Associations of sodium and potassium consumption with the gut microbiota and host metabolites in a population-based study in Chinese adults

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ABSTRACT

Background: There is increasing evidence that sodium consumption alters the gut microbiota and host metabolome in murine models and small studies in humans. However, there is a lack of population-based studies that capture large variations in sodium consumption as well as potassium consumption.

Objective: We examined the associations of energy-adjusted dietary sodium (milligrams/kilocalorie), potassium, and sodium-to-potassium (Na/K) ratio with the microbiota and plasma metabolome in a well-characterized Chinese cohort with habitual excessive sodium and deficient potassium consumption.

Methods: We estimated dietary intakes from 3 consecutive validated 24-h recalls and household inventories. In 2833 adults (18–80 y old, 51.2% females), we analyzed microbial (genus-level 16S ribosomal RNA) between-person diversity, using distance-based redundancy analysis (dbRDA), and within-person diversity and taxa abundance using linear regression, accounting for geographic variation in both. In a subsample ($n = 392$), we analyzed the overall metabolome (dbRDA) and individual metabolites (linear regression). *P* values for specific taxa and metabolites were false discovery rate adjusted (*q*-value).

Results: Sodium, potassium, and Na/K ratio were associated with microbial between-person diversity (dbRDA $P < 0.01$) and several specific taxa with large geographic variation, including pathogenic *Staphylococcus* and *Moraxellaceae*, and SCFA-producing *Phascolarctobacterium* and *Lachnospiraceae* (q-value < 0.05). For example, sodium and Na/K ratio were positively associated with *Staphylococcus* and *Moraxellaceae* in Liaoning, whereas potassium was positively associated with 2 genera from *Lachnospiraceae* in Shanghai. Additionally, sodium, potassium, and Na/K ratio were associated with the overall metabolome (dbRDA $P \leq 0.01$) and several individual metabolites, including butyrate/isobutyrate and gut-derived phenolics such as 1,2,3-benzenetriol sulfate, which was negatively associated with sodium in Guizhou (q-value < 0.05).

Conclusions: Our findings suggest that sodium and potassium consumption is associated with taxa and metabolites that have been implicated in cardiometabolic health, providing insights into the potential roles of gut microbiota and host metabolites in the pathogenesis of sodium- and potassium-associated diseases. More studies are needed to confirm our results. *Am J Clin Nutr* 2020;112:1599–1612.

Keywords: diet, population-based cohort, Chinese, adult, CVD, sodium, potassium, sodium-to-potassium ratio, gut microbiota, host metabolome

Introduction

Excessive dietary sodium intake and inadequate dietary potassium intake contribute to hypertension and cardiovascular disease (CVD) (1–3), through mechanisms involving the renin-angiotensin-aldosterone system and oxidative stress (4, 5). Recent advances in high-throughput sequencing have revealed that intestinal microbes are dependent on diet and may have fundamental impacts on host metabolome and physiology, including blood pressure regulation (6, 7). Therefore, elucidating the relations between key diet risk factors, such as sodium and potassium, with the gut microbiota and circulating metabolites is essential in understanding the roles of microbiota and related metabolites in diet-associated diseases.

Evidence from murine models suggests that a high-sodium diet changes fecal microbiota composition and function, including depletion of *Lactobacillus* and increases in fecal SCFAs and microbial-dependent intestinal T-helper 17 cells (7–10). Concomitant treatment with *Lactobacillus murinus* prevented sodium-induced hypertension in mice (7), indicating that the gut microbiome could be a potential therapeutic target for

sodium-associated diseases. In addition, metabolomics studies have revealed potential pathways underlying the sodium–health relations. In 119 US adults from the crossover sodium-intake feeding trial within the Dietary Approaches to Stop Hypertension (DASH)–Sodium trial, reduced sodium intake was associated with increased plasma metabolites from the microbiota-mediated tryptophan and benzoate metabolic pathways (11), such as 4-ethylphenylsulfate, which has been linked to lean body mass in adults (12). In a double-blinded, crossover trial of 64 untreated UK patients with hypertension, sodium reduction was associated with elevated serum methionine sulfone and β-hydroxyisovalerate, which were associated with reduced diastolic and systolic blood pressure in the same sample, respectively (13). However, there is a lack of population-based studies that capture large variations in sodium consumption with paired microbiome and metabolomics data for a more comprehensive investigation of these complex relations. There has also been a lack of microbiome and metabolomics studies examining dietary sodium and potassium in Asians, who have higher sodium intakes and different sodium sources than Whites, Hispanics/Latinos, and Blacks (14).

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Supplemental Tables 1–13 and Supplemental Figures 1–11 are available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/ajcn/.

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Diet, microbiota, and metabolome data described in the manuscript, code book, and analytic code will be made available upon request pending application and approval. Other questionnaire data (e.g., education, physical activity, smoking) are publicly and freely available without restriction at https://www.cpc.unc.edu/projects/china.

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Abbreviations used: CHNS, China Health and Nutrition Survey; CNTCS, China Nutritional Transition Cohort Study; CVD, cardiovascular disease; DASH, Dietary Approaches to Stop Hypertension; dbRDA, distance-based redundancy analysis; FCT, food-composition table; MDS, multiple dimension scaling; Na/K ratio, sodium to potassium ratio; NSAID, nonsteroidal anti-inflammatory drug; OTU, operational taxonomic unit; PPI, proton pump inhibitor; RMSE, root mean square error; rRNA, ribosomal RNA.

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To address the above knowledge gaps, we used data from a population-based cohort of Chinese adults with habitual high sodium and low potassium intakes (1, 15) to study 2 questions. First, we examined the association between sodium and potassium consumption with gut microbiota in 2833 adults from 12 provinces and 3 megacities. Second, to further understand potential biological responses to sodium and potassium, we examined the association between sodium and potassium consumption with plasma metabolites in a subsample of 392 adults from 2 southern provinces.

Methods

Study sample

We used data from the China Health and Nutrition Survey (CHNS) collected in 2015 during the fall (primarily) and winter. The CHNS is a household-based, longitudinal study across 12 provinces (Heilongjiang, Liaoning, Shaanxi, Henan, Hubei, Jiangsu, Shandong, Zhejiang, Guangxi, Guizhou, Hunan, Yunnan) and 3 megacities (Beijing, Shanghai, Chongqing) that varied substantially in geography, economic development, public resources, and health indicators, as previously described (16). The study met the standards for the ethical treatment of participants and was approved by the Institutional Review Boards of the University of North Carolina at Chapel Hill and the National Institute for Nutrition and Health, Chinese Center for Disease Control and Prevention. Informed consents were obtained for all participants.

For this study, eligible participants were adults aged 18–80 y who had gut microbiome and diet data ($n = 3156$; **Supplemental Figure 1**). Participants were excluded if they had used antibiotics within the past 6 mo, ever had inflammatory bowel disease, irritable bowel syndrome, or bowel removal, or currently had diarrhea ($n = 217$). Participants were further excluded if they were pregnant ($n = 1$) or had extreme energy intake (<500 kcal; $n = 8$), implausible sodium consumption (>10 milligrams/kilocalorie; $n = 1$), or were missing covariates ($n = 96$), resulting in a microbiota analysis sample of 2833 adults, among whom 905 were from the China Microbiome Study and 1928 were from the China Nutritional Transition Cohort Study (CNTCS), which are 2 subcohorts of the CHNS. A subset of 392 adults living in adjacent provinces (Hunan and Guizhou) were included in the metabolomics analysis sample.

