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Sodium Reduction, Metabolomic Profiling and CVD risk in Untreated Black Hypertensives: a Randomized, Double-Blind, Placebo-Controlled Trial

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Abstract

Dietary sodium restriction has multiple beneficial effects on cardiovascular health. The underlying mechanisms are not fully understood and the roles of metabolomics have been rarely studied. We aimed to test the hypothesis that the reduction in dietary sodium intake would induce changes in metabolomic profiling among black hypertensives, and the changes would be associated with reduced blood pressure and improved skin capillary density. A total of 64 untreated black hypertensives were included from a randomized crossover trial of sodium reduction. The participants were given either nine slow sodium tablets (10 mmol sodium per tablet) or placebo tablets daily for six weeks while on reduced-sodium diet aiming at achieving daily sodium intake around 2.0 grams. They then crossed over to receive the other tablets for another six weeks. Untargeted metabolomic profiling was performed in paired serum samples, which were collected at the end of each period, so as blood pressure and capillary density. Mixed-effects models were used. There were 34 metabolites identified with raw ps<0.05. Among those, two metabolites including beta-hydroxyisovalerate and methionine sulfone were significantly increased with sodium reduction (false discovery rate = 0.006 and 0.099, respectively). Increased beta-hydroxyisovalerate was associated with reduced office systolic blood pressure and ambulatory daytime systolic blood pressure; whereas increased methionine sulfone was associated with reduced 24-hour diastolic blood pressure, ambulatory nighttime diastolic blood pressure and increased skin capillary density. Our results suggest that dietary sodium reduction increases the circulating levels of beta-hydroxyisovalerate and methionine sulfone. Further studies are warranted.

Clinical Trial Registration: URL: http://www.clinicaltrials.gov. Unique identifier: NCT00152074.

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Disclosures
None.

Keywords

metabolite; sodium intake; hypertension; blood pressure; capillary

High sodium intake is recognized as one of the most important risk factors for hypertension, left ventricular hypertrophy, chronic kidney disease and heart failure. Modest dietary sodium reduction lowers blood pressure (BP), cardiovascular disease (CVD) and mortality.[1–3] In spite of these well-established relationships, the underlying biological mechanisms are not well understood.

Metabolomic profiling can detect small molecule metabolic products in response to environmental changes, which could serve as biomarkers or underlying mechanisms for biological response to dietary intervention. Recent studies showed that both Dietary Approaches to Stop Hypertension (DASH) and DASH-Sodium feeding trials were able to alter circulating metabolites.[4,5] We previously conducted a randomized double-blind, placebo-controlled crossover trial of modest dietary sodium reduction in untreated hypertensive patients living in the United Kingdom, showing that modest reduction in sodium intake reduced BP, improved large artery compliance[6] and both functional and structural capillary rarefactions.[7] In this study, we tested the hypothesis that the modest reduction in dietary sodium intake would induce changes in metabolomic profiles in an independent cohort of untreated hypertensive subjects. We further tested the hypothesis that the changes in metabolomic levels by modest sodium reduction would be associated with reduced BP, improved large artery compliance and increased skin capillary density.

Materials and Methods

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

Participants

The present study utilized stored serum samples from a previously conducted randomized double-blind, placebo-controlled, crossover trial of dietary sodium reduction in untreated hypertensives. The inclusion criteria were population aged 30 to 75 years, with sitting systolic blood pressure (SBP) 140 to 170 mmHg or diastolic blood pressure (DBP) 90 to 105 mmHg, and with no previous treatment for raised BP.[6] We focused on black hypertensives who tend to have a higher prevalence of salt-sensitive hypertension. All 64 black participants with serum samples available at both time points (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

A randomized, double-blind, placebo-controlled crossover trial was carried out (Figure S1, Supporting Information). In the first two week run-in period, participants were given

detailed advice by specially trained nurses on how to reduce their sodium intake, with an aim of achieving an intake of 2000 mg sodium/day (85 mmol/day). They were advised not to add salt at the table or during cooking, and avoid foods that contained large amount of sodium. Nurses went through with the participants on what foods they usually ate and identified items with high sodium content, and advised them to use low sodium alternatives. In appropriate cases, the spouse or whoever cooked in the household was also seen. 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, participants were given, in a random order, either nine slow sodium tablets (10 mmol sodium per tablet) or placebos daily for six weeks. They then crossed over to receive the other tablets for another six weeks.[6] Reduced-sodium diet plus slow sodium tablets represented usual sodium intake, while reduced-sodium diet plus placebo represented a reduced-sodium intake.

