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Dietary Sodium Restriction Reverses Vascular Endothelial Dysfunction in Middle-Aged/Older Adults With Moderately Elevated Systolic Blood Pressure

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Objectives This study sought to determine the efficacy of dietary sodium restriction (DSR) for improving vascular endothelial dysfunction in middle-aged/older adults with moderately elevated systolic blood pressure (SBP) (130–159 mm Hg) and the associated physiological mechanisms.

Background Vascular endothelial dysfunction develops with advancing age and elevated SBP, contributing to increased cardiovascular risk. DSR lowers BP, but its effect on vascular endothelial function and mechanisms involved are unknown.

Methods Seventeen subjects (11 men and 6 women; mean age, 62 ± 7 years) completed a, randomized crossover study of 4 weeks of both low (DSR) and normal sodium intake. Vascular endothelial function (endothelium-dependent dilation; EDD), nitric oxide (NO)/tetrahydrobiopterin (BH_4) bioavailability, and oxidative stress-associated mechanisms were assessed following each condition.

Results Urinary sodium excretion was reduced by ~50% (to 70 ± 30 mmol/day), and conduit (brachial artery flow-mediated dilation [FMD_{BA}]) and resistance (forearm blood flow responses to acetylcholine [FBF_{ACh}]) artery EDD were 68% and 42% (peak FBF_{ACh}) higher following DSR ($p < 0.005$). Low sodium markedly enhanced NO-mediated EDD (greater ΔFBF_{ACh} with endothelial NO synthase inhibition) without changing endothelial NO synthase expression/activation (Ser 1177 phosphorylation), restored BH_4 bioactivity (less ΔFMD_{BA} with acute BH_4), abolished tonic superoxide suppression of EDD (less ΔFMD_{BA} and ΔFBF_{ACh} with ascorbic acid infusion), and increased circulating superoxide dismutase activity (all $p < 0.05$). These effects were independent of ΔSBP. Other subject characteristics/dietary factors and endothelium-independent dilation were unchanged.

Conclusions DSR largely reversed both macro- and microvascular endothelial dysfunction by enhancing NO and BH_4 bioavailability and reducing oxidative stress. Our findings support the emerging concept that DSR induces "vascular protection" beyond that attributable to its BP-lowering effects. (J Am Coll Cardiol 2013;61:335–43) © 2013 by the American College of Cardiology Foundation

Cardiovascular disease (CVD) remains the leading cause of death in the United States, and the risk for CVD increases progressively with age (1). Vascular endothelial dysfunction, as reflected by impaired endothelium-dependent dilation (EDD) of conduit arteries and/or resistance vessels (2), is a predictor of CVD and CV events (3,4). EDD is reduced in middle-aged and older adults in the absence of major risk factors or clinical CVD (5). Systolic blood pressure (SBP) also increases with advancing age (6), resulting in ~65% of adults 50 years of age and older with SBP ≥130 mm Hg (7). Importantly, the age-associated impairment of EDD is even greater in middle-aged and older adults with elevated compared with normal SBP (2,8).

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Dietary sodium restriction is a commonly recommended lifestyle modification for reducing the risk for CVD (9). Based on recent analyses, the projected health

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Abbreviations and Acronyms

BH_4 = tetrahydrobiopterin
CTRC = Clinical and Translational Research Center
CVD = cardiovascular disease
DSR = dietary sodium restriction
EDD = endothelium-dependent dilation
eNOS = endothelial nitric oxide synthase
FBF_{ACh} = forearm blood flow to acetylcholine
FMD_{BA} = brachial artery flow-mediated dilation
NO = nitric oxide
SBP = systolic blood pressure
SOD = superoxide dismutase

and societal benefits resulting from population-wide dietary sodium restriction (DSR) would be substantial (10). To date, such projections have focused on the effects of DSR on BP. However, high dietary sodium intake has adverse cardiovascular effects independent of BP (11,12). Evidence from animal models and acute salt loading or cross-sectional observations in humans suggests a negative association between sodium intake and vascular endothelial function (11,13–16). However, presently it is unknown whether DSR can reverse endothelial dysfunction in the common clinical setting of aging and elevated SBP.

The physiological mechanisms by which DSR may improve vascular endothelial function in humans are also unknown. Available data from rodent models suggest that high-sodium diets may impair EDD by reducing nitric oxide (NO) bioavailability as a result of oxidative stress (14,17). This may be mediated via oxidation and reduced bioavailability of tetrahydrobiopterin (BH_4) (18), an essential cofactor for endothelial nitric oxide synthase [eNOS]-mediated NO production, which causes uncoupling of the enzyme (14,18). Limited data in rodents suggest that high sodium also may reduce the expression of eNOS (19) and suppress the activity of superoxide dismutase (SOD), an important antioxidant enzyme (20).

The aim of this study was to determine whether DSR improves vascular endothelial dysfunction in middle-aged/older adults with moderately elevated SBP by increasing NO and BH_4 bioavailability and reducing oxidative stress.

