Associations between untargeted plasma metabolomic signatures and gut microbiota composition in the *Milieu Intérieur* population of healthy adults

Valentin Partula[1,2]†, Mélanie Deschasaux-Tanguy[1]*†, Stanislas Mondot[3], Agnès Victor-Bala[4], Nadia Bouchemal[4], Lucie Lécuyer[1], Christine Bobin-Dubigeon[5,6], Marion J. Torres[7], Emmanuelle Kesse-Guyot[1], Bruno Charbit[8], Etienne Patin[9], Karen E. Assmann[1], Paule Latino-Martel[1], Chantal Julia[1,10], Pilar Galan[1], Serge Hercberg[1,10], Lluis Quintana-Murci[9], Matthew L. Albert[11], Darragh Duffy[12], Olivier Lantz[13,14], Philippe Savarin[4], Mohamed Nawfal Triba[4], Mathilde Touvier[1] and The Milieu Intérieur Consortium[15]

[1]*Sorbonne Paris Nord University, INSERM, INRAE, CNAM, Nutritional Epidemiology Research Team (EREN), Epidemiology and Statistics Research Center – University of Paris (CRESS), 93000 Bobigny, France*
[2]*University of Paris-VII Denis Diderot, Université de Paris, 75000 Paris, France*
[3]*PhylHom Team, MICALIS Institute (Inrae/AgroParisTech), 78350 Jouy-en-Josas, France*
[4]*Chemistry, Structure, and Properties of Biomaterials and Therapeutic Agents CSPBAT, Nanomedicine, Biomarkers and Detection Team (CNRS U7244/Université Sorbonne Paris Nord), 93000 Bobigny, France*
[5]*Department of Biopathology, Institut de Cancérologie de l'Ouest, 44800 Saint-Herblain, France*
[6]*Sea, Molecules, Health MMS EA2160 (CNRS FR3473/Université de Nantes), 44000 Nantes, France*
[7]*Nutritional Surveillance and Epidemiology Team (ESEN), French Public Health Agency, Sorbonne Paris Nord University, Epidemiology and Statistics Research Center – University of Paris (CRESS), 93000 Bobigny, France*
[8]*Centre for Translational Research, Institut Pasteur, 75000 Paris, France*
[9]*Human Evolutionary Genetics Laboratory (CNRS URA3012/Institut Pasteur), 75000 Paris, France*
[10]*Department of Public Health, AP-HP Paris Seine-Saint-Denis University Hospital System, 93000 Bobigny, France*
[11]*Department of Immunology and Infectious Diseases, Insitro, San Fransisco, CA 94080, USA*
[12]*Immunobiology of Dendritic Cells Laboratory (Inserm U1223/Institut Pasteur), 75000 Paris, France*
[13]*Curie Institute, PSL University, Inserm U932, 75000 Paris, France*
[14]*Clinical Investigation Center CIC-BT1428 (Institut Gustave Roussy/Institut Curie), Inserm, 75000 Paris, France*
[15]*The Milieu Intérieur Consortium, Pasteur Institute, 75000 Paris, France*

Abstract

Host–microbial co-metabolism products are being increasingly recognised to play important roles in physiological processes. However, studies undertaking a comprehensive approach to consider host–microbial metabolic relationships remain scarce. Metabolomic analysis yielding detailed information regarding metabolites found in a given biological compartment holds promise for such an approach. This work aimed to explore the associations between host plasma metabolomic signatures and gut microbiota composition in healthy adults of the *Milieu Intérieur* study. For 846 subjects, gut microbiota composition was profiled through sequencing of the 16S rRNA gene in stools. Metabolomic signatures were generated through proton NMR analysis of plasma. The associations between metabolomic variables and α- and β-diversity indexes and relative taxa abundances were tested using multi-adjusted partial Spearman correlations, permutational ANOVA and multivariate associations with linear models, respectively. A multiple testing correction was applied (Benjamini–Hochberg, 10 % false discovery rate). Microbial richness was negatively associated with lipid-related signals and positively associated with amino acids, choline, creatinine, glucose and citrate ($-0.133 \leq$ Spearman's $\rho \leq 0.126$). Specific associations between metabolomic signals and abundances of taxa were detected (twenty-five at the genus level and nineteen at the species level): notably, numerous associations were observed for

creatinine (positively associated with eleven species and negatively associated with *Faecalibacterium prausnitzii*). This large-scale population-based study highlights metabolites associated with gut microbial features and provides new insights into the understanding of complex host–gut microbiota metabolic relationships. In particular, our results support the implication of a 'gut–kidney axis'. More studies providing a detailed exploration of these complex interactions and their implications for host health are needed.

Key words: Gut microbiota: Host–gut microbiota relationships: NMR metabolomics: Cross-sectional population-based studies: Epidemiology

The gut metagenome has been estimated to be 150–400 times larger than the human genome[1,2], yielding a colossal metabolic potential[3]. Products of the microbial metabolism comprise a vast range of molecules involved in a variety of biological functions[4,5] and integrated in proposed 'host–microbial metabolic axes'. These molecules complement the host metabolism and subsequently influence the host health through potential beneficial or harmful effects[5–9]. Consequently, the gut microbiota is now fully considered as an endocrine pseudo-organ within the mammalian holobiont superorganism[6,10].

Specific studies investigating targeted molecules – such as SCFA, bile acids, choline metabolites, etc. – have considerably increased the mechanistic knowledge of microbial–human co-metabolism[4,11–13]. However, studies addressing more comprehensively the highly complex microbial–host metabolic interactions remain scarce[9]. Metabolomics – the systematic identification and quantification of the low-molecular-weight metabolic products of a biological system at a specific point in time – enables the simultaneous detection and relative quantification of hundreds of molecules and thus holds promise to elucidate microbial–host interactions[14]. Studies integrating a metabolomic approach have started to expand. Notably, gut microbiota has been reported to associate with the serum metabolome. Pedersen *et al.*[15] indeed showed that the serum metabolome of insulin-resistant individuals presents increased levels of branched-chain amino acids, which were further correlated with gut microbial genetic characteristics (namely the potential for biosynthesis of branched-chain amino acids and lack of genes encoding bacterial inward transporters branched-chain amino acids). Additionally, comparisons of the host metabolomes (in plasma or urine) between diseased individuals[16–18] or subjects included in nutritional interventions[19–22], and controls have detected molecules stemming exclusively from microbial activity (e.g. hippurate or *p*-cresol) as discriminant metabolites. However, in these studies, neither the gut microbiota composition nor its activity was analysed and direct associations between the gut microbiota and the host metabolome were not investigated. In fact, research investigating such associations is scarce and often has been conducted on limited sample sizes ($n \leq 100$)[9,23–27] or focused on subjects with specific characteristics or conditions like chronic heart failure[23], spleen-yang-deficiency syndrome[26], obesity[24,27], physiological and metabolic stress[25], etc. More recently, studies were performed on larger population-based samples, including several hundreds of participants and reporting associations between the composition and diversity of the gut microbiota and circulating metabolites, using metabolomics[28–32].

Following these approaches, our objective was to investigate the associations between untargeted plasma metabolomic signatures and gut microbiota composition in a large sample of healthy French subjects.

