Statin Use and Adrenal Aldosterone Production in Hypertensive and Diabetic Subjects

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Background—Statins substantially reduce cardiovascular mortality and appear to have beneficial effects independent of their lipid-lowering properties. We evaluated the hypothesis that statin use may modulate the secretion of aldosterone, a well-known contributor to cardiovascular disease.

Methods and Results—We measured adrenal hormones in 2 intervention studies. In study 1 in hypertensive subjects, aldosterone was analyzed at baseline and after angiotensin II stimulation on both high- and low-sodium diets (1122 observations, 15% on statins for >3 months). Statin users had 33% lower aldosterone levels in adjusted models (P<0.001). Cortisol was not modified by statins. In secondary analyses, the lowest aldosterone levels were seen with lipophilic statins and with higher doses. Statin users had lower blood pressure and reduced salt sensitivity of blood pressure (both P<0.001). In study 2, aldosterone was measured in diabetic patients on a high-sodium diet, before and after angiotensin II stimulation (143 observations, 79% statin users). Again, statin users had 26% lower aldosterone levels (P=0.006), particularly those using lipophilic statins. Ex vivo studies in rat adrenal glomerulosa cells confirmed that lipophilic statins acutely inhibited aldosterone, but not corticosterone, in response to different secretagogues.

Conclusions—Statin use among hypertensive and diabetic subjects was associated with lower aldosterone secretion in response to angiotensin II and a low-sodium diet in 2 human intervention studies. This effect appeared to be most pronounced with lipophilic statins and higher doses. Future studies to evaluate whether aldosterone inhibition may partially explain the robust cardioprotective effects of statins are warranted. (Circulation. 2015;132:1825-1833. DOI: 10.1161/CIRCULATIONAHA.115.016759.)

Key Words: adrenal cortex hormones ■ aldosterone ■ glucocorticoids ■ hypertension ■ sodium ■ statin

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and reduce inflammation. Interestingly, these statin effects are not consistently observed with low-cholesterol diets, nor with other lipid-lowering medications such as niasin, ezetimibe, cholesterol ester transfer protein inhibitors, or fibrates. To date, the multifactorial mechanisms involved in statin’s anti-thrombotic, anti-inflammatory, and cardioprotective effects have not been fully elucidated and the hypothetical pleiotropic effect of statins are still a matter of debate.

In addition to dyslipidemia, there is strong evidence supporting a role for renin-angiotensin-aldosterone system (RAAS) dysregulation and mineralocorticoid receptor activation in the pathogenesis of atherosclerosis, coronary disease, atrial fibrillation, and heart failure. These effects are in addition to the well-known role of aldosterone signaling on the salt sensitivity of BP and hypertension. For instance, in patients with coronary disease, aldosterone levels predict ischemic events and long-term mortality. Lower aldosterone levels consistently predict better cardiovascular health in a non–high-risk community study such as the Framingham cohort. Furthermore, several trials have shown that the inhibition of RAAS, in particular, mineralocorticoid receptor blockade, has remarkable similarities with the described pleiotropic effects of statins, specifically related to improvements in atherosclerotic plaque, cardiovascular remodeling, BP, and endothelial dysfunction. Despite these clinical similarities, there is insufficient information related to a potential aldosterone inhibitory effect of statins.

In the present study, our primary aim was to evaluate the hypothesis that chronic statin use is associated with lower aldosterone levels. Furthermore, using ex vivo experiments in adrenal zona glomerulosa (ZG) cells, we provide complementary information demonstrating a novel role of statins in modulating aldosterone secretion.

Materials and Methods

Human Studies

Study 1: Discovery Cohort
Participants were studied within the Hypertensive Pathotype (HyperPATH) Protocol, consisting of individuals with mild to moderate hypertension evaluated in response to sodium intake and adrenal secretagogues. The protocol includes rigorous control of several factors that influence RAAS, incorporating antihypertensive medication washout, body positioning, and diurnal variation under strictly controlled diets.

We excluded from this study participants with known or suspected secondary hypertension, such as primary hyperaldosteronism, Cushing syndrome, or renovascular hypertension. Participants with coronary disease, stroke, psychiatric illness, drug abuse, and severe hypertension were also excluded as previously described. The users of other nonstatin medication for dyslipidemia were excluded. Also, to avoid confounding by indication, we excluded all diabetic subjects in study 1. Each institutional review board approved the protocol and informed consent was obtained before enrollment.

Chronic statin use was considered if participants were on a statin for at least 3 months before the study interventions. Because lipophilic statins are taken up by many tissues, including adrenal cells, we determined whether lipophilicity influenced adrenal secretion by classifying subjects in 3 groups: no statin use, low to moderate lipophilic statin (atorvastatin, fluvastatin, lovastatin), or high lipophilic statin (simvastatin). Hydrophilic statins were excluded in this categorization because of the small sample size in study 1. To explore a dose-dependent effect, statin users were classified according to their low-density lipoprotein (LDL) reduction capacity. (See online-only Data Supplement for expanded methods section.)

Human Protocol
Details of this protocol have been published previously. For the run-in phase, all recruited subjects completed a screening visit. To control for the influence that medications may play in aldosterone secretion, all angiotensin-converting enzyme inhibitors (ACEIs), angiotensin receptor blockers, or mineralocorticoid receptor antagonists were discontinued 1 month and all other antihypertensive medications were discontinued at least 2 weeks before the start of the study. Only if necessary, subjects were placed on amlodipine for BP control because of its neutral effect on aldosterone.