For compliance, 24h urinary sodium excretion (the gold standard method to estimate dietary sodium intake) was monitored at baseline, after two weeks on the reduced sodium diet, and at each clinic visit every 3 weeks during the crossover period using two consecutive 24h urine samples. Based on 24h urinary sodium excretion, the average sodium intake was reduced to 2640 mg/day after 2 weeks on the reduced sodium diet. This was slightly higher than the originally set target. During the crossover periods, the mean sodium intake was reduced from 3800 mg/day on slow sodium tablets to 2680 mg/day on placebo. The reduction was smaller than the originally set target. This is likely to be due to the poor compliance in some participants. Among the 64 participants, 13 (20%) either did not comply with the low sodium diet or failed to take the tablets as their 24-hour urinary sodium was not reduced from slow sodium to placebo period.

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 sitting position after 5 to 10 minutes rest. Three readings were taken and the average of the last two readings was used. Twenty-four-hour ambulatory blood pressure monitoring (ABPM) was performed using SpaceLabs 90207 devices (SpaceLabs, InC, Washington, DC) as previously described.[8] Briefly, monitoring was set to take measurements at half hourly intervals during the day and hourly intervals overnight. Recordings were analyzed with the ABPM report manager system software package.[8] Pulse pressure (PP) was calculated as the difference between SBP and DBP. Blood samples were taken at the end of each six-week period for measurements of routine biochemistry. Two consecutive 24h urine samples were collected during the last two days of each study period for measurements of urinary sodium excretion. The mean of the 2 urine measurements was used in the analysis. Carotid-femoral pulse wave velocity (cfPWV) was measured noninvasively using an automatic device Complior.[6]

Skin Capillary Density Measurement

Intravital Capillaroscopy Skin capillary density is a well-established dynamic method for studying skin capillaries and venous congestion is the most effective method for visualization of the maximal number of perfused skin capillaries.[9,10] Microscopic images were obtained with a charge-coupled device camera (Sony model XC-75CE) and were stored using a video recorder (JVC model HR-S6600). The skin of the dorsum and the side of the middle phalanx of the left hand were examined. Four microscopic fields (0.66 mm^2 per field) centered on an ink spot at each site were recorded continuously for 5 minutes to detect intermittently perfused capillaries. The number of capillaries per field was counted online and by running the recorded tapes using computer software (CapiScope, KK-Technology). To maximize the number of visible capillaries, venous congestion was carried out. A miniature neonatal BP cuff was applied to the base of the left middle finger. The cuff was inflated and maintained at 60 mmHg for 2 minutes. During the venous congestion, further images were recorded using 1 of the 4 microscopic fields chosen at random. Measures were averaged across the microscopic fields.

Metabolomic Profiling

Paired 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 64 participants. Global untargeted metabolomics profiling was performed in 128 serum samples in total by Metabolon. The sample preparation process was carried out using the automated MicroLab STAR® system (Hamilton Company, Salt Lake City, UT, USA). The extract was divided into five fractions: two for analysis by two separate reverse phase (RP)/ ultra-performance liquid chromatography (UPLC)- Mass Spectroscopy (MS)/MS methods with positive ion mode electrospray ionization (ESI), one for analysis by RP/UPLC-MS/MS with negative ion mode ESI, one for analysis by hydrophilic interaction liquid chromatography (HILIC)/ UPLC-MS/MS with negative ion mode ESI, and one sample was reserved for backup. All methods utilized a Waters ACQUITY UPLC and a Thermo Scientific Q-Exactive high resolution/accurate mass spectrometer interfaced with heated electrospray ionization (HESI-II) source and Orbitrap mass analyzer operated at 35,000 mass resolution. Raw data were extracted, peak-identified and quality control (QC) processed using Metabolon's hardware and software. Compounds were identified by comparison to library entries of purified standards or recurrent unknown entities. Peaks were quantified using area-under-the-curve.

