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Dietary Patterns Are Associated with Serum Metabolite Patterns and Their Association Is Influenced by Gut Bacteria among Older German Adults

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ABSTRACT

Background: Although dietary intakes and dietary intake patterns (DPs) have been associated with single metabolites, it is unclear whether DPs are also reflected in specific metabolite patterns (MPs). Moreover, the influence of groups of gut bacteria on the relationship between DPs and MPs is underexplored.

Objectives: We aimed to investigate the association of DPs and serum MPs and also the modifying effect of the gut bacteria compositional patterns (BCPs).

Methods: This is a cross-sectional investigation among 225 individuals (median age: 63 y; 53% women) from the European Prospective Investigation into Cancer and Nutrition study. Dietary intakes were assessed by three 24-h dietary recalls, gut bacteria composition was quantified by 16S rRNA gene sequencing, and the serum metabolome was profiled by an untargeted approach. We identified DPs and BCPs by the treelet transform analysis. We modeled associations between DPs and 8 previously published MPs and the modifying effect of BCPs by fitting generalized linear models using DataSHIELD R.

Results: We identified 5 DPs and 7 BCPs. The "bread, margarine, and processed meat" and "fruiting vegetables and vegetable oils" DPs were positively associated with the "amino acids" ($\beta = 0.35$; 95% CI: 0.02, 0.69; $P = 0.03$) and "fatty acids" MPs ($\beta = 0.45$; 95% CI: 0.16, 0.74; $P = 0.01$), respectively. The "tea and miscellaneous" was inversely associated with the "amino acids" ($\beta = -0.28$; 95% CI: −0.52, −0.05; $P = 0.02$) and "amino acid derivatives" MPs ($\beta = -0.21$; 95% CI: −0.39, −0.02; $P = 0.03$). One BCP negatively modified the association between the "bread, margarine, and processed meat" DP and the "amino acids" MP (P-interaction = 0.01).

Conclusions: In older German adults, DPs are reflected in MPs, and the gut bacteria attenuate 1 DP–MP association. These MPs should be explored as biomarkers of these jointly consumed foods while taking into account a potentially modifying role of the gut bacteria. *J Nutr* 2019;00:1–10.

Keywords: dietary intake patterns, gut bacteria compositional patterns, serum metabolite patterns, treelet transform analysis, DataSHIELD

Introduction

Foods consumed are metabolized into various small molecules or metabolites, and the presence and concentration of these metabolites in body fluids are valuable in the assessment of dietary intake (1, 2). Although it is vital to ascertain the effect of single nutrients or foods, the need to explore the dietary intake patterns (DPs) due to the complexity of consumed foods has been emphasized (3). Similarly, exploring metabolite patterns (MPs) is relevant due to the complex metabolism associated with matrices of dietary exposures and the high intercorrelations among metabolites (4). Numerous human observational studies have addressed the relation between DPs and several single metabolites (5–15), but few, if any, have investigated whether DPs are reflected in specific MPs. Because DPs represent a more comprehensive description of dietary intake, investigating their association with MPs might reveal groups of metabolites that could be explored

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as biomarkers of overall dietary intake and jointly consumed foods. The relation between the DPs and MPs will also provide further understanding and new insights into dietary interactions.

The gut microbiota plays an integral role in influencing metabolism through modulating the uptake, bioavailability, and excretion of nutrients (16–21). Furthermore, it has been reported that the gut bacteria exist in groups or communities based on their complementary metabolic actions (18, 19). Therefore, differences in populating groups of gut bacteria may influence the relation between DPs and MPs.

In this study, we sought to evaluate independent associations between DPs and previously published serum MPs and investigate whether groups of gut bacteria modify the independent associations between DPs and serum MPs.

Methods

Study population

The study population comprised 225 participants from a substudy set up between August 2010 and December 2012 (22) within the European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam cohort (23, 24). These 225 individuals are also the first set of participants who provided blood samples from this substudy. The participants answered a comprehensive questionnaire on dietary intake, sociodemographic and lifestyle factors, and medical history, and they underwent physical examinations. The standard procedures of the EPIC-Potsdam study were used to obtain anthropometric measurements. Physical activity was objectively assessed using the activity-monitoring instrument Actiheart. We verified self-reported diseases through medical record reviews and from participants' physicians. Except for educational attainment, which was the status of the participants at baseline recruitment in the EPIC-Potsdam study, all variables considered in the current analysis were based on participant status between August 2010 and December 2012.

Assessment of dietary intake

We obtained dietary intake through three 24-h dietary recalls. The first recall was collected during the visit to the study center, and the remaining 2 recalls were collected over the telephone on random days that included weekends. We aggregated the dietary intake into 39 food groups, and the average intake of the food groups over the 3 recalls as well as total energy intake were calculated.

Laboratory analysis

Serum metabolome.

The serum samples from the 225 individuals in this study were profiled by an untargeted metabolomic approach. Details can be found elsewhere (25). In brief, small polar metabolites and lipids were measured by 2-dimensional GC coupled with time-of-flight MS and by ultraperformance LC, respectively. A total of 587 small polar metabolites and 1039 lipids were detected, out of which 134 small polar metabolites and 592 lipids were identified.

Fecal DNA extraction, 16S ribosomal RNA gene sequencing, and bioinformatics.

Participants provided fecal samples for quantification of gut bacteria. Feces were sampled at home, immediately frozen at −18°C, and delivered within 7 d to the study center, where they were stored at −80°C until DNA extraction. Participants were asked to avoid the use of antibiotics for at least 7 d prior to fecal sampling.

