Aldosterone Dysregulation With Aging Predicts Renal Vascular Function and Cardiovascular Risk

Jennifer M. Brown, Patricia C. Underwood, Claudio Ferri, Paul N. Hopkins, Gordon H. Williams, Gail K. Adler, Anand Vaidya

Abstract—Aging and abnormal aldosterone regulation are both associated with vascular disease. We hypothesized that aldosterone dysregulation influences the age-related risk of renal vascular and cardiovascular disease. We conducted an analysis of 562 subjects who underwent detailed investigations under conditions of liberal and restricted dietary sodium intake (1124 visits) in the General Clinical Research Center. Aldosterone regulation was characterized by the ratio of maximal suppression to stimulation (supine serum aldosterone on a liberal sodium diet divided by the same measure on a restricted sodium diet). We previously demonstrated that higher levels of this Sodium-modulated Aldosterone Suppression-Stimulation Index (SASSI) indicate greater aldosterone dysregulation. Renal plasma flow (RPF) was determined via p-aminohippurate clearance to assess basal renal hemodynamics and the renal vascular responses to dietary sodium manipulation and angiotensin II infusion. Cardiovascular risk was calculated using the Framingham Risk Score. In univariate linear regression, older age ($\beta$=-4.60; $P<0.0001$) and higher SASSI ($\beta$=-58.63; $P=0.001$) predicted lower RPF and a blunted RPF response to sodium loading and angiotensin II infusion. We observed a continuous, independent, multivariate-adjusted interaction between age and SASSI, where the inverse relationship between SASSI and RPF was most apparent with older age ($P<0.05$). Higher SASSI and lower RPF independently predicted higher Framingham Risk Score ($P<0.0001$) and together displayed an additive effect. Aldosterone regulation and age may interact to mediate renal vascular disease. Our findings suggest that the combination of aldosterone dysregulation and renal vascular dysfunction could additively increase the risk of future cardiovascular outcomes; therefore, aldosterone dysregulation may represent a modifiable mechanism of age-related vascular disease. 

Key Words: aging • aldosterone • renal plasma flow • renin-angiotensin system

Aldosterone is a key hormonal mediator of cardiovascular and renal disease, independent of its effect on blood pressure. In overtly dysregulated states such as primary aldosteronism, there is an increased incidence of adverse vascular outcomes, including left ventricular hypertrophy and proteinuria, compared with essential hypertensive controls. Human clinical trials have demonstrated that mineralocorticoid blockade results in robust improvements in cardiovascular mortality and renal vascular outcomes such as albuminuria, further supporting the pathological role of aldosterone on the cardiovascular system. A better understanding of the spectrum of aldosterone dysregulation and its interaction with other cardiovascular disease risk factors may improve efforts to prevent adverse cardiovascular outcomes and target individuals at highest risk.

Even within the normal range, higher aldosterone levels have been associated with increased rates of hypertension and cardiovascular mortality. However, in the majority of cases, it is not a single measurement of aldosterone that indicates whether regulation is normal or abnormal, but rather it is the appropriateness of aldosterone concentration in the context of the specific environmental and physiological milieu. High aldosterone levels may be pathological in some circumstances, but may be adaptive and physiological in others. For example, under conditions of high dietary sodium intake, a relative failure to suppress aldosterone has been associated with obesity, insulin resistance, and dyslipidemia. Conversely, a relative inability to stimulate aldosterone in response to severely restricted sodium intake, or after infusion of exogenous angiotensin II (AngII), has also been associated with unfavorable cardiovascular risk. Human and animal studies have previously reported that the process of aging may be associated with a progressive impairment in adrenal aldosterone stimulation, with such impairment possibly related to increased tissue exposure to AngII, thereby providing 1 potential mechanism to account for age-related vascular diseases.

We have previously reported the development of a comprehensive index to integrate aldosterone responses in an effort to characterize the spectrum of aldosterone regulation. The

Received January 21, 2014; first decision January 31, 2014; revision accepted February 21, 2014.

From the Division of Endocrinology, Diabetes, and Hypertension (J.M.B., P.C.U., G.H.W., G.K.A., A.V.) and Center for Adrenal Disorders (G.H.W., G.K.A., A.V.), Brigham and Women’s Hospital and Harvard Medical School, Boston, MA; University of L’Aquila, San Salvatore Hospital, Coppito, Italy (C.F.); and Cardiovascular Genetics, Department of Internal Medicine, University of Utah School of Medicine, Salt Lake City, UT (P.N.H.).

