Impaired Endothelium-Dependent Flow-Mediated Vasodilation in Hypertensive Subjects With Hyperaldosteronism

Mari K. Nishizaka, MD; M. Amin Zaman, MD; Sharon A. Green, RN; Kerry Y. Renfroe, RN; David A. Calhoun, MD

**Background**—Recent studies suggest that aldosterone may impair endothelium-dependent vascular function through suppression of nitric oxide formation. Assessments of forearm blood flow or arterial compliance suggest a similar effect in humans. The present study was designed to determine whether chronic aldosterone excess in subjects with resistant hypertension impairs endothelium-dependent vascular reactivity as indexed by direct assessment of brachial artery flow-mediated dilation (FMD).

**Methods and Results**—Consecutive subjects (n=80) with resistant hypertension were prospectively evaluated with an early-morning ratio of plasma aldosterone to plasma renin activity and 24-hour urinary aldosterone and sodium. Changes in brachial artery diameter during reactive hyperemia were measured by high-resolution ultrasound. Hyperaldosteronism was diagnosed on the basis of a renin activity <1.0 ng · mL⁻¹ · h⁻¹, urinary aldosterone >12 µg/24 h, and urinary sodium >200 mEq/24 h. FMD was significantly lower in 36 subjects with hyperaldosteronism (1.8±1.3% versus 3.9±1.9% from baseline; P<0.0001) compared with the 44 subjects without hyperaldosteronism. FMD was negatively and significantly correlated with plasma aldosterone (r=-0.38, P=0.0006), 24-hour urinary aldosterone (r=-0.49, P<0.0001), and ratio of plasma aldosterone to plasma renin activity (r=-0.43, P<0.0001) but was independent of blood pressure, age, and body mass index. In 30 subjects, 3 months of treatment with spironolactone significantly increased FMD (2.5±1.7 versus 6.0±2.0%; P<0.0001) independently of blood pressure change.

**Conclusions**—These data demonstrate a strong association between aldosterone excess and impaired endothelial function in human subjects as indexed by flow-mediated arterial vasodilation. These results suggest that chronic aldosteronism may have a blood-pressure–independent effect on cardiovascular disease progression in subjects with resistant hypertension. (*Circulation.* 2004;109:2857-2861.)

**Key Words:** aldosterone • hypertension • renin • ultrasonics • vasodilation

A growing body of evidence suggests that aldosterone, independently of its effects on blood pressure, impairs vascular function through suppression of nitric oxide (NO) formation.

Aldosterone added to rat vascular smooth muscle cells in vitro inhibits expression of inducible NO synthase induced by interleukin-1β in a dose-dependent fashion.1 In a rat model of heart failure, spironolactone, a nonselective mineralocorticoid antagonist, added to an ACE inhibitor improves endothelium-dependent aortic ring relaxation, presumably by suppressing formation of superoxide anion (O₂⁻), a potent scavenger of NO.2 Eplerenone, a selective mineralocorticoid antagonist, has a similar effect on O₂⁻ production in experimental models of atherosclerosis.3

Assessments of vascular compliance or forearm blood flow link chronic aldosterone excess with endothelium-dependent vascular dysfunction in humans. In subjects with hypertension4 or heart failure,5 plasma aldosterone correlates negatively with systemic arterial compliance as calculated from intra-arterial blood pressure and cardiac output. In subjects with primary and secondary (renovascular hypertension) hyperaldosteronism, forearm blood flow response to acetylcholine, an endothelium-dependent vasodilator, as assessed by venous occlusion plethysmography is reduced.6 Similarly, use of venous plethysmography to assess forearm blood flow in subjects with chronic heart failure suggests that spironolactone, added to chronic ACE inhibition, improves endothelial function secondary to increased NO availability.7

Ultrasonographic measurement of flow-mediated dilation (FMD) of the brachial artery allows direct and noninvasive assessment of endothelial-dependent vascular function. Arterial vessel dilation occurs in response to postischemic increases in blood flow and shear stress via release of NO. Impairment of FMD has been demonstrated in subjects with uncomplicated hypertension8 and high-oxidative-stress conditions such as diabetes and hypercholesterolemia.9-11

