Plasma aldosterone levels and aldosterone-to-renin ratios are associated with endothelial dysfunction in young to middle-aged subjects

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A B S T R A C T

Objective: Small clinical studies suggested a role for aldosterone in the development of endothelial dysfunction. We investigated whether the plasma aldosterone concentration (PAC) or the aldosterone-to-renin ratio (ARR) were associated with decreased endothelial function as measured by flow-mediated dilation (FMD) of the brachial artery in the general population.

Methods: Our study population comprised 972 participants from the Study of Health in Pomerania, who were not treated with antihypertensive medication. We performed age-stratified (<50 and ≥50 years) ordinal logistic regression analyses. FMD was categorised as decreased (1st quintile), moderate (2nd–4th quintile), or increased (5th quintile). PAC and ARR were divided into low, moderate, and high values according to age- and sex-specific tertiles. All models were re-calculated for 871 subjects with PAC and ARR within the study-specific reference ranges. Odds ratios (OR) and 95% confidence intervals (CI) are presented.

Results: Subjects <50 years with high PAC (OR 1.60; 95% CI 1.07–2.38) or ARR (OR 1.81; 95% CI 1.21–2.73) had higher odds for decreased FMD than subjects with low PAC or ARR, respectively. Similar results were obtained in analyses restricted to subjects with PAC and ARR within the reference range. High-normal PAC (OR 1.62; 95% CI 1.07–2.47) or ARR (OR 1.62; 95% CI 1.05–2.50) was associated with higher odds for decreased FMD when compared with low-normal PAC or ARR, respectively. These associations were not observed in subjects ≥50 years.

Conclusions: High and high-normal PAC or ARR contribute to an impaired FMD and subsequently the progression of subclinical atherosclerosis in young to middle-aged subjects.

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1. Introduction

Impaired endothelial function represents an early stage of arterial wall damage and is a surrogate marker of subclinical atherosclerosis [1–3]. Characteristics of endothelial dysfunction include alterations in vascular tone, impaired endogenous endothelial repair, increased vascular inflammation, and thrombosis [4].

Established cardiovascular risk factors, such as obesity or diabetes mellitus, are major causes of endothelial dysfunction. Furthermore, age-dependent impairment of endothelial function has been reported [5–7] and is assumed to be secondary to the diminished bioavailability of nitric oxide and the accumulation of cardiovascular risk factors throughout life [8–10]. Impaired function of the vascular endothelium may reflect individual predisposition to arterial hypertension, which may subsequently cause endothelial dysfunction [11].

Small clinical studies [12,13] of patients with primary aldosteronism suggested a key role for aldosterone excess in the development of endothelial dysfunction as assessed by flow-mediated dilation (FMD) of the brachial artery. Hypertensive patients with primary aldosteronism had significantly lower FMD values than those without primary aldosteronism [13]. Furthermore, surgical or pharmacological treatment of primary aldosteronism significantly enhanced FMD [12,13]. Another study in 130 volunteers found an impairment of nitric oxide-mediated
dilation in hypertensive subjects with low-renin status or high aldosterone-to-renin ratios (ARR) as compared to normotensives [14].

While it is generally accepted that endothelial function is impaired in primary aldosteronism, there is little known about the associations between the plasma aldosterone concentration (PAC) or the ARR and endothelial function in the general population. Thus, the aim of our study was to determine whether PAC or ARR were associated with decreased brachial artery FMD in a large population-based cohort. Furthermore, we investigated the associations of PAC or ARR in a subset of our study population that had PAC and ARR within the study-specific reference ranges.

2. Materials and methods

2.1. Study population

The Study of Health in Pomerania (SHIP) is a longitudinal population-based study in northeastern Germany. The study design and sampling methods have been previously described [15]. Briefly, 4308 men and women from a representative population sample of 7008 subjects participated in baseline examinations between October 1997 and May 2001. Five years later, between March 2003 and July 2006, the first follow-up examinations (designated as SHIP-1) were conducted in 3300 participants. All participants gave written, informed consent. The study conformed to the principles of the Declaration of Helsinki as reflected by an a priori approval of the Ethics Committee of the Board of Physicians Mecklenburg-West Pomerania at the University of Greifswald.