Diet assessment

Dietary data were collected by trained interviewers using 3 consecutive 24-h diet recalls during home visits randomly from Monday to Sunday to ensure a mixture of weekdays and weekends were collected. Additionally, household-level consumption was determined by measuring the changes in all household foods and condiments, using digital kitchen scales (graduation: 1 g), during the 3-d period of 24-h recall collection. First, proportions of household-level consumption of foods and condiments were allocated to each household member based on the proportions reported in individual 24-h recalls. Then, individual-level diet data were linked with a Chinese food-composition table (FCT) that includes >2500 foods (1) to

estimate daily sodium and potassium intakes (i.e., averages across 3 d). The sodium and potassium contents of a few imported foods were estimated using Taiwan, Hong Kong, Japan, or the USDA FCTs (1). The protocol for total energy measurement was validated by doubly labeled water (Pearson correlation coefficient: males, 0.56; females, 0.60) (17). We also conducted a validation test for sodium and potassium consumption derived from our 3-d household food inventories and 24-h recalls in an independent sample using 24-h urinary sodium and potassium excretions across 3 consecutive days and para-aminobenzoic acid as a marker for completion of 24-h urine collection (1). We found strong accuracy of our dietary measures for sodium (correlation = 0.58) and potassium (correlation = 0.59) (1). We standardized sodium and potassium by total energy intake in units of milligrams per kilocalorie to account for over- and underreporting and the correlation with energy intake, and divided sodium by potassium to calculate the sodium to potassium ratio (Na/K ratio). We defined excessive sodium ($\geq$2 g) and deficient potassium ($<$3.5 g) consumption according to the WHO (18). We dichotomized fried-food intake by any/no consumption and calculated percentage of calories (%kcal) from animal-source foods by dividing energy intake from animal-source foods by the total energy intake.

Gut microbiome data collection and processing

Stool samples were collected at home from participants who had been trained to use the QIAGEN collection kit following standardized procedures from a modified Human Microbiome Project protocol (19). Samples were temporarily stored in foam boxes with frozen cold packs and brought to the local village or community clinics immediately, where the samples were stored at −20°C. Then, samples were transported to the laboratory in cold chain shipping and stored at −80°C until processing. Samples were randomized by provinces and megacities for sequencing at Novogene Bioinformatics Technology Co, Ltd. Bacterial DNA was extracted using TIANGEN DNA extraction kits (TIANGEN Biotech), and 16S ribosomal RNA (rRNA) sequencing targeting the V4 hypervariable region was performed using primers 515F/806R on the Illumina HiSeq PE-250 platform (Illumina, Inc). The sequencing generated 12,528–77,104 sequences in CNTCS and 21,648–89,427 sequences in the China Microbiome Study. The raw sequencing reads were processed using the QIIME pipeline (20), with forward and reverse reads merged with fastq-join and filtered using a minimum quality score of 20. Operational taxonomic units (OTUs) were identified using the open-reference method based on a threshold of 0.97. Chimeric OTUs were detected by ChimeraSlayer (21) and removed. Taxonomy was assigned based on the SILVA databases (release 128). A total of 1472 genera were detected. We normalized and $\log_{10}$ transformed the raw taxonomic counts as follows to correct for different sequencing depth (22):

$$\log_{10}\left(\frac{\text{taxa } j \text{ count for sample } i}{\text{total taxa count in sample } i} \times \text{average number of taxa count per sample} + 1\right) \quad (1)$$

No sample was filtered out due to low quality.

Plasma metabolomics profiling

Fasting blood samples were collected by certified technicians at each field center within 3 d of fecal sample collection using venipuncture with EDTA as an anticoagulant, centrifuged for 15 minutes at 3000 × *g* to prepare plasma, and stored at −80°C until analyzed. All sites followed the same protocol for the collection, processing, and storage. The nontargeted metabolomics analysis was performed using an integrated, ultra-high-performance LC-tandem MS (UPLC-MS/MS) consisting of Waters ACQUITY UPLC coupled to a Thermo Scientific Q-Exactive high-resolution MS (ThermoFisher) at Metabolon's joint DIAN campus in China. More detailed information on the Metabolon platform has been described elsewhere (23). Briefly, plasma samples were extracted using methanol solvent and analyzed with several types of controls, including extracted water samples as process blanks and pooled experimental samples as technical replicates. Signals in the metabolomics data were extracted and peak identified, with quality control processed using Metabolon's software and hardware. Chemicals were identified and differentiated by matching to the mass-to-charge ratio, retention time/index, and chromatographic data in the Metabolon reference library of authenticated standards, which was created by acquiring data for >3300 purified standard compounds analyzed under the same conditions as the study samples. The metabolomics analysis resulted in the detection of 1108 compounds in our sample. Metabolon rescaled the raw area under the peaks of each metabolite within the same run day to a median of 1 (i.e., median-normalization) to correct for differences in instrument interday tuning, with values below detection limits imputed by the minimum value. We $\log_2$ transformed the metabolite abundance to ensure normality.

Measurements of other host factors

Sociodemographic and behavioral data were collected using interviewer-administered questionnaires. Urbanization was measured using a validated urbanization index that encompasses 12 dimensions of urbanization, such as population density, health infrastructure, and transportation (24). Household income was estimated by income activities of all household members. Total physical activity in metabolic-equivalents/week was estimated from 7-d recalls of occupational, transportation, domestic, and leisure activities. We categorized urbanization index, per-capita household income, and physical activity by tertiles. We grouped occupation into 5 categories: not working, agricultural workers (e.g., farmer, fisherman, hunter), laborers (e.g., craftsman, logger), professional (e.g., doctor, teacher), and manager (e.g., government official, director). We dichotomized educational attainment by high school completion. We defined smokers as individuals who ever smoked cigarettes and alcohol consumers as individuals who drank alcohol during the past year.

Statistical analysis

In descriptive analysis, we compared continuous variables and categorical variables across provinces and megacities using ANOVA and chi-square test, respectively. Primary outcomes

were gut microbial measures at the genus level. We first examined the associations of sodium density, potassium density, and Na/K ratio with microbial diversity measured using the R package vegan (25). For within-person diversity (α-diversity) assessed by Shannon index and richness (number of distinct genera per subject), we used a linear regression. For between-person diversity (β-diversity), we used distance-based redundancy analysis (dbRDA) (26) based on Bray-Curtis distance, a multivariate analysis that did not provide the direction of associations, followed by an ANOVA test with 999 permutations to estimate P value. We then used a linear regression to assess the associations of sodium density, potassium density, and Na/K ratio with 159 specific taxa, after filtering rare taxa presented in <25% of participants to account for spurious findings. All analyses were adjusted for the following potential confounders based on a priori knowledge: age, sex, provinces or megacities (1), batch or plate runs, urbanization (19), occupation, education, income, total energy intake, %kcal from animal-source foods (the strongest indicator of a westernized diet in China) (27), fried-food intake, physical activity (28), smoking (29), alcohol, pro-/prebiotic intake in the past month, nonsteroidal anti-inflammatory drug (NSAID) intake in the past 2 wk, and proton pump inhibitor (PPI) intake in the past month (30). In addition, we conducted a secondary analysis at the OTU level using linear regression for individual OTUs ($n = 256$ after excluding rare OTUs) to contribute to understanding the variation in genus-level results.

Given that another study of our group found large geographic variation (provinces and megacities) in the CHNS gut microbiota sample (unpublished data), we accounted for this geographic variation by including province/megacity (categorical variable) and an interaction term of sodium density, potassium density, or Na/K ratio with province/megacity in the model. In addition to a test for interaction, we assessed the overall association using a joint analysis that simultaneously tests the main effect (sodium density, potassium density, or Na/K ratio) and interaction term for province/megacity (31). This joint test is common in genetic studies with potential interactions since it offers more statistical power than other methods in the presence of interaction and comparable statistical power to other methods when there is no interaction (31). The interaction and joint analyses were examined using the Wald test in linear regression and using partial dbRDA conditioned on the rest of the model variables. For α- and β-diversity measures, the interaction term was removed if the P value for interaction was >0.10. Additionally, to test the difference in province- and megacity-specific coefficients (32), we conducted random-effects meta-analysis and a test of heterogeneity (33), adjusted by the Hartung-Knapp-Sidik-Jonkman method (34).

In the subsample, we repeated the above analyses for metabolomics data (secondary outcome) using dbRDA for the overall metabolome and linear regression for individual metabolites, with adjustment of batch run. We conducted exploratory analysis of the associations between sodium density–, potassium density–, and/or Na/K ratio–associated taxa and metabolites using multivariable-adjusted linear regression. To assess which data had the strongest association with sodium density, potassium density, and Na/K ratio intakes, we compared prediction accuracies of these dietary outcomes by host factor (18 model covariates), microbiota, metabolite data, all possible pairs of these 3 datasets, and the combination of all 3 datasets, using random forest regressions (100 trees) (35). We conducted pairwise comparisons of root mean squared errors (RMSEs) of each model using the 5 iterations of a 2-fold cross-validation modified paired t test, which is powered to compare the performance of learning algorithms with acceptable type I error (36).

