Dietary Sodium Restriction and Association with Urinary Marinobufagenin, Blood Pressure, and Aortic Stiffness

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Summary

Background and objectives Systolic BP and large elastic artery stiffness both increase with age and are reduced by dietary sodium restriction. Production of the natriuretic hormone marinobufagenin, an endogenous α1 Na+, K+-ATPase inhibitor, is increased in salt-sensitive hypertension and contributes to the rise in systolic BP during sodium loading.

Design, setting, participants, & measurements The hypothesis was that dietary sodium restriction performed in middle-aged/older adults (eight men and three women; 60±2 years) with moderately elevated systolic BP (139±2/83±2 mmHg) would reduce urinary marinobufagenin excretion as well as systolic BP and aortic pulse-wave velocity (randomized, placebo-controlled, and crossover design). This study also explored the associations among marinobufagenin excretion with systolic BP and aortic pulse-wave velocity across conditions of 5 weeks of a low-sodium (77±9 mmol/d) and 5 weeks of a normal-sodium (144±7 mmol/d) diet.

Results Urinary marinobufagenin excretion (weekly measurements; 25.4±1.8 versus 30.7±2.1 pmol/kg per day), systolic BP (127±3 versus 138±5 mmHg), and aortic pulse-wave velocity (700±40 versus 843±36 cm/s) were lower during the low- versus normal-sodium condition (all P<0.05). Across all weeks, marinobufagenin excretion was related with systolic BP (slope=0.61, P<0.001) and sodium excretion (slope=0.46, P<0.001). These associations persisted during the normal- but not the low-sodium condition (both P<0.005). Marinobufagenin excretion also was associated with aortic pulse-wave velocity (slope=0.70, P=0.02) and endothelial cell expression of NAD(P)H oxidase-p47phox (slope=0.64, P=0.006).

Conclusions These results show, for the first time in humans, that dietary sodium restriction reduces urinary marinobufagenin excretion and that urinary marinobufagenin excretion is positively associated with systolic BP, aortic stiffness (aortic pulse-wave velocity), and endothelial cell expression of the oxidant enzyme NAD(P)H oxidase. Importantly, marinobufagenin excretion is positively related to systolic BP over ranges of sodium intake typical of an American diet, extending previous observations in rodents and humans fed experimentally high-sodium diets.


Introduction

Both systolic BP (SBP) and salt (NaCl) sensitivity rise progressively with advancing age (1–4). Recent evidence supports the concept that large elastic artery stiffening precedes the development of hypertension with aging (5,6). Dietary sodium restriction is an effective lifestyle intervention for reducing both SBP and arterial stiffness in middle-aged and older adults (7–9). However, the physiologic mechanisms contributing to the reduction in SBP and arterial stiffness with reduced dietary sodium intake are incompletely understood.

Marinobufagenin (MBG) is an endogenous α1 Na+, K+-ATPase inhibitor that is produced primarily by the adrenal cortex in response to NaCl ingestion (10,11). By inhibiting renal tubular Na+,K+-ATPases, MBG prevents renal reabsorption of filtered sodium in the proximal tubule and promotes natriuresis; it also inhibits vascular smooth muscle Na+-K+-ATPases, inducing vasoconstriction, and promoting a rise in SBP (10,12). Na+-K+-ATPases inhibition also may increase oxidative stress by vascular endothelial cell membrane depolarization, which promotes activation of NAD(P)H oxidase (13,14). It is currently unknown if dietary sodium restriction modulates MBG production in humans and whether plasma or urinary MBG level is related to SBP or arterial stiffness in this setting.

Using a randomized, placebo-controlled crossover study design, we recently showed that dietary sodium restriction improves vascular endothelial function in adults with moderately elevated SBP (15). Subjects in this study completed a total of 14 consecutive 24-hour urine collections, allowing a unique opportunity to examine changes in urinary MBG in response to dietary sodium restriction. Our objectives were to (1)
examine the effect of dietary sodium restriction on urinary MBG excretion and aortic pulse-wave velocity, both of which were prespecified end points, and (2) across the study duration, assess associations between urinary MBG excretion, BP, aortic pulse-wave velocity, and markers of oxidative stress.

