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ORIGINAL RESEARCH

Metabolic Signatures of Blood Pressure and Risk of Cardiovascular Diseases

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BACKGROUND: The underlying biological mechanisms linking blood pressure (BP) and cardiovascular diseases (CVD) are only partly understood. We aimed to identify metabolic signatures associated with systolic and diastolic BP and investigate their subsequent association with risk of CVD.

METHODS AND RESULTS: The study included 201 742 UK Biobank participants with measurements on 249 metabolic biomarkers. A multistep adaptive elastic net penalized regression with 10-fold cross-validation was employed to identify metabolic signatures for systolic BP and diastolic BP. External validation was conducted on 848 participants from the EHS (Epirus Health Study). We further assessed the associations between BP metabolic signatures and incident composite CVD (N=6742), myocardial infarction (N=4192), and stroke (N=2757) in the UK Biobank, using multivariable Cox regression models. The metabolic signatures comprised 31 and 25 metabolites, robustly correlated with systolic BP and diastolic BP, respectively, in both the UK Biobank and the EHS. Following adjustments (including BP), the metabolic signature for systolic BP was positively associated with incident myocardial infarction (hazard ratio [HR], 1.11 [95% CI, 1.07–1.15]) and CVD (HR, 1.07 [95% CI, 1.04–1.10]). Similarly, the metabolic signature for diastolic BP was associated with a higher risk of myocardial infarction (HR, 1.16 [95% CI, 1.12–1.20]) and CVD (HR, 1.09 [95% CI, 1.05–1.12]). The associations between the signatures and stroke were not significant. The metabolic signatures partly mediated the total effect of the BP traits on the risk of myocardial infarction and CVD.

CONCLUSIONS: Our findings may enhance our understanding of the biological mechanisms through which BP affects CVD.

Key Words: blood pressure ■ cardiovascular disease ■ metabolic signatures ■ metabolomics ■ NMR ■ UK Biobank

Cardiovascular diseases (CVD) remain the leading cause of disease burden worldwide, responsible for 17.9 million deaths in 2019.[1,2] Blood pressure (BP) is one of the most thoroughly investigated risk factor for CVD[3] and substantial evidence from Mendelian randomization studies[4,5] and randomized clinical trials[6–8] suggests that even small increments in systolic (SBP) and diastolic blood pressure (DBP) are associated with an increased risk of CVD.[9,10]

Although several mechanisms have been proposed, including vascular inflammation, endothelial dysfunction, and structural remodeling,[11] the molecular dysregulations linking BP and CVD are still incompletely understood. Recent advances in metabolomics technology have facilitated metabolomic profiling of large-scale population-based studies. This information can provide insights into the mechanisms underlying associations between exposures and disease risk.[12] Several metabolomic approaches have been applied to uncover the pathophysiology of high BP[13] and different pathways have been suggested in relation to BP including the possible role of the gut microflora,

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CLINICAL PERSPECTIVE

What Is New?

- Using nuclear magnetic resonance metabolic data from the UK Biobank, we discovered and validated in a Greek cohort, metabolic signatures for systolic and diastolic blood pressure using machine-learning algorithms.
- The identified metabolic signatures were independently associated with higher risk of myocardial infarction and cardiovascular disease, and partly mediated the relationships of the blood pressure traits with myocardial infarction and cardiovascular disease risk.

What Are the Clinical Implications?

- This study highlights the potential of metabolomic profiling in deciphering the biological mechanisms that link blood pressure with adverse cardiovascular events.

Nonstandard Abbreviations and Acronyms

CETP	cholesteryl ester transfer protein
EHS	Epirus Health Study
NMR	nuclear magnetic resonance
RMSE	root mean squared error

inflammatory, and lipid pathways.[14–17] However, most studies suffer from limitations including small sample sizes, cross-sectional designs, and lack of replication.

Here, we provide a comprehensive assessment of plasma metabolic signatures associated with SBP and DBP in the large UK Biobank study with external replication of our findings in an independent cohort. To provide deeper insights into the mechanisms that link BP with CVD, we further examined whether the identified signatures mediate the associations between BP traits and CVD risk.

METHODS

The authors declare that analytic code employed in the analyses is available within the article (Data S1).

Data in the article, code book, and analytic code will not be publicly available. The UK Biobank data set used to conduct the research in this paper is available via application directly to the UK Biobank. Applications are assessed to meet the required access criteria, including legal and ethical standards. More information regarding data access can be found here: access@ukbiobank.ac.uk.

Data Sources

UK Biobank

The design of the UK Biobank has been described in detail elsewhere.[18] In brief, from 2006 to 2010 the UK Biobank recruited 501 359 participants aged 37 to 73 years in 22 assessment centers across the United Kingdom. Extensive phenotypic and genotypic data have been collected, including self-report data from questionnaires, physical measurements, sample assays, genome-wide genotyping, and longitudinal follow-up for a wide range of health-related outcomes. The National Information Governance Board for Health and Social Care and the National Health Service North West Centre for Research Ethics Committee granted ethical approval (Ref: 21/NW/0157). The study was conducted in accordance with the Helsinki Declaration, and all subjects provided informed written consent.

Epirus Health Study

The EHS (Epirus Health Study) is a population-based prospective cohort study that was launched in June 2019 and, to date, over 2500 participants have been recruited. The overall aim is to improve the general health of the Greek population by shedding light on the complicated cause of multiple chronic diseases. The EHS cohort includes individuals who live permanently in Greece's Epirus region between the ages of 25 and 70 and who did not have any signs of an active infection at the time of recruitment. The design and methods of this study have been described in detail elsewhere.[19–21] The University of Ioannina's Research Ethics Committee gave its approval for the study, which is carried out in compliance with the Helsinki Declaration. Before taking part in the study, each subject provided written informed consent.