Methods

Study population

This study was conducted in the framework of the *Milieu Intérieur* project, which enrolled 1000 healthy French adults with primary aim to assess the determinants of immunological variance within the general healthy population. The objectives of the present study are secondary objectives to the *Milieu Intérieur* project, for which the rationale, design and methods have been extensively described elsewhere[33]. Briefly, 500 females and 500 males equally distributed across five classes of age (from 20 to 69 years old), and of mainland French descent for at least three generations, were recruited in the suburban area of Rennes (Ille-et-Vilaine, Bretagne, France), between September 2012 and August 2013. Participants were considered 'healthy' upon recruitment, as defined per stringent exclusion criteria comprising any chronic disease or condition involving the immune system, abnormal physical examination or any abnormal clinical–biological analysis[33]. Questionnaires and biological samples were administered and collected by trained medical investigators supported by a full clinical team. The study is sponsored by Institut Pasteur (Pasteur ID-RCB number: 2012-A00238-35). It was conducted as a single centre study and without any investigational product and was approved by Comité de Protection des Personnes – Ouest 6 on 13 June 2012 (CPP Ouest 6-728/MS2) and by Agence Nationale de Sécurité du Médicament on 22 June 2012 (ID-RCB number: 2012-A00238-35, reference ANSM: B120239-70). The protocol, which is registered under ClinicalTrials.gov (NCT01699893), was designed and conducted in accordance with the Declaration of Helsinki and good clinical practice as outlined in the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use Guidelines for Good Clinical Practice.

Data collection

Covariates. During the medical visit, investigators measured BMI using standardised methods and collected information related to age, sex, smoking status, educational level, employment status, occupational category, income level and physical activity.

Gut microbiota profiling from stool samples. Gut microbiota composition was determined through the sequencing of the 16S ribosomal RNA gene in stool samples produced at home maximum 24 h before the scheduled medical visit, collected in a double-lined sealable bag maintaining strict anaerobic conditions and aliquoted and stored at −80°C upon reception at the clinical site. Detailed information regarding DNA preparation, barcoding PCR protocol, sequencing and computation of microbial diversity indexes are provided in online Supplementary Material and Methods 1.

Untargeted NMR metabolomics analysis in plasma samples. Fasting whole blood was collected in heparin tubes and centrifuged (300 **g**, 10 min, room temperature) maximum 6 h after blood draw, and supernatants were stored at −80°C maximum 10 min after centrifugation. Plasma samples underwent two freeze-thaw cycles before proton NMR analysis, the additional freeze-thaw cycle being done for all samples in order to prepare internal quality control samples necessary for the study[34,35] and so that all samples would go through the same process. The NMR analysis was performed blindly on samples (randomised for age and sex) according to a previously published protocol[36]. Briefly, two complementary one-dimensional NMR sequences were acquired per plasma sample, on a 500 MHz-Bruker Avance III spectrometer (Bruker) at 300 K – namely the ^{1}H one-dimensional NMR pulse sequence nuclear Overhauser effect spectroscopy (NOESY1D) with z-gradient (Bruker pulse program noesygppr1d) and the Carr–Purcell–Meiboom–Gill (CPMG) sequences. Consequently, to acquisition, all spectra were pre-processed, calibrated and ultimately 'sliced' into 'buckets' with pre-defined limits so as to maximise recovery of peak entities. The buckets were then scaled to the total summed integrals for each spectrum, and the integrals of peak entities were calculated to obtain continuous NMR variables that were used in further statistical analyses. Finally, NMR signals were assigned using *ad hoc* literature[37]. CHENOMX software (CHENOMX) and the Human Metabolome Database[38] were used to confirm the assignments using the medium spectrum (online Supplementary Fig. S3)[37]. After the statistical analyses, the assignments of the buckets that was associated with the gut microbiota were newly carefully looked and confirmed to be unambiguously assigned by gathering information from S-total correlated spectroscopy, additional two-dimensional NMR experiments (recorded on random samples) such as ^{1}H–^{1}H total correlated spectroscopy and J-resolved experiments. The detailed NMR metabolomic analysis protocol (sample preparation, post-acquisition spectra pre-processing and intelligent bucketing of spectra and obtaining of NMR variables) is provided in online Supplementary Material and Methods 2.

Statistical analyses

Associations between each NMR variable and each α-diversity index were tested using non-parametric partial Spearman correlations. Associations between each NMR variable and inter-individual dissimilarities in gut microbiota composition (Bray–Curtis index of β-diversity) were tested using permutational ANOVA (PERMANOVA) with 999 permutations. Associations between

each NMR variable and the relative abundances of genera and species were tested using multivariate associations with linear models (MaAsLin). This statistical framework performs boosted, additive general linear models and is suited to test the associations between the relative abundance of microbial community members and metadata (here the NMR variables and covariates). Relative abundances are first transformed through a variance-stabilising arcsin-square root transformation. Genera and species were included only if their relative abundance and prevalence were higher than 0·01 and 1 %, respectively.

Partial Spearman correlations, PERMANOVA and MaAsLin were all multi-adjusted for age (20 to <30, 30 to <40, 40 to <50, 50 to <60, and 60 to <70 years old), sex, BMI (continuous variable, in kg/m^2), smoking status (non-smoker, ex-smoker, current smoker) and physical activity (continuous variable, in h/week). These covariates were indeed reported in the literature to have a potential impact on gut microbiota composition or metabolomic signature, or significantly associated with microbial or metabolomic features in our analyses. These associations between covariates and gut microbial and metabolomic features were tested using PERMANOVA and principal component partial R^2 analysis, respectively, enabling to measure the percentage of variation in microbial composition or metabolomic data explained by the factors tested. Additionally, α- and β-diversity models (partial Spearman correlations and PERMANOVA) were adjusted for sequencing depth. P-values were adjusted for multiple testing using a Benjamini–Hochberg correction with a 10 % false discovery rate.

To better visualise the inter-individual variation in metabolomics signatures in our study sample, principal component analysis of the metabolomic data sets was plotted and colour-coded based on age, sex and BMI.

Spearman correlations were performed using SAS 9.4; PERMANOVA, MaAsLin, principal component partial R^2 and principal component analysis representations of metabolomic data sets were performed using R 3.3.2 (packages *vegan*, *Maaslin*, *FactoMineR* and *FactoExtra*).

Results

Characteristics of the study sample

Among 1000 participants to the *Milieu Intérieur* study, 138 were excluded from the study sample because of missing gut microbiota composition data – due to failure during PCR, insufficient number of detected reads or insufficient quantity of stool aliquot or DNA extracted. Among the 862 participants with available gut microbiota data, sixteen were further excluded because their metabolomic signatures could not be determined – due to insufficient quantity of plasma aliquot or failure during NMR spectra acquisition. Participant flow chart is presented in online Supplementary Fig. 1. Characteristics of the 846 subjects are presented in Table 1. Mean BMI was 24·3 (sd 3·3) kg/m^2, and mean physical activity (including both professional and leisure physical activity) was 5·4 (sd 6·3) h/week. The proportion of smokers was 19·9 %.

Characteristics of the gut microbiota in the *Milieu Intérieur* study have been previously described[39,40]. Microbiota analysis

Table 1. Characteristics of the study population, *Milieu Intérieur* study, France, 2012 (*n* 846)
(Numbers and percentages; mean values and standard deviations)

	n	%
Sex		
Male	433	51·2
Female	413	48·8
Age (years)		
20–29	159	18·8
30–39	165	19·5
40–49	174	20·6
50–59	171	20·2
60–69	177	20·9
Smoking status		
Non-smoker	442	52·2
Ex-smoker	236	27·9
Current smoker	168	19·9
BMI (kg/m^2)		
Mean	24·3	
SD	3·3	
Physical activity (h/week)		
Mean	5·4	
SD	6·3	
Simpson's index		
Mean	0·92	
SD	0·05	
Observed richness		
Mean	193·4	
SD	55·1	
Chao1 richness estimate		
Mean	298·3	
SD	117·0	

yielded sequencing depth ranging from 5064 to 240 472 reads per sample (mean sequencing depth 21 427 (SD 19 180) reads). Detected reads clustered into 8422 operational taxonomic units were classified into eleven phyla, 328 genera and 698 species. Firmicutes (mean relative abundance 0·68 (SD 0·13)), Bacteroidetes (0·29 (SD 0·13)), Proteobacteria (0·02 (SD 0·03)), Actinobacteria (0·007 (SD 0·01)) and Verrucomicrobia (0·004 (SD 0·008)) were the five most abundant phyla. Online Supplementary Table S1 presents the twenty-two genera constituting the 'core microbiota' – defined as the genera shared by at least 95 % of samples[41] – in our study population. A complete description of genera and species composing the gut microbiota of our sample is available in online Supplementary Table S2. Regarding α-diversity indexes, Simpson's index ranged from 0·41 to 0·98 (mean 0·92 (SD 0·05)); observed richness from 56 to 346 (mean 193·4 (SD 55·1)) and Chao1 richness estimate from 62 to 1039 (mean 298·3 (SD 117·0)). Univariate associations measuring the percentage of variation in inter-individual dissimilarities in the gut microbial composition (Bray–Curtis index of β-diversity) that is explained by different covariates (age, sex, BMI, smoking status, physical activity and sequencing depth) are shown in online Supplementary Table S3.

