High dietary sodium reduces brachial artery flow-mediated dilation in humans with salt-sensitive and salt-resistant blood pressure

Evan L. Matthews,1 Michael S. Brian,1 Meghan G. Ramick,1 Shannon Lennon-Edwards,1,2 David G. Edwards,1* and William B. Farquhar1*

1Department of Kinesiology and Applied Physiology, University of Delaware, Newark, Delaware; and 2Department of Behavioral Health and Nutrition, University of Delaware, Newark, Delaware

Submitted 7 January 2015; accepted in final form 31 March 2015

Matthews EL, Brian MS, Ramick MG, Lennon-Edwards S, Edwards DG, Farquhar WB. High dietary sodium reduces brachial artery flow-mediated dilation in humans with salt-sensitive and salt-resistant blood pressure. J Appl Physiol 118: 1510–1515, 2015. First published April 2, 2015; doi:10.1152/japplphysiol.00023.2015.—Recent studies demonstrate that high dietary sodium (HS) impairs endothelial function in those with salt-resistant (SR) blood pressure (BP). The effect of HS on endothelial function in those with salt-sensitive (SS) BP is not currently known. We hypothesized that HS would impair brachial artery flow-mediated dilation (FMD) to a greater extent in SS compared with SR adults. Ten SR (age 42 ± 5 yr, 5 men, 5 women) and 10 SS (age 39 ± 5 yr, 5 men, 5 women) healthy, normotensive participants were enrolled in a controlled feeding study consisting of a run-in diet followed by a 7-d low dietary sodium (LS) (20 mmol/day) and a 7-d HS (300 mmol/day) diet in random order. Brachial artery FMD and 24-h BP were assessed on the last day of each diet. SS BP was individually assessed and defined as a change in 24-h mean arterial pressure (MAP) of >5 mmHg between the LS and HS diets (MAP: SS BP 0.6 ± 1.2, SS LS 7.7 ± 0.4 mmHg). Brachial artery FMD was lower in both SS and SR individuals during the HS diet (P < 0.001), and did not differ between groups (P > 0.05) (FMD: SS LS 10.6 ± 1.3%, SS HS 7.2 ± 1.5%, SS LS 12.5 ± 1.7%, SS HS 7.8 ± 1.4%). These data indicate that an HS diet impairs brachial artery FMD to a similar extent in adults with SS BP and SR BP.

SODIUM INTAKE IN INDUSTRIALIZED NATIONS is in excess of recommendations (2) and is an independent risk factor for cardiovascular disease (35). Understanding how excess dietary sodium affects the cardiovascular system has wide-reaching implications for public health because cardiovascular disease accounts for about one-third of all deaths in the United States (25). Traditionally, the link between sodium consumption and cardiovascular disease has been thought to be related to the sodium-induced increase in blood pressure (BP). However, BP responds heterogeneously to a dietary sodium manipulation; individuals who have a large change in BP in response to a sodium manipulation are salt-sensitive (SS). Those who have little or no change in BP are salt-resistant (SR). Approximately 25% of young to middle-aged normotensive adults are SS (37). Salt sensitivity among normotensive adults is clinically relevant because it predicts future hypertension risk and has been linked to increased mortality (1, 26, 33). A 27-yr longitudinal study found that SS normotensive individuals had poorer survival rates than SR normotensive individuals (36). Additionally, when those who had died of cardiovascular disease were compared with those who had died of other causes or were still alive, the group whose death was due to cardiovascular disease was more likely to be SS. Given the link between endothelial dysfunction and cardiovascular disease (15), the study of vascular function in SS adults is warranted.

Our laboratory has previously demonstrated that high dietary sodium (HS) impairs brachial artery flow-mediated dilation (FMD), an index of endothelial function, in SR normotensive individuals (8). These results demonstrate that HS negatively affects vascular function independent of BP, because endothelial function declined in the absence of an acute increase in BP, or hypertension. Findings such as these are clinically relevant because vascular endothelial dysfunction occurs before arterial wall structural changes and is believed to be a primary event in the development of atherosclerosis (15).