The present study was designed to determine whether chronic aldosterone excess in subjects with resistant hypertension impairs endothelium-dependent vascular reactivity as indexed by brachial artery FMD.
Methods

Subjects
Consecutive patients referred to the University of Alabama at Birmingham Hypertension Clinic for resistant hypertension were enrolled over a 23-month period. The University’s Institutional Review Board approved this study, and all subjects provided written informed consent before study participation. Resistant hypertension was defined as uncontrolled hypertension (≥140/90 mm Hg) determined at ≥2 clinic visits despite the use of ≥3 antihypertensive medications, including an ACE inhibitor and/or an angiotensin receptor blocker (ARB), at pharmacologically effective doses. Subjects were required to have been on the same antihypertensive regimen for ≥4 weeks before biochemical evaluation. Spironolactone, triamterene, or amiloride was discontinued for ≥6 weeks before biochemical evaluation. Serum potassium levels were corrected with oral supplementation to be >3.5 mEq/L before evaluation if necessary. Secondary causes of hypertension other than hyperaldosteronism such as renovascular hypertension, pheochromocytoma, or Cushing’s syndrome had been excluded by laboratory evaluation. Subjects with hyperaldosteronism such as renovascular hypertension, pheochromocytoma, or Cushing’s syndrome had been excluded by laboratory analysis and/or radiological imaging as clinically indicated. All subjects underwent 24-hour ambulatory blood pressure monitoring (Spacelabs 90207). The monitor recorded systolic and diastolic blood pressures every 20 minutes during the daytime (6 AM to 8 PM) and every 30 minutes at night (8 PM to 6 AM). Subjects with a history of atherosclerotic disease (myocardial infarction, stroke, or peripheral vascular disease), congestive heart failure, current smoking, diabetes on insulin treatment, or chronic kidney disease (creatinine clearance <60 mL/min) were excluded from study participation.

Laboratory Assessment
Biochemical evaluation was done in all subjects on an outpatient basis. An early-morning plasma aldosterone concentration (PAC) to plasma renin activity (PRA) ratio (ARR), serum potassium, blood urea nitrogen, and creatinine were determined. A 24-hour urine collection for aldosterone (Ualdo), sodium (UNa), and creatinine was obtained during the subject’s ad libitum diet. If the PRA was suppressed (<1.0 ng · mL⁻¹ · h⁻¹) and Ualdo was elevated (>12 µg/24-hour) with UNa <200 mEq/24 h, the urine collection was repeated after 3 days of dietary salt supplementation. Biochemical hyperaldosteronism was determined if the PRA was suppressed (<1.0 ng · mL⁻¹ · h⁻¹) and the 24-hour urinary aldosterone excretion was high (>12 µg/24 h) during high dietary sodium ingestion (>200 mEq/24 h).¹²⁻¹⁴ PAC, PRA, Ualdo, and UNa were measured by commercial laboratories using standard techniques. PAC and PRA levels were measured by radioimmunoassay (Quest Diagnostics). The reference range for PAC is 4.0 to 31.0 ng/dL. The reference range for an upright PRA is 1.31 to 3.95 ng · mL⁻¹ · h⁻¹. Ualdo was measured by radioimmunoassay (Mayo Clinic Laboratories). The reference range for Ualdo is 2 to 16 µg/24 h.

Assessment of Endothelial Function
Changes in brachial artery diameter during reactive hyperemia were measured by high-resolution ultrasound as described in recent reports.¹⁵⁻¹⁶ The patient rested 30 minutes in a supine position in a quiet, air-conditioned room, ultrasound evaluation was done with a 7.5-MHz linear-array ultrasound probe (Accuson 128XP/10, Accuson) by a single dedicated physician. Scans of the brachial artery were obtained 5 cm proximal from the elbow in the longitudinal section in the right arm, and the probe was maintained in a fixed position at a fixed angle during the evaluation. Arterial flow was determined with a pulsed-Doppler signal at the beginning, 10 to 15 seconds after cuff release, and at the end of the study. Increased blood flow was induced by a blood pressure cuff placed around the forearm, with a 5-minute inflation at 50 mm Hg above the subject’s systolic blood pressure, followed by rapid deflation. Baseline images before cuff inflation and then for 2 minutes after cuff deflation were recorded.

