Identification of Serum Metabolites Associated With Incident Hypertension in the European Prospective Investigation into Cancer and Nutrition–Potsdam Study

Stefan Dietrich, Anna Floegel, Cornelia Weikert, Tobias Pischon, Heiner Boeing, Dagmar Drogan

**Abstract**—Metabolomics is a promising tool to gain new insights into early metabolic alterations preceding the development of hypertension in humans. We therefore aimed to identify metabolites associated with incident hypertension using measured data of serum metabolites of the European Prospective Investigation Into Cancer and Nutrition (EPIC)–Potsdam study. Targeted metabolic profiling was conducted on serum blood samples of a randomly drawn EPIC-Potsdam subcohort consisting of 135 cases and 981 noncases of incident hypertension, all of them being free of hypertension and not on antihypertensive therapy at the time of blood sampling. Mean follow-up was 9.9 years. A validated set of 127 metabolites was statistically analyzed with a random survival forest backward selection algorithm to identify predictive metabolites of incident hypertension taking into account important epidemiological hypertension risk markers. Six metabolites were identified to be most predictive for the development of hypertension. Higher concentrations of serine, glycine, and acyl-alkyl-phosphatidylcholines C42:4 and C44:3 tended to be associated with higher and diacyl-phosphatidylcholines C38:4 and C38:3 with lower predicted 10-year hypertension-free survival, although visualization by partial plots revealed some nonlinearity in the above associations. The identified metabolites improved prediction of incident hypertension when used together with known risk markers of hypertension. In conclusion, these findings indicate that metabolic alterations occur early in the development of hypertension. However, these alterations are confined to a few members of the amino acid or phosphatidylcholine metabolism, respectively. 

**Key Words:** glycine ■ hypertension ■ incidence ■ metabolomics ■ phosphatidylcholines ■ serine

Essential hypertension is among the most important preclinical conditions of metabolic syndrome and affects nearly 1 billion people worldwide. The risk to develop essential hypertension seems to be a function of age, triggered by an unhealthy lifestyle with obesity, and physical inactivity as major risk factors. Furthermore, dyslipidemia, inflammatory processes, and oxidative stress have been closely linked to this preclinical condition. Although many pathophysiological mechanisms of hypertension have been elucidated, knowledge is scarce about individual metabolic alterations promoting the development of essential hypertension in healthy subjects or subjects in early stages of this condition.

Application of metabolomics can contribute to fill this gap and generate further insights into the pathogenesis of hypertension development. Metabolites represent intermediates and end products of cellular processes and are substantial for signaling, structuring of membranes, and catalytic activity. Metabolic alterations associated with development of hypertension, therefore, may be present years before hypertension diagnosis. Hence, investigating metabolic profiles in prospective cohorts is a promising opportunity to improve our knowledge of incident hypertension and to discover novel biomarkers that elucidate early changes in potential pathways.

In the US cohort, of 204 metabolites, the metabolite 4-hydroxyhippurate and a metabolic sex steroids pattern were associated with incident hypertension. Another US study using metabolic profiling revealed an association of diacylglycerols, in general, and of the 2 diacylglycerols 16:0/22:5 and 16:0/22:6, in particular, with blood pressure (BP) and incident hypertension. However, to our knowledge, only a few prospective studies have used metabolic profiling to investigate metabolic alterations associated with incident hypertension, and thus further studies are necessary to elucidate this promising approach.