Study Populations

Discovery Population

In the UK Biobank cohort we used individuals with available metabolomic data (N=274 353). We excluded pregnant women (N=167) and those individuals who were taking lipid-lowering medication (N=43 314) or insulin (N=567). Furthermore, we excluded individuals with mismatch in genetic sex and self-reported sex (N=142). Prevalent cases of myocardial infarction (MI) (N=760) and stroke (N=1263) were also not included in the analysis. Finally, individuals with missing values on SBP and DBP were excluded (N=385) (Figure 1). After applying the aforementioned criteria, 201 742 participants were included in further analyses.

External Population

We used baseline data from the EHS (N=997) and employed the same exclusion criteria as the discovery

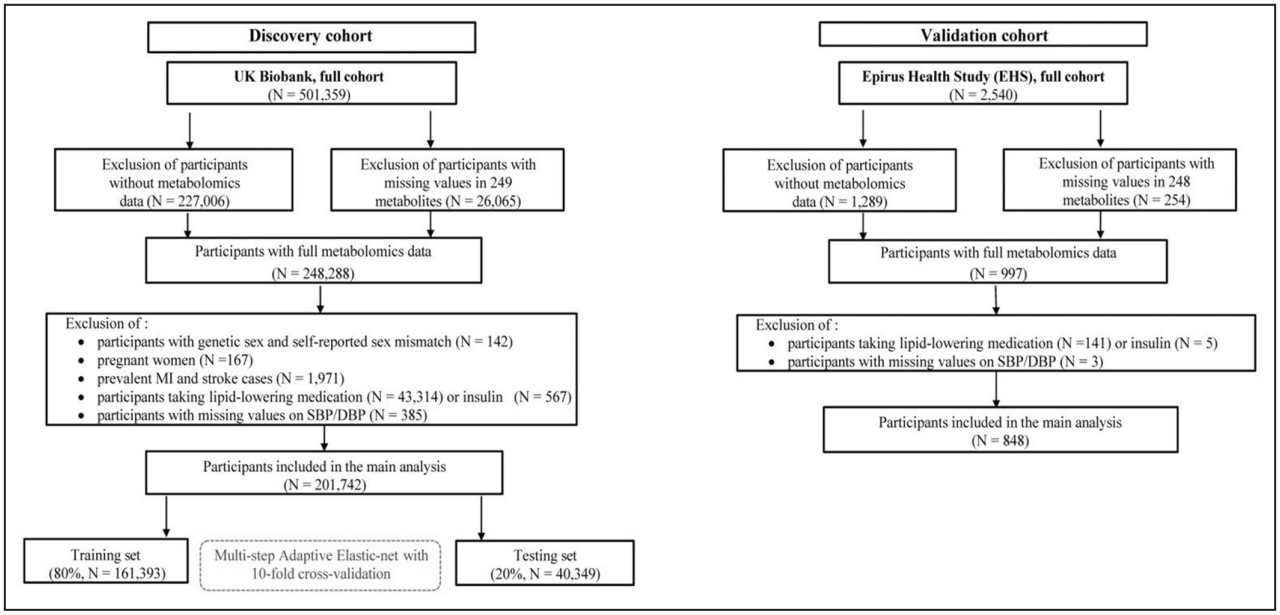

Figure 1. Flow chart of study design.
DBP indicates diastolic blood pressure; MI, myocardial infarction; and SBP, systolic blood pressure.

population in the participant selection process. Specifically, we excluded individuals who were taking lipid-lowering medication (N=141) or insulin (N=5). Missing values on SBP and DBP were excluded (N=3) (Figure 1). Finally, 848 participants were included in the replication analyses.

Measurements

Metabolomic Profiling

A random subset of nonfasting baseline plasma samples from 274 353 UK Biobank individuals has been analyzed using a high-throughput nuclear magnetic resonance (NMR) platform (Nightingale Health Plc; biomarker quantification version 2022). A set of 168 quantitatively profiled metabolites (in molar concentration units), including lipids, lipoprotein particle subclasses, cholesterol subtypes, amino acids, and inflammation markers, and 81 ratios derived from their combinations were further included.[22,23] Data S2 provides further details on metabolomic biomarker measurements. In the EHS, fasting plasma samples from 1251 individuals were also analyzed using the same metabolomic profiling platform employed in the UK Biobank. The median and interquartile ranges of concentrations for the metabolic markers for all participants of the 2 different studies are given in Table S1 and Table S2, respectively.

Blood Pressure

In the UK Biobank, 2 automatic (N=187 378) or 2 manual (N=14 364) BP measurements were used to calculate the mean SBP and DBP. Automatic measurements were performed using an Omron-7015IT digital BP monitor. We took the mean of the 2 readings for participants with manual and automatic BP readings. We used the single BP measurement available for each individual (a manual or an automated BP readout). We corrected SBP and DBP by adding 15 and 10 mm Hg, respectively,[24] among participants who reported taking any BP-lowering medication (N=24 469). In the EHS, 3 BP measurements (MicroLife A6 PC-AFIB PC monitor) were used to calculate the mean SBP and DBP. We also corrected SBP and DBP by adding 15 and 10 mm Hg, respectively, among participants who reported taking any BP-lowering medication (N=85).

Ascertainment of Cardiovascular Events

In the UK Biobank, the clinical outcomes included stroke, MI, and, CVD (defined as the composite of MI and stroke). Incident cases of stroke (ischemic, intracerebral hemorrhage, and subarachnoid hemorrhage) and MI (ST-segment–elevation MI and non–ST-segment–elevation MI) were accessible through algorithms made publicly available by the UK Biobank.[25,26] Using data from hospital and death registers, algorithms were created to determine the incidence of selected diseases accurately. A censoring date of September 12, 2021, corresponding to the last event, was used for all outcomes.

