWV (R -0.433, $p < 0.001$, Figure 5). There were also significant correlations between most blood pressure variables and both AD and PWV. The Spearman correlation co-efficient for AD and blood pressure variables were as follows; systolic blood pressure (R -0.533, $p < 0.001$), diastolic blood pressure (R -0.230, $p = 0.067$) and pulse pressure (R -0.540, $p < 0.001$). The Spearman correlation co-efficient for PWV and blood pressure variables were as follows; systolic blood pressure (R 0.317, $p = 0.013$), diastolic blood pressure (R 0.297, $p = 0.02$) and pulse pressure (R 0.245, $p = 0.057$). Alx correlated significantly with AD (Spearman R -0.378, $p = 0.002$), systolic blood pressure (R 0.426, $p < 0.001$) and pulse pressure (R 0.407, $p = 0.001$) but not diastolic blood pressure or PWV.

Discussion

In this study, PA is associated with increased aortic stiffness manifest as reduced aortic distensibility (AD) and higher pulse wave velocity compared to both healthy
310 controls and subjects with EH. This finding persists after adjustment for age, the other significant predictor of AD. These findings offer further insights into mechanisms involved in blood-pressure independent increased cardiovascular risk associated with aldosterone excess. Importantly, this is the first study to use CMR to assess AD in PA subjects, although this well-validated technique has been used to explore vascular morbidity in other populations at increased cardiovascular risk^{17, 18}.

Our findings that PWV is increased in PA subjects compared to EH and normotensive cohorts, confirm findings of other similar studies^{15, 24}. Central PWV is an established method for assessment of central arterial compliance, with higher
320 PWV being a marker of stiffer central vessels. *In vitro* and animal studies have demonstrated that aldosterone-mediated MR activation leads directly to vascular smooth muscle cells proliferation and excess collagen deposition resulting in vascular remodelling and fibrosis^{10, 11}. In addition, longer term clinical studies have demonstrated that amelioration of aldosterone excess either by adrenalectomy or MR blockade reduces PWV as well as carotid intima medial thickness (a surrogate marker of atherosclerosis) at one year following treatment^{16, 25}.

In addition to the observation that AD is reduced in PA subjects compared to other groups, a significant correlation was observed between AD and both age and PWV.

330 The correlation between AD and age across the groups reflects increasing severity of arteriosclerosis with age which persists irrespective of the study group²⁶. The observed correlation between PWV and AD (as well as the correlation between AD and AIx), to an extent demonstrates a validation of both techniques as measures of arterial stiffness.

There were no differences in left ventricular masses between the groups (all of which were in the normal range) despite suboptimal blood pressure control in both the PA and EH patients. We were very surprised not to find higher LV mass in the PA population particularly compared to healthy controls, although the mean LV mass in
340 controls was a little lower. This probably reflects the fact that participants had been screened to exclude significant cardiac abnormalities prior to participation. This may reflect relatively 'mild' hypertension demonstrated by the PA patients in this study as some patients had commenced specific therapy, which may have already begun to induce regression of LV hypertrophy. The implication from our results is that impairment of vascular function occurs earlier in the course of PA, prior to the onset of left ventricular hypertrophy as end organ damage and may regress more slowly. Additionally, most other studies of LV dimensions in PA and hypertensive patients rely on echocardiography data; CMR provides a more sensitive method of structural

analysis and it is unclear how well CMR and echocardiographic findings correlate. In
350 one previous study, LVH using CMR was found in only 83 of 440 subjects (18%) with
pre-identified LVH using echocardiography²⁷. It is also significant that in the only
other study using relating aldosterone status with CMR findings, there was no
significant difference in LVMI between EH subjects with high versus low aldosterone
production²⁸. The higher LV ejection fraction and smaller LV end systolic volume in
the PA group is intriguing and contrasts with a recent echocardiographic study,
which showed the reverse with larger LV end systolic and end diastolic volumes in
PA patients²⁹. We speculate that in our study, the small end systolic volume may
lead to impaired LV filling and may be a risk factor for diastolic dysfunction and/or
heart failure with preserved ejection fraction. We were surprised that there were no
360 differences in oxidative stress or C-reactive protein between groups, although this
may simply reflect the relatively small size of the groups as excess aldosterone has
been associated with both oxidative stress and inflammation³⁰.