Materials and Methods
The details of the parent study, a randomized, placebo-controlled crossover design conducted from February of 2009 to January of 2012, have been published previously (15). The study was conducted in the University of Colorado Boulder Clinical and Translational Research Center (CTRC), and most of the blood assays were performed by the Colorado Clinical Translational Sciences Institute CTRC Core Laboratory at the University of Colorado Denver Anschutz Medical Campus. The MBG plasma and urine assays were performed in the Laboratory of Cardiovascular Science in the Intramural Research Program of the National Institute on Aging.

Subjects
The inclusion and exclusion criteria have been described previously (15). For the present analyses, we excluded subjects taking antihypertensive medications that may have independently influenced MBG as well as one subject who had numerous missing urine samples for follow-up analyses. Thus, a total of 11 subjects from the parent study (of 17 subjects) was included in these additional analyses. All subjects had a resting SBP within 130–159 mmHg (i.e., high normal or stage 1 systolic hypertension) and diastolic BP<99 mmHg, which was verified on a minimum of two occasions (7,8), but were otherwise free of cardiovascular disease, diabetes, kidney disease, and other clinical disorders as assessed by medical history, physical examination, ankle brachial index (≤0.9), blood chemistries, and resting and exercise electrocardiogram. Salt sensitivity was not assessed for inclusion in the study. All subjects were non-smokers, had a body mass index (BMI) <40 kg/m², and were not taking dietary supplements known to influence vascular function, including those supplements with antioxidant properties. Postmenopausal women (n=3) were not taking hormone replacement therapy. Subjects were either sedentary or recreationally active, but none were performing regular, vigorous exercise. All procedures were approved by the Institutional Review Board of the University of Colorado at Boulder and conform to the Declaration of Helsinki. The nature, benefits, and risks of the study were explained to the volunteers, and their written informed consent was obtained before participation.

Experimental Design and Dietary Sodium Restriction
In the original study, we used a double-blind, placebo-controlled, randomized, crossover design, which was described previously (15). Briefly, a low-sodium intake of ~1,500 mg/d (65 mmol/d) was compared with a normal US sodium intake of 3,600 mg/d (150 mmol/d). During the entire 10-week intervention period, subjects reduced dietary sodium (target was 50 mmol/d; it was anticipated that the actual mean intake would be ~65 mmol/d) and were instructed to take a total of 10 tablets spread across the day with meals. For 5 weeks, the tablets were placebo pills, whereas for the other 5 weeks the tablets were slow-release NaCl tablets (10 mmol [0.23 g] per tablet; HK Pharma, United Kingdom). The slow-release NaCl tablets aimed to return sodium intake to the ~150 mmol/d target. Subjects were provided with comprehensive dietary education and weekly counseling by CTRC bionutritionists to reduce dietary sodium intake without changing caloric intake, dietary composition, or potassium intake (these data have all been published previously) (15). The investigators were blinded to sodium condition in the analysis and recording of all variables (including BP). Six and five subjects were randomized to begin with the low- and normal-sodium conditions, respectively, and there was no evidence of a carryover effect (15).

BP and Blood/Urine Assays
Resting BP measurements and 24-hour urine collections were performed at baseline (two times) and then weekly throughout the 10-week dietary intervention (two times during the final week of each condition) for a total of 14 measurements per subject as previously described in detail for this study (15). Urine MBG excretion (corresponding to each of the 14 24-hour collections) and plasma MBG levels (last week of each sodium condition) were assessed using a competitive fluororimmunoassay as reported previously in detail (10,16–18). Briefly, urine and plasma samples were extracted using C18 SepPak cartridges (Waters Inc., Cambridge, MA), and MBG was measured using a fluororimmunoassay (Dissociation-Enhanced Lanthanide Fluorescent Immunoassay) based on a murine anti-MBG 4G4 monoclonal antibody recently described in detail (19). This assay is based on a competition between immobilized antigen (MBG-glycoside-thyroglobulin) and MBG, other crossreactants, or endogenous cardiotonic steroids within the sample for a limited number of binding sites on an anti-MBG mAbs. Secondary (goat anti-mouse) antibody labeled with nonradioactive europium was obtained from Perkin-Elmer (Waltham, MA). Data on crossreactivity of the MBG antibody have been reported previously in detail (19).

Creatinine clearance, MBG clearance, and sodium-filtered and fractional excretion of sodium were calculated as described previously (16). Other blood assays were performed using standard methodology. Blood samples were not available from one of the subjects because of a failed blood draw.