Covariates Assessment

In the UK Biobank, the Townsend deprivation index, a composite measure of deprivation based on unemployment, nonownership of a car, nonownership

of a home, and household overcrowding, was estimated using data from the previous national census.[27] Body mass index was calculated using weight and height recorded during the recruitment. Information about smoking status (never/previous/current smoker) and alcohol drinking (never/previous/current alcohol drinker) were collected through touch-screen questionnaires. Data on physical activity were obtained using the International Physical Activity Questionnaire, which measured total moderate and vigorous physical activity over the previous 7 days, including walking. Based on a set of scoring criteria,[28] participants were divided into 3 groups that are mutually exclusive: low intensity (600 metabolic equivalent of task-min/week), moderate intensity (600 to 3000 metabolic equivalent of task-min/week), and high intensity (3000 metabolic equivalent of task-min/week). The threshold at 600 metabolic equivalent of task-min/week is equivalent to meeting the advised guidelines (150 min/week) for moderate-intensity physical activity.[29] Participants who had hemoglobin A1c >48 mmol/mol, were on antidiabetic medication (Table S3), and had self-reported or a previous medical diagnosis of diabetes were classified as having diabetes. Participants who answered "do not know" or "prefer not to answer" to any of the self-reported questions were considered missing values. Data field identifiers for all features used are described in Table S4.

Statistical Analysis

Mean±SD for continuous variables and percentages for categorical variables were used to describe the characteristics of study participants.

In the discovery set, none of the metabolites had missing values up to 20% (Figure S1). Participants with missing values in their metabolite data of up to 5% were excluded from the analysis, resulting in the exclusion of 26 065 participants due to at least 1 missing metabolite value. The inverse normal transformation was applied to the 249 metabolites.[30] SBP and DBP measurements were ln-transformed to approach normality (Figures S2 and S3). In the external validation set, 2 out of the 250 plasma metabolites, namely glycerol and beta hydroxybutyrate, were removed because of the high number of missing values (>20%). Missing values (Figure S4) of the remaining metabolites were excluded (N=254). The same transformation approach was applied to the 248 metabolites, whereas both BP measures were ln-transformed.

Identification of Metabolic Signatures for BP Traits

We randomly split 80% of the discovery data into a training set (N=161 393) and the remaining 20% sample into a testing set (N=40 349) to develop and test the SBP and DBP metabolic signatures-related prediction models. We applied multistep adaptive elastic-net[31] (Data S3, Figure S5) with 10-fold cross-validation on the training set, and we computed the root-mean-squared error (RMSE) on the testing set. We specified a range of alpha hyperparameter values from 0.05 to 0.95 in 0.05 increments. This allows the algorithm to explore a range of combinations between L1 (Lasso) and L2 (Ridge) regularization. The algorithm underwent 10 tuning steps. The optimal regularization parameter (lambda) for the model was 1 SE, meaning that the cross-validated error is within 1 SE of the minimum cross-validated error. We selected the optimal value of alpha based on the combination that yielded a low RMSE in the testing set. We applied the selected alpha and lambda values to each multistep adaptive elastic-net for the BP traits in the training set. Finally, the coefficients from multistep adaptive elastic-net were applied to the selected metabolites as weights (positive or negative coefficients) to estimate the metabolic signatures of the ln-transformed SBP and DBP as the weighted sum. Cross-validated Pearson correlations and R^2 were calculated to evaluate the performance of the metabolic signatures in assessing SBP and DBP in the original scale, respectively. A Venn diagram was constructed to visualize the overlapping metabolites associated with SBP and DBP. We then applied the discovery model to the external validation set to calculate the metabolic signatures and evaluate their performance by calculating RMSE, Pearson correlation coefficient and, R^2.

Associations Between BP Traits and the Metabolic Signatures With CVD Risk

The metabolic signature was transformed to a *Z* score (mean=0; SD=1) before Cox regression analysis in the UK Biobank. For our analyses of stroke, MI and CVD incidence, the time-to-event variable was the interval between the date of enrolment and the date of the cardiovascular event, death, or end of follow-up, whichever occurred first.

By examining the relationship between standardized Schoenfeld residuals and time, we tested the proportional hazards assumption and found no evidence of its violation. We fitted 3 multivariable Cox proportional hazards models to examine the associations of the 2 BP traits and the standardized metabolic signatures with stroke, MI, and CVD risk. Age and sex adjustments were applied to the first model (age- and sex-adjusted model). Further covariate adjustments were made to the second model (multivariable-adjusted model), including diabetes, body mass index, Townsend deprivation index, physical activity, smoking, and alcohol use. In the third model (multivariable-adjusted model+mutual adjustments), we further

included each BP measure and the corresponding *Z* score of the metabolic signatures simultaneously to examine association independence. We did not further adjust for cholesterol and glucose as these biomarkers are already represented within the NMR metabolomics panel through detailed measures of lipoprotein subclasses (including cholesterol-related fractions) and glucose levels.

Causal mediation analysis was performed[32,33] to evaluate whether the metabolic signatures (mediators) mediated the relationship between SBP or DBP (exposure) and CVD (outcome), using multivariable-adjusted Cox regressions and the R package *mediator*.[34] The process facilitated the estimation of key mediation effects, encompassing controlled and natural direct effects, natural indirect effects, total effects, and the proportion of mediation, which provides an estimate of the extent to which the total effect is accounted for by the pathway through the mediating variable. When no interaction between the exposure and the mediator exists, the controlled direct effect and natural direct effects are the same. The median value of the metabolic signatures was used to indicate the level of the mediator, and the mean value of SBP (137.3 mm Hg) or DBP (82.8 mm Hg) (on a natural logarithmic scale) presented the exposure level. CIs for the proportion mediated were computed via the bootstrap resampling method[35] applied to 1000 samples using the R package *boot*. The same covariates that were incorporated into the second multivariable-adjusted Cox model were used in the mediation analysis.

