Blood pressure interactions with the DASH dietary pattern, sodium, and potassium: The International Study of Macro-/Micronutrients and Blood Pressure (INTERMAP)

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ABSTRACT

Background: Adherence to the Dietary Approaches to Stop Hypertension (DASH) diet enhances potassium intake and reduces sodium intake and blood pressure (BP), but the underlying metabolic pathways are unclear.

Objectives: Among free-living populations, we delineated metabolic signatures associated with the DASH diet adherence, 24-hour urinary sodium and potassium excretions, and the potential metabolic pathways involved.

Methods: We used 24-hour urinary metabolic profiling by proton nuclear magnetic resonance spectroscopy to characterize the metabolic signatures associated with the DASH dietary pattern score (DASH score) and 24-hour excretion of sodium and potassium among participants in the United States ($n = 2164$) and United Kingdom ($n = 496$) enrolled in the International Study of Macro- and Micronutrients and Blood Pressure (INTERMAP). Multiple linear regression and cross-tabulation analyses were used to investigate the DASH-BP relation and its modulation by sodium and potassium. Potential pathways associated with DASH adherence, sodium and potassium excretion, and BP were identified using mediation analyses and metabolic reaction networks.

Results: Adherence to the DASH diet was associated with urinary potassium excretion (correlation coefficient, $r = 0.42$; $P < 0.0001$). In multivariable regression analyses, a 5-point higher DASH score (range, 7 to 35) was associated with a lower systolic BP by 1.35 mmHg (95% CI, −1.95 to −0.80 mmHg; $P = 1.2 \times 10^{-5}$); control of the model for potassium but not sodium attenuated the DASH-BP relation. Two common metabolites (hippurate and citrate) mediated the potassium-BP and DASH-BP relationships, while 5 metabolites (succinate, alanine, *S*-methyl cysteine sulfoxide, 4-hydroxyhippurate, and phenylacetylglutamine) were found to be specific to the DASH-BP relation.

Conclusions: Greater adherence to the DASH diet is associated with lower BP and higher potassium intake across levels of sodium intake. The DASH diet recommends greater intake of fruits, vegetables, and other potassium-rich foods that may replace sodium-rich processed foods and thereby influence BP through overlapping metabolic pathways. Possible DASH-specific pathways are speculated but confirmation requires further study. INTERMAP is registered as NCT00005271 at www.clinicaltrials.gov. *Am J Clin Nutr* 2022;116:216–229.

Keywords: biomarkers, blood pressure, DASH dietary pattern, 24-hour dietary recalls, hypertension, metabolic pathways, potassium, sodium, urinary metabolites

Introduction

Elevated blood pressure (BP), defined as systolic blood pressure (SBP) of ≥120 mmHg or diastolic blood pressure (DBP) of ≥80 mmHg, remains a key independent risk factor for developing cardiovascular disease (CVD) and for mortality (1, 2). The major underlying causes of elevated BP are attributed to the independent and additive effects of several diet and lifestyle factors, including an adverse calorie balance, physical inactivity, poor diet quality, excess sodium intake, inadequate potassium intake, and excess alcohol intake (1, 2). Adherence to

the evidence-based, "heart-healthy" Dietary Approaches to Stop Hypertension (DASH) diet, characterized by increased protein intake and reduced sodium intake, is recommended for the prevention and management of hypertension (3). Studies also report that dietary potassium is inversely related to BP (4), with higher potassium consumption shown to attenuate the adverse effect of sodium on BP (5).

The DASH dietary pattern is rich in fruits, vegetables, low-fat dairy, fish, nuts, and whole grains, and is limited in red and processed meat, sodium-rich processed foods, and sugar-sweetened beverages. The DASH feeding trial reported SBP was lowered by 5.5 mmHg more in the DASH arm compared with the control-diet arm (typical of what many Americans eat) (6). The BP reduction occurred despite sodium being fixed at 3000 mg/day, thus demonstrating benefits of dietary modification even at sodium levels higher than the recommended guidelines. Sodium reduction has shown additive BP-lowering effects over the DASH diet alone (7), but results of combining the DASH diet with reduced sodium intake proved less robust than expected, potentially due to shared mechanistic pathways or counter-regulatory mechanisms involving these 2 different but interrelated dietary factors. Data from feeding trials reported *N*-methylproline, chiro-inositol, proline betaine, and theobromine as potential biomarkers of the DASH dietary pattern (8, 9). However, the specific nutrients and metabolic pathways of the DASH dietary pattern in free-living populations are yet to be elucidated.

In this paper, we analyzed 24-hour urine specimens collected in the International Study of Macro-/Micronutrients and Blood Pressure (INTERMAP) to investigate relationships of BP and urinary metabolites [characterized by proton nuclear magnetic resonance (^{1}H NMR) spectroscopy], urinary excretion of sodium and potassium (objective measures of intake), and adherence to the DASH dietary pattern using a validated scoring system (10). Data from free-living INTERMAP participants in the United States and United Kingdom were analyzed. The aims were: *1*) to delineate urinary metabolic signatures associated with the DASH dietary pattern score (DASH score), objectively measure levels of sodium and potassium, and test the reproducibility of these relationships; and *2*) to discover possible metabolic pathways underlying the diet-BP relationships that might be in common with both the DASH score and urinary measures of potassium, collectively or specifically. Our primary outcome was the association of BP with the DASH score, and secondary outcomes were associations of the DASH score with urinary excretion of sodium and potassium.

Methods

Population sample

The INTERMAP is a cross-sectional, epidemiological study of 4680 men and women aged 40 to 59 years from 4 countries (China, Japan, the United Kingdom, and the United States) (11). From 1996 to 1999, participants were randomly recruited from community or workplace population lists, and arrayed into 4 age and sex strata. Each participant attended 4 visits, with the first 2 visits occurring on consecutive days and followed 3 weeks later by the second 2 visits (**Supplemental Figure 1**). Institutional ethics committee approval was obtained for each site; all participants provided written informed consent. INTERMAP was approved by the Institutional Review Board of Northwestern University (STU00204462-CR0002) and the Research Ethics Committee of the Health Research Authority (United Kingdom, #EC3169). INTERMAP is registered at www.clinicaltrials.gov as NCT00005271. The present study relates to 2164 of the 2195 US participants, used in the discovery and replication phase, and 496 of the 501 UK participants, used in the validation phase, who had complete dietary data as well as ^{1}H NMR data from the two 24-hour urine samples (**Supplemental Figure 2**).

Clinical measurements

BP was measured 8 times (2 per visit) according to a standardized protocol implemented by trained staff at each visit, following at least 5 minutes of rest, with a random-0 sphygmomanometer (11). The mean of the 8 BP measurements was calculated and used in the analyses. Hypertension status was defined as an SBP $\geq$ 140 mmHg or DBP $\geq$ 90 mmHg, with or without antihypertensive treatment, in alignment with the sixth report of the Joint National Committee on Detection, Evaluation, and Treatment of High Blood Pressure (12) recommendations at the time of sampling. The mean of 4 measurements of height and weight was used to calculate BMI as weight/height^2 (kg/m^2). Education, occupation, race, physical activity, smoking, medical history, family history of high BP, and current medication data were collected using interviewer-guided questionnaires.

Dietary data

Dietary intake was assessed based on four 24-hour dietary recalls administered by staff trained and certified according to a standardized protocol following the tri-phasic method (13). The recall data for the US samples were entered electronically via the Nutrition Data System for Research, Nutrition Coordinating Centre (Version 2.91, University of Minnesota), and data for

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Supplemental Figures 1–3 and Supplemental Tables 1–18 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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Abbreviations used: AI, adequate intake; BCAA, branched-chain amino acid; BP, blood pressure; CDRR, chronic disease risk reduction; CVD, cardiovascular disease; DASH, Dietary Approaches to Stop Hypertension; DBP, diastolic blood pressure; INTERMAP, International Study of Macro-/Micronutrients and Blood Pressure; K, potassium; KEGG, Kyoto Encyclopaedia of Genes and Genomes; Na, sodium; NMNA, *N*-methyl nicotinate; PAG, phenylacetylglutamine; QC, quality control; SBP, systolic blood pressure; SMCSO, *S*-methyl cysteine sulfoxide; SUCNR1, succinate receptor 1; TCA, tricarboxylic acid; TSP, trimethylsilyl propionate; ^{1}H NMR, proton nuclear magnetic resonance; 2PY, *N*-methyl-2-pyridone-5-carboxamide.