During the intervention phase, each subject was provided with a caffeine–alcohol–free diet containing 100 mEq/d potassium, 1000 mg/d calcium, and 200 mEq/d sodium. On the sixth day of this high-sodium (HS) diet, participants were admitted to an inpatient research unit. Blood samples were obtained at 8:00 am to measure aldosterone, cortisol, plasma renin activity, electrolytes, lipid profile, glucose, and insulin by using standardized and validated methods as previously described (HS baseline, intervention 1).

To examine the adrenal response of aldosterone to the physiological secretagogue, angiotensin II infusion (AngII, Bachem AG, Switzerland) was then administered (3 ng/kg per min for 60 minutes, HS stimulation, intervention 2). After completion of 5 to 7 days on HS, the same subject was placed on the same diet with reduced sodium to 10 mEq/d (low-sodium [LS] diet 10 mEq/d) in a crossover intervention (LS baseline, intervention 3), that also increases aldosterone secretion. After 1 week of LS diet, we measured adrenal steroids after AngII infusion (LS stimulation, intervention 4).

Sodium balance was confirmed by urinary sodium (HS >150 mEq/24 h and LS <30 mEq/24 h). Also, 24-hour urinary aldosterone and cortisol were collected as an estimation of adrenal daily production. The protocols were standardized across all study sites (Boston, Salt Lake City, and Paris).

Analysis of BP and Other Hemodynamic Measures
BP was examined using systolic arterial pressure in repeated-measures analysis. Also, we analyzed the effect of statins on the salt sensitivity of BP by comparing the difference between baseline HS and LS measurements. We calculated pulse pressure (systolic BP minus diastolic BP) as a surrogate marker of peripheral vascular compliance on HS and LS diets.

Study 2: Replication Cohort
Enrolled subjects were part of a randomized trial evaluating the role of mineralocorticoid receptor antagonists in diabetic cardiovascular disease (ClinicalTrials.gov NCT00865124). We included these participants, previous spironolactone randomization, because they were evaluated by using the same protocol regarding sodium and AngII interventions.

Description of this protocol has been published previously. The main differences in comparison with the protocol in study 1 are that all subjects had well-controlled type 2 diabetes mellitus and were on an ACEIs. At the start of the 3-month run-in phase, subjects started enalapril 20 mg/d, other antihypertensive medications were tapered off, and amlodipine 5 to 10 mg/d was added if needed. Simvastatin was started if the subject was not on a statin and LDL was >100 mg/dL at the screening visit. In the context of the run-in phase of a randomized trial, all subjects were evaluated with the same criteria for...
starting statin therapy, avoiding a selection bias. Diabetic medications were adjusted to target hemoglobin A1c level ≤7%. Study participants received the same controlled HS diet described in study 1 five days before admission to an inpatient unit. Measurements of baseline adrenal steroid (HS baseline, intervention 1) and after AngII infusion (HS stimulation, intervention 2) were performed as described in study 1. Subjects were categorized as no statin use and hydrophilic (pravastatin and rosuvastatin) or lipophilic statin use (atorvastatin, fluvastatin, lovastatin, and simvastatin). Statins were again classified according to their LDL reduction capacity, now including rosuvastatin users. 

Laboratory Analysis
Brigham and Women’s Hospital served as the central laboratory for all laboratory processing, and all assays used have been extensively reported previously. All subjects had plasma renin activity, aldosterone, and cortisol measured in triplicate in each of the intervention points.

Animal Protocol and In Vitro Studies
Ex vivo adrenal studies were designed to determine whether statins would acutely modulate aldosterone production. Adrenals were isolated from male Wistar rats maintained on HS (N=165%) for 1 week. ZG cells were isolated from adrenal tissues as previously described by carefully dissecting the capsular (glomerulosa) portion. In brief, ZG cells were incubated for digestion at 37°C for 50 minutes in modified Krebs-Ringer bicarbonate solution, centrifuged for 10 minutes at 4°C, washed, and resuspended for another 30 minutes at 37°C. Final cell suspension with pellet dilution obtained 1 to 2×10^5 isolated cells/0.5 mL with a potassium content of 3.7 mmol/L. Cells were incubated in duplicate, measuring basal and stimulated aldosterone in the presence of AngII (10^–5 mol/L, n=20 experiments) and potassium (4.7 mmol/L, n=6 experiments). Samples were preincubated for 15 minutes with 1 of 3 different statins, and pravastatin (10^–3 mol/L), atorvastatin (10^–3 mol/L) obtained from Sigma and hydroxy acid simvastatin (active metabolite of simvastatin, 10^–5 mol/L, Santa Cruz Biotechnology). To the best of our knowledge, this is the first study to explore the effect of statins on aldosterone secretion in adrenal cells ex vivo. Thus, the statin concentrations used in our studies were based on those concentrations used in previously published in vitro studies in other tissues and on our initial observation that different statin doses had similar effects on aldosterone production; thus, we chose an intermediate dose. Of note, this ex vivo protocol was an exploratory study to help understand the potential mechanism of our human results and was not intended to mimic the usual plasma levels of AngII or statin levels achieved in humans.