We extracted DNA from 180–220 mg fecal sample with the QIAamp DNA Stool Mini Kit (Qiagen) according to the manufacturer's protocol (details are provided in **Supplemental Methods**). DNA quality was evaluated by running samples on a gel and DNA quantity by Qubit quantification. After PCR, the V3–V4 variable region of the 16S rRNA gene was sequenced and then made into operational taxonomic units (OTUs) (see Supplemental Methods). DNA samples of 186 individuals were sequenced. We assigned OTUs up to the genus level.

Statistical analyses

Participant characteristics.

Participant characteristics (n = 225) were presented as median and IQR for continuous variables due to their non-normal distribution, and categorical variables were expressed as counts and percentages. This statistical analysis was conducted remotely from the home base of the EPIC-Potsdam study using DataSHIELD-specific R functions in R version 3.4.4 (26).

Identification of DPs and bacteria compositional patterns.

We used treelet transform (TT) analysis—a method that combines principal component analysis (PCA) and hierarchical clustering analysis (27)—to identify DPs and gut bacteria compositional patterns (BCPs). TT was implemented according to the procedure described by Rasmussen (27) using Stata SE software version 14 (StataCorp). TT was carried out on the correlation matrices of intake of 39 food groups (n = 225) and relative abundance of 317 identified genus-level OTUs (n = 186). Prior to TT, the food groups were standardized, and OTUs were zero-imputed by the count zero Bayesian-multiplicative replacement, centered, and log-ratio-transformed. A range of 2–7 treelet components for DPs was deemed favorable for the food groups, and 2–10 treelet components for BCPs was considered favorable for the genus-level OTUs. The corresponding cluster tree levels (cut-levels) of each number of components were determined in 3 successive 10-fold cross-validations in 5 and 10 Monte Carlo repetitions. We chose the number of DPs or BCPs with the most stable cut-level (optimal cut-level). Importantly, we assessed the stability of the DPs and BCPs using a subsampling approach, randomly sampling 80% of the data in 100 bootstrap replications (see Supplemental Methods).

Therefore, we performed TT on 39 food groups for 5 DPs at a cut-level 10. We named the DPs according to the food groups contributing to "large" (≥0.4) loadings. We computed the DP scores for each individual by summing the standardized intake of the food groups weighted by their loadings, across all food groups. This quantifies the degree to which dietary intake reflected the DPs. Thus, individuals with high scores for a DP have a greater tendency to follow that specific DP compared with individuals with low scores. For internal validity of our DPs, we reran TT for dietary intake in a subset of the study sample (individuals with bacteria compositional data; n = 186), and we compared these DPs with the original DPs in terms of their cumulative explained variance, their interpretation, and stability. We also computed the Pearson correlations between the DPs from the full study sample (n = 225) and the DPs from the subset of the sample (n = 186).

Similarly, we performed TT on 317 genus-level OTUs for 7 BCPs at cut-level 94. We named the BCPs based on the dominating bacteria

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Abbreviations used: BCP, bacteria compositional pattern; CBA, carboxylic acid; DP, dietary pattern; EPIC, European Prospective Investigation into Cancer and Nutrition; MP, metabolite pattern; OTU, operational taxonomic unit; PC, phosphatidylcholine; PCA, principal component analysis; PE, phosphatidylethanolamine; TG, triglyceride; TT, treelet transform.

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family. We computed the BCP scores using a similar procedure as that for DPs.

Identification of serum MPs.

TT was used to identify MPs as previously published (25). MPs were generated on 121 small polar metabolites and 353 lipids, producing 8 MPs: "amino acids," "fatty acids," "sugar compounds and carboxylic acids (CBAs)," "amino acid derivatives," "sugar alcohols," "saturated and monounsaturated triglycerides (TGs)," "polyunsaturated TGs," and "phosphatidylethanolamines (PCs) and phosphatidylcholines (PEs)." The cumulative explained variance of the first 5 MPs was 18.9% and that of the last 3 MPs was 17.8%. The stability of "amino acids," "fatty acids," "sugar compounds and CBAs," "amino acid derivatives," "sugar alcohols," "saturated and monounsaturated TGs," "polyunsaturated TGs," and "PCs and PEs" was 76%, 77%, 45%, 76%, 63%, 49%, 7%, and 66%, respectively.

Multivariable adjusted linear regression.

To test the cross-sectional associations between the DP scores (main independent variables) and MP scores (dependent variables), we built generalized linear models (Gaussian family and identity link) using DataSHIELD-specific R functions (26) in R version 3.4.4. We interpreted p values <0.05 as statistically significant. Regression models were computed separately for each DP–MP association. We calculated 3 models with different adjustments for covariates. Model 1 was unadjusted, and model 2 was adjusted for the remaining DPs because treelet component scores are sometimes correlated. Model 3 was further adjusted for the minimal sufficient set of covariates: age; sex; BMI (in kg/m^2); smoking status; educational attainment; occupation; total energy intake; physical activity-induced energy expenditure; and prevalent hypertension, myocardial infarction, stroke, type 2 diabetes mellitus, cancer, or gastrointestinal diseases. This minimal sufficient set of covariates was obtained after entering a number of variables (or factors) that have been reported in previous studies to be associated with the DPs and MPs into a causal directed acyclic graph. This set of covariates thus represents the relations among the DP, MP, and the covariates. The factors that have been reported to be associated with DPs in general are age, sex, BMI, smoking status, educational attainment, occupation, physical activity, and prevalent diseases. In addition to diet, the factors that are related to MPs and metabolite profiles in general are postprandial interval, time of blood sampling, age, sex, BMI, smoking status, alcohol intake, educational attainment, occupation, physical activity, and prevalent diseases.