We conducted statistical analyses in R 3.6.0 (http://www.r-project.org) and Python 3.5.1 (https://www.python.org). All statistical tests were 2-sided. For comparisons across all taxa and all metabolites in linear regression, P values were adjusted using the Benjamini-Hochberg method (false discovery rate, q-value) (37) for sodium density, potassium density, and Na/K ratio separately as part of each test of 3 separate hypotheses for sodium density, potassium density, and Na/K ratio.

Results

Sample characteristics

The microbiota analysis sample had 51.2% females and a mean age of 51.6 y (**Tables 1** and **2**). Gut microbial α-diversity (Shannon index and richness), physical activity, urbanization, income, education, occupation, and intakes of sodium (3372.8–4775.4 mg), potassium (1335.6–1884.0 mg), Na/K ratio (2.3–3.7), energy, animal-source foods, fried food, and pre-/probiotics were different across provinces and megacities ($P < 0.001$). The megacity Chongqing had the highest Shannon index and richness and the lowest potassium intake, whereas Shanghai, a megacity with 59.2% and 53.1% of participants at a high urbanization and income level, respectively, had the highest potassium and the lowest Na/K ratio intake. Yunnan, one of the least urbanized provinces (55.4% at the low urbanization level), had the lowest Shannon index and animal-source food intake, but the highest Na/K ratio intake, while Zhejiang, one of the provinces with the highest income (54.5% at the high income level), had the highest sodium intake. In the metabolomics analysis sample, Hunan had higher urbanization and intakes of sodium and fried food but lower microbial richness than Guizhou ($P < 0.001$; **Supplemental Table 1**).

Microbiota analysis

First, we evaluated the overall measures of the gut microbiota composition and found that sodium density, potassium density, and Na/K ratio were not associated with α-diversity measures (**Table 3**) but were associated with β-diversity, which varied across provinces and megacities (interaction and joint test dbRDA, $P < 0.01$; **Table 4**). The microbial β-diversity was visualized with multiple dimension scaling (MDS) in **Supplemental Figures 2–4**, which show no clear separation of microbiota by sodium density, potassium density, or Na/K ratio.

Then, we examined specific taxa and found that at a joint test q-value <0.10, sodium density was associated with 8 taxa, including *Staphylococcus, Moraxellaceae, Phascolarctobacterium, Salinicoccus*, and *Jeotgalicoccus* (**Figure 1**A); potassium density was associated with 30 taxa, including *Pseudomonas*, *Staphylo-*

TABLE 1 Characteristics of the gut microbiota analysis sample by provinces and megacities, part 1[1]

	Total	Beijing	Heilongjiang	Liaoning	Shaanxi	Henan	Jiangsu	Shandong	Shanghai
n	2833	112	206	127	110	325	134	118	130
Shannon index[2]	2.6 (0.3)	2.5 (0.3)	2.6 (0.3)	2.7 (0.3)	2.5 (0.3)	2.5 (0.3)	2.6 (0.3)	2.6 (0.3)	2.5 (0.4)
Richness[3]	94.6 (40.2)	79.3 (24.1)	91.6 (34.9)	91.3 (25)	87.5 (21.2)	91.3 (37.2)	86.2 (14.8)	84.4 (34.7)	112.7 (66.6)
Age, y	51.6 (12.6)	50.8 (12.8)	51.3 (12.7)	52.2 (14.7)	50.1 (14.6)	52.2 (11.3)	52.5 (14.5)	52.1 (13.9)	50.6 (13.9)
Females, *n* (%)	1450 (51.2)	53 (47.3)	111 (53.9)	63 (49.6)	54 (49.1)	181 (55.7)	66 (49.3)	56 (47.5)	65 (50)
Sodium,[4] mg	4188.1 (2176.9)	4163.9 (2553.5)	4619.6 (2379.4)	4123.2 (2267.5)	3948.1 (2293.9)	4526.1 (2307.2)	4294.8 (2102.5)	4274.4 (2020.5)	3913.9 (2041.1)
Sodium density,[4] mg/kcal	2.4 (1.4)	2.6 (1.9)	2.7 (1.4)	2.6 (1.5)	2.1 (1.4)	2.5 (1.5)	2.3 (1.1)	2.2 (1.1)	2.2 (1.1)
Excessive sodium,[5] *n* (%)	2517 (88.8)	97 (86.6)	189 (91.8)	106 (83.5)	92 (83.6)	301 (92.6)	122 (91.0)	103 (87.3)	116 (89.2)
Potassium,[4] mg	1575.5 (682.7)	1633.1 (734.9)	1545.6 (605.9)	1535 (643.1)	1582.7 (765.0)	1420.0 (636.0)	1675.5 (679.2)	1678.1 (707.2)	1884.0 (856.9)
Potassium density,[4] mg/kcal	0.8 (0.3)	1.0 (0.6)	0.9 (0.2)	0.9 (0.3)	0.8 (0.3)	0.7 (0.3)	0.9 (0.3)	0.8 (0.2)	1.0 (0.4)
Deficient potassium,[5] *n* (%)	2775 (97.9)	110 (98.2)	203 (98.5)	126 (99.2)	107 (97.3)	319 (98.2)	132 (98.5)	113 (95.8)	123 (94.6)
Na/K ratio[4]	3.0 (2.0)	2.8 (1.8)	3.4 (2.3)	3.0 (1.9)	3.0 (2.4)	3.7 (2.7)	2.9 (1.5)	2.8 (1.5)	2.3 (1.3)
Energy intake,[4] kcal	1906.2 (623.5)	1709.8 (573.3)	1829.3 (631.0)	1644.4 (566.6)	2104.0 (724.8)	2040.0 (682.9)	1941.8 (632.1)	2051.9 (624.7)	1837.8 (533.4)
Animal-source foods,[4] %kcal	18.2 (13.4)	12.6 (8.7)	9.3 (8.8)	12.5 (11.3)	25.7 (13.5)	7.0 (8.9)	16.6 (12.6)	11.9 (9.2)	20.1 (10.8)
Fried-food intake,[4] *n* (%)	720 (25.4)	60 (53.6)	62 (30.1)	36 (28.4)	34 (30.9)	82 (25.2)	39 (29.1)	49 (41.5)	55 (42.3)
Ever smoked, *n* (%)	1109 (39.2)	39 (34.8)	80 (38.8)	52 (40.9)	36 (32.7)	131 (40.3)	54 (40.3)	43 (36.4)	41 (31.5)
Alcohol use past year, *n* (%)	850 (30.0)	38 (33.9)	53 (25.7)	40 (31.5)	45 (40.9)	94 (28.9)	34 (25.4)	41 (34.7)	23 (17.7)
Pre-/probiotics use past month, *n* (%)	18 (0.6)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	11 (3.4)	0 (0.0)	0 (0.0)	0 (0.0)
NSAID use past 2 wk, *n* (%)	35 (1.2)	3 (2.7)	2 (1.0)	1 (0.8)	1 (0.9)	5 (1.5)	0 (0.0)	2 (1.7)	3 (2.3)
PPI use past month, *n* (%)	18 (0.6)	0 (0.0)	2 (1.0)	2 (1.6)	0 (0.0)	2 (0.6)	1 (0.7)	1 (0.8)	1 (0.8)
Physical activity,[6] *n* (%)									
Low	944 (33.3)	42 (37.5)	74 (35.92)	44 (34.65)	24 (21.8)	124 (38.2)	42 (31.3)	46 (39.0)	52 (40.0)
Middle	949 (33.5)	46 (41.1)	71 (34.5)	42 (33.1)	41 (37.3)	86 (26.5)	43 (32.1)	43 (36.4)	56 (43.1)
High	940 (33.2)	24 (21.4)	61 (29.6)	41 (32.3)	45 (40.9)	115 (35.4)	49 (36.6)	29 (24.6)	22 (16.9)
Urbanization,[7] *n* (%)									
Low	937 (33.1)	0 (0.0)	109 (52.9)	35 (27.6)	37 (33.6)	175 (53.8)	28 (20.9)	26 (22.0)	0 (0.0)
Middle	957 (33.8)	45 (40.2)	16 (7.8)	36 (28.4)	52 (47.3)	120 (36.9)	40 (29.9)	83 (70.3)	53 (40.8)
High	939 (33.2)	67 (59.8)	81 (39.3)	56 (44.1)	21 (19.1)	30 (9.2)	66 (49.2)	9 (7.6)	77 (59.2)
Income,[8] *n* (%)									
Low	948 (33.5)	12 (10.7)	50 (24.3)	24 (18.9)	34 (30.9)	171 (52.6)	25 (18.7)	25 (21.2)	6 (4.6)
Middle	943 (33.3)	38 (33.9)	76 (36.9)	40 (31.5)	32 (29.1)	92 (28.3)	43 (32.1)	51 (43.2)	55 (42.3)
High	942 (33.2)	62 (55.4)	80 (38.8)	63 (49.6)	44 (40.0)	62 (19.1)	66 (49.2)	42 (35.6)	69 (53.1)
High school completion, *n* (%)	1030 (36.4)	88 (78.6)	80 (38.8)	55 (43.3)	43 (39.1)	85 (26.2)	48 (35.8)	54 (45.8)	90 (69.2)
Occupation,[9] *n* (%)									
Not working	1410 (49.8)	55 (49.11)	109 (52.9)	51 (40.2)	41 (37.3)	213 (65.5)	62 (46.3)	61 (51.7)	59 (45.4)
Agricultural worker	343 (12.1)	0 (0.0)	30 (14.6)	19 (15.0)	20 (18.2)	36 (11.1)	4 (3.0)	4 (3.4)	0 (0.0)
Laborer	673 (23.8)	20 (17.)	24 (11.6)	35 (27.6)	28 (25.5)	50 (15.5)	52 (38.8)	26 (22.0)	37 (28.5)
Professional	203 (7.2)	22 (19.6)	20 (9.7)	11 (8.7)	13 (11.8)	18 (5.5)	5 (3.7)	7 (5.9)	7 (5.4)
Manager	204 (7.2)	15 (13.4)	23 (11.2)	11 (8.7)	8 (7.3)	8 (2.5)	11 (8.2)	20 (17.0)	27 (20.8)