All analyses were performed using R version 4.3.1 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Baseline Characteristics

The mean age was 55.6 years (SD=8.1) in the UK Biobank and 46.1 years (SD=10.2) in the EHS (Table 1, Table S5). In UK Biobank, during a median follow-up period of 12.7 years (interquartile range=11.9–13.3) a total of 6742 incident CVD events, 4192 incident MI (median=12.7 years, interquartile range=11.9–13.3) events, and 2757 incident stroke events (median=12.7 years, interquartile range=11.9–13.3) occurred.

Associations of Metabolites With SBP and DBP

Overall, the multistep adaptive elastic-net selected 31 and 25 metabolites and metabolites' ratios after 10-fold cross-validation for SBP (RMSE=0.131) and DBP (RMSE=0.121), respectively (Figure 2 and Table S6). Nineteen metabolites and ratios had positive associations with SBP, and the remaining had inverse associations. Those metabolites with the highest positive coefficient value were cholesteryl esters in very large very low-density lipoprotein (VLDL), triglycerides to total lipids in medium VLDL, and free cholesterol to total lipids in medium VLDL, whereas those with the highest negative coefficient value were cholines and cholesteryl esters in large VLDL. Among the 13 identified metabolites positively associated with DBP, cholesteryl esters in very large VLDL, free cholesterol in small high-density lipoprotein (HDL), and cholesterol in very large HDL had the highest coefficients, whereas cholesteryl esters in large VLDL had the highest negative coefficient. The identified distinct and common metabolites covered a wide range of metabolic classes, such as lipoprotein subclasses, amino acids, ketone bodies, fatty acids, and metabolites associated with fluid balance and glycolysis (Figure 3, Tables S7 and S8). These metabolites and metabolites' ratios explained 13.9% and 14.8% of the total variance of SBP and DBP, respectively. The derived metabolic signatures were significantly correlated with SBP (Pearson correlation coefficient [r=0.37, 95% CI, 0.36–0.38] and DBP [r=0.38, 95% CI, 0.37–0.39]). In the external validation set (EHS), the metabolites included in the SBP- and DBP-related metabolic signatures accounted for 14.0% (RMSE=0.133) and 14.6% (RMSE=0.166) of the total variance, respectively. The external validation set showed similar magnitudes of correlation for SBP (r=0.37, 95% CI, 0.32–0.43) and DBP (r=0.38, 95% CI, 0.32–0.44).

Associations With Cardiovascular Events

SBP and DBP were associated with a higher risk of stroke (adjusted hazard ratio [HR] for SBP, 1.18 [95% CI, 1.15–1.21] and for DBP, 1.19 [95% CI, 1.15–1.22]), even after further adjustment for the respective metabolic signatures (Figure 4, Table S9). On the other hand, the associations between the metabolic signatures for the BP traits and risk of stroke were attenuated and became insignificant after further adjustment for SBP (HR, 1.03 [95% CI, 0.98–1.08]) and DBP (HR, 1.00 [95% CI, 0.95–1.05]) (Figure 4, Table S9). SBP and DBP were associated with a higher risk of MI (adjusted HR for SBP, 1.20 [95% CI, 1.18–1.23] and for DBP, 1.19 [95% CI, 1.16–1.22]) and CVD (adjusted HR for SBP, 1.18 [95% CI, 1.16–1.20] and for DBP, 1.17 [95% CI, 1.15–1.19]), even after further adjustment for the respective metabolic signatures (Figure 4, Table S9). The metabolic signature for SBP was associated with higher risk of incident MI (adjusted HR per SD increment in metabolic signature, 1.11 [95% CI, 1.07–1.15]) and CVD (adjusted HR per SD increment in metabolic signature, 1.07 [95% CI, 1.04–1.10]). Similar associations were observed between metabolic signatures and DBP.

Table 1. Baseline Characteristics of 201 742 UK Biobank Participants Stratified by Incident CVD Status

Characteristics	Overall	Incident CVD	
		Yes	No
	No.=201 742	No.=8634 (4.3)	No.=193 108 (95.7)
Age, y, mean±SD	55.6±8.1	59.6±7.2	55.4±8.1
Sex			
Men	88 517 (43.9)	5277 (61.1)	83 240 (43.1)
Women	113 225 (56.1)	3357 (38.9)	109 868 (56.9)
TDI, mean±SD	−1.5±3.0	−1.2±3.1	−1.5±3.0
Smoking status			
Never	114 641 (56.8)	4053 (46.9)	110 588 (57.3)
Previous	65 427 (32.4)	3042 (35.2)	62 385 (32.3)
Current	20 723 (10.3)	1484 (17.2)	19 239 (10.0)
Unknown	951 (0.5)	55 (0.7)	896 (0.4)
Alcohol status			
Never	8279 (4.1)	401 (4.6)	7878 (4.1)
Previous	6544 (3.2)	385 (4.5)	6159 (3.2)
Current	186 461 (92.4)	7822 (90.6)	178 639 (92.5)
Unknown	458 (0.3)	26 (0.3)	432 (0.2)
Physical activity			
Low	30 016 (14.9)	1330 (15.5)	28 686 (14.9)
Moderate	66 129 (32.8)	2593 (30.0)	63 539 (32.9)
High	67 391 (33.4)	2878 (33.3)	64 513 (33.4)
Unknown	38 208 (18.9)	1833 (21.2)	36 373 (18.8)
BMI (kg/m^2), mean±SD	27.1±4.6	27·9±4.5	27·1±4.5
Systolic BP (mm Hg), median (IQR)	135.5 (124.6–148.5)	143.5 (131.5–157.0)	135.0 (123.5–148.0)
Diastolic BP (mm Hg), median (IQR)	82.0 (75.5–89.0)	85.0 (78.0–92.0)	82.0 (75.0–89.0)
Diabetes	3991 (2.0)	376 (4.4)	3615 (1.9)

BMI indicates body mass index; BP, blood pressure; CVD, cardiovascular disease; IQR, interquartile range; and TDI, Townsend deprivation index. The number of missing values was 243 for TDI, 582 for BMI, and 200 for diabetes. All figures are expressed as absolute number (and percentages, %) unless otherwise specified.