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the UK were collected on paper forms and coded from an INTERMAP codebook, derived from FOODBASE food codes. Intakes of food groups and nutrients were estimated from the means of the four 24-hour dietary recalls. The DASH score was calculated as previously described by Fung et al. (10) and was based on the intakes of the following 7 key food components: fruits, vegetables, nuts and legumes, low-fat dairy products, and whole grains, and low intakes of sweetened beverages and red meat (including high-sodium processed meats and other processed foods) (**Supplemental Table 1**). For each component, individuals were classified into quintiles according to their intake. Quintile 1 was assigned 1 point and Quintile 5 was assigned 5 points for desirable food groups. For less desirable food groups, reverse scoring was applied. The component scores were summed to attain an overall DASH score ranging from 7 to 35, with a higher score corresponding with a healthier, DASH-like dietary pattern. The correlation between the DASH score and the previously reported DASH nutrient-based score was 0.59 for INTERMAP US participants (14). We used the dietary reference intakes set by the National Academies of Sciences, Engineering and Medicine—that is, a chronic disease risk reduction (CDRR) level of 2300 mg/day of sodium and adequate intakes (AIs) of 2600 mg/day for women and 3400 mg/day for men of potassium—in our analyses to assess intakes of sodium and potassium of our participants (15).

Urine collection and analysis

Two borate-preserved, timed 24-hour urine collections were obtained from individuals on visits 2 and 4; aliquots of urine were frozen on site and air-freighted frozen to the Central Laboratory (Leuven, Belgium) for biochemical analyses (11). Participants were excluded from the study if 2 urine specimens were not available; urine collections were rejected if the participants reported a protocol violation: that is, if "more than a few drops" were missing from the collection, the 24-hour urinary volumes were <250 ml, or the timing of the collection fell outside the 20-hour to 28-hour range. Sodium and potassium were measured using flame emission photometry, and excretion values were corrected to 24 hours with internal and external quality controls. As part of the quality control (QC) procedures, 8% random samples were split at the clinical center and sent to the laboratory with different identification numbers for external assessments of measurement precision (16). The mean and median CVs were 0.75% and 0.28%, respectively, for urinary sodium measurements and 0.60% and 0.35%, respectively, for urinary potassium measurements. As excretion measurements of sodium and potassium are considered more objective and have greater validity than self-reported dietary data for individuals, the mean of the 2 samples was calculated and used throughout the present study as a proxy for dietary intake (17).

^{1}H NMR spectroscopy

Urine specimens were prepared for high-resolution ^{1}H NMR spectroscopy, with the inclusion of 144 QC samples. QC samples were obtained from 3 healthy volunteers, with 2 QC aliquots from each volunteer; 6 QC samples was added to each well plate as described previously (18). Analytical reproducibility was high (CV < 5%). ^{1}H NMR spectra of the urine specimens were obtained at 300 K using a Bruker (Bruker Biospin) Avance 600 MHz spectrometer (19). Spectra were acquired using a standard 1-dimensional pulse sequence (recycle delay-90°-t1-90°-tm-90°-acquisition) with water suppression (20) achieved with a saturation pulse during recycle delay (2s), a mixing time (tm, 100ms), and t1 set to 3μs. Fourier transformation was applied to the free induction decays, spectra were referenced to deuterated trimethylsilyl propionate (TSP), and baseline and phase were corrected. Spectra were automatically phased and baseline-corrected using Bruker Topspin 3.5 software (Bruker BioSpin). Preprocessing was performed using in-house scripts and software, implemented in MATLAB (R2020a, MathWorks) as previously described (19). The spectral regions containing water and urea (δ, 6.4 to 4.5), TSP (δ, 0.2 to −0.2), and regions containing predominantly noise (δ, −0.2 to −4.5; δ, 0.5 to 0.2; and δ 15.5 to 9.5) were removed. The remaining variables were normalized using probabilistic quotient normalization (21) and "binned" to 7100 variables, with bin widths of 0.001 ppm. The urine specimens were randomized for the analysis, and 8% of samples were split at source and metabolite profiles analyzed blindly using hierarchical cluster analysis, with 98% of the split samples correctly identified (18).

Metabolite identification

Urinary metabolites were assigned against reference spectra using in-house databases and the Human Metabolome Database (22). Further confirmation of metabolite identification was performed using a range of 2-dimensional nuclear magnetic resonance experiments, including Total Correlation Spectroscopy and Heteronuclear Single Quantum Coherence, on a QC sample. Additionally, Statistical Total Correlation Spectroscopy (23) was applied to the ^{1}H NMR spectral data set to provide information on the molecular structural correlation.

Statistical methods

All statistical analyses were undertaken using SAS version 9.4 (SAS Institute Inc.) or R programming software packages, version 3.5. Independent ^{1}H NMR data analyses were performed for each study visit (Supplemental Figure 1) to align with each 24-hour timed urine collection. Using this methodological approach, we have previously shown reproducible patterns of metabolite excretion (24). Baseline characteristics of the study population are presented with the use of descriptive statistics, and we also compared mean (SD) DASH scores according to sex, weight status, and race (self-reported). Partial correlation coefficients (r) between the DASH score and its 7 components, urinary excretion of sodium and potassium, and the sodium-to-potassium (Na/K) ratio were calculated. Reliability, as a measure of possible regression dilution bias (25) for the DASH score and BP variables (expressed as the observed univariate regression coefficient as a percentage of the theoretical "true" coefficient), was estimated by the following formula: $1/[1+(\text{ratio}/2)]\times 100$. The ratio is the intraindividual variance divided by the interindividual variance, calculated from mean intakes and BP levels of the first and second sets of 2 visits to account for higher correlation between intakes and BP levels on consecutive days (26).

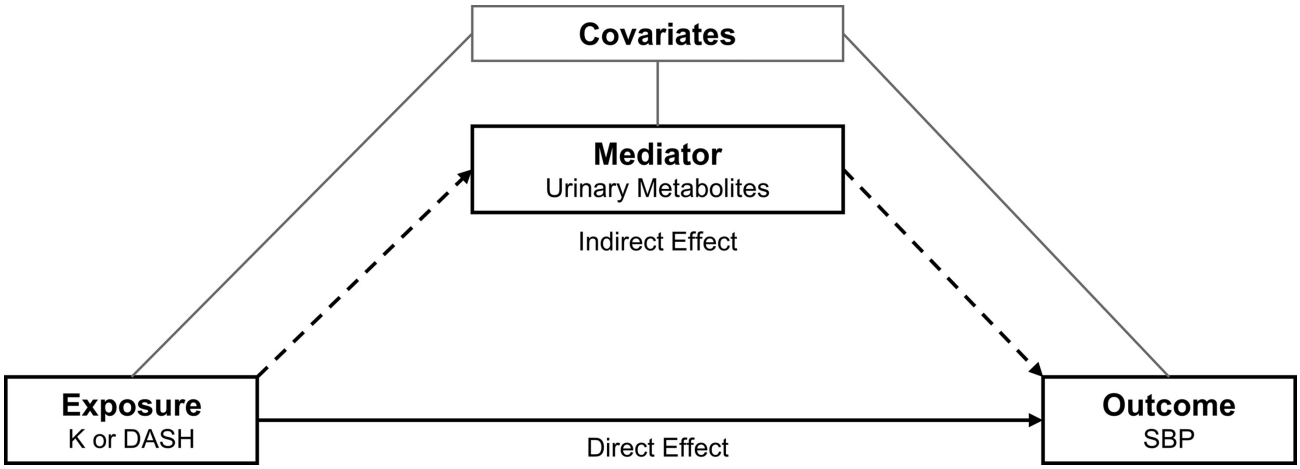

FIGURE 1 Mediation analysis model of associations between dietary exposures (the DASH diet or potassium) and systolic blood pressure, as mediated by urinary metabolites. The indirect effect is represented by dashed arrows and the direct effect by a continuous arrow. Mediator and outcome models were adjusted for several potential covariates. DASH, Dietary Approaches to Stop Hypertension; K, potassium; SBP, systolic blood pressure.