Methods

Using a double-blind, placebo-controlled, randomized crossover design, both conduit artery (macrocirculatory) and resistance vessel (microcirculatory) EDD, as were the putative physiological mechanisms involved, were determined under well-controlled conditions of low (DSR) and normal dietary sodium intake. Vascular NO and BH_4 bioavailability and oxidative stress were assessed by "pharmaco-dissection" using agents that either inhibited or enhanced these processes, whereas eNOS protein expression and activation (phosphorylation at Ser 1177) were measured in endothelial cells obtained from arterial sampling.

The study was conducted in the University of Colorado-Boulder Clinical and Translational Research Center (CTRC), and blood and urine assays were performed at the Colorado Clinical Translational Sciences Institute CTRC Core Lab, University of Colorado–Denver Anschutz Medical Campus.

Subjects. Enrolled subjects were 50 to 79 years of age and had a SBP of 130 to 159 mm Hg (i.e., high-normal or stage I systolic hypertension), with diastolic BP <99 mm Hg, verified on a minimum of 2 occasions, as described previously (21,22). These criteria were chosen to encompass the resting BP values of the majority of middle-aged and older adults at higher BP-related risk for CVD (7,23). Enrolling subjects with high-normal and stage I hypertension also increased the likelihood of assessing adults who were more salt-sensitive at baseline, as salt sensitivity increases with increasing resting BP (24). All subjects were otherwise free of CVD, diabetes, kidney disease, and other clinical disorders. Details of inclusion and exclusion criteria can be found in the Online Appendix.

Twenty middle-aged and older men and women were enrolled in the study (see Online Figure for CONSORT diagram). Three subjects withdrew after beginning DSR (see subsequent discussion), but prior to completing the first set of vascular measurements (1 was uncomfortable with study procedures; 2 decided that they could not comply with the diet). The remaining 17 subjects (ages 51 to 77 years; 11 men and 6 post-menopausal women) completed the entire 10-week protocol. All procedures were approved by the institutional review board of the University of Colorado at Boulder. The nature, benefits, and risks of the study were explained to the volunteers and their written informed consent was obtained prior to participation.

Experimental design and dietary sodium restriction. A double-blind, placebo-controlled, randomized crossover design, originally described by Cappuccio et al. (25) and more recently by our group (21), was used. A low sodium target of 50 mmol/day (1,200 mg/day) was set, with the anticipation that actual mean intake would be ~65 mmol/day (1,500 mg/day), consistent with the DASH (Dietary Approaches to Stop Hypertension) diet (26). This was compared to the mean ("normal") U.S. sodium intake of 150 mmol/day (3,600 mg/day) based on recent National Health and Nutrition Examination Survey dietary intake data (27). During the entire 10-week intervention period, subjects were instructed to reduce their dietary sodium intake to a target of ~50 mmol/day and to take a total of 10 tablets throughout the day, with meals. For 5 of the weeks, the tablets were placebo pills, whereas for the other 5 weeks, the tablets were slow-release sodium chloride tablets (10 mmol [0.23 g] per tablet) (HK Pharma Ltd., Hitchin, United Kingdom). The slow-release salt tablets aimed to return sodium intake to the ~150 mmol/day target.

Following screening, subjects were provided with comprehensive dietary education and counseling by CTRC bionutritionists to reduce dietary sodium intake without changing caloric intake, dietary composition, or potassium intake. Vascular measurements were performed after 4 weeks (i.e., during the fifth week) of each condition. Details

of the experimental design can be found in the Online Appendix.

Blood pressure, clinical characteristics, assays, and diet analysis. Brachial artery BP was assessed under quiet resting conditions, with the subject in the supine position, using a semiautomated device (Dynamap XL, Johnson & Johnson, Raritan, New Jersey) in accordance with the Joint National Committee guidelines (28), as described previously (21). SOD activity was measured in plasma samples (n = 12 of 13) using a commercially available colorimetric kit (Cell Technology, Inc., Mountain View, California) (29). Twenty-four-hour urinary sodium and potassium excretion values were measured weekly as a quantitative maker of dietary intake (Fig. 1). CTRC bionutritionists analyzed a 3-day diet record logged by subjects at baseline and at the end of each sodium condition, as described previously (21). For details of these measurements, as well as assessments of other subject characteristics, please see the Online Appendix.

Conduit artery endothelium-dependent and -independent dilation. Brachial artery flow-mediated dilation (FMD_{BA}), estimated peak shear rate, and endothelium-independent dilation (brachial artery dilation to 0.4 mg of sublingual nitroglycerin) were determined using duplex ultrasonography (Power Vision 6000, Toshiba Corp., Tokyo, Japan) with a 7.5-MHz adjustable (6 to 11 MHz) linear array transducer, as described previously (30,31). BH_4 modulation of EDD was determined by measuring FMD_{BA} ~3 hrs after oral administration of BH_4 or placebo tablets (32) (n = 14/15), and the influence of oxidative stress on FMD_{BA} was assessed by infusing a supraphysiological dose of ascorbic acid or isovolumic saline during each of these visits (BH_4 and placebo days), as described previously (31,33) (n = 12 to 17). See the Online Appendix for details.