(*Continued*)

TABLE 1 (*Continued*)

	Total	Beijing	Heilongjiang	Liaoning	Shaanxi	Henan	Jiangsu	Shandong	Shanghai
High blood pressure,[10] *n* (%)	1844 (65.1)	70 (62.5)	144 (69.9)	94 (74.0)	65 (59.1)	256 (78.8)	99 (73.9)	86 (72.9)	81 (62.3)
Overweight,[10] *n* (%)	1412 (50.0)	64 (57.1)	129 (62.6)	78 (61.4)	45 (40.9)	191 (59.32)	72 (53.7)	69 (58.5)	61 (47.3)

[1]Values are means (SDs) unless noted as *n* (%). Provinces and megacities were compared using ANOVA for continuous variables and chi-square test for categorical variables. MET, metabolic equivalent; Na/K ratio, sodium to potassium ratio; NSAID, nonsteroidal anti-inflammatory drug; PPI, proton-pump inhibitor.

[2]Shannon index at the genus level was calculated using $-\sum p_i ln p_i$, where p_i is the proportional abundance of genera i.

[3]Richness measured the number of distinct genera per subject.

[4]Nutrient intakes estimated by 3 consecutive dietary recalls, household food inventories, and a Chinese food-composition table. Sodium density and potassium density were calculated using absolute sodium and potassium intakes divided by total energy intake, respectively.

[5]Excessive sodium (≥2 g) and deficient potassium (<3.5 g) consumption was defined according to the WHO recommendation (18).

[6]Physical activity measured via 7-d recalls in METs/week was categorized by tertiles to represent low (≤40.8 METs/wk), medium (40.8–144.5 METs/wk), and high (>144.5 METs/wk) levels of physical activity.

[7]Urbanization index, a 12-component scale that includes population density, economic activity, transportation infrastructure, sanitation, etc., to define and distinguish urbanicity, was categorized by tertiles to represent low (≤63), medium (63.4–84.3), and high (>84.3) levels of urbanization.

[8]Per capita household income was categorized by tertiles to represent low (≤9.4k Yuan), medium (9.4–22.4k Yuan), and high (>22.4k Yuan) levels of income.

[9]Occupation was categorized into the following types: unemployed, agricultural worker (e.g., farmer, fisherman, hunter), laborer (e.g., craftsman, logger), professional (e.g., doctor, teacher), manager (e.g., government official, director), and other (e.g., athlete, artist).

[10]High blood pressure was defined as systolic blood pressure or diastolic blood pressure ≥130/80 mmHg or self-reported high blood pressure. Overweight was defined as BMI (weight/height^2) ≥24 kg/m^2. Blood pressure, weight, and height were measured by trained examiners.

coccus, Dorea, *Leuconostocaceae*, and *Oscillospira* (Figure 1B); and Na/K ratio was associated with 54 taxa, including *Moraxellaceae, Pseudomonas, Lactobacillales, Staphylococcus*, and *Microbacterium* (Figure 1C). These associations showed large geographic variations. Province- and megacity-specific model estimates and scatterplots are shown in **Supplemental Tables 2–4** and **Supplemental Figures 5–7**, respectively. For example, sodium density was negatively associated with *Moraxellaceae* in Beijing (coefficient: −0.10; 95% CI: −0.17, −0.04), but positively associated with it in Liaoning (0.14; 0.07, 0.21) and Shanghai (0.18; 0.09, 0.27). Next, we estimated the combined province- and megacity-specific model estimates and tested the heterogeneity across these estimates using meta-analysis and tests of heterogeneity, respectively. We found that most of the combined estimates were not statistically significant ($0.070 \leq P \leq 0.997$; **Supplemental Table 5**) with large heterogeneity across provinces and megacities ($P \leq 0.039$, $I^2 \geq 50\%$), except for *Eggerthella* (Na/K ratio: −0.01; −0.03, −0.00), *Blautia* (Na/K ratio: −0.01; −0.02, −0.00), and *Propionibacteriaceae* (potassium density: −0.06; −0.11, −0.01; Na/K ratio: 0.01; 0.00, 0.02).

Secondary analysis using OTU-level data was consistent with genus-level results. We observed large geographic variation in the following associations at a joint test *q*-value <0.10, sodium density with *Phascolarctobacterium* (**Supplemental Table 6**); potassium density with 6 OTUs, including *Dorea, Ruminococcaceae_UCG-014*, and *Weissella* (**Supplemental Table 7**); and Na/K ratio with 36 OTUs, including *Ruminococcus_2*, *Lachnospiraceae*, *Blautia*, *Phascolarctobacterium*, *Megamonas*, *Ruminococcaceae_UCG-014*, *Catenibacterium*, *Coprococcus_2*, *Clostridium_sensu_stricto_1*, *Akkermansia*, *Ruminococcus_1*, and *Prevotella* (**Supplemental Table 8**).

Metabolomics analysis

We first examined the overall metabolome and found that sodium density, potassium density, and Na/K ratio were associated with the overall metabolome at $P \leq 0.01$ (**Table 5**), which were visualized with MDS in **Supplemental Figures 8–10**. Then, for individual metabolites, we found that at a joint test *q*-value <0.10, sodium density was associated with N6-methyladenosine from the purine metabolic pathway and 3 gut-derived phenolics: 1,2,3-benzenetriol sulfate, 3-methoxycatechol sulfate, and 4-methylcatechol sulfate (**Figure 2**A); potassium density was associated with 6-oxopiperidine-2-carboxylate from the lysine metabolic pathway (Figure 2B); and Na/K ratio was associated with 15 metabolites, including a fibrinogen cleavage peptide (DSGEGDFXAEGGGVR), N6-methyladenosine, thyroxine, 2 eicosanoids (5-HETrE, 5-HETE), and the microbiota-mediated SCFAs butyrate/isobutyrate and isovalerate (also a branched-chain amino acid intermediary) (Figure 2C). We show province-specific estimates and scatterplots in **Supplemental Tables 9–11** and **Supplemental Figure 11**, respectively. Additionally, we found that the combined province-specific estimates were not statistically significant in meta-analysis ($0.312 \leq P \leq 0.958$; **Supplemental Table 12**), with large heterogeneity between provinces ($P \leq 0.009$, $I^2 \geq 85.2\%$), except for the association between potassium density and 6-oxopiperidine-2-carboxylate (test of heterogeneity, $P = 0.061$).