Sociodemographic and behavioural characteristics of study participants were assessed by personal interviews. The participants’ medication was categorised according to the anatomical–therapeutic–chemical (ATC) classification code. During the physical examination, standardised measurements of height, weight, waist circumference, and blood pressure were performed. Systolic and diastolic blood pressures were measured three times on the right arm of the seated participant using a digital blood pressure monitor (HEM-705CP, OMRON Corporation, Tokyo, Japan). For statistical analyses, the mean of the second and third measurements was used.

FMD measurement was offered to all SHIP-1 participants, and 1692 subjects opted to receive this examination. A median time of 16.5 days (1st–3rd quartile ranged from 0 to 75 days) passed between basic examination and FMD measurement. Following a standardised protocol, certified examiners performed the FMD measurement in response to post-ischemic forearm hyperaemia using a 10 MHz linear array transducer (Cypress Acuson, Siemens, Erlangen, Germany). Details on the study protocol and quality assurance procedures have been described elsewhere [16]. Here, we present FMD as percent dilation occurring during the maximum response normalised to baseline brachial artery diameter. Of the 1692 FMD participants, we excluded 174 because of low-quality of sonographic images. We further excluded subjects with renal insufficiency (defined as creatinine clearance <50 ml/min, n = 21), pregnant women (n = 4), and participants with missing PAC or ARR values (n = 11). We further excluded 508 participants taking PAC-, ARR- or FMD-altering medication, including antagonodreners agents (ATC C02), diuretics (ATC C03), beta blockers (ATC C07), calcium channel blockers (ATC C08), agents acting on the renin-angiotensin system (ATC C09), peripheral vasodilators (mainly purine deriva- tives, ATC C04), and vasodilators used in cardiac diseases (mainly nitrates, ATC C01D). An additional two subjects were excluded due to insufficient information on confounders. Our final study population comprised 972 subjects.

For our analyses in the subgroup of subjects with normal PAC and ARR, we further excluded all subjects with PAC, plasma renin concentration (PRC) or ARR outside the previously established study-specific reference ranges (n = 101) [17]. This resulted in a study population of 871 subjects.

2.2. Laboratory measurements

For SHIP-1, blood samples were taken between 8:00 a.m. and 7:30 p.m. from the cubital vein of non-fasting subjects in the supine position. Potassium was measured in serum by indirect poten- tiometry with ion-selective electrodes (QuikLYTE, Dade Behring, Eschborn, Germany). Hypokalemia was defined as serum potas- sium concentrations <3.5 mmol/l. PAC and PRC were measured in EDTA plasma (PAC was measured with Coat-A-Count Aldosterone, Siemens Healthcare Diagnostics, Eschborn, Germany; and PRC was measured with Renin III Generation, Cisbio Bioassay, Bagnols-sur- Cèze Cedex, France), as previously reported [17].

2.3. Statistical analyses

To describe the study population, we report medians (1st, 3rd quartile) for continuous variables and proportions for categorical variables. For group comparisons, we used Kruskal–Wallis and Chi-squared tests. p values <0.05 were considered statistically significant.

Given the lack of standardised and generally accepted cut-offs for normal FMD [18], we applied a distribution-based definition to categorise FMD. FMD in the lowest age- (25–34, 35–44, 45–54, 55–64, and over 64 years of age) and sex-specific quintile (n = 196) was defined as decreased, FMD in the 2nd–4th quintiles was defined as moderate (n = 583), and FMD in the highest quintile (n = 193) was defined as increased.

Ordinal logistic regression using the proportional odds model was used to examine the associations of PAC or ARR with FMD in the whole study population, as well as in subjects with PAC and ARR within the reference ranges. In these models, a single odds ratio (OR) was calculated that represents the association of a predictor variable with all higher vs. lower risk combinations of the outcome variable categories. In our case the ordinal outcome has three levels (decreased, moderate, and high FMD). The two resulting higher vs. lower risk combinations are: (1) decreased and moderate vs. high FMD and (2) decreased vs. moderate and high FMD. To con- firm the validity of the ordered logistic regression model, we tested the proportional odds assumption using a Chi-squared score test, which indicated that the assumption was valid.

