Modest Sodium Reduction Increases Circulating Short-Chain Fatty Acids in Untreated Hypertensives

A Randomized, Double-Blind, Placebo-Controlled Trial

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Abstract—High-sodium diet may modulate the gut microbiome. Given the circulating short-chain fatty acids (SCFAs) are microbial in origin, we tested the hypothesis that the modest sodium reduction would alter circulating SCFA concentrations among untreated hypertensives, and the changes would be associated with reduced blood pressure and improved cardiovascular phenotypes. A total of 145 participants (42% blacks, 19% Asian, and 34% females) were included from a randomized, double-blind, placebo-controlled cross-over trial of sodium reduction with slow sodium or placebo tablets, each for 6 weeks. Targeted circulating SCFA profiling was performed in paired serum samples, which were collected at the end of each period, so as all outcome measures. Sodium reduction increased all 8 SCFAs, among which the increases in 2-methylbutyrate, butyrate, hexanoate, isobutyrate, and valerate were statistically significant (*P*s<0.05). Also, increased SCFAs were associated with decreased blood pressure and improved arterial compliance. There were significant sex differences of SCFAs in response to sodium reduction (*P*s<0.05). When stratified by sex, the increases in butyrate, hexanoate, isobutyrate, isovalerate, and valerate were significant in females only (*P*s<0.05), not in males (*P*s>0.05). In females, changes in isobutyrate, isovalerate, and 2-methylbutyrate were inversely associated with reduced blood pressures (*P*s<0.05). Increased valerate was associated with decreased carotid-femoral pulse wave velocity (*P*=0.040). Our results show that dietary sodium reduction increases circulating SCFAs, supporting that dietary sodium may influence the gut microbiome in humans. There is a sex difference in SCFA response to sodium reduction. Moreover, increased SCFAs are associated with decreased blood pressures and improved arterial compliance.

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Key Words: blood pressure ■ short-chain fatty acids ■ hypertension ■ phenotype ■ sodium

Hypertension is a serious health challenge and imposes a huge burden. Elevated blood pressure (BP) increases the risk of stroke, heart attack, and heart failure.[1] Excess sodium intake is recognized as one of the most important risk factors for hypertension.[2,3] However, the underlying causes are not well understood.

Recent research supports the concept that gut microbiota is involved in BP control and hypertension.[4] In particular, animal studies suggest that microbiome is a key linkage in the causal relationship between sodium intake and elevated BP.[5–7] A study found that high-sodium diet altered the gut microbiome in mice, and the treatment of *L. murinus* prevented salt-induced hypertension.[8] High-sodium diet modulates the gut microbial composition and alters fecal short-chain fatty acids (SCFAs) production in salt-sensitive animal models.[8–10] SCFAs are also involved in BP regulation.[11] SCFAs are products from the fermentative activity of gut bacteria, subsequently absorbed into the bloodstream of the host, which can bind to and activate host receptors, thereby acting as a messenger between gut microbial metabolism and host physiology.[11] Thus, the relationships among sodium intake, SCFAs, and BP would provide important information on the cause of hypertension. However, evidence in humans is scarce. Given the fact that virtually all SCFAs in the circulation are microbial in origin, we tested the hypotheses that (1) modest reduction in dietary sodium intake would alter circulating SCFA levels among the untreated hypertensive participants; (2) whether there was race or sex interaction in response to sodium reduction; (3) the altered SCFAs would be associated with reduced BP and improved large artery compliance.

Methods

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Participants

The present study used stored serum samples from a previously conducted randomized, double-blind, placebo-controlled cross-over trial (randomized controlled trial [RCT]) of modest dietary sodium reduction in untreated hypertensives as described before.[12,13] The inclusion criteria were population aged 30 to 75 years, with sitting systolic BP (SBP) 140 to 170 mm Hg or diastolic BP (DBP) 90 to 105 mm Hg, and with no previous treatment for raised BP.[12] A total of 145 participants (39% whites, 42% blacks, 19% Asian, and 34% females) with serum samples available at both time points (at the end of slow sodium and placebo) were included in this study. The study was approved by the Wandsworth Local Research Ethics Committee and the Institutional Review Board of Augusta University and adhered to the principles of the Declaration of Helsinki. Written informed consent was obtained from all participants.