This study aimed to identify metabolites associated with incident hypertension using data of 127 serum metabolites (Biocrates AbsoluteIDQ p150) determined within the European Prospective Investigation Into Cancer and Nutrition (EPIC)–Potsdam study. The statistical analyses were performed with random survival
Hypertension index as described previously.12 Alcohol intake from bever-
quency questionnaire11 and used to calculate dietary approaches to stop
Baseline blood serum samples were analyzed to determine metabo-
isis (\textit{International Classification of Diseases Tenth Revision}
assisted interviews and questionnaires on medi-
cal conditions, diet and lifestyle, and underwent examinations. The
examinations were conducted by qualified medical staff following
standardized protocols and included anthropometric and 3 BP mea-
surements and collection of blood samples (30 mL). Blood samples
were immediately fractionated, aliquoted into straws (0.5 mL), and
stored at –196°C until the measurement of serum metabolites.
This study was conducted in a subcohort embedded within the EPIC-
Potsdam study. The subcohort was implemented for case–cohort analy-
es in 2005 and included 2500 randomly drawn participants.9 Baseline
blood serum samples of the subcohort members were used for targeted
metabolic profiling. After exclusion of participants with prevalent hyper-
tension, diagnosed hypertension within first year of follow-up time, myo-
cardial infarction and stroke at baseline, with missing or nonverified data
on incident hypertension, missing data on covariates and metabolites, the
analyzed study population included initially 1116 participants free of
high BP at baseline of which 135 participants developed hypertension
during follow-up. Among female cases, there were no pregnancies.

Assessment of Prevalent and Incident Hypertension
Participants with (1) systolic BP ≥140 mm Hg or diastolic BP ≥90
mm Hg, or both, as defined by the mean of the second and third mea-
surements, (2) self-reported hypertension diagnosis, or (3) the use of
antihypertensive medication at baseline were classified as cases of
prevalent hypertension. Information about potential cases of incident
hypertension and hypertension-specific medication during the past
month was recorded every 2 to 3 years by self-administered question-
naires with response rates of 95% on average. For all self-reports, the
treating physician was contacted for verification and only confirmed
diagnosed cases by the treating physician were included in the analy-
sis (\textit{International Classification of Diseases Tenth Revision}; 110).

Assessment of Baseline Covariates
Body mass index was calculated as the ratio of weight (kg) to height
squared (m²). Education at attainment, physical activity, and smoking
were acquired by a standardized interview. For analyses, education at
attainment was categorized as no degree/vocational training, trade/technical
college, and university degree. Smoking behavior as never
smoker, former smoker, and current smoker (≤20 cigarettes/d and >20
cigarettes/d). To account for physical activity, the Improved Physical
Activity Index adjusted for sex and age was calculated as described pre-
nviously.10 Dietary habits were assessed by the use of a validated food fre-
cquency questionnaire11 and used to calculate dietary approaches to stop
hypertension index as described previously.12 Alcohol intake from bever-
ages was categorized into nonconsumer and consumer (women >0–6,
6–12, and >12 g/d and men >0–12, 12–24, and >24 g/d). Cases of preva-
 lent type 2 diabetes mellitus were assessed during a standardized inter-
view at baseline and verified by the treating physician. Diabetes mellitus
status was categorized as existence of type 2 diabetes mellitus or not.

Assessment of Serum Metabolite Concentrations
Baseline blood serum samples were analyzed to determine meta-
bolite concentrations by using AbsoluteIDQ p150 Kits (Biocrates Life
Sciences AG, Innsbruck, Austria), which is based on flow injection analysis tandem mass spectrometry technique.13 Analysis was done
by the Genome Analysis Center at the Helmholtz Zentrum München.
Please refer to the study by Römisch-Margl et al13 for analytic details.
Metabolites with concentrations below the detection limit or high analytic variance (n=36) were excluded,14 leaving the following 127 quantified metabolites for statistical analyses (Table S1 in the online-
only Data Supplement): hexose (sum of 6-carbon monosaccharides
without distinction of isomers), 14 amino acids, 14 spingomyelins,
17 acylcarnitines (C\textsubscript{x}y; with x indicating carbon atoms and y indicating
double bonds), and 81 glycerophospholipids (37 acyl-alkyl-, 34
diacyl-, and 10 lyso-phosphatidylcholines).

