Sex differences in salt sensitivity to nitric oxide dependent vasodilation in healthy young adults

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Dietary sodium intake influences the pathogenesis and treatment of hypertension (12, 18). Endothelial dysfunction—abnormalities in the regulatory functions of vascular endothelium—is one of the earliest detectable stages in the pathophysiology of atherosclerosis, arterial stiffening, and hypertension. Even in healthy individuals, endothelial dysfunction occurs earlier in men than in women (2). A hallmark of endothelial dysfunction is reduced endothelium-dependent vasodilation, which is commonly quantified by the responsiveness before and after competitive inhibition of endothelial NO (NO) (28). Another mechanism is the production of prostacyclin (PGI2) via metabolism of arachidonic acid by cyclooxygenase (1). Additional pathways being elucidated involve endothelium-derived hyperpolarizing factors (EDHF) and activation of calcium-dependent potassium channels (20). The endothelial NO component of ACh-induced vasodilation in vivo is estimated by the difference in ACh vasodilator responsiveness before and after competitive inhibition of eNOS with N5-monomethyl-L-arginine (L-NMMA). Multiple investigations incorporating this experimental paradigm in healthy humans suggest that the contribution of NO to ACh-induced vasodilation ranges from 30% to 50% (7, 9, 11, 20, 23).

One mechanism responsible for endothelial dysfunction is decreased bioavailability of NO. Recent evidence suggests that supplemental salt tablets decrease endothelial NO in healthy, normotensive young men (27). Conversely, blood pressure regulation in normotensive young women is generally insensitive to salt (21). A potential explanation for sex differences in salt sensitivity and vascular function is endothelial NO, but this idea has not been explored. Therefore, the purpose of this study was to test the hypothesis that the endothelial NO component of ACh-evoked forearm vasodilation based on low and high dietary sodium intake is influenced by sex. By measuring the vasodilator response to ACh before and immediately after inhibition of eNOS with L-NMMA, we were able to quantify the effect of high and low dietary sodium intake on the NO component of endothelium-mediated vasodilation.

METHODS

Subjects. Protocol 1 (low sodium) and protocol 2 (high sodium) were conducted in accordance with the Declaration of Helsinki and were approved by the Mayo Institutional Review Board. Both protocols were subsets of a larger investigation focusing on adrenergic receptor gene variation, dietary sodium intake, and cardiovascular control. Subjects were recruited based on common single-nucleotide polymorphisms in the beta-2 adrenergic receptor gene (Arg16/Gly) to explore the influence of gene variation on forearm beta-2 adrenergic receptor-mediated vasodilation. Measures in this report include forearm vasodilator responses to agents not mediated by adrenergic receptors (ACh and nitroprusside, NTP). In protocol 1, a separate analysis has been published without respect to sex (8), and for the present analysis vasodilator responses to ACh and NTP were available from 14 women and 16 men. From protocol 2, similar data were available for 23 women and 13 men. Genotype breakdown was not different between sexes and protocol groups, and no subject participated in both protocols.
Each participant gave written informed consent. Men were under age 40, women were under age 50 and premenopausal, and neither had a history of tobacco use or any acute or chronic disorder associated with alterations in cardiovascular structure or function. Enrollment screening included a physical examination and resting blood pressure which was measured by manual random zero sphygmomanometry in protocol 1 and automated oscillimetry in protocol 2. Females were required to have a negative pregnancy test and were studied during the low-hormone phase of the menstrual cycle or oral contraceptives to minimize the influence of progesterone and high levels of estrogen on renal and systemic parameters (21).

Diets. In protocol 1, subjects consumed a 5-day prestudy diet containing 10 mmol of sodium (0.23 g Na; 0.6 g salt) per day from the Mayo CTSA Clinical Research Unit (CRU) (8). Food items provided constant daily amounts of protein (1.4 g·kg body wt

\[ \text{Protocol 1} \]

Subjects consumed a 5-day prestudy diet in the exact fashion as protocol 1 except subjects received 400 mmol of sodium per day (9.2 g Na; 23 g salt). Similarly, a 24-h urine collection was obtained and subjects fasted until completion of the study.