Arterial diameter was measured at end-diastolic phase (as confirmed by the incident R wave on synchronized ECG monitoring) from super-VHS recordings by a single observer blinded to the subject’s identity. Measurements were taken from the anterior to the posterior interface between the media and adventitia. Every 5 cardiac cycles of measurement from 60 to 120 seconds for baseline and from 30 to 120 seconds after cuff deflation (hyperemia) were taken. For the reactive hyperemia response, measurements with the 5 largest diameters were averaged, and the percentage increase from baseline was determined as FMD.

In a subset of subjects (8 subjects with hyperaldosteronism, 8 control subjects), endothelium-independent vasodilation was evaluated after sublingual administration of nitroglycerin 0.4 mg, an exogenous NO donor. Brachial diameter and blood pressure were measured before and 3 to 4 minutes after nitroglycerin administration.

Assessment of Spironolactone Effect
In 30 subjects, FMD was reevaluated after 3 months of treatment with spironolactone 12.5 to 25 mg/d. In addition to spironolactone, all subjects remained on a stable antihypertensive regimen that included both a diuretic and an ACE inhibitor orARB.

Statistical Analysis
Values are expressed as mean±SD. Values between groups were compared by 2-tailed Student’s t test or paired test for matched data. A potential correlation between biochemical values and FMD was evaluated by linear regression analysis. A value of P<0.05 was considered significant.

Results
A total 80 subjects were evaluated: 36 with and 44 without hyperaldosteronism. Baseline demographics, including clinic and ambulatory blood pressure and medication use, were similar in subjects with and without hyperaldosteronism, except for a greater number of male subjects in the former group (Table 1). Thirty subjects or 38% of total subjects (36% of high aldosterone and 39% of control group) were on medical or diet therapy for known type 2 diabetes. Subjects with hyperaldosteronism had higher mean plasma aldosterone, urinary aldosterone excretion, and ARR than control subjects (Table 2). Of the 44 control subjects, 23 had low renin hypertension based on a PRA <1.0 ng · mL⁻¹ · h⁻¹.

Vascular Function
Brachial artery diameter at baseline was similar in high-aldosterone and control subjects (5631±778 versus 5308±703 µm; P<0.05). Brachial artery diameter increased in response to reactive hyperemia in both groups; however, this increase was significantly less in subjects with hyperaldosteronism compared with control subjects (1.8±1.3% versus 3.9±1.9% from baseline; P<0.0001; Figure 1). This difference was independent of blood pressure (both clinic and 24-hour ambulatory blood pressure monitoring), age, and body mass index. Nitroglycerin-induced vasodilation was similar in the 2 groups (10.1±1.4% versus 10.8±0.7% from baseline; P>0.05). In all subjects, there was a significant negative correlation between FMD and plasma aldosterone (r = −0.38, P = 0.0006), 24-hour Ualdo (r = −0.49, P < 0.0001), and ARR (r = −0.43, P < 0.0001) (Figure 2). No significant relationship was observed between FMD and PRA, blood pressure, age, body mass index, or baseline brachial artery diameter.
Effect of Aldosterone Blockade With Mineralocorticoid Receptor Blockade

In 30 subjects, including 18 with hyperaldosteronism, FMD was reevaluated after 3 months of treatment with spironolactone 12.5 to 25 mg/d added to their existing antihypertensive regimen (Table 3). Systolic and diastolic blood pressures were significantly reduced in all subjects with spironolactone treatment (157±21/92±15 versus 131±14/77±10 mm Hg; P<0.0001), with high-aldosterone and control subjects having similar reductions in blood pressure (−26±17/−16±12 versus −26±31/−12±16 mm Hg; P>0.05). Baseline diameter of the studied artery was not significantly affected by spironolactone therapy.

For all subjects, mean FMD was significantly increased with spironolactone treatment (2.5±1.7 versus 6.0±2.0; P<0.0001; Table 3). A significant increase in FMD was observed in both high-aldosterone and control subjects, but the former subjects, who had lower mean FMD at baseline, showed greater increases in FMD with spironolactone treatment (4.4±2.3 versus 2.2±1.6; P=0.008; Figure 3). Overall, increases in FMD did not correlate with baseline aldosterone status or with blood pressure reduction.