NMR analysis on plasma samples generated 260 CPMG and 269 NOESY1D variables. Spectral regions most likely corresponding to noise (i.e. CPMG regions 0–0·5, 4·3–5·0 and 8·55–10·0 ppm; and NOESY1D regions 4·3–5·0 and 9·5–11·0 ppm) were excluded from further analysis. A list of the resulting 424 NMR continuous variables used in our analyses (202 CPMG signals and 215 NOESY1D: spectral regions with their metabolite

assignments, as well as mean and SD) is presented in online Supplementary Tables S4 and S5. Results of the principal component analysis performed on the CPMG and NOESY1D metabolomic data sets are shown in Fig. 1 and online Supplementary Fig. S2, respectively. Although no clear clustering appears, grouping patterns along gradient of sex, age and BMI can be observed. Results of the principal component partial R^2 analysis measuring the percentage of variation in the metabolomic data set that is explained by the covariates (age, sex, BMI, smoking status and physical activity) are shown in online Supplementary Table S6.

Associations between plasma metabolomic signals and α- and β-diversity indexes

The associations between metabolomic variables and α-diversity indexes are presented in Fig. 2. Overall, and after correcting for multiple testing with a 10 % false discovery rate, observed richness was associated with twenty-four CMPG and eight NOESY1D signals. Negative associations were observed with lipid-related signals (namely lipoproteins or unsaturated lipids) as well as with ester- and ketone-related metabolites (partial Spearman's ρ range −0·133 to −0·097), while positive associations were detected with signals pertaining to amino acids (polar glutamine and histidine and aromatic tyrosine, as well as related metabolites) and proteins, creatinine, as well as with choline- and glycolysis-related metabolites (glucose and citrate) (partial Spearman's ρ range 0·087 to 0·126). Similar results were obtained with Chao1 richness estimate, but no association was detected with Simpson's α-diversity index. Likewise, no association was observed between NMR metabolomic variables and inter-individual dissimilarities in gut microbiota composition (Bray–Curtis β-diversity index).

Associations between plasma metabolomic signals and bacterial taxa (relative abundances)

The associations (significant at 10 % false discovery rate) between NMR metabolomic variable and the relative abundance of gut microbiota genera and species are presented in Table 2. At the genus and species levels, twenty-five and nineteen associations were detected, respectively. Associated taxa comprised genera and species with prevalence ranging from 20 to 100 % of the study population. Creatinine was the metabolite associated with most taxa (sixteen genera and eleven species). Notably, consistent positive associations were found between the CPMG and the NOESY1D analyses for genera *Catabacter*, *Saccharofermentans* and *Oscillibacter*, and a negative association was observed for *Faecalibacterium prausnitzii*.

Discussion

In the present work, we investigated the associations between characteristics of the gut microbiota – namely α-diversity, inter-individual dissimilarities and the relative abundances of genera and species – and plasma NMR metabolomic signatures of the host in a large population-based sample of 846 healthy French adults. Overall, our results suggest that gut bacterial features are associated with the systemic metabolism of the host. In
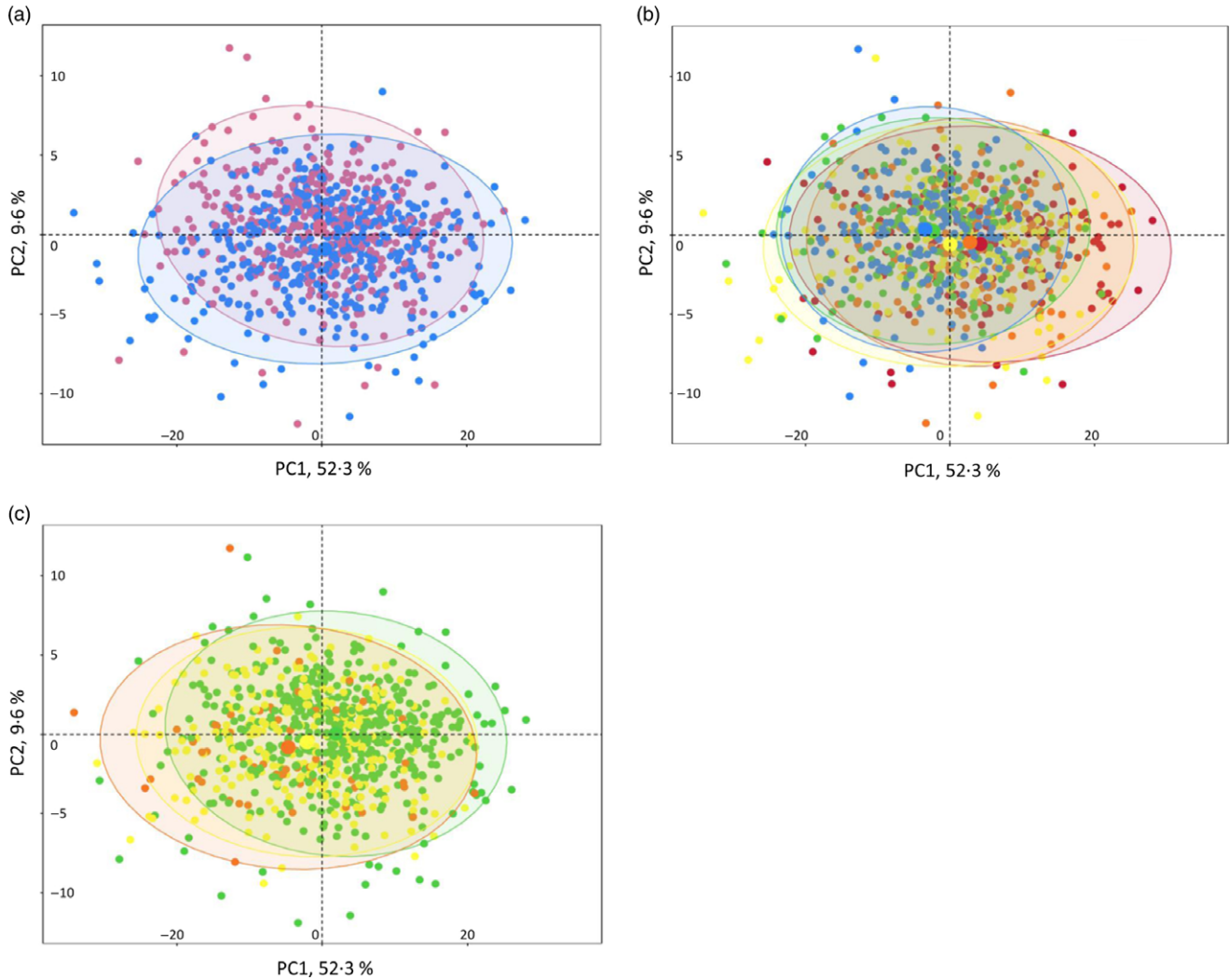
Fig. 1. Interindividual variation in metabolomic signatures represented by principal component (PC) analysis (PCA) of the Carr–Purcell–Meiboom–Gill (CPMG) metabolomic data set, *Milieu Intérieur* study, France, 2012 (*n* 846). Each point represents an individual from the study sample. PCA was obtained via the *PCA* function (package *FactoMineR*) and plotted and colour-coded based on sex (a), age (b) and BMI (c) via the *fviz_pca_ind* function (package *FactoExtra*). Concentration ellipses (95 %) are shown. Percentages on the axes represent the proportion of variation explained by the two first components of the PCA. (a) ●, Female; ●, male. (b) ●, 20 to <30 years; ●, 30 to <40 years; ●, 40 to <50 years; ●, 50 to <60 years; ●, 60 to <70 years. (c) ●, 18·5 to <25 kg/m^2; ●, 25 to <30 kg/m^2; ●, 30 to <35 kg/m^2.