Resistance vessel endothelium-dependent and -independent dilation. Forearm blood flow (FBF) was measured in the experimental (catheterized) and the control forearm with strain-gauge venous occlusion plethysmography (AI6 Arterial Inflow System, D.E. Hokanson Inc., Bellevue, Washington), as previously described (n = 12 of 13) (34). All FBF values are presented as percentage increases in flow (ml/100 ml forearm tissue/min) in response to intrabrachial artery drug infusion (acetylcholine [ACh], sodium nitroprusside, ACh + *N*G monomethyl-L-arginine or ACh + ascorbic acid) compared with saline control. Forearm vascular conductance (FVC) was calculated from intra-arterial BP and FBF (FVC = FBF/mean arterial pressure) to account for any differences in arterial BP between sodium conditions. See the Online Appendix for details.

Endothelial cell protein assessments. The procedures for collection and analysis of arterial endothelial have been described in detail previously (30,31). Vascular endothelial cells were obtained from the brachial artery and analyzed for expression of total eNOS and eNOS phosphorylated at Ser 1177 using immunofluorescence imaging (n = 12/13). For details, please see the Online Appendix.

Statistics. Changes in EDD in response to dietary sodium restriction (DSR vs. normal sodium condition) were analyzed using a series of linear mixed-effects models (Online Table 1; see Online Appendix for details and power calculations). Differences in other variables were assessed using paired *t* tests (between sodium conditions) or repeated measures analysis of variance with post hoc Bonferonni corrected comparisons (sodium conditions vs. baseline). Bivariate relations were determined using the Pearson correlation coefficient. All data are reported as mean ± SD.

Results

Clinical characteristics and efficacy of DSR. Subjects were non-Hispanic Caucasian (88%) or Asian (12%) with a mean age of 62 ± 7 years (Table 1). They successfully reduced sodium intake without altering potassium intake based on both urinary excretion (sodium: 151 ± 37 mmol/day, baseline; 153 ± 27 mmol/day, normal sodium condition; 70 ± 30 mmol/day, DSR [week-5 values]) (Fig. 1) and dietary analysis (Table 2). Other than sodium intake, diet composition was unchanged (Table 2). As anticipated, SBP was reduced during DSR, but other clinical characteristics, including diastolic BP, were unaffected (Table 1). Circulating humoral factors also were unchanged (Table 3).

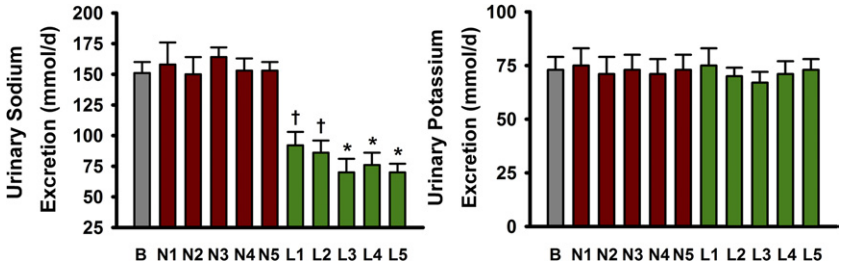

Figure 1 Twenty-Four-Hour Urinary Sodium and Potassium Excretion

The 24-h urinary excretion of sodium **(left)** and potassium **(right)** at baseline (B), weeks 1-5 of the normal sodium condition (N1 to N5) and weeks 1 to 5 of the low sodium condition (dietary sodium restriction [DSR]) (L1 to L5). Values are mean ± SE. *p < 0.001; †p < 0.005 versus baseline or normal sodium of same week (repeated measures analysis of variance with post hoc Bonferonni corrected comparisons; n = 17).

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Table 1 Clinical Characteristics

Characteristic	Baseline	Low Sodium	Normal Sodium
Mass (kg)	80.5 ± 17.5	79.3 ± 17.1	79.8 ± 17.4
Body mass index (kg/m^2)	27.1 ± 4.1	26.4 ± 4.2	26.6 ± 4.3
Systolic blood pressure (mm Hg)	138 ± 7	128 ± 10*	140 ± 15
Diastolic blood pressure (mm Hg)	83 ± 7	79 ± 6	82 ± 6
Total cholesterol (mg/dl)	196 ± 27	187 ± 27	194 ± 22
LDL cholesterol (mg/dl)	123 ± 23	118 ± 21	127 ± 23
HDL cholesterol (mg/dl)	52 ± 16	49 ± 16	50 ± 15
Triglycerides (mg/dl)	104 ± 61	98 ± 39	88 ± 42
Fasting glucose (mg/dl)	80 ± 16	81 ± 15	78 ± 15
Plasma sodium (mmol)	139.4 ± 2.7	138.1 ± 2.8	139.0 ± 1.8
Creatinine clearance (ml/min/1.73 m^2)	96 ± 16	92 ± 26	99 ± 29
MDRD eGFR (ml/min/1.73 m^2)	81 ± 13	82 ± 15	86 ± 19
PA (ActiGraph) (kcal/week)	N/A	3242 ± 1285	3016 ± 1320
PA (questionnaire) (MET h/week)	N/A	27 ± 29	28 ± 28

Values are mean ± SD. ActiGraph (accelerometer) data are on a subsample of 10 subjects. *p < 0.01 versus baseline or normal sodium.

eGFR = estimated glomerular filtration rate (Modification of Diet in Renal Disease Study Equation); HDL = high-density lipoprotein; LDL = low-density lipoprotein; MET = metabolic equivalent; N/A = not applicable; PA = physical activity.