Discussion

These data demonstrate a direct relation between endogenous aldosterone excess and impaired endothelial function in human subjects as indexed by FMD. In the present study, flow-mediated, endothelium-dependent vascular reactivity was significantly correlated with both plasma aldosterone and 24-hour urinary aldosterone excretion, supporting the hypothesis that aldosterone excess contributes to endothelial dysfunction. This impairment in endothelium-dependent vascular reactivity was reversed, at least in part, with chronic mineralocorticoid receptor blockade. This improvement with spironolactone treatment occurred in subjects maintained on an ACE inhibitor or ARB, indicating endothelial benefit in

### Table 1. Baseline Characteristics of All Subjects and Subjects With and Without Hyperaldosteronism

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All Subjects (n=80)</th>
<th>High-Aldosterone Subjects (n=36)</th>
<th>Control Subjects (n=44)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>53±10</td>
<td>50±11</td>
<td>55±9</td>
</tr>
<tr>
<td>Male/female, n</td>
<td>53/27</td>
<td>29/7</td>
<td>24/20*</td>
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<tr>
<td>Race, black/nonblack, n</td>
<td>39/41</td>
<td>19/17</td>
<td>20/24</td>
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<tr>
<td>BMI, kg/m²</td>
<td>34.1±7.1</td>
<td>33.6±5.7</td>
<td>34.5±8.1</td>
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<tr>
<td>Clinic BP, mm Hg</td>
<td>161±25/92±16</td>
<td>159±20/95±12</td>
<td>163±28/91±18</td>
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<tr>
<td>24-h Ambulatory BP, mm Hg</td>
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<td>150±16/91±9</td>
<td>146±18/86±14</td>
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<td>Antihypertensive medications, n</td>
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<td>4.3±1.2</td>
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</tr>
<tr>
<td>ACE-I use, %</td>
<td>64</td>
<td>53</td>
<td>73</td>
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<tr>
<td>ARB use, %</td>
<td>55</td>
<td>64</td>
<td>48</td>
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<tr>
<td>Diuretic use, %</td>
<td>78</td>
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<tr>
<td>CCB use, %</td>
<td>71</td>
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<td>68</td>
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<tr>
<td>β-Blocker use, %</td>
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<td>64</td>
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<tr>
<td>Aspirin use, %</td>
<td>86</td>
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<td>85</td>
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<tr>
<td>Statin use, %</td>
<td>25</td>
<td>28</td>
<td>23</td>
</tr>
</tbody>
</table>

BMI indicates body mass index; BP, blood pressure; ACE-I, ACE inhibitor; CCB, calcium channel blocker; and statin, HMG-CoA reductase inhibitor. Values are mean±SD.

*Different from high-aldosterone subjects (P<0.05).

### Table 2. Baseline Values of All Subjects and Subjects With and Without Hyperaldosteronism

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All Subjects</th>
<th>High-Aldosterone Subjects</th>
<th>Control Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAC, ng/dL</td>
<td>14.4±11.3</td>
<td>20.4±12.8</td>
<td>9.5±6.6*</td>
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<tr>
<td>PRA, ng·mL⁻¹·h⁻¹</td>
<td>3.2±8.4</td>
<td>0.5±0.2</td>
<td>5.4±10.8*</td>
</tr>
<tr>
<td>ARR</td>
<td>25.9±27.1</td>
<td>44.7±29.5</td>
<td>10.5±10.2*</td>
</tr>
<tr>
<td>Ualdo, μg/24 h</td>
<td>14.7±9.5</td>
<td>22.4±8.2</td>
<td>8.6±5.0*</td>
</tr>
<tr>
<td>Brachial arterial diameter, μm</td>
<td>5453±750</td>
<td>5631±778</td>
<td>5308±703</td>
</tr>
<tr>
<td>Flow-mediated vasodilation, μm</td>
<td>161±104</td>
<td>107±79</td>
<td>206±102*</td>
</tr>
<tr>
<td>Flow-mediated vasodilation, %</td>
<td>3.0±1.9</td>
<td>1.8±1.3</td>
<td>3.9±1.9*</td>
</tr>
</tbody>
</table>

*Values are mean±SD.