For the discovery analysis (US samples, first urine collection), we identified urinary metabolites significantly associated with the DASH score (and its components) and excretion of sodium and potassium by partial Pearson correlation. *Q* values were calculated using the Benjamini-Hochberg false discovery rate to correct for multiple testing for the 7100 ^{1}H NMR variables. To further avoid false positive associations, we employed a similar method to those previously described (24). For these discovery analyses, spectral variables were deemed significant if the *Q* value was <0.01 and the sign of the two adjacent spectral variables was in the same direction. Where several spectral variables with significant *Q* values refer to the same metabolite, the variable with the most significant correlation (smallest *P* value) was selected (usually the apex of the peak), as was the peak with the least overlap. The selected spectral variable was used for subsequent mediation and correlation analyses. Significantly associated variables were visualized in a Manhattan plot, depicting -$\log_{10}$ *Q* values multiplied by the sign of the association (r). The urinary metabolites identified as putative biomarkers of the DASH dietary pattern were used in a further partial correlation analysis against the 7 key DASH food components.

The relationship of the DASH dietary pattern to BP and its modulation by other dietary factors were investigated using multivariable linear regression, and the associations between each 5-point increase in DASH score with BP were examined. A multiple cross-tabulation analysis, based on quintiles of DASH scores and urinary excretion of sodium and potassium, was used to further examine the relationships between DASH, sodium, potassium, and BP.

Adjustments were made for the following covariates in the multivariable models. Model 0 was adjusted for age (years, continuous), sex, and race. Model 1 was additionally adjusted for education (years), physical activity (hours/day), alcohol (g/day, continuous), smoking (yes or no), vitamin supplement usage (yes or no), special diet (including salt or fat reduction) reported (yes or no), history of CVD (yes or no), antihypertensive medication (yes or no), family history of hypertension (yes or no), and mean energy intake (kcal/day, continuous). Models 2, 3, and 4 were model 1 plus additional adjustments for excretion of sodium, excretion of potassium, and the Na/K ratio, respectively. As obesity may lie on the causal pathway between diet and BP, all models were computed with and without adjustments for BMI (continuous variable).

Mediation analysis

A mediation analysis shows whether some, or all, of the association between an independent and dependent variable is statistically explained by the influence of a mediator variable. Here, a mediation analysis was performed to investigate whether the association between excretion of potassium and BP and between the DASH score and BP is mediated by urinary metabolites. The total effect of potassium or DASH on BP consists of the direct effect (the effect of potassium or DASH on BP) and the indirect effect (the effect of potassium or DASH on BP mediated by urinary metabolites; **Figure 1**). Note that mediation on its own does not signify that the mediator lies on the causal pathway between the exposure and outcome. The ratio of the indirect to total effect was calculated to estimate the proportion mediated by urinary metabolites, identified from the ^{1}H NMR partial correlation analysis. For example, if the proportion mediated is found to be 10%, the mediator variable can be interpreted as mediating 10% of the effect of the independent variable on the dependent variable. The significance of the indirect effect ($P < 0.05$) was tested using bootstrapping procedures, with 1000 bootstrap samples. Mediation analyses were implemented using the R package "mediation," using Baron and Kenny's approach (27, 28). Urinary metabolite signals were standardized by calculating the z-score for that signal, to facilitate interpretation and comparability. All mediation models were adjusted for model 1 covariates, with and without BMI.

Metabolic reaction network

MetaboNetworks software (29) was used to create an integrated metabolic reaction network, showing the shortest metabolic paths connecting the DASH-associated metabolites that were identified in the ^{1}H NMR partial correlation analysis. Reconstructions were generated of the symbiotic and co-metabolic reactions occurring between the host and different gut microbial species and of the connectivity between metabolites, using information from the Kyoto Encyclopaedia of Genes and Genomes (KEGG). A metabolic reaction database was created that includes those reactions occurring in Homo Sapiens and the gut microbiota that are the most common endosymbionts—*Firmicutes, Bacteroidetes, Alphaproteobacteria, Betaproteobacteria, Deltaproteobacteria, Gammaproteobacteria*, and *Actinobacteria*—as these make up 99% of the phyla found in the human gut (30). The constructed database considers that 2 metabolites are linked if a biochemical reaction entry in KEGG indicates they are a main reactant pair and the reaction is mediated by: *1*) an enzyme linked to human genes; *2*) an enzyme linked to microbial genes; or *3*) part of a spontaneous process. The shortest path (number of reactions) between metabolites is calculated, and a network diagram is drawn for all compounds needed to link observed metabolites. The network consists of nodes (metabolites), and each line connecting 2 nodes represents a biochemical reaction. Dotted lines indicate the closest related metabolite in the network based on literature for metabolites that are not present in the KEGG database. The network was superimposed on a colored map, where the background shading indicates different areas of metabolism.

Replication and validation of urinary metabolite associations assayed by ^{1}H NMR in independent samples

Urinary metabolite associations identified in the discovery analysis that used the first set of urine samples were replicated using the second set of urine samples from the US cohort, obtained on average 3 weeks later. This allowed us to assess the reproducibility of our findings in the same individuals over time. The findings were then validated using the INTERMAP UK cohort as an external data set, to evaluate reproducibility in an independent population. The same methods and protocol were used for the US and UK INTERMAP sample data collections (11). For the replication analyses (US cohort's second urine collection), a threshold of Q values < 0.01 was used to indicate significant associations. For the validation analyses (UK ^{1}H NMR data), a threshold of Q values < 0.05 was used due to the smaller sample size in the UK cohort.

Results

Descriptive statistics

For the INTERMAP US cohort, the mean DASH scores from 4 dietary recalls were similar in women (21.0; SD, 5.0) and men (20.9; SD, 4.7). Participants in Quintile 5 of DASH adherence (highest DASH score), compared to those in DASH score Quintile 1 (lowest DASH score), were older, were more likely to be White, completed more years of education, were less likely to be current smokers, had a lower BMI, were more likely to take vitamin or mineral supplements, and indicated they followed a special diet. They also had higher levels of urinary potassium (**Table 1**). Excretion of sodium was similar across the DASH quartiles, and 270 US participants (12.5%) met the CDRR level for sodium (i.e., sodium excretion <2300 mg/day). Excretion of potassium was 46.2 mmol/24-hr (SD, 16.9 mmol/24-hr) for participants in DASH score Quintile 1, compared to 72.1 mmol/24-hr (SD, 21.6 mmol/24-hr) for those in DASH score Quintile 5. Based on estimates from their 24-hour urine excretion, 344 participants (16%) met the AI level of potassium consumption.

Reliability

Reliability estimates for the averages of two 24-hour urinary potassium measurements were 62% (US cohort) and 53% (UK cohort); for urinary sodium, the estimates were 42% (US cohort) and 40% (UK cohort). Reliability estimates for DASH scores were 65% (US cohort) and 70% (UK cohort), and BP reliability estimates were uniformly high (both US and UK cohorts: 91% for SBP and 90% for DBP).

Association of the DASH score and its components with excretion of sodium and potassium

With adjustments for model 1 covariates and BMI, the DASH score showed a stronger partial Pearson correlation ($P < 0.0001$) with excretion of potassium ($r = 0.42$) than with the urinary Na/K ratio ($r = -0.28$; **Supplemental Table 2**). Self-reported intakes of DASH-recommended vegetables, fruits, whole grains, nuts, legumes, and low-fat dairy were positively correlated with excretion of potassium; intakes of red meat and sugar-sweetened beverages were negatively correlated with urinary potassium ($P < 0.0001$).