The predictor variables (PAC and ARR) entered the model as con- tinuous or categorical variables. PAC and ARR were categorised as low, moderate or high according to the age- (25–34, 35–44, 45–54, 55–64, and over 64 years of age) and sex-specific tertiles of the respective distribution.

In addition to the ordinal regression model we calculated a quantile regression model [19] to explore the changes in median FMD as a function of PAC or ARR. In these models, FMD as well as PAC and ARR were used as continuous variables. As the quantile regression analysis does not require normal distribution of the data, a transformation of the dependent variable was not necessary. Coefficient estimates for the median with 95% confidence intervals (CI) are presented.

Previous studies [5–7,9,10] demonstrated that FMD decreases with age. In older subjects with already impaired endothelial function, high PAC or ARR may not have the same effects as in younger subjects with normal endothelial function. We tested whether the participants’ age (younger than 50 years of age vs. 50 years or older) was a potential effect modifier in our ordinal regression models. In three out of four models significant interactions (p < 0.10) with the
exposure variable were detected. We therefore decided to perform age-stratified analyses in subjects younger than 50 years of age and in subjects 50 years or older.

All models were adjusted for sex (male, female), age (in years), systolic and diastolic blood pressure (in mmHg), waist circumference (in cm), smoking (yes, no), diabetes mellitus (yes, no), physical activity (yes, no), and time (in days) between blood sampling and FMD measurement. In a sensitivity analysis we further adjusted all models for time of blood sampling. OR and 95% CI for all models are presented. All statistical analyses were performed with SAS 9.1 (SAS Institute Inc., Cary, NC, USA).

3. Results

Systolic and diastolic blood pressures, waist circumference, as well as proportions of hypertension and diabetes mellitus were significantly higher in subjects 50 years of age or older than in subjects younger than 50 years of age. We observed lower ARR as well as higher FMD and PRC in the younger than in older subjects (Table 1).

We detected higher systolic and diastolic blood pressures and higher proportions of hypertension in subjects with high ARR than in subjects with low ARR. Differences in blood pressure levels and proportions of hypertension were not detected between the PAC tertiles. Furthermore, proportions of current smokers and physically active subjects did not significantly vary between the PAC or ARR tertiles (see Supplementary Material for details).

Descriptive statistics further revealed that subjects younger than 50 years of age with high PAC or ARR had significantly lower FMD than subjects with low PAC or ARR (Fig. 1). These observations were not confirmed in subjects 50 years of age or older.

In fully adjusted ordinal regression models, subjects younger than 50 years of age with high PAC had higher odds for decreased FMD than subjects with low PAC (Table 2). Likewise, subjects younger than 50 years of age with moderate or high ARR had higher odds for decreased FMD than subjects with low ARR. When ARR entered the model as a continuous variable, we observed higher odds for decreased FMD with every increase of one standard deviation in ARR. Following the proportional odds model, these associations hold over the three FMD categories (from decreased to increased FMD). Similar results were obtained when the analyses were restricted to subjects with PAC and ARR within the reference ranges. Subjects younger than 50 years of age with high-normal PAC had higher odds for decreased FMD than subjects with low-normal PAC. Subjects with high-normal ARR had higher odds for decreased FMD than subjects with low-normal ARR. We further observed higher odds for decreased FMD with every increase of one standard deviation of ARR. When PAC entered the model as a continuous variable, statistical significance was barely missed. There was no relation between PAC or ARR and FMD in subjects 50 years of age or older. For our sensitivity analysis we additionally adjusted all models for time of blood sampling, which yielded the same results as in our main analyses (data not shown).

Our fully adjusted quantile regression model revealed a significant association between ARR and FMD in subjects younger than 50 years of age. A one unit increase in ARR resulted in a 0.09 decrease in median FMD [coefficient estimate −0.09 (95% CI −0.16, −0.02)]. We found no statistically significant association between PAC and FMD. These findings confirm the results obtained with the ordinal regression analyses with PAC and ARR as continuous variables.