particular, we found that bacterial richness was positively associated with signals pertaining to amino acids (glutamine, histidine, tyrosine), as well as with creatinine-, choline- and glycolysis-related metabolites, while negative associations were observed with lipid-, as well as ester- and ketone-related signals. Finally, specific associations between metabolomic signals and bacterial members of the gut microbiota were detected.

The metabolic relevance and importance of the gut microbiota for the host are now widely admitted, and research scrutinising host–gut microbial metabolic relationships is an active area of investigation[42]. However, studies investigating direct associations between gut microbiota composition and host blood metabolome in large population-based samples are scarce, while more available studies have been conducted on a limited number of participants or focused on specific populations[15,23,26,27]. In particular, a study by Org *et al.*[28] assessed the relationships between gut microbiota composition and

circulating metabolites in 531 healthy Finnish males from the METSIM cohort. Consistent with their study, we reported positive correlations between bacterial richness and glutamine, histidine and creatinine – with similar Spearman correlation coefficients. Furthermore, a study on 399 subscribers to a US Scientific Wellness programme was able to explain 45 % of the gut microbiota α-diversity with forty plasma metabolites, among which a high frequency of phenylalanine and tyrosine metabolites (including *p*-cresol sulphate, a potentially uraemic toxin) and hippurate[32], while a study in 1529 females from the TwinsUK cohort found five metabolites that consistently correlated with gut microbiota α-diversity (Shannon index) including hippurate, *p*-cresol sulphate, phenylacetylglutamine, 3-phenylpropionate and hyodeoxycholate[31].

In a study conducted on 893 participants from the Dutch LifeLines-DEEP cohort, Fu *et al.*[13] measured specific lipid levels through targeted colorimetric methods and found that TAG and

		Richness	
Sequence	Shift (ppm)	Observed	Chao1 index
CPMG	0·567–0·607 Unassigned signal	*	–
	1·207–1·311 Lipoproteins, –CH₂	**	*
	1·521–1·527 CH₂CH₂COOC	**	*
	2·151–2·166 Lipid, –CH₂=C=C–	*	*
	2·166–2·167	**	*
	2·167–2·264 Acetone, CH₂CO	**	*
	2·551–2·571 Methylamine	**	*
	2·831–2·848 Citrate/=CH–CH₂–CH=	*	–
	3·071–3·111 Creatinine	**	*
	3·167–3·269 Choline and glucose	*	–
	3·311–3·312 Glucose	*	–
	3·616–3·711 Glutamine	*	*
	5·127–5·167	**	*
	5·864–5·889 Unassigned signal	*	–
	5·889–5·969	**	–
	6·492–6·527	**	*
	6·728–6·767 Proteins	**	*
	6·857–6·943 Tyrosine	*	*
	6·912–6·943 Proteins	**	*
	6·943–6·968	**	*
	7·508–7·539 3-methylhistidine	**	–
	7·629–7·647	**	*
	7·647–7·687 1-methylhistidine	**	*
	7·687–7·772 Histidine	**	*
NOESY1D	4·191–4·214	**	**
	5·830–5·835	**	*
	5·861–5·887 Unassigned signal	**	**
	5·887–5·965	**	**
	5·965–5·966	**	**
	6·486–6·526	*	–
	6·726–6·766 Proteins	**	**
	7·646–7·686 1-methylhistidine	*	–

Fig. 2. Associations between NMR variables and α-diversity indexes (observed richness and Chao1 estimate of richness) from Spearman partial correlations adjusted for age, sex, BMI, smoking status, physical activity and sequencing depth, *Milieu Intérieur* study, France, 2012 (*n* 846). *Q*-values were obtained applying a multiple testing correction (Benjamini–Hochberg false discovery rate method). Only associations with *Q*-value ≤0·1 for observed richness, as well as subsequent associations with Chao1 richness are presented. ** *P*-value ≤ 0·003 and *Q*-value ≤ 0·05; * *P*-value ≤ 0·01 and *Q*-value ≤ 0·1; –, *P*-value ≤ 0·05 and *Q*-value >0·1. Corresponding values are shown in online Supplementary Table S7. Spearman ρ: ▬, 0·13; ▬, −0·13. ppm, Parts per million; CPMG, Carr–Purcell–Meiboom–Gill; NOESY1D, ¹H one-dimensional NMR pulse sequence nuclear Overhauser effect spectroscopy.

HDL were, respectively, negatively and positively associated with both bacterial richness and diversity. Consistently, a more recent study using NMR metabolomics in 2309 individuals from the Rotterdam Study and the LifeLines-DEEP cohort observed inverse associations between the diversity of the gut microbiota and serum levels of not only VLDL particles, TAG, total fatty acids, MUFA and SFA but also glycoprotein acetyl, alanine, isoleucine and lactate and positive associations with HDL particles. In addition, Le Chatelier *et al.*[43] associated a decreased gut microbial richness with metabolic impairments such as increased fat mass and body weight, inflammation of the adipose tissue and dyslipidaemia. In the present work, we consistently found that lipid-related metabolomic signals were negatively associated with bacterial richness. However, we were unable to further characterise these lipid-related signals and discriminate between the different lipid classes potentially hampering the detection of specific associations previously reported[13,28]. Indeed, long-chain metabolites such as fatty acids result in an overlap of signals of protons in NMR, limiting a more specific assignment[44]. Still, it should be noted that, in our sample, NMR signals assigned to lipids demonstrated excellent correlation with blood TAG (Spearman's ρ > 0·65, *P*-value < 0·0001 for spectral regions 1·207–1·311, 2·151–2·166 and 2·166–2·167 ppm), adding support to the consistency of our results with those previous findings. Furthermore, high-fat Western diet has consistently been associated with gut microbiota impairments[45,46], and lipid-related signals in our study correlated positively with dietary intakes of processed meat (Spearman's ρ = 0·09, *P*-value = 0·006 for spectral regions 2·166–2·167 ppm and ρ = 0·08, *P*-value = 0·02 for 1·207–1·311 and 2·151–2·166 ppm) and fried products (ρ = 0·07, *P*-value = 0·04 for 2·166–2·167 ppm), and negatively correlated with fruit (ρ = −0·09, *P*-value = 0·01 for 1·207–1·311 and 2·166–2·167 ppm and ρ = −0·08, *P*-value = 0·02 for 2·151–2·166 ppm) and vegetables (ρ = −0·09, *P*-value = 0·007 for 2·166–2·167 ppm and ρ = −0·07, *P*-value = 0·04 for 2·151–2·166 ppm). Nonetheless, these assumptions should be considered with caution as additional exploration and targeted identification of these lipid signals using MS or lipidomics are necessary to get more detailed insights into the associations we observed.