Aldosterone was measured at baseline and 1 hour after stimulation by using radioimmunoassay kit (Siemens, Los Angeles, CA) as described by our group. Data were normalized to the number of cells in each incubate and reported as the percentage of change. Zona fasciculata cells were isolated by using the same protocol measuring basal and stimulated corticosterone in the presence of adrenocorticotropic (10^–12 mol/L). The Animal Care Committee at Harvard Medical School approved all of the experimental procedures.

Statistical Analyses
Baseline analyses included the Student t test and χ^2; continuous variables are presented as mean±standard deviation and categorical variables as a percentage of the total sample. Because of the unique characteristics of our protocol where several measurements are available in the same subject in 4 different intervention settings, we performed a mixed-model linear regression analysis, thus allowing adjustment for confounders and addressing the effect of statin within and between subjects. Two models were tested and these covariates (fixed effects) were chosen for their clinical importance and significance in univariate analysis, whereas identity was the random effect (see online-only Data Supplement). In study 2, similar repeated-measures analyses were performed (see online-only Data Supplement). P values of ≤0.05 were considered statistically significant. All human analyses were performed by using STATA 13. Animal data were analyzed by using 1-way ANOVA, followed by Tukey post hoc analysis, with the use of the GraphPad Prism 6 software.

Results
Study 1: Adrenal Secretion in Hypertensive Subjects by Statin Use

Characteristics of Hypertensive Participants
After applying the inclusion-exclusion criteria, a total of 1122 aldosterone measurements were available from the HyperPATH cohort. These measurements represent available repeated analyses from 317 subjects at 4 different intervention points (26% on HS baseline, 18% HS AngII stimulation, 28% LS baseline, and 28% LS AngII stimulation). One hundred seventy-five of the 1122 aldosterone measurements (15.6%)

Table 1. Baseline Characteristics of Hypertensive Subjects Categorized by Statin Use on High- and Low-Sodium Diets

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No Statin Group (85%)</th>
<th>Statin Group (15%)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>49.1±7.1</td>
<td>53.9±6.6</td>
<td>0.001*</td>
</tr>
<tr>
<td>Female, %</td>
<td>43.5</td>
<td>46.3</td>
<td>0.76</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>28.6±4.4</td>
<td>30.8±4.2</td>
<td>0.001*</td>
</tr>
<tr>
<td>Systolic blood pressure on HS</td>
<td>146.6±18.6</td>
<td>141.0±18.4</td>
<td>0.07</td>
</tr>
<tr>
<td>Systolic blood pressure on LS</td>
<td>131.4±16.1</td>
<td>127.9±14.4</td>
<td>0.16</td>
</tr>
<tr>
<td>Urinary sodium on HS diet (24 h mmol)</td>
<td>232.9±68.4</td>
<td>228.6±70.2</td>
<td>0.70</td>
</tr>
<tr>
<td>Urinary sodium on LS diet (24 h mmol)</td>
<td>13.9±7.6</td>
<td>14.7±7.8</td>
<td>0.54</td>
</tr>
<tr>
<td>HS plasma potassium, mmol/L</td>
<td>4.15±0.3</td>
<td>4.14±0.3</td>
<td>0.87</td>
</tr>
<tr>
<td>LS plasma potassium, mmol/L</td>
<td>4.17±0.3</td>
<td>4.12±0.3</td>
<td>0.32</td>
</tr>
<tr>
<td>HS plasma renin activity, ng/mL×h</td>
<td>0.51±0.4</td>
<td>0.35±0.3</td>
<td>0.18</td>
</tr>
<tr>
<td>LS plasma renin activity, ng/mL×h</td>
<td>2.49±2.8</td>
<td>1.91±1.7</td>
<td>0.34</td>
</tr>
<tr>
<td>HS LDL cholesterol, mmol/L</td>
<td>3.21±0.9</td>
<td>3.16±1.1</td>
<td>0.80</td>
</tr>
</tbody>
</table>

Data reported as mean±SD, except as noted. HS indicates high sodium; LDL, low-density lipoprotein; LS, low sodium; and SD, standard deviation.
*Indicates P value is significant.
were obtained in individuals with confirmed chronic statin use. The proportions of patients on the different statins were simvastatin 43%, atorvastatin 23%, lovastatin 19%, pravastatin 9%, and fluvastatin 6%. When categorized by type of treatment, 84.8% of the observations were in individuals without statin treatment, 8.3% of the observations were in those on a low to moderate lipophilic statin (atorvastatin, fluvastatin, or lovastatin), and 6.9% of the patients were on high lipophilic statin treatment (simvastatin). When categorized by dose, an 84% of aldosterone measurements were categorized as no statin use, 7.8% were categorized as a low statin dose, and 7.7% were categorized as a moderate to high statin dose.

Subjects on statins were similar to those without statin treatment regarding sex, race, sodium intake, potassium, and plasma renin activity on HS and LS diet (Table 1). Subjects using statins were slightly older and heavier; thus, age and body mass index were included as covariates in the adjusted models.

In comparison with participants with no treatment, statin users had significantly lower values of unadjusted baseline aldosterone, AngII-stimulated aldosterone, and 24-hour urinary aldosterone on a HS diet (all P<0.001). While on a LS diet, the effect of statins resembled the effect seen on a HS diet (Table 2 and online-only Data Supplement Figure). The aldosterone-to-renin ratio was not different when analyzed by statin use on both HS and LS diet. It is noteworthy that cortisol levels had no significant changes when comparing by statin use groups in all measurements (baseline, stimulated, and 24-hour urinary collection on both diets (Table 2).

