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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CONFLICTS OF INTEREST

None of the authors report a conflict of interest related to research presented in this article.

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SHORT RUNNING HEAD

DASH diet, sodium, potassium and blood pressure

ABBREVIATIONS

^{1}H NMR, proton nuclear magnetic resonance; 2PY, *N*-methyl-2-pyridone-5-carboxamide; 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; FDR, false discovery rate; INTERMAP, International Study of Macro-/Micronutrients and Blood Pressure; K/Na, potassium to sodium; KEGG, Kyoto Encyclopaedia of Genes and Genomes; NMNA, *N*-methyl nicotinate; PAG, phenylacetylglutamine; Q1, quintile 1; Q5, quintile 5; QC, quality control; SBP, systolic blood pressure; SMCSO, *S*-methyl cysteine sulfoxide; SUCNR1, succinate receptor 1; TCA, tricarboxylic acid; TSP, trimethylsilyl propionate; UK, United Kingdom; US, United States.

CLINICAL TRIAL REGISTRY

The INTERMAP is registered as NCT00005271 at www.clinicaltrials.gov.

DATA AVAILABILITY

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

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.

Objective: Among free-living populations, delineate metabolic signatures associated with the DASH diet adherence, 24-hr urinary sodium and potassium excretions and the potential metabolic pathways involved.

Design: 24-hr urinary metabolic profiling by proton nuclear magnetic resonance spectroscopy was used to characterize the metabolic signatures associated with the DASH dietary pattern score (DASH score) and 24-hr excretion of sodium and potassium among participants in the United States (n=2,164) 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 DASH diet was associated with urinary potassium excretion (correlation coefficient, r = 0.42, P<0.0001). In multivariable regression analyses, a five-point higher DASH score (range 7 to 35) was associated with a lower systolic BP by 1.35 mmHg (95% confidence interval: -1.95, -0.80, P=1.2x10^{-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 five metabolites (succinate, alanine, *S*-methyl cysteine sulfoxide, 4-hydroxyhippurate, phenylacetylglutamine) were found specific to the DASH-BP relation.

Conclusions: Greater adherence to 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.

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

INTRODUCTION

Elevated blood pressure (BP), defined as systolic blood pressure (SBP) of ≥120 mm Hg or diastolic blood pressure (DBP) of ≥80 mm Hg, remains a key independent risk factor for developing cardiovascular diseases (CVD) and 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 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 wholegrains, and is limited in red/processed meat, sodium rich processed foods and sugar-sweetened beverages. The DASH feeding trial, reported SBP lowered by 5.5 mm Hg more in the DASH vs. control diet (typical of what many Americans eat) (6). The BP reduction occurred despite sodium being fixed at 3,000 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 two different but interrelated dietary factors. Data from feeding trials reported *N*-methylproline, chiro-inositol, proline betaine, and theobromine (as potential biomarkers of 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-hr 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 (US) and United Kingdom (UK) were analyzed. The aims were: 1) to delineate urinary metabolic signatures associated with the DASH dietary pattern score (DASH score), objectively measured levels of sodium and potassium and test the reproducibility of these relationships, 2) to discover possible metabolic pathways underlying the diet-BP relationships that might be in common with both DASH score and urinary measure of potassium collectively or specifically. Our primary outcome was association of BP with DASH score and secondary outcomes were DASH score with urinary excretion of sodium and potassium.

METHODS

Population sample

The INTERMAP is a cross-sectional epidemiological study of 4,680 men and women aged 40 to 59 years from four countries (China, Japan, UK, and US) (11). From 1996 to 1999, participants were randomly recruited from community or workplace population lists, arrayed into four age/sex strata. Each participant attended four visits with the first two visits occurring on consecutive days and followed three weeks later by the second two visits (**Supplementary 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 2,164 of

the 2,195 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-hr urine samples (**Supplementary Figure 2)**.

Clinical measurements

BP was measured eight times (two per visit) according to a standardized protocol implemented by trained staff at each visit following at least five minutes rest with a random-zero sphygmomanometer (11). The mean of the eight BP measurements was calculated and used in the analyses. Hypertension status was defined as SBP ≥ 140 mm Hg or DBP ≥ 90 mm Hg, with/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 four 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-hr 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 the UK were collected on paper forms and coded from an INTERMAP codebook derived from FOODBASE food codes. Intake of food groups and nutrients were estimated from the mean of the four 24-hr dietary recalls. The DASH score was calculated as previously described by Fung *et al.* (10) and was based on the intake of the following seven key food components; fruits, vegetables, nuts and legumes, low-fat dairy products, and whole grains

