



OPEN ACCESS

ORIGINAL RESEARCH

Mediterranean diet intervention in overweight and obese subjects lowers plasma cholesterol and causes changes in the gut microbiome and metabolome independently of energy intake

Victoria Meslier,¹ Manolo Laiola,² Henrik Munch Roager ,³ Francesca De Filippis,^{2,4} Hugo Roume,¹ Benoit Quinquis,¹ Rosalba Giacco,⁵ Ilario Mennella,² Rosalia Ferracane,² Nicolas Pons,¹ Edoardo Pasoli,^{2,4} Angela Rivellesse,^{4,6} Lars Ove Dragsted ,³ Paola Vitaglione,^{2,4} Stanislav Dusko Ehrlich ,¹ Danilo Ercolini ^{2,4}

► Additional material is published online only. To view please visit the journal online (<http://dx.doi.org/10.1136/gutjnl-2019-320438>).

For numbered affiliations see end of article.

Correspondence to

Professor Danilo Ercolini and Dr Paola Vitaglione, Department of Agricultural Sciences, University of Naples Federico II, Portici 80055, Italy; ercolini@unina.it, paola.vitaglione@unina.it and Professor Stanislav Dusko Ehrlich, Metagenopolis, INRAE, 78350 Jouy en Josas, France; stanislav.ehrlich@inra.fr

VM, ML and HMR contributed equally.

Received 10 December 2019
Revised 17 January 2020
Accepted 21 January 2020
Published Online First
19 February 2020



► <http://dx.doi.org/10.1136/gutjnl-2019-319654>
► <http://dx.doi.org/10.1136/gutjnl-2020-320781>



© Author(s) (or their employer(s)) 2020. Re-use permitted under CC BY-NC. No commercial re-use. See rights and permissions. Published by BMJ.

To cite: Meslier V, Laiola M, Roager HM, et al. *Gut* 2020;**69**:1258–1268.

ABSTRACT

Objectives This study aimed to explore the effects of an isocaloric Mediterranean diet (MD) intervention on metabolic health, gut microbiome and systemic metabolome in subjects with lifestyle risk factors for metabolic disease.

Design Eighty-two healthy overweight and obese subjects with a habitually low intake of fruit and vegetables and a sedentary lifestyle participated in a parallel 8-week randomised controlled trial. Forty-three participants consumed an MD tailored to their habitual energy intakes (MedD), and 39 maintained their regular diets (ConD). Dietary adherence, metabolic parameters, gut microbiome and systemic metabolome were monitored over the study period.

Results Increased MD adherence in the MedD group successfully reprogrammed subjects' intake of fibre and animal proteins. Compliance was confirmed by lowered levels of carnitine in plasma and urine. Significant reductions in plasma cholesterol (primary outcome) and faecal bile acids occurred in the MedD compared with the ConD group. Shotgun metagenomics showed gut microbiome changes that reflected individual MD adherence and increase in gene richness in participants who reduced systemic inflammation over the intervention. The MD intervention led to increased levels of the fibre-degrading *Faecalibacterium prausnitzii* and of genes for microbial carbohydrate degradation linked to butyrate metabolism. The dietary changes in the MedD group led to increased urinary urolithins, faecal bile acid degradation and insulin sensitivity that co-varied with specific microbial taxa.

Conclusion Switching subjects to an MD while maintaining their energy intake reduced their blood cholesterol and caused multiple changes in their microbiome and metabolome that are relevant in future strategies for the improvement of metabolic health.

INTRODUCTION

Diet is a fundamental factor affecting gut health. Mounting evidence highlights that diets richer in plant-based rather than animal-based foods could represent healthier choices to prevent disease.^{1 2}

Significance of this study

What is already known about this subject?

- Diet fundamentally influences gut health.
- Mediterranean diet (MD) is a recommended nutritional pattern with known beneficial effects including the prevention of several types of disease.
- Current knowledge of the effect of diet on microbiome-mediated health outcomes in humans relies mainly on observational studies.

What are the new findings?

- An isocaloric 8-week intervention with an MD in obese and overweight subjects leads to a decrease in total, low-density lipoprotein and high-density lipoprotein plasma cholesterol and faecal bile acids levels independently of energy intake, the decrease in cholesterol being proportional to MD adherence rates.
- The MD intervention causes microbiome changes with increased gene richness in individuals with reduced inflammation, a rise in the fibre-degrading *Faecalibacterium prausnitzii* accompanied by a decrease of the potentially proinflammatory *Ruminococcus gnavus*.
- Increased MD adherence generates consistent metabolome changes, with lower plasma and urinary carnitine levels and protein degradation products concomitant with the increase of dietary biomarkers of plant-based foods.
- The consumption of plant foods typical of an MD can determine increase in urolithin production, decreased insulin resistance and bile acid levels, and such changes are all consistently related to baseline levels and variations of the microbial species involved in these specific metabolic features.

The Mediterranean diet (MD) is a recommended nutritional pattern with evidence of beneficial effects including the prevention of several types of disease, such as cardiovascular disease (CVD), type

Significance of this study

How might it impact on clinical practice in the foreseeable future?

- The results of this study clearly show that a change in dietary behaviour without any concomitant change in individual energy intake, macronutrient intake and physical activity can lower blood cholesterol, already after 4 weeks, in a population with cardiometabolic risk for unhealthy lifestyle.
- The beneficial dietary changes are in line with an increased adherence to MD and include a reduction of intake of meat and refined cereal products and increased intakes of fruit, vegetables, wholegrain cereal products, legumes and fish, along with a daily consumption of nuts.
- Some individuals harbour a gut microbiome that is more susceptible to MD-induced changes and experience further clinical advantages such as amelioration of insulin sensitivity and of inflammatory status.

2 diabetes, obesity, inflammatory diseases, degenerative diseases and cancer.^{3–5}

The microbiome partly but significantly affects individual metabolism and how one responds to changes in dietary habits.^{6,7} Host health is influenced by microbiome composition and by microbial metabolites that can be produced from host metabolic intermediates or from dietary precursors.⁸ Therefore, current trends in personalised nutrition suggest that diet can be used to modulate microbiome composition and function.^{9,10} Indeed, the production of beneficial microbial metabolites can be increased, and the production of detrimental metabolites can be reduced by modulating nutrient intake and supplying a beneficial pattern of key precursors to the microbiome.

The current knowledge of the role of diet on microbiome-mediated health outcomes in humans mainly relies on observational studies in which confounding factors affect the conclusions.¹¹ Intervention studies to address the causal effects of diet on microbiome functions are still scarce or have been performed in animal models,¹² and this lack of knowledge also applies to the MD.¹³ Despite their cost and labour-intensiveness, randomised controlled trials (RCTs) are the gold standard for evidence-based medicine and are an appropriate tool for identifying a causal relationship of a specific nutrient or diet on a health outcome in humans.^{14,15}

A Western diet is characterised by an excessive intake of foods with a high energy density and that are rich in fats, sugars and animal proteins, as well as a very low intake of fruits and vegetables. Such a dietary style, accompanied by low levels of physical activity, promotes inflammation¹⁶ and predisposes individuals to obesity, CVD, type 2 diabetes and metabolic syndrome.^{17,18} Because obesity is highly prevalent worldwide and is recognised as an independent risk factor for metabolic-driven chronic diseases, efforts need to be made urgently to provide evidence-based recommendations for healthy dietary patterns.

The aim of this study was to evaluate the effect of an individually tailored MD intervention in subjects at increased risk of cardiovascular disease.

MATERIALS AND METHODS**Study design and population**

We investigated the gut microbiome, faecal, blood and urinary metabolomic profiles in 82 overweight/obese subjects in response to an 8-week isocaloric dietary intervention with an MD or

a control diet. Each participant provided written informed consent and received no financial compensation. The trial was registered at ClinicalTrials.gov (number NCT03071718). The protocol ended when the last group of participants completed the protocol (Study Start Date: June 2016; Actual Primary Completion Date: July 2017; Actual Study Completion Date: February 2019).

The study design, selection criteria and participant flow throughout the study are reported in the online supplementary materials (online supplementary figure 1). Plasma lipids (including plasma cholesterol and triglycerides) and faecal levels of short-chain fatty acids (SCFAs) were registered as primary outcomes of the study, while changes in gut microbiota and some intermediate markers of metabolic disease, such as blood pressure, fasting blood glucose, serum high sensitivity C reactive protein (hs-CRP), urinary and plasma trimethylamine oxide (TMAO), plasma gastrointestinal peptides and urinary polyphenols, were secondary outcomes (detection methods described in the online supplementary material). Briefly, 334 potentially eligible adults were screened on the basis of the inclusion/exclusion criteria, including medical and lifestyle conditions (ie, habitual diet and physical activity) (detailed criteria are in the online supplementary material). Adherence to the MD was estimated by using the 11-unit dietary score and is reported as the Italian Mediterranean Index (MD index).¹⁹

Eighty-two subjects (43 female and 39 male, average body mass index (BMI) 31.1 ± 4.5 kg/m², age 43 ± 12 years, further baseline features in the online supplementary table 1) were selected, enrolled and randomised between the two intervention arms of the parallel study design, that is, MedD or ConD.

Dietary intervention

Each participant in the MedD group consumed an individually tailored diet that maintained the daily energy and macronutrient intake of the habitual diet and guaranteed a dietary pattern typical of the MD. Participants in the ConD group were asked to maintain their habitual diet. Individual compliance with the protocol was assessed every 2 weeks by self-recorded 7-day food diaries and physical activity questionnaires. Visits and sample collection were performed at baseline, 4 weeks and 8 weeks (full details reported in the online supplementary material).

Metabolomics

Untargeted urine, serum and faeces metabolomics,^{20,21} as well as targeted quantification of bile acids (BAs) and SCFAs in the faeces,^{22,23} were performed by ultra-high-performance liquid chromatography mass spectrometry. Trimethylamine N-oxide (TMAO), carnitine, choline, creatinine, betaine in plasma and urine, as well as urinary urolithins, were also determined by targeted metabolomics using liquid chromatography tandem mass spectrometry. Details are available in the online supplementary material and online supplementary tables 2–3.