AD has been utilised in several other patient groups as either a marker of increased
vascular risk or for identifying patients at risk of poorer long term cardiovascular
survival²¹. In otherwise healthy young individuals, obese subjects have been shown
by CMR to have decreased aortic compliance³¹. We observed a significant
correlation between increasing BMI and reduced AD and PWV in our cohort. AD has
been demonstrated to be reduced in heart failure¹⁸, ischaemic heart disease, end

370 stage renal disease¹⁷, and diabetes³². AD may represent a surrogate marker for clinical trials and has been shown to improve following significant weight loss in obese subjects³³. Interestingly in patients with chronic kidney disease, the addition of spironolactone to conventional antihypertensive therapy improved AD significantly compared to placebo, hinting at beneficial effects of MR antagonism on the vasculature in a non-PA patient cohort³⁴.

Our study has some limitations. As a cross sectional study, our study demonstrates an association between increased PA and increased arterial stiffness and do not imply causation. The size of the overall cohort is relatively small, as is each of the
380 individual subgroups. Despite our aim to match the hypertensive groups, blood pressure was higher in the PA group, which may have some bearing on the results of vascular function studies. Despite our attempts to match the groups as much as possible for factors which may influence AD (age, renal function, BMI), with this small study it is possible that other unrecognised factors may influence AD across the groups. Measurement of brachial rather than central blood pressure is a potential issue when using AD as a marker of vascular stiffness. Calculation of AD is based on cross-sectional volume of the vessel wall and pulse pressure. For practical reasons, direct aortic blood pressure measurement has been substituted by non-invasive indirect brachial blood pressure measurement. Therefore it is difficult to
390 dissociate any clinical effect associated with changes in aortic distensibility from that

due directly to pulse pressure. Nonetheless, as it is otherwise impossible to truly assess aortic stiffness, without documentation of pressure within the vessel lumen, the approach used in this study has been used widely in other patient groups. Finally, whilst CMR is becoming an increasingly widely used technique, it remains expensive and time consuming, and currently remains established as a research tool rather than as routine clinical measure of vascular health. The major advantage of using CMR over other imaging modalities, is its ability to provide comprehensive assessment of the vasculature and detailed analysis of myocardial tissue composition in patients at elevated cardiovascular risk in one examination without
400 the use of ionising radiation³⁵. Progress is still required to facilitate more rapid scanning and analysis at lower cost. Better automated analysis software should facilitate straightforward assessment of the acquired images.

In summary, these results demonstrate that PA patients demonstrate increased arterial stiffness compared to comparable degrees of EH, independent of normal vascular aging. This has been illustrated using both applanation tonometry and, uniquely, by assessment of aortic distensibility using CMR. The implication is that aldosterone *per se* rather than subsequent hypertension invokes arterial remodelling by direct effects on vascular smooth muscle cells leading to functional impairment of
410 arterial conduit function. Both CMR and PWV are potential surrogate markers of vascular health, which may represent targets for clinical trials of interventions in PA

patients. The long-term prognostic implications of increased arterial stiffening in association with aldosterone excess require further study.

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Conflict of Interest. The authors have no conflicts of interest to declare.

Summary Table

What is known about the topic

- Excess aldosterone is associated with increased cardiovascular risk
- Cardiovascular magnetic resonance imaging (CMR) is a novel tool for studying vascular function by measuring aortic distensibility
- Patients with primary aldosteronism (PA) have been shown to have increased arterial stiffness measured by pulse wave velocity. This may improve after adrenalectomy in adenomatous PA

What this study adds

- PA patients displayed increased arterial stiffness with both CMR and PWV compared to comparable degrees of essential hypertension, independent of aging
- The long term implications of increased arterial stiffening in association with aldosterone excess require further study