and low intakes of sweetened beverages, red meat, high sodium processed meats and other processed foods (**Supplementary Table 1**). For each component, individuals were classified into quintiles according to their intake. Quintile 1 (Q1) was assigned 1 point and quintile 5 (Q5) was assigned 5 points for desirable food groups. For less desirable food groups a 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 DASH score and 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, i.e., chronic disease risk reduction (CDRR) level of 2,300 mg/day of sodium and adequate intake (AI) of 2,600 mg/day for women and 3,400 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-hr urine collections were obtained from individuals on visits two and four; 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 protocol violation, i.e., "more than a few drops" were missing from the collection, the 24-hr urinary volumes were <250 ml, or the timing of the collection fell outside the 20-hr to 28-hr range. Sodium and potassium were measured using flame emission photometry and excretion values were corrected to 24-hr with internal and external quality control. As part of quality control (QC) procedures, 8% random sample were split at the clinical center and sent to the laboratory with different identification numbers for external assessment of measurement precision (16). The mean and median coefficient of variation were 0.75% and 0.28% for urinary sodium measurements and 0.60% and 0.35% for urinary

potassium measurements. As excretion of sodium and potassium are considered more objective and have greater validity than self-reported dietary data for individuals, the mean of the two samples was calculated and used throughout the present study as a proxy for dietary intake (17).

Proton nuclear magnetic resonance spectroscopy

Urine specimens were prepared for high-resolution ^{1}H NMR spectroscopy with the inclusion of 144 QC samples. QC samples were obtained from three healthy volunteers with two QC aliquots from each volunteer; 6 QC samples was added to each well-plate as described previously (18); analytical reproducibility was high (coefficient of variation <5%). ^{1}H NMR spectra of the urine specimens were obtained at 300K using a Bruker (Bruker Biospin, Rheinstetten, Germany) Avance 600MHz spectrometer (19). Spectra were acquired using a standard one-dimensional pulse sequence (recycle delay -90°-t1-90°-tm-90° acquisition) with water suppression (20). Fourier transformation was applied to the free induction decays, spectra were referenced to deuterated trimethylsilyl propionate (TSP), and baseline and phase corrected. Spectra were automatically phased and baseline corrected using Bruker Topspin 3.5 software (Bruker BioSpin, Germany). Pre-processing 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 7,100 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 were 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 two-dimensional NMR experiments including, Total Correlation Spectroscopy and Heteronuclear Single Quantum Coherence, on a QC sample. Additionally, Statistical Total Correlations Spectroscopy (23) was applied to the ^{1}H NMR spectral dataset to provide information on the molecular structural correlation.

Statistical methods

All statistical analyses were undertaken using SAS version 9.4 (SAS Institute Inc., Cary, North Carolina, USA) or R programming software packages version 3.5. Independent ^{1}H NMR data analyses were performed for each study visit (**Supplementary Figure 1**) to align with each 24-hr 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 score according to sex, weight status, and race (self-reported). Partial correlation coefficients (r) between DASH score and its seven components and urinary excretion of sodium, potassium and sodium to potassium (Na/K) ratio were calculated respectively. Reliability as a measure of possible regression dilution bias (25) for 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+(ratio/2)]x100. The ratio is intraindividual variance divided by interindividual variance, calculated from mean intakes/BP levels of the first and second 2 visits to account for higher correlation between intakes/BP levels on consecutive days (26).

For the discovery analysis (US samples, first urine collection), we identified urinary metabolites significantly associated with the DASH score (and its components), excretion of

sodium and potassium by partial Pearson correlation. *Q* values were calculated using the Benjamini-Hochberg false discovery rate (FDR) to correct for multiple testing for the 7,100 ^{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) and a 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 further partial correlation analysis against the seven key DASH food components.

The relationship of the DASH dietary pattern to BP and its modulation by other dietary factors was investigated using multivariable linear regression, and the associations between each one-point increase in DASH score with BP were examined. Multiple cross-tabulation analysis, based on quintiles of DASH score and urinary excretion of sodium and potassium, was used to further examine the relationship between DASH, sodium, potassium, and BP. Adjustments were made for the following covariates in the multivariable models: Model 0 adjusted for age (years, continuous), sex, and race; Model 1 additionally adjusted for education (years), physical activity (hours/day), alcohol (g/day, continuous), smoking (yes/no), vitamin supplement usage (yes/no), special diet (including salt/fat reduction) reported (yes/no), history of CVD (yes/no), anti-hypertensive medication (yes/no), family history of hypertension (yes/no), and mean energy intake (kcal/day, continuous). Models 2, 3, 4 were Model 1 plus additional adjustment for excretion of sodium, potassium, Na/K ratio,

respectively. As obesity may lie on the causal pathway between diet and BP, all models were computed without and with adjustment for BMI (continuous variable).