Although there is evidence that elevated dietary sodium can impair endothelial function in SR adults (8), it is not clear whether the magnitude of the decline is similar or greater in SS adults. Because of the worse cardiovascular outcomes in SS adults (36), we speculated that dietary sodium may have more deleterious effects on the vasculature in SS compared with SR adults. In addition, the acute increase in BP associated with sodium loading in SS adults may further decrease endothelial function beyond the direct effects of dietary sodium. Indeed, normotensive adults subjected to an acute increase in limb BP (∼15 mmHg) via vertically hanging the arm below the plane of the body for 3 h demonstrate a lower brachial artery FMD (30). Accordingly, the purpose of this study was to examine the effect of HS on brachial artery FMD in normotensive SS and SR adults; our hypothesis was that HS would lead to a greater decline in brachial artery FMD in SS compared with SR normotensive adults. To test this hypothesis, salt sensitivity was individually assessed to classify adults as SS or SR, and brachial artery FMD was measured under low dietary sodium (LS) and HS conditions.

METHODS

Participants. All procedures and protocols employed conformed with the Declaration of Helsinki of 1975 (as revised in 1983), and were approved by the University of Delaware Institutional Review Board. All participants signed a written, informed consent before participating. Forty-one healthy normotensive adults completed the study; none of whom had been reported in the previous analysis examining SR BP and brachial artery FMD (8). Ten of these participants were SS. Ten SR participants matched for sex and race with similar ages were included in this analysis. The remaining SR participants displayed similar responses to vascular tests but are not presented in this manuscript to maintain group similarity.

* D. G. Edwards and W. B. Farquhar contributed equally to this work.
The screening visit included completion of a 12-lead electrocardiogram; medical history; resting BP; height and weight measurements; and a 24-h fasted venous blood sample for assessment of hemoglobin, hematocrit, electrolytes, renal function, and lipid levels. All individuals were free of cardiovascular disease, hypertension, diabetes mellitus, pulmonary disease, neurological disease, cancer, and renal impairment. Additional exclusion criteria included the use of tobacco or nicotine products and body mass index >30 kg/m².

**Dietary sodium perturbation.** Details on dietary sodium perturbation have been published previously (8). A controlled feeding study was employed to assess salt sensitivity and to establish LS and HS conditions for brachial artery FMD testing. A registered dietitian prepared all food and designed all the diets to contain 50% carbohydrate, 30% fat, and 20% protein. The diet phases were isocaloric and adjusted using the Mifflin-St Jeor equation (9). All participants began with a 7-day diet of 100 mmol sodium/day as a standardization period. This was immediately followed by a two-phase, randomized crossover 7-day dietary sodium manipulation: LS (20 mmol/day) and HS (300 mmol/day). Participants were instructed to maintain normal activity levels, to drink water ad libitum, and maintain a fluid log.

**Salt sensitivity classification.** Twenty-four-hour ambulatory BP assessments were performed on the final day of each phase (model 90207; Spacelabs Medical, Issaquah, WA). The ambulatory monitor was programmed to measure BP every 20 min during waking hours and every 30 min during sleep. Participants with an increase in 24-h ambulatory mean arterial pressure (MAP) of >5 mmHg from the LS to the HS were classified as SS (8, 11, 32). Conversely, those with an increase in 24-h ambulatory MAP on the HS of ≤5 mmHg were classified as SR. The classification of salt sensitivity has been shown to be reproducible (13, 38).

**Twenty-four-hour urine.** Twenty-four-hour urine was collected and assed on the final day of each phase. Urine volume, collection time, electrolytes (Easy Electrolyte Analyzer; Medica, Bedford, MA), and osmolality (3D3 Osmometer; Advanced Instruments, Norwood, MA) were measured. Free water clearance and sodium, chloride, and potassium excretion were determined.

**Blood analysis.** Venous blood samples were obtained at each visit and used to determine hemoglobin (Hb 201 + model; HemoCue, Lake Forest, CA), hematocrit (Clay Adams Brand, Readacrit Centrifuge; Becton Dickinson, Sparks, MD), serum electrolytes (Easy Electrolyte Analyzer; Medica, Bedford, MA), and plasma osmolality (3D3 Osmometer; Advanced Instruments, Erkrath, Germany). Plasma renin activity (PRA) and serum aldosterone were measured via radioimmunoassay at Wake Forest University Baptist Medical Center. The interassay and intra-assay coefficients of variation (CVs) were as follows: PRA, interassay, 14.8% CV for a mean of 25 ng/dl; intra-assay, 10.0% CV for a mean of 1.6 ng·ml⁻¹·h⁻¹; aldosterone, interassay precision of 9.0% CV for a mean of 35 ng/dl, and intra-assay precision of 4.0% CV for a mean of 25 ng/dl.