Because both DP scores and MP scores are standardized values, the coefficients obtained from these regression analyses are standardized coefficients. Also, because the relationship between DPs and MPs may be nonlinear, we fitted second-order polynomial regression models for the associations between the DPs and MPs that are nonsignificant in model 1 and adjusting for all covariates.

To address our second objective—determining whether BCPs modified the associations between the DPs and MPs—we constructed interaction models of the product variable of each DP and BCP pair and included covariates. These covariates were the DP and the BCP, the remaining DPs and BCPs, the minimal adjustment set for DP–MP association, and the minimal adjustment set of covariates for BCP–MP association. Except for bacterial diversity, both minimal adjustment sets of covariates were identical. Therefore, the interaction model of DP1 and BCP1 included their product variable; DP1; BCP1; remaining DPs and BCPs; age; sex; BMI; smoking status; educational attainment; occupation; total energy intake; physical activity-induced energy expenditure; and prevalent hypertension, myocardial infarction, stroke, type 2 diabetes mellitus, cancer, gastrointestinal diseases, and bacterial diversity (Shannon diversity index); this was also the case for other DP–BCP pairs.

Effect modification was present if the coefficient of the product term was statistically significant at $P < 0.05$. To exclude the mediating role of BCP in the association between DPs and MPs, we regressed the BCPs on DPs as one of the causal steps to confirm mediation.

TABLE 1 Basic characteristics of the study population comprising 225 older German adults[1]

Characteristics	Values
Women, *n*	119 (53)
Age, y	63 [15.2]
BMI, kg/m^2	26.7 [5]
Education, university	93 (41)
Occupational status, full-time[2]	81 (36)
Smoking, current smokers	29 (13)
Energy intake, kcal/d	1949 [740]
Energy expenditure,[3] kcal/d	578 [393]
Hypertension	114 (51)
Type 2 diabetes	17 (8)
Myocardial infarction	5 (2)
Stroke	4 (2)
Cancer	21 (9)
Gastrointestinal diseases	53 (24)

[1] Values are number (%) or median [IQR].
[2] Full-time = ≥35 h/wk.
[3] Physical activity-induced energy expenditure.

Results

Participant characteristics

The basic characteristics of our study population ($n = 225$) are presented in **Table 1**. There were relatively more women than men. Our study participants, on average, were aged 63 y, moderately overweight, consumed 1949 kcal/d, and expended 578 kcal/d through physical activity. Approximately 1 in 10 were smokers, and ~2 in 5 had a university education as well as full-time jobs. Hypertension and gastrointestinal diseases were more prevalent than other diseases.

Dietary intake and identification of DPs

Supplemental Table 1 shows the description of the 39 food groups, which are similar to the food groups that have been previously published (28). The study participants habitually consumed relatively low amounts of leafy vegetables, root vegetables, cabbage, legumes, nuts and seeds, other fruits, pasta and rice, other cereals, poultry, offal, fish, other fats, soft drinks, and tea. However, they often consumed potatoes, fruiting vegetables, other vegetables, and fruits (**Supplemental Table 2**).

Five unique and distinct DPs accounting for 17.7% of the variance in food intake were derived. All food groups were positively correlated with their DPs. Spirits, beer, red meat, soft drinks, and other fats characterized DP1, whereas DP2 was characterized by bread, margarine, processed meat, and sugar and confectionery. DP3 had contributions from fruiting vegetables and vegetable oils, DP4 from tea and miscellaneous (yeast, spices, herbs and flavorings, condiments, soya products, dietetic products, and artificial sweeteners), and DP5 from pasta and rice, and sauces. These DPs were named as "alcohol and red meat" (DP1), "bread, margarine, and processed meat" (DP2), "fruiting vegetables and vegetable oils" (DP3), "tea and miscellaneous" (DP4), and "pasta and rice, and sauce" (DP5), respectively. The "bread, margarine, and processed meat," "fruiting vegetables and vegetable oils," and "tea and miscellaneous" DPs were quite stable, appearing in >70% of the

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subsampling repetitions. The amount of variation accounted for by each of these DPs, ordered loadings of the food groups on the DPs, and stability of the DPs are presented in Table 2. Except for a new DP, "fish and juice," DPs and the cumulative variance among the subset of the study population with microbiome data ($n = 186$) were generally similar to those in the original study sample, although they were generally less stable (**Supplemental Table 3**). Furthermore, the DPs from the full study sample and those from the subset of the sample were significantly correlated at $P < 0.05$ (**Supplemental Table 4**).

Gut BCPs

We identified 7 BCPs loaded by 93 OTUs, explaining 19.91% of the overall variance of the 317 OTUs. All OTUs were positively correlated with their BCPs. BCP1 was loaded by 66 OTUs; BCP2 by 9 OTUs; BCP3 by 7 OTUs; BCP4, BCP5, and BCP6 by 3 OTUs each; and BCP7 by 2 OTUs. BCP1 was named as "Veillonellaceae, Comamonadaceae, and Family XI-dominated"; BCP2 as "Erysipelotrichaceae, Coriobacteriaceae, and Lachnospiraceae"; BCP3 as "Ruminococcaceae-dominated"; BCP4 as "*Anaerovibrio*, Uncultured genus in family Rhodospirillaceae, and *Brachyspira* genera"; BCP5 as "*Prevotella* 6, *Ezakiella*, and *Porphyromonas* genera"; BCP6 as "*Butyrivibrio*, Unidentified genus in family uncultured organism, order NB1-n, and *Victivallis* genera"; and BCP7 as "Enterobacteriaceae." The "*Anaerovibrio*, Uncultured genus in family Rhodospirillaceae, and *Brachyspira* genera" and "*Prevotella* 6, *Ezakiella*, and *Porphyromonas* genera" were quite stable, appearing in >70% of the subsampling repetitions. The ordered loadings and stability for the BCPs are presented in Table 3.