The online-only Data Supplement is available with this article at http://hyper.ahajournals.org/lookup/suppl/doi:10.1161/HYPERTENSIONAHA.114.03231/-/DC1.

Correspondence to Anand Vaidya, MD, MMSc, Center for Adrenal Disorders, Division of Endocrinology, Diabetes, and Hypertension, Brigham and Women’s Hospital, Harvard Medical School, 221 Longwood Ave, RFB, Boston, MA 02115. E-mail avaidya1@partners.org.

© 2014 American Heart Association, Inc.

Hypertension is available at http://hyper.ahajournals.org DOI: 10.1161/HYPERTENSIONAHA.114.03231

1205
Sodium-modulated Aldosterone Suppression-Stimulation Index (SASSI) is the ratio of aldosterone measured on a liberal sodium diet (where adrenal aldosterone should be suppressed) to aldosterone measured on a restricted sodium diet (where adrenal aldosterone should be stimulated). A failure to suppress or stimulate appropriately results in a high SASSI, indicating dysregulated aldosterone physiology. We previously showed that the SASSI strongly predicted cardiometabolic abnormalities and the metabolic syndrome and provided a more comprehensive assessment compared with single aldosterone concentrations when evaluating individuals with only a few cardiometabolic risk factors. We, therefore, hypothesized that aldosterone dysregulation, as represented by SASSI, would predict renal vascular dysfunction and cardiovascular risk. Given the previous observations linking age with dysregulated aldosterone physiology, we further hypothesized that SASSI and age may interact to predict vascular risk. Herein, we present the analyses to test these hypotheses on a large population of individuals (n=562) without baseline kidney or cardiac disease, who completed a strictly controlled study protocol to characterize SASSI and renal vascular hemodynamics.

**Methods**

**Study Population**

Cross-sectional analyses from the International Hypertensive Pathotype (HyperPATH) data set were conducted. The HyperPATH data set consists of a multicenter cohort of adults who underwent detailed profiling of the renin-angiotensin-aldosterone system (RAAS) under strict control of known confounders, including medication use, dietary sodium intake, posture, and time of day. Participants were studied at Brigham and Women’s Hospital (Boston, MA), University of Utah Medical Center (Salt Lake City, UT), Hospital Broussais (Paris, France), University of Rome (Rome, Italy), and Vanderbilt University (Nashville, TN). For the analyses presented herein, subjects were only included if they completed both study visits (1 on liberal [LIB] and 1 on restricted [RES] sodium intake) with available data for serum aldosterone measures as well as renal plasma flow (RPF) obtained under both LIB and RES dietary sodium conditions. We excluded from our analyses those participants with inadequate sodium balance under each dietary condition (urine sodium <150 mmol/24 hours on LIB diet or urine sodium >40 mmol/24 hours on RES diet) and those with evidence suggestive of primary hyperaldosteronism (aldosterone to renin ratio >30, serum aldosterone >12 ng/dL, and urinary aldosterone excretion rate >15 mcg/24 hours on LIB diet). With these criteria, our study population included 562 subjects who completed a total of 1124 study visits.

The detailed inclusion and exclusion criteria for the HyperPATH cohort in general have been outlined in detail and are described briefly in the online-only Data Supplement. All subjects provided informed consent, and all study procedures were approved by the institutional review board of each study site.

**Study Protocol**

The HyperPATH protocol has been described in detail previously. In brief, if applicable, antihypertensive medications were weaned during a 1- to 3-month washout period before study procedures to ensure interpretability of RAAS measurements. Participants completed study procedures under 2 controlled dietary sodium conditions: LIB (200 mmol per day) and RES (10 mmol per day). After 5 to 7 days of study diet, subjects were admitted to the General Clinical Research Center for overnight supine rest before beginning study procedures in the morning. Blood pressure was measured while supine in the morning after overnight rest using the average of 5 readings from a Dinamap automated device (Critikon, Tampa, FL). Sodium balance was confirmed with a 24-hour urine collection, and body mass index (BMI) was recorded. On the morning after admission, blood pressure and fasting baseline measures, including glucose, total cholesterol, and high-density lipoprotein, were obtained at the initiation of weight-based para-aminohippurate (PAH) infusion for determination of RPF.

