# Original Article

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# An exploratory analysis of comparative plasma metabolomic and lipidomic profiling in salt-sensitive and salt-resistant individuals from The Dietary Approaches to Stop Hypertension Sodium Trial

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**Objective:** This study conducted exploratory metabolomic and lipidomic profiling of plasma samples from the DASH (Dietary Approaches to Stop Hypertension) Sodium Trial to identify unique plasma biomarkers to identify salt-sensitive versus salt-resistant participants.

**Methods:** Utilizing plasma samples from the DASH-Sodium Trial, we conducted untargeted metabolomic and lipidomic profiling on plasma from salt-sensitive and salt-resistant DASH-Sodium Trial participants. Study 1 analyzed plasma from 106 salt-sensitive and 85 salt-resistant participants obtained during screening when participants consumed their regular diet. Study 2 examined paired within-participant plasma samples in 20 salt-sensitive and 20 salt-resistant participants during a high-salt and low-salt dietary intervention. To investigate differences in metabolites or lipidomes that could discriminate between salt-sensitive and salt-resistant participants or the response to a dietary sodium intervention Principal Component Analysis and Orthogonal Partial Least Square Discriminant Analysis was conducted. Differential expression analysis was performed to validate observed variance and to determine the statistical significance.

**Results:** Differential expression analysis between salt-sensitive and salt-resistant participants at screening revealed no difference in plasma metabolites or lipidomes. In contrast, three annotated plasma metabolites, tocopherol alpha, 2-ketoisocaproic acid, and citramalic acid, differed significantly between high-sodium and low-sodium dietary interventions in salt-sensitive participants.

**Conclusion:** In DASH-Sodium Trial participants on a regular diet, plasma metabolomic or lipidomic signatures were not different between salt-sensitive and salt-resistant participants. High-sodium intake was associated with changes in specific circulating metabolites in salt-sensitive participants. Further studies are needed to validate the identified metabolites as potential biomarkers that are associated with the salt sensitivity of blood pressure.

**Keywords:** Dietary Approaches to Stop Hypertension–Sodium Trial, lipidomics, metabolomics, salt-resistant, salt-sensitive, sodium intake

**Abbreviations:** AHA, American Heart Association; BP, blood pressure; CSH, charged surface hybrid; CUDA, 12-[[(cyclohexylamino)carbonyl]amino]-dodecanoic acid; DASH, Dietary Approaches to Stop Hypertension; DSS, Dahl salt-sensitive; ESI, electrospray ionization; HILIC, hydrophilic interaction liquid chromatography; HS, high salt; LS, low salt; MeOH, methanol; MTBE, methyl-tertiary butyl ether; NHLBI, National Heart, Lung and Blood Institute; OPLS-DA, Orthogonal Partial Least Square Discriminant Analysis; PCA, principal component analysis; RI, retention index; SERFF, Systemic Error Removal by Random Forest; TG, triacylglycerol

## INTRODUCTION

Hypertension is a critical global health issue that is associated with concomitant increases in cardiovascular and renal disease and morbidity. According to the 2017 American Heart Association (AHA) guidelines, the prevalence of hypertension among United States adults is 46% [1]. Several clinical studies have presented strong evidence that excess dietary salt causes an increase in blood pressure (BP) that fosters an increased risk of premature cardiovascular morbidity and mortality [2–8]. Although the BP responses to salt modestly affect the population as a whole, some individuals exhibit an exaggerated BP response to salt intake and are characterized as salt-sensitive individuals. Salt-

sensitive hypertension occurs in approximately 50% of hypertensive patients, and salt-sensitive individuals are at an increased risk of adverse cardiovascular outcomes [7]. Despite the increasing evidence of adverse effects of excess dietary salt, 90% of United States adults consume an excess of dietary sodium intake that exceeds the AHA recommended level of less than 2300 mmol/day of sodium for most adults and a target intake of 1500 mmol/day of sodium that is recommended for hypertensive individuals [9]. Thus, identifying individuals that are salt-sensitive is critical. Currently, the only method of identifying salt-sensitive individuals is monitoring the BP changes with a carefully performed time-consuming dietary protocol that is not feasible for large-scale clinical diagnosis [10]. Therefore, there remains a critical need to identify alternative approaches to determine the salt sensitivity of BP.

Genetic, lifestyle, and environmental factors may influence the development of salt sensitivity. However, the exact underlying physiological and metabolic factors that drive the salt sensitivity of BP are not known. The profiling of biological analytes, including metabolites (metabolomics) and lipids (lipidomics), offers a unique approach to measure physiological and biochemical effects associated with a disease state [11,12]. Metabolomics and lipidomics have been increasingly used to identify disease biomarkers for cardiovascular diseases, including hypertension [13–16]. Thus, studying plasma metabolomic and lipidomic profiling in salt-sensitive individuals may help establish unique biomarkers to determine the salt sensitivity of BP.

In this study, we conducted exploratory untargeted plasma metabolomic and lipidomic profiling on salt-sensitive and salt-resistant participants from the Dietary Approaches to Stop Hypertension 2 Sodium (DASH-Sodium) Trial. The DASH-Sodium clinical trial examined the impact of dietary sodium on BP in individuals via a control diet, which models the typical American consumption, or the DASH diet, both delivered at high, intermediate, and low levels of sodium [17,18]. This carefully monitored dietary trial study provided the opportunity to identify salt-sensitive versus salt-resistant individuals by their BP response to dietary sodium intervention. The novelty of our current study is that we examined plasma metabolic and lipidomic traits among salt-sensitive and salt-resistant participants at screening when maintained on their regular diet (i.e. prior to a dietary intervention) and in a subset of participants their response to dietary salt intervention on the DASH control diet. Our primary hypothesis was that salt-sensitive participants would exhibit altered metabolic and lipidomic profiling at baseline screening on their regular diet. Our secondary hypothesis was that any differences in the metabolic and lipidomic profile between salt-sensitive and salt-resistant will be exaggerated by a high sodium diet.