As shown in **Supplemental Table 5**, the prevalence of the 317 OTUs did not influence their loading on the BCP. In fact, out of the 24 OTUs detected in all 186 individuals, only *Bacteroides*, *Blautia*, *Lachnoclostridium*, and Ruminococcaceae UCG-002 loaded on the BCPs.

Multivariable adjusted linear regression

Association between DPs serum MPs.

For the 225 individuals with DP and MP scores, we observed that after adjusting for other DPs and covariates, a 1 SD increase in "bread, margarine, and processed meat" score was associated with an increase in the score of "amino acids" by 0.35 SD (β: 0.35; 95% CI: 0.02, 0.69; $P = 0.03$). A 1 SD increase in "fruiting vegetables and vegetable oils" score was associated with an increase of 0.45 SD in the "fatty acids" score (β: 0.45; 95% CI: 0.16, 0.74; $P = 0.01$). Finally, a 1 SD increase in "tea and miscellaneous" score was associated with a 0.28 SD decrease in the "amino acids" score (β: −0.28; 95% CI: −0.52, −0.05; $P = 0.02$) and with a 0.21 SD decrease in the "amino acid derivatives" score (β: −0.21; 95% CI, −0.39, −0.02; $P = 0.03$) (Table 4). There were no nonlinear associations between any DPs and MPs after full multivariable adjustment (data not shown).

Modifying effect of BCPs on the associations between DPs and MPs

Among 186 individuals, we investigated whether any BCPs modified the previously discussed statistically significant DP–MP associations ("bread, margarine, and processed meat"–"amino acids"; "fruiting vegetables and vegetable oils"–"fatty acids"; "tea and miscellaneous"–"amino acids"; or "tea and miscellaneous"–"amino acid derivatives").

There was a significant negative modifying effect of "bread, margarine, and processed meat" DP and "*Butyrivibrio*, Unidentified genus in family uncultured organism, order NB1-n, and *Victivallis* genera" BCP on "amino acids" MP (β-interaction: −0.31; 95% CI: −0.55, −0.07; P-interaction = 0.01). The association between "bread, margarine, and processed meat" and "amino acids" was lower by 0.31 SD for a 1 SD increase in "*Butyrivibrio*, Unidentified genus in family uncultured organism, order NB1-n, and *Victivallis* genera" score. The significant interaction indicates that at any 2 values of "*Butyrivibrio*, Unidentified genus in family uncultured organism, order NB1-n, and *Victivallis* genera," the associations between "bread, margarine, and processed meat" and "amino acids" will always be significantly different from each other. Moreover, the "bread, margarine, and processed meat" DP does not predict the "*Butyrivibrio*, Unidentified genus in family uncultured organism, order NB1-n, and *Victivallis* genera" BCP, and in fact no DP significantly predicted any BCP (**Supplemental Table 6**). Consequently, we can exclude the mediating role of the BCPs.

Discussion

In this study, we identified DPs and BCPs by TT, evaluated the relationship between the DPs and 8 previously published serum MPs, and investigated the modifying effect of the BCPs. We identified 5 DPs and 7 BCPS; 3 DPs were associated with 3 MPs after adjustment for covariates, and 1 BCP modified a DP–MP association. We observed that greater intake of the "bread, margarine, and processed red meat" DP was associated with higher values of the "amino acids" MP, greater intake of the "fruiting vegetables and vegetable oils" DP was associated with higher values of the "fatty acids" MP, and greater intake of the "tea and miscellaneous" DP was associated with lower values of the "amino acids" and "amino acid derivatives" MPs. The "*Butyrivibrio*, Unidentified genus in family uncultured organism, order NB1-n, and *Victivallis* genera" BCP negatively modified the relation between the "bread, margarine, and processed red meat" DP and "amino acids" MP.

Our DPs clearly reflect jointly consumed foods, and because we applied the same procedure to derive the DPs and BCPs, it is therefore reasonable to conclude that our BCPs are naturally populating bacterial groups in the gut and thus biologically relevant. DPs are difficult to compare across studies due to heterogeneous study and sample characteristics, dietary assessment instruments and timing, and statistical methods. However, some of our DPs are broadly comparable to those previously reported within the EPIC-Potsdam cohort (29, 30) and other cohorts (5, 8, 31–34). BCPs are even more challenging to compare due to varying populations, the effect of fecal sampling, and methodologies used in gut microbiota analyses (20). Nonetheless, "*Prevotella* 6, *Ezakiella*, and *Porphyromonas*" and "Ruminococcaceae-dominated" are comparable to *Prevotella* and *Ruminococcus* enterotypes (35) and to the *Prevotella*-dominated community previously reported (36, 37). The fact that most of the bacteria in our BCPs are distantly related bacteria reveals that the interrelationship among bacteria was not restricted to only closely related bacteria (38, 39). In fact, our BCPs comprise mixtures of dominant and scarce bacteria families that have been reported in other populations (20). In addition, the "Enterobacteriaceae-*Klebsiella* and *Enterobacter*" and "*Prevotella* 6, *Ezakiella*, and *Porphyromonas*" BCPs confirm that pathogenic genera do co-occur (38). Compared with other phyla, bacteria belonging