TABLE 2 Characteristics of the gut microbiota analysis sample by provinces and megacities, part 2[1]

	Zhejiang	Chongqing	Guangxi	Guizhou	Hubei	Hunan	Yunnan	*P*
n	123	125	412	283	117	390	121	
Shannon index[2]	2.5 (0.3)	2.7 (0.3)	2.6 (0.3)	2.6 (0.3)	2.5 (0.3)	2.6 (0.3)	2.4 (0.3)	<0.001
Richness[3]	87.7 (43.7)	174.1 (59.2)	92.8 (27.2)	96.8 (45)	91.2 (25.7)	85.5 (27.7)	90.8 (26.9)	<0.001
Age, y	52 (13.3)	52 (14.7)	51.3 (10.6)	50.8 (12.0)	52.2 (14.4)	52 (11.2)	51.1 (14.3)	0.911
Females, *n* (%)	63 (51.2)	60 (48.0)	207 (50.2)	148 (52.3)	53 (45.3)	208 (53.3)	62 (51.24)	0.873
Sodium,[4] mg	4775.4 (2620.4)	3524.5 (1642.1)	4148 (2018.4)	3372.8 (1472.0)	4102.2 (1864.3)	4387.7 (2193.0)	4518.3 (2654.4)	<0.001
Sodium density,[4] mg/kcal	2.9 (1.6)	2.3 (1.3)	2.1 (1.0)	2 (1.0)	2.3 (1.1)	2.5 (1.5)	2.8 (1.9)	<0.001
Excessive sodium,[5] *n* (%)	114 (92.7)	104 (83.2)	383 (93.0)	234 (82.67)	109 (93.2)	345 (88.5)	102 (84.3)	<0.001
Potassium,[4] mg	1578.2 (585.6)	1335.6 (554.6)	1625.9 (623.7)	1449.8 (709.5)	1690.3 (758.1)	1687.9 (685.0)	1379.5 (610.8)	<0.001
Potassium density,[4] mg/kcal	0.9 (0.4)	0.8 (0.3)	0.8 (0.3)	0.8 (0.3)	0.9 (0.3)	0.9 (0.3)	0.8 (0.3)	<0.001
Deficient potassium,[5] *n* (%)	122 (99.2)	125 (100.0)	405 (98.3)	279 (98.6)	113 (96.6)	379 (97.2)	119 (98.3)	0.177
Na/K ratio[4]	3.3 (1.9)	3.1 (1.8)	2.8 (1.4)	2.8 (1.8)	2.7 (1.6)	2.9 (1.9)	3.7 (3.0)	<0.001
Energy intake,[4] kcal	1752.7 (533.0)	1670.4 (519.0)	2069.8 (622.6)	1843.4 (533.6)	1894.3 (565.3)	1931.7 (649.2)	1764.9 (558.2)	<0.001
Animal-source foods,[4] %kcal	19.1 (11.0)	22.5 (12.3)	26.1 (12.9)	25.4 (13.2)	14.4 (9.5)	24.7 (11.3)	6.4 (8.1)	<0.001
Fried-food intake,[4] *n* (%)	39 (31.7)	18 (14.4)	22 (5.3)	43 (15.2)	36 (30.8)	118 (30.3)	27 (22.3)	<0.001
Ever smoked, *n* (%)	35 (28.5)	52 (41.6)	174 (42.2)	112 (39.6)	55 (47.0)	164 (42.1)	41 (33.9)	0.117
Alcohol use past year, *n* (%)	44 (35.8)	44 (35.2)	126 (30.6)	89 (31.4)	45 (38.5)	98 (25.1)	36 (29.8)	0.002
Pre-/probiotics use past month, *n* (%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	7 (1.8)	0 (0.0)	<0.001
NSAID use past 2 wk, *n* (%)	1 (0.8)	0 (0.0)	1 (0.2)	7 (2.5)	2 (1.7)	7 (1.8)	0 (0.0)	0.254
PPI use past month, *n* (%)	2 (1.6)	1 (0.8)	2 (0.5)	0 (0.0)	1 (0.9)	3 (0.8)	0 (0.0)	0.855
Physical activity,[6] *n* (%)								<0.001
Low	29 (23.6)	49 (39.2)	104 (25.2)	98 (34.6)	39 (33.3)	145 (37.2)	32 (26.4)	
Middle	56 (45.5)	34 (27.2)	132 (32.0)	96 (33.9)	40 (34.2)	122 (31.3)	41 (33.9)	
High	38 (30.9)	42 (33.6)	176 (42.7)	89 (31.5)	38 (32.5)	123 (31.5)	48 (39.8)	
Urbanization,[7] *n* (%)								<0.001
Low	29 (23.6)	51 (40.8)	159 (38.6)	125 (44.2)	17 (14.5)	79 (20.3)	67 (55.4)	
Middle	62 (50.4)	17 (13.6)	75 (18.2)	100 (35.3)	40 (34.2)	202 (51.8)	16 (13.2)	
High	32 (26.0)	57 (45.6)	178 (43.2)	58 (20.5)	60 (51.3)	109 (28.0)	38 (31.4)	
Income,[8] *n* (%)								<0.001
Low	19 (15.5)	56 (44.8)	225 (54.6)	85 (30.0)	27 (23.1)	138 (35.4)	51 (42.2)	
Middle	37 (30.1)	29 (23.2)	133 (32.3)	94 (33.2)	49 (41.9)	136 (34.9)	38 (31.4)	
High	67 (54.5)	40 (32.0)	54 (13.1)	104 (36.8)	41 (35.0)	116 (29.7)	32 (26.6)	
High school completion, *n* (%)	52 (42.3)	38 (30.4)	101 (24.5)	80 (28.3)	41 (35.0)	137 (35.1)	38 (31.4)	<0.001
Occupation,[9] *n* (%)								<0.001
Not working	63 (51.2)	66 (52.8)	162 (39.3)	130 (45.9)	50 (42.7)	222 (56.9)	66 (54.6)	
Agricultural worker	3 (2.4)	12 (9.6)	80 (19.4)	57 (20.1)	18 (15.4)	40 (10.3)	20 (16.5)	
Laborer	28 (22.8)	25 (20.0)	161 (39.1)	55 (19.4)	34 (29.1)	81 (20.8)	17 (14.1)	
Professional	15 (12.2)	15 (12.0)	4 (1.0)	20 (7.1)	9 (7.7)	27 (6.9)	10 (8.3)	
Manager	14 (11.4)	7 (5.6)	5 (1.2)	21 (7.4)	6 (5.1)	20 (5.1)	8 (6.6)	

(*Continued*)

TABLE 2 (*Continued*)

	Zhejiang	Chongqing	Guangxi	Guizhou	Hubei	Hunan	Yunnan	*P*
High blood pressure,[10] *n* (%)	68 (55.3)	76 (60.8)	266 (64.6)	166 (58.7)	66 (56.4)	243 (62.3)	64 (52.9)	<0.001
Overweight,[10] *n* (%)	55 (44.7)	53 (42.4)	152 (37.3)	150 (53.0)	52 (44.8)	189 (48.5)	52 (43.0)	<0.001

[1]Values are means (SDs) unless noted as *n* (%). Provinces and megacities were compared using ANOVA for continuous variables and chi-square test for categorical variables. MET, metabolic equivalent; Na/K ratio, sodium to potassium ratio; NSAID, nonsteroidal anti-inflammatory drug; PPI, proton-pump inhibitor.

[2]Shannon index at the genus level was calculated using $-\sum p_i ln p_i$, where p_i is the proportional abundance of genera *i*.

[3]Richness measured the number of distinct genera per subject.

[4]Nutrient intakes estimated by 3 consecutive dietary recalls, household food inventories, and a Chinese food-composition table. Sodium density and potassium density were calculated using absolute sodium and potassium intakes divided by total energy intake, respectively.

[5]Excessive sodium (≥2 g) and deficient potassium (<3.5 g) consumption was defined according to the WHO recommendation (18).

[6]Physical activity measured via 7-d recalls in METs/week was categorized by tertiles to represent low (≤40.8 METs/wk), medium (40.8–144.5 METs/wk), and high (>144.5 METs/wk) levels of physical activity.

[7]Urbanization index, a 12-component scale that includes population density, economic activity, transportation infrastructure, sanitation, etc., to define and distinguish urbanicity, was categorized by tertiles to represent low (≤63), medium (63.4–84.3), and high (>84.3) levels of urbanization.