Mediation analysis

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 indirect to total effect was calculated to estimate the mediated proportion 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 1,000 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, identified in the ^{1}H NMR partial correlation analysis. A reconstruction of the symbiotic and

co-metabolic reactions occurring between the host and different gut microbial species was generated, and the connectivity between metabolites using information from the Kyoto Encyclopaedia of Genes and Genomes (KEGG). A metabolic reaction database was created including 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 two metabolites are linked if a biochemical reaction entry in KEGG indicates they are a main reactant pair and the reaction is mediated by; a) an enzyme linked to human genes, b) an enzyme linked to microbial genes or, c) 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 two 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 which used the first set of urine samples, were replicated using the second set of urine samples from the US cohort, obtained on average three 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 dataset, to evaluate reproducibility in an independent population. The same methods and protocol were used for the US and UK INTERMAP sample data collection (11). For the replication analyses (US, second urine collection), a

threshold of $Q < 0.01$ was used to indicate significant associations. For the validation analyses (UK ^{1}H NMR data), a threshold of $Q < 0.05$ was used due to the smaller sample size in the UK cohort.

RESULTS

Descriptive statistics

For the INTERMAP US cohort, the mean DASH score from four dietary recalls was similar in females (21.0, SD 5.0) and males (20.9, SD 4.7). Participants in Q5 of DASH adherence (DASH Q5, highest DASH score) compared to DASH Q1 (lowest DASH score) were older, more likely to be white, completed more years in education, were less likely to be current smokers, had a lower BMI, more likely to take vitamin/mineral supplements and indicate they followed a special diet. They also had higher levels of urinary potassium (**Table 1**). Excretion of sodium was similar across the DASH quartiles, 270 US participants (12.5%) met CDRR level for sodium (i.e. sodium excretion <2,300 mg/day). Excretion of potassium was 46.2 (SD 16.9) mmol/24-hr for participants in DASH Q1 compared to 72.1 (SD 21.6) mmol/24-hr for those in DASH Q5 and 344 participants (16%) consumed AI level of potassium estimated from their 24-hr urine excretion.

Reliability

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

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

With adjustment for Model 1 covariates and BMI, DASH score showed a stronger partial Pearson correlation (P<0.0001) with excretion of potassium (r = 0.42) than urinary Na/K ratio

(r = -0.28) (**Supplementary Table 2**). Self-reported intakes of DASH recommended vegetables, fruits, wholegrains, nuts, legumes, and low-fat dairy were positively correlated with excretion of potassium; red meat and sugar sweetened beverages were negatively correlated with urinary potassium ($P < 0.0001$).

Association of DASH score and its components with blood pressure

In multivariable regression analyses with adjustment for covariates known to affect BP (Model 1) plus BMI, a five-point higher DASH score was associated with lower SBP by 1.35 mmHg (95% CI: -1.95, -0.80, $P = 1.2 \times 10^{-5}$), and DBP, lower by 0.40 mmHg (95% CI: -0.85, 0.03, $P = 0.07$) (**Table 2**); additional adjustment for potassium attenuated the association between DASH and SBP (from 1.35 mm Hg in Model 1 to 1.05 mm Hg in Model 3). However additional adjustment for sodium (Model 2) did not attenuate this association, a five-point higher DASH score was associated with lower SBP by 1.40 mm Hg (95% CI: -2.00, -0.80). With excretion of sodium and DASH score stratified into quintiles, adjusted mean SBP remained similarly higher for people in the DASH Q1 (lowest), compared with those in the DASH Q5 (**Figure 2A**). With excretion of potassium and DASH score stratified into quintiles, adjusted mean SBP was consistently lower for those in Q5 (highest potassium), compared to those in Q1 (lowest potassium), at all quintiles of DASH score (**Figure 2B**). For example, SBP was 117.7 (95% CI: 115.7, 119.6) mm Hg for potassium Q5 and DASH Q5 vs. 122.1 (95% CI: 120.0, 124.1) mm Hg for potassium Q1 and DASH Q1.