A total of 870 serum metabolites were detected, among which 231 (27%) biochemicals have not been named and confirmed. Among the named 639 metabolites, we further excluded 44 (7%) metabolites for which more than 50% of samples had metabolite values below the detection limit. Finally, 595 identified metabolites with satisfying detection rates were entered into analysis.

Statistical Analysis

The general characteristics of the subjects are presented as mean ± standard deviation (SD) for continuous variables and N (%) for categorical variables. Two-tailed paired t-test was conducted to examine the differences in variables between placebo and sodium tablets.

Before statistical analysis, metabolomic data were log-transformed and standardized to unit variance and zero mean. Based on the intent-to-treat principle,[11] mixed-effects linear regression was used to assess the differential profiling of metabolites between sodium and placebo tablets while incorporating repeated measured data and controlling for age, sex and BMI as confounding variables. To correct for multiple testing, the raw *p*s were converted to false discovery rates (FDRs) according to the Bonferroni-Hochberg's correction.[12] As Bonferroni-Hochberg's correction for multiple hypotheses has been considered very conservative and may lead to a high number of false negatives, so we used a FDR of 0.10 for statistical significance.[4]

We further tested whether the changes in the identified metabolites were associated with the changes in BPs, PP, cfPWV and capillary density measurements. Mixed-effects model was used to examine the association between the levels of identified metabolites and phenotypes, which included SBP, DBP, PP 24-hour SBP, DBP, and PP, daytime SBP, DBP and PP, nighttime SBP, DBP and PP, cfPWV and skin capillary density measurements. $P < 0.05$ was considered statistically significant. We further conducted a sensitivity analysis to test whether identified serum metabolites are also associated with 24h urinary sodium excretion. All analyses were performed using Stata version 12.0 (StataCorp., College Station, Texas, USA).

Results

General Characteristics of the Participants

All black subjects with serum samples available were included (N=64), with mean age of 50.2 ± 9.5 years, mean BMI 30.9 ± 5.3 kg/m^2 and 50% males. Sodium reduction was associated with lowered BPs and cfPWV. In addition, sodium reduction was associated with increased skin basal capillaries at the side of the fingers, and associated with increased maximal capillary density (with venous congestion) at both dorsum and side of fingers (*p*s < 0.05), as previously reported (Table 1).[6]

Changes in Metabolites Associated with Sodium Reduction

There were 34 metabolites that responded to sodium reduction with raw *p*s < 0.05 (Table S1). The top 10 metabolites were altered by sodium reduction with raw *p*s < 0.01 (Table 2, Figure 1). Among those, six were upregulated by sodium reduction. The top two metabolites beta-hydroxyisovalerate (beta-hydroxy-beta-methylbutyric acid or HMB) and methionine sulfone remained significant after FDR correction (FDR = 0.006, 0.099, respectively).

Association of 24h urinary sodium excretion with changes in serum metabolites

We conducted a sensitivity analysis to test the associations between the changes in 24h urinary sodium excretion (the gold standard measure for dietary sodium intake) and the changes in serum metabolites, given the compliance of 80%. Reduced urinary sodium excretion was associated with increased HMB (β=0.45, p=0.001) and increased methionine sulfone (β=0.21, p=0.007).