Our finding of a positive association between glutamine and gut bacterial richness echoes a result from De Souza *et al.*[47] who suggested that glutamine might have an anti-inflammatory effect through a modulation of specific gut bacterial members following the observation that an oral supplementation with L-glutamine decreased the Firmicutes:Bacteroidetes ratio in obese and overweight adults.

In our study, creatinine was the metabolomic signal most associated with the relative abundances of bacterial taxa. This by-product of muscle metabolism is exclusively excreted through the kidneys, and a high creatinine level in blood is notably used as an indicator of kidney dysfunction[48]. Our finding of positive associations between plasma creatinine and the relative abundance of various representatives of the Clostridiales order and the Ruminococcaceae family matches results from Org *et al.*[28]. We also reported a negative association between creatinine and *Faecalibacterium prausnitzii*, a species drawing a particular attention from the research community as it has been shown to exert anti-inflammatory properties through its ability to produce anti-inflammatory metabolites (e.g. butyrate), or to inhibit of the production of pro-inflammatory cytokines[49]. Various studies have reported that *F. prausnitzii* is reduced in conditions such as ulcerative colitis, inflammatory bowel disease, colorectal cancer, diabetes, psoriasis, etc.[49–51]. Consequently, this species has been proposed as a potential biomarker of gut health[52] and is even considered as an interesting probiotic candidate[53]. In particular, a decrease in *F. prausnitzii* was previously reported in chronic kidney disease patients compared with healthy controls[54,55]. This echoes our result of a negative association between *F. prausnitzii* and creatinine obtained in a healthy sample without renal dysfunction. Overall, the numerous associations we detected between bacterial taxa and creatinine are in support of a gut-kidney conversation[56,57]. In this previously described bi-directional organ axis, the gut contributes to inflammation and renal injury through microbiota dysbiosis and dysregulations in microbial metabolite production. Conversely, kidney perturbations lead to dysbiosis and disruptions in tight junction function therefore promoting inflammatory

Table 2. Associations between NMR variables and relative abundances of taxa with a *Q*-value ≤ 0·1 after multiple testing correction, using multivariate associations with linear models (MaAsLins), *Milieu Intérieur* study, France, 2012 (*n* 846)

	Shift (ppm)	Signal assignation	Phylum | Class | Order | Family|	Genus	Species	MaAsLin coefficient	Coverage*	P†	Q‡
CPMG	1·021–1·051	Unassigned signal	Firmicutes| Negativicutes | Selenomonadales | Veillonellaceae|	Sporomusa§		−68·5	35·22	<0·0001	0·07
	1·527–1·631	CH₂CH₂COOC	Firmicutes| Clostridia | Clostridiales | Lachnospiraceae | Clostridium XIVa|	Clostridium	C. bolteae	74·2	99·17	<0·0001	0·06
	2·831–2·848	Citrate/=CH–CH₂–CH=	Firmicutes | Bacilli | Bacillales | Bacillaceae 1|	Caldibacillus		−198·2	35·34	<0·0001	0·07
	2·967–3·071	Creatine/albumin lysyl	Firmicutes | Clostridia | Clostridiales | Ruminococcaceae|	Hydrogenoanaerobacterium	H. saccharovorans	−31·8	90·07	<0·0001	0·07
	3·071–3·111	Creatinine	Firmicutes | Clostridia | Clostridiales | Ruminococcaceae|	Faecalibacterium		−777·4	99·41	<0·0001	0·02
			Firmicutes | Clostridia | Clostridiales | Ruminococcaceae|	Faecalibacterium	F. prausnitzii	−797·3	99·41	<0·0001	0·03
			Firmicutes | Negativicutes | Selenomonadales | Veillonellaceae|	Sporomusa§		31·8	35·22	<0·0001	0·06
			Firmicutes | Negativicutes | Selenomonadales | Veillonellaceae|	Sporomusa	S. termitida	31·5	34·75	<0·0001	0·06
			Tenericutes | Mollicutes | Acholeplasmatales | Acholeplasmataceae|	Acholeplasma		42·0	32·98	0·0001	0·09
			Firmicutes | Clostridia | Clostridiales | Clostridiaceae 1|	Caloramator		63·9	58·63	<0·0001	0·06
			Firmicutes | Clostridia | Clostridiales | Catabacteriaceae|	Catabacter|		67·0	68·56	<0·0001	0·001
			Firmicutes | Clostridia | Clostridiales | Catabacteriaceae|	Catabacter	C. hongkongensis	64·0	68·56	<0·0001	0·002
			Firmicutes | Clostridia | Clostridiales | Defluviitaleaceae|	Defluviitalea		70·2	81·32	<0·0001	0·07
			Firmicutes | Clostridia | Clostridiales | Defluviitaleaceae|	Defluviitalea	D. saccharophila	71·1	81·32	<0·0001	0·07
			Firmicutes | Clostridia | Clostridiales | Peptococcaceae 2|	Pelotomaculum		74·5	52·36	<0·0001	0·07
			Firmicutes | Clostridia | Clostridiales | Peptococcaceae 2|	Pelotomaculum	P. propionicium	82·6	51·30	<0·0001	0·01
			Firmicutes| Clostridia | Clostridiales | Christensenellaceae|	Christensenella		138·5	93·14	<0·0001	0·06
			Firmicutes| Clostridia | Clostridiales | Christensenellaceae|	Christensenella	C. minuta	170·2	93·14	<0·0001	0·01
			Firmicutes| Clostridia | Clostridiales | Ruminococcaceae|	Saccharofermentans§||		187·6	65·13	<0·0001	0·005
			Firmicutes| Clostridia | Clostridiales | Ruminococcaceae|	Saccharofermentans	S. acetigenes||	189·3	65·13	<0·0001	0·01
			Firmicutes | Clostridia | Clostridiales | Ruminococcaceae|	Sporobacter		305·1	93·50	<0·0001	0·02
			Firmicutes | Clostridia | Clostridiales | Ruminococcaceae|	Sporobacter	S. termitidis	273·1	93·50	<0·0001	0·07
			Firmicutes | Clostridia | Clostridiales | Ruminococcaceae|	Intestinimonas		320·8	98·70	<0·0001	0·003
			Firmicutes | Clostridia | Clostridiales | Ruminococcaceae|	Intestinimonas	I. butyriciproducens	352·4	98·70	<0·0001	0·002
			Firmicutes | Clostridia | Clostridiales | Ruminococcaceae|	Oscillibacter||		396·2	99·76	<0·0001	0·03
			Firmicutes | Clostridia | Clostridiales | Ruminococcaceae|	Oscillibacter	O. valericigenes	460·2	95·98	<0·0001	0·03
			Firmicutes | Clostridia | Clostridiales | Eubacteriaceae|	Eubacterium		447·6	99·88	<0·0001	0·06
			Firmicutes | Clostridia | Clostridiales | Eubacteriaceae|	Eubacterium	E. coprostanoligenes	580·5	94·44	<0·0001	0·01
	3·111–3·129	Unassigned signal	Firmicutes | Clostridia | Clostridiales | Lachnospiraceae | Clostridium XIVa|	Clostridium	C. viride	−133·2	90·31	<0·0001	0·03
	3·367–3·437		Firmicutes | Clostridia | Clostridiales | Eubacteriaceae|	Eubacterium	E. rectale§	266·4	98·94	<0·0001	0·1
	3·437–3·496		Firmicutes | Clostridia | Clostridiales | Eubacteriaceae|	Eubacterium	E. rectale§	−470·9	98·94	<0·0001	0·03
	3·496–3·587	Glucose	Firmicutes | Clostridia | Clostridiales | Eubacteriaceae|	Alkalibaculum		−20·1	74·35	<0·0001	0·07
	5·687–5·727	Urea	Firmicutes | Clostridia | Clostridiales | Ruminococcaceae|	Saccharofermentans§||		−219·2	65·13	<0·0001	0·07
			Firmicutes | Clostridia | Clostridiales | Ruminococcaceae|	Saccharofermentans	S. acetigenes||	−207·7	65·13	<0·0001	0·09
NOESY1D	3·071–3·111	Creatinine	Firmicutes | Clostridia | Clostridiales | Catabacteriaceae|	Catabacter§		79·7	68·56	<0·0001	0·01
			Firmicutes | Clostridia | Clostridiales | Ruminococcaceae|	Saccharofermentans§||		265·0	65·13	<0·0001	0·03
			Firmicutes | Clostridia | Clostridiales | Ruminococcaceae|	Oscillibacter||		567·1	99·76	<0·0001	0·09
	3·532–3·588	Glucose	Firmicutes | Clostridia | Clostridiales | Ruminococcaceae|	Pseudobacteroides§		28·9	19·62	<0·0001	<0·0001
			Firmicutes | Clostridia | Clostridiales | Ruminococcaceae|	Pseudobacteroides	P. cellulosolvens	31·9	19·62	<0·0001	<0·0001
			Firmicutes | Clostridia | Clostridiales | Clostridiaceae 4|	Salimesophilobacter		41·9	24·82	<0·0001	0·03
			Firmicutes | Clostridia | Clostridiales | Clostridiaceae 4|	Salimesophilobacter	S. vulgaris	44·5	24·82	<0·0001	0·03
	3·588–3·611		Firmicutes | Clostridia | Clostridiales | Ruminococcaceae|	Pseudobacteroides§		−21·0	19·62	<0·0001	0·0001
	9·471–9·511	Unassigned signal	Firmicutes | Clostridia | Clostridiales | Ruminococcaceae|	Clostridium III		1038·8	98·11	<0·0001	0·08