Metagenomics

A full description of the sampling, sequencing and data analysis procedures is reported in the online supplementary material. DNA libraries were sequenced using the Ion Proton Sequencer (ThermoFisher Scientific, Waltham, USA), with a minimum of 20 million 150bp high-quality reads generated per library. Metagenomic species pangenome (MSP)²⁴ was used to identify and quantify species associated with the 9.9-million-gene integrated reference catalogue. The functional potentials of the intestinal gut microbiota were determined by using the in-house

FAnToMet pipeline as described in the online supplementary material.

Statistical analysis

Statistical analysis and visualisation were carried out in R environment V.3.4.2 (<https://www.r-project.org>). *ggpubr* and *PMCMR* R packages were used to assess significant differences. Variations in dietary and clinical variables at specific time points compared with baseline values between the MedD and ConD groups were evaluated by two-way analysis of variance with repeated measures and Tukey's post hoc test. Non-parametric Wilcoxon signed-rank test (*testRelations* function of *momr* R package) was performed to compare means between ConD and MedD subjects at each time point, while the post hoc Nemenyi test for multiple comparisons following the Friedman test was used within each group.

Pairwise Spearman's rank correlations were used to estimate the overall similarity of the microbiome and metabolome within the MedD and ConD groups and between time points (baseline vs 4 weeks and 4 weeks vs 8 weeks). The same test was applied to the microbiome, dietary variables, clinical markers and targeted metabolome datasets. Adjustments were performed using the Benjamini-Hochberg procedure. Correlations were visualised using the *ComplexHeatmap* package.²⁵

Machine learning-based classification²⁶ of metabolomics data and further details on data analysis and visualisation are provided in the online supplementary material.

Patient and public involvement

Patient and public involvement was not considered in this research.

Data availability

Metagenomic reads generated in this study are available (without conditions of reuse) under the accession number PRJEB33500 at the European Nucleotide Archive (ENA) in EBI (<https://www.ebi.ac.uk/ena/data/view/PRJEB33500>).

RESULTS

MD lowered plasma cholesterol in the overall population

No significant differences in anthropometric measures or clinical variables monitored in blood and urine samples were observed between the ConD (n=39) and MedD (n=43) groups at baseline (online supplementary table 1). Regarding the primary outcomes, as a consequence of the intervention, the participants in the MedD showed a significant decrease in total plasma cholesterol (figure 1) and high-density lipoprotein (HDL)-cholesterol after 4 weeks compared with the ConD group (online supplementary table 1). No changes in any of the secondary outcomes such as blood glucose, serum hs-CRP, plasma insulin, TMAO or any intermediate markers of metabolic disease (glucagon, ghrelin, GIP, GLP-1, leptin, C-peptide, resistin, visfatin and PAI-1) were observed (online supplementary table 1).

Successful compliance to the protocol and cholesterol decrease proportional to MD adherence rates

Adherence to the MD significantly increased in the MedD group at 4 and 8 weeks compared with the baseline (figure 1A) and was highly correlated with the Healthy Food Diversity (HFD) index²⁷ (online supplementary figure 2). Significant percentage changes in dietary and metabolic variables are shown in figure 1B. Participants in the MedD group significantly increased their daily intake of dietary fibre by twofold and their dietary vegetable:animal protein ratio by 2.5-fold over the intervention compared with the ConD group ($p<0.001$, figure 1C,D). A significant reduction in saturated fat intake and an increase in polyunsaturated fat intake was also achieved ($p<0.001$, figure 1E). These changes in nutrient intake in the MedD versus the ConD group were due to increased consumption of fruits, vegetables, nuts, wholegrain cereals and fish products concurrent with reduced consumption of refined cereals, dairy and meat products. The reduced consumption of meat products was confirmed by the reduction of the dietary intake biomarker of these foods in the MedD versus ConD group, that is, the concentration of carnitine in the plasma (14% and 11% reductions after 4 and 8 weeks,

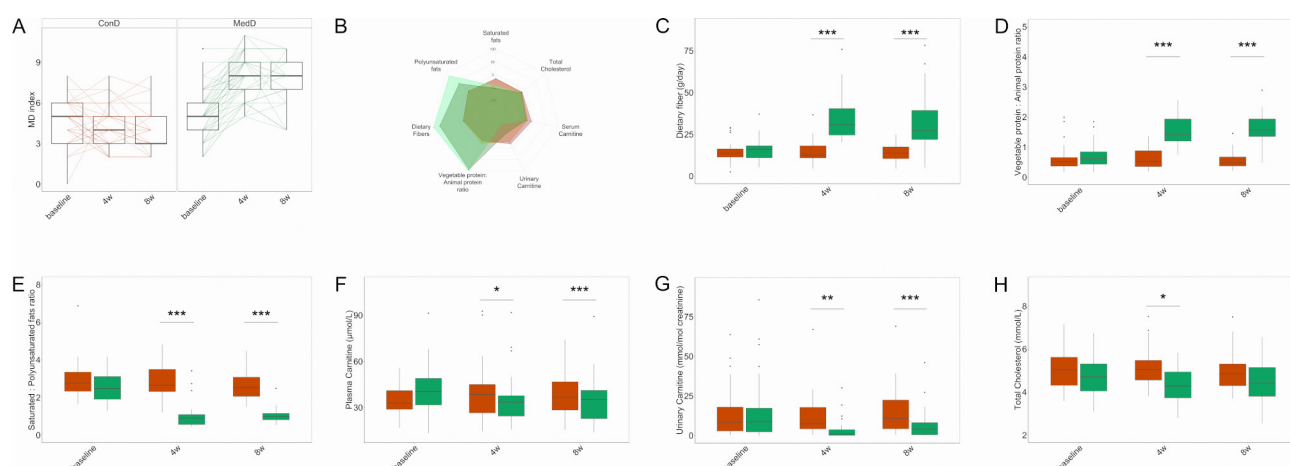


Figure 1 Adherence to the Mediterranean diet (MD) and changes in dietary and metabolic variables. (A) Box plots showing MD index score for controls (ConD) or treated subjects (MedD) during the intervention, the significance was tested by applying the post hoc Friedman-Nemenyi test for pairwise test of multiple comparisons within each group. (B) Percentage changes in dietary and metabolic variables are represented as spider chart. Changes in levels of dietary components consumption including (C) dietary fibre, (D) vegetable proteins/animal proteins ratio, (E) saturated to polyunsaturated fats ratio. Reduction in serum and urinary markers such as (F) plasma carnitine, (G) urinary carnitine and (H) total cholesterol. The significance was tested by applying unpaired Wilcoxon rank-sum tests for variation at the specific time point compared with baseline in MedD versus ConD. Orange boxes refer to controls and green boxes to Mediterranean subjects, respectively. Baseline, 0 weeks; 4w, 4 weeks; 8w, 8 weeks of nutritional intervention (* $p<0.05$, ** $p<0.01$ and *** $p<0.001$).

$p < 0.05$ and $p < 0.001$, respectively, figure 1F) and urine (75% and 51% reductions after 4 and 8 weeks, $p < 0.01$ and $p < 0.001$, respectively, figure 1G).

During the run-in period, some subjects (12 in the MedD group and 6 in the ConD group) undesirably increased their intake of fruit and vegetables above three servings/day compared with their consumption at the time of enrolment. We decided to strictly focus on the population who maintained a baseline dietary intake of fruits and vegetables < 3 servings/day. In addition, two subjects were not considered because not all the faecal samples were available. Therefore, the subsequent data analyses were carried out with a subgroup of 62 subjects, 32 in the ConD group and 30 in the MedD group. High compliance with the intervention was confirmed in both groups. Changes in dietary intake of nutrients from several food categories are shown in online supplementary figure 3. The effect of MedD on plasma cholesterol was confirmed in this subgroup. Indeed, following 4 weeks of intervention with an MD, a significant reduction ($p = 0.03$) in plasma LDL-cholesterol from 2.90 ± 0.13 mmol/L at baseline to 2.66 ± 0.12 mmol/L at 4 weeks was observed in the MedD group compared with the change in the ConD group (3.24 ± 0.13 mmol/L at baseline to 3.25 ± 0.12 mmol/L at 4 weeks), and a significant reduction ($p = 0.02$) in plasma HDL-cholesterol from 1.26 ± 0.05 mmol/L at baseline to 1.18 ± 0.04 mmol/L at 4 weeks was observed in the MedD group compared with the change in the ConD group (1.21 ± 0.05 mmol/L at baseline to 1.25 ± 0.05 mmol/L at 4 weeks).

Interestingly, decrease in cholesterol levels was proportional to MD adherence rate. By applying a linear model, it was found that each unit increase in the MD index corresponded to $\approx 2\%$ reduction in total plasma cholesterol ($p = 0.003$, online supplementary figure 4), a 2% reduction in plasma LDL-cholesterol ($p = 0.01$) and 1% reduction in plasma HDL-cholesterol ($p = 0.04$) after adjustment for age, sex, BMI and energy intake.

Diet-induced metabolome changes with release of biomarkers of MD consumption

We measured approximately 11 000 molecular features in all our participants during the intervention (2200 in faeces, 4125 in blood and 4645 in urine). A list of annotated metabolites is provided in online supplementary table 4 and the evidence substantiating the annotation of diet-responsive metabolites is provided in online supplementary material (online supplementary figures 5–25). Clear shifts in the metabolomic profiles were observed in the MedD group after the intervention compared with the baseline conditions and the ConD group (figure 2). Decreasing Spearman's correlation coefficients (4 weeks vs baseline; 8 weeks vs baseline) indicated a significant change in the urine metabolic profiles after 4 ($p = 0.01$) and 8 weeks ($p = 0.01$) of intervention in the MedD group versus the ConD group. In order to validate the robustness of the shifts observed, we used a machine learning-based classification approach²⁶ (area under the curve (AUC) = 0.88 and 0.87 between the ConD and MedD groups at 4 and 8 weeks, respectively; as a control, AUC = 0.52 was observed at baseline), which supported the metabolome changes found. In agreement with the replacement of refined cereal with wholegrain products and the replacement of meat, eggs and dairy products with fishery products, legumes and provided nuts, we found increased levels of the biomarkers of wholegrains (3-(3,5-dihydroxyphenyl) propanoic acid-glucuronide),²⁸ legumes (tryptophan betaine),²⁹ vegetables/berries (oxindole-3-acetic acid)³⁰ and nuts (urolithins)³¹ in the MedD group, while biomarkers of meat (carnitine),³² BAs, leucine and isoleucine were more closely linked to the ConD group (figure 2). Notably, no change in urine or serum TMAO was observed, possibly due to contrasting effects of increasing intake of fish and lowering meat-derived proteins in the MedD group.