**Brachial artery FMD assessment.** Previously established guidelines for assessing endothelial function as assessed by brachial artery FMD were followed (5, 34). Brachial artery FMD testing was performed at the same time of day ±1 h on the final day of both the LS and HS conditions. Participants fasted for at least 4 h, and remained supine for at least 15 min before testing. Participants refrained from using over-the-counter medications, alcohol, caffeine, and exercising for 12 h before the study. The right arm was extended perpendicular to the body at heart level. An occlusion cuff connected to a rapid cuff inflator (AG101 Rapid Cuff Inflator; Hokanson, Bellevue, WA) was place on the forearm ~3 cm from the antecubital crease. A 12-MHz linear phased-array ultrasound transducer (GE P5; Healthcare, Waukesha, WI) was used to acquire longitudinal images of the brachial artery and continuous Doppler blood velocity. Following baseline acquisition, the cuff was inflated to 200 mmHg for 5 min. Data recording continued through the inflation period and ended 2 min following cuff deflation.

An S-Video connection between the ultrasound and a recording computer transferred images to a National Instruments MAQ PCI-1411 image acquisition board at 30 frames/s. Brachial artery diameter and blood velocity were determined from the saved video using automated edge detection software in National Instruments LabVIEW version 13.0. A 3s-wide median filter was applied to each diameter data point prior to assessing baseline and post cuff deflation peak diameter. Our laboratory has previously found reproducibility of this technique to have a CV of 1.3 ± 1.1% and 1.9 ± 1.6% for baseline and peak diameters, respectively (8). Brachial artery FMD was calculated as a percent change from baseline to peak diameter. Shear rate was calculated from Doppler data as 4Vpeak·Vessel diameter¹⁻², where Vpeak = centerline velocity.

**Statistical analysis.** Baseline group (SS vs. SR) characteristics were compared using a two-tailed, unpaired t-test. A univariate repeated-measures ANOVA (group × diet phase) was used to assess differences in urinary and blood markers and brachial artery FMD. A continuous shear rate sequence was constructed for the calculation of area under the curve (AUC) (31). Shear rate AUC was determined from cuff deflation to peak brachial artery diameter (31) via shear rate/sampling frequency, and analyzed with a univariate repeated-measures ANOVA. Tukey’s post hoc comparisons were made when appropriate. Data are reported as means ± SE with the alpha level set at 0.05. Statistics were performed using SigmaPlot 11.0 (Systat Software, Erkrath, Germany).

### RESULTS

Table 1 displays baseline demographic and biochemical characteristics obtained at the screening visit during participants’ habitual sodium intake. By design, SS and SR participants were similar in sex, race, and age. Fasting triglycerides...
were greater in SR than in SS participants, but did not correlate with brachial artery FMD ($P > 0.05$). All other demographic and biochemical parameters were similar between groups.

**Dietary sodium perturbation.** HS resulted in increased body mass and decreased hemoglobin and hematocrit compared with LS ($P < 0.05$ for all; see Table 2), likely due to fluid retention. Serum sodium and plasma osmolality were higher during HS compared with LS (both $P < 0.05$). Plasma renin activity and serum aldosterone were suppressed during HS in both groups (both $P < 0.05$). Adherence to the study diet was confirmed as 24-h urinary sodium excretion increased ($P < 0.001$) as expected during the HS phase without differences between groups ($P > 0.05$) (see Table 2). Increased urine flow rate ($P < 0.001$) during the HS phase occurred without changes in urine osmolality ($P > 0.05$) or free water clearance rates ($P > 0.05$) (see Table 2). By design, all 24-h measures of BP increased ($P < 0.05$) (see Table 2). Increased urine flow rate ($P < 0.001$) or free water clearance rates ($P < 0.05$). Plasma renin activity and serum aldosterone were suppressed during HS in both groups (both $P < 0.05$). Adherence to the study diet was confirmed as 24-h urinary sodium excretion increased ($P < 0.001$) as expected during the HS phase without differences between groups ($P > 0.05$) (see Table 2). Increased urine flow rate ($P < 0.001$) during the HS phase occurred without changes in urine osmolality ($P > 0.05$) or free water clearance rates ($P > 0.05$) (see Table 2). By design, all 24-h measures of BP increased ($P < 0.05$) (see Table 2).