Mediation of the Associations Between BP Traits and Cardiovascular Events

The proportion of the total effect of ln SBP on the risk of MI mediated through the SBP metabolic signature (per SD increment) was 10.7% (95% CI, 7.30–13.47), whereas the total effect of ln SBP on CVD risk was 8.33% (95% CI, 5.51–10.65). The metabolic signature (per SD increment) of DBP mediated 14.5% (95% CI, 10.73–18.01) and 9.41% (95% CI, 6.23–12.21) of the total effect of ln DBP on the risk of MI and CVD, respectively (Figure 5, Table S10).

DISCUSSION

By using machine-learning algorithms, we developed and validated metabolic signatures of SBP and DBP. These metabolic signatures were independently associated with a higher risk of MI and CVD. Furthermore, our study revealed that these metabolic signatures partially mediated the relationships between BP and CVD outcomes. Our findings support the potential value of metabolomic profiling for studying biological mechanisms underlying BP and CVD associations.

The relationship between blood metabolome and BP has been extensively investigated. Several observational studies have identified numerous circulating metabolites associated with BP, such as lipoprotein particles of different sizes and subclass concentrations, fatty acids, lipid species, carnitines, amino acids, energy-related metabolites, urea cycle metabolites, and microbiota-derived metabolites.[13,36,37] To our knowledge, this is the first study to identify metabolic signatures of BP traits using a multistep estimation algorithm built upon adaptive elastic-net regularization, reducing the false positives while maintaining the estimation accuracy.[31] The metabolic signatures for both BP traits were found to be composed mainly of lipoprotein particle subclasses followed by fatty acids, amino acids, and energy metabolism-related metabolites.

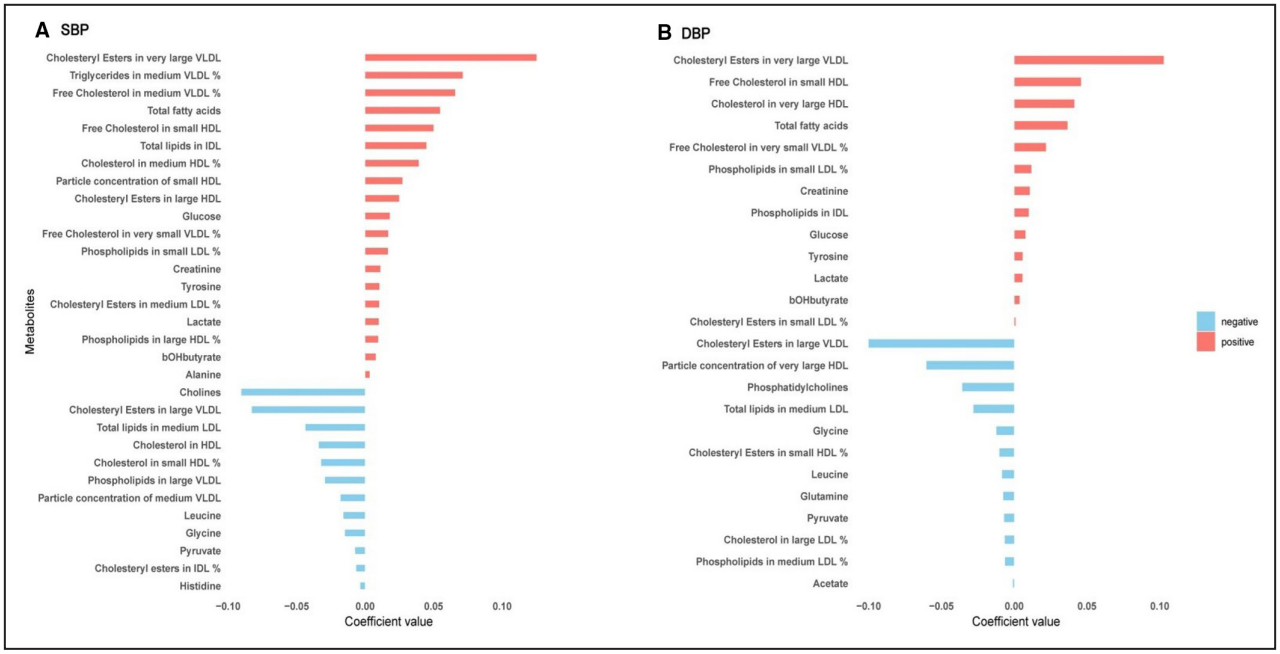

Figure 2. Metabolites ranked from the highest to the lowest MSA-Enet positive and negative regression coefficients for ln SBP (A) and ln DBP (B).
Exposure contrast is per SD/*Z* score increase of the metabolite (N=201 742). DBP indicates diastolic blood pressure; HDL, high-density lipoprotein; IDL, intermediate-density lipoprotein; LDL, low-density lipoprotein; MSA-Enet, multistep adaptive elastic-net; SBP, systolic blood pressure; and VLDL, very low-density lipoprotein.