Random Survival Forest
RSF computes a forest of decision trees based on bootstrap samples,
which can be used to select most predictive variables for event time of
interest.8 For computation of the decision trees, random node splitting
is used and a node is split by a variable, which maximize the survival
differences between daughter nodes determined by a log rank statist-
ic. Please refer the study by Ishwaran et al\textsuperscript{8} for detailed description
of the RSF method.
The predictiveness of a variable for time until event can be deter-
mined by a ranking method called minimal depth.15 To compute the
minimal depth of a variable, the distance from the root node to the
node at which a variable splits first in a decision tree is determined
and is then averaged over all bootstrap decision trees. Variables that
split near the root in the decision trees are more predictive regarding
time until the event and result in lower minimal depth values.
The prediction accuracy of an RSF model is determined by the
RSF prediction error rate, which is based on Harrell concordance in-
dex (C-index).\textsuperscript{8} To calculate the RSF error rate, out of back samples,
representing observations not included in the respective bootstrap
samples, are used and dropped down the decision tree computed by
the respective bootstrap samples. According to Harrell C-index the
probability is than estimated, that within a randomly selected pair of
cases, the case with the shorter follow-up time has the worst pre-
dictive outcome.\textsuperscript{8} The RSF error rate is conform to 1-C-index with
values between 0 and 1, where lower prediction error rate values are
corresponding to RSF models with more precise prediction accuracy.\textsuperscript{8}

Statistical Analysis
Baseline characteristics of noncases and cases of incident hyperten-
sion are presented as means and SD for continuous variables and as
frequencies for categorical variables. Age, body mass index, sex,
Improved Physical Activity Index, dietary approaches to stop hyper-
tension index, alcohol intake from beverages, smoking behavior,
education at attainment, and prevalent diabetes mellitus were used
as covariates.
For metabolite selection, the following RSF backward selection algo-
rithm, recently suggested for variable selection in the context of Random
Forest\textsuperscript{16} and subsequently adapted to RSF, was used: (1) compute an
RSF with the data set that contains the covariates and the metabolites
to be tested, (2) remove the metabolite with the worst minimal depth
rank from the data set, (3) use the data set with all the covariates and
the remaining metabolites to compute a new RSF, (4) repeat steps 2 and 3
till only 1 metabolite remains, and (5) choose the set of metabolites with
the smallest predicted error rate. The RSF backward selection algorithm
was applied on data consisting of covariates and all 127 metabolites
to identify the most predictive metabolites.
After the variable selection procedure, data of identified metabolites
and covariates were used to compute a new RSF model. The RSF mod-
el was further used to calculated partial plots of identified metabolites.\textsuperscript{17}
Partial plots represent the effect of each metabolite on predicted 10-
year hypertension-free survival after accounting for the average effects
of the other selected metabolites and the covariates. Finally, to prove
whether the selected metabolites improve RSF prediction error rates
4 different RSF models were computed and compared. The following
data sets were used to compute the respective RSF models: (1) covari-
ates only, (2) covariates and selected metabolites, (3) all metabolites,
and (4) all metabolites and covariates. Hundred repetitions of each RSF
Results

Baseline characteristics of the EPIC-Potsdam subcohort sample are presented in Table 1. Of 127 analyzed metabolites, a set of 6 metabolites resulted in the smallest RSF prediction error rate during the variable selection process and was most predictive for incident hypertension (Figure 1). Ranked by the minimal depth measure, identified metabolites were serine, acyl-alkyl-phosphatidylcholines C42:4, C44:3, diacyl-phosphatidylcholines C38:4, glycine, and diacyl-phosphatidylcholines C38:3. Serine was the most predictive metabolite for incident hypertension, whereas diacyl-phosphatidylcholines C38:3 was less predictive. The identified metabolites seem to represent no common metabolic pathway (Figure S1).

From the minimal depth ranking (Figure 1), it was also found that the covariates body mass index, age, and Improved Physical Activity Index had an even smaller minimal depth than serine, whereas the other covariates had higher minimal depth than the 6 selected metabolites. This suggests that the identified metabolites are more predictive than some traditionally used risk markers of hypertension. No metabolite from the acylarnitines, sphingomyelins, and lysosphatidylcholines class were selected to be predictive for incident hypertension. In addition, the covariate sex was ranked with a relative high minimal depth value suggesting low information content about the prediction of incident hypertension because of sex differences.