Association of Changes in Metabolites with Changes in Cardiovascular Phenotypes

HMB and methionine sulfone, which were upregulated by sodium reduction, were associated with reduced BPs (ps < 0.05). In particular, HMB was associated with both casual and daytime ambulatory SBPs, whereas methionine sulfone was only associated with 24h ambulatory DBP and nighttime ambulatory DBP. In addition, increased HMB was associated with reduced PP. Moreover, upregulated methionine sulfone was associated with increased basal capillary density at the side of the fingers and maximal capillary density at the dorsum of the fingers. These results suggest that increased methionine sulfone was associated with increased microcirculation (ps<0.05, Table 3).

Discussion

The present study shows that modest dietary sodium reduction could change serum metabolite levels in untreated black hypertensives. In particular, HMB and methionine sulfone were significantly upregulated by sodium reduction. Moreover, upregulated HMB and methionine sulfone were associated with reduced BPs and PP. Increased methionine sulfone was also associated with increased skin capillary density.

HMB is a metabolite of the amino acid leucine and is produced endogenously through oxidation of the ketoacid of L-Leucine in both animals and humans. In healthy adults, supplementation with HMB also increased exercise-induced gains in muscle size, muscle strength, and lean body mass, reduced muscle damage from exercise.[13–16] Another RCT in highly-trained males in combat sport found that HMB not only increased muscle, but also reduced fat mass simultaneously.[15] HMB is a precursor for cellular cholesterol synthesis especially in tissues such as muscle that rely on *de novo* synthesis of cholesterol. In a series of small randomized placebo-controlled trials conducted in men and women, young and old, exercising or nonexercising, HMB supplementation 3 g/day for 3 to 8 weeks decreased total cholesterol by 5.8% and LDL cholesterol by 7.3%. In addition, HMB supplementation decreased SBP by 4.4 mmHg, suggesting having cardio-protective effects.[17] Our results showed that increased HMB levels by sodium reduction were associated with both reduced office SBP and daytime ambulatory SBP in untreated black hypertensives, which provide further evidence linking HMB to BP regulation. More independent RCTs of HMB supplementation on BP in humans are warranted.

The underlying mechanisms between HMB and BP regulation are not well understood. In a previous study, supplementation of HMB is found to be able to increase the levels of circulating insulin-like growth factor (IGF-1),[18] which is inversely related to SBP.[19] IGF-1 possesses a protective effect on the endothelium through anti-inflammatory and nitric oxide generation- a potential BP-lowering effect.[20, 21] Moreover, HMB also attenuates the circulating tumor necrosis factor-α (TNF-α) and angiotensin II (AngII) expression post-exercise,[22,23] both of which are pro inflammation factors and could elevate BP.[24,25]

Methionine sulfone is the irreversible final oxidation product of methionine. We also found that sodium reduction led to increased methionine sulfone, which was associated with decreased BP and improved capillary density. There is no clear conclusion about the biochemical function of methionine sulfone in previous studies. The DASH study

also found plasma methionine sulfone was increased when changing sodium intake from high to low, but the association failed to pass the Bonferroni's correction.[4] Another study found that sodium reduction was associated with increased urinary methionine sulfoxide in humans,[26] which was the intermediate oxidation product of methionine.[27] Protein methionine oxidation has recently been recognized as a potential molecular mechanism of redox regulation of protein function in vascular biology. Emerging data suggest that protein methionine oxidation may play a pathogenic role in atherosclerosis, ischemic heart disease, hypertension, and thrombosis.[27] Several proteins important in vascular biology have been shown to contain oxidation-sensitive methionine residues including calcium/calmodulin-dependent protein kinase II, apolipoprotein A-I, thrombomodulin (TM), and von Willebrand factor.[27]

In addition, methionine is also one of the methyl donors that increase DNA methylation.[28] Because of the circular nature of the methionine cycle, methionine excess may actually impair DNA methylation by inhibiting remethylation of homocysteine.[28] A study found that dietary methionine restriction induced secretion of cardio-protective hormones, such as adiponectin and fibroblast growth factor 21 (FGF21).[29] Methionine-restricted mice were also found to be resistant to diet-induced obesity and insulin resistance.[30] High methionine diet posed a cardiac threat by increasing oxidative stress, inflammatory manifestations, matrix/vascular remodeling, and decreased cardiac function in mice.[31] Methionine sulfone is an irreversible oxidation production of methionine.[27] The increase in methionine sulfone induced by sodium reduction may stimulate oxidation of methionine and therefore decreased methionine, which would be beneficial for cardiovascular health.