ppm, Parts per million; CPMG, Carr–Purcell–Meiboom–Gill; NOESY1D, ¹H one-dimensional NMR pulse sequence nuclear Overhauser effect spectroscopy.

* Prevalence of bacterial taxa in the study sample.

† *P*-value for MaAsLin adjusted for age, sex, BMI, smoking status and physical activity before Benjamini–Hochberg correction; computed using the MaAsLin package on R.

‡ Corrected *P*-value (Benjamini–Hochberg method, 10 % false discovery rate). Only associations with a *Q*-value ≤ 0·1 are presented.

§ Taxa found in association with at least two NMR signals.

\|\| Taxa found with both CPMG and NOESY1D.

conditions in the gut. Notably, Jiang *et al.*[55] reported that indicators of the renal function such as serum creatinine, estimated glomerular filtration rate and cystatin C were the most important environmental parameters to influence the overall microbial communities in end-stage renal disease patients. In our study, a link between the gut and the kidneys seemed to be already observed even in a healthy setting where individuals were free of kidney or gut impairments. However, further exploration of renal function indicators (e.g. estimated glomerular filtration rate, cystatin C and urine creatinine) would be of utmost importance to confirm such a link. If we hypothesise a gut–kidney axis to interpret our results, alternate interpretations could also be proposed. Indeed, negative associations have also been reported between trimethylamine *N*-oxide and *F. prausnitzii*[28,58], and creatinine is a precursor for the gut bacterial synthesis of trimethylamine, which is further oxidised into trimethylamine *N*-oxide in the liver[48]. Therefore, we cannot rule out that the association we observed between creatinine and *F. prausnitzii* may actually reflect the overall trimethylamine *N*-oxide metabolic pathway instead of being a true association. However, because trimethylamine *N*-oxide could not be detected by NMR in our study, it was not possible to verify this assumption.

An important strength of this work pertains to its design – namely the acquisition of both detailed metabolomics data and gut microbiome data in a large population-based sample of healthy adults. While individual or specific approaches consider biomolecules separately, metabolomics allows for the simultaneous detection and relative quantification of hundreds of molecules, hence the monitoring of subtle system-wide metabolic interactions[59]. In this study, we used NMR metabolomics with standardised and optimised protocols yielding high-quality data[60]. This robust and stable technology has demonstrated its suitability for long-term epidemiological studies[61], notably by allowing to analyse large samples quickly with a high reproducibility[62]. From a statistical standpoint, the analyses implemented here allowed to explore detailed associations between gut bacterial and metabolomic data sets, beyond the sole assessment of global connections obtained through multivariate correlation-based approaches (e.g. co-inertia or Procrustes analyses). In addition, we were able to take into account a range of factors reported to influence the gut microbiota composition or the systemic metabolism of the host in our models, thus mitigating potential confounding. Nonetheless, residual confounding cannot be ruled out. For instance, intestinal transit time was shown to be a major confounding factor of gut microbiota composition[63], and we were not able to collect this information. Another important strength and originality of this work is that it was based on a large sample from the general population including only healthy men and women selected using strict criteria. Hence, our results were not confounded by underlying disease and reflected normal range. Yet, this may also have induced less variations in plasma metabolites levels or in gut microbiota composition and therefore resulted in associations of smaller magnitude. Some limitations should also be acknowledged for this study. First, the cross-sectional design of our study, in which blood and stool samples were taken at the same time, prevents from drawing any conclusion regarding the sequential causality of events between host metabolism (e.g. creatinine levels) and

gut microbiota composition (e.g. *F. prausnitzii*). Further, plasma metabolomic signatures only give a metabolic 'snapshot' that results from both the endogenous and the gut microbial metabolisms, which makes it not possible to discriminate signals from human or microbial origin. In future studies, subsequent time points with both metabolomic and microbiota data collected would be of utmost interest to investigate the stability over time and the chronology of the associations observed in the present cross-sectional work. In addition, the fact that most detected metabolites result from multiple metabolic pathways that may be related to gut microbiota or not may also explain the modest correlation coefficients observed between plasma metabolites and gut microbial richness. It should also be acknowledged that, although highly informative, the use of 16S rRNA gene sequencing and NMR metabolomics presents technical limitations. Shotgun metagenomics for instance would give a more precise characterisation of microbiota features and also bring additional information regarding the functional metabolic capacity of the gut microbiota. This would allow to go beyond the compositional approach, limited by the redundancy of gene pool and metabolic functionalities between distinct gut microbial members[64,65], but still relevant according to recent studies arguing that some metabolic pathways pertain to a handful of species[66]. Likewise, while NMR detects only the most abundant metabolites (μM to mM range), MS has lower detection limits and higher sensitivity for signal assignment and would therefore allow the detection of metabolites undetectable with NMR[67]. This technical limitation of NMR may explain why several low-concentration metabolites deriving specifically from gut microbiota were not detected in our study. As already mentioned, the precision of NMR bucket assignment was also limited for some metabolites, especially for lipids and proteins. For instance, the unsaturation corresponds to a chemical function in the aliphatic chain of various lipids, and it is impossible to differentiate among the different lipid types within the plasma sample. Hence, this limits the comparability of our results to prior studies. In addition, plasma samples underwent two freeze-thaw cycles prior to NMR analyses (necessary to prepare internal quality controls) so that the possibility of increased metabolites cleavage cannot be ruled out. Still, this would similarly affect all samples as they all went through the same process.

Although the *Milieu Intérieur* population is somewhat representative of the French Ille-et-Vilaine region[33], our study sample is not representative of the whole French adult population – caution is therefore needed in the generalisation of our results. However, it is important to acknowledge that from a gut microbiota composition standpoint, our study sample was consistent with other studies performed in Western settings[41,68], with the same five most abundant phyla and shared 'core genera' (online Supplementary Table S1). Finally, the results highlighted here were mostly exploratory as they were obtained from a single population. These results will need to be replicated and confirmed in the future, using independent samples and/or analytical techniques allowing a better refinement of the detected metabolites (e.g. MS, lipidomics). In addition, the hypotheses we proposed to interpret our results were based on existing

literature and would need to be developed and confirmed in experimental settings *in vitro* and/or *in vivo*. In particular, detailed mechanistic studies comprehensively elucidating the metabolic capability of gut microorganisms are needed to enlighten the associations we detected.