**Vascular function.** Baseline brachial artery diameter did not differ between groups or diets (see Table 3). Brachial artery FMD was lower in both SS and SR groups during the HS phase ($P < 0.001$), and did not differ between groups ($P > 0.05$) (SR LS 10.6 ± 1.3%Δ, SS LS 12.5 ± 1.7%Δ, SS HS 7.8 ± 1.4%Δ) (see Fig. 1). The shear stimulus for dilation as indexed by the shear rate AUC did not differ between groups or diets (see Table 3).

**DISCUSSION**

The major finding of the current study is that a HS intervention impaired brachial artery FMD to a similar degree in both SS and SR normotensive adults. These results support the idea that excess dietary sodium has adverse effects on the vasculature, independent of BP. Documenting the adverse BP-independent effects of dietary sodium (8) has public health implications because the majority of studies tend to focus on the relationship between dietary sodium and hypertension, rather than between dietary sodium and vascular health. Elevated BP (25) and reduced endothelial function (12) are both associated with an increased risk of cardiovascular events; therefore, a concomitant increase in BP and decline in vascular function as a result of HS intake may be important in the poor cardiovascular outcomes observed in SS adults.

Several methodological aspects of this study strengthen these findings. These include 1) the use of a controlled feeding design in which all foods were prepared, 2) the use of a crossover, randomized design for optimal within-participant...
comparisons between diets, 3) individual assessment of salt sensitivity using 24-h ambulatory BP, and 4) the use of SS and SR adults who were comparable in age, sex, and race. We chose to focus on normotensive adults rather than hypertensive adults because several recent rodent studies (10, 19, 20, 27, 39) and human studies (8, 11, 21) suggest that dietary sodium has adverse vascular effects even in the absence of hypertension. Studying the salt sensitivity of BP is clinically relevant because this trait has been associated with worse cardiovascular outcomes (36).

Endothelial dysfunction is believed to be a primary event in the development of atherosclerosis and occurs prior to structural changes to the arterial wall (15). The endothelium is an active autocrine, paracrine, and endocrine tissue that releases substances related to the control of vasoconstriction, vasodilation, thrombogenesis, and inflammation (14). Nitric oxide (NO) is one of the key substances released by the endothelium that is involved in regulating the aforementioned processes. Endothelial dysfunction is often associated with a lack of NO bioavailability. The current study focused on the brachial artery using the predominantly NO-mediated technique of FMD, which is an established index of endothelial function (5), and relates to both current risk factors (4) and future cardiovascular events (12).

Evidence of impaired endothelial function in SS hypertensive (3) and SS normotensive (23) adults during habitual sodium intake has been demonstrated. However, these studies did not examine endothelial function during controlled dietary sodium conditions, thereby making it difficult to draw comparisons with the present data. We did not assess brachial artery FMD during habitual conditions due to the high variability of sodium intake (16), and because this would not allow for assessment of salt sensitivity. Therefore, although information on habitual values is important, our study focused on the effects of a dietary sodium perturbation utilizing a within-participant design to optimize the LS vs. HS comparison.

Salt sensitivity and its effect on endothelial function during HS and LS conditions in humans has been examined in a group of Japanese hypertensive men (24). Forearm blood flow responses to arterial infusion of acetylcholine, a stimulus for NO synthesis, was examined. The researchers found that men in the SS group had impaired dilation to acetylcholine infusion on both diets. Our brachial artery FMD data are not consistent with these findings and may be due to the difference in clinical populations studied or differences in the size of vessels studied.

The physiological rationale underlying our hypothesis was based on the finding that acute increases in BP cause a decline in brachial artery FMD (30). Thus we hypothesized that the combination of HS with an increase in BP (i.e., a SS response) would lead to a greater decline in brachial artery FMD compared with HS alone. Because this was not the case, we conclude that the magnitude of the dietary sodium-induced decline in brachial artery FMD overwhelmed any potential effect that may occur as a result of an increase in BP. Sodium restriction has been shown to improve endothelial function. Jablonski et al. (17) found that 4 wk of a reduced dietary sodium diet reversed decrements in endothelial function in middle-aged/older adults with elevated systolic blood pressure. Although BP declined in this study, the dietary sodium-induced improvements in brachial artery FMD and acetylcholine-induced vasodilation were still evident after statistically controlling for BP. Similar to the findings of Jablonski et al. (17), a reduction in salt intake of 3 g/day improved brachial artery FMD in normotensive overweight and obese participants by 1.5% after 2 days and 2.1% after 6 wk (6). The authors found the improvement in brachial artery FMD was independent of changes in BP. On the basis of findings by others (6, 17) and our current and previous results with SR adults (8), it is likely that dietary sodium, independent of BP, can directly alter endothelial function.