It is worth mentioning that changes in HMB were associated with changes in office SBP, and ambulatory daytime SBP, whereas changes in methionine sulfone were associated with changes in 24h ambulatory DBP and nighttime DBP in our study, suggesting these two metabolites may be involved in different pathophysiology as we discussed above. The mechanisms for elevated SBP differ from elevated DBP and mechanisms for elevated nocturnal BPs differ from overall or/and daytime BPs. Sodium reduction inducing BP reduction may be through various pathways.

Increased HMB was also associated with reduced office PP by sodium reduction. PP is a surrogate measure for proximal aortic stiffness. A meta-analysis shows that PP, not mean arterial pressure, is the major determinant of CVD risk.[32] PP is also an important predictor of incident atrial fibrillation in the 2 decades of prospective follow-up in the Framingham original and offspring cohorts.[33] PWV is another surrogate marker of arterial stiffness. Interestingly, we did not observe an association between HMB and PWV, suggesting these two markers may be involved in different mechanisms of arterial stiffness.

Derkach et al. examined the effect of sodium intake on metabolites in 119 participants from the DASH-Sodium feeding study including 73 participants at the end of their high- and low-sodium interventions and 46 participants at the end of high- and medium-sodium interventions using the Metabolon platform.[4] The participants in the DASH-Sodium study randomly received low- (1150 mg/day), medium- (2300 mg/day), and high-sodium (3450 mg/day) diet for 30 days each. There were 82 metabolites associated linearly with sodium

intake at a FDR ≤ 0.10 mostly when participants switched from high- to low- sodium intervention. Very few associations were found when participants switched from high sodium to medium sodium intervention; only three metabolites were identified when comparing high- to medium-sodium diet at raw *p*s<0.05, and none of which passed FDR correction. No participants were studied from the group switched from medium sodium to low sodium intervention. Our sodium reduction intervention is more comparable to the high- to medium sodium intervention in the DASH-Sodium study. There were 34 metabolites associated with sodium reduction with a raw p-value <0.05, 2 of them had FDR<0.1 in our study. In addition, methionine sulfone was also identified in the DASH-Sodium study. Characteristics of the participants and the intervention methods are the major differences between the DASH-Sodium study and ours. First, the DASH-Sodium study included 50% black participants, while our study examined black participants only. Second, 47% of the DASH-Sodium study participants were hypertensive, while all our participants were hypertensive. Third, the DASH-Sodium study was a feeding study that controlled both for sodium level and energy intake; whereas our participants were instructed to reduce sodium in their own diet, however, the sodium reduction was monitored and confirmed by 2 consecutive collections of 24-hour urinary sodium excretion. It is possible that the modest reduction was not sufficient to induce or observe significant changes in metabolites. However, as stated in the DASH-Sodium study, there was not enough evidence to confidently reject the hypothesis that sodium intake was linearly related to log-metabolite levels.[4]

The strengths of our study include that we utilized the sample from a well-controlled, randomized, double-blind clinical trial of dietary sodium reduction with well-characterized CVD phenotypes. The intervention was a cross-over design: each participant served as his/her own control, diminishing the inter-person variations. In addition, we focused on black hypertensives, in whom salt sensitivity and salt-sensitive hypertension are common. Compared to previous studies, our study was able to link identified metabolites with CVD phenotypes, providing mechanistic insights into cardio-beneficial effects of modest sodium reduction. The study is limited by its relatively modest sample size, and the lack of an independent replication sample. This study is also limited by the modest sodium reduction scale, which may reduce power for significant discovery. However, we replicate the previous findings based on DASH and DASH-Sodium samples that sodium reduction increases methionine oxidation pathway activities.[4,26]

In conclusion, our results show that dietary sodium reduction increases the circulating levels of HMB and methionine sulfone in untreated black hypertensive patients. Moreover, increased HMB or methionine sulfone is associated with reduced BP, arterial stiffness and increased capillary density.