PAH was given initially as a bolus (8 mg/kg) followed by a continuous infusion of 12 mg/min, as previously described. PAH clearance was used to calculate RPF. On measuring baseline plasma renin activity and serum aldosterone, AngII was infused at 5 ng/kg per minute for 60 minutes, after which PAH and serum aldosterone were measured again. Identical procedures were performed on both LIB and RES diets with a 1-week interval between studies. Because the LIB diet more closely approximates a typical ad lib Western diet, basal RPF under LIB conditions was used in all analyses of RPF, consistent with previous studies. Because dietary salt loading and restriction influence circulating blood volume and thus RPF, the RPF response to dietary salt intake was also regarded as salt sensitivity of the renal vasculature. This was calculated as the difference between RPF on the LIB diet and RPF on the RES diet (ΔRPF = RPF on LIB diet – RPF on RES diet). AngII infusion increases adrenal aldosterone secretion and also causes direct glomerular arteriolar vasoconstriction and thus reduces RPF. This change in RPF in response to AngII (ΔRPF AngII) serves as an indicator of renal vascular health and the local renal vascular RAAS, where a blunted ΔRPF AngII indicates greater local RAAS activity and poorer renal vascular health.3,13,20,21 ΔRPF AngII was calculated as the difference between RPF before and after AngII infusion on the LIB diet (ΔRPF AngII = change in RPF in response to AngII infusion on LIB diet). To confirm our RPF findings using a more common clinical measure of renal function, we calculated the estimated glomerular filtration rate (eGFR) using the CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration)22 and MDRD (Modification of Diet in Renal Disease) formulas,23 which incorporate age, sex, race, and serum creatinine. Because our results did not differ based on which formula was used, only the results of analyses using eGFR calculated by the CKD-EPI equation are included herein.

To characterize dynamic aldosterone regulation, we calculated the SASSI using the ratio of supine serum aldosterone on the LIB diet to supine serum aldosterone on the RES diet. Impaired suppression of aldosterone on LIB diet or impaired stimulation on RES diet is manifested in a high SASSI. As previously described, higher values of SASSI indicate more abnormal regulation of aldosterone and are better predictors of severity of the metabolic syndrome compared with single aldosterone measures alone.

In addition, we further confirmed our findings using 2 complementary indices: Sodium-modulated Aldosterone Urinary Suppression-to-Stimulation Index (SAUSSI) and Sodium-modulated Aldosterone Suppression-to-Stimulation Index with AngII infusion (SASSI-I). The ratio of 24-hour urinary aldosterone excretion rates on LIB and RES diets was used to calculate the corresponding urinary index: the SAUSSI-I. A second serum index, the SASSI-II, was calculated using the ratio of supine serum aldosterone on the LIB diet to supine serum aldosterone after AngII infusion on the RES diet. AngII infusion while on a RES diet is expected to provide maximal adrenal aldosterone stimulation, thereby providing an extreme physiological extension of SASSI. In addition, AngII infusion reduces renal vascular vasoconstriction to reduce RPF, thereby SASSI-II is a useful correlate to AngII AngII. The values of both SAUSSI and SASSI-II are also indicative of impaired physiological regulation of aldosterone.

Framingham Risk Score (FRS) was selected as a well-validated, age-dependent measure of cardiovascular risk. Using FRS, estimated 10-year risk of coronary heart disease was calculated from a composite of age, sex, smoking status, total cholesterol, high-density lipoprotein, and untreated systolic blood pressure (SBP). Smoking status was established by self-report at the initial screening visit. Average SBP and measures of total and high-density lipoprotein-cholesterol obtained on the LIB diet were used in FRS calculation because the LIB diet approximates the typical ad lib Western diet.

**Laboratory Assays**

Full details of the relevant laboratory assays are included in the online-only Data Supplement.
Statistical Analysis

We assessed the relationships between aldosterone regulation (SASSI), age, and renal vascular function (RPF) using linear regression with 3 additive models. Univariate regression was used to analyze the relationship between SASSI and RPF. Because age is a prominent risk factor for renal vascular disease,25 multivariate model 1 included adjustment for age. Model 2 included additional adjustments for known vascular disease risk factors, namely race, sex, BMI, and presence of diabetes mellitus. Model 3 further adjusted for SBP, which is known to correlate with SASSI17 and is strongly associated with age, renal function, and vascular disease. Because all subjects were studied off blood pressure medications, there was a continuous range of SBP values within the study population. Given the possibility of a distinct effect of hypertension status on renal function or cardiovascular risk, model 3 analyses were repeated using the categorical variable of hypertension (yes/no) in place of SBP. However, because this did not change the results appreciably, only the results of analyses using continuous SBP in model 3 are described below. Continuous interaction modeling adjusted for all covariates (model 3 plus interaction term) was used to assess for effect modification between SASSI and age.