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TABLE 2 Characteristics, loading patterns, and stability of the 5 extracted DPs generated by treelet transform analysis of 39 food groups in 225 older German adults[1]

DP	Explained variance (%)	Original variables, food groups loaded (and loadings)	Description	Stability (%)
DP1	4.42	Spirits (0.54), beer (0.54), red meat (0.41), soft drinks (0.36), and other fats (0.34)	"Alcohol and red meat"	60
DP2	4.12	Bread (0.59), margarine (0.59), processed meat (0.43), sugar and confectionery (0.36)	"Bread, margarine, and processed meat"	81
DP3	3.13	Fruiting vegetables (0.71) and vegetable oils (0.71)	"Fruiting vegetables and vegetable oils"	83
DP4	3.06	Tea (0.71) and miscellaneous (0.71)	"Tea and miscellaneous"	73
DP5	2.95	Pasta and rice (0.71), and sauces (0.71)	"Pasta and rice, and sauces"	31
Cumulative explained variance, %	17.68			

[1] DP, dietary pattern.

to Firmicutes are more dispersed across our BCPs. This is in accordance with findings from another study (40).

Importantly, our study reveals that DPs are reflected in patterns of serum metabolites. The first finding is the positive relation between the "bread, margarine, and processed meat" and the "amino acids" MP. This relation seems to be primarily driven by the meat component because meat and its products provide essential amino acids with high bioavailability (41) and, to a lesser extent, by the protein in bread (42). Epidemiological studies have reported associations between intake of red meat and some amino acids (43), bread consumption and serum tryptophan and its associated amino acid metabolites (44), animal food-based diets (32), and a Western dietary pattern (45) and higher concentrations of plasma amino acids. Although the association between "fruiting vegetable and vegetable oil" and "fatty acids" might appear to be mainly driven by vegetable oils, fruiting vegetables that include tomatoes, sweet pepper, and avocados also contain some amount of fatty acids. Furthermore, correlations between dietary and serum fatty acids have been documented (46), and intake of mixed meals containing vegetable oils correlates with free fatty acids (47, 48). Overall, these findings suggest that "amino acids" and "fatty acids" MPs should be further explored as biomarkers for intake of the "bread, margarine, and processed meat" and "fruiting vegetable and vegetable oil" DPs, respectively.

In addition, because the "amino acids" and "amino acid derivatives" MPs were negatively correlated with the "tea and miscellaneous" DP, this suggests that these amino acid metabolites represent a negative effect biomarker of this DP. Similar to tea, the miscellaneous food group that includes spices, herbs and flavorings, condiments, and soy products is rich in polyphenols. The inhibitory effect of this polyphenol-rich DP on protein digestion and absorption is a possible explanation. The strong effect of polyphenols on protein digestion and absorption via its strong affinity for endogenous proteins and dietary proteins has been elegantly reviewed (49). However, little is documented in humans on either associations or effects of this DP or its components on serum amino acids. Therefore, further studies are needed to replicate and confirm this finding.

Remarkably, the "*Butyrivibrio*, Unidentified genus in family uncultured organism, order NB1-n, and *Victivallis* genera" BCP weakens the relation between the "bread, margarine, and processed meat" DP and the "amino acids" MP. In addition to the well-known butyrate and lactate-producing properties of the genus *Butyrivibrio* (50), *Butyrivibrio fibrisolvens* possesses active proteolytic enzymes (51), and *Butyrivibrio crossotus* shows amino acid inward transport action (52). This indicates that *Butyrivibrio* utilizes amino acid substrates to generate butyrate and lactate. However, rather little is known about the metabolizing activities of *Victivallis* and order NB1-n, except for their sugar fermenting properties (53, 54). Because *Victivallis* is also capable of metabolizing lactose (55), this suggests that the lactose produced by *Butyrivibrio* serves as a nutrient source for *Victivallis*, implying that cross-feeding may exist between these bacteria. Cross-feeding among gut bacteria is well reported (21). Furthermore, the fact that bread (and its ingredients) contains naturally occurring and added sugars also suggests assimilation of nutrients by *Victivallis* and order NB1-n from this DP.

Most nutrients, including peptides and amino acids, are absorbed in the small intestine. Nevertheless, substantial amounts enter the colon, where trivial quantities are absorbed, some are used by the microbiota, and the remaining are excreted (16, 56). The abundance of "*Butyrivibrio*, Unidentified genus in family uncultured organism, order NB1-n, and *Victivallis* genera" suggests that dietary amino acids that reach the colon are utilized by this BCP, thereby further reducing their meager absorption from the colon.

The first strength of the current study is the use of TT to generate DPs and BCPs. The classical PCA is arguably the most widely used data-driven method for defining patterns from data sets with a high degree of multicollinearity and/or high dimension. PCA is known to produce a wide range of possible components to retain based on several "rules of thumb." This and other steps in the PCA are prone to subjectivity. The lack of sparsity of loading of original variables on components that often makes interpretability of components challenging also warrants mentioning (57, 58). TT is one of the methods that address these aforementioned drawbacks. Furthermore, to the best of our knowledge, this is the first study to apply TT for identifying patterns within a gut bacteria compositional data set. Of note, we could have also addressed a priori defined DPs from established dietary guidelines for identifying their related metabolites because posteriori defined or exploratory DPs are less meaningful with respect to health benefits. However, our study was prioritized to explore foods actually consumed together in a diet within our study population in order to identify biomarkers for these diets.