4. Discussion

High and high-normal PAC or ARR were associated with decreased FMD in subjects from the general population. Moreover, we observed a linear association between ARR and FMD but not between PAC and FMD. All detected associations were present in young to middle-aged subjects below the age of 50 years but not in older subjects. The lack of associations in older subjects may be explained by the accumulation of cardiovascular risk factors throughout life. The accumulating risk factors may enforce functional and structural changes in the vascular endothelium, triggering endothelial dysfunction and preceding the development of atherosclerotic disease [1,5,20]. Furthermore, increased age alone, independent of atherosclerotic disease, may lead to vascular dysfunction [5,6]. In older subjects whose vessels are stiffer and endothelial function is already reduced, high PAC or ARR may not have the same effects on FMD as in younger subjects. This might explain our observations of associations of PAC and ARR with decreased FMD as a subclinical phenotype in young to middle-aged subjects only.

The adverse impact of high PAC or ARR on the endothelium was previously demonstrated in patients with primary aldosteronism [12,13]. FMD was significantly lower in hypertensive patients with primary aldosteronism compared to those without primary aldosteronism [12,13]. Furthermore, FMD increased after surgical

Table 1

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Age &lt;50 years</th>
<th>Age ≥50 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>566</td>
<td>406</td>
</tr>
<tr>
<td>Women (%)</td>
<td>51.6</td>
<td>49.5</td>
</tr>
<tr>
<td>Age (years)</td>
<td>39.0 (34.0–44.0)</td>
<td>59.0 (54.0–64.0)</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>122.0 (112.5–133.5)</td>
<td>132.5 (122.0–144.0)</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>80.0 (74.5–86.0)</td>
<td>83.0 (76.0–89.5)</td>
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<tr>
<td>Waist circumference (cm)</td>
<td>85.8 (77.0–95.5)</td>
<td>92.4 (84.1–100.0)</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>20.9</td>
<td>38.2</td>
</tr>
<tr>
<td>Current smokers (%)</td>
<td>38.7</td>
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<td>Physical activity (%)</td>
<td>47.7</td>
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<td>Diabetes mellitus (%)</td>
<td>0.7</td>
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<td>Hypokalemia (%)</td>
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<td>0.0</td>
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<tr>
<td>Potassium (mmol/l)</td>
<td>4.34 (4.12–4.57)</td>
<td>4.35 (4.14–4.60)</td>
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<tr>
<td>FMD (%)</td>
<td>5.6 (3.2–8.6)</td>
<td>3.9 (2.1–6.3)</td>
</tr>
<tr>
<td>PAC (ng/l)</td>
<td>43.0 (26.0–62.0)</td>
<td>41.0 (24.0–58.0)</td>
</tr>
<tr>
<td>PRC (ng/l)</td>
<td>8.4 (5.5–12.8)</td>
<td>7.3 (4.9–10.1)</td>
</tr>
<tr>
<td>ARR</td>
<td>5.0 (2.9–8.0)</td>
<td>5.3 (3.1–9.5)</td>
</tr>
</tbody>
</table>

BP, blood pressure; FMD, flow-mediated dilatation of the brachial artery; PAC, plasma aldosterone concentration; PRC, plasma renin concentration; ARR, aldosterone-to-renin ratio.

To convert PAC in pmol/l multiply by 2.775. To convert PRC in mU/l multiply by 1.66. Continuous variables are given as median (1st–3rd quartile), and dichotomous variables are given as percentages.

* p < 0.05.

Fig. 1. Median (1st, 3rd quartile) levels of FMD according to tertiles of PAC and ARR in subjects younger than 50 years of age. * p < 0.05; FMD, flow-mediated dilation of the brachial artery; PAC, plasma aldosterone concentration; ARR, aldosterone-to-renin ratio.
Table 2
Odds ratios and 95% confidence intervals for decreased FMD by age-group in the whole study population and in a subgroup of subjects with PAC and ARR within the reference range.