Effects of DSR on conduit artery (macrocirculatory) endothelial function. FMD_{BA} was 68% greater during DSR versus normal sodium condition (%Δ: 6.01 ± 2.31 vs. 3.57 ± 1.69; p < 0.001) (Fig. 2A). The response was consistent with all but 2 subjects demonstrating higher FMD_{BA} with DSR versus the normal sodium state (Fig. 2B). When the 2 sodium conditions were pooled, there was a significant inverse correlation between sodium excretion and FMD_{BA} (r = −0.48; p < 0.01). The difference in FMD_{BA} between sodium conditions remained significant (p < 0.001) after corrections for SBP and medication use, and ΔSBP with DSR did not correlate with ΔFMD_{BA} (r = −0.05; p = 0.85). No trends for sex differences were observed, although the study was not powered to detect such differences. Baseline brachial artery diameter and shear rate did not differ across sodium conditions (Table 4) or with ascorbic acid with or without BH_4 administration, and the difference in FMD_{BA} between conditions remained significant (p < 0.001) when covarying for these factors. Endothelium-independent dilation to sublingual nitroglycerin was unaffected by DSR (Table 4). The associated mixed-effects regression models are shown in Online Table.

Table 2 Diet Composition

Component	Baseline	Low Sodium	Normal Sodium
Total intake (kcal/day)	2191 ± 562	2045 ± 509	2019 ± 466
Carbohydrates (% of total kcal)	47 ± 8	52 ± 9	47 ± 12
Protein (% of total kcal)	16 ± 3	15 ± 3	17 ± 3
Fat (% of total kcal)	34 ± 6	33 ± 9	33 ± 9
Calcium (mmol/day)	23 ± 8	19 ± 6	19 ± 5
Magnesium (mmol/day)	11 ± 5	12 ± 5	11 ± 3
Potassium (mmol/day)	63 ± 18	64 ± 17	64 ± 18
Sodium (mmol/day)†	136 ± 48	56 ± 15*	57 ± 12*
Fruits and vegetables (cups/day)	3.7 ± 1.9	3.6 ± 1.6	3.7 ± 1.8

Values are mean ± SD. *p < 0.01 versus baseline. †Sodium intake was based on 3-day diet records and excludes sodium added back in pill form.

Table 3 Circulating Humoral Factors

Factor	Low Sodium	Normal Sodium	p Value
Log C-reactive protein (mg/l)	0.27 ± 0.53	0.15 ± 0.51	0.53
Log interleukin-6 (pg/ml)	0.13 ± 0.25	0.08 ± 0.32	0.59
Log tumor necrosis factor α (pg/ml)	0.05 ± 0.10	0.03 ± 0.17	0.66
Angiotensin II (pg/ml)	4.7 ± 2.7	3.9 ± 2.3	0.42
Plasma renin activity (ng/ml/h)	1.48 ± 1.2	0.84 ± 0.54	0.36
Aldosterone (ng/dl)	5.7 ± 2.6	4.1 ± 1.6	0.32
Endothelin-1 (pg/ml)	6.3 ± 1.4	5.9 ± 1.3	0.58
Norepinephrine (pg/ml)	400 ± 170	392 ± 140	0.88
Cystatin C (mg/l)	0.76 ± 0.15	0.77 ± 0.13	0.93
Insulin (μU/ml)	11.4 ± 4.7	9.3 ± 3.9	0.19

Values are mean ± SD.

Effects of DSR on resistance vessel (microcirculatory) endothelial function. DSR improved EDD to ACh, whether expressed as FBF (+42% at peak dose) or as BP-corrected forearm vascular conductance (+52% at peak

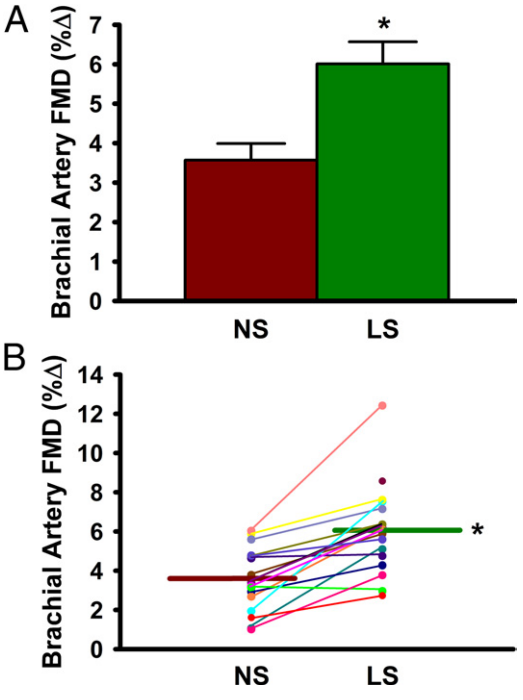

Figure 2 Brachial Artery Flow-Mediated Dilation

Mean percentage changes in group **(A)** and individual subject **(B)** brachial artery flow-mediated dilation (FMD) during normal-sodium (NS) conditions versus low-sodium (LS) conditions (DSR). Values are mean ± SE. *p < 0.001 versus normal-sodium condition (linear mixed-effects models [see Online Appendix for details]); n = 16/17). All 17 subjects are shown in **B,** but due to similarities, some subjects' data overlap and may not be individually distinguishable.