Disturbances in the proportion of specific lipid fractions related to hypertension have been reported. A prospective study of 17 527 healthy women followed for 8 years found that elevated levels of LDL, HDL, especially small particles, and VLDL, particularly large triglyceride-rich particles, were associated with incident hypertension.[38] A recent small cohort study conducted among 100 healthy individuals demonstrated a

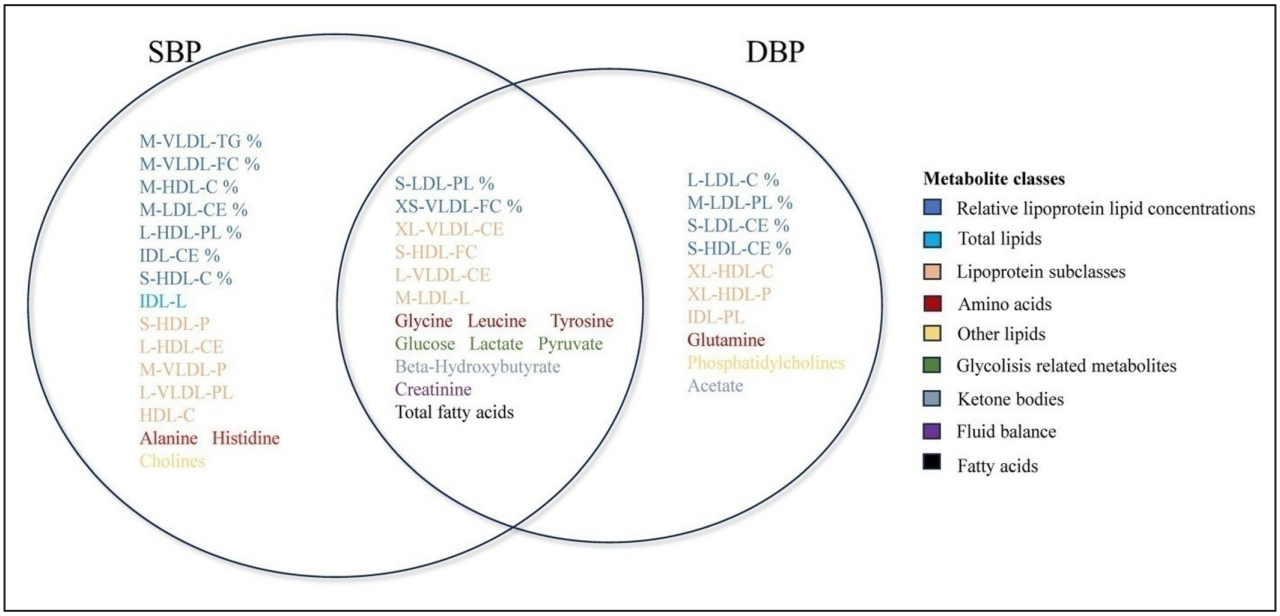

Figure 3. Venn diagram comparing metabolites profiles of ln SBP and ln DBP.
C indicates cholesterol; CE, cholesteryl esters; DBP, diastolic blood pressure; FA, fatty acids; FC, free cholesterol; HDL, high-density lipoprotein; IDL, intermediate-density lipoproteins; L, large; L, total lipids; LDL, low-density lipoprotein; M, medium; P, lipoprotein particle concentrations; PL, phospholipids; S, small; SBP, systolic blood pressure; TG, triglycerides; VLDL, very low-density lipoprotein; XL, very large; and XS, very small.