<table>
<thead>
<tr>
<th>Model*</th>
<th>Categorization of PAC and ARR</th>
<th>Age &lt;50 years</th>
<th>Age ≥50 years</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PAC</td>
<td>ARR</td>
<td>PAC</td>
</tr>
<tr>
<td>All subjects (n = 972)</td>
<td>Moderate vs. low</td>
<td>1.12 (0.74–1.66)</td>
<td>1.64 (1.10–2.46)</td>
</tr>
<tr>
<td></td>
<td>High vs. low</td>
<td>1.60 (1.07–2.38)</td>
<td>1.81 (1.21–2.73)</td>
</tr>
<tr>
<td></td>
<td>Continuous (unit = 1SD)</td>
<td>1.14 (0.96–1.34)</td>
<td>1.22 (1.04–1.44)</td>
</tr>
<tr>
<td>PAC and ARR within</td>
<td>Moderate vs. low</td>
<td>1.07 (0.70–1.63)</td>
<td>1.51 (0.99–2.31)</td>
</tr>
<tr>
<td>the reference range (n = 871)</td>
<td>High vs. low</td>
<td>1.62 (1.07–2.47)</td>
<td>1.62 (1.05–2.50)</td>
</tr>
<tr>
<td></td>
<td>Continuous (unit = 1SD)</td>
<td>1.17 (0.97–1.40)</td>
<td>1.22 (1.02–1.45)</td>
</tr>
</tbody>
</table>

FMD, flow-mediated dilation of the brachial artery; PAC, plasma aldosterone concentration; ARR, aldosterone-to-renin ratio.
FMD was categorized in decreased (1st quintile), moderate (2nd–4th quintile), or increased (5th quintile); PAC and ARR entered the models as continuous or categorical variables (low 1st tertile, moderate 2nd tertile, and high 3rd tertile).
All models were adjusted for sex (male, female), age (in years), systolic and diastolic blood pressure (in mmHg), waist circumference (in cm), smoking (yes, no), diabetes mellitus (yes, no), physical activity (yes, no), and time between blood sampling and FMD measurement (in days).

* Ordinal logistic regression using the proportional odds model.

or pharmacological treatment of primary aldosteronism [12,13]. Also in hypertensive patients without primary aldosteronism, associations between renin activity or ARR and FMD were reported [14]. Duffy and colleagues [14] found that a low renin status or high ARR, but not PAC, were associated with impaired responses to methacholine in hypertensive subjects [14]. Associations of plasma renin with brachial artery FMD were also found in hypertensive and normotensive subjects in the Framingham Heart Study [21]. No association was found between serum aldosterone and FMD [21]. In our study, high PAC was associated with decreased FMD in young individuals but there was no linear association between PAC and FMD. Then again, we identified a linear association between ARR and FMD. This suggests that relative PAC values, seen in relation to PRC, may be more closely linked to FMD than absolute PAC values. Unfortunately, in the report analysing data from the Framingham Heart Study [21], the association between ARR and PAC was not investigated.

Strengths of our study include the various quality assurance methods employed and the large study population. These methods included periodic certification processes of readers and examiners before and during data collection [15,16], ensuring high-quality FMD measurements. However, four limitations of our investigation merit comment. First, due to voluntary participation, the subsample of FMD participants is not representative of the whole SHIP population. FMD participants were younger and probably healthier than non-participants. Second, although the median time interval between blood sampling and FMD measurement was only 16.5 days, it was longer than 90 days in about 20% of our participants.

This long interval in a fraction of our subjects may have introduced bias. Third, circadian rhythms with peak secretion in the mornings were described for the aldosterone and renin secretion [22,23]. As in SHIP blood samples were taken at varying time points during day, we recalculated all ordinal regression models adjusted for the median of blood sampling. Since our results remained unchanged, we do not suspect a relevant influence of the circadian secretion on our results. Fourth, our epidemiological study cannot provide causal explanations for the observed associations between PAC or ARR with FMD.

5. Conclusion

In summary, we identified associations of high and high-normal PAC or ARR with decreased FMD in young to middle-aged subjects from the general population. Our data show that high and high-normal PAC or ARR contribute to an impaired FMD and subsequently the progression of subclinical atherosclerosis in young to middle-aged subjects.

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Conflict of interest

There are no conflicts of interest that could be perceived as prejudicing the impartiality of the research reported.

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Appendix A. Supplementary data


References