Sodium Reduction Protocol

The RCT of modest sodium reduction was carried out (Figure S1 in the Data Supplement), the study protocol and compliance were described in detail elsewhere.[12–15] Briefly, in the first 2 weeks run-in period, participants were given detailed advice by specially trained nurses on how to reduce their sodium intake, with the aim of achieving a sodium intake of 2000 mg/day (85 mmol/day). They were advised not to add salt at the table or during cooking, and avoid foods that contained a large amount of sodium. Nurses went through with the participants on what foods they usually ate, identified items with high sodium content and advised them to use lower sodium alternatives. In appropriate cases, the spouse or whoever cooked in the household was also seen. The advice was reinforced at each visit for the whole duration of the study. Sodium-free bread was provided for those who had no easy access to it. While continuing on the reduced-sodium diet, the participants were given, in a random order, either 9 slow sodium tablets (10 mmol sodium per tablet) or placebos daily for 6 weeks. They then crossed over to receive the other tablets for another 6 weeks.[12] Reduced-sodium diet plus slow sodium tablets represented usual sodium intake, while a reduced-sodium diet plus placebo represented a reduced-sodium intake.

Anthropometric and Laboratory Measurements

Measurements were performed at the end of each 6-week study period. Height and body weight were measured with light clothing and without shoes. Body mass index (BMI) was calculated as weight (kg) per square of height (m^2). BP was measured by a validated automatic digital BP monitor (Omron HEM-705CP) in the sitting position after 5 to 10 minutes rest. Three readings were taken, and the average of the last 2 readings was used. Twenty-four-hour ambulatory BP monitoring was performed using SpaceLabs 90207 devices (SpaceLabs, Inc, Washington, DC) as previously described.[16] Briefly, the monitor was set to take BP measurements at half-hourly intervals during the daytime and hourly intervals during the nighttime. Recordings were analyzed with the ambulatory BP monitoring report manager system software package.[16] Pulse pressure (PP) was calculated as the difference between SBP and DBP. Carotid-femoral pulse wave velocity (cf-PWV) was measured noninvasively using an automatic device Complior.[12] Blood samples were collected at the end of each 6-week period for the measurements of biochemistry.

Targeted Metabolomics Analysis of SCFAs in Serum

Serum samples were collected at the end of 6-week slow sodium tablets and at the end of 6-week placebo tablets periods from each of the 145 participants. Targeted SCFA panel profiling was performed by Metabolon in paired serum samples from 145 participants. Eight SCFAs: acetic acid (C2), propionic acid (C3), isobutyric acid (C4), butyric acid (C4), 2-methyl-butyric acid (C5), isovaleric acid (C5), valeric acid (C5), and caproic acid (hexanoic acid, C6) were quantified by liquid chromatography with tandem mass spectrometry (Metabolon Method TAM148: liquid chromatography with tandem mass spectrometry method for the quantitation of SCFA [C2–C6] in human plasma and serum). The serum samples were spiked with stable labeled internal standards and were homogenized and subjected to protein precipitation with an organic solvent. After centrifugation, an aliquot of the supernatant was derivatized. The reaction mixture was injected onto an Agilent 1290/AB Sciex QTrap 5500 liquid chromatography with tandem mass spectrometry system equipped with a C18 reversed phase ultrahigh performance liquid chromatography column. The mass spectrometer was operated in negative mode using electrospray ionization. The peak area of the individual analyte productions was measured against the peak area of the productions of the corresponding internal standards. Quantitation was performed using a weighted linear least squares regression analysis generated from fortified calibration standards prepared immediately before each run. Liquid chromatography with tandem mass spectrometry raw data were collected and processed using AB SCIEX software Analyst 1.6.2. Data reduction was performed using Microsoft Excel 2016.

Three levels of QC were prepared in serum by diluting with PBS and spiking with stock solutions to obtain the appropriate concentrations for each level. Sample analysis was performed in a 96-well plate format containing 2 calibration curves and 6 QC samples (per plate) to monitor assay performance. Precision was evaluated using the corresponding QC replicates in the sample runs. Targeted acceptance criteria are at least 50% of QC samples at each concentration level per analyte should be within ±20.0% of the running mean, and at least 2/3 of all QC samples per analyte should fall within ±20.0% of the corresponding running mean. QCs met acceptance criteria at all levels for all analytes. Detailed results and coefficients of variations are presented in Table S1.