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

Partial Pearson correlation analysis (first urine collection) of 7,100 ^{1}H NMR spectral variables with DASH score, adjusted for Model 1 covariates and BMI showed that 37 urinary metabolites were significantly associated with DASH score at a *Q* value threshold of < 0.01 (**Figure 3**). Correlation coefficient (r) values between these urinary metabolites and DASH

score ranged from -0.18 for glutamine to 0.21 for proline betaine (**Supplementary Table 3**). Significant associations with DASH score of all 37 ^{1}H-NMR-identified metabolites from 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 three weeks later (**Supplementary Table 4**). Independent data from the INTERMAP UK cohort replicated 27 of the identified significant DASH-metabolite associations at a *Q* value threshold of < 0.05, this may reflect lower statistical power for these analyses because of the smaller sample size in the UK cohort (**Supplementary 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 (**Supplementary Table 6**). Three metabolites were negatively associated with wholegrains; 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), 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; five metabolites including phenylacetylglutamine (PAG) (r = 0.16) were associated with sugar sweetened beverages. Further information on metabolite identification can be found in **Supplementary Table 7**.

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

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 of < 0.01 (**Supplementary 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 analysis of the first set of urine samples could be replicated in the second set of urine samples (**Supplementary Table 9**). Validation using independent data from the INTERMAP UK cohort also showed similar results (**Supplementary Table 10**).

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

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

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

There was a total of 28 shared metabolites present in the metabolic signatures relating to both DASH score and excretion of potassium. For both the DASH score 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 score and excretion of potassium, inverse associations were found with metabolites involved in branched-chain amino acid (BCAA) metabolism (isoleucine, leucine, and 3-hydroxyisovalerate), amino acids (alanine, glutamine, and glycine), gut-microbial co-metabolites (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 DASH score are in the opposite direction, including direct markers of meat intake (*O*-acetyl carnitine, 3-methylhistidine, alanine, dimethylglycine, and glutamine; direct associated with sodium), markers of fruit and vegetable intake (hippurate, proline betaine, and citrate; direct associated with DASH score), a metabolite involved in one-carbon metabolism of gut microbial origin (formate; direct associated with sodium), and a precursor of distal colonic microbial protein putrefaction (tyrosine; direct associated with sodium).

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

With adjustment 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, 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 **Supplementary Table 14**). The second US urine collection data replicated seven of the nine mediatory metabolites, the exceptions were *N*-acetylglycoproteins and 2PY (**Supplementary 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 **Supplementary Table 16**). The second US urine collection data replicated three of the five mediatory metabolites, the exception were fatty acids (C5-C10) and *N*-acetylglycoproteins (**Supplementary Table 17**). Excretion of sodium was excluded from the mediation analysis of urinary metabolites as in the present study, sodium had no effect on the DASH-BP relation.

Identification of pathway associations using metabolic reaction networks

The multi-compartmental reaction network (**Supplementary Figure 3** and **Supplementary 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 aromatic amino acid metabolism. The urinary metabolic profile was also suggestive of enhanced metabolic activity relating to vitamin metabolism (specifically vitamin B_3 and B_5), and reduced activity relating to 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-hr dietary DASH score 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 strongly associated with the DASH score and potassium adjustment attenuated the DASH-BP relationship. The connection between DASH score and urinary potassium is likely derived from increased fruit, vegetable, and dairy intake 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 meeting the CDRR level for sodium), or the fact that the high potassium or possibly 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 score and potassium excretion are recognized biomarkers of healthy diet patterns (40, 41). Additionally, 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 CVD risk, but 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). Metabolic profiling studies of spontaneously hypertensive rats excreted less hippurate, citrate, and succinate, but more phenylacetylglycine and 4-cresyl glucuronide (45), consistent with the diet-dependent association of gut microbial and TCA-related metabolic with BP found in this study. Additionally, citrate facilitates absorption of calcium and magnesium, which have known BP benefits (46).

We found five metabolites that may be specifically involved in the metabolic pathway linking DASH score and BP: succinate, alanine, SMCSO, and two 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 content of alkaline foods, e.g. 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 metabolic intermediates 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 nor-epinephrine 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 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 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 (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 large sample size, multiple high-quality 24-hr urinary synchronized with interviewer administered 24-hr dietary measurements, standardized measurement of urinary metabolites by ^{1}H NMR, and use of objective measures of weight, height, and 24-hr 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 validations sets. Limitations of the study include the cross-sectional design thereby prohibiting causal inferences, self-reported dietary data subject to recall bias, regression-dilution bias due to imprecise measurements, 24-h recalls may not reflect true dietary intake and reliability estimates of DASH score was moderate since a food-based scoring algorithm was used. We used mediation analysis and network mapping, to reconstruct the metabolic relationships. However, mediation analysis relies on the assumption that there is no uncontrolled confounding. The potential difficulty of generalizability to non-US/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 score, consistent with the higher plant-based content of individuals adherent to the DASH dietary pattern and associated 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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AUTHORS' CONTRIBUTIONS

ARD, LMS, LJA, BLR, PE, JS, LVH: designed the INTERMAP Study, conducted the fieldwork and collected data; QC, GW: 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 and LVH: were responsible for final content and all authors: read and approved the final manuscript.