### Table 2. Unadjusted Effects of Statin Use on Aldosterone and Cortisol Levels on Sodium Diet and Angiotensin II Interventions

<table>
<thead>
<tr>
<th>Hormonal Measurements for 317 Subjects</th>
<th>Control Group on HS (85%)</th>
<th>Statin Group on HS (15%)</th>
<th>P Value</th>
<th>Control Group on LS (85%)</th>
<th>Statin Group on LS (15%)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline aldosterone, nmol/L (ng/mL)</td>
<td>0.14±0.09 (5.0±3.3)</td>
<td>0.09±0.04 (3.6±1.5)</td>
<td>0.008</td>
<td>0.47±0.27 (17.3±9.9)</td>
<td>0.29±0.16 (10.5±5.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Baseline cortisol, nmol/L (μg/dL)</td>
<td>278.7±113.1 (10.1±4.1)</td>
<td>289.7±93.8 (10.5±3.4)</td>
<td>0.69</td>
<td>314.5±93.4 (11.4±3.8)</td>
<td>10.1±4.1 (11.1±3.1)</td>
<td>0.70</td>
</tr>
<tr>
<td>Stimulated aldosterone, nmol/L (ng/mL)</td>
<td>0.39±0.21 (13.9±7.7)</td>
<td>0.30±0.13 (11.0±4.8)</td>
<td>0.003</td>
<td>1.08±0.54 (39.0±19.5)</td>
<td>0.70±0.33 (25.3±11.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Stimulated cortisol, nmol/L (μg/dL)</td>
<td>259.3±124.2 (9.4±4.5)</td>
<td>251.1±91.0 (9.1±3.3)</td>
<td>0.74</td>
<td>262.1±113.1 (9.5±3.2)</td>
<td>223.8±66.2 (9.10±2.4)</td>
<td>0.26</td>
</tr>
<tr>
<td>Urinary aldosterone, μg/24 h</td>
<td>11.7±7.8 (10.1±4.1)</td>
<td>8.3±4.6 (10.5±3.4)</td>
<td>&lt;0.001</td>
<td>38.4±23.3 (11.4±3.8)</td>
<td>27.0±13.7 (11.1±3.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Urinary cortisol, μg/24 h</td>
<td>68.5±32.2 (9.4±4.5)</td>
<td>66.2±48.3 (9.1±3.3)</td>
<td>0.69</td>
<td>49.4±24.8 (5.0±34.5)</td>
<td>50.0±34.5 (11.1±3.1)</td>
<td>0.90</td>
</tr>
</tbody>
</table>

Data reported as mean±SD, except as noted. HS indicates high sodium; LS, low sodium; and SD, standard deviation.

### Table 3. Effect of Statins on Aldosterone and Cortisol Levels in Repeated-Measures Analysis Adjusted by Confounders in Hypertensive Subjects

<table>
<thead>
<tr>
<th>Model 1*</th>
<th>Model 2†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adrenal Hormones (1122 Observations)</td>
<td>Statin effect: β (Predicted Change)</td>
</tr>
<tr>
<td>Aldosterone, ng/dL (HSb, HStim, Lsb, LStim)‡</td>
<td>−6.41 (−33%)</td>
</tr>
<tr>
<td>Cortisol, μg/dL (HSb, HStim, Lsb, LStim)‡</td>
<td>−0.23</td>
</tr>
<tr>
<td>Aldosterone, ng/dL on HS (b, stim)§</td>
<td>−1.72 (−18%)</td>
</tr>
<tr>
<td>Aldosterone, ng/dL on LS (b, stim)</td>
<td></td>
</tr>
</tbody>
</table>

* Mixed model 1: adjusted by age, body mass index, sex, and urinary sodium.
† Mixed model 2: covariates in model 1 plus race, site, and sodium diet order.
‡ HSb, HStim, Lsb, LStim (1122 observations).
§ HSb, HStim (494 observations).
|| Lsb, LStim (628 observations).
The use of statins was significantly associated with a decrease in aldosterone when included in the model as a sensitivity analysis. No interaction between statin use on aldosterone with age, body mass index, sex, or race was observed.

Noninvasive Hemodynamic Measurements by Statin Use

The use of statins was significantly associated with a decrease in systolic BP in repeated-measures analysis (BP at baseline and after AngII on both HS and LS, combined effect of 5.57 mmHg, \( P = 0.001 \)) even when adjusted by confounders and when analyzed on each diet. Furthermore, statin users had reduced salt sensitivity of BP (systolic HS – systolic LS, \( \beta = -5.85 \text{ mmHg}, P < 0.001 \), Table 4). Also, pulse pressure was significantly decreased in statin users when combining the 4 intervention measurements (\( \beta = -2.95 \text{ mmHg}, P = 0.004 \), Table 4).