[8]Per capita household income was categorized by tertiles to represent low (≤9.4k Yuan), medium (9.4–22.4k Yuan), and high (>22.4k Yuan) levels of income.

[9]Occupation was categorized into the following types: unemployed, agricultural worker (e.g., farmer, fisherman, hunter), laborer (e.g., craftsman, logger), professional (e.g., doctor, teacher), manager (e.g., government official, director), and other (e.g., athlete, artist).

[10]High blood pressure was defined as systolic blood pressure or diastolic blood pressure ≥130/80 mmHg or self-reported high blood pressure. Overweight was defined as BMI (weight/height^2) ≥24 kg/m^2. Blood pressure, weight, and height were measured by trained examiners.

Integrated analysis of microbiota and metabolite data

We examined the associations between sodium density–, potassium density–, and/or Na/K ratio–associated taxa ($n = 67$) and metabolites ($n = 18$) and found that *Coriobacteriaceae* and *Ruminococcaceae* were positively associated with 4-methylcatechol sulfate (q-value < 0.10; **Supplemental Table 13**). In random forest analysis assessing microbiota or metabolite data as a whole, we found that metabolite data and the combinations of metabolite + host factor data, microbiota + metabolite data, and microbiota + metabolite + host factor data had higher accuracy (lower RMSE, $P < 0.05$) than microbiota data in predicting sodium density and Na/K ratio (**Figure 3**). Metabolite data and host factor data had comparable accuracies in predicting sodium density, potassium density, and Na/K ratio, and adding microbiota data to the combination of metabolite + host factor data made no difference in the prediction accuracy of these 3 diet outcomes.

Discussion

In this study, we investigated the association of dietary sodium and potassium consumption with the gut microbiota and host metabolites in a population-based cohort of Chinese adults with habitual excessive sodium intake and deficient potassium intake (1, 15). We found that, independent of a wide range of sociodemographic and behavioral factors and after accounting for geographic variations, energy-adjusted sodium (i.e., density in milligrams/kilocalorie), potassium, and Na/K ratio were associated with the microbial between-person diversity (β-diversity) and several microbial groups, including infectious pathogens, such as *Staphylococcus* (38) and *Pseudomonas* (39), and taxa that have been linked to CVD risk factors, such as *Dorea* (40), *Ruminococcus*, *Ruminococcaceae* (41), and *Lachnospiraceae* (42). In subsample analysis, we found that dietary sodium, potassium, and Na/K ratio were associated with the overall metabolome and several metabolites involved in inflammation and etiology of CVD, including 3 gut-derived phenolics (1,2,3-benzenetriol sulfate, 3-methoxycatechol sulfate, and 4-methylcatechol sulfate) (43) and 2 SCFAs (butyrate/isobutyrate and isovalerate) (44). These results suggest that the gut microbiota and related metabolites may play important roles in sodium- and potassium-associated diseases.

Our findings add evidence to the sodium–microbiota associations in a large, free-living human population and were consistent with previous studies (7–10). A high-sodium diet has been found to alter the gut microbiota composition and function in murine models, as reflected by decreases in *Lactobacillus* and increases in *Lachnospiraceae*, *Ruminococcus*, and fecal SCFA concentrations (7–10). Moreover, there is little known about microbiota associated with potassium, another well-established dietary risk factor for CVD (1–3). The sodium- and potassium-associated taxa we found have been implicated in CVD risk. For example, *Dorea* and *Ruminococcus* were positively associated with BMI in Swedish adults (40), *Lachnospiraceae* and *Ruminococcaceae* were related to lower long-term weight gain in females from TwinsUK (41), *Lachnospiraceae* and *Blautia* were correlated with metabolic impairment in Austrian older adults (42), and *Eggerthella* and *Prevotella* were associated with hypertension in Chinese adults (45, 46). Furthermore,

TABLE 3 Associations of sodium density, potassium density, and Na/K ratio with within-person gut microbial diversity measures[1]

	Sodium density		Potassium density		Na/K ratio	
	Coefficient (95% CI)	*P*	Coefficient (95% CI)	*P*	Coefficient (95% CI)	*P*
Shannon index[2]	0.00 (−0.01, 0.01)	0.554	0.02 (−0.02, 0.05)	0.419	0.00 (−0.00, 0.01)	0.566
Richness[3]	0.38 (−0.68, 1.44)	0.483	− 2.28 (−6.72, 2.17)	0.316	0.29 (−0.41, 0.98)	0.418

[1] $n = 2833$. The linear regression model was adjusted for age, sex, provinces or megacities, batch or plate run, urbanization, occupation, income, education, total energy intake, %kcal from animal-source foods, fried-food intake, physical activity, smoking, alcohol intake, pro-/prebiotic intake, nonsteroidal anti-inflammatory drug intake, and proton-pump inhibitor intake. Interaction with province/megacity was removed from all models because *P* values were >0.10. Na/K ratio, sodium to potassium ratio.

[2] Shannon index at the genus level was calculated using $-\sum p_i ln p_i$, where p_i is the proportional abundance of genera i.

[3] Richness measured the number of distinct genera per subject.

we found that, in Liaoning, Henan, and Shanghai, sodium consumption was positively associated with pathogenic bacteria including *Staphylococcus*, which causes a wide variety of severe infections (38), and *Moraxellaceae*, a biomarker for Crohn disease (47), indicating that high sodium intake may increase the susceptibility to gut infection and inflammation. Indeed, sodium exposure has been shown to enhance proinflammatory cytokine production in human intestinal mononuclear cells and a high-sodium diet exacerbated colitis in mice (48). That we did not observe a clear separation of the gut microbiota composition by sodium and potassium consumption, but found an association between sodium and potassium consumption with the overall microbiome after accounting for potential confounders, was likely because the gut microbiome of our free-living participants was determined by numerous host factors, among which dietary sodium and potassium only explained 0.07–0.11% of variance in the microbiome.

A probable mechanism of how the gut microbiota influence host physiology is through SCFAs, such as acetate, butyrate, and propionate, which are primarily produced from bacterial fermentation of carbohydrates (44). Animal studies suggest that SCFAs are potentially beneficial to cardiometabolic health by modulating gut barrier function, immunomodulation, glucose homeostasis, and blood pressure (44, 49). However, human observational studies suggest that higher concentrations of fecal and plasma SCFAs are associated with poorer gut health and higher obesity and hypertension risk (50, 51). We found that Na/K ratio consumption was positively associated with isovalerate and butyrate/isobutyrate in Guizhou. Similarly, the DASH crossover sodium-intake feeding trial showed that plasma isovalerate was reduced after a month-long sodium-restricted diet (11). In addition, we found that, in Guizhou Province, sodium consumption was negatively associated with 1,2,3-benzenetriol sulfate, 3-methoxycatechol sulfate, and 4-methylcatechol sulfate, which are phenolics derived from microbiota conversion of dietary polyphenols that potentially have anti-inflammatory bioactivity (43). These results are consistent with studies showing high-sodium–induced inflammation in mice (7, 48). Our integrated analysis of microbiota and metabolites showed that the Na/K ratio–associated *Coriobacteriaceae* and *Ruminococcaceae* were positively associated with 4-methylcatechol sulfate. *Coriobacteriaceae* has been shown to be involved in phenolic conversion (52). However, we may lack statistical power to detect associations for other microbiota-mediated metabolites, such as SCFAs, in the subsample. Larger population samples with diet, microbiota, and metabolite data are needed to allow a more complex integrated analysis.

The strengths of our study include the well-characterized, population-based cohort with large variations in sodium and potassium consumption. Host factors collected from standardized and validated instruments allowed us to control for a wide range of potential confounders. Our paired microbiota and metabolite data enabled us to compare the association between sodium and potassium consumption with microbiota versus

TABLE 4 Associations of sodium density, potassium density, and Na/K ratio with between-person gut microbial diversity[1]

	Sodium density		Potassium density		Na/K ratio	
	Interaction[2]	Joint[3]	Interaction[2]	Joint[3]	Interaction[2]	Joint[3]
R^2,[4] %	0.07		0.11		0.10	
Sum of squares	0.88	0.93	1.02	1.06	1.18	1.25
F	1.32	1.31	1.53	1.50	1.78	1.76
P value	0.002	0.003	0.001	0.001	0.001	0.001

[1] $n = 2833$. The distance-based redundancy analysis (dbRDA) model was adjusted for age, sex, provinces or megacities, batch or plate run, urbanization, occupation, income, education, total energy intake, %kcal from animal-source foods, fried-food intake, physical activity, smoking, alcohol intake, pro-/prebiotic intake, nonsteroidal anti-inflammatory drug intake, and proton-pump inhibitor intake. Na/K ratio, sodium to potassium ratio.