In analyses of aldosterone dysregulation and cardiovascular risk (FRS), we evaluated a subset of 352 individuals from the original population of 562 because 210 individuals had missing data for total cholesterol. When FRS was the outcome, multivariate regression models included adjustment only for race, BMI, and presence of diabetes mellitus because all other relevant covariates were components of FRS itself. A \( P \) value <0.05 was considered statistically significant. All statistical analyses were performed using SAS 9.3 (SAS Institute, Cary, NC).

Results

Study Populations

The study population was characterized by a mean age of 45.8±0.5 years and mean eGFR of 86.8±0.8 mL/min. Other baseline characteristics are shown in Table 1. There were no appreciable differences with regard to demographic characteristics, blood pressure, SASSI, or kidney function between the total study population (n=562) and the subset used with complete data available for subsequent FRS assessments (n=352).

Aldosterone Dysregulation, Age, and Renal Vascular Dysfunction

Lower RPF was predicted by higher SASSI (\( \beta = -58.63; \ P = 0.001 \)) and by older age (\( \beta = -4.60; \ P < 0.0001 \)) in univariate analyses. eGFR, a more common clinical metric of renal clearance based on serum creatinine measures, was correlated with RPF measured under experimental conditions (\( r = 0.339; \ \beta = +2.16; \ P < 0.0001 \)) and was similarly predicted by higher SASSI (\( \beta = -9.93; \ P = 0.0006 \)). When aldosterone dynamics were represented by urinary measures (SAUSSI) instead of serum measures (SASSI), we confirmed that lower RPF was also predicted by higher SAUSSI (\( \beta = -61.98; \ P = 0.009 \)).

Because both higher SASSI17 and declining renal function25 have been previously associated with older age, we closely examined the role that aging may play in this relationship. We first crudely stratified the population by median age (47 years). For those younger than or equal to the median, there was no significant relationship between SASSI and RPF (Table 2); however, for those with age greater than the median, a significant relationship between SASSI and RPF was present, which persisted despite multivariate adjustments for all variables, except SBP (Table 2). Fully adjusted multivariate interaction modeling (model 3 plus interaction term) confirmed that SASSI

### Table 1. Baseline Characteristics

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>All Subjects (n=562)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>45.8±0.5</td>
</tr>
<tr>
<td>Sex, No. (%)</td>
<td></td>
</tr>
<tr>
<td>Women</td>
<td>225 (40.0)</td>
</tr>
<tr>
<td>Men</td>
<td>337 (60.0)</td>
</tr>
<tr>
<td>Race, No. (%)</td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>462 (82.4)</td>
</tr>
<tr>
<td>Black</td>
<td>77 (13.7)</td>
</tr>
<tr>
<td>Other</td>
<td>22 (3.9)</td>
</tr>
<tr>
<td>Diabetic, No. (%)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>52 (9.3)</td>
</tr>
<tr>
<td>Hypertensive, No. (%)</td>
<td>334 (59.4)</td>
</tr>
<tr>
<td>Current smoker, No. (%)</td>
<td>31 (5.5)</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>182.7±2.0</td>
</tr>
<tr>
<td>HDL-cholesterol, mg/dL</td>
<td>43.0±0.7</td>
</tr>
<tr>
<td>LDL-cholesterol, mg/dL</td>
<td>107.3±1.6</td>
</tr>
<tr>
<td>Screening serum creatinine, mg/dL</td>
<td>0.98±0.01</td>
</tr>
<tr>
<td>eGFR, mL/min (CKD-EPI)</td>
<td>86.8±0.8</td>
</tr>
<tr>
<td>eGFR, mL/min (MDRD)</td>
<td>79.7±0.8</td>
</tr>
<tr>
<td>SASSI</td>
<td>0.36±0.01</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td></td>
</tr>
<tr>
<td>LIB Diet</td>
<td>27.7±0.2</td>
</tr>
<tr>
<td>RES diet</td>
<td>27.1±0.2</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td></td>
</tr>
<tr>
<td>LIB Diet</td>
<td>133.1±1.0</td>
</tr>
<tr>
<td>RES diet</td>
<td>120.3±0.8</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td></td>
</tr>
<tr>
<td>LIB Diet</td>
<td>79.7±0.6</td>
</tr>
<tr>
<td>RES diet</td>
<td>73.5±0.5</td>
</tr>
<tr>
<td>Urine sodium, mmol/24 h*</td>
<td>231 (88)</td>
</tr>
<tr>
<td>LIB Diet</td>
<td>11 (11.1)</td>
</tr>
<tr>
<td>RES diet</td>
<td></td>
</tr>
<tr>
<td>Aldosterone, ng/dL*</td>
<td></td>
</tr>
<tr>
<td>LIB Diet</td>
<td>3.2 (3.3)</td>
</tr>
<tr>
<td>RES diet</td>
<td>14.5 (12.9)</td>
</tr>
<tr>
<td>PRA, ng/mL per hour*</td>
<td></td>
</tr>
<tr>
<td>LIB Diet</td>
<td>0.3 (0.4)</td>
</tr>
<tr>
<td>RES diet</td>
<td>1.9 (2.4)</td>
</tr>
<tr>
<td>Basal RPF, mL/min</td>
<td></td>
</tr>
<tr>
<td>LIB Diet</td>
<td>518.4±4.9</td>
</tr>
<tr>
<td>RES diet</td>
<td>497.7±5.1</td>
</tr>
</tbody>
</table>