Effect of Type and Dose of Statin

We observed a significant decrease in aldosterone levels related to higher lipophilic characteristics (1050 observations, \( \beta = -3.11 \text{ ng/mL}, P \text{ trend} \leq 0.001 \)). In addition, we observed a significant decrease in aldosterone on adjusted repeated-measures analysis, comparing high lipophilic statin users with nonstatin users (\( \beta = -6.26 \text{ ng/mL}, P \leq 0.001 \)) and low to moderate lipophilic statin to nonstatin users (\( \beta = -3.04 \text{ ng/mL}, P = 0.001 \)) as observed in Figure 1. Also, high lipophilic statin users had lower aldosterone levels than low to moderate lipophilic statin users (\( \beta = -3.23 \text{ ng/mL}, P = 0.015 \); Figure 1). Regarding a dose effect, we observed a consistent effect on aldosterone levels (1045 observations, \( \beta = -2.87 \text{ ng/mL}, P \text{ trend} \leq 0.001 \)) and in the comparison of moderate to high dose with no statin use (\( \beta = -5.81 \text{ ng/mL}, P = 0.001 \)) and low dose with no statin use (\( \beta = -2.71 \text{ ng/mL}, P = 0.004 \)). Interestingly, when both lipophilicity and dose were included as explanatory variables in the adjusted model, only lipophilicity remained significant. Neither type nor dose of statin was associated with changes in cortisol levels.

Study 2: Adrenal Secretion in Diabetic Subjects on Liberal Sodium Diet by Statin Use

Characteristics of Participants With Type 2 Diabetes Mellitus

After the inclusion-exclusion criteria were applied, a total of 143 observations in 79 diabetic subjects were available for analysis. As expected in this population, chronic statin use was highly prevalent (69%). The proportion of subjects on the different statins were similar to those in study 1 with simvastatin being the most commonly used (65%), followed by atorvastatin (14%), rosuvastatin (8%), pravastatin (5%), and lovastatin (3%). Subjects on statins were comparable to those without statin treatment regarding age, sex, race, body mass index, sodium intake, and years of diabetes mellitus (Table I in the online-only Data Supplement).

Adrenal Hormones Secretion on Liberal Sodium Intake in Diabetic Patients by Statin Use

Consistent with the findings in study 1, statin use was associated with lower unadjusted aldosterone levels at baseline and after AngII stimulation on HS diet (Table I in the online-only Data Supplement). Because all subjects in study 2 were on an ACEI, these results suggest that statins may act downstream of the angiotensin II receptor (AT1R). Again, cortisol levels were similar (baseline and stimulated) in the comparison of diabetic subjects with or without statin use (online-only Data Supplement Table 1). Plasma renin levels and potassium levels were not different by statin use.

Repeated-Measures Analysis in Diabetic Patients by Statin Use

As in study 1, we tested the effect of statin use on aldosterone levels by using repeated measurements of aldosterone (baseline aldosterone and AngII-stimulated aldosterone) for each subject on a HS diet. Statin use was associated with decreased

![Figure 1. Adjusted mean±SD changes in aldosterone levels categorized by type of statins in hypertensive and diabetic subjects. HyperPATH: 1050 observations; Diabetes Trial: 143 observations. NS indicates not significant; and SD, standard deviation.](image-url)
Table 5. Effect of Statins on Aldosterone and Cortisol Levels in Repeated-Measures Analysis Adjusted by Confounders (Diabetes Replication Study)

<table>
<thead>
<tr>
<th></th>
<th>Model 1*</th>
<th></th>
<th>Model 2†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Statin Effect: β</td>
<td>(Predicted Change)</td>
<td>P Value</td>
</tr>
<tr>
<td>Aldosterone, ng/dl (baseline, Ang II stim)</td>
<td>−2.13 (26%)</td>
<td>0.009</td>
<td>−2.20 (26%)</td>
</tr>
<tr>
<td>Cortisol, μg/dl (baseline, Ang II stim)</td>
<td>−0.24</td>
<td>0.78</td>
<td>0.14</td>
</tr>
</tbody>
</table>

Ang II stim indicates angiotensin II stimulation.
*Mixed model 1: adjusted by age, body mass index, sex, and urinary sodium.
†Mixed model 2: covariates in model 1 plus race, diabetes mellitus duration, and amloidine use for hypertension control.

Discussion

In these short-term RAAS intervention studies, we demonstrate that chronic statin use is associated with reduced aldosterone levels in both hypertensive and diabetic subjects. This association is evident on both HS and LS diets, in response to an infusion of the aldosterone secretagogue Ang II and when measured in 24-hour urine samples. The effect on aldosterone was specific, because cortisol levels were not modified in any intervention setting. Interestingly, both human cohort data suggest that lipophilic statins could be more efficient in blocking aldosterone secretion, results that were confirmed ex vivo.