NOTES

Supplementary Tables 1-18 and Supplementary Figures 1-3 are available from the "Supplementary data" link in the online posting of the article.

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Table 1. Baseline characteristics of the INTERMAP-US cohort by quintiles of DASH score (n = 2,164).[1]

	DASH Q1*[2] *(n=410)		***DASH Q2 (n=437)***		***DASH Q3 (n=477)***		***DASH Q4 (n=420)***		***DASH Q5*[2] *(n=420)***		***P for trend***
DASH score, Median (range)	**15**	**(8-16)**	**18**	**(17-19)**	**21**	**(20-22)**	**24**	**(23-25)**	**28**	**(26-35)**	
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
Asian, n (%)	53	(12.9)	46	(10.5)	77	(16.1)	66	(15.7)	65	(15.5)	
Black, n (%)	173	(42.2)	148	(33.9)	105	(22.0)	70	(16.7)	44	(10.5)	
White, n (%)	176	(42.9)	241	(55.2)	290	(60.8)	280	(66.7)	306	(72.9)	
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, mm Hg	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, mm Hg	73.7	(9.8)	74.7	(9.9)	73.7	(9.8)	73.5	(9.5)	71.5	(9.2)	<0.0001
Education, years	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/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
Wholegrain, g/day	9.8	(16.9)	18.3	(25.2)	28.0	(31.7)	42.6	(47.7)	61.9	(50.1)	<0.0001
Vegetable 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 legume, 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-hr	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. Abbreviations: 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 Q1 = lowest DASH score; DASH Q5 = highest DASH score.

[3] Chronic disease risk reduction (CDRR) level for sodium: 2,300 mg/day.

[4] Adequate intake (AI) for potassium: women 2,600 mg/day and men 3,400 mg/day.

Table 2 Relation of five-point higher DASH score to systolic and diastolic blood pressure difference (mm Hg) in the INTERMAP-US cohort (n = 2,164).[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, -2.05)	9.2×10^{-16}	-1.65 (-2.25, -1.10)	4.3×10^{-8}	-0.95 (-1.35, -0.60)	5.8×10^{-7}	-0.45 (-0.85, -0.04)	0.03
1[3]	-2.05 (-2.65, -1.45)	2.8×10^{-10}	-1.35 (-1.95, -0.80)	1.2×10^{-5}	-0.75 (-1.20, -0.30)	7.0×10^{-4}	-0.40 (-0.85, 0.03)	0.07
2[4]	-2.05 (-2.65, -1.45)	1.9×10^{-10}	-1.40 (-2.00, -0.80)	1.3×10^{-5}	-0.75 (-1.20, -0.30)	7.0×10^{-4}	-0.40 (-0.85, 0.04)	0.07
3[5]	-2.05 (-0.54, -0.28)	2.1×10^{-8}	-1.05 (-1.75, -0.40)	0.001	-0.80 (-1.25, -0.30)	0.001	-0.30 (-0.75, 0.20)	0.26
4[6]	-1.75 (-0.48, -0.23)	3.2×10^{-7}	-1.20 (-1.85, -0.60)	1.0×10^{-4}	-0.65 (-1.15, -0.20)	0.004	-0.35 (-0.85, 0.08)	0.11

[1] ΔBP indicates blood pressure difference, calculated by multivariable linear regression. Abbreviations: 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 1 variables plus education, physical activity, alcohol, smoking, vitamin supplement usage, special diet reported, history of cardiovascular disease, anti-hypertensive 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.

LEGENDS FOR FIGURES

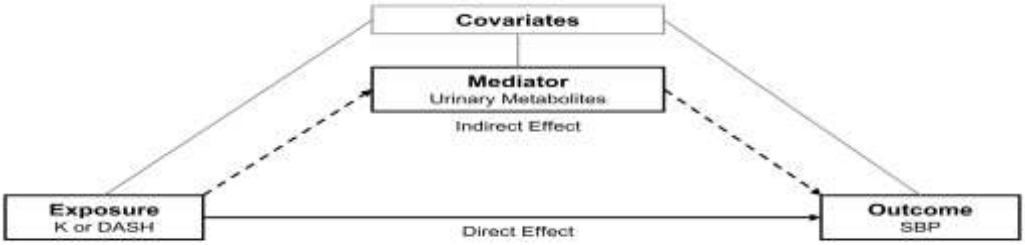

Figure 1 Mediation analysis model of associations between dietary exposures (the DASH diet or potassium) and systolic blood pressure, as mediated by urinary metabolites. The indirect effect is represented by dashed arrows and the direct effect by a continuous arrow. Mediator and outcome models were adjusted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