Association of DASH score and its components with BP

In multivariable regression analyses with adjustments for covariates known to affect BP (model 1) plus BMI, a 5-point higher DASH score was associated with a lower SBP (by 1.35 mmHg; 95% CI: −1.95 to −0.80 mmHg; $P = 1.2 \times 10^{-5}$) and DBP (lower by 0.40 mmHg; 95% CI, −0.85 to 0.03 mmHg; $P = 0.07$; **Table 2**); an additional adjustment for potassium attenuated the association between DASH and SBP (from 1.35 mmHg in model 1 to 1.05 mmHg in model 3). However, an additional adjustment for sodium (model 2) did not attenuate this association, and a 5-point higher DASH score was associated with a lower SBP (by 1.40 mmHg; 95% CI, −2.00 to −0.80 mmHg). With the excretion of sodium and DASH score stratified into quintiles, the adjusted mean SBP values remained similarly higher for people in the DASH score Quintile 1 (lowest) compared with those in the DASH score Quintile 5 (**Figure 2**A). With the excretion of potassium and DASH score stratified into quintiles, the adjusted mean SBP was consistently lower for those in Quintile 5 (highest potassium) compared to those in Quintile 1

TABLE 1 Baseline characteristics of the INTERMAP US cohort by quintiles of DASH scores ($n = 2164$)[1]

	DASH score Q1[2] ($n = 410$)		DASH score Q2 ($n = 437$)		DASH score Q3 ($n = 477$)		DASH score Q4 ($n = 420$)		DASH score Q5[2] ($n = 420$)		
DASH score, median (range)	15	(8–16)	18	(17–19)	21	(20–22)	24	(23–25)	28	(26–35)	*P* for trend
Male, *n* (%)	193	(47.1)	235	(53.8)	252	(52.8)	203	(48.3)	202	(48.1)	0.17
Female, *n* (%)	217	(52.9)	202	(46.2)	225	(47.2)	217	(51.7)	218	(51.9)	—
Age, years	47.9	(5.2)	48.8	(5.5)	49.2	(5.3)	49.8	(5.5)	50.0	(5.1)	<0.0001
Race: American Indian and Alaska Native, *n* (%)	2	(0.5)	2	(0.5)	2	(0.4)	1	(0.2)	1	(0.2)	<0.0001
Race: Asian, *n* (%)	53	(12.9)	46	(10.5)	77	(16.1)	66	(15.7)	65	(15.5)	—
Race: Black, *n* (%)	173	(42.2)	148	(33.9)	105	(22.0)	70	(16.7)	44	(10.5)	—
Race: White, *n* (%)	176	(42.9)	241	(55.2)	290	(60.8)	280	(66.7)	306	(72.9)	—
Race: Other, *n* (%)	6	(1.5)	0		3	(0.6)	3	(0.7)	4	(1.0)	—
BMI, kg/m^2	30.5	(6.3)	29.5	(5.6)	29.0	(6.0)	28.5	(5.8)	26.9	(5.0)	<0.0001
Systolic blood pressure, mmHg	120.8	(13.6)	120.9	(14.2)	118.2	(13.5)	118.3	(14.2)	114.8	(13.3)	<0.0001
Diastolic blood pressure, mmHg	73.7	(9.8)	74.7	(9.9)	73.7	(9.8)	73.5	(9.5)	71.5	(9.2)	<0.0001
Education, y	13.7	(3.1)	14.3	(2.7)	15.2	(3.0)	15.3	(2.9)	16.2	(2.9)	<0.0001
Current smoker, *n* (%)	128	(31.2)	102	(23.3)	65	(13.6)	44	(10.5)	23	(5.5)	<0.0001
Taking vitamin/mineral supplement, *n* (%)	143	(34.9)	191	(43.7)	251	(52.6)	252	(60.0)	286	(68.1)	<0.0001
Following a special diet, *n* (%)	54	(13.2)	65	(14.9)	71	(14.9)	93	(22.1)	109	(26.0)	<0.0001
Diagnosed with cardiovascular disease, *n* (%)	173	(42.2)	187	(42.8)	195	(40.9)	163	(38.8)	142	(33.8)	0.05
Taking antihypertensive medication, *n* (%)	89	(21.7)	116	(26.5)	110	(23.1)	91	(21.7)	72	(17.1)	0.02
Family history of hypertension, *n* (%)	272	(66.3)	302	(69.1)	320	(67.1)	299	(71.2)	274	(65.2)	0.36
Moderate or heavy physical activity, hours/day	4.0	(3.7)	3.4	(3.1)	2.9	(3.0)	2.9	(3.0)	3.0	(2.9)	<0.0001
Alcohol intake, g/day	7.3	(16.4)	8.5	(16.7)	7.3	(14.0)	5.6	(10.1)	6.0	(9.4)	0.01
Energy intake, kcal/day	2228	(719)	2246	(744)	2285	(744)	2227	(674)	2221	(599)	0.64
Whole grain, g/day	9.8	(16.9)	18.3	(25.2)	28.0	(31.7)	42.6	(47.7)	61.9	(50.1)	<0.0001
Vegetables, excluding potato, g/day	110.7	(76.9)	147.6	(110.0)	180.6	(105.2)	203.5	(114.3)	256.8	(128.9)	<0.0001
Fruit, g/day	85.8	(116.4)	153.0	(153.4)	225.8	(208.8)	272.9	(208.0)	369.3	(227.8)	<0.0001
Nuts and legumes, g/day	33.7	(43.1)	38.8	(45.5)	42.0	(43.6)	46.4	(40.6)	67.4	(56.6)	<0.0001
Low fat dairy, g/day	40.3	(89.2)	101.6	(173.5)	155.3	(199.3)	204.5	(214.7)	287.1	(240.2)	<0.0001
Red meat, including processed, g/day	122.6	(66.7)	100.9	(71.7)	87.9	(67.1)	63.4	(48.6)	47.2	(43.1)	<0.0001
Sugar-sweetened beverages, g/day	619.1	(465.9)	453.8	(397.9)	332.6	(381.0)	238.0	(294.5)	136.3	(266.0)	<0.0001
Urinary sodium, mmol/24-hrs	158.4	(62.4)	166.2	(61.4)	163.6	(58.4)	162.8	(58.4)	161.6	(55.8)	0.41
<CDRR sodium estimated from 24-hour urine excretion,[3] *n* (%)	63	(15.4)	52	(11.9)	63	(13.2)	41	(9.8)	51	(12.1)	0.17
Urinary potassium, mmol/24-hr	46.2	(16.9)	51.3	(16.9)	56.9	(19.5)	62.3	(19.8)	72.1	(21.6)	<0.0001
>AI potassium estimated from 24-hour urine excretion,[4] *n* (%)	18	(4.4)	27	(6.2)	56	(11.7)	87	(20.7)	156	(37.1)	<0.0001

[1]Mean ± (SD) or *n* (%) shown, unless otherwise specified. AI, adequate intake; CDRR, chronic disease risk reduction; DASH, Dietary Approaches to Stop Hypertension; INTERMAP, International Study of Macro-/Micronutrients and Blood Pressure; Q, quintile.

[2]DASH score Q1 = Quintile 1, lowest DASH score; DASH score Q5 = Quintile 5, highest DASH score.

[3]CDRR level for sodium: 2300 mg/day.

[4]AIs for potassium: 2600 mg/day in women and 3400 mg/day in men.

TABLE 2 Relation of 5-point higher DASH score to systolic and diastolic blood pressure difference (mmHg) in the INTERMAP US cohort ($n = 2164$)[1]

	SBP		SBP + BMI		DBP		DBP + BMI	
Model	ΔBP (95% CI)	*P*	ΔBP (95% CI)	*P*	ΔBP (95% CI)	*P*	ΔBP (95% CI)	*P*
0[2]	−2.65 (−3.25 to −2.05)	9.2×10^{-16}	−1.65 (−2.25 to −1.10)	4.3×10^{-8}	−0.95 (−1.35 to −0.60)	5.8×10^{-7}	−0.45 (−0.85 to −0.04)	0.03
1[3]	−2.05 (−2.65 to −1.45)	2.8×10^{-10}	−1.35 (−1.95 to −0.80)	1.2×10^{-5}	−0.75 (−1.20 to −0.30)	7.0×10^{-4}	−0.40 (−0.85 to 0.03)	0.07
2[4]	−2.05 (−2.65 to −1.45)	1.9×10^{-10}	−1.40 (−2.00 to −0.80)	1.3×10^{-5}	−0.75 (−1.20 to −0.30)	7.0×10^{-4}	−0.40 (−0.85 to 0.04)	0.07
3[5]	−2.05 (−0.54 to −0.28)	2.1×10^{-8}	−1.05 (−1.75 to −0.40)	0.001	−0.80 (−1.25 to −0.30)	0.001	−0.30 (−0.75 to 0.20)	0.26
4[6]	−1.75 (−0.48 to −0.23)	3.2×10^{-7}	−1.20 (−1.85 to −0.60)	1.0×10^{-4}	−0.65 (−1.15 to −0.20)	0.004	−0.35 (−0.85 to 0.08)	0.11

[1] ΔBP indicates a blood pressure difference, calculated by multivariable linear regression. BP, blood pressure; DASH, Dietary Approaches to Stop Hypertension; DBP, diastolic blood pressure; INTERMAP, International Study of Macro-/Micronutrients and Blood Pressure; SBP, systolic blood pressure.

[2] Model 0: adjusted for age, sex, and race.