In past decades, accumulating evidence has shown that aldosterone dysregulation participates in hypertension, coronary disease, vascular injury, and heart failure. Because statins are one of the most studied drugs in cardiovascular prevention and treatment, there is growing literature discussing the potential interaction between statins, the RAAS, and related medications, beyond the well-described anti-inflammatory and cardioprotective effects of statins. For instance, a synergistic and beneficial effect of statins with both Ang II blockers and ACEIs has been described in patients with aldosterone levels even when adjusted by confounders in both models 1 and 2 (Table 5). This effect was maintained when including LDL levels and systolic BP in the model as a sensitivity analysis. Cortisol levels were consistently not modified by statin use (Table 5). Similar to the results in study 1, lower aldosterone levels were related in a stepwise fashion to higher lipophilicity (adjusted β=−1.11 ng/mL, P trend=0.003). Furthermore, aldosterone levels in diabetic subjects were lower in those receiving a lipophilic statin in comparison with those not receiving a statin (adjusted β=−2.19 ng/mL, P=0.002; Figure 1), but not in the comparison of those using a hydrophilic statin with nonusers (adjusted β=−1.63 ng/mL, P=0.15; Figure 1). We also observed a dose-response effect when subjects were classified into the following 3 groups: no statin, low-dose statin, or moderate- to high-dose statin (β=−1.15 ng/mL, P trend=0.002). In the comparison of only the moderate- to high-dose statin users with nonusers, we observed 30% lower adjusted aldosterone levels (P=0.002), but again there was no effect on cortisol.

Ex Vivo Studies

To confirm whether the lower aldosterone levels observed in statin users could be explained by a direct effect of statins on adrenal secretion (as opposed to an increased aldosterone metabolism in response to statins), we performed ex vivo studies in rodent adrenal cells. In this system, ZG aldosterone production is increased in response to various secretagogues, including Ang II (which uses the AT1R pathway) and potassium (which acts via an AT1R-independent mechanism). Notably, we observed that the acute statin effect on aldosterone secretion was dependent on the type of statin. It is noteworthy that the lipophilic statins, simvastatin and atorvastatin, reduced both potassium- and AngII-stimulated aldosterone production, whereas the hydrophilic statin pravastatin did not have a significant effect on aldosterone acute secretion (Figure 2). In the potassium-stimulated aldosterone studies, simvastatin had significantly greater blocking effect than pravastatin and atorvastatin.

In addition, we verified that statins did not acutely affect corticosterone production in zona fasciculata cells. Furthermore, statins did not decrease the precursor of aldosterone (corticosterone) in ZG cells, thus suggesting that statins could be modulating the late pathway of aldosterone steroidogenesis.
with hypertension, diabetes mellitus, and heart failure.\textsuperscript{33–35} Furthermore, atorvastatin and simvastatin in humans, both lipophilic statins, reduced AngII sensitivity and downregulated AT1R.\textsuperscript{36} Thus, it has been assumed that this synergistic effect is secondary to an effect mediated directly by AngII. However, previous studies have not considered the possibility that this potential synergism may be secondary to an effect on aldosterone production.

To the best of our knowledge, our study is the first to analyze adrenal secretion of aldosterone and cortisol by statin use in the setting of prolonged washout of medications that affect the RAAS, strictly controlled sodium diet, and AngII infusion. However, our results showing a specific effect on aldosterone secretion but not on cortisol, as shown by our human and ex vivo data, are supported by several previous studies. An early report, evaluating whether statins impaired adrenal steroidogenesis showed that 9 months of simvastatin significantly decreased aldosterone but not cortisol levels.\textsuperscript{37} Moreover, in an animal intervention study, long-term simvastatin treatment on a HS diet significantly reduced plasma aldosterone levels.\textsuperscript{38} More recently, Palmer et al\textsuperscript{39} observed that starting a statin post–myocardial infarction was associated with a significant decrease in aldosterone levels, although values were not adjusted by sodium intake or medications affecting the RAAS. Of note, torcetrapib, a cholesterol ester transfer protein inhibitor for dyslipidemia, was halted from clinical use because of higher mortality. Treatment with the cholesterol ester transfer protein inhibitor led to increased aldosterone levels, and subsequent studies showed that the cholesterol ester transfer protein inhibitor induces aldosterone synthase in adrenal cells.\textsuperscript{40} On the other hand, fenofibrate did not change aldosterone levels in a similar protocol of HS diet intervention.\textsuperscript{41} In relation to cortisol, there are numerous well-designed studies, including cortisol levels after adrenocorticotropin stimulation, that demonstrate that typical statin use does not affect cortisol levels.\textsuperscript{42} Intriguingly, renin levels did not change despite a consistent decrease in aldosterone levels. Even though a moderate decrease in aldosterone could be insufficient to upregulate renin secretion, the specific mechanism(s) for the apparent impaired negative feedback warrant further assessment.

There are several novel and exciting results in our work that support a potential role for statins in modulating aldosterone secretion. First, the results are consistent in different intervention settings within the same subjects, and in different clinical settings, as well, such as hypertension and diabetes mellitus. Also, we found a dose-response effect for statin use with lower aldosterone levels at higher doses, providing greater evidence for causality. In relation to the type of statins, our data suggest that lipophilic statins, particularly simvastatin, rather than hydrophilic statins, are associated with lower aldosterone levels. The biological plausibility of this finding is supported by the following: (1) different affinity for adrenal tissue described by type of statin showing that radiolabeled simvastatin has a high adrenal uptake (even higher than liver) compared with pravastatin\textsuperscript{23}; (2) previous preliminary reports showing that aldosterone levels were modified by simvastatin\textsuperscript{37} but not pravastatin\textsuperscript{43}; (3) our ability to replicate our initial findings in a second cohort; and (4) our demonstration that only certain types of statins acutely reduce aldosterone secretion ex vivo.