Aortic Pulse-Wave Velocity
Aortic pulse-wave velocity was measured during the final week (week 5) of each sodium condition. Two identical transcutaneous Doppler flow meters (model 810-A; Parks Medical) were used to obtain the pulse wave between the carotid and femoral artery, which has been described in detail previously (8,20). The distance from the suprasternal notch to the carotid was subtracted from the distance between the two recording sites, and pulse-wave velocity was calculated as distance divided by time between the foot of waveforms recorded at each site, which has been described previously (21,22). Pulse-wave velocity was assessed at simultaneous recording sites (the carotid and femoral arteries) by two blinded
investigators (one at each site), and the intraobserver coefficient of variation for aortic pulse-wave velocity in our laboratory is $7.2\pm 2.1\%$.

**Endothelial Cell Protein Assessments**

Endothelial cells were collected from the brachial artery during the final week of treatment (week 5) of each sodium condition for subsequent analysis of protein expression of NAD(P)H oxidase-p47phox (cells available from nine subjects; Abcam) using immunofluorescence. These procedures have been previously described (15,23–25). The values for each protein are reported as a ratio of arterial endothelial cell to human umbilical vein endothelial cell average pixel intensity, which minimizes the possible influence of differences in staining intensity among different staining sessions.

**Statistical Analyses**

The changes in urinary MBG excretion in response to dietary sodium restriction (baseline and normal- and low-sodium conditions) were analyzed using a linear mixed effects model. The model was fit across all 5 weeks of each condition in the intervention period using the covariance structure (toeplitz) with the smallest information criteria value. Details of this statistical method have also been previously provided (15). Differences in other variables were assessed using paired $t$ tests (between dietary sodium conditions) or repeated measures ANOVA with posthoc Bonferroni corrected comparisons (sodium conditions) or repeated measures ANOVA with posthoc Bonferroni corrected comparisons (sodium conditions) or repeated measures ANOVA with posthoc Bonferroni corrected comparisons (sodium conditions) or repeated measures ANOVA with posthoc Bonferroni corrected comparisons (sodium conditions). Other clinical characteristics and dietary components did not change, which is shown in Table 1 and/or published previously (15). Mean BMI of participants at baseline was $27.2\pm 1.3$ kg/m$^2$, with 45% ($n=5$), 27% ($n=3$), and 27% ($n=3$) classified as normal weight, overweight, and obese, respectively.

### Table 1. Clinical characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline</th>
<th>LS</th>
<th>NS</th>
</tr>
</thead>
<tbody>
<tr>
<td>$N$ (women/men)</td>
<td>11 (3/8)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Race (% [n] Caucasian, Asian)</td>
<td>91 [10], 9 [1]</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Urine sodium (mmol/24 h)</td>
<td>$159\pm 13$</td>
<td>$77\pm 9^a$</td>
<td>$144\pm 7$</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>$139\pm 2$</td>
<td>$127\pm 3^b$</td>
<td>$138\pm 5$</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>$83\pm 2$</td>
<td>$77\pm 2$</td>
<td>$81\pm 2$</td>
</tr>
<tr>
<td>Pulse pressure (mmHg)</td>
<td>$57\pm 2$</td>
<td>$49\pm 3$</td>
<td>$57\pm 5$</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>$81.8\pm 5.4$</td>
<td>$80.7\pm 5.2$</td>
<td>$80.7\pm 5.3$</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>$59\pm 4$</td>
<td>$59\pm 3$</td>
<td>$59\pm 3$</td>
</tr>
<tr>
<td>Plasma Na (mEq/L)</td>
<td>$139.1\pm 0.9$</td>
<td>$138.7\pm 1.0$</td>
<td>$139.1\pm 0.6$</td>
</tr>
<tr>
<td>Plasma K (mmol/L)</td>
<td>$4.27\pm 0.16$</td>
<td>$4.08\pm 0.08$</td>
<td>$4.00\pm 0.09$</td>
</tr>
<tr>
<td>Plasma Ca (mmol/L)</td>
<td>$9.26\pm 0.15$</td>
<td>$9.00\pm 0.17$</td>
<td>$8.98\pm 0.16$</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>$44.8\pm 0.6$</td>
<td>$43.4\pm 0.8$</td>
<td>$43.7\pm 0.6$</td>
</tr>
<tr>
<td>Creatinine clearance (ml/min per 1.73 m$^2$)</td>
<td>$93.9\pm 5.9$</td>
<td>$87.0\pm 8.8$</td>
<td>$92.0\pm 7.3$</td>
</tr>
<tr>
<td>Na filtered (mmol/min)</td>
<td>$13.01\pm 0.67$</td>
<td>$12.03\pm 1.25$</td>
<td>$12.62\pm 0.91$</td>
</tr>
<tr>
<td>Aortic pulse-wave velocity (cm/s)</td>
<td>N/A</td>
<td>$700\pm 40^c$</td>
<td>$843\pm 36$</td>
</tr>
</tbody>
</table>

Data are mean $\pm$ SE. LS, low sodium; NS, normal sodium; SBP, systolic BP; DBP, diastolic BP.