Measurements, analysis, and statistics. Subjects were positioned supine and a 20-gauge brachial arterial catheter was placed, under local anesthesia, in the nondominant arm for blood pressure (BP) measurement and drug infusions. FBF was measured with venous occlusion plethysmography as previously described (8). After baseline measurement and drug infusions, FBF was measured with venous occlusion plethysmography as previously described (8). FBF was reached. After 10 min and return of FBF to baseline levels, a bolus dose of L-NMMA (50 mg) was infused over 10 min, followed by a “maintenance” dose of L-NMMA (1 mg/min). The dose of L-NMMA administered into the forearm relative to systemic infusions was consistent with previous forearm investigations of eNOS inhibition (5–7, 9, 20, 27). Because L-NMMA is a competitive inhibitor of eNOS and the measurement of NO inhibition in intact human regional models is problematic, the complete inhibition of eNOS in this study can only be inferred based on the large dose of L-NMMA administered into the forearm relative to systemic infusions (3, 13, 25). Importantly, the dose of ACh was based on forearm volume, and was identical in the dose and time infused immediately before and after L-NMMA infusion. The baseline recording and ACh infusion was repeated in the presence of L-NMMA. To determine an effect of dietary sodium on endothelium-independent vasodilation, NTP was administered for 2 min at 1.0 g·100 ml forearm tissue

\[ \text{Protocol 2} \]

In protocol 2, subjects consumed a 5-day prestudy diet in the exact fashion as protocol 1 except subjects received 400 mmol of sodium per day (9.2 g Na; 23 g salt). Similarly, a 24-h urine collection was obtained and subjects fasted until completion of the study.

RESULTS

As shown in Table 1, the age of the low-sodium cohort was greater, prediet screening SBP was lower, and prediet screening DBP tended to be higher than the high-sodium cohort. Because prediet blood pressure was measured manually by random zero sphygmomanometry in protocol 1, and by computer-automated oscillometric cuff in protocol 2, the screening blood pressure discrepancies were likely an effect of the screening method of measurement because the prediet MAP values were similar between groups. Furthermore, compared with before the diet, resting HR or BP was not affected by diet in either cohort. As expected, there was a significant effect of dietary sodium intake on the 24-h urine volume and sodium excretion. Potassium excretion was increased in the low- vs. high-sodium cohort. Interestingly, this was dependent on sex, as potassium excretion in men was 103 ± 4.3 and 76 ± 5 mmol in the low- and high-sodium cohorts, respectively (P < 0.001). Potassium excretion in women was 77.5 ± 6 and 77.4 ± 5 mmol in the low- and high-sodium cohorts (P = 0.99; P = 0.02, sex-by-sodium interaction).

For all subjects in the low-sodium cohort, ACh increased FBF from 2.8 ± 0.2 at baseline to 19.5 ± 1.9 ml/100 ml forearm tissue

\[ \text{ACh increased FBF from 3.2} \]

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\[ \text{ACh increased FBF from 4.3} \]

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Low Sodium (n = 30)</th>
<th>High Sodium (n = 36)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, F/M</td>
<td>14/16</td>
<td>23/13</td>
<td>0.2</td>
</tr>
<tr>
<td>Age</td>
<td>30 ± 1</td>
<td>23 ± 1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>23 ± 0.5</td>
<td>24 ± 0.5</td>
<td>0.6</td>
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<tr>
<td>Height, cm</td>
<td>173 ± 2</td>
<td>174 ± 1</td>
<td>0.9</td>
</tr>
<tr>
<td>Screening wt, kg</td>
<td>70 ± 2</td>
<td>72 ± 2</td>
<td>0.6</td>
</tr>
<tr>
<td>Δwt, kg</td>
<td>–0.2 ± 0.1</td>
<td>0.5 ± 0.3</td>
<td>0.01</td>
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<tr>
<td>Screening HR, beats/min</td>
<td>67 ± 1</td>
<td>68 ± 2</td>
<td>0.5</td>
</tr>
<tr>
<td>Δ HR, beats/min</td>
<td>0.7 ± 2</td>
<td>–0.6 ± 0.2</td>
<td>0.7</td>
</tr>
<tr>
<td>Screening SBP, mmHg</td>
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<td>121 ± 2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Δ SBP, mmHg</td>
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<td>–4 ± 2</td>
<td>0.5</td>
</tr>
<tr>
<td>Screening DBP, mmHg</td>
<td>70 ± 1</td>
<td>67 ± 1</td>
<td>0.1</td>
</tr>
<tr>
<td>Δ DBP, mmHg</td>
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<td>0.4</td>
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<tr>
<td>Screening MAP, mmHg</td>
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<tr>
<td>Δ MAP, mmHg</td>
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<td>0.4</td>
</tr>
<tr>
<td>24-h urine volume, ml</td>
<td>1841 ± 128</td>
<td>2535 ± 291</td>
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<td>24-h Na⁺ excretion, mmol</td>
<td>31 ± 3</td>
<td>311 ± 18</td>
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<tr>
<td>24-h K⁺ excretion, mmol</td>
<td>91 ± 4</td>
<td>77 ± 4</td>
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</tr>
<tr>
<td>24-h Cr excretion, mmol</td>
<td>1169 ± 83</td>
<td>1538 ± 105</td>
<td>0.12</td>
</tr>
</tbody>
</table>

Values are means ± SE. Hemodynamic values were recorded during a screening visit prior to beginning the 5-day diet. Following the diet, the changes in vital signs from the screen visit are listed as Δ. Urinary indexes were obtained from a 24-h urine collection on the final day of the diets. BMI, body mass index; HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure.

\[ \text{Table 1. Subject characteristics} \]
respectively (main effect of sodium, \(P = 0.019\)). However, the sodium effect was larger for the men, with values of 7.9 ± 2.0 and 2.2 ± 1.4 for men vs. 3.1 ± 1.3 and 2.7 ± 1.0 ml·100 ml forearm tissue\(^{-1}\)·min\(^{-1}\) for the women (\(P = 0.034\), sex-by-sodium interaction). As shown in Fig. 2, for men in the low-sodium condition, \(\text{L-NMMA}\) caused a significant blunting in the vasodilator response to \(\text{ACh}\) compared with men in the high-sodium diet, or compared with women in either diet. Finally, infusions of \(\text{ACh}\) and \(\text{L-NMMA}\) into the brachial artery did not evoke a systemic effect on hemodynamics, as HR and BP were unaffected by the forearm infusions in either dietary sodium group (data not shown).

For endothelium-independent vasodilation in the low-sodium cohort, NTP increased FBF from 2.1 ± 0.1 to 15.1 ± 0.9; after \(\text{L-NMMA}\), NTP increased FBF from 2.1 ± 0.1 to 15.9 ± 0.9 ml·100 ml forearm tissue\(^{-1}\)·min\(^{-1}\). In the high sodium cohort NTP increased FBF from 2.2 ± 0.2 to 15.8 ± 0.8; after \(\text{L-NMMA}\) NTP increased FBF from 1.7 ± 0.1 to 16.2 ± 0.9 ml·100 ml forearm tissue\(^{-1}\)·min\(^{-1}\) (\(P < 0.001\) for all). There was no evidence to suggest that the responses to NTP were dependent on dietary sodium or sex.

**DISCUSSION**

This is the first report to compare endothelium-dependent vasodilation in healthy normotensive young men and women following a controlled 5-day diet consisting of either low or high dietary sodium. There were three major findings. First, the significant vasodilator response to \(\text{ACh}\) (before eNOS inhibition with \(\text{L-NMMA}\)) was not affected by dietary sodium in either sex. Second, following inhibition of eNOS, the magnitude of the reduction in the vasodilator response to \(\text{ACh}\) was significantly affected by diet in the men but not the women. Compared with the low-sodium diet, high dietary sodium reduced the ability of \(\text{L-NMMA}\) to blunt the vasodilator response, indicating that the NO component of \(\text{ACh}\)-mediated vasodilation was reduced after high sodium intake in the men. Finally, there were no sex differences in the endothelial NO
component of vasodilation after high sodium intake. Therefore, the overall vasodilator response to ACh is not affected by extremes of dietary sodium in this healthy population, but there are sex differences in the mechanisms responsible for how vasodilation is achieved that are influenced by dietary sodium.

Endothelium-dependent vasodilation involves several mechanisms. ACh evokes increases in endothelial generation of NO, EDHF, and PGI2, and each component is likely modulated by dietary sodium (17). In male Sprague-Dawley rats fed a high-salt diet (8%) for 4 wk vs. those fed a low-salt diet (0.4%), mesenteric vessels displayed a similar vasodilator response to ACh but the mechanism appeared to shift from endothelium-derived NO in the low-salt condition to endothelium-derived EDHF in the high-salt condition (24). Moreover, in mice lacking the PGI2 receptor gene, sodium loading increased blood pressure and increased the urinary excretion of prostacyclin metabolites (29). Taken together, these animal models suggest that sodium loading causes a reduction in endothelial NO with a compensatory increase in EDHF and PGI2, which appears consistent with our findings in men.

A recent randomized double-blind crossover study of 16 healthy normotensive young men receiving a 5-day course of placebo or salt tablets (200 mmol/day) demonstrated that the forearm vasodilator response to brachial arterial administration of ACh was blunted after salt loading; moreover, the vasoconstrictor response to incremental doses of L-NMMA was less pronounced after salt loading, indicating a reduction in endothelial levels of NO (27). Our data are consistent with a reduction in endothelial NO, but we were unable to demonstrate an effect of dietary sodium on the vasodilator response to ACh (before eNOS inhibition), which may be due to protocol differences such as controlled dietary potassium intake (19), and may also represent the aforementioned redundant components of ACh-evoked vasodilation.

Sex differences in our findings may be interpreted in several ways. First, men may have a greater capacity to increase NO bioavailability during sodium restriction compared with high sodium intake, suggesting that improvements in endothelial NO from dietary sodium restriction may be achieved to a greater extent in men than women. Second, endothelial NO in healthy women may be less responsive to extremes of dietary sodium intake. Taken together, this would suggest that women have alternative means of endothelium-dependent vasodilation that are less dependent on NO compared with men. Indeed, serum NO metabolite levels are reportedly greater in young healthy men than women (10), supporting the idea of sex differences in NO regulation.

An additional interpretation may relate to the sex differences in potassium excretion. Dietary sodium restriction activates the renin-angiotensin system, which increases potassium excretion compared with sodium loading (22). This effect was seen in men only, with a significantly elevated potassium excretion during low sodium compared with men in high sodium or in women of either dietary cohort. Normotensive men have higher renin activity than normotensive women at any age (16). Whether potassium balance directly affected endothelial NO in men during low sodium is speculative, as linear regression analysis revealed no suggestive correlation between urinary potassium excretion and the NO component of endothelium-dependent vasodilation.

The Dietary Approaches to Stop Hypertension (DASH) eating plan includes a recommended daily sodium intake of 1.5 to 2.3 g per day. Recently, Hodson and colleagues administered a 30-day DASH diet to 14 middle-aged adults (8 men, 6 women) and found a reduction in BP but no change in brachial artery flow-mediated dilation compared with matched controls (14). While that study may have been underpowered to detect a diet effect flow-mediated dilation (FMD) in both sexes, our findings suggest that exploration of a sex-by-sodium interaction specifically targeting the contribution of NO to vasodilation may be necessary to provide mechanistic insight into the reduction in BP following reduced dietary sodium regimens such as DASH. Importantly, in our cohorts resting BP was not changed after sodium loading or restriction, an effect that was likely due to the shorter duration of the study diet or the notion that subjects were young and free of disorders or lifestyle habits that affect cardiovascular and renal function. Furthermore, potassium supplementation to levels similar to what we provided our subjects has been shown to essentially eliminate salt sensitivity of blood pressure in normotensive black and white men (19). Conversely, a low-sodium diet improves markers of endothelial function in normotensive overweight and obese normotensive adults (4) and hypertensive adults (15), whereas high-sodium diet impairs vascular function (pulse wave velocity) in hypertensive adults (26). Few if any studies have addressed sex differences in these physiological markers.

The limitations of this report center on the comparison of data obtained in a present cohort (high sodium) with data from a separate cohort (low sodium) using similar methodology except for dietary sodium intake. However, the present findings clearly raise important questions about the interaction of dietary sodium, sex, and endothelial function that warrant detailed, prospective trials within individuals of both sexes to further elucidate how dietary sodium alters vascular function in humans.

Conclusions. In summary, this is the first report of a sex-by-dietary sodium interaction with regard to the NO component of forearm vasodilator mechanisms in healthy young men and women. From a population standpoint, men have higher blood pressure than age-matched premenopausal women, putting men at greater risk of cardiovascular and renal disease earlier in life. Furthermore, blood pressure in young normotensive women is generally less sensitive to salt (21). In this context, our findings raise important new questions about sex differences in salt sensitivity and vascular phenotypes in healthy young adults.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).
AUTHOR CONTRIBUTIONS


REFERENCES