[3] Model 1: adjusted for model 0 variables plus education, physical activity, alcohol, smoking, vitamin supplement usage, special diet reported, history of cardiovascular disease, antihypertensive medication, family history of hypertension, and mean energy intake.

[4] Model 2: adjusted for model 1 variables plus excretion of sodium.

[5] Model 3: adjusted for model 1 variables plus excretion of potassium.

[6] Model 4: adjusted for model 1 variables plus urinary sodium-to-potassium ratio.

(lowest potassium) at all quintiles of DASH scores (Figure 2B). For example, the SBP was 117.7 mmHg (95% CI, 115.7–119.6 mmHg) for potassium Quintile 5 and DASH score Quintile 5 compared with 122.1 mmHg (95% CI, 120.0–124.1 mmHg) for potassium Quintile 1 and DASH score Quintile 1.

Association of the DASH score and its components with the ^{1}H NMR metabolic profile

A partial Pearson correlation analysis (first urine collection) of 7100 ^{1}H NMR spectral variables with DASH scores, adjusted for model 1 covariates and BMI, showed that 37 urinary metabolites were significantly associated with the DASH score at a *Q*-value threshold < 0.01 (**Figure 3**). Correlation coefficient (r) values between these urinary metabolites and DASH scores ranged from −0.18 for glutamine to 0.21 for proline betaine (**Supplemental Table 3**). Significant associations with DASH scores of all 37 ^{1}H NMR–identified metabolites from the analysis of the first set of urine samples could be replicated in the second set of urine samples from the US cohort, obtained on average 3 weeks later (**Supplemental Table 4**). Independent data from the INTERMAP UK cohort replicated 27 of the identified significant DASH-metabolite associations at a *Q*-value threshold < 0.05; this may reflect lower statistical power for these analyses because of the smaller sample size in the UK cohort (**Supplemental Table 5**).

Correlation analyses of ^{1}H NMR data, adjusted for model 1 covariates and BMI, showed that 32 known metabolites were significantly associated with specific DASH food components (**Supplemental Table 6**). Three metabolites were negatively associated with whole grains; 11 metabolites, including citrate (r = 0.12) and *S*-methyl cysteine sulfoxide (SMCSO; r = 0.12), were associated with vegetables; 22 metabolites were associated with fruits, including proline betaine (r = 0.53), citrate (r = 0.26), 4-hydroxyproline betaine (r = 0.42), and 2-hydroxy-2-(4-methyl cyclohex-3-en-1-yl)propoxyglucuronide (r = 0.30); *N*-methyl nicotinate (NMNA) was positively associated with legumes and nuts; valine and succinate were positively associated with low-fat dairy; glutamine, *O*-acetyl carnitine, dimethylglycine, taurine, and 3-methylhistidine were positively associated with red meat; and 5 metabolites, including phenylacetylglutamine (PAG; r = 0.16), were associated with sugar-sweetened beverages. Further information on metabolite identification can be found in **Supplemental Table 7**.

Association of potassium with the ^{1}H NMR metabolic profile

A partial correlation analysis (first urine collection) of ^{1}H NMR spectral variables with potassium excretion, adjusted for model 1 covariates and BMI, showed that 34 urinary metabolites were significantly associated with potassium at a *Q*-value threshold < 0.01 (**Supplemental Table 8**). Correlation coefficient (r) values between these urinary metabolites and potassium excretion ranged from −0.18 for dimethylamine to 0.25 for citrate. Significant associations with potassium excretion of all 34 ^{1}H NMR–identified metabolites from an analysis of the first set of urine samples could be replicated in the second set of urine samples (**Supplemental Table 9**). Validation using

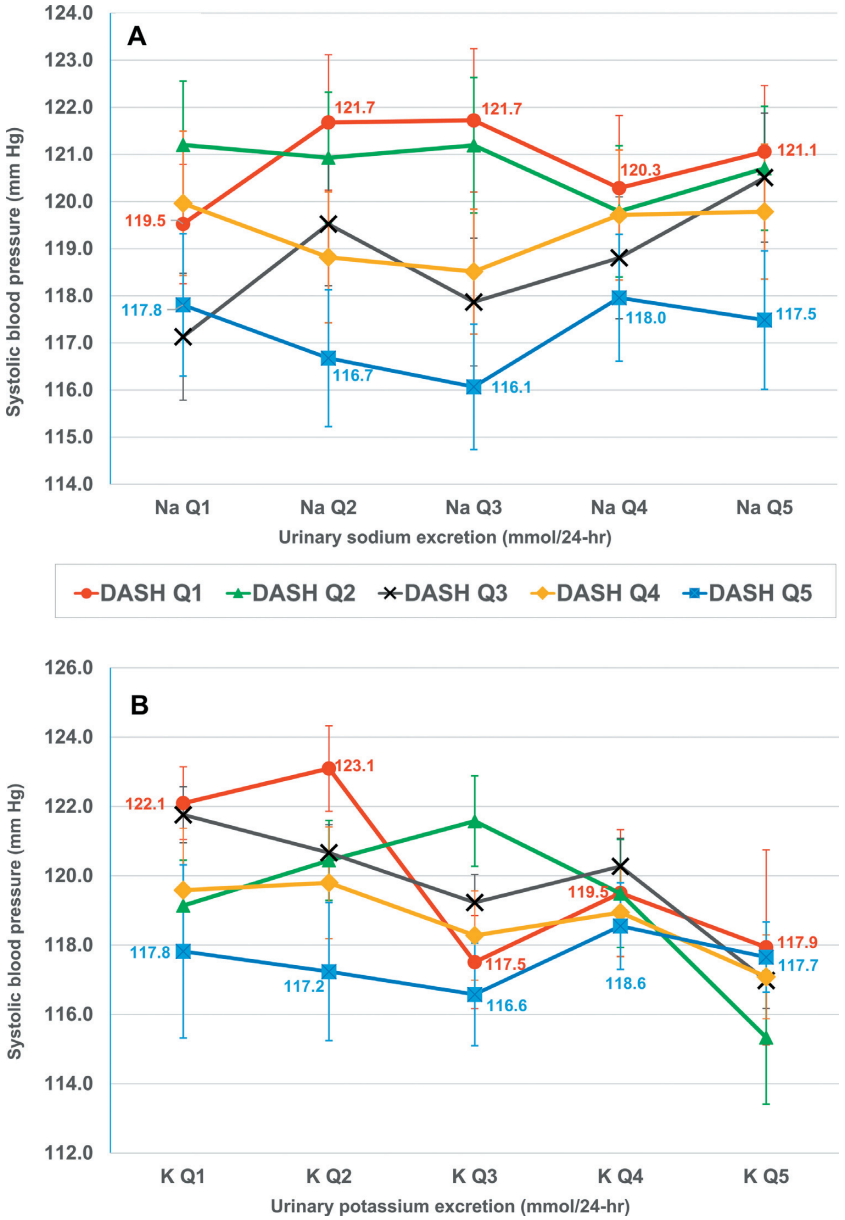

FIGURE 2 Mean systolic blood pressure (mmHg) by sex-specific quintiles of DASH score and (A) excretion of sodium or (B) excretion of potassium in the INTERMAP US cohort ($n = 2164$). Adjusted for age, sex, race, education, physical activity, alcohol, smoking, vitamin supplement usage, special diet reported, history of cardiovascular disease, antihypertensive medication, family history of hypertension, mean energy intake, and BMI. Quintile cutoffs for DASH scores were 17 (Q1), 20 (Q2), 23 (Q3), and 26 (Q4). Quintile cutoffs for excretion of sodium (mmol/24-hrs) were 122.5 (Q1), 157.9 (Q2), 192.5 (Q3), and 234.5 (Q4) for men and 94.6 (Q1), 125.0 (Q2), 150.4 (Q3), and 186.2 (Q4) for women. Quintile cutoffs for excretion of potassium (mmol/24-hrs) were 46.0 (Q1), 56.5 (Q2), 67.4 (Q3), and 82.6 (Q4) for men and 34.9 (Q1), 44.1 (Q2), 53.1 (Q3), and 63.8 (Q4) for women. DASH, Dietary Approaches to Stop Hypertension; INTERMAP, International Study of Macro-/Micronutrients and Blood Pressure; K potassium; Na, sodium; Q, quintile.

independent data from the INTERMAP UK cohort also showed similar results (**Supplemental Table 10**).