Perspectives

Modest dietary sodium reduction is beneficial in preventing CVD. However, the underlying mechanisms are not well understood. Our global untargeted metabolomic profiling study shows that sodium reduction increases serum HMB and methionine sulfone levels, which

are also associated with reduced BP, arterial stiffness and increased skin capillary density in untreated black hypertensive patients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Novelty and Significance

What Is New?

- Dietary sodium reduction increases the serum levels of beta-hydroxyisovalerate (HMB) and methionine sulfone, which are further associated with decreased blood pressure (BP) in untreated black hypertensive individuals.
- In addition, increased HMB is associated with reduced pulse pressure, a surrogate marker for conduit arterial stiffness. Increased methionine sulfone is also associated with increased skin capillary density.

What Is Relevant?

- Our results provide new evidence linking both HMB and methionine sulfone to BP regulation. In addition, our results suggest that HMB and methionine sulfone play a role in the regulation of arterial stiffness and skin microcirculation.

Summary

Dietary sodium reduction increases the circulating levels of HMB and methionine sulfone, which may mediate the beneficial effects of modest sodium reduction on cardiovascular health such as BP, arterial stiffness and microcirculation.

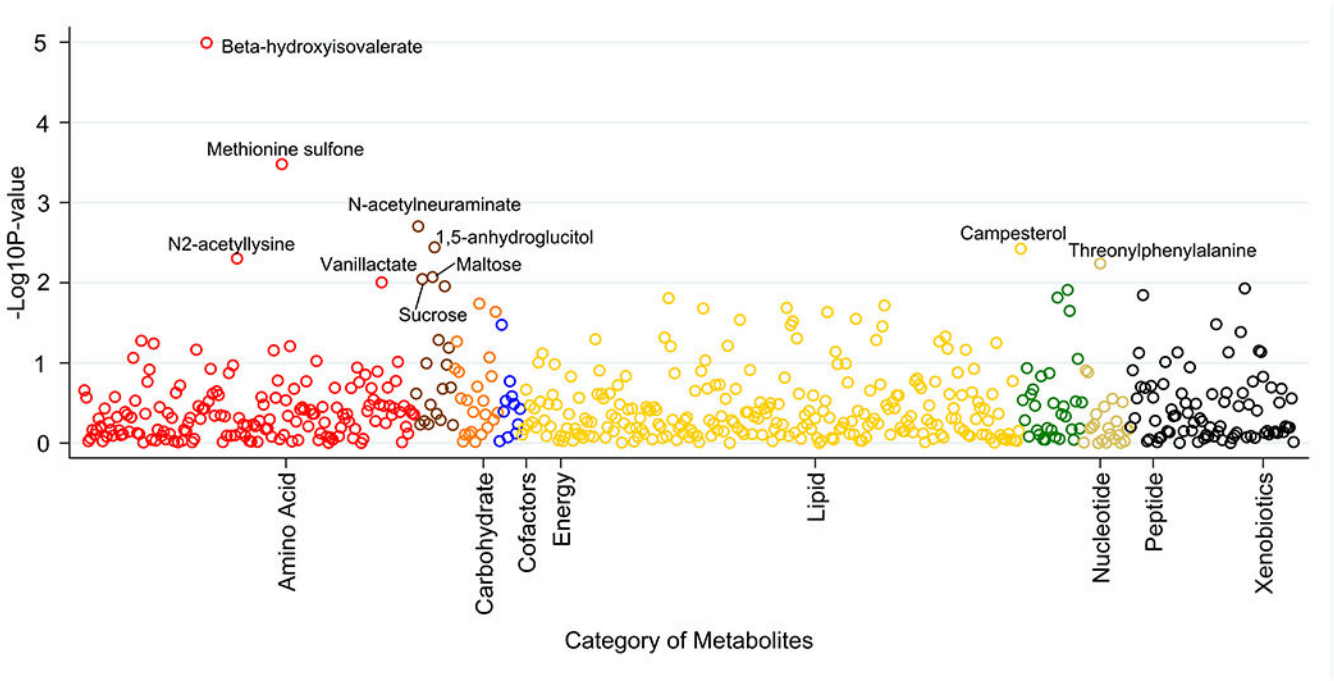

Figure 1. Plot of $-\log_{10}P$s for the metabolomic profiles in response to the sodium reduction.