## METHODS

The DASH-Sodium Trial, a multicenter randomized control trial sponsored by the National Heart, Lung and Blood Institute (NHLBI) was conducted to test the effects of dietary sodium on blood pressure. The details of the DASH-Sodium Trial (ClinicalTrials.gov Identifier NCT00000608; Clinical Trial Registry https://clinicaltrials.gov/ct2/show/NCT00000608) study design have been previously described in detail [17]. In brief, the trial

was conducted in 412 healthy adult individuals aged 22 years or older with a SBP of 120–159 mmHg and DBP of 80–95 mmHg (range normal to Stage 1 hypertension). After a screening phase (during which participants were consuming their regular diet) and a 2-week run-in period, by using a parallel study design method, participants were randomized to receive a control diet representing a typical American diet or a DASH diet that is rich in fruits, vegetables, and low-fat dairy food. Using a crossover design, the participants in each dietary arm (control or DASH) were further randomized to receive three different sodium levels – low (50 mmol/day – optimal recommended daily intake), intermediate (100 mmol/day – the upper limit of currently recommended sodium levels), or high-salt (150 mmol/day – current typical US sodium intake) sodium content, for 30 days.

Twenty-four-hour ambulatory blood pressure recordings and overnight fasted plasma samples were obtained during the screening period, when participants were consuming their regular diet, and during the last week of each dietary sodium intake period. The NHLBI Biologic Specimen and Data Repository Information Coordinating Center (BioLINCC) approved our request to obtain stored plasma samples from the DASH-Sodium Trial. In the DASH-Sodium Trial, participants who exhibited an SBP increase of 5 mmHg or higher on the high-salt diet compared with the SBP value recorded on a low-salt intake were classified as salt-sensitive, whereas participants with less than 5 mmHg change in SBP between high-salt and low-salt diet were considered salt-resistant. To characterize the metabolomic and lipidomic responses, we used plasma samples collected at the time of screening from patients consuming their standard daily intake (referred to as baseline) from 106 salt-sensitive and 85 salt-resistant participants (total 192 participants) who were subsequently assigned to a control diet in the DASH-Sodium low-salt or high-salt intervention. This represents the total number of participants that were randomly assigned to the control diet in DASH-Sodium for which plasma samples were available at screening and during the low-salt and high-salt interventions. Additionally, to address our secondary hypothesis, in a subset of these participants (20 salt-sensitive and 20 salt-resistant) who were randomly selected from the pool of 106 salt-sensitive and 85 salt-resistant participants, plasma samples from within the same participants, during both a high-salt and low-salt dietary intervention, while participants were maintained on a control diet, were also examined. An untargeted metabolic and lipidomic screen of plasma samples was performed by the West Coast Metabolomics Center at UC Davis (http://metabolomics.ucdavis.edu/) as described below. Raw data files are stored at the NIH metabolomics database (www.metabolomics-workbench.org) and KEGG identifiers were obtained from the community database KEGG LIGAND DB.

### Metabolomic profiling

#### Sample extraction, data acquisition and processing
Full details of sample preparation and data acquisition have been previously published [19]. Data processing was done using ChromaTOF versus 2.32 with the following settings: no smoothing, 3 s peak width, baseline subtraction just above the noise level, and automatic mass spectral deconvolution and peak detection at signal/noise levels of 5 : 1 throughout

the chromatogram. Result were exported to a data server with absolute spectra intensities and further processed by the BinBase filtering algorithm (rtx5) with the following settings: validity of chromatogram (<10 peaks with intensity $>10^7$ counts/s), unbiased retention index marker detection (MS similarity >800, the validity of intensity range for high m/z marker ions), retention index calculation by fifth order polynomial regression. Spectra were cut to 5% base peak abundance and matched to database entries from most to least abundant spectra using the following matching filters: retention index window ± 2000 units (equivalent to about ± 2 s retention time), validation of unique ions and apex masses (based on unique ion inclusion in apexing masses and presentation at >3% of base peak abundance), mass spectrum similarity fit criteria dependent on peak purity and signal/noise ratios, and a final isomer filter. Quantification was reported as peak height (*mz* value) using the unique ion as default at a specific retention index. A quantification report table was produced for all database entries that were positively detected in more than 10% of the samples of a study design class (as defined in the miniX database) for unidentified metabolites.

### Metabolomic data normalization and quality control
Data was normalized by vector normalization in which the sum of all peak heights for all identified metabolites for each sample was calculated and termed 'mTIC'. Subsequently, significant differences between the treatment groups or cohorts mTIC averages were determined. If these averages were different by *P* less than 0.05, the data were normalized to each group's average mTIC. If averages between treatment groups or cohorts were not different, data was normalized to the total average mTIC. Blanks were run as negative quality controls to evaluate contamination and background noise. Additionally, a quality control sample of National Institute of Standards and Technology standard plasma was run after each 11th sample injection.

## Lipidomic profiling

### Sample extraction
A 20 μl aliquot of each sample was added to 975 μl of premixed, ice-cold, $N_2$ purged 3 : 10 MeOH (methanol): MTBE (methyl-tertiary butyl ether) and quality control mix [22 : 1CE (cholesteryl esters)] and 188 μl of LC-MS grade water and gently shaken for 6 min at 4 °C. Following centrifugation (2 min at 14 000*g*), the upper organic phase was divided into two 350 μl aliquots and the bottom aqueous phase was divided into two 110 μl aliquots, one aliquot from each organic phase was dried down by centrivap. The upper phase was reconstituted in 110 μl of a MeOH/toluene (9 : 1) mixture containing an internal standard 12-[[(cyclohexylamino)carbonyl]amino]-dodecanoic acid (CUDA) at 50 ng/ml. The lower phase was reconstituted in 100 μl of acetonitrile : $H_2O$ (80 : 20).

## Data acquisition

### Charged surface hybrid analysis
Extracts were separated using a charged surface hybrid (CSH) C18 column (Waters). For ESI (electrospray ionization) positive mode the Mobile phase A constituted 60 : 40 acetonitrile : water and 10 mmol/l ammonium formate with 0.1% formic. Mobile phase B solvent constituted 90 : 10 isopepanolol : acetonitrile with 10 mmol/l ammonium formate and 0.1% formic acid at a flow rate of 0.6 ml/min. For ESI-negative mode, the composition of mobile phases was identical but 10 mmol/l ammonium acetate was used in place of ammonium formate. The quadrupole/time-of-flight (QTOF) mass spectrometers are operated with ESI performing a full scan in positive mode (Agilent 6530) and negative mode (Agilent 6550).