Although rarely reported in other studies, we have shown how much trust to place in our patterns by reporting the stability of the DPs and BCPs. Moreover, our DPs showed both internal and external validity. Internal validity is demonstrated

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TABLE 3 Characteristics, loading patterns, and stability of the 7 gut BCPs generated by treelet transform of 317 genus-level OTUs in 186 older German adults[1]

BCP	Explained variance (%)	Original variables, genus-level OTUs loaded (and loadings)	Description	Stability (%)
BCP1	14.55 (66)	*Coprococcus 3* (0.15); *Atopostipes* (0.15); *Cellulophaga* (0.15); *Sarcina* (0.15); *Asaccharobacter* (0.15); Unnamed genus in unnamed family, order uncultured bacterium (0.15); Uncultured genus in family Comamonadaceae (0.15); *Anaerosinus* (0.15); *Snodgrassella* (0.15); *Thauera* (0.15); Uncultured bacterium in family GR-WP33-58 (0.15); Uncultured genus in family Alcaligenaceae (0.15); Unnamed genus in uncultured bacterium, order B38 (0.15); *Rickettsiella* (0.15); *Marinomonas* (0.15); *Achromobacter* (0.15); *Prosthecobacter* (0.15); *Sedimentibacter* (0.15); *Acidovorax* (0.14); dgA-11 gut group (0.14); *Cellulosilyticum* (0.14); *Variovorax* (0.14); *Eikenella* (0.14); *Succinatimonas* (0.14); Unnamed genus in family uncultured bacterium adhufec202 (0.14); *Chryseobacterium* (0.14); *Carnobacterium* (0.14); *Candidatus arthromitus* (0.14); *Mobiluncus* (0.13); *Brumimicrobium* (0.13); *Negativicoccus* (0.13); *Selenomonas 4* (0.13); *Vibrio* (0.13); *Morganella* (0.13); *Cobetia* (0.13); Uncultured bacterium in family gir-aah93h0 (0.12); Uncultured organism in family Lachnospiraceae (0.12); *Bulleidia* (0.12); (*Anaerorhabdus*) *furcosa* group (0.12); *Synergistes* (0.12); *Erysipelotrichaceae*(0.11); *Natranaerovirga* (0.11); *Sphingomonas* (0.11); *Butyricicoccus* (0.10); *Fastidiosipila* (0.10); *Syntrophomonas* (0.10); Uncultured bacterium in family Anaeroglobus (0.10); *Gardnerella* (0.10); *Sneathia* (0.10); *Geobacillus* (0.10); Uncultured genus in family Actinomycetaceae (0.09); Uncultured genus in family Corynebacteriaceae (0.09); *Cryptobacterium* (0.09); *Bacteroides* (0.09); Unnamed genus in family uncultured rumen bacterium (0.09); *Lysinibacillus* (0.09); Uncultured genus in family Clostridiaceae *1*(0.09); *Anaerococcus* (0.09); *Finegoldia* (0.09); *Peptoniphilus* (0.09); *Aeromonas* (0.09); *Selenomonas 3* (0.09); *Cardiobacterium* (0.09); *Burkholderia* (0.08); *Comamonas* (0.08); *Acinetobacter* (0.08)	Veillonellaceae, Comamonadaceae, and Family XI-dominated	2
BCP2	1.37 (9)	*Erysipelatoclostridium* (0.35); *Holdemania* (0.35); *Eggerthella* (0.34); *Incertae sedis* in family Erysipelotrichaceae (0.33); *Gordonibacter* (0.33); *Anaerostipes* (0.32); *Blautia* (0.33); *Lachnoclostridium* (0.31); (*Eubacterium*) *hallii* group (0.33)	Erysipelotrichaceae, Coriobacteriaceae, and Lachnospiraceae	67
BCP3	1.33 (7)	Christensenellaceae R-7 group (0.40); Ruminococcaceae UCG-005 (0.40); Ruminococcaceae UCG-010 (0.39); Uncultured bacterium in family vadinBB60 group (0.39); Ruminococcaceae UCG-002 (0.38); Ruminococcaceae UCG-014 (0.35); Unidentified genus in family uncultured bacterium, order Mollicutes RF9 (0.34)	Ruminococcacea-dominated	49
BCP4	0.76 (3)	*Anaerovibrio* (0.61); Uncultured genus in family Rhodospirillaceae (0.50); *Brachyspira* (0.61)	*Anaerovibrio*, Uncultured genus in family Rhodospirillaceae, and *Brachyspira* genera	80
BCP5	0.70 (3)	*Prevotella 6* (0.58); *Ezakiella* (0.58); *Porphyromonas* (0.57)	*Prevotella 6*, *Ezakiella*, and *Porphyromonas* genera	92
BCP6	0.64 (3)	*Butyrivibrio* (0.59); Unidentified genus in family uncultured organism, order NB1-n (0.59); *Victivallis* (0.56)	*Butyrivibrio*, Unidentified genus in family uncultured organism, order NB1-n, and *Victivallis* genera	22
BCP7	0.56 (2)	*Enterobacter* (0.71); *Klebsiella* (0.71)	Enterobacteriaceae	35
Cumulative explained variance, %	19.91			

[1] BCP, bacteria compositional pattern; OTU, operational taxonomic unit.

in terms of similar cumulative variance and interpretation of DPs and correlation of DPs in a subset of the study participants. External validity is demonstrated in terms of similar variance to those reported by others (29, 33, 59). Furthermore, the fact that the DPs and MPs that are significantly related to each other are very stable suggests that our findings are unlikely to be spurious.