Association of sodium with the ^{1}H NMR metabolic profile

A partial correlation analysis (first urine collection) showed that 24 urinary metabolites were significantly associated with sodium excretion at a Q-value threshold < 0.01 when adjusted for model 1 covariates. With an additional adjustment for BMI, 21 metabolites were significant, with coefficient values ranging from −0.17 for citrate to 0.19 for formate (**Supplemental Table 11**). Of the significant ^{1}H NMR–identified sodium-metabolite associations, 14 could be replicated in the second set of urine samples without a BMI adjustment. With an additional BMI adjustment, only the associations with ethyl glucuronide, *N*-acetylneuraminate, 3-methylhistidine, and formate could be replicated (**Supplemental Table 12**). Validation using independent data from the INTERMAP UK cohort produced similar results to the discovery analysis (**Supplemental Table 13**).

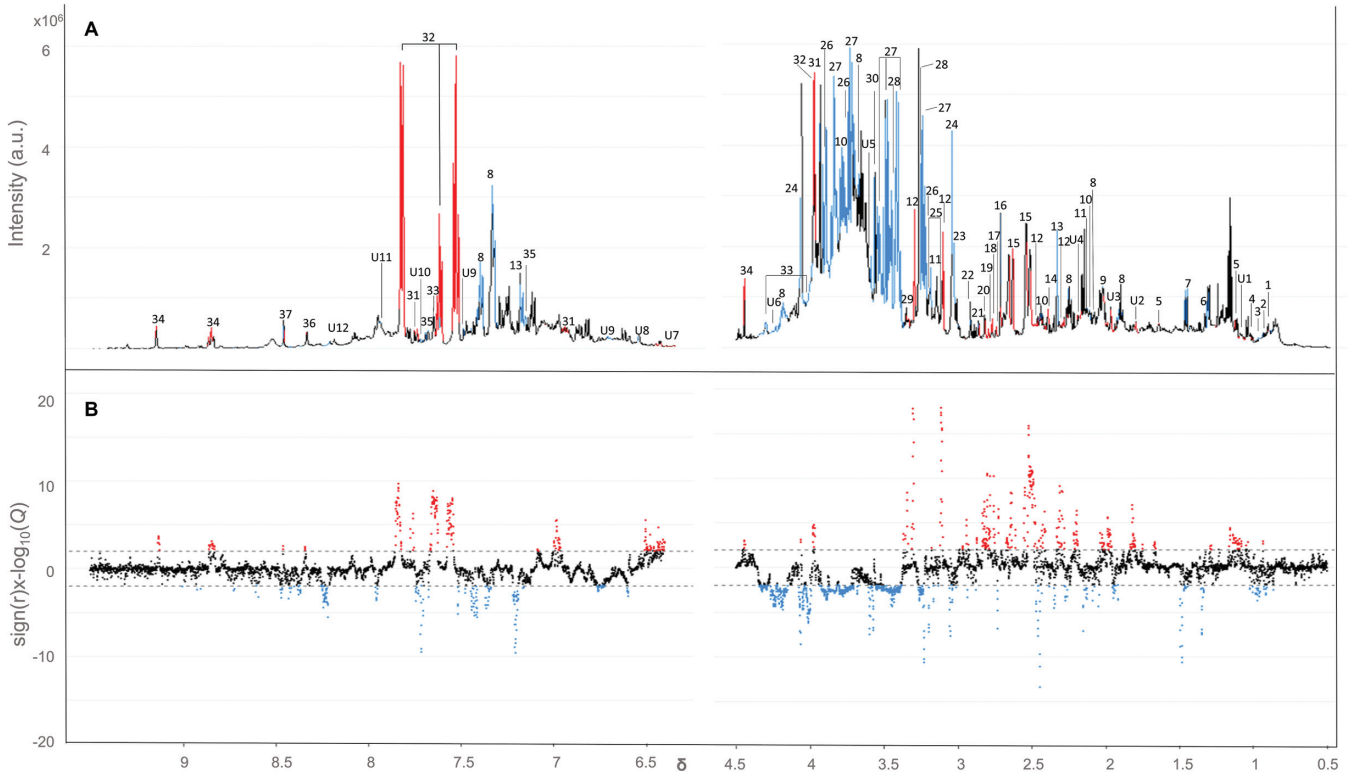

FIGURE 3 Associations of urinary metabolites with DASH dietary pattern score in the INTERMAP US cohort ($n = 2164$). Partial correlation with 7100 ^{1}H NMR variables adjusted for age, sex, race, education, physical activity, alcohol, smoking, vitamin supplement usage, special diet reported, history of cardiovascular disease, antihypertensive medication, family history of hypertension, mean energy intake, and BMI. (A) Median 600 MHz ^{1}H NMR spectrum, showing significant DASH-associated metabolites in the first urine collection. (B) Manhattan plot showing -$\log_{10}$ (Q) × sign of partial correlation coefficient (r) for each of the spectral variables. Significance was determined based on a Q-value threshold < 0.01. Significant variables are colored red if directly associated and blue if inversely associated. Significant metabolites are numbered as follows: 1: pantothenate; 2: isoleucine; 3: leucine; 4: valine; 5: 2-hydroxy-2-(4-methyl cyclohex-3-en-1-yl) propoxy glucuronide; 6: 2-hydroxyisobutyrate; 7: alanine; 8: phenylacetylglutamine; 9: *N*-acetylglycoproteins; 10: glutamine; 11: *O*-acetyl carnitine; 12: proline betaine; 13: 4-cresyl sulfate; 14: succinate; 15: citrate; 16: dimethylamine; 17: *S*-methyl cysteine sulfoxide metabolite; 18: *N*-acetyl-*S*-methyl cysteine sulfoxide; 19: *S*-methyl cysteine sulfoxide metabolite; 20: *S*-methyl cysteine sulfoxide; 21: trimethylamine; 22: dimethylglycine; 23: creatine; 24: creatinine; 25: histidine; 26: 3-methylhistidine; 27: glucose; 28: taurine; 29: 4-hydroxyproline betaine; 30: glycine; 31: 4-hydroxyhippurate; 32: hippurate; 33: pseudouridine; 34: *N*-methyl nicotinate; 35: 2-furoyl glycine; 36: *N*-methyl-2-pyridone-5-carboxamide; and 37: formate, U1 to U12 unidentified metabolites. Data are tabulated in Supplemental Table 3. Further information on metabolite identification can be found in Supplemental Table 7. a.u., arbitrary unit, DASH, Dietary Approaches to Stop Hypertension; INTERMAP, International Study of Macro-/Micronutrients and Blood Pressure; ^{1}H NMR, proton nuclear magnetic resonance.

Comparison of ^{1}H NMR spectral variables associated with DASH scores, sodium, and potassium

There was a total of 28 shared metabolites present in the metabolic signatures relating to both DASH scores and excretion of potassium. For both the DASH scores and potassium, direct associations were found with tricarboxylic acid (TCA) intermediates (succinate and citrate), metabolites involved in vitamin metabolism [NMNA; *N*-methyl-2-pyridone-5-carboxamide (2PY), and pantothenate], markers of citrus fruit consumption (proline betaine and 4-hydroxyproline betaine), markers of gut microbial activity (hippurate, 4-hydroxyhippurate, and trimethylamine), and markers of cruciferous vegetable intake (SMCSO). For both DASH scores and excretion of potassium, inverse associations were found with metabolites involved in the branched-chain amino acid (BCAA) metabolism (isoleucine, leucine, and 3-hydroxyisovalerate), amino acids (alanine, glutamine, and glycine), gut-microbial cometabolites (PAG, 2-hydroxyisobutyrate, dimethylamine, and dimethylglycine), metabolites relating to inflammation [*N*-acetyl signals from urinary glycoproteins fragments, predominantly α-1-acid glycoprotein with contributions from other glycoproteins (31)], a metabolite relating to nucleic acid turnover (pseudouridine), and 2-furoylglycine [a marker of coffee consumption (32)]. In addition, several shared spectral variables associated with both excretion of sodium and the DASH score are in the opposite direction, including direct markers of meat intake (*O*-acetyl carnitine, 3-methylhistidine, alanine, dimethylglycine, and glutamine; directly associated with sodium), markers of fruit and vegetable intakes (hippurate, proline betaine, and citrate; directly associated with the DASH score), a metabolite involved in 1-carbon metabolism of the gut microbial origin (formate; directly associated with sodium), and a precursor of distal colonic microbial protein putrefaction (tyrosine; directly associated with sodium).