*P*s were calculated based on the mixed effects models adjusted for age, sex and BMI as confounding variables. Associations with raw *p*s<0.01 are labeled with chemical names.

Table 1.

General characteristics of the participants

Characteristic	Sodium	Placebo	p
Age (years) *	50.2 ± 9.5	--	--
Male (%)	32 (50)	--	--
BMI (kg/m^2) *	30.9 ± 5.3	--	--
Urinary sodium (mmol/24 hours)	163.1 ± 60.7	115.2 ± 43.6	< 0.001
Office BP and pulse rate			
SBP (mmHg)	148.9 ± 13.5	143.7 ± 11.8	< 0.001
DBP (mmHg)	90.7 ± 9.1	88.2 ± 9.1	< 0.001
MAP (mmHg)	110.1 ± 9.4	106.7 ± 9.0	< 0.001
cfPWV	11.8 ± 2.1	11.2 ± 1.8	0.003
Ambulatory BP			
24-hour SBP (mmHg)	144.5 ± 8.4	140.1 ± 10.1	< 0.001
24-hour DBP (mmHg)	87.8 ± 8.9	85.4 ± 9.0	< 0.001
Day SBP (mmHg)	150.1 ± 9.3	144.9 ± 10.4	< 0.001
Day DBP (mmHg)	92.7 ± 9.7	89.9 ± 9.6	< 0.001
Night SBP (mmHg)	138.2 ± 9.7	134.1 ± 12.1	< 0.001
Night DBP (mmHg)	82.4 ± 9.3	80.4 ± 9.8	0.017
Pulse pressure			
Office PP (mmHg)	58.2 ± 11.3	55.4 ± 9.9	0.007
24-hour PP (mmHg)	56.8 ± 6.6	54.7 ± 6.7	<0.001
Day PP (mmHg)	57.3 ± 7.4	55.0 ± 7.4	<0.001
Night PP (mmHg)	55.8 ± 7.4	53.8 ± 7.5	0.004
Skin temperature (°C)	30.6 ± 2.4	30.8 ± 2.1	0.536
Capillary density by capillaroscopy			
Dorsum of the fingers			
At resting (capillaries/mm^2)	67.6 ± 15.0	70.5 ± 16.0	0.053
With venous congestion (capillaries/mm^2)	70.4 ± 14.8	76.6 ± 16.2	<0.001
Side of the fingers			
At resting (capillaries/mm^2)	63.9 ± 16.2	68.3 ± 17.8	<0.001
With venous congestion (capillaries/mm^2)	69.0 ± 17.4	73.4 ± 18.8	0.005

*Age and BMI were only measured once.

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Table 2.

Top ten metabolites associated with sodium reduction

Rank	Metabolites	β*	p	FDR	Super pathway	Sub pathway
1	Beta-hydroxyisovalerate	0.56	<0.001	0.006	Amino Acid	Leucine, Isoleucine and Valine Metabolism
2	Methionine sulfone	0.23	<0.001	0.099	Amino Acid	Methionine, Cysteine, SAM and Taurine Metabolism
3	N-acetylneuraminate	0.30	0.002	0.393	Carbohydrate	Aminosugar Metabolism
4	1,5-anhydroglucitol	0.11	0.004	0.448	Carbohydrate	Glycolysis, Gluconeogenesis, and Pyruvate Metabolism
5	Campesterol	−0.26	0.004	0.448	Lipid	Sterol
6	N2-acetyllysine	0.30	0.005	0.489	Amino Acid	Lysine Metabolism
7	Threonylphenylalanine	−0.36	0.006	0.489	Peptide	Dipeptide
8	Maltose	−0.35	0.008	0.562	Carbohydrate	Glycogen Metabolism
9	Sucrose	−0.39	0.009	0.562	Carbohydrate	Disaccharides and Oligosaccharides
10	Vanillactate	0.23	0.009	0.562	Amino Acid	Tyrosine Metabolism