Another strength of this study is that the use of non-fasting serum samples reflects the ideal (postprandial) serum metabolome that comprises both the endogenous metabolome and the food metabolome. In addition, the untargeted profiling of the serum indicates that the complexity of human metabolism is captured. Furthermore, our approach of investigating patterns of food groups, serum metabolites, and gut microbiota prevents multiple hypothesis testing of single food groups, individual bacteria, and serum metabolites. Most studies that have investigated the association of DPs with single metabolites correct for multiple hypothesis testing. Nonetheless, important DP–metabolite associations might have been missed because multiple hypothesis testing correction may increase the number of false negatives (60). Finally, ethicolegal challenges of data sharing are well known, and we have shown in this study that it is possible to perform remote statistical analyses of individual-level data through DataSHIELD and obtain valid inferences.

There are limitations in our study. Our study participants are a convenience sample that is based on availability of untargeted metabolomics data, so the disadvantages of such sampling also

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TABLE 4 Standardized regression coefficients (β) and 95% CIs of the association between dietary patterns and serum metabolite patterns in 225 older German adults[1]

	Amino acids	Fatty acids	Sugar compounds and CBAs	Amino acid derivatives	Sugar alcohols	Saturated and monounsaturated TGs	Polyunsaturated TGs	PEs and PCs
Model 1[2]								
Alcohol and red meat	0.18 (–0.14, 0.49)	–0.21 (–0.49, 0.07)	0.07 (–0.20, 0.33)	–0.02 (–0.26, 0.22)	–0.04 (–0.24, 0.17)	0.43 (–0.29, 1.15)	0.76 (0.23, 1.29)	–0.90 (–1.27, –0.54)
Bread, margarine, and processed meat	0.35 (0.11, 0.58)	–0.30 (–0.51, –0.10)	–0.12 (–0.32, –0.10)	0.06 (–0.12, 0.24)	0.05 (–0.10, 0.21)	–0.27 (–0.81, 0.28)	0.14 (–0.27, 0.55)	–0.69 (–0.97, –0.41)
Fruiting vegetables and vegetable oils	–0.09 (–0.41, 0.22)	0.48 (0.21, 0.75)	–0.16 (–0.10, 0.42)	0.02 (–0.22, 0.25)	0.01 (–0.19, 0.22)	–0.20 (–0.92, 0.52)	–0.29 (–0.83, 0.24)	0.34 (–0.04, 0.72)
Tea and miscellaneous	–0.30 (–0.53, –0.07)	0.16 (–0.05, 0.36)	0.09 (–0.11, 0.28)	–0.19 (–0.37, –0.01)	–0.08 (–0.23, 0.08)	0.19 (–0.35, 0.72)	0.25 (–0.15, 0.65)	0.07 (–0.22, 0.35)
Pasta and rice, and sauces	–0.06 (–0.37, 0.25)	–0.20 (–0.47, 0.07)	–0.14 (–0.41, 0.12)	0.08 (–0.16, 0.32)	–0.02 (–0.23, 0.18)	–0.27 (–0.99, 0.44)	0.07 (–0.47, 0.60)	–0.17 (–0.55, 0.21)
Model 2[3]								
Alcohol and red meat	0.05 (–0.27, 0.37)	–0.05 (–0.33, 0.22)	0.14 (–0.13, 0.41)	–0.06 (–0.30, 0.19)	–0.06 (–0.28, 0.16)	0.54 (–0.21, 1.28)	0.76 (0.20, 1.31)	–0.71 (–1.08, –0.34)
Bread, margarine, and processed meat	0.33 (0.08, 0.57)	–0.28 (–0.49, –0.08)	–0.14 (–0.35, 0.064	0.07 (–0.12, 0.26)	0.06 (–0.10, 0.22)	–0.39 (–0.95, 0.18)	0.02 (–0.40, 0.43)	–0.59 (–0.87, –0.31)
Fruiting vegetables and vegetable oils	–0.06 (–0.37, 0.25)	0.43 (0.17, 0.70)	0.16 (–0.11, 0.42)	0.02 (–0.23, 0.26)	0.01 (–0.21, 0.22)	–0.18 (–0.90, 0.55)	–0.18 (–0.72, 0.36)	0.17 (–0.19, 0.53)
Tea and miscellaneous	–0.29 (–0.52, –0.06)	0.18 (–0.02, 0.38)	0.11 (–0.09, 0.31)	–0.20 (–0.38, –0.03)	–0.08 (–0.23, 0.08)	0.24 (–0.30, 0.78)	0.28 (–0.12, 0.68)	0.04 (–0.23, 0.31)
Pasta and rice, and sauces	0.04 (–0.27, 0.35)	–0.26 (–0.53, 0.01)	–0.18 (–0.45, 0.09)	0.13 (–0.11, 0.37)	0.002 (–0.21, 0.21)	–0.39 (–1.11, 0.34)	0.01 (–0.53, 0.54)	–0.26 (–0.62, 0.10)
Model 3[4]								
Alcohol and red meat	–0.06 (–0.45, 0.34)	0.04 (–0.31, 0.38)	0.18 (–0.17, 0.52)	0.01 (–0.29, 0.32)	–0.09 (–0.35, 0.18)	0.18 (–0.69, 1.05)	0.59 (–0.03, 1.22)	–0.32 (–0.76, 0.13)
Bread, margarine, and processed meat	0.35 (0.02, 0.69)	–0.21 (–0.51, 0.09)	–0.11 (–0.41, 0.19)	0.20 (–0.06, 0.47)	0.15 (–0.08, 0.38)	–0.65 (–1.39, 0.10)	0.13 (–0.41, 0.67)	–0.35 (–0.73, 0.03)
Fruiting vegetables and vegetable oils	0.05 (–0.28, 0.38)	0.45 (0.16, 0.74)	0.12 (–0.17, 0.42)	0.12 (–0.14, 0.37)	0.01 (–0.22, 0.24)	0.16 (–0.57, 0.89)	–0.05 (–0.58, 0.48)	0.18 (–0.20, 0.55)
Tea and miscellaneous	–0.28 (–0.52, –0.05)	0.22 (–0.02, 0.43)	0.15 (–0.06, 0.37)	–0.21 (–0.39, –0.02)	–0.04 (–0.21, 0.12)	0.17 (–0.36, 0.70)	0.40 (–0.02, 0.78)	0.05 (–0.22, 0.32)
Pasta and rice, and sauces	0.15 (–0.20, 0.51)	–0.21 (–0.52, 0.10)	–0.23 (–0.55, 0.08)	0.19 (–0.09, 0.46)	0.05 (–0.20, 0.29)	–0.27 (–1.05, 0.52)	0.28 (–0.28, 0.84)	–0.19 (–0.59, 0.21)