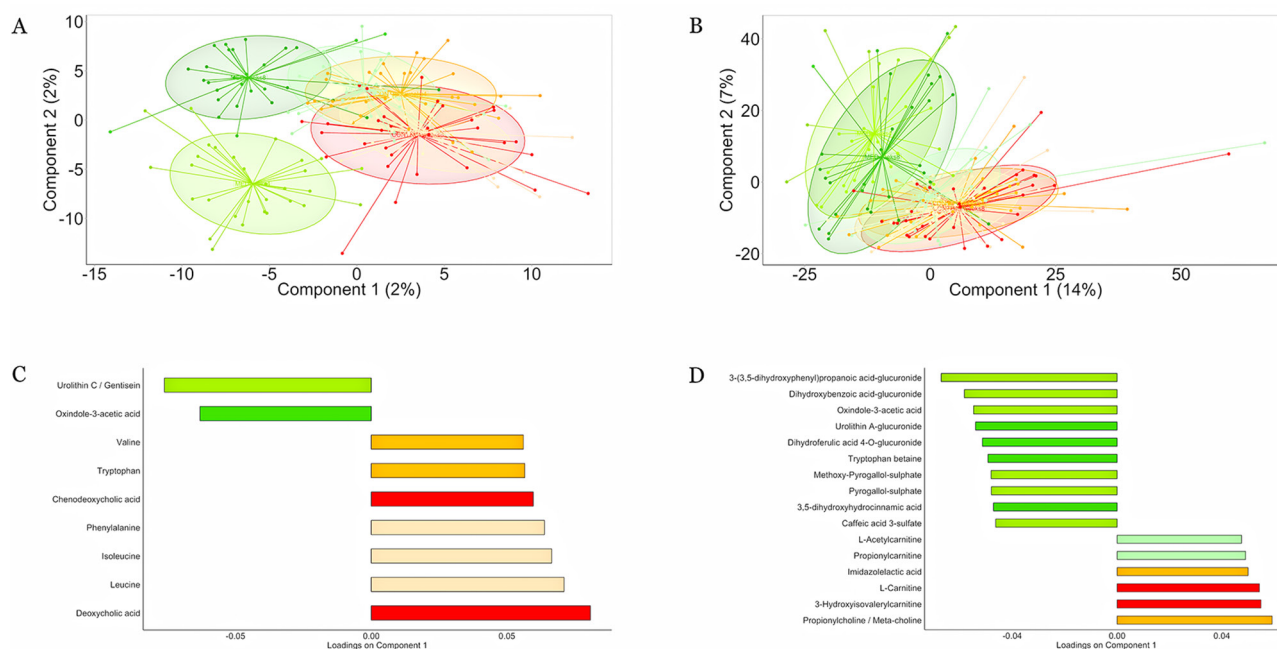


Figure 2 Mediterranean diet changes the intestinal and systemic metabolome. Partial least squares discriminant analysis plots based on molecular features detected in (A) faeces and (B) urine. Subjects belonging to different categories were coloured according to diet and time points: MedD subjects at baseline (light green), after 4 weeks (green) and 8 weeks of intervention (dark green). ConD subjects at baseline (light orange), after 4 weeks (orange) and 8 weeks (dark orange) of intervention. The loading plots display vectors that contributed the most to variability of individual dataset; variables explaining the variance between the groups in (C) faecal and (D) urine metabolome are reported as bar plots.

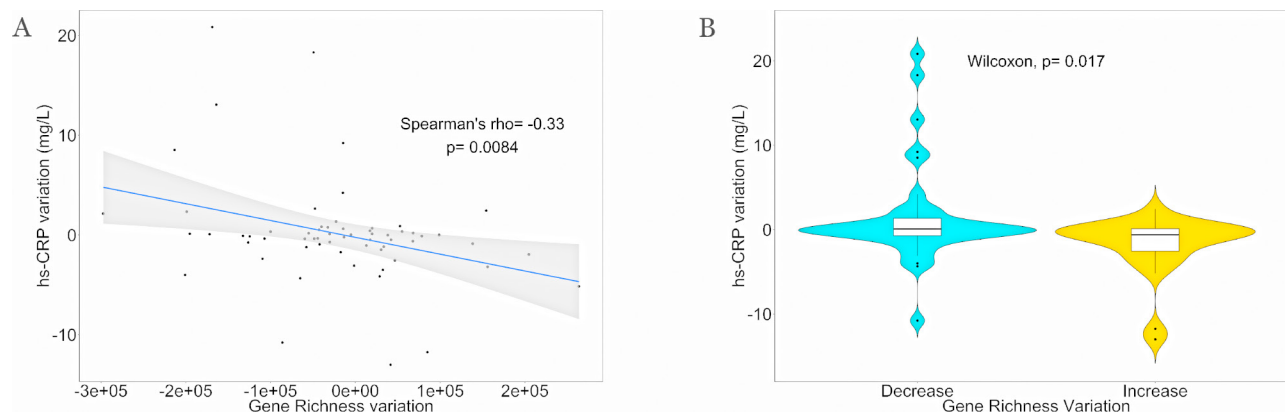


Figure 3 Microbial diversity richness anticorrelates with inflammation. (A) Spearman's correlation between variation of gut microbial gene richness and individual inflammatory status (serum hs-CRP) variation at the end of trial; n observation=62. (B) Violin plot showing differences in serum hs-CRP variation between subjects increasing ($n=25$, yellow) compared with subjects decreasing ($n=37$, light blue) gene richness at the end of trial. Statistical differences between groups were determined using unpaired Wilcoxon rank-sum tests. hs-CRP, high sensitivity C reactive protein.

MD-mediated increase in biomarkers of wholegrain (benzoxazinoids, pipercolic acid betaine), vegetable (oxindole-3-acetic acid), legume (tryptophan betaine, pyrogallol-sulfate), nuts (urolithins) and fish (3-carboxy-4-methyl-5-propyl-2-furanpropionic acid) consumption and decrease in meat (carnitine) and protein degradation products such as branched-chain amino acids (BCAAs), aromatic amino acids, *N*-acetylcadaverine and microbial-derived proteolysis products (*p*-cresol sulfate, indoxyl sulfate, phenylacetylglutamine) was further confirmed by their significant links with the MD index (online supplementary figure 26). Finally, a range of host-derived short-chain and medium-chain acylcarnitines was significantly reduced in the urine following the MD intervention, indicating a shift in substrates for energy metabolism from fat to complex carbohydrates and protein.³³

Gut microbiome composition is modulated by adherence to the MD

Gene (average= $5\,551\,310.5 \pm 120\,191$) and MSP richness (average= 230.9 ± 53.1) metrics were maintained during the intervention. However, a significant inverse correlation was found between the variation in gut microbial gene richness and individual inflammatory status evaluated by serum hs-CRP variations (figure 3A). Subjects showing increased gene richness displayed significantly lower levels of serum hs-CRP after 8 weeks of the dietary intervention (figure 3B).

The increased adherence to the MD in the first 4 weeks corresponded to a decrease in the microbiome similarity in the MedD group during the same time interval, suggesting a MD-induced rearrangement of the gut microbiome composition (figure 4). This change was not observed either in the ConD group over the entire intervention or in the MedD group between 4 weeks and 8 weeks, that is, in intervention conditions when participants did not change their adherence to the MD.

While a negligible number of differentially abundant MSPs was found at baseline between the ConD and MedD groups ($n=27$ MSPs, online supplementary figure 27 and online supplementary table 5), more contrasting species were observed at 4 ($n=77$ MSPs) and 8 weeks ($n=44$ MSPs, online supplementary figures 28 and 29, online supplementary table 5), with the proportion of contrasting species consistently linked to the MD adherence evaluated by MD index (online supplementary tables 5 and 6).

During the increasing MD adherence phase (baseline–4 weeks), *Ruthenibacterium lactatiformans*, *Flavonifractor plautii*,

Parabacteroides merdae, *Ruminococcus torques* and *Ruminococcus gnavus* were significantly reduced in the MedD compared with the ConD group, along with *Streptococcus thermophilus*, a well-known marker of dairy product consumption. In contrast, five members of the *Faecalibacterium prausnitzii* clade were enriched in the MedD compared with the ConD group at either 4 or 8 weeks (online supplementary tables 5 and 6), along with several members of the *Roseburia* and *Lachnospiraceae* taxa. Consistently, MSPs enriched in the MedD group after 4 weeks were significantly linked to MD food biomarkers (online supplementary figure 30).

While only five gut metabolic modules (GMMs) were different between the diets (4% of functional potential variation; online supplementary table 7) at baseline, 18% variation in the metabolic potential captured by GMM was observed after 4 weeks. Several GMMs ($n=19$) were enriched in the MedD group, mainly including pathways related to amino acid and carbohydrate degradation. The pathways also included triglyceride and glycoprotein degradation and conversion of acetyl-CoA and glutamate degradation, both leading to crotonyl-CoA, a possible precursor of butyrate metabolism (online supplementary table 7). Although only 6% variation was observed after 8 weeks, enrichment in glutamate degradation to crotonyl-CoA was maintained in the MedD group. This pathway was significantly linked to the levels of *F. prausnitzii* msp_0388 (Spearman's $\rho=0.73$, $p<10^{-6}$, online supplementary figure 30).

Altogether, by integrating the three meta-omics datasets,³⁴ we observed a separation of the ConD and MedD groups on the basis of microbiome diversity, functional modules and metabolomic profiles (Hotelling $T^2=40.95$, $p<7.038 \times 10^{-12}$; online supplementary figure 31) corroborating the changes induced by the MD intervention.