Statistical Analysis

The changes in cardiovascular risks and SCFAs from slow sodium to placebo in all participants are presented as mean±SD. Two-tailed paired *t* test was conducted to examine the differences in variables between placebo and sodium tablets. In multiple regression models, circulating SCFA levels were square-root-transformed and standardized to unit variance and zero mean. Based on the intent-to-treat principle,[17] two-level mixed-effects linear regression models were used to assess the differences in SCFAs between sodium and placebo tablets while incorporating repeated measured data and controlling for age, sex, race, and BMI as confounding variables. We further tested whether the changes in the SCFAs were associated with the changes in BPs, PPs, and cf-PWV using mixed-effects models. A $P<0.05$ was considered statistically significant. All analyses were performed using Stata version 12.0 (StataCorp, College Station, TX).

Results

General Characteristics of the Participants

A total of 145 participants with serum samples available were included, with a mean age of 50.7±10.7 years, mean BMI 29.1±5.1 kg/m^2, 34% females, 42% blacks, and 19% Asians. Overall, sodium reduction was associated with lowered BPs and cf-PWV (Ps<0.05), as previously reported (Table S2).[12] Table 1 presents the changes in cardiovascular disease (CVD) phenotypes by sex. The results remained the same, except that the decreases in nighttime DBP and office PP were not significant in the female, and the decrease in cf-PWV was not significant in the male.

Effects of Sodium Reduction on the Circulating Levels of SCFAs

As shown in Table 2, modest sodium reduction increased the circulating levels of all the 8 SCFAs, among which the increases in 2-methylbutyrate, butyrate, hexanoate, and isobutyrate were statistically significant (raw Ps<0.05). Moreover, as shown in Table 3, these SCFAs remained significant after adjustment for age, sex, race, and BMI, the increase in acetate became borderline significant (P=0.065), and the increase in valerate became significant (P=0.027). Then we tested whether there was a race/ethnicity, sex, or BMI interaction.

Table 1. Changes in CVD Variables From Slow Sodium to Placebo in All Participants

	Female (N=49)			Male (N=96)		
Variable	Slow Sodium	Placebo	*P* Value	Slow Sodium	Placebo	*P* Value
Urinary sodium (mmol/24 h)	152.3±52.4	95.8±40.1	<0.001	177.2±60.8	120.3±51.2	<0.001
Office BP and pulse rate, mm Hg						
SBP	148.4±13.7	144.9±14.0	0.036	145.7±13.0	139.6±11.7	<0.001
DBP	89.7±7.9	87.9±8.4	0.014	90.9±8.4	88.1±9.1	<0.001
MAP	109.3±8.8	106.9±9.3	0.014	109.2±8.9	105.3±8.9	<0.001
Ambulatory BP, mm Hg						
24-hour SBP	143.6±9.2	138.2±10.6	<0.001	139.8±10.4	136.5±10.9	<0.001
24-hour DBP	84.8±8.4	82.7±8.7	0.006	86.9±8.7	85.3±8.4	0.002
Day SBP	149.1±9.7	143.1±11.4	<0.001	146.9±10.6	142.8±11.3	<0.001
Day DBP	89.7±9.1	87.3±9.3	0.003	92.7±9.6	90.6±9.4	0.001
Night SBP	137.0±9.7	132.3±11.7	0.001	131.9±11.4	129.3±12.3	0.006
Night DBP	79.4±8.4	77.7±8.9	0.115	80.3±9.1	79.0±9.4	0.047
cf-PWV, m/s	11.7±1.9	11.1±1.6	0.002	11.7±2.5	11.3±2.0	0.062
PP, mm Hg						
Office PP	58.7±11.0	57.1±10.9	0.201	54.8±10.4	51.5±9.9	<0.001
24-hour PP	58.8±6.8	55.5±6.8	<0.001	52.9±7.6	51.3±7.2	<0.001
Day PP	59.4±7.9	55.8±7.9	<0.001	54.2±8.2	52.3±7.7	<0.001
Night PP	57.7±6.9	54.6±7.2	<0.001	51.5±7.7	50.3±7.6	0.049

BP indicates blood pressure; cf-PWV, carotid-femoral pulse wave velocity; CVD, cardiovascular disease; DBP, diastolic BP; MAP, mean arterial pressure; PP, pulse pressure; and SBP, systolic BP.