[1] CBA, carboxylic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; TG, triglyceride.
[2] Unadjusted model.
[3] Adjustment for other dietary patterns.
[4] Further adjustment for age (continuous), sex, BMI (continuous), smoking status (3 groups: never, former, or current), educational attainment (3 groups: no vocational training or vocational training, technical school, and university), occupation [6 categories: full-time (≥35 h/wk), part-time (15 to <35 h/wk), hourly (<15 h/wk), jobless/retraining, (early) retirement/invalidity pension, and unemployed], physical activity-induced energy expenditure (continuous), total energy intake (continuous), and prevalent diseases (hypertension, myocardial infarction, stroke, type 2 diabetes mellitus, cancer, and gastrointestinal diseases: two categories—yes/no).

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apply to this study. Overall, our DPs and BCPs did not account for a "large" cumulative explained variance in their original data sets. Nevertheless, the trade-off between maximizing variance and offering interpretable patterns is a worthwhile advantage of TT. Furthermore, being purely data-driven, our DPs or BCPs may never be exactly the same in other studies and populations. In investigating the associations between the DPs and MPs, we adjusted for several potential confounders, such as age, sex, BMI, smoking status, and alcohol intake; however, we cannot exclude residual confounding due to unmeasured or unaccounted factors. Possible mechanisms through which age, sex, BMI, smoking status, and alcohol intake directly influence metabolites include altered functions associated with aging (61), endogenous sex hormones regulating metabolism (62), adipose tissue exerting an impact on metabolism (63), and compounds in cigarette smoke (64) and alcohol intake (65) inducing metabolizing enzymes. The "*Butyrivibrio*, Unidentified genus in family uncultured organism, order NB1-n, and *Victivallis* genera" BCP having a low percentage of explained variance and a low stability suggests caution in interpreting their modifying effect. In addition, it should be considered that due to their untargeted nature, our metabolome and gut microbiota sequencing analyses might suffer from biases. Based on our tools, we could only classify our 16S rRNA gene sequences up to the genus level; species-level classification and direct measure of the functional gene pool of the gut bacterial community should be considered in future studies. In addition, this study is limited in determining the precise contribution of this BCP to the serum metabolome. Further studies should consider these analyses with a focus on bacteria-specific serum metabolites. Due to the limited DataSHIELD-specific R functions, we were only able to explore nonlinear associations using linear regression. Therefore, we may have missed other nonlinear associations. Exclusion of individuals with chronic diseases would have been optimal. However, this study comprises older adults with a high prevalence of chronic diseases. Thus, excluding individuals with chronic diseases from this relatively small sample would greatly underpower our analysis. Therefore, rather than an outright exclusion of this confounder, we controlled for it.

The identified MPs should be further explored as biomarkers of the DPs, serving as their objective measures or as their adjuncts. Elevated serum amino acids and fatty acids are related to health outcomes such as cardiovascular diseases (66, 67). Therefore, moderate intake of "bread, margarine, and processed red meat" and "fruiting vegetables and vegetable oils," increasing the intake of "tea and miscellaneous," and increasing the gut abundance of "*Butyrivibrio*, Unidentified genus in family uncultured organism, order NB1-n, and *Victivallis* genera" might yield cardiovascular health gains. Our additional statistical analysis suggests that there are no significant predictors of the "*Butyrivibrio*, Unidentified genus in family uncultured organism, order NB1-n, and *Victivallis* genera" BCP; however, other factors that were not explored in this study might predict this BCP. Factors that potentially alter this BCP should be explored in intervention studies.

An important next step will be to examine whether our DPs and BCPs are reproducible within this study sample over time. Furthermore, it will be necessary to investigate the impact of these findings by linking them to pathophysiology and health outcomes.

In conclusion, we have demonstrated that 3 DPs are reflected in 3 MPs, and "*Butyrivibrio*, Unidentified genus in family uncultured organism, order NB1-n, and *Victivallis* genera" modifies the relation between "bread, margarine, and processed meat" and serum "amino acids." This study highlights the interactions between dietary intake, the gut microbiota, and host metabolism. These MPs should be explored as biomarkers of these jointly consumed foods, here captured as DPs, while taking into account a potentially modifying role of the gut bacteria.

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