### HILIC analysis
Extracts were separated using a liquid chromatography gradient with a 0.6 ml/min linear velocity flow rate. Mobile phase followed the following gradient: 0 min 15% (B), 0–2 min 30% (B), 2–2.5 min 48% (B), 2.5–11 min 82% (B), 11–11.5 min 99% (B), 11.5– 12 min 99% (B), 12–12.1 min 15% (B), 12.1–15 min 15% (B). Acquisition speed was 2 spectra/s, and the mass range was *m/z* 60–1200 Da.

## Data processing and quality control
Data processing was done using MS-DIAL [20], followed by a blank subtraction in Microsoft Excel and cleanup of data using MS-FLO [21]. Peaks were annotated in manual comparison of MS/MS spectra and accurate masses of the precursor ion to spectra given in the Fiehn laboratory's LipidBlast spectral library [22]. Blanks were run as negative quality controls to evaluate both contamination and background noise and CUDA was used as internal standard in all samples. Additionally, a quality control sample of National Institute of Standards and Technology NIST standard plasma was run after 11th sample injection.

## Data analysis

### Data
The metabolomics and lipidomics data obtained from the UC Davis West Coast Metabolomics core was provided to GeneVia technologies for analysis. For metabolomics data, normalization was applied as described above. However, for lipidomics data since no normalization had been previously applied and as no internal standards were used as untargeted analysis was conducted, the data was assessed raw, and with two different normalization methods; quantile normalization and Systemic Error Removal by Random Forest (SERFF) normalization [23].

### Quality control
Principal component analyses (PCA) were performed on the data following $log_2$ transformation and the results were visualized using R packages *ggfortify* [24] and *ggplot2* [25] with the samples colored according to group. Separate PCAs were carried out on the raw data as well as the two different normalization methods for lipidomics data. Additional PCA plots were also produced, coloring the samples according to race and sex, to investigate any potential relationships. Orthogonal Partial Least Square Discriminant Analysis (OPLS-DA) was also performed as a secondary control indicator on the dataset, using the MetaboAnalyst R R package [26].

**TABLE 1. Baseline demographics of Dietary Approaches to Stop Hypertension–Sodium Trial participants**

| | Salt-resistant | | | | Salt-sensitive | | | |
|---|---|---|---|---|---|---|---|---|
| | Non-African Americans | | African American | | Non-African Americans | | African American | |
| | Male | Female | Male | Female | Male | Female | Male | Female |
| **Age [% (n)]** | | | | | | | | |
| 18–30 years | 3.7 (1) | 7.1 (1) | 5.0 (1) | 0.0 (0) | 3.8 (1) | 0.0 (0) | 0.0 (0) | 1.9 (1) |
| 31–55 years | 81.5 (22) | 71.4 (10) | 75.0 (15) | 87.5 (21) | 50.0 (13) | 50.0 (5) | 72.2 (13) | 75.0 (39) |
| ≥56 years | 14.8 (4) | 21.4 (3) | 20.0 (4) | 12.5 (3) | 46.2 (12) | 50.0 (5) | 27.8 (5) | 23.1 (12) |
| **Income [% (n)]** | | | | | | | | |
| ≤ $29 999 | 11.1 (3) | 53.3 (8) | 47.4 (9) | 33.3 (8) | 23.1 (6) | 40.0 (4) | 27.8 (5) | 42.3 (22) |
| $30 000–$59 999 | 25.9 (7) | 20.0 (3) | 31.6 (6) | 45.8 (11) | 38.5 (10) | 40.4 (4) | 50.0 (9) | 48.1 (25) |
| ≥ $60 000 | 63.0 (17) | 26.7 (4) | 21.1 (4) | 20.8 (5) | 34.6 (9) | 0.0 (0) | 22.2 (4) | 5.8 (3) |
| **Employment [% (n)]** | | | | | | | | |
| Full time | 29.6 (8) | 33.3 (5) | 42.1 (8) | 54.2 (13) | 34.6 (9) | 70.0 (7) | 61.1 (11) | 51.9 (27) |
| Part time | 25.9 (7) | 26.7 (4) | 26.3 (5) | 12.5 (3) | 30.8 (8) | 0.0 (0) | 27.8 (5) | 19.2 (10) |
| Retired | 7.4 (2) | 13.3 (2) | 10.5 (2) | 8.3 (2) | 7.7 (2) | 10.0 (1) | 5.6 (1) | 11.5 (6) |
| Other | 33.3 (9) | 26.7 (4) | 15.8 (3) | 25.0 (6) | 23.1 (6) | 20.0 (2) | 5.6 (1) | 15.4 (8) |
| **Education [% (n)]** | | | | | | | | |
| High school | 3.7 (1) | 26.7 (4) | 26.3 (5) | 16.7 (4) | 11.5 (3) | 20.0 (2) | 27.8 (5) | 30.8 (16) |
| Some college | 25.9 (7) | 20.0 (3) | 36.8 (7) | 37.5 (9) | 15.4 (4) | 40.0 (4) | 27.8 (5) | 42.3 (22) |
| College graduate | 37.0 (10) | 20.0 (3) | 31.6 (6) | 20.8 (5) | 26.9 (7) | 20.0 (2) | 22.2 (4) | 17.3 (9) |
| Postgraduate/degree | 33.3 (9) | 33.3 (5) | 5.3 (1) | 25.0 (6) | 46.2 (12) | 20.0 (2) | 22.2 (4) | 9.6 (5) |
| **Hypertension (SBP >140) [% (n)]** | | | | | | | | |
| No | 81.5 (22) | 86.7 (13) | 73.7 (14) | 75.0 (18) | 73.1 (19) | 70.0 (7) | 50.0 (9) | 55.8 (29) |
| Yes | 18.5 (5) | 13.3 (2) | 26.3 (5) | 25.0 (6) | 26.9 (7) | 30.0 (3) | 50.0 (9) | 44.2 (23) |
| Weight (kg) | 93.8 ± 13.3 | 75.8 ± 16.9 | 92.0 ± 12.1 | 85.1 ± 13.4 | 91.4 ± 12.7 | 76.0 ± 16.0 | 87.2 ± 13.3 | 80.5 ± 17.2 |
| Height (cm) | 179.0 ± 4.3 | 163.1 ± 7.0 | 179.1 ± 5.4 | 164.8 ± 6.1 | 176.6 ± 6.3 | 160.9 ± 5.1 | 174.9 ± 6.5 | 164.0 ± 6.7 |
| BMI (kg/m²) | 29.3 ± 4.4 | 28.3 ± 5.2 | 28.7 ± 3.9 | 31.4 ± 5.4 | 29.4 ± 4.1 | 29.3 ± 5.4 | 28.5 ± 4.1 | 29.8 ± 5.4 |
| Waist circumference (cm) | 103.8 ± 10.9 | 95.7 ± 14.0 | 99.3 ± 10.0 | 99.9 ± 14.8 | 106.1 ± 12.9 | 96.9 ± 16.2 | 99.7 ± 11.9 | 96.2 ± 15.7 |