In relation to our exploratory ex vivo experiments, we observed in adrenal cells that the type of statin affected acute aldosterone production. Lipophilic statins had a greater effect in blocking aldosterone production than pravastatin, a hydrophilic statin. Furthermore, our finding that potassium-stimulated aldosterone could be blocked acutely by statins (up to a 33% reduction), with simvastatin as the most potent and pravastatin the least potent, suggests that statins act downstream from the AT1R to decrease aldosterone production. Consistent with this concept, we found that statin use was associated with lower aldosterone levels in our replication cohort where all individuals were being treated with the same type and dose of ACEI drug. Although we did not find previous studies examining the effect of statins in adrenal cells, our results are consistent with an in vitro study in human mesangial cells showing that atorvastatin suppressed acute aldosterone production induced by AngII.\textsuperscript{44} The specific mechanism for aldosterone inhibition, which is beyond the scope of this initial study, remains to be elucidated, but in rats long-term pitavastatin treatment suppressed the expression of aldosterone synthase in the kidney.\textsuperscript{45} Reduced cholesterol availability and isoprenylation of signaling proteins have been postulated as potential mechanisms for statins modulating steroidogenesis.\textsuperscript{46} Consistent with the concept that statins affect steroidogenesis, a recent meta-analysis showed that statins could lower testosterone in men and in women with polycystic ovary syndrome.\textsuperscript{47} The mechanisms by which statins affect aldosterone production remain to be elucidated and these studies will need to examine and contrast the acute effects of statins on adrenal steroidogenesis, and of chronic in vivo effects, as well.

We additionally showed that statins were associated with lower BP, as previously reported, but also with less salt sensitivity of BP and pulse pressure. Because both sodium reabsorption and arterial stiffness have been related to aldosterone dysregulation, these observations could be, at least in part, secondary to aldosterone secretion modulation by statins.\textsuperscript{48} A recent study showed that simvastatin outperformed pravastatin in reducing AngII-induced hypertension, which is consistent with our human and ex vivo results.\textsuperscript{49}

From a clinical perspective, we believe that even a moderate decrease of aldosterone may be relevant. For example, the Ludwigshafen Risk and Cardiovascular Health (LURIC) study showed that a quartile increase in normal aldosterone levels significantly increased cardiovascular mortality after 7 years of follow-up.\textsuperscript{50} It could be that a modest decrease in aldosterone secretion may be a new and nontraditional effect of statins that may partially explain their robust cardioprotective actions not consistently observed with other lipid-lowering interventions.

Despite novel findings, our study has some limitations. First, statin intervention was not randomized, and unmeasured confounding variables associated with statin users cannot be ruled. Second, the acute dietary interventions and AngII infusions have no long-term follow-up, despite the fact our protocols are the ideal setting in which to measure aldosterone because they were designed to control all factors that could modulate adrenal secretion. Future studies should make sure...
that there is not a compensatory increase in adrenocorticotropin to maintain cortisol levels. In addition, although initial data suggest that statins may modulate the late pathway of aldosterone synthesis, the specific mechanism for the aldosterone-decreasing effect of chronic statin use needs to be fully elucidated. Finally, these results should be validated in prospective randomized studies with controlled RAAS modulators and in different populations where statins are routinely used.

Conclusions

In the present study, we demonstrate that chronic statin users have lower aldosterone levels in 2 studies that evaluate RAAS modulation by sodium diet and AngII. The effect of statins on adrenal secretion appears to be specific for aldosterone and related to the lipophilicity and dose of statin. Future confirmation studies and the assessment of potential mechanisms for this novel finding are warranted.

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Disclosures

None.

References


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CLINICAL PERSPECTIVE

Statins are the most widely prescribed class of drug in the world and they substantially reduce cardiovascular mortality. In addition, there is strong evidence implicating aldosterone dysregulation in cardiovascular disease and mortality. In the present study, we observe that chronic statin use results in lower aldosterone levels in 2 independent human protocols that evaluated aldosterone modulation in response to dietary sodium manipulation and the infusion of angiotensin II. The chronic use of lipophilic statins was associated with lowered aldosterone secretion in comparison with hydrophilic statins, but cortisol levels were unaffected. Our findings suggest that lipophilic statins may specifically inhibit adrenal cortical aldosterone secretion, and therefore may potentially identify a new and nontraditional mechanism by which statins influence cardiovascular health.
Statin Use and Adrenal Aldosterone Production in Hypertensive and Diabetic Subjects

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SUPPLEMENTAL MATERIAL

1. Supplemental Methods

A. Study 1: Discovery Cohort

Statins users were classified according to their LDL reduction capacity in three groups, no statin use (Group 1), low dose use (Group 2: atorvastatin 5 mg, lovastatin <20 mg, pravastatin <20 mg, simvastatin <10 mg and fluvastatin 20mg) or regular-high dose use (Group 3: atorvastatin ≥ 10 mg, lovastatin ≥ 40 mg, pravastatin ≥ 40 mg, simvastatin ≥ 20 mg and fluvastatin ≥ 40 mg) \(^1\).

In the regression model 1 in HyperPATH cohort, statin use effect was adjusted by age, BMI, gender, 24h urinary sodium (LS and HS) and including the effect of time (intervention points) in the model (1=HS baseline, 2=HS AngII stimulation, 3=LS baseline, 4=LS AngII stimulation). The second model included more potential confounders including those in model 1 plus race (Caucasian, African-American, Other), site (Boston, Salt Lake City, Paris) and intervention order (LS vs HS first). We also perform sensitivity analysis including LDL levels and systolic BP in the model (probably intermediary variables and not confounders). In order to test the robustness of the model we searched for effect measure modification introducing a priori interaction terms and tested for eventual collinearity.