430 **Legend**

Table 1. Demographics and clinical data of the subjects. Values are mean plus standard deviation in parentheses for all values except gender and diabetes where number and percentage are shown, duration of hypertension where median and inter-quartile range is shown and number of antihypertensives where median and range are displayed. Tests of significance for non-categorical variables are t-test except duration of hypertension, plasma aldosterone and number of AHT where Mann-Whitney U is used. Abbreviations- BMI- body mass index, SBP- systolic blood pressure; DBP- diastolic blood pressure, LDL –low density lipoprotein; CRP - C – reactive protein; eGFR – estimated glomerular filtration rate by 4 variable MDRD
 440 formula; AHT – antihypertensives; BSA- body surface area; A.u. Arbitrary units. * p<0.05 PA vs. EH, † p<0.001 PA vs. EH, ‡ p<0.01 Controls vs. PA/EH subjects, § p<0.001 Controls vs. PA/EH subjects

Table 2

Results of vascular function and left ventricular dimensions and function. Abbreviations- * p<0.05 PA vs. EH, † p<0.001 PA vs. EH, ‡ p<0.01 Controls vs. PA/EH subjects, § p<0.001 Controls vs. PA/EH subjects, || p<0.05 Controls vs. EH subjects, # p<0.01 Controls vs. PA subjects, ** p<0.05 Controls vs. PA subjects

Table 3

Results of analysis of covariance. β represents differences between mean AD
 450 between groups for PA and EH groups compared to healthy volunteers as the

reference, and the relative effect of age on AD when clinical group is controlled as a covariate.

Figure 1. Schematic diagram of the cross sectional aortic area being defined (volume is calculated by multiplication by the CMR slice thickness). AA- ascending aorta, DA- descending aorta

Figure 2. Box and whisker plots of AD in groups. The central line represents the median with the boxes representing the 25th and 75th percentiles. The whiskers represent 95% confidence intervals for normally distributed data. The open circles are outlying subjects.

460 **Figure 3.** Scatter plot of AD vs. age. R^2 is for Pearson correlation co-efficient

Figure 4. Box and whisker plots of PWV by groups. Format is as per Figure 2

Figure 5. Scatter plot of AD vs. PWV. R^2 is for Pearson correlation co-efficient