MD intervention affects microbiome functions

We measured a number of metabolites associated with gut microbial metabolism to investigate the effect of the MD dietary intervention on health-related microbial activities. Urinary levels of urolithin glucuronides increased in the MedD compared with the ConD group (table 1). Such increase was consistently linked with the levels of urolithin producers in the microbiome, including, among others, members of the *Eggerthellaceae* family (online supplementary table 8), and with the consumption of nuts that were the sole dietary source of ellagitannins significantly increased in the MedD group. Interestingly, urolithin production

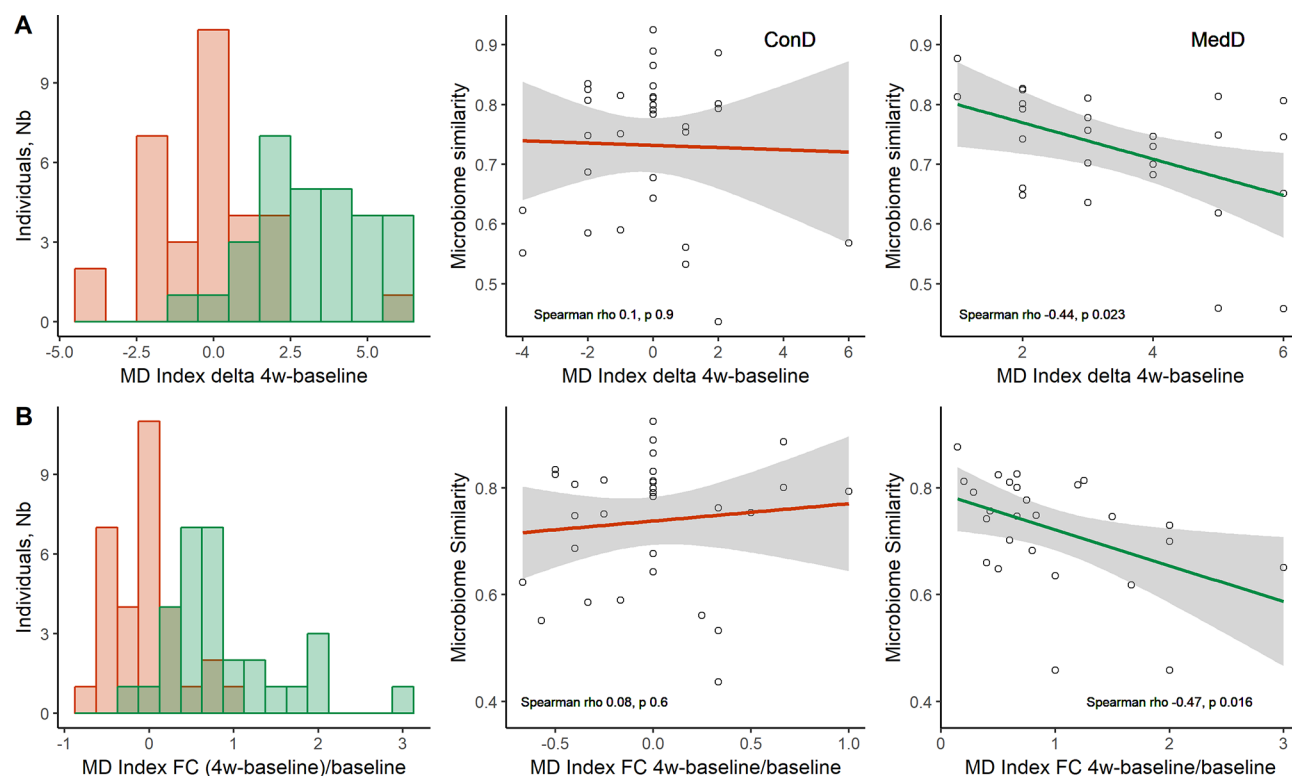


Figure 4 Mediterranean diet (MD) affects gut microbiome composition. (A) Total delta MD index changes over the 4w-baseline period. Left: histograms of delta (4w-baseline) MD index ($n=62$). Right: linear regressions of microbiome similarity compared with delta 4w-baseline MD index. Microbiome similarity was estimated by Spearman correlations between microbial composition at 4 weeks and baseline within each individual. (B) Total MD index fractional changes (FCs) (4w-baseline)/baseline, used as proxy to measure the effort of adherence ($n=62$). Left: distribution of individuals relative to MD index FC. Right: linear regressions of microbiome similarity and FC. Spearman correlations (ρ and p values) are reported, excluding outliers, for ConD ($n=31$) and MedD ($n=26$) groups, respectively. 4w, 4 weeks.

was negatively correlated with serum hs-CRP, triglycerides, body fat mass, body weight, BMI and urinary carnitine (false discovery rate (FDR) <0.05 , online supplementary figure 32).

Compared with the baseline values, a significant reduction in faecal concentrations of total BAs, including both primary and secondary BAs, was observed in the MedD group on the MD intervention (figure 5A–C). In addition, faecal deoxycholic acid was significantly reduced after 4 ($p<0.01$) and 8 weeks of the intervention ($p<0.01$) along with faecal lithocholic acid ($p<0.05$ and $p<0.01$ after 4 and 8 weeks, respectively) within the MedD group. Paired Wilcoxon rank-sum tests of faecal BA concentrations within each intervention group are shown in online supplementary figure 33. A comparison of faecal BA concentrations between the MedD and ConD groups after 8 weeks showed a significant reduction in faecal chenodeoxycholic acid ($p<0.05$). Accordingly, primary and secondary BAs in the faeces were positively linked to proteins and fats from

animal-based food products, as well as systolic blood pressure, BMI, body weight and urinary carnitine (FDR <0.05 , online supplementary figure 32).

It was also noteworthy that subjects showing the highest reduction in total BAs and the secondary/primary BA ratio had higher baseline levels of *Bilophila wadsworthia*, which decreased significantly after 4 weeks of the intervention ($p<0.05$, figure 5D).

Despite the twofold increase in dietary fibre intake, no changes in faecal concentrations of the main SCFAs acetate, butyrate and propionate were observed. However, significant reductions in branched-chain fatty acids (BCFAs), such as valerate, isovalerate, isobutyrate and 2-methylbutyrate, were observed in the faeces of the participants in the MedD group over the intervention (figure 6A–D), and these changes mirrored the increased intake of plant-based foods (FDR <0.05 , online supplementary figure 32). Moreover, subjects in the quartile of the highest faecal butyrate increase at 4 weeks showed

Table 1 Urinary urolithins-glucuronides levels (ng/ μ mol creatinine) detected over the study period

	MedD			ConD			P values	
	Baseline	4	8w	Baseline	4w	8w	$\Delta_{(4w - baseline)}$	$\Delta_{(8w - baseline)}$
Urolithin-A-glucuronide	30.8 \pm 37.7	139.8 \pm 296.8	214.4 \pm 358	5.4 \pm 30.5	6.9 \pm 35.7	5 \pm 28.3	0.013	0.025
Urolithin-B-glucuronide	0.1 \pm 0.7	21.7 \pm 60.6	74.1 \pm 243.4	0.2 \pm 0.9	0.1 \pm 0.5	5.4 \pm 30.6	0.0073	0.086
Urolithin-C-glucuronide	1.6 \pm 8.8	46.8 \pm 107.2	43.2 \pm 176.6	0 \pm 0	0 \pm 0	0 \pm 0	0.021	0.16
Total urolithins (A+B+C)	32.6 \pm 91.8	208.3 \pm 373.8	336.7 \pm 594.3	5.5 \pm 30.5	7 \pm 35.7	10.4 \pm 41	0.00034	0.033

Data are expressed as mean \pm SD. P values refer to variation at the specific time point compared with baseline in MedD versus ConD measured by unpaired Wilcoxon rank-sum test.

4w, 4 weeks; 8w, 8 weeks.