Indeed, there were significant sex interactions on isovalerate and valerate (*P*s=0.028 for both) but no race/ethnicity nor BMI interaction (*P*s>0.05). The increases in butyrate, hexanoate, isobutyrate, isovalerate, and valerate induced by sodium reduction were significant in females (*P*s<0.05), not in males (*P*s>0.05; Table 3). Changes in SCFAs from slow sodium to placebo were presented separately by sex in Tables S3 and S4.

Associations of Changes in SCFAs With Changes in Cardiovascular Phenotypes

Table 4 presents the associations between the changes in SCFAs and the changes in cardiovascular phenotypes. Increased circulating isovalerate was associated with reduced 24-hour DBP and night DBP (*P*s<0.05). Hexanoate was positively associated with daytime DBP (*P*=0.044).

Associations of Changes in SCFAs With Changes in Cardiovascular Phenotypes in Women

Because the increases in SCFAs induced by sodium reduction were significant only in females, we further examined the associations between changes in circulating SCFAs and improved CVD measurements in this gender group (Table 5). Increased isobutyrate was associated with reductions of daytime PP, and all the ambulatory BP measurements except daytime DBP (*P*s<0.05). Increased isovalerate was also associated with reductions of all the ambulatory BP measurements (*P*s<0.05) as well as office

Table 2. Changes in SCFAs From Slow Sodium to Placebo in All Participants

Metabolites	Slow Sodium	Placebo	Changes	*P* Value
2-Methylbutyrate, ng/mL	76.5±35.5	81.5±34.3	5.0±27.8	0.032
Acetate, ng/mL	1704.7±1077.5	2320.8±4862.7	616.1±4948.1	0.136
Butyrate, ng/mL	28.3±15.8	32.8±25.1	4.5±24.6	0.031
Hexanoate, ng/mL	33.5±11.6	36.0±12.3	2.5±13.6	0.031
Isobutyrate, ng/mL	50.2±19.4	53.3±18.1	3.1±16.1	0.021
Isovalerate, ng/mL	66.5±31.6	69.7±31.0	3.2±31.1	0.217
Propionate, ng/mL	39.7±29.5	49.9±54.9	9.2±52.3	0.266
Valerate, ng/mL	6.0±3.5	6.6±3.7	0.6±4.1	0.065

P values were calculated from 2-tailed paired *t* test by comparing the differences in SCFAs between placebo and sodium tablets. SCFA indicates short-chain fatty acid.

Table 3. Effects of Sodium Reduction on Serum SCFAs

	All		Male (N=96)		Female (N=49)	
Metabolites	β	*P* Value	β	*P* Value	β	*P* Value
2-Methylbutyrate	0.16	0.020	0.15	0.101	0.19	0.083
Acetate	0.20	0.065	0.26	0.093	0.08	0.402
Butyrate	0.20	0.029	0.12	0.253	0.35	0.036
Hexanoate	0.21	0.027	0.12	0.299	0.38	0.017
Isobutyrate	0.19	0.009	0.11	0.225	0.34	0.005
Isovalerate	0.11	0.156	–0.01	0.907	0.36	0.023
Propionate	0.09	0.563	–0.07	0.675	0.32	0.264
Valerate	0.21	0.027	0.06	0.564	0.49	0.003

Regression coefficients (β) and significance levels (*P*) of intervention were estimated using the mixed-effects model adjusted for age, sex, race, and body mass index, and reflect changes in SCFAs from sodium tablets to placebo tablets. SCFA indicates short-chain fatty acid.

SBP and mean arterial pressure (MAP) (*P*s<0.05). Moreover, increased valerate was associated with decreased cf-PWV (*P*=0.040). Table S5 presented the R^2 values of the full models (SCFAs, age, race, and BMI) and the reduced models (age, race, and BMI) among females. Differences in R^2 values between full models and reduced models are estimations of the proportions of variances in CVD phenotypes explained by the changes in SCFAs. Increase in isobutyrate explained 4.44% of the reduction in daytime PP. Increase in isovalerate explained 7.03%, 4.19%, and 6.90% of the reduction in office SBP, DBP, and MAP, and increase in valerate explained 6.21% of the reduction in cf-PWV.