Characteristics are shown for the total population used in the primary analyses. Parameters that varied depending on liberal sodium (LIB) or restricted sodium (RES) diets are shown in the corresponding rows below each parameter that applied. Values are presented as mean±SEM unless otherwise indicated. BMI indicates body mass index; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MDRD, Modification of Diet in Renal Disease; PRA, plasma renin activity; RPF, renal plasma flow; SASSI, Sodium-modulated Aldosterone Suppression-Stimulation Index; and SBP, systolic blood pressure.

*Median (interquartile range) is presented for variables that are not normally distributed.
interacted continuously and independently with age to predict RPF (P interaction=0.027); higher SASSI in young individuals did not appreciably influence RPF, but this relationship was increasingly evident with advancing age (Figure 1).

We further examined the relationships between SASSI, RPF, and age by evaluating them under experimental conditions where physiological provocations were used to dynamically characterize SASSI and RPF. Examination of these relationships under extreme physiological manipulation was used to confirm existing associations. We first assessed the relationship between SASSI and RPF response to dietary sodium manipulation ($\Delta RPF_{\text{sodium}}$, RPF on LIB diet–RPF on RES diet), where a blunted $\Delta RPF_{\text{sodium}}$ indicates an abnormal renal vascular response to sodium manipulation. Consistent with our initial findings, higher SASSI is associated with a lower $\Delta RPF_{\text{sodium}}$ ($\beta=-23.43; \; P=0.06$), though this relationship did not reach statistical significance. However, when stratified by median age, we confirmed that higher SASSI predicted a blunted $\Delta RPF_{\text{sodium}}$ in older, but not younger, individuals (age $\leq$ median: $\beta=+2.94; \; P=0.90$; age $> $ median: $\beta=-28.25; \; P=0.037$). We then evaluated the relationship between SASSI-II (ratio of supine serum aldosterone on LIB diet to supine serum aldosterone after ANGII infusion on RES diet) and $\Delta RPF_{\text{ANGII}}$ ($\Delta RPF_{\text{ANGII}}$, change in RPF in response to ANGII infusion on LIB diet) where ANGII infusion provides a maximal stimulus to raise serum aldosterone and to reduce RPF. Consistent with our aforementioned findings, higher SASSI-II predicted a blunted $\Delta RPF_{\text{ANGII}}$ in univariate analyses ($\beta=+57.49; \; P=0.020$), and this relationship was also predominant with older, but not younger, age (age $\leq$ median: $\beta=-3.74; \; P=0.93$; age $> $ median: $\beta=+62.91; \; P=0.015$).

**Aldosterone Dysregulation, Renal Vascular Hemodynamics, and Cardiovascular Disease Risk**

Given our findings of a relationship between aldosterone regulation and renal vascular hemodynamics modified continuously by age, we explored whether these findings could be extended to cardiovascular disease risk. FRS, an age-dependent measure of 10-year risk of coronary heart disease, was calculated for participants with all available data. In our current study population, which was recruited to safely tolerate antihypertensive withdrawal and ANGII infusion, median FRS was 9 for both men and women, corresponding to a median 10-year coronary heart disease risk of 5% and 1%, respectively. Despite this relatively low-risk population, lower RPF and lower calculated eGFR each predicted higher FRS (FRS: $\beta=-0.02; \; P<0.0001$; eGFR: $\beta=-0.12; \; P<0.0001$), as expected. More notably, higher FRS was also significantly and independently predicted by higher SASSI in multivariate modeling adjusted for race, BMI, and presence of diabetes mellitus ($\beta=+6.95; \; P<0.0001$). Together, higher SASSI ($P<0.0001$) and lower RPF ($P<0.0001$) appeared to display an additive effect in predicting FRS in our multivariate adjusted model (Figure 2). However, neither discrete nor continuous interaction modeling revealed an interaction between renal function and SASSI in predicting FRS.