Mediation of urinary metabolites in the relationship between DASH scores and SBP and between potassium and SBP

With adjustments for model 1 covariates and BMI, a mediation analysis of DASH-associated urinary metabolites on the DASH-SBP association found significant mediation effects for alanine,

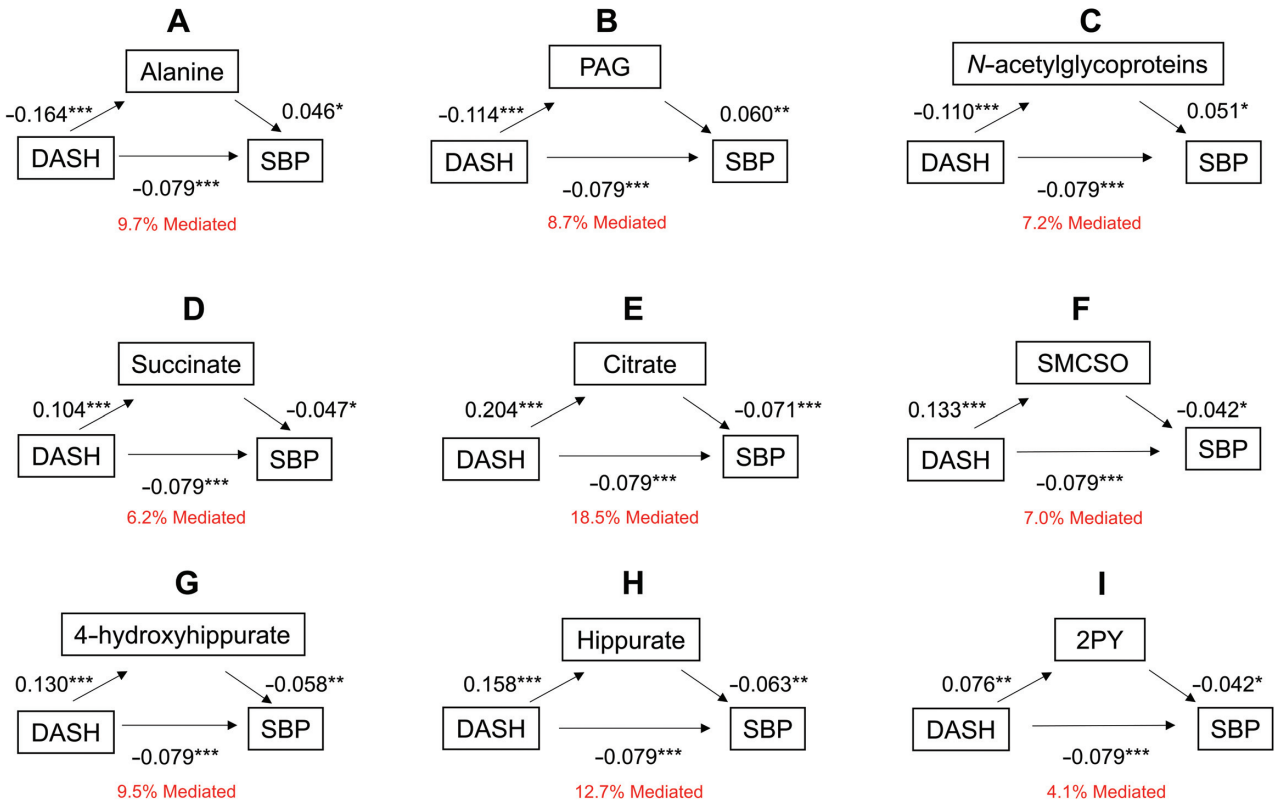

FIGURE 4 Mediation analysis for association between DASH score and SBP (mmHg) as mediated by (A) alanine, (B) PAG, (C) *N*-acetylglycoproteins, (D) succinate, (E) citrate, (F) SMCSO, (G) 4-hydroxyhippurate, (H) hippurate, and (I) 2PY in the INTERMAP US cohort ($n = 2164$). $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$. Adjusted for age, sex, race, education, physical activity, alcohol, smoking, vitamin supplement usage, special diet reported, history of cardiovascular disease, antihypertensive medication, family history of hypertension, mean energy intake, and BMI. DASH, Dietary Approaches to Stop Hypertension; INTERMAP, International Study of Macro-/Micronutrients and Blood Pressure; PAG, phenylacetylglutamine; SBP, systolic blood pressure; SMCSO, *S*-methyl cysteine sulfoxide; 2PY, *N*-methyl-2-pyridone-5-carboxamide.

PAG, *N*-acetylglycoproteins, succinate, citrate, SMCSO, 4-hydroxyhippurate, hippurate, and 2PY, with mediated proportions ranging from 4.1% for 2PY to 18.5% for citrate (**Figure 4** and **Supplemental Table 14**). The data from the second set of US urine samples replicated 7 of the 9 mediatory metabolites; the exceptions were *N*-acetylglycoproteins and 2PY (**Supplemental Table 15**). An additional mediation analysis was performed between potassium and SBP, as mediated by urinary metabolites. Five metabolites were found to have a significant mediation effect. Fatty acids (C5–C10), *N*-acetylglycoproteins, citrate, hippurate, and formate mediated 10.2%, 9.9%, 20.2%, 11.5%, and 6.0% of the association, respectively (**Figure 5** and **Supplemental Table 16**). The data from the second set of US urine samples replicated 3 of the 5 mediatory metabolites; the exceptions were fatty acids (C5–C10) and *N*-acetylglycoproteins (**Supplemental Table 17**). Excretion of sodium was excluded from the mediation analysis of urinary metabolites because, in the present study, sodium had no effect on the DASH-BP relation.

Identification of pathway associations using metabolic reaction networks

The multicompartmental reaction network (**Supplemental Figure 3** and **Supplemental Table 18**) shows that TCA intermediates (succinate and citrate) were positively associated with the DASH score, and that the amino acids alanine and glutamine feed into the TCA cycle. Notably, metabolites derived from the gut microbiome and related to the DASH score mapped onto several pathways. Dimethylamine, trimethylamine, dimethylglycine, and formate mapped onto choline metabolism, a pathway that has previously been linked with the development of CVD (33), whilst hippurate, 4-hydroxyhippurate, 4-cresyl sulfate, PAG, and 3-hydroxymandelate were clustered with the aromatic amino acid metabolism. The urinary metabolic profile was also suggestive of enhanced metabolic activity relating to the vitamin metabolism (specifically, vitamins B_3 and B_5) and reduced activity relating to the BCAA metabolism and muscle turnover. In support, 3-methylhistidine was related to meat intake and muscle mass, and was found here to be inversely associated with the DASH diet (34).

Discussion

Urinary metabolic signatures associated with the 24-hour dietary DASH scores and urinary excretion of sodium and potassium were characterized, and their interactions and possible nutrient-related pathways proposed. Consistent with previous reports, an inverse association between higher adherence to the DASH diet and BP was found (35). Excretion of potassium was

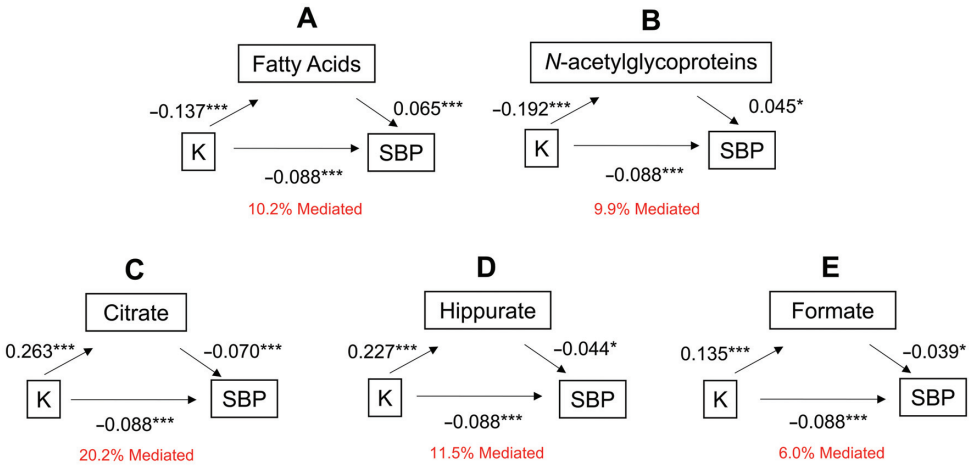

FIGURE 5 Mediation analysis for association between excretion of potassium and systolic blood pressure (mmHg), as mediated by (A) fatty acids (C5–C10), (B) *N*-acetylglycoproteins, (C) citrate, (D) hippurate, and (E) formate in the INTERMAP US cohort ($n = 2164$). $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$. Adjusted for age, sex, race, education, physical activity, alcohol, smoking, vitamin supplement usage, special diet reported, history of cardiovascular disease, antihypertensive medication, family history of hypertension, mean energy intake, and BMI. INTERMAP, International Study of Macro-/Micronutrients and Blood Pressure; K, potassium; SBP, systolic blood pressure.