Discussion

The present study, for the first time, shows that modest dietary sodium reduction increases circulating SCFA levels in untreated hypertensive patients, supporting that dietary sodium may influence the gut microbiome in humans. There were sex differences in SCFAs' response to sodium reduction. Moreover, the increased circulating SCFAs were associated with reduced BPs and cf-PWV.

SCFAs are a class of gut microbial metabolites and virtually all the SCFAs in the circulation are microbial in origin.[18] It is well recognized that dietary intakes influence the gut microbiome.[19] However, the effects of sodium intake on SCFAs, which mediate the effects of the microbiome on host physiology, have been rarely studied in humans, and there are contradictory findings in animal studies. To the best of our knowledge, our study is the first to address the question that whether changes in dietary sodium intake alter circulating SCFAs in hypertensive patients. By leveraging a completed RCT, we were able

Table 4. Association Between Changes in Serum SCFAs and Changes in Cardiovascular Phenotypes in All Participants

Phenotypes	2-Methylbutyrate	Acetate	Butyrate	Hexanoate	Isobutyrate	Isovalerate	Propionate	Valerate
Office BP								
SBP	0.60	–0.25	1.28	0.59	0.24	–0.66	1.90	–0.07
DBP	0.59	0.15	0.45	0.42	0.22	–0.02	1.12	–0.17
MAP	0.58	0.03	0.74	0.48	0.22	–0.24	1.35*	–0.14
Ambulatory BP								
24-hour SBP	–0.02	0.33	0.66	0.42	–0.12	–0.74	0.73	0.28
24-hour DBP	–0.25	–0.10	0.16	0.31	–0.37	–0.78*	0.54	0.05
Day SBP	0.31	0.57	0.70	0.88	0.15	–0.19	1.15	0.15
Day DBP	0.20	0.43	0.12	0.81*	0.16	–0.23	0.96	0.15
Night SBP	–0.27	0.37	0.89	0.11	–0.18	–0.98	0.98	0.51
Night DBP	–0.54	–0.57	0.34	0.19	–0.65	–1.15*	0.93	0.17
cf-PWV	0.09	0.01	0.05	0.03	0.01	0.08	–0.14	–0.13
PP								
Office PP	–0.33	–0.52	0.85	0.21	–0.28	–0.74	0.78	0.19
24-hour PP	0.08	0.32	0.36	0.06	0.05	–0.08	–0.26	0.15
Day PP	–0.06	0.08	0.36	0.08	–0.17	–0.05	–0.23	–0.07
Night PP	0.22	0.82	0.46	–0.11	0.36	0.05	–0.10	0.28

BP indicates blood pressure; cf-PWV, carotid-femoral pulse wave velocity; DBP, diastolic BP; MAP, mean arterial pressure; PP, pulse pressure; SBP, systolic BP; and SCFA, short-chain fatty acid.

*P<0.05.

Table 5. Association Between Changes in Serum SCFAs and Changes in Cardiovascular Phenotypes Among the Females

Phenotypes	2-Methylbutyrate	Acetate	Butyrate	Hexanoate	Isobutyrate	Isovalerate	Propionate	Valerate
Office BP								
SBP	−2.45	−0.81	0.68	0.27	−2.36	−2.84*	1.51	−0.20
DBP	−0.30	−0.34	−0.10	−0.58	−0.79	−1.00	0.61	−0.80
MAP	−1.01	−0.45	0.15	−0.33	−1.31	−1.65*	0.86	−0.61
Ambulatory BP								
24-hour SBP	−2.26	−1.97	−0.37	−1.02	−2.89†	−2.50†	0.58	−1.38
24-hour DBP	−1.81*	−0.25	−0.51	0.26	−2.03†	−1.97‡	0.36	−0.32
Day SBP	−1.62	−2.23	−0.59	−0.24	−2.59*	−2.07*	0.40	−1.58
Day DBP	−0.91	0.13	−0.84	0.55	−1.08	−1.56*	0.69	−0.53
Night SBP	−2.35	−1.10	0.42	−1.82	−2.65*	−2.70†	1.09	−0.96
Night DBP	−1.70	0.23	0.28	0.11	−2.11*	−2.34‡	0.78	0.05
cf-PWV	−0.09	0.22	0.04	0.07	−0.12	−0.04	0.02	−0.28*
PP								
Office PP	−2.44*	−0.54	0.78	0.86	−1.78	−1.62	0.90	0.61
24-hour PP	−0.87	−1.84	−0.15	−1.24*	−1.20	−0.50	−0.70	−1.07
Day PP	−0.96	−1.92	−0.13	−0.75	−1.65*	−0.38	−1.01	−1.05
Night PP	−0.81	−1.44	0.02	−1.89†	−0.77	−0.47	0.09	−1.02