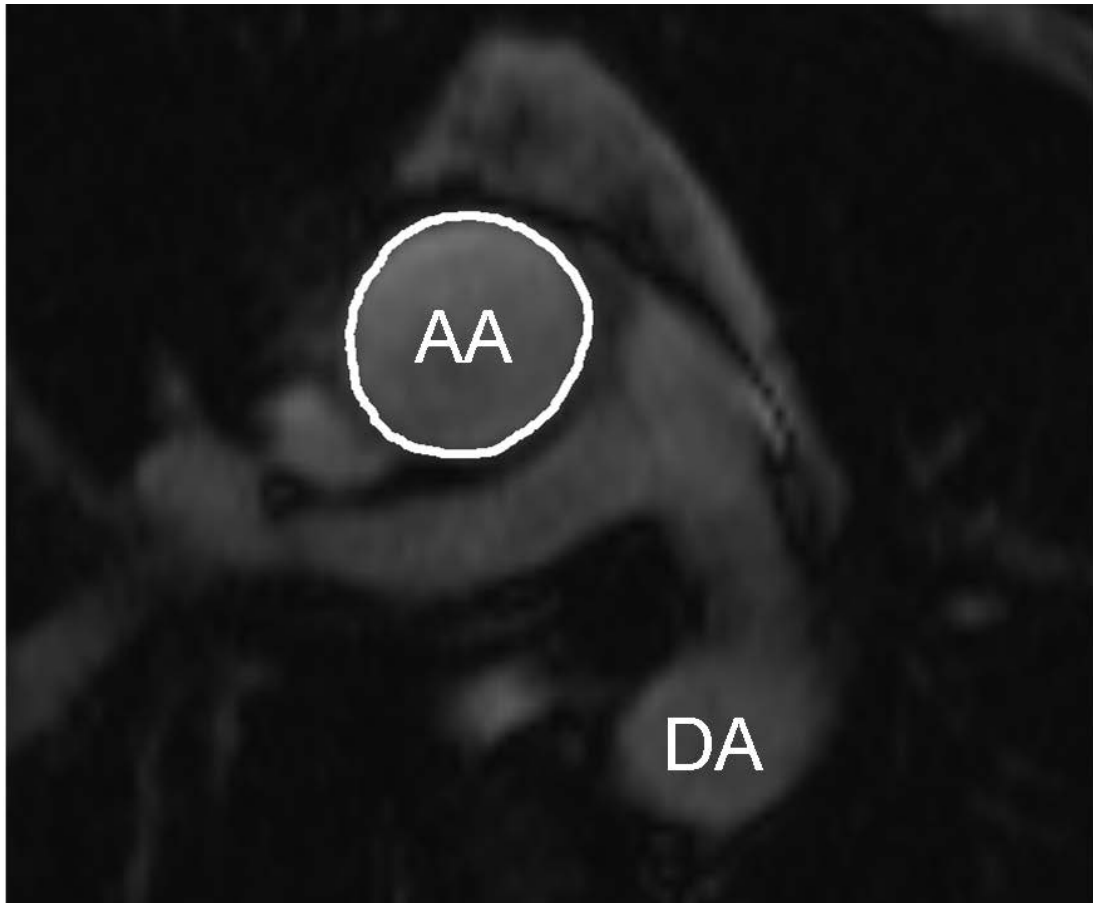
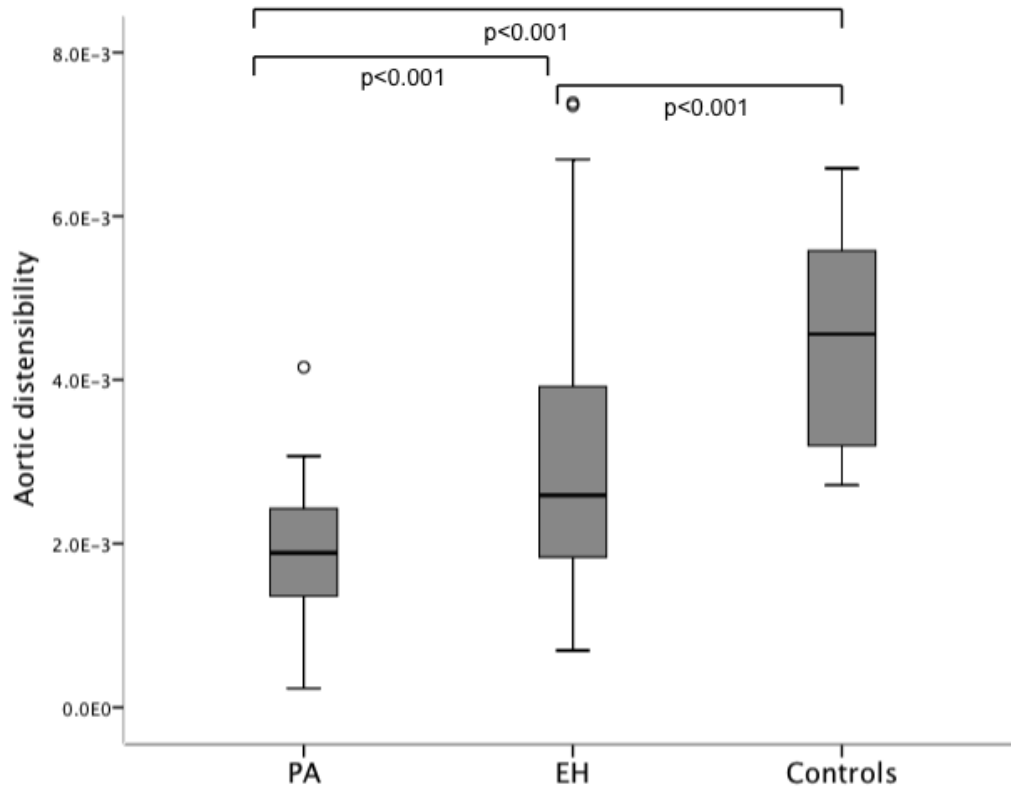