[2] The interaction of sodium density, potassium density, or Na/K ratio with province/megacity.

[3] The joint tests of sodium density, potassium density, or Na/K ratio with its interaction with province/megacity. Pseudo *F* statistics and *P* values of the interaction and joint test were obtained from partial dbRDA conditioned on the rest of the model variables followed by an ANOVA test with 999 permutations.

[4] R^2 was estimated in a univariate dbRDA model for sodium density, potassium density, or Na/K ratio.

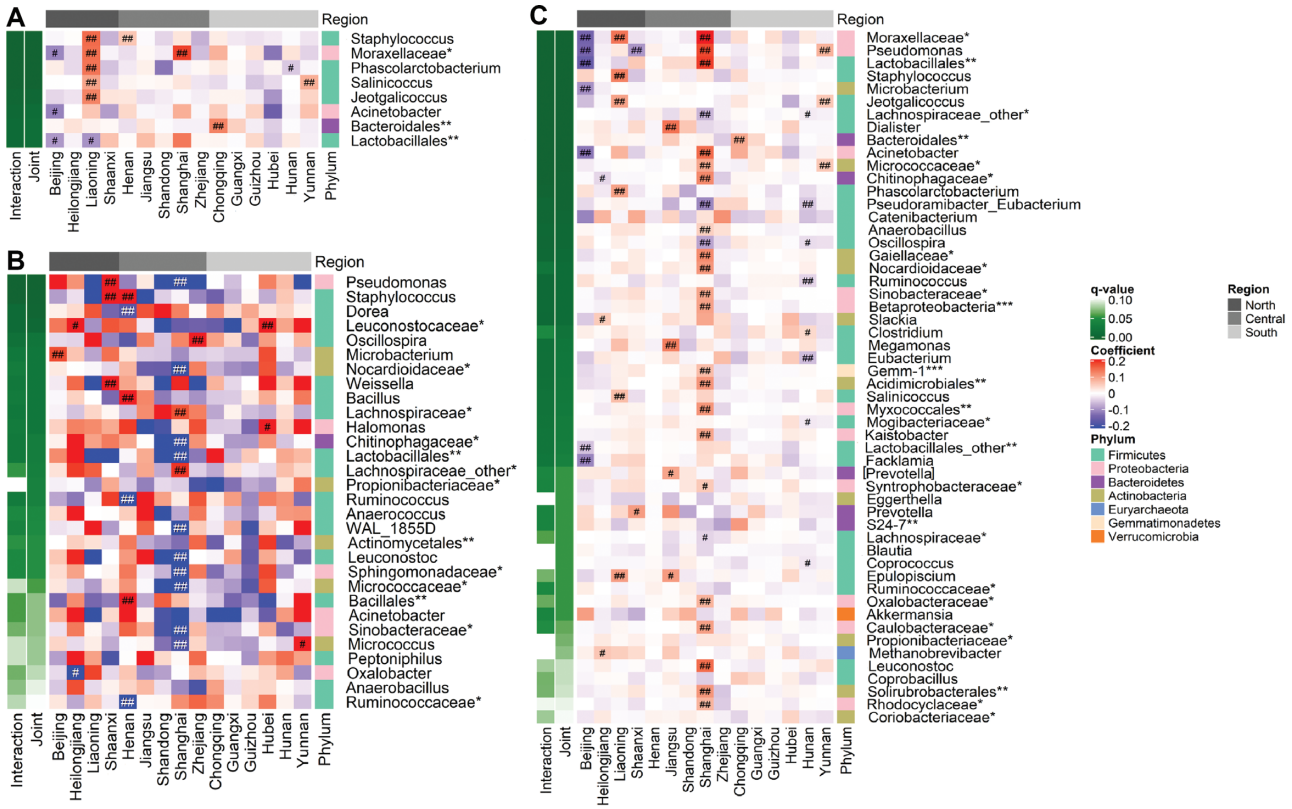

FIGURE 1 Heatmap of associations between sodium density (A), potassium density (B), and Na/K ratio (C) with specific taxa. $n = 2833$. Color and shading of the heatmap indicate the direction and magnitude of model coefficient. Taxa were ordered by joint test q-value (false discovery rate–adjusted P value) and provinces and megacities were ordered by region. The linear regression model was adjusted for age, sex, provinces or megacities, batch or plate run, urbanization, occupation, education, income, total energy intake, %kcal from animal-source foods, fried-food intake, physical activity, smoking, alcohol intake, pro-/prebiotic intake, nonsteroidal anti-inflammatory drug intake, and proton pump inhibitor intake. "Interaction" indicates interaction with province/megacity; "Joint" indicates joint test of the main and interaction effects. *Unknown genera from family; **unknown genera from order; ***unknown genera from class. $^{\#}q$-values <0.10 and $^{\#\#}q$-values <0.05 for province- and megacity-specific estimates. Na/K ratio, sodium to potassium ratio.

metabolites. We found that, while the combination of microbiota and metabolite data had higher predictive accuracy for sodium and Na/K ratio than microbiota data alone, adding microbiota data to metabolite data did not improve the predictive accuracy for sodium, potassium, and Na/K ratio, indicating that these dietary factors had stronger associations with plasma metabolites than with gut microbiota. Additionally, our large and diverse cohort allowed us to examine potential effect modification by geographic locations, which explained the largest variation (17.9%) in gut microbiota in our sample compared with all other

TABLE 5 Associations of sodium density, potassium density, and Na/K ratio with the overall plasma metabolome[1]

	Sodium density		Potassium density	Na/K ratio	
	Interaction[2]	Joint[3]		Interaction[2]	Joint[3]
R^2,[4] %	0.53		0.47	0.34	
Sum of squares	0.13	0.24	0.17	0.14	0.24
F	1.55	1.44	2.01	1.70	1.42
P value	0.010	0.009	0.002	0.007	0.007

[1] $n = 392$. The distance-based redundancy analysis (dbRDA) model was adjusted for age, sex, provinces or megacities, batch run, urbanization, occupation, income, education, total energy intake, %kcal from animal-source foods, fried-food intake, physical activity, smoking, alcohol intake, pro-/prebiotic intake, nonsteroidal anti-inflammatory drug intake, and proton-pump inhibitor intake. Interaction of potassium density and province/megacity was removed because P values were >0.10. Na/K ratio, sodium to potassium ratio.

[2] The interaction between sodium density or Na/K ratio and province/megacity.

[3] The joint test of sodium density or Na/K ratio and its interaction with province/megacity. Pseudo F statistics and P values of the interaction and joint test were obtained from partial dbRDA conditioned on the rest of the model variables followed by an ANOVA test with 999 permutations.

[4] R^2 was estimated in a univariate dbRDA model for sodium density, potassium density, or Na/K ratio.