Baseline demographics across race and sex in salt-sensitive and salt-resistant DASH-Sodium Trial participants at the time of screening on their regular diet. The values are shown as percentage (n) for categorical variables and mean ± SDs for continuous variables. Demographic information regarding income, education, and employment were missing for a few of the participants.

## Differential expression analysis

The metabolite and lipidome profile was compared between sample groups; salt-sensitive versus salt-resistant individuals, salt-sensitive high-salt diet versus salt-sensitive low-salt diet, salt-resistant high-salt diet versus salt-resistant low-salt diet, salt-sensitive high-salt diet versus salt-resistant high-salt diet, and salt-sensitive low-salt diet versus salt-resistant low-salt diet. Statistical testing between sample groups was performed with *limma* [27] using log transformed data. Adjusted P values were also calculated by correcting for multiple testing using the Benjamin–Hochberg method with a false discovery rate of 0.05 [28]. Results were obtained both unfiltered and filtered for high-salt diet versus low-salt diet in salt-sensitive and salt-resistant groups and unfiltered for salt-sensitive versus salt-resistant groups, with metabolites presenting an adjusted P value less than 0.05 and absolute log₂ fold change greater than 1 being considered as differentially expressed. Further analysis was carried out using the MetaboAnalyst R R package [26] for salt-sensitive versus salt-resistant groups. Due to consistent results obtained with *Limma,* remaining analysis were done using *limma* alone.

## RESULTS

### Participant demographics

We analyzed fasted plasma samples from 191 DASH–sodium trial participants for untargeted metabolomic and lipidomic profiling. The demographic characteristics of these participants are summarized in Table 1. Overall, there

was an equivalent distribution of men and women in both the salt-resistant and salt-sensitive groups. While the distribution of ethnic backgrounds among men was generally similar across the salt-sensitive and salt-resistant groups, the number of African-American women in salt-sensitive group was higher compared with salt-resistant group. The demographic characteristics for education, income, and education levels were largely similar across the salt-sensitive and salt-resistant groups.

### Metabolomic baseline plasma profiling of salt-sensitive versus salt-resistant participants

PCA showed no difference in the metabolomics profile between salt-sensitive and salt-resistant participants at baseline consuming their standard daily intake. Further evaluation via PCA for independent groups constituting of sex and race also showed no difference between metabolites between salt-sensitive versus salt-resistant participants (Supplementary Figures 1, http://links.lww.com/HJH/B670 and 2, http://links.lww.com/HJH/B670, respectively). Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA) showed no significant difference between salt-sensitive and salt-resistant groups (Fig. 1).

The differential expression analysis on the comparisons between salt-sensitive versus salt-resistant (Fig. 2 and Supplementary Table 1, http://links.lww.com/HJH/B670) showed no annotated metabolites to be differentially expressed. However, one nonannotated metabolite labeled 390144 was present at a significantly higher level in the salt-sensitive group (Supplementary Table 2, http://links.lww.com/HJH/B670).
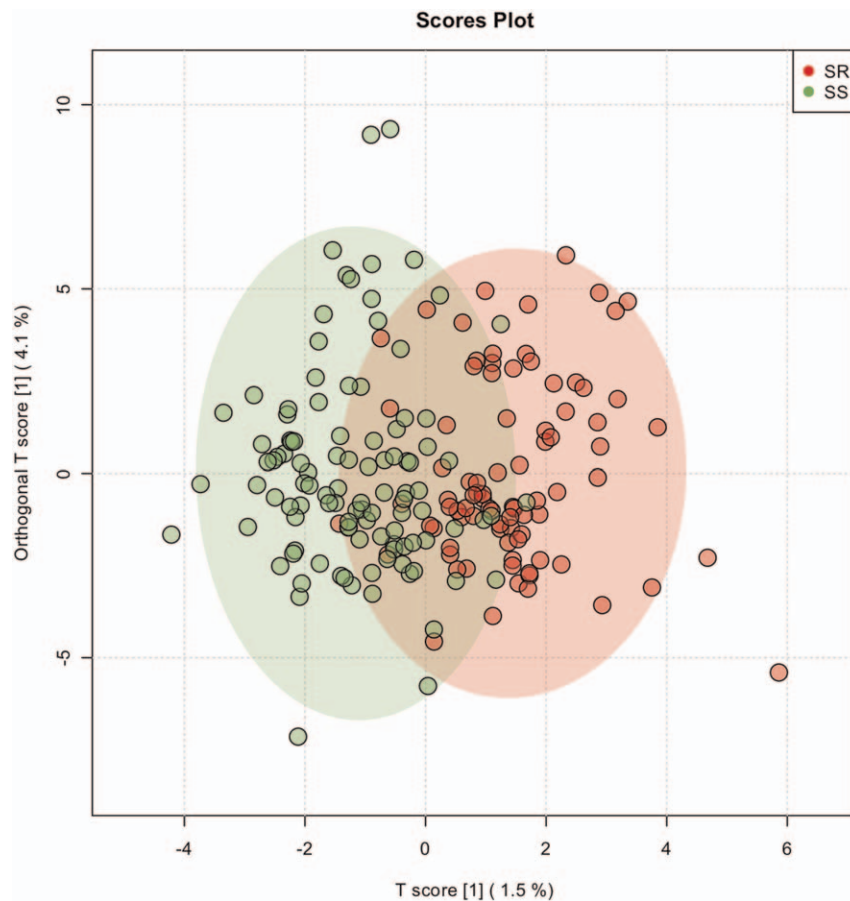
**FIGURE 1** Orthogonal Partial Least Square Discriminant Analysis score plot of baseline plasma metabolites for salt-sensitive (SS) (*n* = 106) and salt-resistant (SR) (*n* = 85) participants from Dietary Approaches to Stop Hypertension–Sodium Trial. Plots were created from a partial least squares discriminant analysis of 191 fasted plasma samples collected at the time of screening from participants maintained on their regular diet (i.e. prior to a dietary intervention).