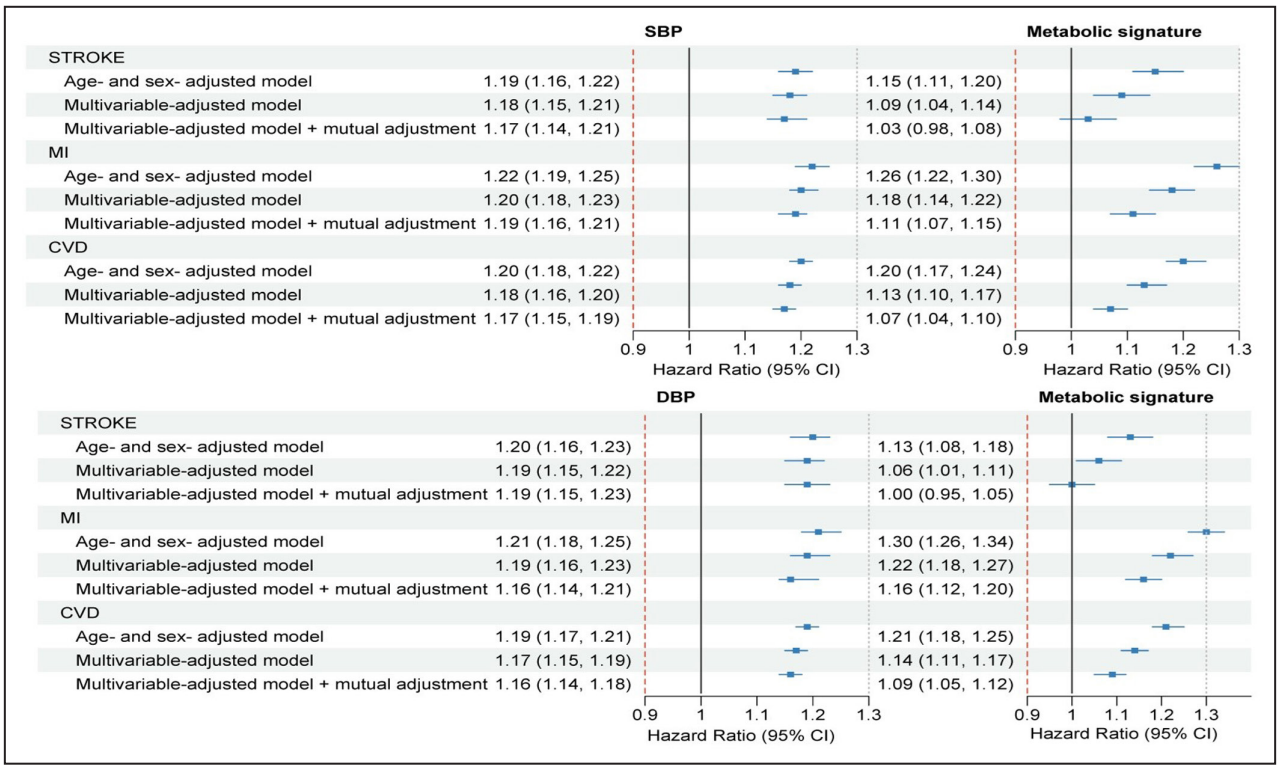

Figure 4. Associations of BP and metabolic signatures with cardiovascular diseases.
Hazard ratio and 95% CI per 10% increment of BP; hazard ratio and 95% CI per SD increment in metabolic signatures; multivariable-adjusted model, based on an age- and sex-adjusted model, further adjusted for body mass index, Townsend deprivation index, alcohol status, physical activity, diabetes, and smoking status; multivariable-adjusted model+mutual adjustment, included each BP measure and the corresponding standardized metabolic signature simultaneously in the multivariable-adjusted model to examine association independence. The *Z* score of metabolic signature of SBP consists of 31 identified metabolites, whereas the *Z* score of metabolic signature of DBP consists of 25 identified metabolites. There were 6742 CVD events, 4192 MI events, and 2757 stroke events. BP indicates blood pressure; CVD, cardiovascular disease; DBP, diastolic blood pressure; MI, myocardial infarction; and SBP, systolic blood pressure.

positive association, though not independent, between small, dense HDL-3 concentrations and hypertension.[14] Our findings are in line with these observations by showing that small HDL and LDL particles were positively associated with SBP and DBP. Results from our study also suggest a higher activity of CETP (cholesteryl ester transfer protein), which leads to the transfer of cholesteryl esters from HDL to apolipoprotein B-containing lipoproteins, including very large VLDL and small LDL particles.[39] The enhanced capacity of larger VLDL particles to accumulate cholesteryl esters may contribute to elevated BP by inducing endothelial dysfunction and vascular inflammation.[15] We used a statistical technique designed to control for high multicollinearity in metabolomics data and association independencies, which might have influenced the direction of the associations of very large and large VLDL rich in cholesteryl esters with BP. Biologically, very large VLDL particles are typically richer in triglycerides and cholesteryl esters compared with smaller VLDL particles. Due to their size and composition, these larger particles play a distinct role in lipid metabolism and are considered more atherogenic. They are more likely to contribute to the production of small dense LDL particles,[40] which are linked to an increased risk of cardiovascular diseases. Conversely, large VLDL particles, although still part of lipid transport, appear to have a different impact, possibly reflecting a less direct role in atherogenic processes, which might account for their negative association with BP. The observed associations between total fatty acids and both BP traits are in line with previous findings[13] and could reflect the activation of hepatic de novo lipogenesis.[41]

Although numerous epidemiological studies investigated the association of amino acids with BP or hypertension, the evidence remains inconclusive.[42] Our findings are consistent with previous research that has reported higher concentrations of tyrosine and lower concentrations of glycine, histidine, and glutamine to be associated with higher BP.[43–46] Moreover, circulating tyrosine concentrations have been positively correlated with inflammatory markers,[47] potentially contributing to elevated BP. Furthermore, higher circulating concentrations of branched-chain amino acids

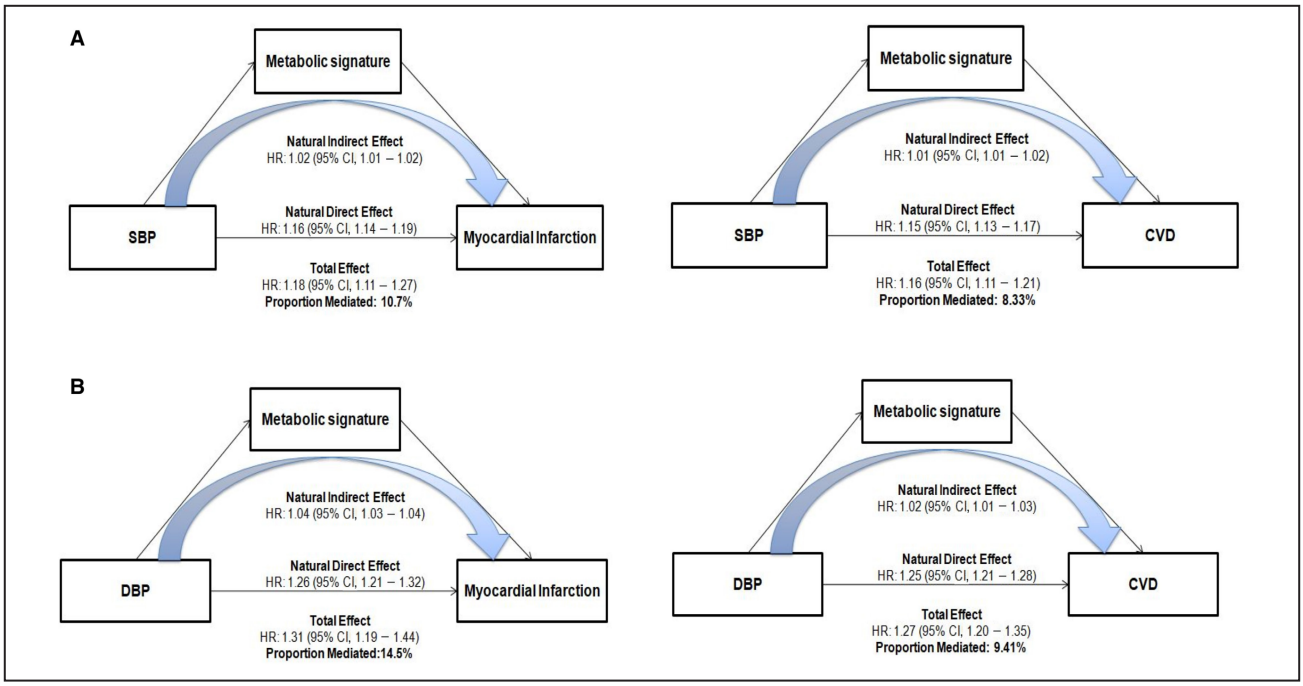

Figure 5. Causal mediation analysis.
A, Causal mediation analysis is shown for the presence of metabolic signature of SBP as a mediator in the relation between SBP and MI/CVD. **B**, Causal mediation analysis is shown for the presence of metabolic signature of DBP as a mediator in the relation between DBP and MI/CVD. Mediation analysis for MI (N=4192) and CVD (N=6742) risk per 1 unit increase in ln SBP and ln DBP; proportion mediated at the median metabolic signatures. All models are adjusted for age, sex, body mass index, Townsend deprivation index, alcohol status, physical activity, diabetes, and smoking status. CVD indicates cardiovascular disease; DBP, diastolic blood pressure; HR, hazard ratio; MI, myocardial infarction; and SBP, systolic blood pressure.