As visualized in the partial plots (Figure 2), nonlinear associations between concentrations of identified metabolites and predicted 10-year hypertension-free survival were observed. Higher concentrations of the metabolites serine, acyl-alkyl-phosphatidylcholines C42:4, C44:3, and glycine were associated with a higher 10-year predictive hypertension-free survival, suggesting lower risk to develop hypertension within 10 years of follow-up for individuals with the respective metabolite concentrations. In contrast, higher concentrations of the metabolites diacyl-phosphatidylcholines C38:4 and C38:3 were associated with a lower 10-year predictive hypertension-free survival. However, because of the nonlinear nature of the observed associations, the partial plots show a peak at given metabolite concentrations which were associated with the highest 10-year predictive hypertension-free survival. In particular, for serine, glycine, and diacyl-phosphatidylcholines C38:4 peaks at ≈100, 250, and 100 μmol/L were, respectively, observed to be associated with the highest predicted 10-year hypertension-free survival.

An RSF model computed based on data including only covariates resulted in a prediction error rate of 0.3168 (Table 2). A supplement of the covariate data with the 6 identified metabolites resulted in a more precise computed RSF model regarding prediction of incident hypertension with an improved prediction error rate of 0.2789. However, RSF models computed based on data of all 127 metabolites alone or together with the covariates resulted in RSF models with >9% worsened prediction error rates (error rate 0.4444 and 0.3747) compared with an RSF model computed based on data of selected metabolites and covariates.

Discussion

This study is one of the largest prospective cohorts using targeted metabolomics to investigate possible associations between 127 serum metabolites and incident hypertension. By application of an RSF backward selection procedure, 6 metabolites were identified to be most predictive for the development of hypertension within a follow-up time of ≈10 years. As demonstrated by 4 different RSF models, the 6 identified metabolites may contribute to an improvement of the prediction of incident hypertension when used together with known epidemiological risk factors of hypertension. The comparison of the prediction error rate of 4 different RSF models revealed that, especially noise metabolites were removed by the RSF backward selection process resulting in the identification of the most predictive metabolites. Moreover, the visualization by partial plots revealed nonlinear associations between concentrations of identified metabolites and predicted 10-year survival, indicating possible diagnostic cut points for further research.

Two of the identified metabolites were the biochemically closely related amino acids serine and glycine. In general, serine and glycine are known to act as neurotransmitters, to represent essential elements of many lipids (eg, sphingolipids, ceramides, and glycerophospholipids). As far as known, no study has until now demonstrated an association of serum blood levels of serine and glycine with incident hypertension. Nevertheless, the observed results are supported by recent research indicating that supplementation with serine as well as glycine may have BP-lowering effects. Moreover, anti-inflammatory and antioxidant properties were attributed to serine and glycine, which may contribute to protective effects regarding the development of hypertension.

In this study, a nonlinear positive association of serine concentrations with 10-year hypertension-free survival was observed, whereby serine concentrations of 100 μmol/L were most protective regarding 10-year hypertension-free survival. This is confirmed by a study of Mishra et al describing serine-induced vasodilation in endothelium-intact vessels as a result of increasing serine concentrations. As shown by this previous study, the endothelium-dependent vasodilation was probably caused by serine promoted K+ efflux from the endothelium, independent of endothelial nitric oxide release. Moreover, elevation of antioxidant agents by administering serine to human endothelial cell culture were reported reflecting cytoprotective and antioxidant effects of serine. It was also demonstrated that dietary serine intake lowers plasma homocysteine concentrations. Homocysteine is a known risk factor for cardiovascular diseases and arterial endothelial dysfunction confirming the present results.