## Metabolomic profiling comparing the responses to a high-salt versus low-salt diet between salt-sensitive and salt-resistant participants

The differential expression analysis of the metabolomic profile in the high-salt versus low-salt dietary intervention in salt-sensitive participants maintained on a control diet showed a significant difference (an adjusted *P* value <0.05 and absolute $\log_2$ fold change >1) in three annotated metabolites, tocopherol alpha, 2-ketoisocaproic acid, and citramalic acid (Fig. 3 and Table 2) and two nonannotated metabolites labeled 210343 and 390144 (Supplementary Table 4, http://links.lww.com/HJH/B670). In the salt-sensitive group, the high-salt dietary intervention was associated with a significant increase in citramalic acid levels and a significant decrease in the levels of tocopherol alpha, 2-ketoisocaproic acid, 210343 and 390144. The comparative differential expression analysis in salt-resistant participants yielded no significant effect of alterations in dietary salt intake on the metabolomic profile.

## Lipidomic profiling to compare salt-sensitive versus salt-resistant participants

Lipidomics OPLS-DA showed no difference in the baseline lipid profile between salt-sensitive and salt-resistant

participants consuming their regular diet (Fig. 4). Additionally, the lipid differential expression analysis yielded no differences among the plasma lipidomes between salt-sensitive and salt-resistant groups at baseline (Supplementary Table 5, http://links.lww.com/HJH/B670).