$^aP<0.01$ versus baseline or NS ($P=0.002$ versus baseline and $P=0.006$ versus NS).

$^bP<0.001$ versus baseline or NS.

$^cP=0.001$ versus NS.

**Results**

### Effectiveness of Dietary Sodium Restriction and Baseline Clinical Characteristics

The 11 subjects (eight men and three women; $60\pm 2$ years of age, range=51–72 years) reduced sodium excretion and SBP similarly to the entire cohort (Table 1) (15). Using a definition of salt sensitivity of a reduction of mean arterial pressure $\geq 3$ mmHg (26), 7 of 11 subjects (64%) were salt-sensitive. Other clinical characteristics and dietary components did not change, which is shown in Table 1 and/or published previously (15). Mean BMI of participants at baseline was $27.2\pm 1.3$ kg/m$^2$, with 45% ($n=5$), 27% ($n=3$), and 27% ($n=3$) classified as normal weight, overweight, and obese, respectively.

**Dietary Sodium Restriction, Urinary MBG Excretion, and Plasma MBG Levels**

Urinary MBG excretion was reduced during 5 weeks of low sodium (average over 5 weeks: $2.04\pm 0.16$ nmol/d) compared with 5 weeks of high sodium (average over 5 weeks: $2.45\pm 0.17$ nmol/d) (Figure 1, left panels and Table 2). This result was also the case when MBG was expressed normalized to body mass (25.4±1.8 versus 30.7±2.1 pmol/kg per day) (Figure 1, right panels and Table 2). Individual subject paired values for peak changes in MBG excretion are shown in Supplemental Figure 1. Plasma MBG levels, however, did not differ between sodium conditions (low sodium: $0.15\pm 0.02$ nmol/L; normal sodium: $0.17\pm 0.02$ nmol/L; $P=0.37$).

**Aortic Pulse-Wave Velocity and Oxidative Stress**

Aortic pulse-wave velocity was reduced by 17% during the low-sodium condition compared with the normal-sodium...
Arterial endothelial cell NAD(P)H oxidase-p47phox expression was not significantly changed with dietary sodium restriction (NADPH oxidase [relative to human umbilical vein endothelial cell control]: low sodium=1.53±0.30; normal sodium=1.80±0.46; P=0.62).

Association of MBG Excretion with Sodium Excretion and BP

Urinary MBG excretion was related to urinary sodium excretion (r=0.46, P<0.001), as assessed from all urine collections. When examined separately during each dietary sodium condition, this association was stronger during the normal-sodium condition (Figure 2, top panel, closed circles) but not statistically significant during the low-sodium condition (Figure 2, top panel, open circles). These results were almost identical when the three data points with the highest SBP values were excluded from analysis. A similar pattern was observed for the association between urinary MBG excretion and SBP (r=0.39, P<0.001 across all time points) (Figure 2, middle panel).

Table 2. Effect of dietary sodium restriction on urinary marinobufagenin (MBG) excretion (mixed effects regression model)

<table>
<thead>
<tr>
<th>Urinary MBG Excretion Units</th>
<th>Parameters</th>
<th>Slope (β; SE)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nanomoles per 24 hours</td>
<td>NS</td>
<td>Reference</td>
<td>0.005a</td>
</tr>
<tr>
<td>Nanomoles per 24 hours</td>
<td>LS</td>
<td>−0.33 (0.14)</td>
<td>0.031b</td>
</tr>
<tr>
<td>Nanomoles per 24 hours</td>
<td>Baseline</td>
<td>0.43 (0.23)</td>
<td>0.078</td>
</tr>
<tr>
<td>Picomoles per kilogram per 24 hours</td>
<td>NS</td>
<td>Reference</td>
<td>0.004c</td>
</tr>
<tr>
<td>Picomoles per kilogram per 24 hours</td>
<td>LS</td>
<td>−4.50 (1.91)</td>
<td>0.030b</td>
</tr>
<tr>
<td>Picomoles per kilogram per 24 hours</td>
<td>Baseline</td>
<td>4.94 (3.09)</td>
<td>0.12</td>
</tr>
</tbody>
</table>

NS, normal sodium; LS, low sodium.