BP indicates blood pressure; cf-PWV, carotid-femoral pulse wave velocity; DBP, diastolic BP; MAP, mean arterial pressure; PP, pulse pressure; SBP, systolic BP; and SCFA, short-chain fatty acid.

*$P<0.05$, †$P<0.01$, and ‡$P<0.001$.

to establish a causal relationship that is modest dietary sodium reduction increased circulating SCFAs (in particular, butyrate, isobutyrate, 2-methylbutyrate, hexanoate, and valerate) in untreated hypertensives. Our findings were supported by several recent animal studies. Miranda et al reported that 4 weeks of high-salt diet treatment altered gut microbiota composition by decreasing *Lactobacillus* levels and decreased luminal butyrate production in mice.[9] Decreased *Lactobacillus* levels in the gut microbiota by high-salt diet were also observed in other studies.[8,20] However, fecal SCFAs (acetate, propionate, and isobutyrate but not butyrate) were found to be significantly elevated by high-salt diet in male Dahl salt-sensitive rats.[9] Moreover, Wilck et al[8] reported that a high-salt challenge conducted in 12 European adults reduced intestinal survival of *Lactobacillus spp*., increased Th 17 cells, and increased BP.

Recent work generated from SCFA receptor null mice suggests that SCFAs play a role in BP regulation.[18,21] SCFAs have an anti-inflammatory effect on both colonic epithelial and immune cells.[21] SCFAs are products from the fermentative activity of gut bacteria, subsequently absorbed into the bloodstream of the host, which can bind to and activate host receptors such as Olfr78 (olfactory receptor 78) and Gpr41 (G-protein coupled receptor 41), thereby acting as a messenger between gut microbial metabolism and host physiology.[11,18] Olfr78 is localized in the renal afferent arteriole, the site of renin storage and secretion. Olfr78 null mice have lowered plasma renin levels and lowered baseline BP.[22] Whereas Gpr41 is localized in the vascular endothelium, the Gpr41 null mice have isolated systolic hypertension with stiffer vessels.[23]

There is evidence that gut microbiota and serum metabolites are different between black and white American patients with hypertension.[24] However, there was no race/ethnicity interaction with sodium reduction on circulating SCFAs in our study. We did identify a sex difference in the present study. Our results showed that the effects of sodium reduction on SCFAs were significant in females, only not in males. Modest sodium reduction significantly increased serum levels of butyrate, isobutyrate, hexanoate, isovalerate, and valerate in untreated female hypertensives. Moreover, increased SCFAs by sodium reduction were associated with reduced BPs. Among the aforementioned animal studies, only male animals were used. To the best of our knowledge, our study is the only study to examine the effect of dietary sodium on SCFAs in humans. Further replications are needed. It is well recognized that there are sex differences in hypertension and CVD.[25] In line with our findings, some studies found larger BP elevation by sodium loading in women than in men.[26–28] An RCT found that there were no differences in hemodynamics after 1-week salt loading (200 mmol Na^+/day) in men while women had higher 24-hour SBP, daytime SBP, nighttime SBP, nighttime DBP, and mean arterial pressure.[26] The reason that women have greater sensitivity to dietary sodium intake is unclear. Several underlying mechanisms have been identified. For example, hypertensive females may have greater anti-inflammatory immune profiles.[25] Whereas endothelial nitric oxide production is sensitive to dietary sodium changes in men but not women.[29] Recently, sex differences in gut microbiota composition have been observed in several human studies.[30–32] Our findings suggest a novel mechanism that changes in gut microbiota composition leading to changes in circulating SCFAs may underlie the BP responses to dietary sodium reduction in females, but not in males. Other mechanisms, for example, endothelial nitric oxide pathway may play a role in

salt-sensitive hypertension in males. Sex differences in SCFA response to sodium reduction warrant further investigation.