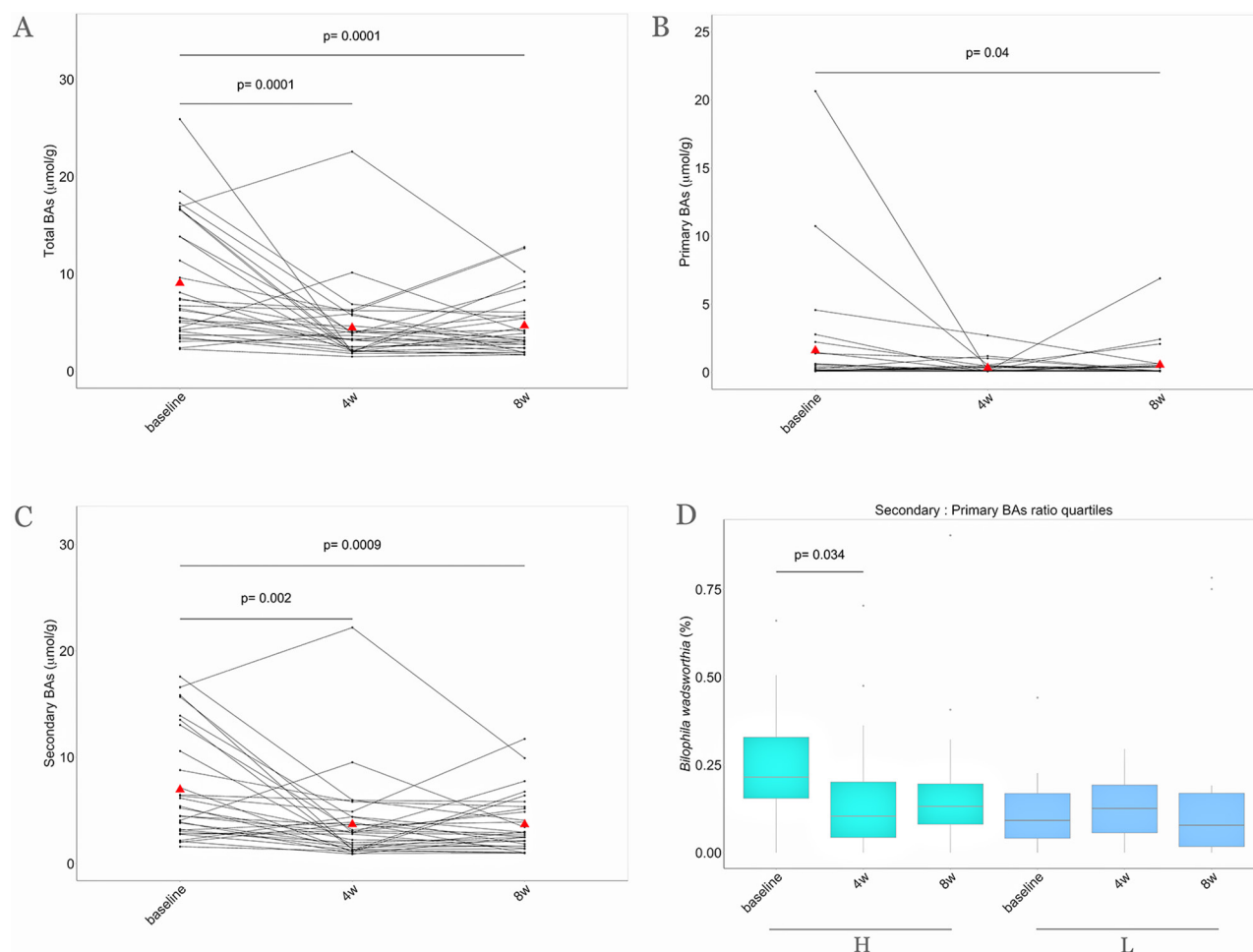


Figure 5 Faecal BA concentrations over the nutritional intervention. Parallel coordinate plot showing variations of faecal (A) total, (B) primary and (C) secondary BA concentrations within the MedD group during the intervention. The red triangles indicate mean values, the lines connecting dots are used to indicate the same sample at each time point. The significance was tested by applying the post hoc Friedman-Nemenyi test for pairwise test of multiple comparisons within each group. (D) In the box plot the relative abundances of *Bilophila wadsworthia* are compared considering subjects falling in the highest quartile ($n=16$, green) and in the lowest quartile of reduction ($n=16$, blue) of secondary to primary BAs ratio after 4 weeks of treatment. Baseline, 0 weeks; 4w, 4 weeks; 8w, 8 weeks of nutritional intervention. H, highest quartile of reduction; L, lowest quartile of reduction; BAs, bile acids.

consistently higher levels of *F. prausnitzii* and *Lachnospiraceae* taxa (figure 6E,F).

Variation in insulin resistance is linked to baseline levels of specific microbial taxa

The Homeostatic Model Assessment for Insulin Resistance (HOMA) was calculated as a measure of insulin resistance, and it did not change as a result of the intervention (online supplementary table 1). However, by stratifying the subjects by the variation in HOMA at 4 weeks compared with baseline, we found that subjects who reduced their HOMA on the MD intervention had significantly higher baseline levels of several *Bacteroides* species (including *B. uniformis* and *B. vulgatus*, $p < 0.05$) and lower *Prevotella* sp. and *P. copri* levels ($p < 0.05$) than subjects who did not exhibit changes in HOMA over time (online supplementary figure 34). Interestingly, *P. copri* baseline levels showed a positive correlation with HOMA variation over the intervention (Spearman's $\rho = 0.28$; $p = 0.031$).

Consistently, when we computed co-abundance groups (CAGs) from 16S rRNA gene sequencing analysis, we found significantly lower levels of CAG2 (including *Prevotella* as the most abundant genus) in subjects who exhibited reduced HOMA, while levels

of CAG4 (including *Faecalibacterium*, *Roseburia*, *Bacteroides*, other Clostridia) were significantly higher at baseline in participants who exhibited reduced HOMA and increased on dietary treatment (online supplementary figure 35).

The pangenome of the HOMA and serum hs-CRP-associated species (*P. copri*, *F. prausnitzii*, *B. uniformis*, *B. vulgatus*) was further investigated. No clear differences were found according to intervention, increase in dietary fibre consumption or decrease in HOMA indicating a high subject specificity at the strain level (online supplementary figure 36).

DISCUSSION

This study clearly shows that a change from a Western diet to a Mediterranean dietary pattern, without any concomitant change in energy intake, macronutrient intake or physical activity, modulates individual clinical outcomes, the gut microbiome and metabolome after 4 weeks of the intervention in a population with cardiometabolic risk due to unhealthy lifestyle.

Each participant in the MedD group received a diet that was tailored to his/her habitual energy and macronutrient intake to increase the adherence to a typical MD pattern. In other words, each subject was instructed on the exact replacements of foods

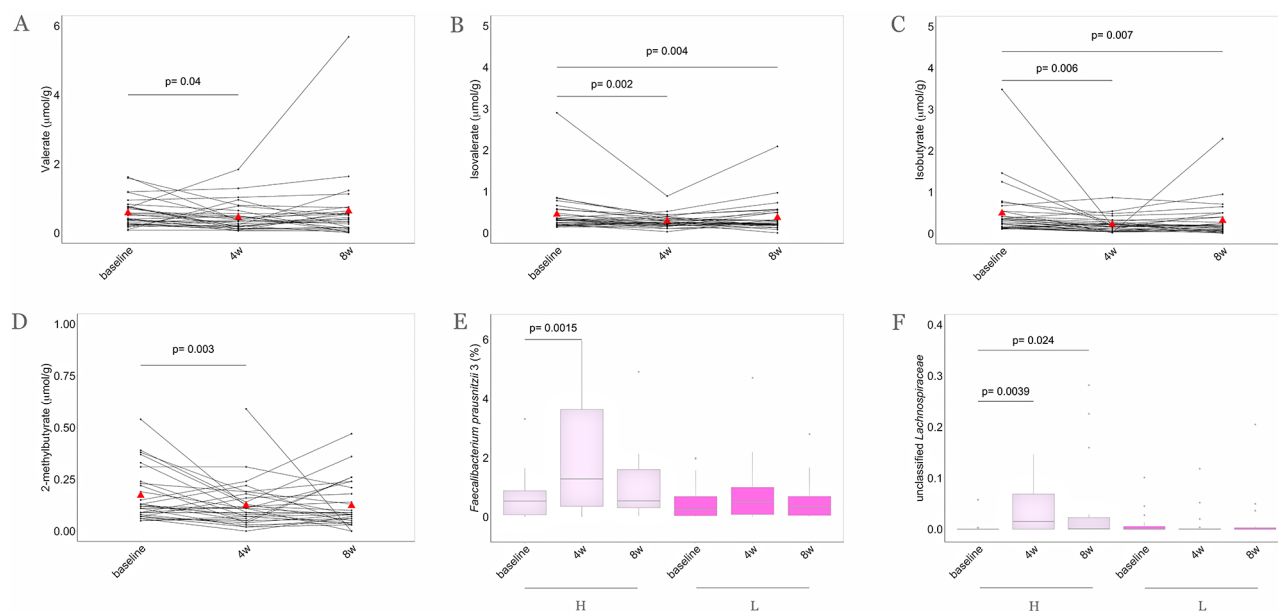


Figure 6 MD intervention determines a reduction of faecal branched-chain fatty acid (BCFA) concentrations and higher levels of *Faecalibacterium prausnitzii* and *Lachnospiraceae* taxa. Parallel coordinate plot showing variations of (A) valerate, (B) isovalerate, (C) isobutyrate and (D) 2-methylbutyrate faecal concentrations within MedD population. The red triangles indicate mean values, the lines connecting dots are used to indicate the same sample at each time point. The significance was tested by applying the post hoc Friedman-Nemenyi test for pairwise test of multiple comparisons within each group. In the box plots, the relative abundances of (E) *F. prausnitzii* 3 and (F) *Lachnospiraceae* family are compared considering subjects falling in the highest quartile (n=16, violet) and in the lowest quartile (n=16, purple) of faecal butyrate increase after 4 weeks of treatment. Statistical differences between groups were determined using Wilcoxon rank-sum tests. Baseline, 0 weeks; 4w, 4 weeks; 8w, 8 weeks of intervention. H, highest quartile of increase; L, lowest quartile of increase.

so that specific amounts of Western diet foods were replaced by the ones typical of an MD. At the best of our knowledge, this approach has not been previously used in intervention studies with the MD. This ensured that changes in metabolic markers, the gut microbiome and systemic metabolome were not biased by variation in energy intake over the nutritional intervention.

From a clinical perspective, the data show that within a short period, consumption of an MD can lower total, LDL-cholesterol and HDL-cholesterol in plasma independently of individual energy intake and physical activity level. The decrease in LDL-cholesterol (by 0.24 mmol/L, -8.3% vs baseline) associated with MD in this study is far from the reduction of 1 mmol/L that has been indicated as clinically relevant towards a reduction in heart disease risk.³⁵ However, it is higher than the average reductions (between 0.11 and 0.23 mmol/L) that have been found in RCTs comparing meat-based diets with plant-protein-based diets including nuts or legumes separately,³⁶ as well as those achievable (~0.1 mmol/L) with diets including ellagitannins or anthocyanins.³⁷ Interestingly, in the current study, the cholesterol-lowering effect was linearly associated with individual adherence to the MD. The relationship between the MD index and plasma cholesterol highlights the importance of the whole MD pattern and of individual dietary compliance in eliciting the hypolipidaemic effect of the MD. We hypothesise that the lower dietary intakes of cholesterol ($p < 0.0001$ at 4 and 8 weeks vs baseline) and saturated fats ($p = 0.005$ at 4 and at 8 weeks vs baseline) on the MD intervention are the main factors responsible for that effect.³⁸

Adherence to the MD was confirmed by comprehensive untargeted metabolic profiling of faeces, serum and urine, as well as targeted quantification of selected biomarkers. In agreement with the MD pattern, we found increased levels of biomarkers of wholegrains, legumes, vegetables and nuts, as well as reduced

concentrations of biomarkers of meat and protein degradation products after the MD intervention. These objective measures substantiated the dietary records obtained by the Food Frequency Questionnaires (FFQ) and the 7-day food diary. The MD-dependent metabolome shift was particularly evident in the urine metabolome due to the accumulation of diet-derived metabolites of wholegrains, nuts and vegetables. In addition, a range of short-chain and medium-chain acylcarnitines were consistently reduced in urine following the MD intervention, suggesting a diet-induced shift in energy production from beta-oxidation to glycolysis in the mitochondria, probably due to an extended period of carbohydrate availability due to a steady release from fibre degradation. In agreement with these findings, plasma short-chain acylcarnitines have been associated with a Western diet³⁹ and have been found in higher concentrations in meat eaters than in vegetarians and vegans,⁴⁰ and urine levels of acylcarnitines were reduced with increased wholegrain intake.⁴¹ Since acylcarnitines have been associated with an increased risk of CVD,⁴² the reduction in acylcarnitines in urine suggests a beneficial MD-induced effect on energy metabolism caused by increased intake of dietary fibre.