have been associated with elevated BP in several studies,[42,48,49] although a previous cross-sectional study did not support these results.[50] In our study, leucine, one of the branched-chain amino acids, was inversely associated with both BP traits. It is of note that circulating concentrations of branched-chain amino acids are influenced by dietary intake and the gut microbiome,[51] which could potentially contribute to the observed discrepancies across studies.

Metabolites related to glucose metabolism, such as glucose and lactate, are higher in individuals with hypertension, whereas those involved in the tricarboxylic acid cycle, such as pyruvate, are lower. These metabolic changes have been suggested to be involved in the inflammatory state and oxidative stress observed in hypertension.[13] Notably, beta-hydroxybutyrate, the most abundant ketone body in circulation, was positively associated with both BP measures, and we could speculate that a ketogenic shift in those individuals with higher BP levels may have occurred.

Previous studies have reported an association between higher BP and plasma creatinine levels,[52] which may reflect reduced renal function. Our findings also support this observation. Furthermore, our study confirms previous research by demonstrating inverse associations between choline levels and SBP.[53]

In our study, the identified metabolic signatures were consistent with adverse profiles for cardiovascular health. The combination of higher concentrations of cholesteryl esters in larger VLDL subclasses enhanced CETP activity, higher numbers of small HDL and LDL particles associated with elevated BP measures, and lower levels of total HDL associated with higher SBP and larger HDL particles associated with higher DBP may contribute to a higher risk of developing atherosclerosis and MI.[54–57] Additionally, the increased concentrations of molecules involved in cardiac energy metabolism, such as glucose, lactate, and ketone bodies, may indicate perturbations in cardiac energy metabolism and a shift to pyruvate oxidation and the breakdown of ketone bodies.[58] Of note, beta-hydroxybutyrate, besides serving as an energy source, may exert signaling effects on inflammation, oxidative stress, and cardiac remodeling that may induce a harmful effect on CVDs.[59–61] Regarding amino acids, several prospective and Mendelian randomization studies suggested that low glycine levels may be related to MI[62] and coronary heart disease risk.[63] Similarly, lower concentrations of glutamine have been reported in individuals with CVD, and its cardioprotection may be attributed to improved myocardial metabolism and antioxidant and anti-inflammatory properties.[64] Another amino acid with

antioxidant and anti-inflammatory properties, histidine, has been suggested to have a protective effect on incident coronary heart disease.[65] In our study, we found inverse associations between glycine and both BP measures, histidine and SBP, and glutamine and DBP. On the other hand, tyrosine was positively associated with BP, and it has previously been associated with an increased risk of CVD.[66]

Our study highlighted BP metabolic signatures that are independently associated with long-term MI and CVD risk. The role of these BP-related metabolic alterations in disease development was further supported by mediation analysis. The metabolic signatures accounted for 10.7% and 14.5% of the association between SBP and DBP with MI, respectively. Additionally, these metabolic signatures contributed to 8.3% and 9.4% of the association between SBP and DBP with CVD risk. However, the associations of the signatures with stroke were attenuated and became nonsignificant after adjusting for BP, suggesting that the metabolites in the signatures do not biologically underlie these associations.

The large sample size, the long follow-up period, the use of a robust NMR metabolomics platform enhancing the potential clinical utility of the identified metabolic signatures,[67] and the exclusion of participants taking lipid-lowering and insulin medication reducing treatment-associated biases are strengths of this study. This study also demonstrates the cross-population reproducibility of the metabolic signature by conducting discovery analyses in a large UK cohort and validating the findings in a Greek cohort. This approach underscores the robustness and generalizability of our approach and findings. Regarding limitations, the metabolite coverage by the NMR assay is narrower than that afforded by mass spectrometry[68] and lipid focused, limiting the identification of new metabolites associated with BP. Another limitation of our study is the use of nonfasting blood samples from the UK Biobank, which may introduce variability in metabolic biomarker levels due to recent food intake. However, replication of findings in fasting samples from EHS adds validity to our results. Furthermore, most of the participants included in the UK Biobank cohort are of White race, which may limit the generalizability of our results to other ethnicities. Although we adjusted the Cox regression models for several confounding factors, we cannot exclude residual and unmeasured confounders.

CONCLUSIONS

In summary, using NMR-based metabolomic data, we discovered and externally validated distinct metabolic signatures associated with SBP and DBP. Our findings indicate that early abnormalities in lipoprotein subclass distributions along with perturbations of lipid, amino acid, and energy metabolism related to higher BP may contribute to the development of MI and CVD. This could shed light on the biological mechanisms driving atherosclerotic processes and the development of these adverse cardiovascular events. These signatures could also be further explored to guide lifestyle or pharmacological interventions targeting the BP-related metabolic abnormalities to prevent CVD.

ARTICLE INFORMATION

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Disclosures

None.

Ethics Statement

The analysis was performed under the UK Biobank application number 79696. The UK Biobank has approval from the Northwest Multi-centre Research Ethics Committee. It has also sought approval from the Patient Information Advisory Group in England and Wales to access information that would allow it to invite potential participants.

Supplemental Material

Data S1
Tables S1–S10
Figures S1–S5
References [69–70]

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