Table 4 Hemodynamic Factors

Factor	Low Sodium	Normal Sodium	p Value
Brachial artery FMD (mm Δ)	0.23 ± 0.07	0.15 ± 0.07	<0.001
Baseline brachial artery diameter (mm)	3.89 ± 0.56	4.07 ± 0.44	0.31
Peak shear rate (s^{-1})	391 ± 104	378 ± 118	0.50
Brachial artery dilation to NTG (% Δ)	24.0 ± 4.5	23.4 ± 2.3	0.54
Brachial artery dilation to NTG (mm Δ)	0.91 ± 0.14	0.91 ± 0.15	0.82
AUC of %Δ FBF_{ACh} vs. saline	1768 ± 1002	1213 ± 890	<0.05
AUC of %Δ FVC_{ACh} vs. saline	1811 ± 1006	1220 ± 873	<0.05
AUC of %Δ FBF_{SNP} vs. saline	1035 ± 377	995 ± 635	0.93
AUC of %Δ FVC_{SNP} vs. saline	1040 ± 465	1000 ± 655	0.93

Values are mean ± SD.

AUC = area under the curve; FBF_{ACh} = forearm blood flow to acetylcholine; FMD = flow-mediated dilation; FVC = forearm vascular conductance; NTG = nitroglycerin; SNP = sodium nitroprusside.

dose) (both, $p < 0.05$) (Figs. 3A and 3B). EDD also was greater with DSR versus the normal sodium state when expressed as area under the dose–response curve (Table 4). Again, the effect of DSR was consistent among the subjects, with all but 2 individuals demonstrating higher values for area under the dose–response curve of FBF_{ACh} compared with the normal sodium state (Fig. 3C). The FBF and FVC responses to the endothelium-independent dilator sodium nitroprusside were unchanged across sodium conditions (Table 4). FBF_{ACh} in the control arm did not change with sodium condition or drug infusions (not shown).

Effects of DSR on EDD: NO bioavailability. Coinfusion of the eNOS inhibitor *N*G monomethyl-L-arginine markedly reduced FBF_{ACh} and BP-corrected FVC with DSR but not during the normal sodium condition (Figs. 4A to 4D). As a result, FBF_{ACh} no longer differed between DSR and the normal sodium condition during eNOS inhibition, indicating increased NO bioavailability during DSR. In vascular endothelial cells sampled from the brachial artery, eNOS protein expression and activation, the latter indicated by phosphorylation at Ser 1177, did not differ between sodium conditions (Figs. 4E and 4F).

Effects of DSR on EDD: oxidative stress and BH_4 bioavailability. Infusion of ascorbic acid increased FMD_{BA} during the normal sodium condition (+37%; $p < 0.05$), but not during DSR (Fig. 5A), indicating reduced oxidative stress–related suppression of EDD during DSR. Similarly, oral BH_4 administration improved FMD_{BA} only in the normal sodium state (+65%; $p < 0.005$) (Fig. 5A). No further improvement in FMD_{BA} was observed when ascorbic acid and BH_4 were coadministered (Fig. 5A). FMD_{BA} no longer differed between DSR and the normal sodium condition following the administration of ascorbic acid and/or BH_4. These responses were independent of the sodium condition–related influence on SBP. Endothelium-independent dilation to nitroglycerin did not change with ascorbic acid + BH_4 during either sodium condition (data not shown). Circulating SOD activity was modestly greater ($p < 0.05$) during DSR versus the normal sodium condition (Fig. 5B).

Coinfusion of ascorbic acid also improved FBF_{ACh} (peak dose: +57%) under the normal sodium condition compared with DSR (Fig. 5C). FBF_{ACh} no longer differed between DSR and the normal sodium condition during coinfusion of ascorbic acid. Results were similar when EDD was expressed as forearm vascular conductance (Fig. 5D).