**Discussion**

Our findings suggest that impaired aldosterone regulation may be an important mediator of the age-related decline in renal vascular function. Using a novel index, SASSI, to characterize the spectrum of aldosterone regulation under fixed environmental conditions, we observed that the degree of aldosterone dysregulation predicted impairments in renal vascular hemodynamics. Notably, our study participants all had normal GFR, thereby indicating the detection of subclinical renal vascular dysfunction in association with aldosterone dysregulation. Older age modified this relationship, suggesting that with advancing age the presence of aldosterone dysregulation could compound the risk of renal dysfunction. We scrutinized these new relationships and interactions and effectively confirmed them, by analyzing the outcomes of provocations that modulated SASSI and RPF in physiologically meaningful manners. Though hard outcomes could not be assessed in this cross-sectional analysis, we demonstrate, for the first time to our knowledge, a clear interaction between aldosterone dysregulation, renal vascular function, and cardiovascular disease risk.
regulation, aging, and renal vascular function and raise the possibility that aldosterone dysregulation may represent a key feature of age-related vascular disease risk.

We here extend the findings of previous studies that have provided evidence for aldosterone as a mediator of endothelial dysfunction and renal and cardiovascular disease. In preclinical in vitro and animal studies, aldosterone has been shown to cause nephropathy and cardiomyopathy via increased oxidative stress, inflammation, and fibrosis. In hyperaldosteronism, inappropriately elevated aldosterone levels have been linked to impaired vascular function and increased cardiovascular disease. Furthermore, clinical studies have demonstrated a mortality benefit of mineralocorticoid receptor blockade in congestive heart failure and a benefit in reducing albuminuria in both diabetic and non-diabetic nephropathy. These previous human studies largely relied on single measures of aldosterone with variable degrees of control for confounders. We here report a robust relationship using an integrated aldosterone measure reflective of responses to dynamic conditions and obtained under strict conditions of medication, postural, and dietary control in a large cohort of subjects. Furthermore, existing clinical studies have largely assessed aldosterone in the context of pathological aldosterone excess and pre-existing vascular disease.

The current knowledge of mineralocorticoid receptor activation permits an intuitive connection between the inability to suppress adrenal aldosterone release appropriately in the setting of high dietary sodium intake and vascular disease. The failure to suppress adrenal aldosterone secretion appropriately in the setting of restricted sodium intake is less intuitive; however, previous studies have shown that blunted aldosterone secretion is also associated with adverse cardiometabolic profiles. We speculate that progressive impairment in the adrenal aldosterone-producing apparatus prevents adequate secretion in times of restricted sodium and adequate suppression in times of plentiful sodium balance, resulting in a limited dynamic range of aldosterone responsiveness. This dynamic range of adrenal aldosterone responses to dietary sodium challenges is effectively represented by SASSI and seems to worsen with older age. The SASSI captures not only individuals with a defect in aldosterone suppression or stimulation but also those in whom both suppression and stimulation are impaired; therefore, despite being a cross-sectional assessment of adrenal function, SASSI provides a comprehensive snapshot of individual adrenal physiology.

We here demonstrate that within a population of individuals without chronic kidney disease or primary hyperaldosteronism, there is a range of subclinical renal vascular dysfunction and aldosterone dysregulation, and that these parameters are correlated. Our demonstration of an interaction between age and aldosterone dysregulation further suggests a potential mechanism underlying age-related decline in vascular function; the relationship between aging and impairment in renal function may be enhanced by dysregulated aldosterone physiology. In this regard, interventions to target aldosterone (such as mineralocorticoid blockade) to mitigate renal vascular disease and nephropathy may be most effective in the subset of older individuals with an identified abnormality in aldosterone regulation.