*β is the regression coefficient of intervention in the mixed-effects model adjusted for age, sex and BMI, reflects metabolomic profiling change from sodium tablets to placebo tablets.

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Table 3.

Association between changes in metabolites with changes in blood pressure and CVD phenotypes

Phenotypes	Beta-hydroxyisovalerate	Methionine sulfone	N-acetylneuraminate	1,5-anhydroglucitol	Campesterol	N2-acetyllysine	Threonylphenylalanine	Maltose	Sucrose	Vanillactate
Office BP										
SBP	−2.37*	−1.67	−1.34	−0.41	3.75†	−3.01†	1.00	1.16	2.04*	−1.13
DBP	−1.00	−1.14	−1.80*	0.30	2.00†	−2.02†	0.48	0.22	0.67	−1.24
MAP	−1.41*	−1.45	−1.77*	0.21	2.72‡	−2.40†	0.65	0.52	1.14	−1.31
cfPWV	−0.17	−0.16	−0.22	−0.26	−0.07	−0.15	−0.13	0.21	0.07	−0.07
Ambulatory BP										
24-hour SBP	−1.14	−1.37	−2.00*	1.11	1.26	−0.84	0.64	0.90	1.60*	−0.56
24-hour DBP	−0.79	−2.03*	−1.88†	0.09	1.50*	−0.50	0.69	0.56	1.09*	−1.44*
Day SBP	−1.78*	−0.92	−2.35*	1.23	1.66	−1.44	1.53	1.09	1.84*	0.27
Day DBP	−1.17	−1.73	−2.10†	0.14	2.01*	−0.67	0.99	0.77	1.17	−1.32
Night SBP	−0.46	−1.42	−1.68	1.13	0.74	−0.58	−0.58	0.98	2.10*	−0.92
Night DBP	−0.45	−2.00*	−1.54*	0.14	0.88	−0.71	−0.42	0.55	1.67†	−1.40
Pulse pressure										
Office PP	−1.56*	−0.65	0.66	−1.23	2.16*	−1.39	1.11	1.15	1.33	0.22
24-hour PP	−0.57	0.32	−0.12	0.83	0.25	−0.39	0.64	0.56	0.41	1.00
Day PP	−0.85	0.4	−0.19	0.73	0.33	−0.74	1.2*	0.54	0.44	1.55*
Night PP	−0.15	0.49	−0.15	0.87	0.15	0.00	0.26	0.57	0.42	0.7
Microcirculation										
Skin temperature	−0.04	0.10	0.28	−0.26	−0.02	−0.02	−0.07	0.30	−0.02	0.05
Capillary density by capillaroscopy										
Dorsum of the fingers										
At resting	1.76	3.26	1.95	0.82	1.61	1.90	−0.47	1.02	1.13	1.67
With venous congestion	2.10	3.93*	2.66	−0.48	1.95	3.24*	−1.26	−0.15	0.66	0.94

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Phenotypes	Beta-hydroxyisovalerate	Methionine sulfone	N-acetylneuraminate	1,5-anhydroglucitol	Campesterol	N2-acetyllysine	Threonylphenylalanine	Maltose	Sucrose	Vanillactate
Side of the fingers										
At resting	1.68	3.38*	3.18†	−0.73	−1.00	0.28	−1.33	−0.71	0.12	1.15
With venous congestion	1.98	3.18	3.65†	−0.65	−0.61	−0.05	−1.79	0.14	0.40	1.39

*$p<0.05$,

†$p<0.01$,

‡$p<0.001$.