Overall, the differences in the faecal metabolome associated with the intervention reflect the replacement of foods of animal origin with plant-based foods following MD adherence. Oxindole-3-acetic acid, a naturally occurring auxin in plants,⁴³ as well as the BCAAs leucine and isoleucine and BAs appeared to be the main drivers. BAs can be implicated in atherosclerosis, diabetes and other cardiometabolic diseases.⁴⁴ Targeted quantification of faecal BAs confirmed a significant reduction in their concentrations within the MedD group coherently with the reduced intake of meat products. In line with these findings, a vegan diet has been found to reduce plasma BCAAs and BAs in comparison with the levels associated with an animal-based diet.⁴⁵

High adherence to an MD has also been associated with increased faecal concentrations of SCFAs.⁴⁶ Despite the fact that participants doubled their intake of dietary fibre, the MD intervention did not significantly increase the faecal concentrations of SCFAs. Faecal SCFA represents the difference between the production and absorption or utilisation of SCFAs in the colon and rectum. We speculate that a possible improved gut epithelial function may have increased SCFAs utilisation and absorption, thus hampering the observation of their increase due to higher fibre intake. This result was corroborated by recent findings that dysbiosis is associated with increased faecal SCFA excretion.⁴⁷ The MD decreased faecal concentrations of BCFAs, including valerate, which is in agreement with previous studies reporting faecal valerate as linked to the consumption of protein-rich animal foods⁴⁶ and not to MD adherence.⁴⁸ These results suggest an altered colonic proteolytic fermentation caused by the replacement of animal-based products with plant-based foods. This finding was substantiated by microbial-derived proteolytic products being reduced with increased MD adherence. An interesting increase in urolithins was observed in the MedD group.

Urolithins are gut microbial metabolites of ellagitannins.⁴⁹ Dietary sources of these polyphenols are berries, pomegranate and walnuts. However, our data indicated that only nut consumption significantly increased over the intervention with MD. Therefore, increase in urinary urolithin glucuronides was most likely attributed to the intake of walnuts in our study as previously reported by others.⁵⁰ Recently, urolithin A has been shown to improve intestinal barrier function in a preclinical model⁵¹ and has also been associated with lower cardiometabolic risk.⁵² In addition, urolithin A has been demonstrated to be involved in the prevention of prostate,⁵³ endometrial⁵⁴ and breast cancer⁵⁵ in vitro. Interestingly, in our intervention study, urolithin levels were negatively correlated with cardiometabolic risk factors such as triglycerides and BMI and these observations further corroborate the hypothesis that an MD dietary pattern might beneficially impacts human health status through gut microbiota metabolism.

It was recently reported that microbiome composition is more associated with specific food choices than with nutritional patterns, that food–microbe interactions are highly personalised, and that these factors might limit the observation of overall microbiome responses to specific diets.⁹ Interestingly, despite such insightful evidence, we observed clear microbiome shifts following our dietary intervention protocol.

Gut microbial taxonomic and functional composition in our isocaloric MD intervention revealed that the overall microbial richness was maintained, which is consistent with recent studies showing similar trends after increased consumption of wholegrain.^{28,56} However, we observed that the MD dynamically modulates the intestinal microbiome composition and that the microbiome variations are proportional to the increase in MD adherence rates.

Even though prior studies addressed the link between diet, gene richness and inflammation markers,^{7,28,56–58} intervention studies describing variation of the microbial genetic richness following an MD dietary pattern have not previously been described. Interestingly, here MD improves the inflammatory status of individuals experiencing an increase in gut microbiome gene richness during controlled energy and modified macronutrient intakes, further supporting the idea that MD might be an efficient dietary strategy to reduce inflammation.^{13,59}

The MD intervention protocol determined a decline in *R. torques* and *R. gnavus*. The latter species has been recently demonstrated as a proinflammatory species due to secretion of

a polysaccharide that induces tumour necrosis factor alpha in dendritic cells,⁶⁰ whereas possible involvement of *R. torques* in inflammation remains largely uncertain and is currently based on associations.^{61,62}

Subjects with the highest reduction in faecal BAs consistently also exhibited reduced relative abundance of *Bilophila wadsworthia*, which was previously linked to higher BA levels,⁶³ animal-based and high fat diets, as well as irritable bowel diseases.^{7,64} This decline was accompanied by an increase in several potentially beneficial species, including the fibre-degrading *F. prausnitzii*, *Roseburia* and members of the *Clostridiales* and *Lachnospiraceae* taxa, linked to butyrate precursor functional pathways. These reportedly beneficial species were previously documented for their anti-inflammatory properties and their role in the development of the intestinal barrier^{65,66} and were, in the present study, found to be boosted by foods recommended as part of a healthy MD nutritional pattern.

Our data also show that an MD-tailored dietary intervention might be helpful in ameliorating insulin sensitivity in individuals harbouring higher levels of several *Bacteroides* species and lower levels of *Prevotella* sp. and *P. copri*. The association of *P. copri* with insulin resistance was already reported by Pedersen *et al.*⁶⁷ and it was recently demonstrated to be strain-dependent and correlated with the occurrence of genes involved in BCAA biosynthesis.⁶⁸

These findings are in line with the concept of personalised responses of individuals to similar diets, and they are of importance for clinical practice in the era of precision medicine and personalised nutrition.^{9,12}

Taken together, our results indicate that an MD may remodel the intestinal microbiome towards a state that promotes metabolic and cardiovascular health. In addition, our observations can be useful to plan baseline stratifications of subjects based on microbiome composition to select specific metabolotypes that could be involved in ad hoc nutritional interventions to potentiate the clinical outcomes.

Author affiliations

- ¹Université Paris-Saclay, INRAE, MGP, Jouy-en-Josas, France
- ²Department of Agricultural Sciences, University of Naples Federico II, Portici, Italy
- ³Department of Nutrition, Exercise and Sports, Faculty of Science, University of Copenhagen, Frederiksberg, Denmark
- ⁴Task Force on Microbiome Studies, University of Naples Federico II, Naples, Italy
- ⁵Institute of Food Science of CNR, Avellino, Italy
- ⁶Department of Clinical Medicine and Surgery, University of Naples Federico II, Naples, Italy

Twitter Manolo Laiola @hroager, Henrik Munch Roager @hroager and Danilo Ercolini @DaniloErcolini

Acknowledgements We would like to thank Agria Spa, Fiammante Icab Spa, Olio Dante Spa and Pastificio Di Martino Spa for kindly providing foods to be included in the food boxes for our participants. The authors would also like to thank Cecilie Fryland Appeldorff, Sofie Skov Frost and Sarah Fleischer Ben Soltane (University of Copenhagen) for excellent technical assistance on ultra-high-performance liquid chromatography mass spectrometry (UHPLC-MS) analyses; Sébastien Fromentin, Magali Berland, Kévin Da Silva and Florence Thirion (MGP Metagenopolis) for their distinguished bioinformatical guidance; and Dr Maria Assunta Gallo and Centro Diagnostico San Ciro (Portici, Italy) for their support with the clinical visits.

Contributors The principal investigators (DE, DSE and LOD) and the other coapplicants (PV) designed the study and obtained funding. The nutritional intervention was carried out at the University of Naples Federico II by AAR, RG, IM and PV. HMR, RF, LOD and PV generated and analysed the metabolomics data. HR and BQ generated the metagenomics data, and ML, VM, FDF, EP and NP analysed the microbiome data and performed the statistical analyses. All authors participated in the data interpretation; DE wrote the paper with contributions by ML, HMR, PV and VM. All authors read, edited and approved the final version of the manuscript. The corresponding authors attest that all listed authors meet authorship criteria and that no others meeting the criteria have been omitted.

Funding The study was conducted within the Diet-Induced Arrangement of the gut Microbiome for the Improvement of Cardiometabolic health (DINAMIC) project funded within the European Joint Programming Initiative “A Healthy Diet for a Healthy Life” (JPI HDHL) – Joint Action “Intestinal Microbiomics”. The DINAMIC national funding organization are (i) the Italian Ministry of Education, University and Research (prot.0000426); (ii) the Innovation Fund Denmark (grant # 5195-00001B) and (iii) the French National Research agency (ANR-15-HDIM-0002-04). The study was also partially supported by a Semper Ardens grant to Lars Ove Dragsted and the Metagenopolis grant ANR-11-DPBS-0001. A full list of the DINAMIC consortium and their affiliations appears in the online supplementary material.

Competing interests None declared.

Patient consent for publication Not required.

Ethics approval The trial was conducted at the University of Naples Federico II and was approved by the related Ethics Committee (Protocol number: 108/16)

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available in a public, open access repository. Metagenomic reads generated in this study are available (without conditions of reuse) under the accession number PRJEB33500 at the European Nucleotide Archive (ENA) in EBI (<https://www.ebi.ac.uk/ena/data/view/PRJEB33500>).

Open access This is an open access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited, appropriate credit is given, any changes made indicated, and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>.