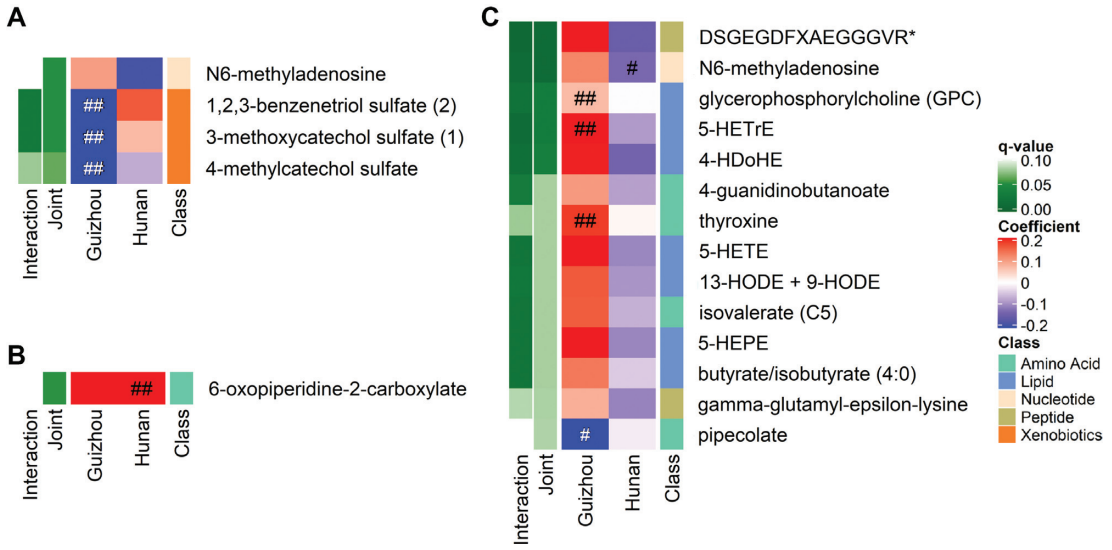

FIGURE 2 Heatmap of associations between sodium density (A), potassium density (B), and Na/K ratio (C) with individual metabolites. $n = 392$. Color and shading of the heatmap indicate the direction and magnitude of model coefficient. Metabolites were ordered by joint test q-value (false discovery rate–adjusted P value). The linear regression model was adjusted for age, sex, provinces or megacities, batch run, urbanization, occupation, education, income, total energy intake, %kcal from animal-source foods, fried-food intake, physical activity, smoking, alcohol intake, pro-/prebiotic intake, nonsteroidal anti-inflammatory drug intake, and proton pump inhibitor intake. "Interaction" indicates interaction with province/megacity; "Joint" indicates joint test of main and interaction effects. *Metabolites at level 2 identification according to the Metabolomics Standards Initiative (53); other metabolites were at level 1 identification. $^{\#}q$-values <0.10 and $^{\#\#}q$-values <0.05 for province-specific estimates. Na/K ratio, sodium to potassium ratio.

host factors, including age and sex (<1%) (unpublished data). We found that large geographic variation in microbiota in our cohort was related to differential dietary traditions, lifestyles, and regional habitats (e.g., climate, soil composition) (unpublished data). We observed that the associations between sodium and potassium consumption with microbiota varied substantially across provinces and megacities, but not by age or sex, indicating that geographical variation should be considered in future microbiota analyses. Our meta-analysis results further suggest large heterogeneity across province- and megacity-specific model estimates. We also observed geographic variation using OTU-level data with more detailed taxa identification than genus-level data. The large geographic variation may relate to basal differences in gut microbiota across provinces and megacities, as well as different dietary sources of sodium and potassium (1, 54). For example, while the contribution of added salt during cooking to sodium intake was lower in megacities (58.6%) than in provinces (65.9–67%), the contribution of processed food to sodium intake tended to be higher in megacities (13.3%) than in provinces (8.8–10.4%) (54). Our analysis is a preliminary step to identify associations across provinces and megacities. Further province- and megacity-specific analyses are needed to delineate mechanisms underlying the observed geographic variation.

A limitation of our study is the potential measurement error in diet assessment tools. Although 24-h dietary recall has shown poorer performance in estimating sodium intake in Chinese adults than 24-h urine (55), our estimation of sodium and potassium consumption was based both on 3 consecutive 24-h recalls and household inventories, and had been validated by 24-h urine (1). Given that a majority of our unique sample consumed a high-sodium and low-potassium diet, we acknowledge that our findings have limited generalizability to populations with lower sodium and higher potassium intakes, common to less urbanized areas. We were unable to adjust for former smoking due to small numbers of male ($n = 89$) and female ($n = 34$) former smokers. Our analyses were limited to microbial community structures using 16S rRNA data, and thus specific pathways of relevant microbial functional genes could not be established. Our study also lacks an independent sample for replication and repeated measures of microbiota and metabolites to model changes and test stability. Whereas it has been previously reported in the CHNS that the fecal microbiota were stable over 2 wk (19), circulating metabolites were dynamic (56).

In conclusion, we provide substantial observational evidence of the associations of sodium and potassium consumption with the gut microbiota and plasma metabolites in a population-based cohort of Chinese adults with habitual excessive sodium intake and inadequate potassium intake. In line with murine models and smaller human studies, we show that sodium, potassium, and Na/K ratio consumption is associated with the microbiota and metabolites related to inflammation and CVD risk factors. Taken together, our findings suggest the roles of the gut microbiota and related metabolites in the diet–health relation. More studies are needed to replicate our results and fully elucidate the biological pathways linking dietary sodium and potassium to CVD outcomes.

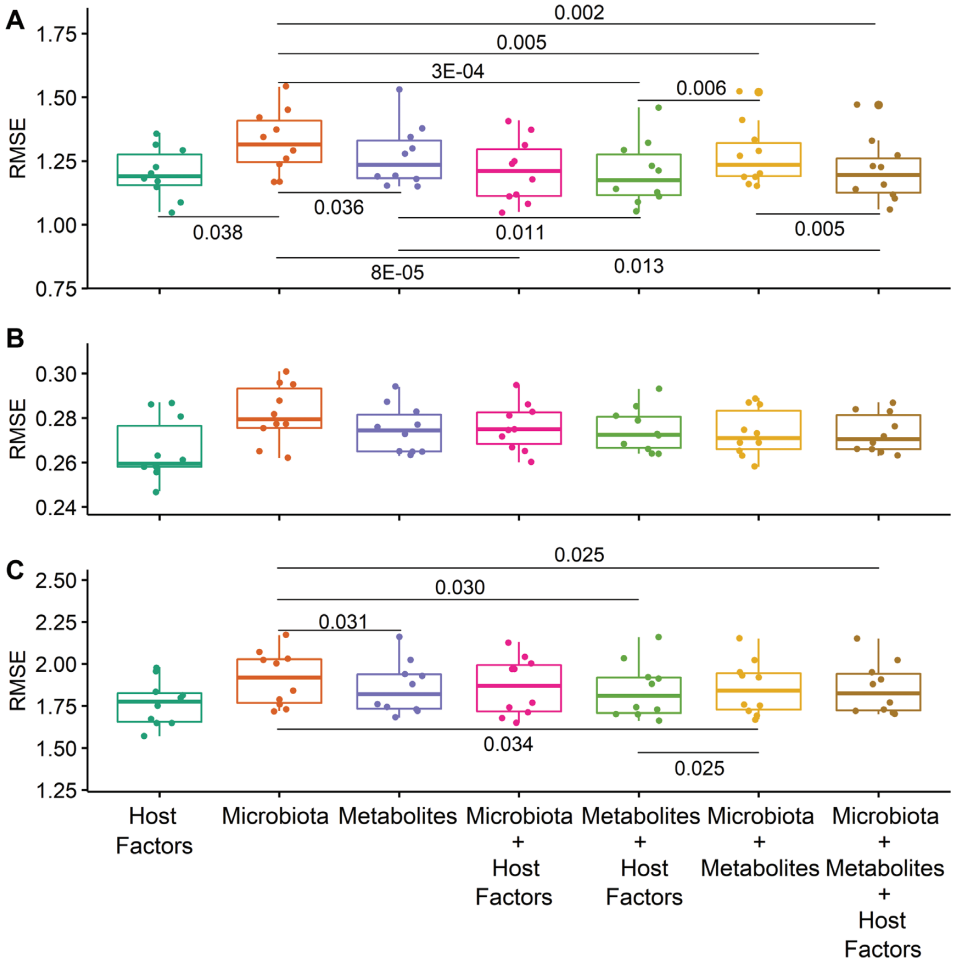

FIGURE 3 Box plots of RMSEs of sodium density (A), potassium density (B), and Na/K ratio (C) estimated by host factors and microbiota and metabolite data, using random forest regression. $n = 392$. P values for 5 iterations of 2-fold cross-validation modified paired t test <0.05 are shown between comparison groups. Na/K ratio, sodium to potassium ratio; RMSE, root mean square error.

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The authors' responsibilities were as follows—BZ, CS, HW, JZ, and ZW: collected the survey data and biospecimens for the research; SS: performed the bioinformatics analysis of the microbiota data; YW and PG-L: designed the study; YW: performed the statistical analysis and wrote the manuscript; AAF and MCBT: supervised the bioinformatics analysis; AGH, MCBT, PG-L, and SS: supervised the statistical analysis; AAF, AGH, CLA, KAM, MCBT, PG-L, SS, and WS: provided intellectual content to revise the manuscript; and all authors: read and approved the final manuscript and assume full responsibility for the final content. The authors report no conflicts of interest.

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