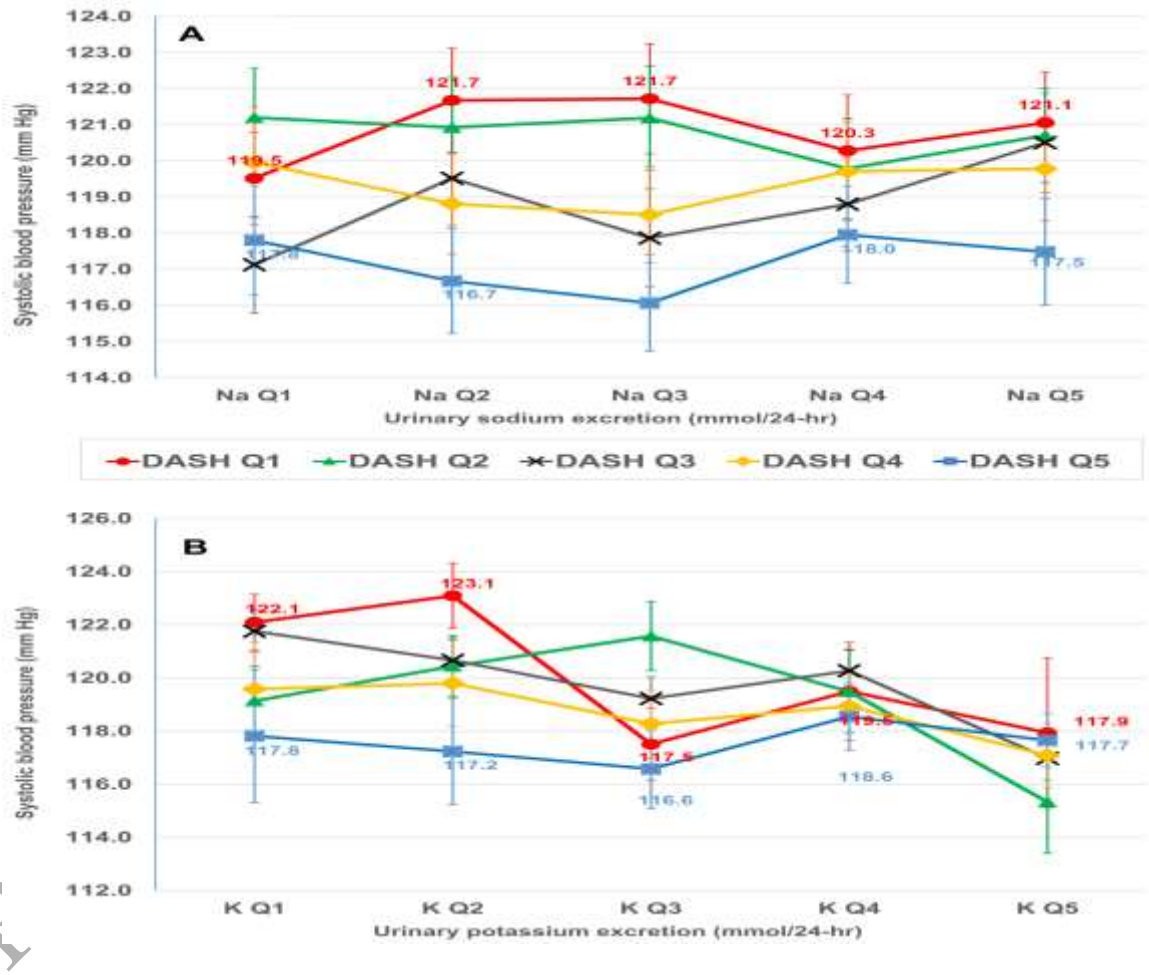

Figure 2 Mean systolic blood pressure (mm Hg) by sex specific quintiles of DASH score and (**A**) excretion of sodium (**B**) excretion of potassium in the INTERMAP-US cohort (*n* = 2,164). Adjusted for age, sex, race, education, physical activity, alcohol, smoking, vitamin supplement usage, special diet reported, history of cardiovascular disease, anti-hypertensive medication, family history of hypertension, mean energy intake, and BMI. Quintile cut-offs for DASH score were 17 (Q1), 20 (Q2), 23 (Q3), and 26 (Q4). Quintile cut-offs for excretion of sodium (mmol/24-hr) were 122.51 (Q1), 157.91 (Q2), 192.48 (Q3), and 234.45 (Q4) for males and 94.57, 125.03, 150.40, and 186.20 for females. Quintile cut-offs for excretion of potassium (mmol/24-hr) were 46.0 (Q1), 56.5 (Q2), 67.4 (Q3), and 82.6 (Q4) for males and 34.9, 44.1, 53.1, and 63.8 for females. DASH, Dietary Approaches to Stop Hypertension; INTERMAP, International Study of Macro-/Micronutrients and Blood Pressure; Q, Quintile.