Figure 1

470 **Figure 2**

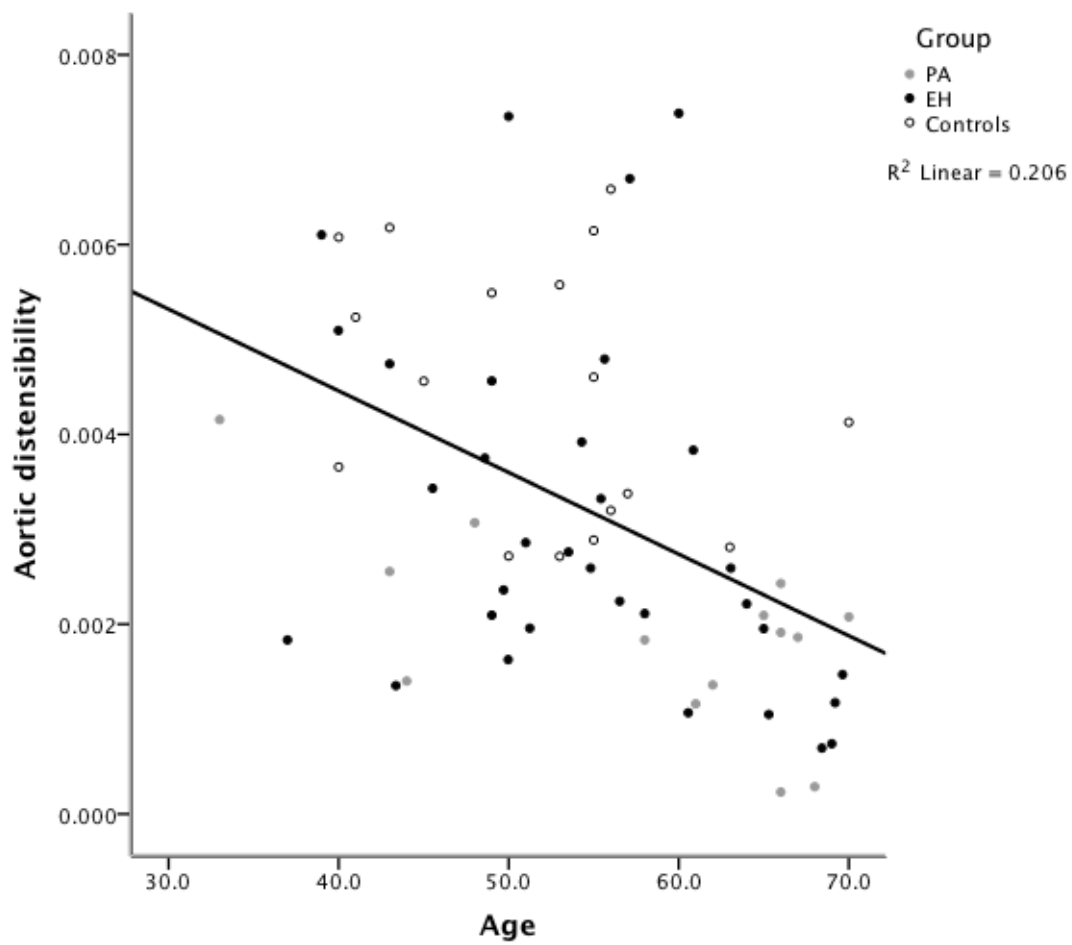


Figure 3