strongly associated with the DASH score, and potassium adjustment attenuated the DASH-BP relationship. The connection between DASH scores and urinary potassium is likely derived from increased fruit, vegetable, and dairy intakes in the DASH diet, resulting in increased dietary potassium. This may explain the overlap between the DASH diet and potassium intake in lowering BP. Although reduced sodium intake has previously been showed to ameliorate the DASH diet's impact on BP (6, 7), in this investigation, sodium did not appear to attenuate the DASH-BP relation (36). Possible explanations include the relatively high sodium intake among US participants (only 12.5% of them met the CDRR level for sodium) and the fact that the high potassium content, or possibly the calcium content, of the DASH diet attenuated the effects of low sodium intake (37, 38). Since sodium and potassium are both key drivers of the renin-angiotensin system, there may be some redundancy when targeting this pathway (39).

Many metabolites associated with potassium excretion were also associated with the DASH score, whereas some metabolites associated with sodium were in the opposite direction. This suggests that potassium (a nutrient) and DASH (a dietary pattern) together form a nutritional complex that fortifies favorable effects on certain metabolic pathways, whilst sodium may have contrasting, adverse effects on these same pathways. Several of the metabolites common to both DASH scores and potassium excretion are recognized biomarkers of healthy diet patterns (40, 41). Additionally, the metabolites associated with a higher DASH score and lower sodium intake included reduced excretion of markers of meat intake and microbial protein putrefaction, suggestive of a predominately plant-based diet. These findings may provide new insight into the interaction of these dietary variables with BP.

A large body of evidence supports the beneficial effects of the DASH dietary pattern and potassium intake on BP and on CVD risks, but the specific nutrients and metabolic pathways involved are largely unknown (4, 6, 10, 42). We found hippurate and citrate mediated both the DASH-BP and potassium-BP relationships. Hippurate is derived from gut microbial fermentation of plant phenolics to benzoic acid (43). An inverse association between hippurate and BP has been reported previously (19) and may reflect dietary modulation of gut microbial activity. Probiotic intake has been shown to lower BP, which suggests a role for the gut microbiota in the development of hypertension (44). In metabolic profiling studies, spontaneously hypertensive rats excreted less hippurate, citrate, and succinate, but more phenylacetylglycine and 4-cresyl glucuronide (45), consistent with the diet-dependent associations found in this study of the gut microbe and TCA-related metabolites with BP. Additionally, citrate facilitates absorption of calcium and magnesium, which have known BP benefits (46).

We found 5 metabolites that may be specifically involved in the metabolic pathway linking DASH scores and BP: succinate, alanine, SMCSO, and 2 gut microbial metabolites (4-hydroxyhippurate and PAG). In certain conditions, such as diabetes, succinate is thought to signal through succinate receptor 1 (SUCNR1), causing renin-induced hypertension (47). A high intake of alkaline foods—that is, fruit and vegetables—could result in increased excretion of TCA intermediates due to decreased utilization in the proximal tubular mitochondria, and could prevent SUCNR1-mediated activation of the renin-angiotensin system (48). Moreover, stimulation of SUCNR1 leads to the production of nitric oxide and prostaglandin E_2, both well-established vasodilators (47), and suggests that these intermediaries of the metabolic pathway regulate several aspects of renal function, consistent with previously reported interactions between the DASH dietary pattern and the renin-angiotensin system (49).

Animal protein is a rich source of alanine (50); thus, our findings of a direct association between alanine and BP is consistent with a high-meat diet. One potential mechanistic link between alanine and BP is through modulation of cardiovascular responses via circulating catecholamines (51). Alanine has been reported to reduce norepinephrine release from cardiac sympathetic nerves in animals, subsequently elevating BP (52).

Another possibility is the interaction between alanine and insulin, which has also been shown to affect arterial BP (53). SMCSO is a sulfur-containing phytochemical found in cruciferous vegetables. Sulfur is important for cardiovascular health, as sulfur deficiency causes abnormalities in the endothelial function (54). Sulfur-containing compounds, such as allicin and *S*-allylcysteine, can lower BP through stimulation of hydrogen sulfide and nitric oxide production, as well as blockage of angiotensin-II production, which subsequently induces vasodilation and reduces BP (55). Thus, it is plausible that SMCSO lowers BP through similar actions on the endothelial function.

Our results suggest that the DASH dietary pattern modulates gut microbiome activity, lowering BP via multiple microbial-medicated pathways, concordant with previous findings (56). Urinary 4-hydroxyhippurate, inversely associated with BP, showed a close connection to hippurate in the metabolic network; both are products of the benzoic acid metabolism, and a strong intercorrelation has been reported previously (57). Increased excretion of phenolics has been associated with improvements in flow-mediated dilation by a mechanism thought to involve enhanced nitric oxide bioavailability (58). Conversely, a direct association between BP and microbially derived PAG was observed, consistent with findings of increased excretion of phenylacetylglycine (the rodent equivalent) in spontaneously hypertensive rats (45). PAG has been associated with an increased risk of coronary artery disease and is suggested to be a mediator of the gut microbiota–CVD relationship (59). In addition to higher potassium intake, this microbial influence could account for the independent benefits that the DASH dietary pattern has on BP.

Particular strengths of this study include the large sample size; the collection of multiple, high-quality, 24-hour urinary samples, synchronized with interviewer-administered 24-hour dietary measurements; the use of standardized measurements of urinary metabolites by ^{1}H NMR; and the use of objective measures of weight, height, and 24-hour excretion of sodium and potassium. Increased confidence in the reproducibility of the urinary metabolite excretion patterns over time and across populations is drawn from the replication and validation sets. Limitations of the study include the cross-sectional design, which prohibits drawing causal inferences; self-reported dietary data that are subject to recall bias; regression-dilution bias due to imprecise measurements; 24-hour recalls that may not reflect true dietary intakes; and moderate-reliability estimates of DASH scores, since a food-based scoring algorithm was used. We used mediation analyses and network mapping to reconstruct the metabolic relationships. However, mediation analyses rely on the assumption that there is no uncontrolled confounding. The potential difficulty of generalizing the results to non-US and non-UK populations should therefore be acknowledged.

In conclusion, this study showed that higher excretion of potassium, but not sodium, was strongly associated with higher DASH dietary pattern scores, consistent with the higher plant-based intakes of individuals adherent to the DASH dietary pattern, and was associated with a lower BP. Specific urinary metabolites, including microbially derived biochemicals, may mediate these associations. Some mediatory metabolites, common to the potassium-BP and DASH-BP relations, suggest partial overlapping mechanisms for lowering BP. Notably, we also found mediatory metabolites specific to the DASH dietary pattern, indicative of distinct DASH-associated pathways. Future interventional and experimental evidence is required to confirm the involvement of the identified metabolites in the causal pathway linking the DASH dietary pattern and BP.

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The authors' responsibilities were as follows – ARD, LMS, LJA, BLR, PE, JS, LVH: designed the International Study of Macro-/Micronutrients and Blood Pressure (INTERMAP), conducted the fieldwork, and collected data; QC, GMW: performed the analysis, interpreted the data, and wrote the paper; C-HEL, RLL, EH: contributed to the biochemical analysis for biomarkers and revised the work critically for important intellectual content; RG, GSA, LVH: contributed to the dietary data analysis and revised the work critically for important intellectual content; TMDE, JMP, ARD: contributed to the statistical analysis and revised the work critically for important intellectual content; LMS, LJA, BLR, MLD, PE, JS: revised the work critically for important intellectual content; EH, LVH: were responsible for the final content; and all authors: read and approved the final manuscript. The authors report no conflicts of interest.

Data Availability

An anonymized data set including data described in the manuscript, code book, and analytic code is available upon request pending application and approval.

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