ORCID iDs

Henrik Munch Roager <http://orcid.org/0000-0002-2504-8313>

Lars Ove Dragsted <http://orcid.org/0000-0003-0609-6317>

Stanislav Dusko Ehrlich <http://orcid.org/0000-0002-7563-4046>

Danilo Ercolini <http://orcid.org/0000-0003-3061-9560>

REFERENCES

- Kelly JT, Palmer SC, Wai SN, *et al.* Healthy dietary patterns and risk of mortality and ESRD in CKD: a meta-analysis of cohort studies. *Clin J Am Soc Nephrol* 2017;12:272–9.
- Chiavaroli L, Viglianiouk E, Nishi SK, *et al.* Dash dietary pattern and cardiometabolic outcomes: an umbrella review of systematic reviews and meta-analyses. *Nutrients* 2019;11:338.
- Martínez-González MA, Gea A, Ruiz-Canela M. The Mediterranean diet and cardiovascular health. *Circ Res* 2019;124:779–98.
- Bendall CL, Mayr HL, Opie RS, *et al.* Central obesity and the Mediterranean diet: a systematic review of intervention trials. *Crit Rev Food Sci Nutr* 2018;58:3070–84.
- Eleftheriou D, Benetou V, Trichopoulou A, *et al.* Mediterranean diet and its components in relation to all-cause mortality: meta-analysis. *Br J Nutr* 2018;120:1081–97.
- Caní PD. Human gut microbiome: hopes, threats and promises. *Gut* 2018;67:1716–25.
- David LA, Maurice CF, Carmody RN, *et al.* Diet rapidly and reproducibly alters the human gut microbiome. *Nature* 2014;505:559–63.
- Roager HM, Dragsted LO. Diet-derived microbial metabolites in health and disease. *Nutr Bull* 2019;44:216–27.
- Johnson AJ, Vangay P, Al-Ghalith GA, *et al.* Daily sampling reveals personalized Diet-Microbiome associations in humans. *Cell Host Microbe* 2019;25:789–802.
- Bashiardes S, Godneva A, Elinav E, *et al.* Towards utilization of the human genome and microbiome for personalized nutrition. *Curr Opin Biotechnol* 2018;51:57–63.
- Grosso G, Micek A, Godos J, *et al.* Health risk factors associated with meat, fruit and vegetable consumption in cohort studies: a comprehensive meta-analysis. *PLoS One* 2017;12:e0183787.
- De Filippis F, Vitaglione P, Cuomo R, *et al.* Dietary interventions to modulate the gut Microbiome-How far away are we from precision medicine. *Inflamm Bowel Dis* 2018;24:2142–54.
- Bailey MA, Holscher HD. Microbiome-mediated effects of the Mediterranean diet on inflammation. *Adv Nutr* 2018;9:193–206.
- Harris RP, Helfand M, Woolf SH, *et al.* Current methods of the US preventive services Task force: a review of the process. *Am J Prev Med* 2001;20:21–35.
- Blumberg J, Heaney RP, Huncharek M, *et al.* Evidence-Based criteria in the nutritional context. *Nutr Rev* 2010;68:478–84.
- Myles IA. Fast food fever: reviewing the impacts of the Western diet on immunity. *Nutr J* 2014;13:61.
- Minihane AM, Vinoy S, Russell WR, *et al.* Low-Grade inflammation, diet composition and health: current research evidence and its translation. *Br J Nutr* 2015;114:999–1012.
- Mozaffarian D, Hao T, Rimm EB, *et al.* Changes in diet and lifestyle and long-term weight gain in women and men. *N Engl J Med* 2011;364:2392–404.
- Agnoli C, Krogh V, Grioni S, *et al.* A priori-defined dietary patterns are associated with reduced risk of stroke in a large Italian cohort. *J Nutr* 2011;141:1552–8.
- Andersen M-BS, Kristensen M, Manach C, *et al.* Discovery and validation of urinary exposure markers for different plant foods by untargeted metabolomics. *Anal Bioanal Chem* 2014;406:1829–44.
- Barri T, Dragsted LO. UPLC-ESI-QTOF/MS and multivariate data analysis for blood plasma and serum metabolomics: effect of experimental artefacts and anticoagulant. *Anal Chim Acta* 2013;768:118–28.
- Christiansen CB, Gabe MBN, Svendsen B, *et al.* The impact of short-chain fatty acids on GLP-1 and PYY secretion from the isolated perfused rat colon. *Am J Physiol Gastrointest Liver Physiol* 2018;315:G53–65.
- Hjerpsted JB, Dragsted LO, Tholstrup T. Cheese intake lowers plasma cholesterol concentrations without increasing bile acid excretion. *J Nutr Intermed Metab* 2016;3:12–17.
- Plaza Oñate F, Le Chatelier E, Almeida M, *et al.* MSPminer: abundance-based reconstitution of microbial pan-genomes from shotgun metagenomic data. *Bioinformatics* 2019;35:1544–52.
- Gu Z, Eils R, Schlesner M. Complex heatmaps reveal patterns and correlations in multidimensional genomic data. *Bioinformatics* 2016;32:2847–9.
- Pasolli E, Truong DT, Malik F, *et al.* Machine learning meta-analysis of large metagenomic datasets: tools and biological insights. *PLoS Comput Biol* 2016;12:e1004977.
- Drescher LS, Thiele S, Mensink GBM. A new index to measure healthy food diversity better reflects a healthy diet than traditional measures. *J Nutr* 2007;137:647–51.
- Roager HM, Vogt JK, Kristensen M, *et al.* Whole grain-rich diet reduces body weight and systemic low-grade inflammation without inducing major changes of the gut microbiome: a randomised cross-over trial. *Gut* 2019;68:83–93.
- Keller BO, Wu BT, Li SSJ, *et al.* Hypophosphine is present in human milk in association with consumption of legumes. *J Agric Food Chem* 2013;61:7654–60.
- Cuparencu CS, Andersen M-BS, Gürdeniz G, *et al.* Identification of urinary biomarkers after consumption of sea buckthorn and strawberry, by untargeted LC–MS metabolomics: a meal study in adult men. *Metabolomics* 2016;12:31.
- Tulipani S, Urpi-Sarda M, García-Villalba R, *et al.* Urolithins are the main urinary microbial-derived phenolic metabolites discriminating a moderate consumption of nuts in free-living subjects with diagnosed metabolic syndrome. *J Agric Food Chem* 2012;60:8930–40.
- Dragsted LO. Biomarkers of meat intake and the application of nutrigenomics. *Meat Sci* 2010;84:301–7.
- Khakimov B, Poulsen SK, Savorani F, *et al.* New Nordic diet versus average Danish diet: a randomized controlled trial revealed healthy long-term effects of the new Nordic diet by GC-MS blood plasma metabolomics. *J Proteome Res* 2016;15:1939–54.
- Singh A, Shannon CP, Gautier B, *et al.* DIABLO: an integrative approach for identifying key molecular drivers from multi-omics assays. *Bioinformatics* 2019;35:3055–62.
- Lewington S, Whitlock G, Clarke R, *et al.* Blood cholesterol and vascular mortality by age, sex, and blood pressure: a meta-analysis of individual data from 61 prospective studies with 55,000 vascular deaths. *Lancet* 2007;370:1829–39.
- Guasch-Ferré M, Satija A, Blondin SA, *et al.* Meta-Analysis of randomized controlled trials of red meat consumption in comparison with various comparison diets on cardiovascular risk factors. *Circulation* 2019;139:1828–45.
- García-Conesa M-T, Chambers K, Combet E, *et al.* Meta-Analysis of the effects of foods and derived products containing ellagitannins and anthocyanins on cardiometabolic biomarkers: analysis of factors influencing variability of the individual responses. *Int J Mol Sci* 2018;19:694.
- Wolff E, Vergnes M-F, Portugal H, *et al.* Cholesterol-absorber status modifies the LDL cholesterol-lowering effect of a Mediterranean-type diet in adults with moderate cardiovascular risk factors. *J Nutr* 2011;141:1791–8.
- Bouchard-Mercier A, Rudkowska I, Lemieux S, *et al.* The metabolic signature associated with the Western dietary pattern: a cross-sectional study. *Nutr J* 2013;12:158.
- Schmidt JA, Rinaldi S, Ferrari P, *et al.* Metabolic profiles of male meat eaters, fish eaters, vegetarians, and vegans from the EPIC-Oxford cohort. *Am J Clin Nutr* 2015;102:1518–26.
- Ross AB, Pere-Trépat E, Montoliu I, *et al.* A whole-grain-rich diet reduces urinary excretion of markers of protein catabolism and gut microbiota metabolism in healthy men after one week. *J Nutr* 2013;143:766–73.
- Guasch-Ferré M, Zheng Y, Ruiz-Canela M, *et al.* Plasma acylcarnitines and risk of cardiovascular disease: effect of Mediterranean diet interventions. *Am J Clin Nutr* 2016;103:1408–16.
- Korver RA, Koevoets IT, Testerink C. Out of shape during stress: a key role for auxin. *Trends Plant Sci* 2018;23:783–93.
- Chávez-Talavera O, Tailleux A, Lefebvre P, *et al.* Bile acid control of metabolism and inflammation in obesity, type 2 diabetes, dyslipidemia, and nonalcoholic fatty liver disease. *Gastroenterology* 2017;152:1679–94.
- Draper CF, Vassallo I, Di Cara A, *et al.* A 48-hour vegan diet challenge in healthy women and men induces a branch-chain amino acid related, health associated, metabolic signature. *Mol Nutr Food Res* 2018;62:1700703.