B) Study 2: Replication cohort

In the regression model 1 in the diabetes trial, aldosterone levels were adjusted by age, BMI, gender, and HS urinary sodium. In model 2, we included model 1 covariates plus race, T2DM duration and amlodipine use for hypertension control. Bootstrapping with 1000 iterations was performed to all regression models in both studies since this non-parametric methodology does not require normality assumptions, prevents false positive results by outliers and indicates our models were not over-fitted. Further analysis to check for residuals normality and non-linearity effect modification were performed.
Supplementary Table. Baseline characteristics of diabetic participants categorized by statin use.

<table>
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<tr>
<th>n=79</th>
<th>No Statin Group (21%)</th>
<th>Statin Group (79%)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>52.2 ± 8.9</td>
<td>54.2 ± 7.5</td>
<td>0.37</td>
</tr>
<tr>
<td>Female (%)</td>
<td>38</td>
<td>37</td>
<td>0.94</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>32.9 ± 5.5</td>
<td>31.8 ± 4.4</td>
<td>0.37</td>
</tr>
<tr>
<td>Years of Diabetes</td>
<td>9.1 ± 7.6</td>
<td>7.6 ± 6.0</td>
<td>0.39</td>
</tr>
<tr>
<td>HS urinary sodium (24h mEq)</td>
<td>270.4 ± 90.2</td>
<td>272.3 ± 71.7</td>
<td>0.94</td>
</tr>
<tr>
<td>Aldosterone ng/dl (baseline)</td>
<td>4.18 ± 2.4</td>
<td>3.12 ± 1.1</td>
<td>0.01</td>
</tr>
<tr>
<td>Plasma renin activity (ng/mL*h)</td>
<td>2.89 ± 4.1</td>
<td>1.48 ± 2.5</td>
<td>0.19</td>
</tr>
<tr>
<td>Aldosterone ng/dl (AngII stim)</td>
<td>12.47 ± 7.8</td>
<td>9.22 ± 3.64</td>
<td>0.02</td>
</tr>
<tr>
<td>Cortisol ug/dL (baseline)</td>
<td>10.73 ± 3.6</td>
<td>10.09 ± 3.44</td>
<td>0.51</td>
</tr>
</tbody>
</table>

Data reported as mean ± SD, except as noted. HS indicates high sodium diet.
Supplementary Figure

Legend:
Aldosterone levels (mean ± SD) categorized by statin use after sodium diet interventions and angiotensin II infusions in 317 hypertensive subjects from the HyperPATH protocol.
고혈압 및 당뇨병 환자에서 스타틴을 사용하면 알도스테론 분비가 억제된다

신 준한 교수 아주대학교병원 순환내과

초록

배경
스타틴은 일반적으로 심혈관계 사망을 감소시키는데, 이는 지질 감소 효과와는 별개의 독립적인 유용한 효과 때문일으므로 알려져 있다. 본 연구는 스타틴이 심혈관계 질환의 범인과 관련 있을 것으로 생각되는 알도스테론의 분비를 변화시킬 것이라는 가설 아래 진행되었다.

방법 및 결과
두 개의 연구를 진행하여 부신호르몬의 측정하였다. 연구 1: 고혈압 환자에서 고염 식이와 저염 식이를 교차 시행하면서 angiotensin II 자극검사 전후의 혈청 및 소변 알도스테론을 측정하였다. 연구 대상 환자는 317명이며, 알도스테론 측정은 1,122회 시행하였고, 대상 환자 중 15%에서 이상 스타틴을 복용하였다. 스타틴 복용군이 변형 모델에서 알도스테론 수치가 33% 적었고, 스타틴에 따른 코티졸의 수치는 변화가 없었다. 스타틴 복용군 중에서는 지질개방성 스타틴 복용용과 고용량 스타틴 복용자의 알도스테론 수치가 가장 낮았다. 스타틴 복용군은 혈압이 낮았고(\(P<0.001\)), 염분감수성이 낮았다(\(P<0.001\)). 연구 2: 고염 식이 상태의 당뇨병 환자에서 angiotensin II 자극검사 전후의 알도스테론을 측정하였다. 대상환자는 79명이며, 알도스테론 측정은 143회 시행하였고, 79%가 스타틴을 복용하였다. 스타틴 복용군이 복용하지 않은군보다 알도스테론 수치가 26% 낮았으며(\(P=0.006\)), 특히 지질개방성 스타틴 복용자가 낮았다. 위의 adrenal glomerulosa 세포를 이용한 ex vivo 연구에서 지질개방성 스타틴은 알도스테론의 분비를 급속하게 억제하지만, corticosterone의 분비를 억제하는 데 못하였다.

결론
스타틴은 고혈압 및 당뇨병 환자에서 angiotensin II 자극과 저염 식이에 대한 알도스테론의 분비를 억제한다. 또한, 이런 효과는 지질개방성 스타틴을 복용하거나 고용량을 복용할 때 더 뚜렷하였다. 스타틴의 심장보호 효과 중 하나로 알도스테론의 분비 억제를 꼽을 수 있을지 향후 연구가 기대된다.