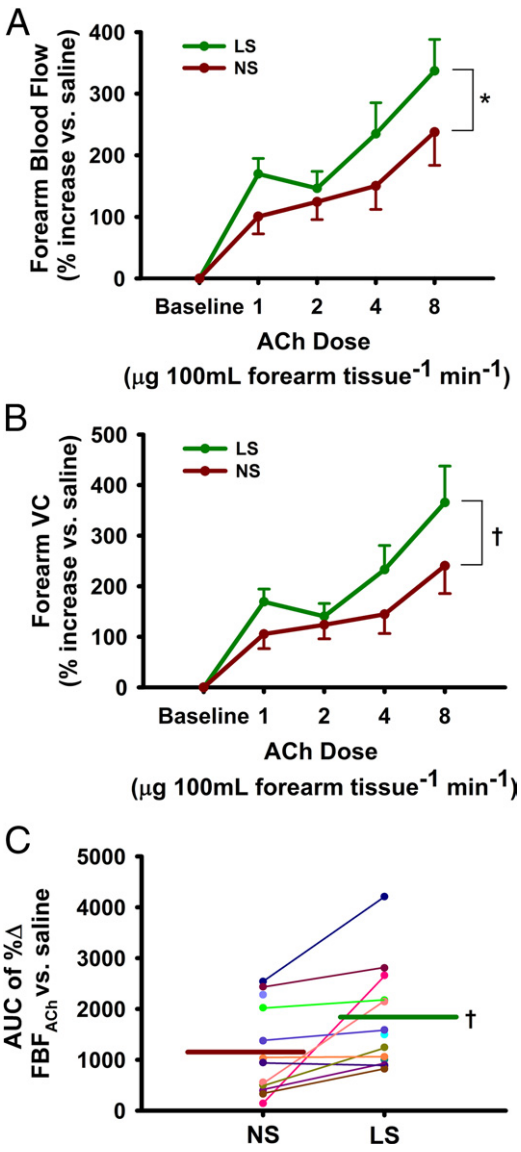

Figure 3 Forearm Blood Flow to Acetylcholine

Forearm blood flow (FBF) **(A)** and vascular conductance (VC) **(B)** responses to acetylcholine (ACh) during NS conditions versus LS (DSR). Individual subject FBF_{ACh} (area under the curve, AUC) **(C)**. Values are mean ± SE. *$p < 0.005$; †$p < 0.05$ LS versus NS (linear mixed-effects model; n = 12 of 13). Abbreviations as in Figure 2.

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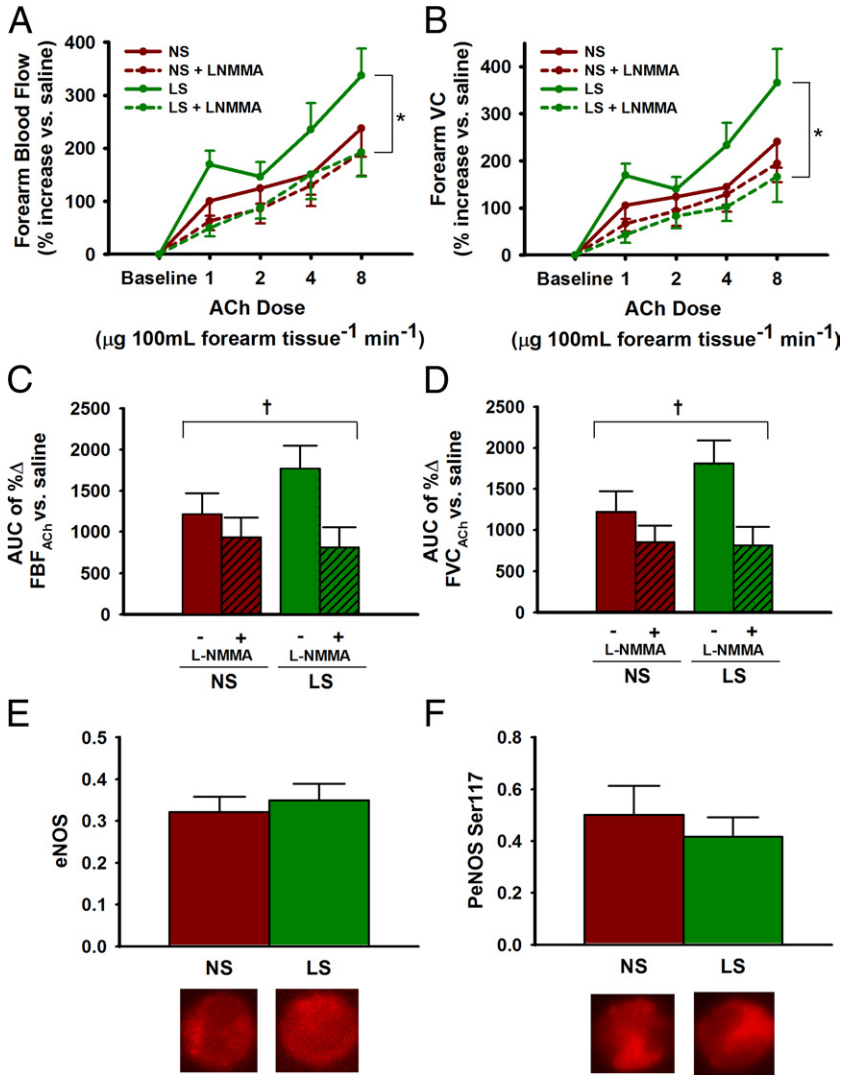

Figure 4 **Contribution of eNOS to EDD**

Dose-response for FBF **(A)** and forearm VC **(B)** to ACh with or without *N*G monomethyl-L-arginine (L-NMMA) during NS and LS (DSR); AUC values **(C and D).** Total endothelial nitric oxide synthase (eNOS) protein **(E)** and eNOS phosphorylated at Ser 1177 (PeNOS) **(F)** in subjects' endothelial cells relative to human umbilical vein endothelial cell (HUVEC) control; representative images shown below. Values are mean ± SE. *$p < 0.001$; †$p < 0.05$ (drug × sodium condition interaction; linear mixed-effects model; n = 12 of 13). Abbreviations as in Figures 2 and 3.