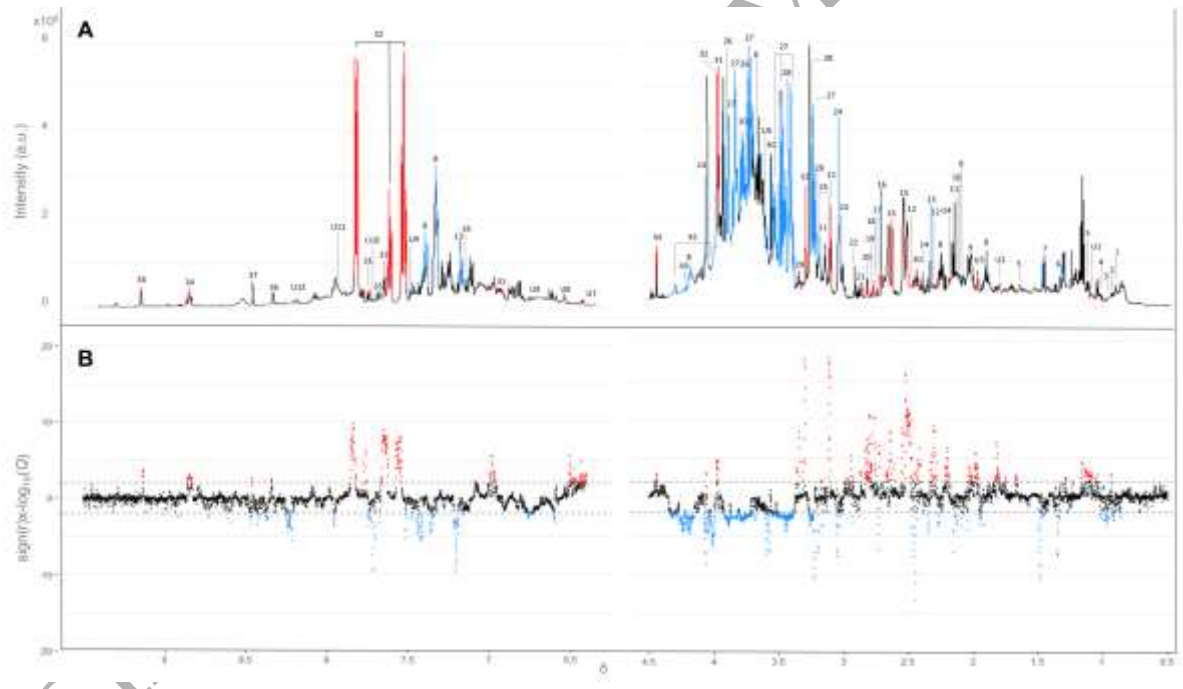

Figure 3 Associations of urinary metabolites with DASH dietary pattern score in the INTERMAP-US cohort (*n* = 2,164). Partial correlation with 7,100 ^{1}H NMR variables adjusted for age, sex, race, education, physical activity, alcohol, smoking, vitamin supplement usage,

special diet reported, history of cardiovascular disease, anti-hypertensive 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 of < 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 (PAG), 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, 37: formate, U1 to U12 unidentified metabolites. Data are tabulated in Supplementary Table 3. Further information on metabolite identification can be found in Supplementary Table 7. DASH, Dietary Approaches to Stop Hypertension; ^{1}H NMR, Proton Nuclear Magnetic Resonance; INTERMAP, International Study of Macro-/Micronutrients and Blood Pressure.