Beyond the implications for renal vascular disease, aldosterone levels within the normal range have been found to predict cardiovascular and all-cause mortality in a prospective study of patients presenting for coronary angiography. We extend this finding now to a population without cardiovascular disease, other than mild to moderate hypertension, showing that those individuals with abnormal physiological aldosterone responses have higher FRS, an estimate of 10-year coronary heart disease risk. Together, aldosterone dysregulation and impaired renal vascular hemodynamics seem to additively compound FRS. This affirms the importance of considering aldosterone dysregulation not only as a renal vascular risk factor but also as a cardiovascular risk factor in its own right, and as a potential target of intervention warranting future study. It should be acknowledged that age is independently associated with aldosterone dysregulation, renal dysfunction, and cardiovascular
risk and, therefore, may be a central bridge linking all of these factors; however, we studied a relatively young and healthy population with a narrow age range (mean, 46 years; interquartile range, 41–54 years), suggesting that age alone is unlikely to account for the observed differences in cardiovascular risk. Furthermore, our findings were apparent despite this narrow age range, suggesting that with a larger range of ages our findings may have been more dramatic. Future intervention studies that modulate aldosterone physiology or action may disentangle the relative contributions of aging alone and aldosterone dysregulation alone on vascular outcomes.

Our study has the benefit of several strengths. First, aldosterone physiology was carefully characterized using measures obtained under standardized conditions of time of day, fixed sodium intake, withdrawal of interfering medications, and overnight supine posture. Second, SASSI incorporates the ability of aldosterone to be stimulated and suppressed, a measure shown to be more sensitive compared with either measure alone for the presence of cardiometabolic risk. Third, in assessing renal vascular function, we determined RPF experimentally using PAH clearance instead of relying on a calculated GFR, which is a crude measure and particularly experimentally using PAH clearance instead of relying on a calculated GFR, which is a crude measure and particularly

unaccurate at the high end of the range. This calculated GFR, which is a crude measure and particularly

experimentally using PAH clearance instead of relying on a calculated GFR, which is a crude measure and particularly

susceptible to inaccuracy at the high end of the range. This permitted assessment of subclinical changes in renal vascular function in individuals with otherwise normal GFR.

However, some limitations should be acknowledged. First, the cross-sectional design of our study provides insights into associations but cannot determine causality. The possibility of reverse causation, such that declining renal vascular function promotes aldosterone dysregulation, must be considered. If this were the case, we would expect to see a similar relationship between RPF and plasma renin activity, an upstream stimulus for aldosterone production. However, we have previously shown that plasma renin activity responses to dietary sodium modulation do not account for the relationship between cardiometabolic risk and SASSI. Second, though our findings were repeatedly affirmed using several related indices and physiological maneuvers, it should be noted that the addition of SBP in the most complete adjusted multivariate model resulted in some loss of statistical significance. This may be because of the fact that there is a close collinear relationship between aldosterone dysregulation and blood pressure, and adjusting for SBP may inappropriately eliminate the observed effect. Third, unlike the aldosterone to renin ratio, the SASSI is defined under fixed and extreme dietary sodium conditions and is, therefore, not practical for clinical use. Instead of focusing on aldosterone suppressibility alone, the SASSI, in contrast to the aldosterone to renin ratio, incorporates experimentally determined aldosterone responses to both suppression and stimulation, thereby providing an experimental tool for characterizing the comprehensive dynamic range of aldosterone regulation to help uncover the underlying pathophysiology. Fourth, although we evaluated renal function using both RPF and eGFR, we lacked data on proteinuria to provide a more complete assessment of the impact of renal vascular abnormalities on renal outcomes. Finally, though the FRS has been extensively validated, it remains an estimate of cardiovascular disease risk, and as expected, the corresponding 10-year coronary heart disease risk in our selected study population was low.

**Perspectives**

In summary, we found associations suggesting that aldosterone dysfunction interacts synergistically with aging to predict renal vascular dysfunction. Furthermore, abnormal aldosterone physiology and reduced renal vascular function in combination seem to additively increase age-dependent future cardiovascular risk. These findings support consideration of aldosterone dysregulation as a mediator of age-related vascular disease and reinforce the need for additional studies examining aldosterone physiology and the benefits of aldosterone-targeted interventions.

**Acknowledgments**

We thank the participants, trainees, and nursing staff who contributed to the studies involving the HyperPath cohort.

**Sources of Funding**

Research reported in this publication was supported by the National Heart, Lung, and Blood Institute of the National Institutes of Health under award numbers K23 HL11177 (to A.V.) and K24 HL103845 (to G.K.A.). Research was also supported by a Brigham and Women’s Hospital Biomedical Research Institute Grant (to A.V.), a William Randolph Hearst Young Investigator Award from the Brigham and Women’s Hospital Department of Medicine (to A.V.), and a Harvard Medical School Research Fellowship (to J.M.B.), and by the following grants: U71HP25059 from the Health Resources and Services Administration; U54LM008748 from the National Library of Medicine; UL1RR025758, Harvard Clinical and Translational Science Center, from the National Center for Research Resources; M01-RR02635, Brigham and Women’s Hospital, General Clinical Research Center, from the National Center for Research Resources; and P50HL055000, Specialized Center of Research in Molecular Genetics of Hypertension. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Center for Research Resources or the National Institutes of Health.