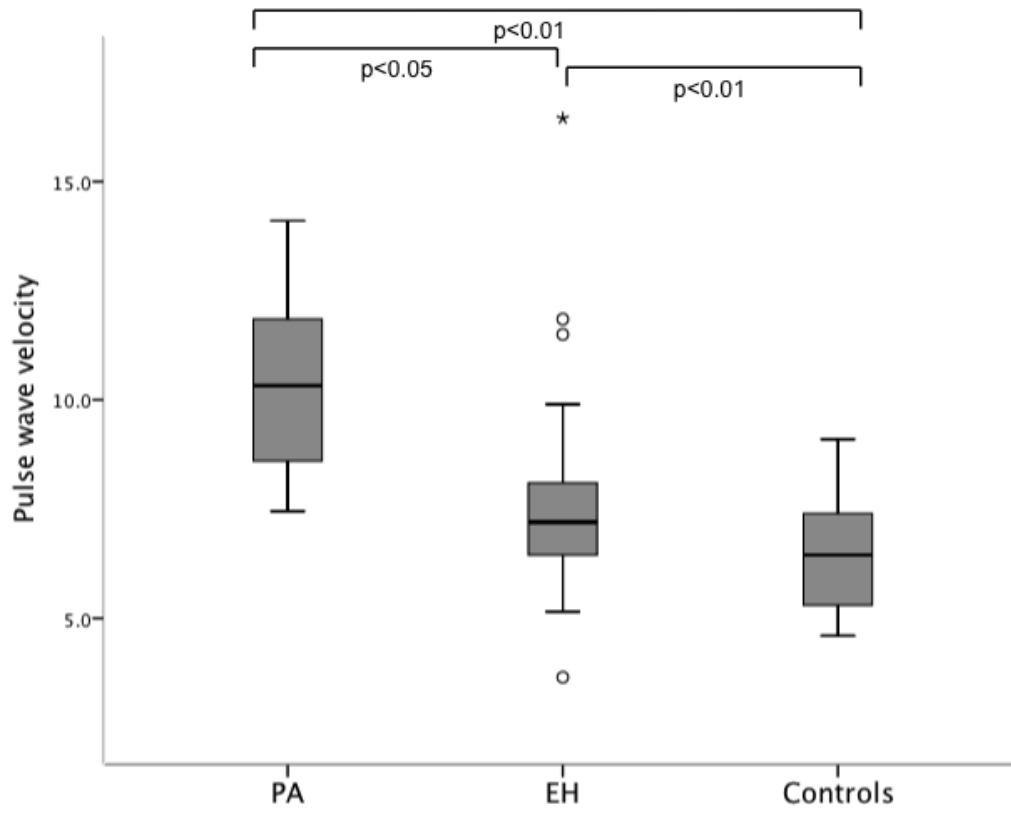
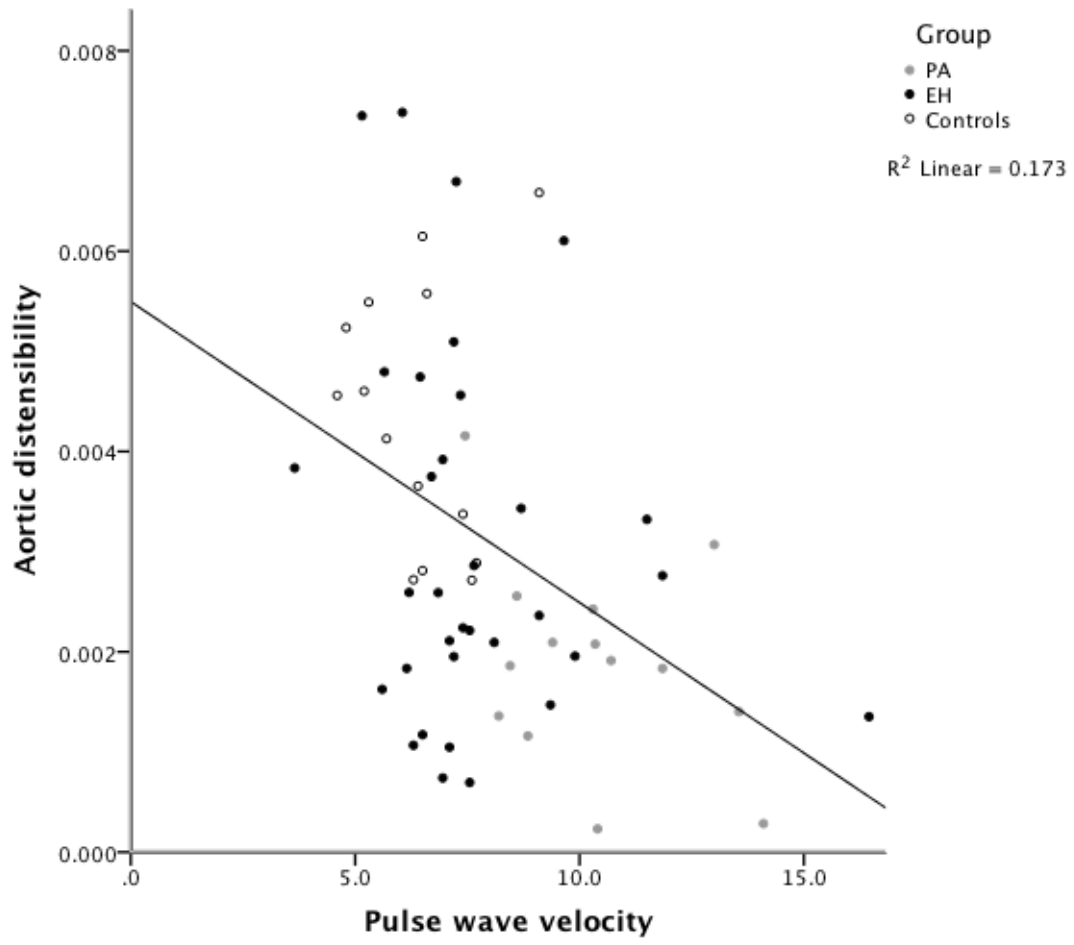