The second identified amino acid glycine is known to reduce oxidative stress because of an enhancement of the
bioavailability of nitric oxide.\textsuperscript{22,23} As seen in the partial plot of glycine, an increase of glycine concentrations of $\approx 150$ to $250 \mu$mol/L was closely associated with an improvement of 10-year hypertension-free survival time. A recent study showed that glycine concentrations of around 200 $\mu$mol/L are required to activate glycine-gated chloride channels which, inter alia, occur in macrophages, monocytes, and endothelial cells.\textsuperscript{29–32} Furthermore, it was shown that glycine may exhibit anti-inflammatory effects, in particular, with regard to endothelial cells.\textsuperscript{25} In addition, reduced glycine concentration levels in urine of women with preeclampsia were previously also detected.\textsuperscript{33} However, preeclampsia is linked with a period of pronounced hormonal and metabolic changes and findings may thus not be comparable with our sample of the general population.

Remarkably, no other analyzed amino acid than serine and glycine was associated with the development of hypertension, to date. In accordance with the present results, the Rotterdam study also showed no association between incident hypertension and several amino acids, such as arginine, lysine, glutamic acid, cysteine, and tyrosine.\textsuperscript{34} In addition, serine and glycine were not considered by the Rotterdam study.\textsuperscript{34}

**Table 1. Baseline Characteristics of the Subcohort Embedded Within EPIC-Potsdam*\textsuperscript{a}

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Noncases (n=981)</th>
<th>Cases of Incident Hypertension (n=135)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>46.9 (8.4)</td>
<td>51.7 (8.7)</td>
</tr>
<tr>
<td>Women, %</td>
<td>71.2</td>
<td>71.7</td>
</tr>
<tr>
<td>BMI (kg/m\textsuperscript{2})</td>
<td>24.3 (3.5)</td>
<td>26.6 (4.2)</td>
</tr>
<tr>
<td>Systolic BP (mm Hg)</td>
<td>117.4 (10.0)</td>
<td>125.1 (8.8)</td>
</tr>
<tr>
<td>Diastolic BP (mm Hg)</td>
<td>76.8 (6.7)</td>
<td>80.7 (5.8)</td>
</tr>
<tr>
<td>Education</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No degree/vocational training, %</td>
<td>33.8</td>
<td>39.1</td>
</tr>
<tr>
<td>Trade/technical school, %</td>
<td>25.5</td>
<td>30.4</td>
</tr>
<tr>
<td>University degree, %</td>
<td>40.7</td>
<td>30.4</td>
</tr>
<tr>
<td>Smoking status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never, %</td>
<td>47.5</td>
<td>56.5</td>
</tr>
<tr>
<td>Former, %</td>
<td>30.2</td>
<td>25.4</td>
</tr>
<tr>
<td>Current, %</td>
<td>22.2</td>
<td>18.1</td>
</tr>
<tr>
<td>Among smokers: number of cigarettes/d</td>
<td>12.4 (8.3)</td>
<td>14.4 (10.6)</td>
</tr>
<tr>
<td>IPAI</td>
<td>37.1 (4.4)</td>
<td>34.9 (4.4)</td>
</tr>
<tr>
<td>Alcohol intake from beverages (g/d)</td>
<td>12.8 (14.7)</td>
<td>11.2 (14.4)</td>
</tr>
<tr>
<td>DASH index</td>
<td>16.0 (4.9)</td>
<td>15.7 (4.8)</td>
</tr>
<tr>
<td>Prevalent T2DM, %</td>
<td>1.7</td>
<td>1.5</td>
</tr>
<tr>
<td>Follow-up time (y)</td>
<td>10.4 (1.9)</td>
<td>6.5 (2.6)</td>
</tr>
</tbody>
</table>