*Different from high-aldosterone subjects, P<0.05.
addition to that accomplished by inhibiting ACE or blocking the angiotensin II type I receptor.

Prior studies have indicated that aldosterone impairs endothelial function through suppression of NO formation. Ikeda et al reported that aldosterone reduces NO bioactivity in cultured smooth muscle cells when stimulated by cytokines. In this study, increases in nitrite production and inducible NO synthase availability in response to interleukin-1 β stimulation were inhibited by aldosterone in a dose-dependent manner.

Based on assessments of systemic resistance or forearm blood flow, studies of human subjects have indicated aldosterone-induced impairment of endothelium-dependent vascular function secondary to probable reductions in endogenous NO availability. Taddei et al observed reduced NO-dependent (acetylcholine-induced) but normal NO-independent (sodium nitroprusside-induced) vasodilation in subjects with aldosteronism, either primary or secondary to renovascular hypertension. Farquharson et al demonstrated that chronic administration of spironolactone increases forearm blood flow induced by acetylcholine in association with an increase in vasoconstriction resulting from N-monomethyl-L-arginine (a competitive inhibitor of NO synthase) in subjects with congestive heart failure maintained on an ACE inhibitor. This improvement was not associated with blood pressure reduction or a change in NYHA functional class.

In the present study, additional benefit of mineralocorticoid receptor antagonism was observed in the setting of chronic ACE inhibition or angiotensin receptor blockade. This incremental benefit is consistent with recent reports from this laboratory that spironolactone further reduces blood pressure when added to an ACE inhibitor or angiotensin receptor blocker in subjects with resistant hypertension. The observations suggest that aldosterone may have detrimental vascular effects distinct from those of angiotensin II. These results may relate to recent studies of heart failure demonstrating mortality benefit with the addition of a mineralocorticoid antagonist to conventional therapy that included, in most subjects, ACE inhibition or ARB.

Interestingly, both groups of subjects, those with and without hyperaldosteronism, manifested a significant improvement in FMD with chronic spironolactone therapy, although the increase was significantly greater in the high-aldosterone subjects. Benefit in control subjects was probably attributable, in some part, to blocking aldosterone, because many of these subjects had high normal aldosterone levels in the setting of suppressed PRA, suggestive of relative aldosterone excess. Alternatively, some benefit in FMD may have occurred independently of aldosterone such as through increases in serum potassium level. Additional studies are necessary to explore these possibilities.

In the present study, addition of spironolactone to existing antihypertensive therapy significantly reduced blood pressure in both high-aldosterone and control subjects. Although the design of the present study does not allow exclusion of the possibility that the improvement in FMD was related to a blood pressure...
effect, several considerations make this unlikely. First, in this study, improvement in FMD with spironolactone was not related to a reduction in either systolic or diastolic blood pressure. Second, other investigators have clearly documented that acute and chronic blood pressure reductions occur without improvement in FMD, depending on the class of antihypertensive agent used, suggesting that blood pressure reduction in itself is not sufficient to improve endothelial function.21

A recent prospective evaluation has shown that incidence of cardiovascular disease is significantly increased in subjects with low FMD.24 Taken with the present results, it suggests that subjects with resistant hypertension and persistent aldosteronism may be at even greater risk of cardiovascular disease than similarly hypertensive subjects with low or normal aldosterone excretion. If so, preferential use of mineralocorticoid receptor antagonists in this high-risk patient group may provide significant long-term benefit when added to conventional antihypertensive therapy. Prospective evaluations of mineralocorticoid receptor antagonist use in subjects with resistant hypertension are necessary to test this possibility.

Acknowledgments

This work was supported by American Heart Association grant-in-aids 005001N and 0355302B (Dr Calhoun) and National Heart, Lung, and Blood Institute grant HL-07457 (Dr Zaman).

References

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_Circulation_. 2004;109:2857-2861; originally published online June 1, 2004;
doi: 10.1161/01.CIR.0000129307.26791.8E
_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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