**Disclosures**

None.

**References**

Novelty and Significance

What Is New?

- We examined the role of aldosterone as a mediator of age-related vascular dysfunction using a novel index to represent the dynamic range or renal blood flow response to aldosterone in human hypertension. Clin Endocrinol (Oxf). 2011;74:294–299.


What Is Relevant?

- Characterization of the dynamic range of aldosterone regulation can reveal important pathways in the renal vascular and cardiovascular decline that occurs with age.

Summary

There is a synergistic interaction between aldosterone dysregulation and aging that predicts reduced renal vascular function, implicating control of aldosterone in the pathophysiology of age-related vascular disease. Even in a relatively young and healthy population, aldosterone dysregulation and renal vascular function seem to increase age-dependent cardiovascular risk.
Aldosterone Dysregulation With Aging Predicts Renal Vascular Function and Cardiovascular Risk

Jenifer M. Brown, Patricia C. Underwood, Claudio Ferri, Paul N. Hopkins, Gordon H. Williams, Gail K. Adler and Anand Vaidya

_Hypertension._ 2014;63:1205-1211; originally published online March 24, 2014;
doi: 10.1161/HYPERTENSIONAHA.114.03231

_Hypertension_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2014 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/63/6/1205

Data Supplement (unedited) at:
http://hyper.ahajournals.org/content/suppl/2014/03/24/HYPERTENSIONAHA.114.03231.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Hypertension_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Hypertension_ is online at:
http://hyper.ahajournals.org//subscriptions/
ONLINE SUPPLEMENT

ALDOSTERONE DYSREGULATION WITH AGING PREDICTS RENAL-VASCULAR FUNCTION AND CARDIO-VASCULAR RISK

AUTHORS: Jenifer M. Brown BA1,3, Patricia C. Underwood NP PhD1,3, Claudio Ferri MD4, Paul N. Hopkins MD MSPH5, Gordon H. Williams MD1,3, Gail K. Adler MD PhD1,3, Anand Vaidya MD MMSc1,3

AFFILIATIONS: 1Division of Endocrinology, Diabetes, and Hypertension, 2Center for Adrenal Disorders, 3Brigham and Women’s Hospital and Harvard Medical School; 4University of L’Aquila, San Salvatore Hospital, Coppito, Italy; 5Cardiovascular Genetics, Department of Internal Medicine, University of Utah School of Medicine.

SHORT TITLE: Aldosterone, Aging, and Vascular Risk

WORD COUNT: 313

CORRESPONDING AUTHOR: Anand Vaidya MD MMSc, Center for Adrenal Disorders, Division of Endocrinology, Diabetes, and Hypertension, Brigham and Women’s Hospital, Harvard Medical School, 221 Longwood Ave, RFB, Boston, MA, 02115. Tel: 617-525-8285, Fax: 617-732-5764. Email: avaidya1@partners.org.
METHODS: Study Population
Briefly, participants were between 18 and 65 years old. They were excluded for coronary artery disease, stroke, renal insufficiency, any secondary causes of hypertension such as hyperaldosteronism, major psychiatric illness, active oral contraceptive use, active tobacco/illicit drug use or moderate-to-heavy alcohol use by history; for abnormal electrolytes, abnormal liver/thyroid function tests, or serum creatinine >1.5mg/dL on screening laboratory evaluation; or for evidence of heart block, arrhythmia, or ischemia on screening electrocardiogram. Subjects were assigned hypertensive status for a seated diastolic blood pressure of ≥90 mmHg on no antihypertensive medications, ≥80 mmHg on at least one medication, or the use of at least two antihypertensive medications.1

METHODS: Laboratory Assays
Serum creatinine was assayed along with other routine measures to establish study eligibility at the initial screening visit. Under both LIB and RES dietary conditions, blood samples drawn after overnight supine rest were used to measure fasting glucose, total cholesterol, LDL, and HDL. Aliquots from the 24-hour urine collection performed during the overnight admission were used to quantify urinary sodium, potassium, and aldosterone excretion rate. Blood samples obtained at baseline and after 60-minute AngII infusion were assayed for PRA (Diasorin, Inc., Stillwater, MN) and serum aldosterone (Siemens, Los Angeles, CA). PAH was measured in triplicate before and after the AngII infusion, and was assayed by autoanalyzer technique.2

REFERENCES: