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Salt loading has a more deleterious effect on flow-mediated dilation in salt-resistant men than women

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Abstract *Background and aims:* Dietary sodium loading has been shown to adversely impact endothelial function independently of blood pressure (BP). However, it is unknown whether dietary sodium loading impacts endothelial function differently in men as compared to women. The aim of this study was to test the hypothesis that endothelial-dependent dilation (EDD) would be lower in men as compared to women in response to a high sodium diet.
Methods and results: Thirty subjects (14F, 31±2y; 16M, 29±2y) underwent a randomized, crossover, controlled diet study consisting of 7 days of low sodium (LS; 20 mmol/day) and 7 days of high sodium (HS; 300–350 mmol/day). Salt-resistance was determined by a change in 24-hr mean arterial pressure (MAP) $\leq$ 5 mm Hg between HS and LS as assessed on day 7 of each diet. Blood and 24-hr urine were also collected and EDD was assessed by brachial artery flow-mediated dilation (FMD). By design, MAP was not different between LS and HS conditions and urinary sodium excretion increased on HS diet ($P < 0.01$). FMD did not differ between men and women on the LS diet (10.2 ± 0.65 vs. 10.7 ± 0.83; $P > 0.05$) and declined in both men and women on HS ($P < 0.001$). However, FMD was lower in men as compared to women on HS (5.7 ± 0.5 vs. 8.6 ± 0.86; $P < 0.01$).
Conclusions: HS reduced FMD in both men and women. In response to an HS diet, FMD was lower in men compared to women suggesting a greater sensitivity of the vasculature to high sodium in men.

Introduction

High dietary sodium consumption is a significant risk factor for the development of hypertension. While many key dietary trials have shown the benefit of sodium reduction on blood pressure (BP) levels [1], more recent work has illustrated the detrimental effects of sodium on the vasculature prior to, or independent of, any change in blood pressure [2–4]. Endothelial dysfunction is thought to be an initial event in the development of atherosclerosis and has been associated with an increased risk of cardiovascular disease [5,6]. Brachial artery flow-mediated dilation (FMD) is a non-invasive research tool used to assess endothelial function [7] and has been shown to be useful in predicting future cardiovascular events [8].

Changes in sodium consumption have been shown to alter endothelial-dependent dilation (EDD) in several populations. Reduction of sodium consumption (50 mmol/

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day) from the typical U.S. intake (150 mmol/day) improved EDD as assessed by FMD in healthy overweight and obese adults with a concurrent reduction in systolic BP [9]. Further, a cross-sectional study on middle-aged and older adults with elevated systolic BP who followed a low sodium diet showed an improvement in FMD [10]. While these studies examined changes in vascular function, BP was noted to change as well. We recently demonstrated that a high sodium diet can impair FMD in healthy salt-resistant adults independent of BP changes [2]. To date, very few studies have studied the impact of salt loading on endothelial function in normotensive salt-resistant adults independent of changes in BP.

While salt loading has been shown to impair FMD, it is not clear if there are sex differences in this response. It is widely known that cardiovascular disease related morbidity and mortality are lower in women than men prior to menopause [11]. He et al. [12] reported sex differences in BP in response to a 7-day high salt intervention in which women illustrated a greater BP sensitivity to the salt loading particularly with aging and in the presence of hypertension. Whether these sex differences apply to the vasculature has not been extensively studied. To date, one study has suggested that healthy young males appear to be more sensitive to sodium loading as sex differences were reported in the contribution of nitric oxide to acetylcholine induced vasodilation in the forearm however no functional difference was found [4].

Therefore, the purpose of the current study was to determine if sex differences exist in the endothelial response to dietary sodium loading in young salt-resistant men and women in a randomized crossover controlled feeding study. We hypothesized that men would have a lower FMD in response to a high sodium diet compared to women.

Methods

Study population

Forty-three subjects qualified for the study. Five dropped out following the screening, 5 subjects started the diet but dropped out before completion, and 3 were salt sensitive. Therefore, thirty healthy salt-resistant individuals aged 22–44 participated in this study: 14 women and 16 men. Four women and six men participated in a previous study conducted in our laboratory [2]; their data were reanalyzed using the methods described in the *'Statistical analysis'* section below to address the novel hypotheses of this study. The study protocol and procedures were approved by the Institutional Review Board of the University of Delaware and conform to the provisions of the Declaration of Helsinki. Informed consent was obtained from all participants prior to enrollment in the study.

Experimental protocol

Subjects reported to the laboratory for an initial screening visit after a 12-h fast and completed a medical history form. A resting 12-lead electrocardiogram, resting BP, and height and weight were determined. A venous blood sample was collected. Because the focus of this study was healthy adults, subjects with a history of cardiovascular disease, hypertension, malignancy, diabetes mellitus, or renal impairment were excluded. Subjects with a BMI of greater than 30 kg/m^2, use of tobacco products, and those taking any type of medication were also excluded. Women were required to have a negative pregnancy test but menstrual cycle was not controlled for. Menopausal women were excluded from the study as salt sensitivity of BP increases with menopause and endothelial function declines [13].

Dietary sodium manipulation

This experiment was a controlled feeding study with all food prepared by a registered dietitian. Participants first completed a 7-day run in diet (100 mmol sodium/day; 2300 mg/day) in order to normalize baseline dietary sodium intake. Following this, subjects were randomized to undergo 7 days of a low-sodium (LS) diet (20 mmol sodium/day; 500 mg/day) and 7 days of a high-sodium [14] diet (300–350 mmol sodium/day; ~7000–8000 mg/day) with no washout period in between. These sodium intakes were selected in order to allow us to accurately classify adults with salt-resistant BP and are consistent with previously published studies [2,4]. Standardized equations was used to adjust the caloric content of the diet to maintain a constant body weight. In all conditions, dietary potassium intake was held constant and averaged 75.4 ± 1.5 mmol/day. The diet consisted of 50% carbohydrates, 30% fat, and 20% protein. Daily fluid intake was monitored and recorded and subjects were instructed to maintain normal activity levels throughout the study.

During the last 24-h period of the LS and HS diets all urine was collected and analyzed for total volume, urinary electrolytes (Easy-Electrolyte Analyzer; Medica) and urine osmolality (Advanced 3D3 Osmometer; Advanced Instruments). Free water clearance and fractional excretion of sodium and chloride were calculated using standard equations. During the same 24-h period, subjects wore an ambulatory BP monitor (Spacelabs Medical) on their arm. BP was measured every 20 min while the subject was awake and every 30 min during sleep. Laboratory BP was also measured by an automated oscillometric sphygmomanometer (Dinamap Dash 2000; GE Medical Systems) during experimental visits.

Hemoglobin (Hb 201 + model; HemoCue), hematocrit (Readacrit Centrifuge; Becton Dickinson), serum electrolytes (EasyElectrolyte Analyzer; Medica), and plasma osmolality (Advanced 3D3 Osmometer; Advanced Instruments) were measured from a venous blood sample obtained during each experimental visit.

Salt resistance classification

Salt resistance was defined as a 5 mm Hg or less change in 24-h MAP determined while on the LS and HS diets [15]