Overall, the present study provides interesting insights regarding the associations between gut microbiota composition and plasma metabolic signatures of the host. Our results contribute to a better understanding of the host–gut microbiota relationships and notably highlight a possible gut–kidney axis in healthy subjects. Because the associations we found in healthy individuals were already reported in chronic kidney disease patients, this builds up to the concept that onset of chronic diseases is a lengthy process that could be detected at early stages. This appears all the more interesting that microbial–host co-metabolites were shown to be excellent prodromal markers of future divergence in metabolic and behavioural outcomes in mice[69]. However, our results remain to be confirmed in independent populations, underlying mechanisms to be elucidated and causal implications for the host health to be established.

Supplementary material

For supplementary materials referred to in this article, please visit https://doi.org/10.1017/S0007114520004870

References

1. MetaHIT Consortium, Qin J, Li R, *et al.* (2010) A human gut microbial gene catalogue established by metagenomic sequencing. *Nature* **464**, 59–65.
2. MetaHIT Consortium, Li J, Jia H, *et al.* (2014) An integrated catalog of reference genes in the human gut microbiome. *Nat Biotechnol* **32**, 834–841.
3. Yadav M, Verma MK & Chauhan NS (2018) A review of metabolic potential of human gut microbiome in human nutrition. *Arch Microbiol* **200**, 203–217.
4. Nicholson JK, Holmes E, Kinross J, *et al.* (2012) Host–gut microbiota metabolic interactions. *Science* **336**, 1262–1267.
5. Schroeder BO & Bäckhed F (2016) Signals from the gut microbiota to distant organs in physiology and disease. *Nat Med* **22**, 1079–1089.
6. Brown JM & Hazen SL (2015) The gut microbial endocrine organ: bacterially derived signals driving cardiometabolic diseases. *Annu Rev Med* **66**, 343–359.
7. Rajani C & Jia W (2018) Disruptions in gut microbial–host co-metabolism and the development of metabolic disorders. *Clin Sci* **132**, 791–811.
8. Postler TS & Ghosh S (2017) Understanding the Holobiont: how microbial metabolites affect human health and shape the immune system. *Cell Metab* **26**, 110–130.
9. Li M, Wang B, Zhang M, *et al.* (2008) Symbiotic gut microbes modulate human metabolic phenotypes. *Proc Natl Acad Sci U S A* **105**, 2117–2122.
10. Kundu P, Blacher E, Elinav E, *et al.* (2017) Our gut microbiome: the evolving inner self. *Cell* **171**, 1481–1493.
11. Louis P, Hold GL & Flint HJ (2014) The gut microbiota, bacterial metabolites and colorectal cancer. *Nat Rev Microbiol* **12**, 661–672.
12. Ryan PM, Stanton C & Caplice NM (2017) Bile acids at the crossroads of gut microbiome–host cardiometabolic interactions. *Diabetol Metab Syndr* **9**, 102.
13. Fu J, Bonder MJ, Cenit MC, *et al.* (2015) The gut microbiome contributes to a substantial proportion of the variation in blood lipids. *Circ Res* **117**, 817–824.
14. Smirnov KS, Maier TV, Walker A, *et al.* (2016) Challenges of metabolomics in human gut microbiota research. *Int J Med Microbiol* **306**, 266–279.
15. Pedersen HK, Gudmundsdottir V, Nielsen HB, *et al.* (2016) Human gut microbes impact host serum metabolome and insulin sensitivity. *Nature* **535**, 376–381.

16. Kelly RS, Sordillo JE, Lasky-Su J, *et al.* (2018) Plasma metabolite profiles in children with current asthma. *Clin Exp Allergy* **48**, 1297–1304.

17. Naviaux RK, Naviaux JC, Li K, *et al.* (2016) Metabolic features of chronic fatigue syndrome. *Proc Natl Acad Sci U S A* **113**, E5472–E5480.

18. Urpi-Sarda M, Almanza-Aguilera E, Llorach R, *et al.* (2019) Non-targeted metabolomic biomarkers and metabotypes of type 2 diabetes: a cross-sectional study of PREDIMED trial participants. *Diabetes Metab* **45**, 167–174.

19. Derkach A, Sampson J, Joseph J, *et al.* (2017) Effects of dietary sodium on metabolites: the Dietary Approaches to Stop Hypertension (DASH)–Sodium Feeding Study. *Am J Clin Nutr* **106**, 1131–1141.

20. Mora-Cubillos X, Tulipani S, Garcia-Aloy M, *et al.* (2015) Plasma metabolomic biomarkers of mixed nuts exposure inversely correlate with severity of metabolic syndrome. *Mol Nutr Food Res* **59**, 2480–2490.

21. Pantophlet AJ, Wopereis S, Eelderink C, *et al.* (2017) Metabolic profiling reveals differences in plasma concentrations of arabinose and xylose after consumption of fiber-rich pasta and wheat bread with differential rates of systemic appearance of exogenous glucose in healthy men. *J Nutr* **147**, 152–160.

22. Tovar J, de Mello VD, Nilsson A, *et al.* (2017) Reduction in cardiometabolic risk factors by a multifunctional diet is mediated via several branches of metabolism as evidenced by nontargeted metabolite profiling approach. *Mol Nutr Food Res* **61**, 1600552.

23. Cui X, Ye L, Li J, *et al.* (2018) Metagenomic and metabolomic analyses unveil dysbiosis of gut microbiota in chronic heart failure patients. *Sci Rep* **8**, 635.

24. Dewulf EM, Cani PD, Claus SP, *et al.* (2013) Insight into the prebiotic concept: lessons from an exploratory, double blind intervention study with inulin-type fructans in obese women. *Gut* **62**, 1112–1121.

25. Karl JP, Margolis LM, Madslien EH, *et al.* (2017) Changes in intestinal microbiota composition and metabolism coincide with increased intestinal permeability in young adults under prolonged physiological stress. *Am J Physiol Gastrointest Liver Physiol* **312**, G559–G571.

26. Lin Z, Ye W, Zu X, *et al.* (2018) Integrative metabolic and microbial profiling on patients with Spleen-yang-deficiency syndrome. *Sci Rep* **8**.

27. Liu R, Hong J, Xu X, *et al.* (2017) Gut microbiome and serum metabolome alterations in obesity and after weight-loss intervention. *Nat Med* **23**, 859–868.

28. Org E, Blum Y, Kasela S, *et al.* (2017) Relationships between gut microbiota, plasma metabolites, and metabolic syndrome traits in the METSIM cohort. *Genome Biol* **18**, 70.

29. Vojinovic D, Radjabzadeh D, Kurilshikov A, *et al.* (2019) Relationship between gut microbiota and circulating metabolites in population-based cohorts. *Nat Commun* **10**, 5813.

30. Menni C, Zhu J, Le Roy CI, *et al.* (2020) Serum metabolites reflecting gut microbiome alpha diversity predict type 2 diabetes. *Gut Microbes* **11**, 1632–1642.

31. Pallister T, Jackson MA, Martin TC, *et al.* (2017) Hippurate as a metabolomic marker of gut microbiome diversity: modulation by diet and relationship to metabolic syndrome. *Sci Rep* **7**, 13670.

32. Wilmanski T, Rappaport N, Earls JC, *et al.* (2019) Blood metabolome predicts gut microbiome α-diversity in humans. *Nat Biotechnol* **37**, 1217–1228.