We found that increased circulating levels of isobutyrate and isovalerate by sodium reduction were associated with decreased BPs in untreated female hypertensives. Gomez-Arango et al[33] observed that in overweight and obese pregnant women at 16 weeks gestation, the abundance of butyrate-producing bacteria and butyrate production in the gut microbiota is significantly negatively associated with BPs. In animal studies, SCFAs are also found to be negatively associated with BP. In rats, the infusion of acetate into the cecum prevented obstructive sleep apnea-induced hypertension.[34] Propionate significantly attenuated cardiac hypertrophy, fibrosis, vascular dysfunction, and hypertension in atherosclerosis mice model.[35] However, de la Cuesta-Zuluaga et al[36] found that higher fecal acetate, propionate, and butyrate were associated with higher BP in humans. Huart et al[37] also found higher stool levels of acetate, butyrate, and propionate in hypertensive versus normotensive individuals in men. The inconsistency of the findings between these studies and ours may be due to the difference in specimens used. Both de la Cuesta-Zuluaga and Huart used fecal SCFA excretion, which cannot be equated to SCFA production or absorption, and the association of higher fecal SCFA concentrations with obesity and cardiometabolic dysregulation may be due to less efficient absorption and utilization of these metabolites.[36] A human study showed that the participants with lower fecal acetate tended to have higher acetate absorption.[38] A recent study suggested that SCFAs measured in circulation might better represent SCFA absorption.[36] Future studies assessing both fecal and circulating SCFAs are needed.

The strengths of our study include that we leveraged a randomized, double-blind, placebo-controlled, cross-over trial of dietary sodium reduction with well-characterized CVD phenotypes. The cross-over RCT design enables each participant to serve as his/her own control, diminishing the inter-person variations. However, our study is limited by its relatively modest sample size and the lack of an independent replication sample. Moreover, it was a post hoc analysis of an RCT, which was not originally designed to test the effect of sodium reduction on SCFAs; therefore, the causal relationships between dietary sodium-driven changes in SCFAs and BP/cf-PWV cannot be established. Future RCTs are needed. Last but not least, raw *P* values were reported without multiple testing correction due to the modest sample size. Evidence shows that gut microbiota is different between hypertensive and normotensive individuals.[36] Our findings that sodium reduction increased circulating SCFAs were based on hypertensive patients. Whether circulating SCFAs have the same response to sodium reduction among normotensive population is unknown. Future replications in large scale RCTs, including both hypertensive and normotensive individuals, are warranted.

In conclusion, our results for the first time show that dietary sodium reduction increases the circulating levels of SCFAs among untreated hypertensive individuals, supporting the concept that dietary sodium may influence the gut microbiome in humans.[8] In addition, there was a sex difference in SCFA response to sodium reduction. Moreover, increased circulating SCFAs are associated with decreased BPs and improved arterial compliance.

Perspectives

Gut microbiota is involved in BP regulation and hypertension. SCFAs are products from the fermentative activity of gut bacteria. Our data show that moderate sodium reduction increases serum SCFAs, which are also associated with reduced BP and arterial stiffness. High dietary sodium intake may play a role in gut microbiota dysbiosis.

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Disclosures

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Novelty and Significance

What Is New?

- Dietary sodium reduction increases circulating short-chain fatty acids (SCFAs), suggesting that dietary sodium may influence the gut microbiome in hypertensives given that almost all circulating SCFAs are microbial in origin.
- There is a sex difference in SCFA response to sodium reduction, the increases are statistically significant only in females.
- Increased circulating SCFAs by sodium reduction are further associated with decreased blood pressure (BP) and improved arterial compliance in untreated patients with hypertension.

What Is Relevant?

- Animal studies suggest that microbiome is a key linkage in the causal relationship between sodium intake and elevated BP. Our study provides the evidence in hypertensives.
- SCFAs, the gut microbial metabolites, play a role in BP regulation in SCFA receptor null mice. Our study shows that changes in circulating SCFAs are associated with changes in BP in hypertensives.
- The sex difference of SCFA in response to sodium reduction observed in our study may contribute to the understanding of sex differences in hypertension and CVD.

Summary

Moderate dietary sodium reduction increases the circulating levels of SCFAs, which may mediate the beneficial effects of sodium reduction on cardiovascular health, such as reducing BP and arterial stiffness.