Figure 4



480 Figure 5

	PA		EH		Controls	
Number	14		33		17	
Age	58.4	(11.6)	54.8	(9.1)	51.8	(8.2)
Male (%)	11	(78.6)	28	(84.8)	10	(58.8)
BMI	29.8	(4.3)	29.1	(4.4)	25.8‡	(3.7)
Diabetes (%)	1	(7.1)	2	(6.1)	0	
SBP (mmHg)	152.9	(30.7)	145.8	(16.3)	117.1§	(10.4)
DBP (mmHg)	92.3	(11.6)	89.2	(8.6)	74.4§	(7.5)
Pulse pressure (mmHg)	60.6	(26.9)	56.6	(15.2)	42.8‡	(6.2)
Duration of hypertension (years)	7.5	(15.0)	10	(13.8)	-	-
Sodium (mmol l ⁻¹)	140.5	(2.7)	139.5	(2.0)	-	-
Potassium (mmol l ⁻¹)	3.6*	(0.7)	3.9	(0.4)	-	-
eGFR (ml min ⁻¹ 1.73m ⁻²)	85.7	(13.4)	95.1	(18.9)	-	-
LDL cholesterol (mmol l ⁻¹)	2.1	(0.2)	2.9	(0.9)	-	-
CRP (mg l ⁻¹)	2.4	(1.7)	2.3	(2.3)	-	-
Number of AHT	2.5	(1-4)	2	(0-5)	-	-
Plasma aldosterone (pmol l ⁻¹)	734.2‡	(311.0)	311.5	(251.1)	-	-
Oxidative stress (A.u.)	0.44	0.20	0.42	0.25	-	-

Table 1

	PA		EH		Controls	
PWV (m s^{-1})	10.3†	(2.1)	7.7	(2.3)	6.4‡	(1.3)
Augmentation Index (%)	27.1	(5.7)	28.2	(6.8)	22.2	(8.2)
AD ($\times 10^{-3} \text{ mmHg}^{-1}$)	1.9*	(1.0)	3.1	(1.9)	4.4§	1.3
LV Mass/BSA (g m^{-2})	65.2.	(11.6)	65.0	(17.2)	64.6	(13.1)
End diastolic volume/BSA (ml m^{-2})	59.9	(11.1)	65.8	(12.3)	-	-
End systolic volume/BSA (ml m^{-2})	14.6*	(6.2)	21.0	(9.4)	-	-
Ejection fraction (%)	76.1*	(6.1)	68.8	(10.7)	-	-

Table 2

	β	95% Confidence Interval for β	p
Intercept	0.008	(0.006, 0.010)	0.000
Age	-6.752×10^{-5}	(0.000, -2.745×10^{-5})	0.001
PA	-0.002	(-0.003, -0.001)	0.000
EH	-0.001	(-0.002, 0.000)	0.010
Controls	Reference		-

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Table 3

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