Although we did not assess the contribution of altered NO bioavailability to the observed changes in brachial artery FMD, NO synthesis and bioavailability is critical for normal arterial function and largely mediates FMD responses (7, 34). Cell culture studies found that increasing the sodium content of the cell culture medium within the physiological range decreased NO synthase activity by >25% in bovine aortic endothelial cells (22) and increased the stiffness of human umbilical vein endothelial cells leading to decreased shear-induced NO production (29). In the current study, both SS and SR adults demonstrated a significant increase in serum sodium in response to HS (see Table 2), suggesting that an increase in endothelial cell stiffness and decreased shear-induced NO production may be at least partially responsible for the current findings. Additionally, rodent models suggest that HS leads to increased reactive oxygen species resulting in decreased NO production and/or availability via an oxidative stress mechanism (20, 27, 28, 40). Using demographically similar participants and the same dietary sodium protocol, our laboratory has previously observed impaired cutaneous microvascular function in normotensive SR adults in response to HS (11). In this previous study, we found that microvascular function during HS was improved by local ascorbic acid administration, a potent antioxidant (11), suggesting that oxidative stress plays a role in high sodium-induced reductions in vascular function.

Assessing differences in brachial artery FMD in SR adults in the present study provided us with an opportunity to determine the reproducibility of our previous findings of dietary sodium-induced declines in brachial artery FMD in SR adults (8). The SR adults recruited for this study did not participate in the previous study, but were demographically similar. This sug-
gests that the finding of dietary sodium-induced declines in brachial artery FMD in SR adults is reproducible.

A limitation to the current study is that we did not measure endothelial-independent dilation to assess smooth muscle function. Indeed, 6 wk of dietary sodium restriction has been shown to improve endothelial-independent dilation to sublingual nitroglycerin administration in overweight and obese normotensive adults (6). However, it appears that smooth muscle effects of sodium require extended periods to develop because two studies—a 4-wk dietary sodium restriction (17) and, in our laboratory, a randomized 1-wk LS and HS intake (8)—did not find a sodium effect on endothelial-independent dilation. Although all participants consumed the recommended daily intake of sodium (100 mmol/day) for 1 wk before initiating the randomized diet, the level of dietary sodium used in the current study for HS (300 mmol/day) is higher than both recommended (100 mmol/day) and typical [148 mmol/day (18)] levels of sodium consumption in the United States. Likewise, the LS diet (20 mmol/day) used in the current study is lower than both the recommended and typical levels of sodium consumption.

Regardless, this study demonstrates that a large change in dietary sodium has a strong effect over a short period of time. Others (6) have shown that a relatively small decrease in dietary sodium over a longer period of time results in an improvement in brachial artery FMD. Examining the effects on endothelial function of dietary sodium across more clinically translatable intake levels in SS vs. SR adults warrants future study. Although brachial artery FMD is a test used to assess NO-mediated vasodilation, a limitation of the current study is the lack of a direct measure of NO.

Conclusions. The results of the present study demonstrate that HS decreases brachial artery FMD in both SR and SS normotensive adults, and that the decrease occurs similarly between the groups. This suggests a deleterious effect of HS on endothelial function that occurs regardless of BP salt sensitivity classification. Therefore, the deleterious effect of HS on endothelial function is independent of its potential effects on BP.

ACKNOWLEDGMENTS

We thank Dr. A. Prettyman, K. Masso, and the dietetics department at the Eugene du Pont Preventive Medicine & Rehabilitation Institute for assistance with this study.

GRANTS

This research was funded by National Institutes of Health Grants 2-P20-RR-016472-11 and R01-HL104106.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS


REFERENCES

6. Dickinson KM, Clifton PM, Keogh JB. A reduction of 3 g/day from a usual 9 g/day salt diet improves endothelial function and decreases endothelin-1 in a randomised cross-over study in normotensive overweight and obese subjects. Atherosclerosis 233: 32–38, 2014.

J Appl Physiol • doi:10.1152/japplphysiol.00023.2015 • www.jappl.org