*aP<0.01.

*bP<0.05.

*cP<0.005.
and urinary MBG excretion and diastolic BP ($r=0.37$, $P<0.001$ across all time points) (Figure 2, lower panel). When adjusted for time point of measurement (i.e., repeated measures), the slopes were almost identical to the unadjusted correlations (urine sodium excretion: slope=0.44, $P<0.001$; SBP: slope=0.38, $P<0.001$; diastolic BP: slope=0.37, $P<0.001$). The age, sex, and BMI (model 1) and model 1 plus 24-hour urinary sodium excretion (model 2) adjusted slopes are shown in Table 3.

**Aortic Pulse-Wave Velocity, Oxidative Stress, and MBG**

Aortic pulse-wave velocity was positively related to urinary MBG excretion across both conditions (Figure 3, upper panel). Because of the smaller number of data points for aortic pulse-wave velocity and oxidative stress markers (one time per sodium condition rather than weekly measures), these associations were not assessed for separate sodium conditions. Although average endothelial cell NADPH oxidase protein expression did not significantly change with dietary sodium restriction, this oxidant

### Table 3. Associations between urinary marinobufagenin (MBG) excretion and sodium excretion, BP, arterial stiffness, and oxidative stress

<table>
<thead>
<tr>
<th>Model</th>
<th>Condition</th>
<th>Slope ($\beta$)</th>
<th>$P$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MBG excretion (pmol/kg per day) and sodium excretion</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Pooled</td>
<td>0.46</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>1</td>
<td>LS</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>1</td>
<td>NS</td>
<td>0.58</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MBG excretion (pmol/kg per day) and SBP</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Pooled</td>
<td>0.61</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>1</td>
<td>LS</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>1</td>
<td>NS</td>
<td>0.71</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>2</td>
<td>Pooled</td>
<td>0.39</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>2</td>
<td>LS</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>2</td>
<td>NS</td>
<td>0.71</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MBG excretion (pmol/kg per day) and DBP</td>
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<td></td>
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<tr>
<td>1</td>
<td>Pooled</td>
<td>0.31</td>
<td>&lt;0.001</td>
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<tr>
<td>1</td>
<td>LS</td>
<td>0.36</td>
<td>0.003</td>
</tr>
<tr>
<td>1</td>
<td>NS</td>
<td>0.28</td>
<td>0.02</td>
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<tr>
<td>2</td>
<td>Pooled</td>
<td>0.18</td>
<td>0.05</td>
</tr>
<tr>
<td>2</td>
<td>LS</td>
<td>0.36</td>
<td>0.003</td>
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<tr>
<td>2</td>
<td>NS</td>
<td>0.28</td>
<td>0.02</td>
</tr>
<tr>
<td>MBG excretion (pmol/kg per day) and aPWV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Pooled</td>
<td>0.70</td>
<td>0.02</td>
</tr>
<tr>
<td>2</td>
<td>Pooled</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>MBG excretion (pmol/kg per day) and NADPH oxidase</td>
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<td></td>
</tr>
<tr>
<td>1</td>
<td>Pooled</td>
<td>0.64</td>
<td>0.006</td>
</tr>
<tr>
<td>2</td>
<td>Pooled</td>
<td>0.33</td>
<td>0.045</td>
</tr>
</tbody>
</table>

Model 1 was adjusted for sex, age, and body mass index. Model 2 was adjusted for all variables in model 1 with the addition of 24-hour urinary sodium excretion. Model 2 is not applicable for the association between urinary MBG excretion and sodium excretion. MBG, marinobufagenin; pooled, all time points combined (baseline [if applicable], low sodium [LS], and normal sodium [NS]); N/A, variable not entered into stepwise regression; SBP, systolic BP; DBP, diastolic BP; aPWV, aortic pulse-wave velocity; NADPH oxidase, arterial endothelial cell NAD(P)H oxidase-p47phox (NADPH oxidase) protein expression.
enzyme was correlated with urinary MBG excretion among individual subjects across both sodium conditions (Figure 3, lower panel). The relations between MBG excretion and each of these variables remained statistically significant when adjusting for age, sex, and BMI (Table 3, model 1), but the relation between MBG excretion and aortic pulse-wave velocity was no longer statistically significant after additional adjustment for 24-hour urinary sodium excretion (model 2; slope = 0.37, P = 0.10).