BMI indicates body mass index; BP, blood pressure; DASH, dietary approaches to stop hypertension; EPIC, European Prospective Investigation into Cancer and Nutrition; IPAI, improved physical activity index; and T2DM, type 2 diabetes mellitus.\textsuperscript{35–38} It was shown that phosphatidylcholines are able to inhibit the upregulation of the inflammatory cytokines tumor necrosis factor-\alpha and interleukin-6 and the actin-assembly in phagosomes and macrophages.\textsuperscript{35,37} Moreover, phosphatidylcholines seem to be required for lipoprotein assembly and hepatic secretion of triglyceride-rich very low-density lipoprotein particles as well as high-density lipoprotein particles.\textsuperscript{39} Through a vinyl-ether bond in the acyl-alkyl-phosphatidylcholines, acyl-alkyl-phosphatidylcholines may be capable to act as blood antioxidants to protect lipoproteins from oxidation.\textsuperscript{40} Moreover, in a previous study, it was shown that acyl-alkyl-phosphatidylcholines were positively correlated with high-density lipoprotein-cholesterol.\textsuperscript{39} These previous findings may support the observed protective association between acyl-alkyl-phosphatidylcholines and predicted 10-year hypertension-free survival of this study and may point to a role as blood antioxidants of the identified acyl-alkyl-phosphatidylcholines. Of note, the metabolites acyl-alkyl-phosphatidylcholines C42:4, diacyl-phosphatidylcholines C38:4 as well as glycine...
were previously also found to be associated in the same direction with incident type 2 diabetes mellitus, suggesting a potential role of identified metabolites in the metabolic syndrome. However, the results obtained suggest that the early stage of hypertension is accompanied by only a few alteration of investigated serum lipid metabolism. Although hypertension is closely linked to dyslipidemia, to date, only a limited number of prospective cohorts exists that investigated blood parameters in lipid metabolism in relation to incident hypertension. A recent study in the US-Hispanic population used serum lipidomic profiling including phosphatidylcholines to elucidate associations with incident hypertension. In the study by Kulkarni et al, of measured phosphatidylcholines, only the diacyl-phosphatidylcholines C34:4 was significantly associated with an increased diastolic BP but not with incident hypertension. In addition, of all the measured metabolites, 1 phosphatidylethanolamin metabolite (C40:6) and 2 diacylglycerol metabolites (DG 16:0/22:5 and DG 16:0/22:6) were associated with incident hypertension. However, phosphatidylcholines with >40 C-atoms were not measured in the study by Kulkarni et al, and diacylglycerols and phosphatidylethanolamins were not measured in this study, which allows only a limited comparison with this study.

In a further study by Zheng et al, the association between metabolites and incident hypertension was investigated resulting in the identification of the metabolite 4-hidroxyhippurate and a sex steroids pattern. Again, only a limited comparability is given, because, except for some amino acids, different metabolites were measured. Serine was not measured in the study by Zheng et al, but glycine. However, glycine was not associated with incident hypertension risk in this study.

In addition, metabolomics was also applied to retrospectively identify metabolites associated with prevalent hypertension and BP. Graessler et al reported altered blood plasma levels of acyl-alkyl-phosphatidylcholines in hypertensive German men, relative to the control group of normotensive men. Notably, identified acyl-alkyl-phosphatidylcholines (C36:4, C36:5, C38:4–C38:6) were highly unsaturated, as the 3 identified phosphatidylcholines metabolites in this study which may be beneficial to enhance membrane fluidity. However, none of the metabolites, reported by Graessler et al, were associated with incident hypertension in this study, suggesting differences in metabolite composition of individuals with incident and prevalent hypertension. Recently, of 280 metabolites, 15 metabolites were identified by Menni et al to be independently associated with BP. However, of identified metabolites, only hexadecanediol, a dicarboxylic acid, showed concordant association with BP in 2 replication cohort (KORA and Hertfordshire). In addition, metabolomics was also applied to retrospectively identify metabolites associated with prevalent hypertension and BP. Graessler et al reported altered blood plasma levels of acyl-alkyl-phosphatidylcholines in hypertensive German men, relative to the control group of normotensive men. Notably, identified acyl-alkyl-phosphatidylcholines (C36:4, C36:5, C38:4–C38:6) were highly unsaturated, as the 3 identified phosphatidylcholines metabolites in this study which may be beneficial to enhance membrane fluidity. However, none of the metabolites, reported by Graessler et al, were associated with incident hypertension in this study, suggesting differences in metabolite composition of individuals with incident and prevalent hypertension. Recently, of 280 metabolites, 15 metabolites were identified by Menni et al to be independently associated with BP. However, of identified metabolites, only hexadecanediol, a dicarboxylic acid, showed concordant association with BP in 2 replication cohort (KORA and Hertfordshire).
summary, the small number of studies also highlights the need for further research to gain a deeper insight into possible association between metabolites and hypertension development.