Discussion

The present findings show that restricting dietary sodium intake to a level consistent with the DASH diet improves both conduit artery (macrovascular) and resistance vessel (microvascular) endothelial function in middle-aged/older adults with moderately elevated SBP, a large clinical demographic with significant CVD risk burden. The results also provide insight into humans regarding the physiological mechanisms mediating improvements in vascular endothelial function with DSR, which include increased NO and BH_4 bioavailability and reduced oxidative stress. None of the vascular effects observed were related to changes in BP, other dietary factors, clinical characteristics, or humoral influences. Overall, the findings support the emerging concept that maintaining low dietary sodium intake exerts "vasculoprotective" effects beyond those attributable to BP lowering.

DSR and vascular endothelial function. The present results demonstrate that DSR improves EDD as assessed by both FMD_{BA}, a measure of peripheral conduit artery dilation to a mechanical (shear) stress, and FBF_{ACh}, an assessment of resistance vessel EDD in response to a chemical stimulus. These findings are in agreement with those from previous reports of impaired EDD in animals (13,18) and younger humans (11,15) following a high sodium load, as well as improved FMD_{BA} after short-term

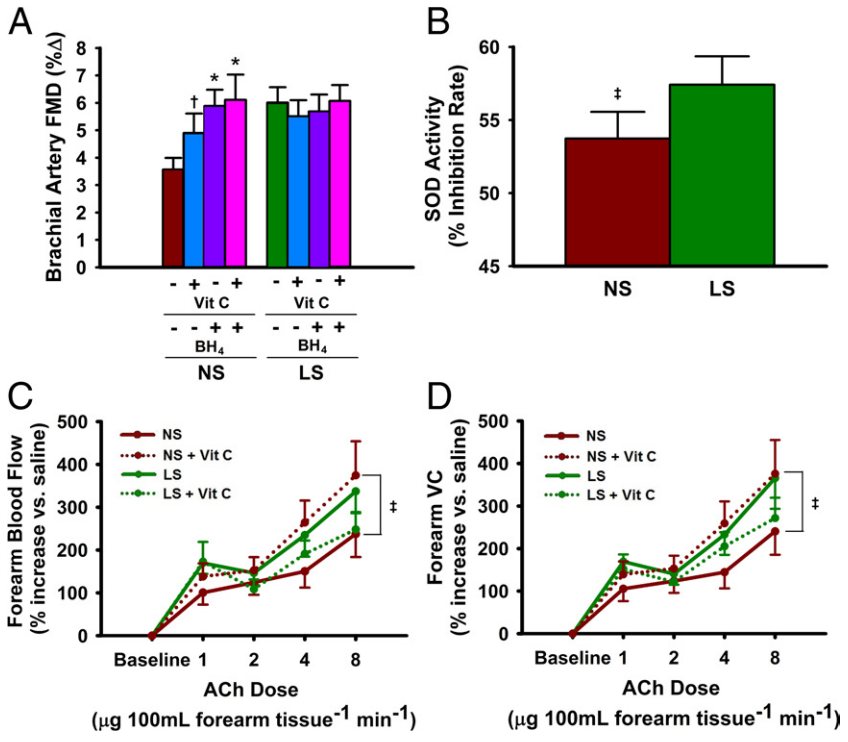

Figure 5 **Contributions of Oxidative Stress and BH4 Bioavailability to EDD**

Brachial artery FMD during NS versus LS with or without coadministration of ascorbic acid (Vit C) and/or tetrahydrobiopterin (BH_4) **(A).** Circulating superoxide dismutase (SOD) activity **(B).** FBF **(C)** and forearm VC **(D)** responses to ACh ± Vit C. Values are mean ± SE. *$p < 0.001$; †$p < 0.005$; ǂ$p < 0.05$ (drug × sodium condition interaction or main effect of sodium condition; linear mixed-effects model; n = 12 to 17). Abbreviations as in Figures 2 and 3.

sodium restriction in overweight/obese, normotensive adults (35). The improvements in both micro- and macrocirculatory EDD with DSR in the present investigation were observed in almost all subjects and did not differ between the men and women studied. We also found that conduit artery EDD (FMD_{BA}) was inversely related to urinary sodium excretion, an objective measure of dietary sodium intake.

The reduction in SBP with low sodium (12 mm Hg) was similar to those from previous studies on dietary sodium restriction (21,22) and was consistent with those from other crossover studies employing well-controlled BP assessments (25,36). The present magnitude of reduction was observed despite the inclusion of subjects on antihypertensive medications. Importantly, the improvements in EDD with DSR remained significant after statistically correcting for SBP, and the changes in EDD were not related to the changes in SBP, whereas diastolic BP was unaffected. Thus, DSR improved vascular endothelial function beyond its influence on BP alone.