Discussion
We have shown, for the first time in humans, that dietary sodium restriction reduces urinary MBG excretion and that MBG excretion is positively associated with SBP and aortic stiffness. Importantly, MBG excretion is positively related to SBP over ranges of sodium intake typical of an American diet, extending previous observations in rodents and humans fed experimentally high-sodium diets (10,16,17).

We cannot discern from these observations the specific sequence of events by which dietary sodium restriction induced reductions in MBG excretion, SBP, and aortic stiffness. In Dahl salt-sensitive rats, 4 weeks of a high-sodium diet (level recommended by the American Heart Association (30) and DASH [Dietary Approaches to Stop Hypertension] diet) (31), was representative of average American dietary sodium intake (27). We posit that this response may be amplified with long-term (a lifetime of) sodium intake typical of an American diet, such that there is sustained promotion of high vascular tone, contributing to the age-associated rise in SBP that does not occur in low-salt cultures (28,32).

Although there was a limited number of subjects included in this analysis, our findings are strengthened by the fact that we obtained 24-hour urine collections from study participants on 14 separate occasions over a 2-week screening and 10-week intervention period. This large number of samples allowed for detailed insight into the MBG response to changes in sodium intake over the course of the dietary intervention. Additional strengths include using a diet that was well controlled without changes in potassium intake or other dietary components between conditions (as documented in the original publication) (15) and using a placebo-controlled crossover design, which allowed for isolation of dietary sodium as the modulating factor. We also have confirmed our previous observation of reduced aortic pulse-wave velocity (the gold standard measure of large elastic artery stiffness) with dietary sodium restriction in postmenopausal women (8) in a cohort of both men and women.

Figure 3. | Relation between urinary marinobufagenin (MBG) excretion and (top panel) aortic pulse-wave velocity (aPWV) and (bottom panel) arterial endothelial cell NAD(P)H oxidase-p47phox (NADPH oxidase) protein expression (ratio to human umbilical vein endothelial cell control) across both sodium conditions (Pearson correlation coefficient).
We acknowledge that we present here associations between MBG and SBP, aortic pulse-wave velocity, and oxidative stress and not a causal role of MBG. We also recognize that we cannot determine the sequencing of these physiologic changes. To definitively show that dietary sodium restriction reduces SBP, arterial stiffness, and oxidative stress through reduced MBG and the ordering of these events, it would be necessary to inhibit MBG experimentally (as in animal studies), which is not currently possible in humans. We also recognize that it would have been of interest to measure plasma MBG in addition to urinary MBG excretion at baseline as well as oxidative stress markers during each of the 10 weeks and at baseline. However, samples were not available for such additional assays.

Although it may seem somewhat surprising that there was no significant decrease in plasma MBG, despite changes in urinary excretion, this finding is consistent with previous work showing that sodium chloride-induced changes in urinary MBG excretion substantially exceeded those changes observed in plasma (10,18,33). We do not fully understand the reason for such dissociation; however, diurnal variations common in steroid hormones may have blunted our ability to detect a change in plasma levels between sodium conditions (34).

In summary, the present results show that dietary sodium restriction reduces urinary MBG excretion and that urinary MBG excretion is positively associated with SBP and arterial stiffness in middle-aged and older adults with moderately elevated SBP. Our findings suggest that this reduction in urinary MBG may contribute, possibly through reduced oxidative stress, to the reductions in large elastic artery stiffness and SBP that accompanied dietary sodium reduction. Given age-associated increases in arterial stiffness, SBP, and salt sensitivity, the role of MBG in these processes merits additional attention in future research.

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Disclosures
None.

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Reductions in Aortic Stiffness and Systolic Blood Pressure with Dietary Sodium Restriction are Related to Lowered Renal Marinobufagenin Excretion

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Figures

Supplemental Figure 1: Peak paired response of individual subjects to 24-hour urinary excretion of marinobufagenin (MBG) expressed in absolute units (left) and normalized to body mass (right), during the normal sodium (NS) and low sodium (LS) conditions.