## DISCUSSION

The salt sensitivity of BP is strongly linked to hypertension risk [2–8]. Clinical identification of the salt sensitivity of BP was conducted in the DASH-Sodium Trial. Utilizing plasma samples from the DASH-Sodium Trial, we employed untargeted metabolomic and lipidomic techniques with the goal to identify potential biomarkers of the salt sensitivity of BP. At the time of screening in DASH-Sodium Trial when participants were consuming their standard daily intake, we observed no difference among annotated metabolites or lipidomes between salt-sensitive and salt-resistant participants. However, sub-group analysis of plasma metabolites in salt-sensitive participants maintained on a control diet in which only the dietary sodium content was modified and all remaining dietary components were unaltered revealed that a high-salt dietary intervention decreased the content of alpha-tocopherol and 2-Ketoisocaproic acid species, and increased the content of citramalic acid.
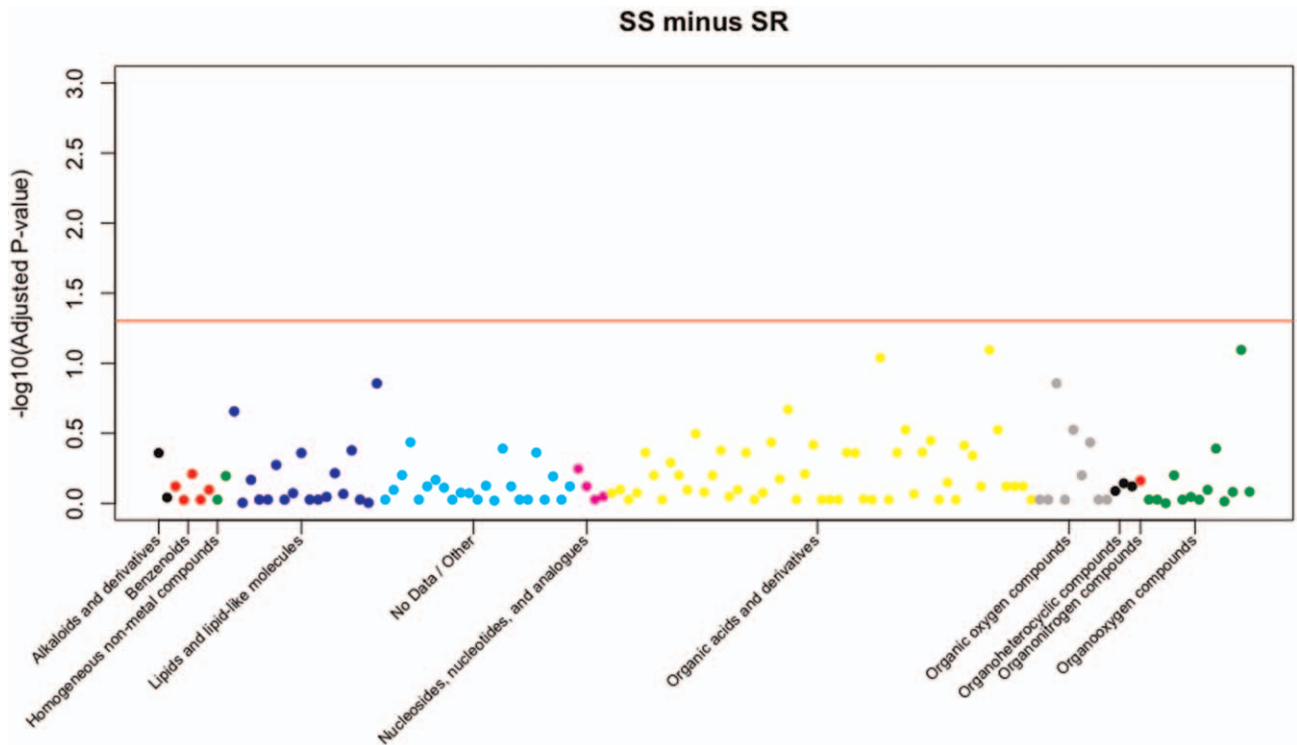
**FIGURE 2** Dot plot showing the −log10 of the adjusted *P* value for all baseline plasma metabolites grouped by superclass for analysis comparing salt-sensitive (SS) (*n* = 106) and salt-resistant (SR) (*n* = 85) participants. The analysis was conducted in the plasma samples collected at the time of screening from Dietary Approaches to Stop Hypertension–sodium trial participants maintained on their regular diet (i.e. prior to a dietary intervention). Only annotated metabolites (KEGG IDs, metabolite names) are shown.
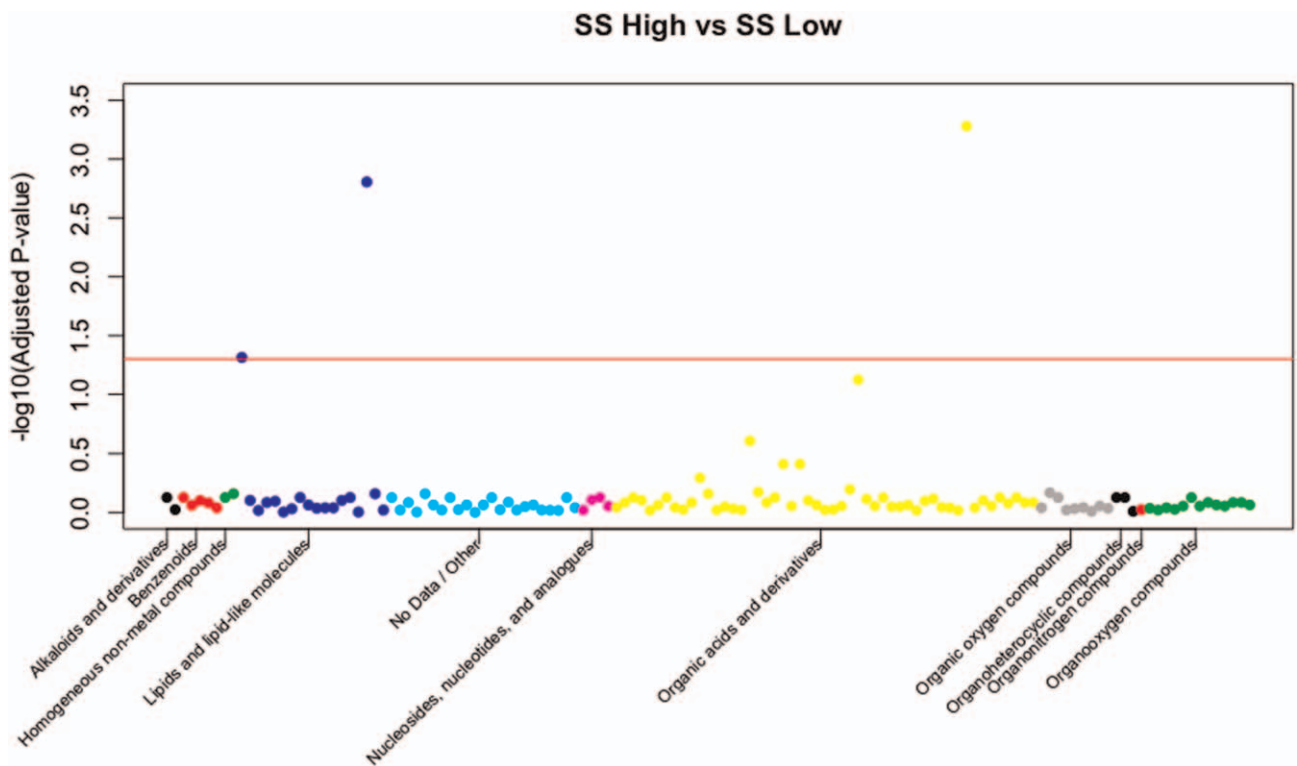