Physiological mechanisms. High sodium intake in younger humans and rodents impairs EDD by reducing NO bioavailability (13–15), and the chronic impairment in EDD in salt-sensitive compared with salt-resistant adults with hypertension is associated with reductions in bioavailable NO (37). Consistent with these observations, in the present study, lowering dietary sodium improved resistance vessel EDD by increasing NO bioavailability. The latter was not obviously associated with changes in eNOS or its activation, as eNOS protein expression and phosphorylation of eNOS at Ser 1177 did not differ between sodium conditions in vascular endothelial cells obtained from brachial artery sampling.

In rodents fed a high-salt diet, impairments in EDD are linked to reduced bioavailability of BH_4, an essential cofactor for NO production by eNOS (18), as well as oxidative stress (14,17), as indicated in part by improved EDD following acute administration of an antioxidant (17). In the present study, a single oral dose of BH_4, previously shown to improve EDD in settings of endothelial dysfunction (32), improved EDD (FMD_{BA}) during normal, but not low sodium intake. Moreover, infusion of supraphysiological doses of ascorbic acid (vitamin C), a well-established method for acutely reducing oxidative stress-related suppression of EDD (8,33), improved both conduit artery (FMD_{BA}) and resistance vessel (FBF_{ACh}) EDD during normal, but not lower, sodium intake. Indeed, the administration of BH_4, ascorbic acid, or both abolished the differences in EDD between the normal- and low-sodium conditions. A modest but significant increase in circulating

SOD activity was also found with DSR, in agreement with the inhibitory effects of high-salt feeding on SOD reported previously in rats (20).

BH_4 bioavailability modulates NO-mediated EDD in the absence of changes in eNOS protein expression or activation (38). Without adequate BH_4, eNOS is "uncoupled," producing more superoxide anion and less NO (39). It is possible that DSR "recoupled" eNOS, increasing NO production without further activation of the enzyme. The apparent increase in BH_4 bioavailability with DSR most likely was mediated by reduced oxidative stress, as reactive oxygen species, particularly peroxynitrite formed by the reaction of superoxide and NO, readily oxidize BH_4 to its inactive form, BH_2 (39). Alternatively, DSR may have increased NO bioavailability by inhibiting superoxide production by oxidant enzymes such as NADPH oxidase or by mitochondria, resulting in less superoxide to react with/reduce the bioavailability of NO.

Clinical significance. The concept that high sodium intake has adverse CV effects independent of BP has been advanced previously (9,12). High dietary sodium impairs EDD even in rodents that are salt-resistant and, thus, do not exhibit increases in BP in response to a high-salt diet (13,18,20). Acute impairment of EDD in normotensive adults after sodium loading also is BP independent (11), and adults with elevated SBP who report lower sodium intake have enhanced EDD independent of BP (16). The present results extend these findings to sodium restriction and lend support to the overall hypothesis that sodium intake not only elevates BP but also exerts other adverse influences (12). The effects of sodium restriction on endothelial function reported here also complement previous findings that reducing sodium intake can rapidly de-stiffen large elastic arteries (21), another independent vascular risk factor for CVD (40). The improvements in these 2 common forms of arterial dysfunction, both predictors of CV events (3,4,40), suggest that DSR has strong potential for reducing CVD risk via broad vasculoprotective effects.

Strengths and limitations. Diet was well-controlled in the present study, with only sodium intake differing between the 2 conditions, as documented by "gold-standard" 24-hr urine collections combined with diet records. This feature, along with the placebo-controlled crossover design employed, allowed for the isolation of dietary sodium as the modulating factor between conditions. Importantly, the results are generalizable to community settings in that sodium restriction was achieved by subjects on their own, guided by dietary counseling, rather than delivered as prepared meals in a research setting. The assessments of macro- and microcirculatory endothelial function, both of which are involved in age- and BP-related CV pathology, as well as the unique insight into mechanisms of action acquired by the use of novel translational research techniques, are additional strengths of the study.

Due to the complexity, burden, and, in some cases, invasiveness of the study design and procedures, only a limited number of subjects could be studied. However, as recently emphasized by Donald et al. (41), a much smaller sample size is required when assessing EDD using a crossover compared with a parallel-group design, as shown in previous investigations (31,42). As such, the study was well powered to assess change in EDD (Online Appendix), and importantly, the approach allowed investigation of the mechanisms underlying the beneficial vascular effects of low sodium intake at a depth and level of detail that would not be possible in large cohorts.

Conclusions

The results of the present study support the hypothesis that DSR to a level consistent with the Dietary Approaches to Stop Hypertension diet improves both conduit artery (macrovascular) and resistance vessel (microvascular) endothelial function in middle-aged/older men and women with elevated SBP by increasing NO and BH_4 bioavailability and reducing oxidative stress. These findings provide further support for the idea that DSR has the potential to improve CV health and reduce the risk for CVD beyond its BP-lowering effects.

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Key Words: aging ■ diet ■ hypertension ■ nitric oxide ■ oxidative stress.

APPENDIX

For a an expanded Methods section, and supplemental table and figure, please see the online version of this article.