The strength of this study is the application of targeted metabolomics in a well-described population-based prospective cohort with strictly standardized study protocols and a long follow-up time. Furthermore, the investigated metabolites have been previously validated and those metabolites below the detection limit and with high analytic variance were excluded.\textsuperscript{14} With exception of acyl-alkyl-phosphatidylcholines C42:4, the identified metabolites showed good reliability during a 4-month period\textsuperscript{14} (Table S2). The exploratory data analysis of complex metabolomic data using traditional statistical regression approaches is accompanied by false-positive detection because of high number of correlated variables, which brings problems such as multiple hypotheses testing, decreased statistical power, and increased risk of multicollinearity. The machine learning method RSF was specifically developed for statistical analysis of complex, right-censored survival data.\textsuperscript{8} RSF is completely data driven, reduces overfitting by bootstrapping and the interruption of intercorrelation structures by random node splitting allows reliable variable selection in the presence of multicollinearity.\textsuperscript{7} Indeed, RSF has been successfully applied to identify risk factors of cancer and cardiovasculardiseases\textsuperscript{31,32} and thus RSF seems appropriate for metabolite selection. Moreover, nonlinear associations between identified metabolites and predicted survival time were visualized allowing definition of possible clinical thresholds and cut points in further scientific studies.

Indeed, this study also has some limitations. Although we included established hypertension risk factors, our findings were obtained from an observational study. As such, we cannot exclude the possibility that our findings have been influenced by additional factors that were not included. Moreover, given the observational nature of our study, causality of present findings cannot be proven. Nevertheless, the observed nonlinear associations in the partial plots are useful to derive possible cut points for future studies. A major limitation of this study is that our findings cannot be validated in an external cohort because there is no similar prospective study of sufficient size with available measurements of Biocrates metabolites that have validated end point of incident hypertension. Although previous studies support the biological plausibility of our findings, validation in external prospective cohorts and proof of causality are necessary to confirm the observed results and to translate them into clinical practice.

In conclusion, the analysis of targeted metabolic data in the EPIC-Potsdam study provided new insights about metabolic alterations that occur early in the development of hypertension. However, these alterations are confined to a few chemically related members of amino acid or phosphatidylcholine metabolism, respectively. Similar studies of this type are essential to elucidate metabolic predictors of hypertension, as interindividual and population-based metabolic differences and their technical measurements may lead to a modified selection of metabolites in other studies.

Perspectives

This study successfully used targeted metabolic profiling to identify 6 metabolites associated with incident hypertension. The gained insights enhance our knowledge about metabolic alterations that influence the development of hypertension. On the basis of this study, further research can be carried out with the objective to confirm the archived results and to improve individual clinical treatment and prevention strategies.

Acknowledgments

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Disclosures

None.

References

Novelty and Significance

What Is New?

This is one of the first studies using targeted metabolomics in a prospective cohort (European Prospective Investigation into Cancer and Nutrition [EPIC]-Potsdam) to identify metabolites associated with incident hypertension.

What Is Relevant?

• Higher concentrations of serine, glycine, and the acyl-alkyl-phosphatidylcholines C42:4 and C44:3 tended to be associated with higher and diacyl-phosphatidylcholine C38:4 with lower predicted 10-year hypertension-free survival.

• Nonlinear associations between concentrations of identified metabolites and predicted 10-year hypertension-free survival time were observed.

Summary

This study indicates that metabolic alterations occur early in the development of hypertension. However, these alterations are confined to a few members of the amino acid or phosphatidylcholine metabolism, respectively.