**FIGURE 3** Dot plot showing the −log10 of the adjusted *P* value for all plasma metabolites grouped by superclass for analysis comparing high-salt dietary intervention relative to low-salt dietary intervention from the salt-sensitive (SS) (*n* = 20) Dietary Approaches to Stop Hypertension–sodium trial participants maintained on a control diet. Only annotated metabolites (KEGG IDs, metabolite names) are shown.

**TABLE 2.** Annotated classes of plasma metabolites featuring significant alterations associated with high-salt dietary intervention relative to low-salt dietary intervention in salt-sensitive individuals ($n = 20$) from Dietary Approaches to Stop Hypertension–sodium trial maintained on a control diet

| SuperClass | Class | Metabolite | Kegg | LogFC | P value | Adjusted P value |
|---|---|---|---|---|---|---|
| Lipids and lipid-like molecules | Fatty Acyls | Citramalic acid | C00815 | 1.5121 | 0.0005 | **0.0485** |
| Lipids and lipid-like molecules | Glycerolipids | 1-Monostearin | D01947 | 0.2730 | 0.2338 | 0.7900 |
| Lipids and lipid-like molecules | Lipids | Palmitoleic acid | C08362 | −0.0347 | 0.8890 | 0.9609 |
| Lipids and lipid-like molecules | Lipids | Oleic acid | C00712 | 0.5602 | 0.2822 | 0.8227 |
| Lipids and lipid-like molecules | Lipids | Myristic acid | C06424 | −0.1843 | 0.2462 | 0.8031 |
| Lipids and lipid-like molecules | Lipids | Linoleic acid | C01595 | −0.0111 | 0.9583 | 0.9917 |
| Lipids and lipid-like molecules | Lipids | Lauric acid | C02679 | 0.1748 | 0.6728 | 0.9306 |
| Lipids and lipid-like molecules | Lipids | Isothreonic acid | C00639 | 0.2791 | 0.0902 | 0.7469 |
| Lipids and lipid-like molecules | Lipids | Dehydroabietic acid | C12078 | −0.1728 | 0.4130 | 0.8633 |
| Lipids and lipid-like molecules | Lipids | Capric acid | C01571 | 0.0892 | 0.6459 | 0.9210 |
| Lipids and lipid-like molecules | Lipids | Azelaic acid | C08261 | 0.1586 | 0.5967 | 0.9120 |
| Lipids and lipid-like molecules | Lipids | Arachidonic acid | C00219 | 0.1318 | 0.6310 | 0.9124 |
| Lipids and lipid-like molecules | Lipids | Arachidic acid | C06425 | −0.2809 | 0.2153 | 0.7900 |
| Lipids and lipid-like molecules | Lipids | 2-Hydroxybutanoic acid | C05984 | 0.3585 | 0.1676 | 0.7469 |
| Lipids and lipid-like molecules | Prenol lipids | Tocopherol gamma- | C02483 | −0.0114 | 0.9592 | 0.9917 |
| Lipids and lipid-like molecules | Prenol lipids | Tocopherol alpha- | C00376 | −0.5610 | 0.0000 | **0.0016** |
| Organic acids and derivatives | Carboxylic acids and derivatives | Fumaric acid | C00122 | 0.1081 | 0.4936 | 0.8827 |
| Organic acids and derivatives | Carboxylic acids and derivatives | Succinic acid | C00042 | 0.4067 | 0.0447 | 0.6365 |
| Organic acids and derivatives | Carboxylic acids and derivatives | Pyruvic acid | C00022 | −1.6831 | 0.0009 | 0.0750 |
| Organic acids and derivatives | Carboxylic acids and derivatives | Oxalic acid | C00209 | −0.8602 | 0.1859 | 0.7690 |
| Organic acids and derivatives | Carboxylic acids and derivatives | N-Acetylglycine | | 0.1198 | 0.5021 | 0.8827 |
| Organic acids and derivatives | Carboxylic acids and derivatives | methionine sulfoxide | C02989 | 0.3899 | 0.1147 | 0.7469 |
| Organic acids and derivatives | Carboxylic acids and derivatives | Malonic acid | C00383 | 0.0821 | 0.5321 | 0.8945 |
| Organic acids and derivatives | Carboxylic acids and derivatives | Maleic acid | C01384 | 0.0758 | 0.5439 | 0.8945 |
| Organic acids and derivatives | Carboxylic acids and derivatives | Isocitric acid | C00451 | 0.3861 | 0.4034 | 0.8633 |
| Organic acids and derivatives | Carboxylic acids and derivatives | Citrulline | C00327 | −0.0524 | 0.8803 | 0.9609 |
| Organic acids and derivatives | Carboxylic acids and derivatives | Citric acid | C00158 | 0.5445 | 0.2391 | 0.7995 |
| Organic acids and derivatives | Carboxylic acids and derivatives | Aminomalonate | C00872 | −0.3394 | 0.1816 | 0.7639 |
| Organic acids and derivatives | Carboxylic acids and derivatives | Alpha-ketoglutarate | C00026 | −0.2666 | 0.5678 | 0.9032 |
| Organic acids and derivatives | Carboxylic acids and derivatives | Aconitic acid | C00417 | 0.1960 | 0.6330 | 0.9126 |
| Organic acids and derivatives | Carboxylic acids and derivatives | 3-Hydroxybutyric acid | C01089 | −0.0473 | 0.8745 | 0.9609 |
| Organic acids and derivatives | Carboxylic acids and derivatives | 2-Ketoisocaproic acid | C00233 | −1.8588 | 0.0000 | **0.0005** |
| Organic acids and derivatives | Carboxylic acids and derivatives | 2-Aminobutyric acid | C02721 | −0.1302 | 0.5944 | 0.9112 |
| Organic acids and derivatives | Carboxylic acids and derivatives | 2,4-Diaminobutyric acid | C03283 | −0.4532 | 0.2124 | 0.7900 |

Major annotated plasma metabolite classes featuring significant changes associated with high-salt dietary intervention relative to low-salt dietary intervention in salt-sensitive individuals ($n = 20$) from DASH–Sodium Trial maintained on a control diet that exhibited significant changes in a specific metabolite within that class. The results are based on the differential expression analysis on the comparisons of metabolites predicted to be differentially expressed based on cut-offs of adjusted $P$ value less than 0.05 and absolute log$_2$ fold change greater than 1. Kegg, *Kyoto Encyclopedia of Genes and Genomes*, log FC, log fold change, $P$ value is calculated per individual comparison, and adjusted $P$ value for multiple testing by Benjamin–Hochberg procedure that takes into account multitest correction.

Alpha-tocopherol, the predominant form of vitamin E in humans, is a lipid-soluble antioxidant [29,30]. Significantly, alpha-tocopherol supplementation in Dahl salt-sensitive (DSS) rats maintained on a high-salt diet (8% NaCl) prevented the development of salt-sensitive hypertension [31]. Given our observation of a reduction in the level of the metabolite alpha-tocopherol during a HS dietary intervention in the salt-sensitive sub-group we speculate that reduced alpha-tocopherol levels may contribute to the sensitivity of blood pressure in human particpants, and, increased dietary intake of vitamin E may potentially reduce the salt sensitivity of blood pressure. 2-Ketoisocaproic acid, a leucine metabolite, suppresses skeletal muscle insulin-mediated glucose transport and is associated with insulin resistance [32,33], which is linked to the salt sensitivity of BP [34,35]. In our study, high-salt diet in salt-sensitive particpants was associated with a decrease in 2-ketoisocaproic acid levels. However, as diabetes was an exclusion criterion for the DASH-Sodium Trial participants, we are unable to examine a potential association between 2-ketoisocaproic and diabetes in salt-sensitive participants in our analysis. In our study, elevated levels of citramalic acid metabolites were observed following high-salt intake in salt-sensitive individuals. Increased levels of citramalic acid metabolites have been linked with obesity [36], suggesting a possible association of obesity and the salt sensitivity of BP. Consequently, the association of citramalic acid with high-salt intake in the salt-sensitive individuals warrants further investigation.