33. Thomas S, Rouilly V, Patin E, *et al.* (2015) The Milieu Intérieur study — an integrative approach for study of human immunological variance. *Clin Immunol* **157**, 277–293.

34. Gika HG, Zisi C, Theodoridis G, *et al.* (2016) Protocol for quality control in metabolic profiling of biological fluids by U(H)PLC-MS. *J Chromatogr B* **1008**, 15–25.

35. Dunn WB, Broadhurst D, Begley P, *et al.* (2011) Procedures for large-scale metabolic profiling of serum and plasma using gas chromatography and liquid chromatography coupled to mass spectrometry. *Nat Protoc* **6**, 1060–1083.

36. Lécuyer L, Victor Bala A, Deschasaux M, *et al.* (2018) NMR metabolomic signatures reveal predictive plasma metabolites associated with long-term risk of developing breast cancer. *Int J Epidemiol* **47**, 484–494.

37. Nicholson JK, Foxall PJD, Spraul M, *et al.* (1995) 750 MHz ^{1}H and ^{1}H-^{13}C NMR spectroscopy of human blood plasma. *Anal Chem* **67**, 793–811.

38. Wishart DS, Jewison T, Guo AC, *et al.* (2012) HMDB 3.0—the human metabolome database in 2013. *Nucleic Acids Res* **41**, D801–D807.

39. Scepanovic P, Hodel F, Mondot S, *et al.* (2019) A comprehensive assessment of demographic, environmental, and host genetic associations with gut microbiome diversity in healthy individuals. *Microbiome* **7**, 130.

40. Partula V, Mondot S, Torres MJ, *et al.* (2019) Associations between usual diet and gut microbiota composition: results from the Milieu Intérieur cross-sectional study. *Am J Clin Nutr* **109**, 1472–1483.

41. Falony G, Joossens M, Vieira-Silva S, *et al.* (2016) Population-level analysis of gut microbiome variation. *Science* **352**, 560–564.

42. Daliri EB-M, Wei S, Oh DH, *et al.* (2017) The human microbiome and metabolomics: current concepts and applications. *Crit Rev Food Sci Nutr* **57**, 3565–3576.

43. MetaHIT consortium, Le Chatelier E, Nielsen T, *et al.* (2013) Richness of human gut microbiome correlates with metabolic markers. *Nature* **500**, 541–546.

44. Khoury S, Canlet C, Lacroix M, *et al.* (2018) Quantification of lipids: model, reality, and compromise. *Biomolecules* **8**, 174.

45. Davis SC, Yadav JS, Barrow SD, *et al.* (2017) Gut microbiome diversity influenced more by the Westernized dietary regime than the body mass index as assessed using effect size statistic. *MicrobiologyOpen* **6**, e00476.

46. De Filippo C, Cavalieri D, Di Paola M, *et al.* (2010) Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. *Proc Natl Acad Sci U S A* **107**, 14691–14696.

47. Zambom de Souza AZ, Zambom AZ, Abboud KY, *et al.* (2015) Oral supplementation with L-glutamine alters gut microbiota of obese and overweight adults: a pilot study. *Nutrition* **31**, 884–889.

48. Chhibber-Goel J, Singhal V, Parakh N, *et al.* (2017) The metabolite trimethylamine-*N*-oxide is an emergent biomarker of human health. *Curr Med Chem* **24**, 3942–3953.

49. Ferreira-Halder CV, Faria AVS & Andrade SS (2017) Action and function of *Faecalibacterium prausnitzii* in health and disease. *Best Pract Res Clin Gastroenterol* **31**, 643–648.

50. Sokol H, Pigneur B, Watterlot L, *et al.* (2008) Faecalibacterium prausnitzii is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of Crohn disease patients. *Proc Natl Acad Sci U S A* **105**, 16731–16736.

51. Machiels K, Joossens M, Sabino J, *et al.* (2014) A decrease of the butyrate-producing species *Roseburia hominis* and *Faecalibacterium prausnitzii* defines dysbiosis in patients with ulcerative colitis. *Gut* **63**, 1275–1283.

52. Lopez-Siles M, Duncan SH, Garcia-Gil LJ, *et al.* (2017) *Faecalibacterium prausnitzii*: from microbiology to diagnostics and prognostics. *ISME J* **11**, 841–852.

53. Martín R, Miquel S, Benevides L, *et al.* (2017) Functional characterization of novel *Faecalibacterium prausnitzii* strains isolated from healthy volunteers: a step forward in the use of *F. prausnitzii* as a next-generation probiotic. *Front Microbiol* **8**, 1226.

54. Jiang S, Xie S, Lv D, *et al.* (2016) A reduction in the butyrate producing species Roseburia spp. and *Faecalibacterium prausnitzii* is associated with chronic kidney disease progression. *Antonie van Leeuwenhoek* **109**, 1389–1396.

55. Jiang S, Xie S, Lv D, *et al.* (2017) Alteration of the gut microbiota in Chinese population with chronic kidney disease. *Sci Rep* **7**, 2870.

56. Chen Y-Y, Chen D-Q, Chen L, *et al.* (2019) Microbiome–metabolome reveals the contribution of gut–kidney axis on kidney disease. *J Transl Med* **17**, 5.

57. Evenepoel P, Poesen R & Meijers B (2017) The gut–kidney axis. *Pediatr Nephrol* **32**, 2005–2014.

58. Sanchez-Alcoholado L, Castellano-Castillo D, Jordán-Martínez L, *et al.* (2017) Role of gut microbiota on cardio-metabolic parameters and immunity in coronary artery disease patients with and without type-2 diabetes mellitus. *Front Microbiol* **8**, 1936.

59. Patti GJ, Yanes O & Siuzdak G (2012) Metabolomics: the apogee of the omics trilogy. *Nat Rev Mol Cell Biol* **13**, 263–269.

60. Beckonert O, Keun HC, Ebbels TMD, *et al.* (2007) Metabolic profiling, metabolomic and metabonomic procedures for NMR spectroscopy of urine, plasma, serum and tissue extracts. *Nat Protoc* **2**, 2692–2703.

61. Dumas M-E, Maibaum EC, Teague C, *et al.* (2006) Assessment of analytical reproducibility of ^{1}H NMR spectroscopy based metabonomics for large-scale epidemiological research: the INTERMAP study. *Anal Chem* **78**, 2199–2208.

62. Emwas A-H, Roy R, McKay RT, *et al.* (2019) NMR spectroscopy for metabolomics research. *Metabolites* **9**, 123.

63. Roager HM, Hansen LBS, Bahl MI, *et al.* (2016) Colonic transit time is related to bacterial metabolism and mucosal turnover in the gut. *Nat Microbiol* **1**, 16093.

64. Abubucker S, Segata N, Goll J, *et al.* (2012) Metabolic reconstruction for metagenomic data and its application to the human microbiome. *PLoS Comput Biol* **8**, e1002358.

65. Holmes E, Li JV, Marchesi JR, *et al.* (2012) Gut microbiota composition and activity in relation to host metabolic phenotype and disease risk. *Cell Metab* **16**, 559–564.

66. Morrison DJ & Preston T (2016) Formation of short chain fatty acids by the gut microbiota and their impact on human metabolism. *Gut Microbes* **7**, 189–200.

67. Riekeberg E & Powers R (2017) New frontiers in metabolomics: from measurement to insight. *F1000Res* **6**, 1148.

68. Rothschild D, Weissbrod O, Barkan E, *et al.* (2018) Environment dominates over host genetics in shaping human gut microbiota. *Nature* **555**, 210–215.

69. Dumas M-E, Rothwell AR, Hoyles L, *et al.* (2017) Microbial–host co-metabolites are prodromal markers predicting phenotypic heterogeneity in behavior, obesity, and impaired glucose tolerance. *Cell Rep* **20**, 136–148.