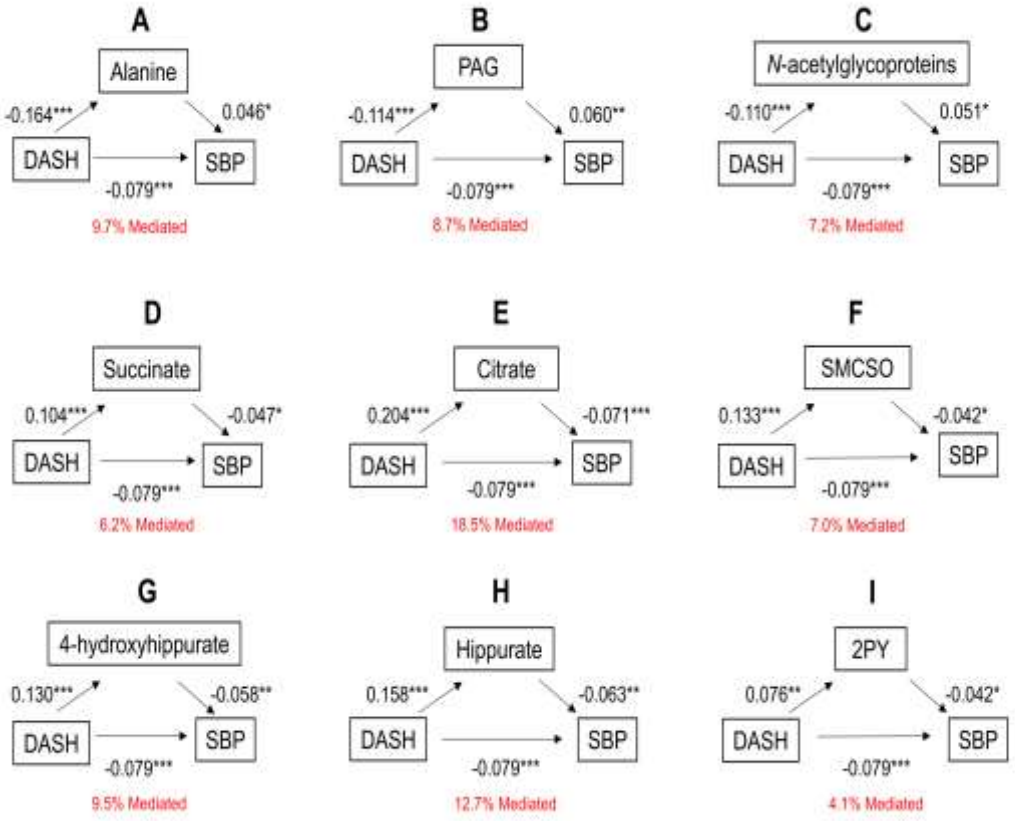

Figure 4 Mediation analysis for association between DASH score and systolic blood pressure (mm Hg) as mediated by alanine **(A)**, PAG **(B)**, *N*-acetylglycoproteins **(C**), succinate **(D)**, citrate **(E)**, SMCSO **(F)**, 4-hydroxyhippurate **(G)**, hippurate **(H)**, and 2PY **(I)** in the INTERMAP-US cohort *(n* = 2,164). Asterisks represent association significance (* $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$). Adjusted for age, sex, race, education, physical activity, alcohol, smoking, vitamin supplement usage, special diet reported, history of cardiovascular disease, anti-hypertensive medication, family history of hypertension, mean energy intake, and BMI. 2PY, *N*-methyl-2-pyridone-5-carboxamide; 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.

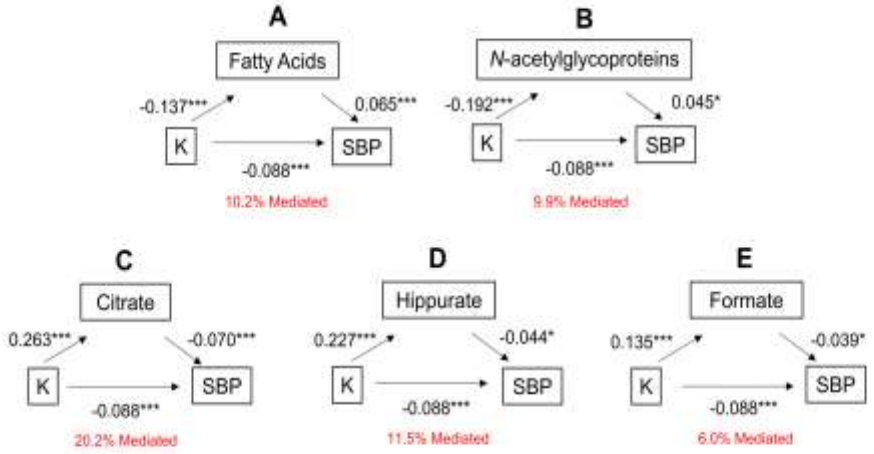

Figure 5 Mediation analysis for association between excretion of potassium and systolic blood pressure (mm Hg) as mediated by fatty acids (C5-C10) **(A)**, *N*-acetylglycoproteins **(B)**, citrate **(C)**, hippurate **(D)**, and formate **(E)** in the INTERMAP-US cohort *(n* = 2,164). Asterisks represent association significance (* $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$). Adjusted for age, sex, race, education, physical activity, alcohol, smoking, vitamin supplement usage, special diet reported, history of cardiovascular disease, anti-hypertensive 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.