Prior targeted plasma metabolic phenotyping of the DASH−sodium trial reported that a low-salt intervention was associated with an increased level of metabolites involved in methionine metabolism, tryptophan and decreased levels of the short-chain fatty acid isovalerate and g-glutamyl amino acids were observed [37]. The altered metabolomic profile observed between our study and prior analyses may be attributed to our untargeted approach, the inclusion of plasma samples from only a small subset of participants with high-salt to low-salt intervention from the control diet group only and analytical separation into salt-sensitive and salt-resistant groups. Additionally, a double-blind crossover study in middle-aged adults with elevated
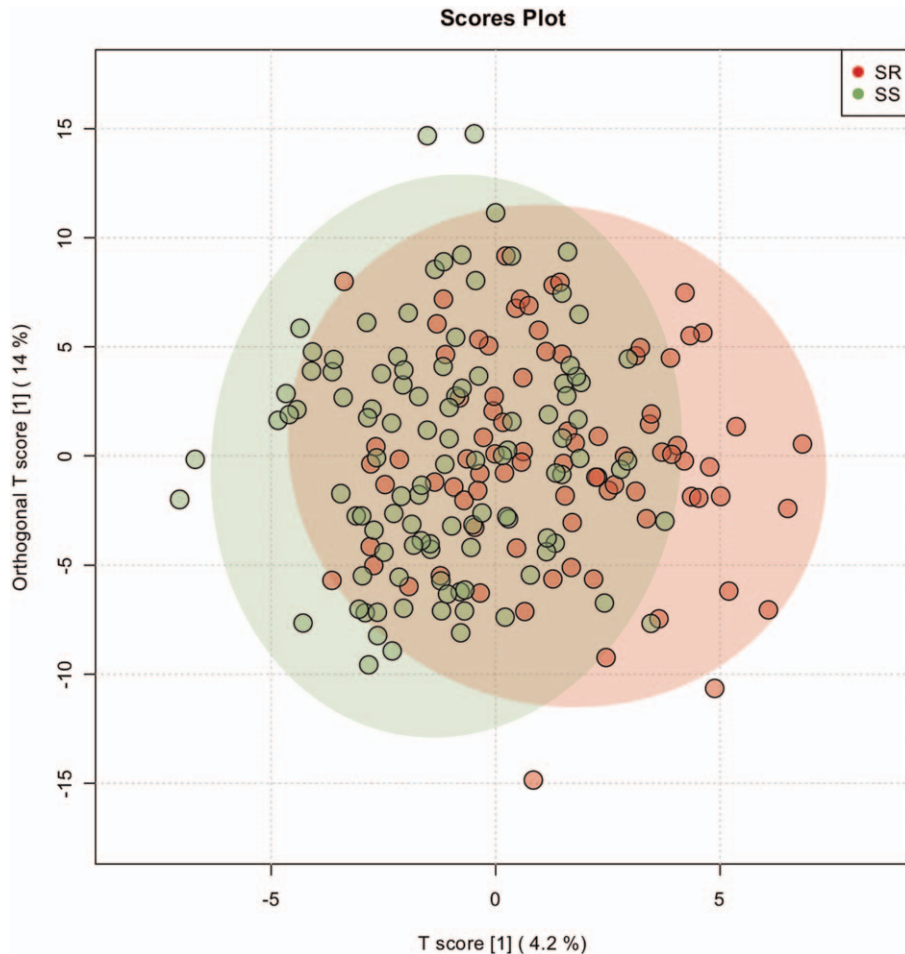
**FIGURE 4** Orthogonal Partial Least Square Discriminant Analysis score plot of baseline plasma lipid profile for salt-sensitive (SS) (*n* = 106) and salt-resistant (SR) (*n* = 85) individuals from Dietary Approaches to Stop Hypertension–sodium trial participants. Plots were created from a partial least squares discriminant analysis of 191 fasted plasma samples collected at the time of screening from participants maintained on their regular diet (i.e. prior to a dietary intervention).

BP that examined urinary metabolite changes in response to dietary sodium restriction reported an increase in succinate, methionine sulfoxide, S adenosylhomocysteine, D gluconate, and aspargine [38]. In our study, the changes in these metabolites were not observed, which may be attributed to the difference between urinary and plasma metabolites. These studies highlight the importance of considering sample type and comorbidities whenever conducting metabolomic and lipidomic analyses.

Hypertension, irrespective of the salt sensitivity of BP has been linked to alterations in metabolomic and lipidomic profiles [13–16]. A plasma metabolomics study comparing young hypertensive and normotensive groups observed differences in the levels of glycine, lysine, and cysteine [39]. In contrast, the urinary metabolite analysis from the INTERMAP study revealed a positive association of alanine and an inverse association of hippurate, formate, and N-methylnicotinate with increased BP [40]. A lipidomic study conducted in hypertensive and normotensive men reported reduced levels of ether phosphatidylcholines and phosphatidylethanolamines with hypertension [41]. In contrast, lipidomic profiling in treated and untreated hypertensive patients showed increased levels of several triacylglycerol

species in hypertension that were reduced in response to antihypertensive drugs [42]. The absence of an altered lipidomic profile between salt-sensitive and salt-resistant participants in the current untargeted exploratory analysis is supported by the prior finding of the DASH-Sodium Trial that changes in dietary sodium intake over the range of 50–150 mmol/day did not affect blood lipid concentrations [43].

The current study has several strengths: the DASH-Sodium Trial was a carefully conducted controlled feeding study with crossover design for dietary sodium intervention that allowed participants to serve as their own control, our current analysis included sub-group analysis of plasma samples from participants who were on the control diet, which is representative of a typical American diet, and salt-sensitive and the salt-resistant groups were preidentified based on the SBP changes observed with high-salt to low-salt diet intervention at the end of the DASH-Sodium Trial. Potential limitations of the current study include: a comparatively small sample size as the study was conducted in a subset of salt-sensitive and salt-resistant groups; despite the use of Benjamin Hochberg correction to correct for a false discovery rate we acknowledge the possibility of false-positive results and that these findings require future

validation and replication in additional independent datasets; and owing to sample normalization without the use of internal standards our data is qualitative not quantitative.

In conclusion, our untargeted metabolomic and lipidomic plasma profiling in participants form the DASH-Sodium trial showed that there was no difference in plasma metabolomic and lipidomic profiles between salt-sensitive and salt-resistant participants at baseline on their regular dietary intake. This outcome does not support our primary hypothesis that salt-sensitive individuals would exhibit altered metabolic and lipidomic profiles. In contrast, our sub-group analysis shows that in salt-sensitive participants, a high-salt intervention was associated with alterations in the levels of tocopherol alpha, 2-ketoisocaproic acid, and citramalic acid, supporting our secondary hypothesis that salt-sensitive participants exhibit an altered metabolomic profile to a high-sodium diet. Further investigations are required to understand the potential physiological significance of these findings and the utility of an association of alterations in these metabolites with the salt sensitivity of BP.

## ACKNOWLEDGEMENTS

### Conflicts of interest
There are no conflicts of interest.

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