- 46 De Filippis F, Pellegrini N, Vannini L, *et al.* High-Level adherence to a Mediterranean diet beneficially impacts the gut microbiota and associated metabolome. *Gut* 2016;65:1812–21.
- 47 de la Cuesta-Zuluaga J, Mueller N, Álvarez-Quintero R, *et al.* Higher fecal short-chain fatty acid levels are associated with gut microbiome dysbiosis, obesity, hypertension and cardiometabolic disease risk factors. *Nutrients* 2019;11:51.
- 48 Mitsou EK, Kakali A, Antonopoulou S, *et al.* Adherence to the Mediterranean diet is associated with the gut microbiota pattern and gastrointestinal characteristics in an adult population. *Br J Nutr* 2017;117:1645–55.
- 49 Cerdá B, Periago P, Espín JC, *et al.* Identification of urolithin A as a metabolite produced by human colon microflora from ellagic acid and related compounds. *J Agric Food Chem* 2005;53:5571–6.
- 50 García-Aloy M, Hulshof PJM, Estruel-Amades S, *et al.* Biomarkers of food intake for nuts and vegetable oils: an extensive literature search. *Genes Nutr* 2019;14:7.
- 51 Singh R, Chandrashekhara S, Bodduluri SR, *et al.* Enhancement of the gut barrier integrity by a microbial metabolite through the Nrf2 pathway. *Nat Commun* 2019;10:89.
- 52 Selma MV, González-Sarrias A, Salas-Salvado J, *et al.* The gut microbiota metabolism of pomegranate or walnut ellagitannins yields two urolithin-metabolites that correlate with cardiometabolic risk biomarkers: comparison between normoweight, overweight-obesity and metabolic syndrome. *Clin Nutr* 2018;37:897–905.
- 53 Stanisławska IJ, Granica S, Piwowarski JP, *et al.* The activity of urolithin A and M4 Valerolactone, colonic microbiota metabolites of polyphenols, in a prostate cancer in vitro model. *Planta Med* 2019;85:118–25.
- 54 Zhang W, Chen J-H, Aguilera-Barrantes I, *et al.* Urolithin A suppresses the proliferation of endometrial cancer cells by mediating estrogen receptor- α -dependent gene expression. *Mol Nutr Food Res* 2016;60:2387–95.
- 55 Teixeira LL, Costa GR, Dörr FA, *et al.* Potential antiproliferative activity of polyphenol metabolites against human breast cancer cells and their urine excretion pattern in healthy subjects following acute intake of a polyphenol-rich juice of grumixama (*Eugenia brasiliensis* Lam.). *Food Funct* 2017;8:2266–74.
- 56 Haro C, Montes-Borrego M, Rangel-Zúñiga OA, *et al.* Two healthy diets modulate gut microbial community improving insulin sensitivity in a human obese population. *J Clin Endocrinol Metab* 2016;101:233–42.
- 57 Haro C, García-Carpintero S, Alcalá-Díaz JF, *et al.* The gut microbial community in metabolic syndrome patients is modified by diet. *J Nutr Biochem* 2016;27:27–31.
- 58 Vanegas SM, Meydani M, Barnett JB, *et al.* Substituting whole grains for refined grains in a 6-wk randomized trial has a modest effect on gut microbiota and immune and inflammatory markers of healthy adults. *Am J Clin Nutr* 2017;105:635–50.
- 59 Vitaglione P, Mennella I, Ferracane R, *et al.* Whole-grain wheat consumption reduces inflammation in a randomized controlled trial on overweight and obese subjects with unhealthy dietary and lifestyle behaviors: role of polyphenols bound to cereal dietary fiber. *Am J Clin Nutr* 2015;101:251–61.
- 60 Henke MT, Kenny DJ, Cassilly CD, *et al.* *Ruminococcus gnavus*, a member of the human gut microbiome associated with Crohn's disease, produces an inflammatory polysaccharide. *Proc Natl Acad Sci U S A* 2019;116:12672–7.
- 61 Le Chatelier E, Nielsen T, Qin J, *et al.* Richness of human gut microbiome correlates with metabolic markers. *Nature* 2013;500:541–6.
- 62 Brahe LK, Le Chatelier E, Prifti E, *et al.* Specific gut microbiota features and metabolic markers in postmenopausal women with obesity. *Nutr Diabetes* 2015;5:e159.
- 63 Devkota S, Wang Y, Musch MW, *et al.* Dietary-fat-induced taurocholic acid promotes pathobiont expansion and colitis in IL10 $^{-/-}$ mice. *Nature* 2012;487:104–8.
- 64 Natividad JM, Lamas B, Pham HP, *et al.* *Bifidobacterium wadsworthii* aggravates high fat diet induced metabolic dysfunctions in mice. *Nat Commun* 2018;9:2802.
- 65 Sokol H, Pigneur B, Watterlot L, *et al.* *Faecalibacterium prausnitzii* is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of Crohn disease patients. *Proc Natl Acad Sci U S A* 2008;105:16731–6.
- 66 Vital M, Karch A, Pieper DH. Colonic butyrate-producing communities in humans: an overview using omics data. *mSystems* 2017;2:e00130–17.
- 67 Pedersen HK, Gudmundsdottir V, Nielsen HB, *et al.* Human gut microbes impact host serum metabolome and insulin sensitivity. *Nature* 2016;535:376–81.
- 68 De Filippis F, Pasolli E, Tett A, *et al.* Distinct genetic and functional traits of human intestinal *Prevotella copri* strains are associated with different habitual diets. *Cell Host Microbe* 2019;25:444–53.

SUPPLEMENTARY MATERIALS

SUPPLEMENTARY METHODS

- Selection criteria
- Dietary intervention
- Metagenomics
- 16S rRNA gene sequencing and data analysis
- Metabolomics untargeted analysis
- Metabolomics targeted analysis by LC/MS/MS
- Analysis of urinary urolithins and derived metabolites by LC-HRMS
- Analysis of markers of inflammation and metabolic disease
- Blood glucose, insulin and HOMA
- Determination of plasma lipids
- Determination of anthropometric measurements and body composition
- Statistical analysis

SUPPLEMENTARY RESULTS

- Pangenome analysis of selected microbial species

SUPPLEMENTARY METHODS

Selection criteria

The selection of volunteers was carried out by a nutritionist and a physician through i) the collection of information concerning personal data, work activity and lifestyle as well as anamnestic data, including alcohol (quantity and type of drink) and/or medication use, ii) assessment of individual nutritional status by measure of body weight and height and calculation of the body mass index (BMI), iii) evaluation of habitual diet through a food consumption frequency questionnaire (FFQ) and the 7-day food diary.

The eligibility of the volunteers to participate into the study was defined on the basis of the inclusion and exclusion criteria listed below.

Inclusion criteria

- Healthy subjects;
- $20 \leq \text{age} \leq 65$ years;
- $\text{BMI} \geq 24 \text{ kg/m}^2$;
- Both genders;
- No consumption of probiotics and functional foods and/or food supplements of any kind;
- Habitual diet characterized by no more than 2 portions a day of whole foods and/or enriched with dietary fiber;
- Habitual diet with no more than 3 servings of fruit and vegetables per day;
- Low level of physical activity (sedentary lifestyle);
- Signature of the informed consent form.

Exclusion criteria

- Gastrointestinal disorders of any kind;
- Pregnancy or breastfeeding;
- Previous abdominal surgery;
- Hypertriglyceridaemia (Triglycerides > 300 mg/dL);
- Hypercholesterolemia (Cholesterol > 220 mg/dL);
- Arterial hypertension;
- Pharmacological treatments of any type at enrollment and in the 2 months prior to the study;
- Habitual diet rich in fruit and vegetables;
- High level of physical activity;
- Consumption of wine or alcohol equivalent beverage greater than 3 glasses of wine per day;
- Contemporary participation in other studies.

Dietary intervention

Eighty-two subjects (43 in the MedD group and 39 in the ConD group) completed the study and were included in the analyses (Supplementary Figure 1). The composition of the habitual diet was assessed through a food consumption frequency questionnaire (FFQ) and a 7-day food diary.[1] Physical activity levels were assessed by the International Physical Activity Questionnaire.[2]

Participants had a 2-week run-in period in which they were asked not to change their habitual diets

or physical activity. After the run-in, the subjects were assigned to the MedD or ConD group on the basis of a randomization sequence that was previously obtained using a computer-generated permuted block (n=5) randomization scheme. The sequence was generated by a statistician.

Each participant in the MedD group consumed a personalized diet that was isocaloric compared to their habitual diet and was based on the inclusion of fruit and vegetables and nuts (at least 5 portions, ~500 g/day) and nuts (30 g/day) as well as calorie-adjusted replacement of refined cereal products with wholegrain products (at least 2 portions, ~200 g/day between wholegrain pasta, bread and breakfast cereal); replacement of meat, eggs and dairy products with fish and legumes (at least 2 portions, ~300 g/week of fish and 3 portions, ~300 g/week of legumes); replacement of butter/margarine with extra-virgin olive oil. Participants in the ConD group were asked to maintain their habitual diet. Participants in both groups received a personalized scheme to follow during the intervention period that advised on the weekly and/or daily consumption frequency and portion size of the main food categories. Participants were advised not to change the level of physical activity over the intervention period. Moreover, the participants and those assessing outcomes were blinded after assignment to interventions.

All the participants received at baseline and every 4 weeks a basket containing some foods they could consume during the dietary intervention. Thus, subjects in MedD group had nuts, wholegrain wheat pasta, legumes, tomato sauce whereas subjects in ConD group had refined wheat pasta and tomato sauce. At each 4 weeks visit, subjects were asked to return the foods they received on the previous month and they did not consume.

During the visits, the food diaries and physical activity questionnaires completed on the previous days were also checked by dietitians, and further indications to improve protocol compliance were given to the subjects if needed. Additionally, assessment of compliance with the protocol and counselling of the volunteers was performed via a phone call every second week after each visit.

At baseline, 4 weeks and 8 weeks, at 8:00 a.m. after at least 10 h of fasting, volunteers attended the laboratory. After measurement of blood pressure and the visit with the physician, blood and urine samples were collected and anthropometric measures were taken (see below). On the same day, the subjects delivered the faecal samples that were self-collected according to the standard operating procedure (SOP 004) of the International Human Microbiome Standards (www.microbiome-standards.org).

Metagenomics

DNA extraction and high throughput sequencing. Faecal sampling was performed following the IHMS SOP 004, for samples handled to the biological laboratory within 24 hours to 7 days from collection. Samples have been kept at 4 °C and transported to the laboratory within 24h, where they have been stored at -80 °C prior to further analyses. Frozen faecal material were aliquoted to 200 mg and DNA extraction was performed following IHMS SOP P7 V2. DNA was quantified using Qubit Fluorometric Quantitation (ThermoFisher Scientific, Waltham, US) and qualified using DNA size profiling on a Fragment Analyzer (Agilent Technologies, Santa Clara, US). Three µg of high molecular weight DNA (>10 kbp) was used to build the library. Shearing of DNA into fragments of approximately 150 bp was performed using an ultrasonicator (Covaris, Woburn, US) and DNA fragment library construction was performed using the Ion Plus Fragment Library and Ion Xpress

Barcode Adapters Kits (ThermoFisher Scientific, Waltham, US). Purified and amplified DNA fragment libraries were sequenced using the Ion Proton Sequencer (ThermoFisher Scientific, Waltham, US), with a minimum of 20 million high-quality reads of 150 bp generated per library.

Read Mapping. An average of 22.2 ± 1.6 million reads was produced and quality filtered to remove any low-quality sequences (6 %, on average) using Alentrimmer software (with params ‘-k 10 -l 45 -m 5 -p 40 -q 20’) and potential human-related reads (0.6 %, on average) using bowtie2 (removing reads with at least 95% identity with Human genome reference GRCh38). Resulting high-quality reads (average of 20.8 million reads \pm 1.8) were mapped onto the 9.9 million gene integrated reference catalog of the human microbiome[3] using the METEOR suite.[4] Mapping was performed using an identity threshold of 95% to the reference gene catalog with Bowtie 2[5] in a two-step procedure. First, unique mapped reads (reads mapped to a unique gene in the catalogue) were attributed to their corresponding genes. Second, shared reads (reads that mapped with the same alignment score to multiple genes in the catalogue) were weighted according to the ratio of unique mapping counts. Gene abundance table was further rarefied, normalized and analyzed using MetaOMineR (*momr*) R package (<https://cran.r-project.org/web/packages/momr/index.html>). Rarefaction was performed by randomly drawn 12 million reads per sample without replacement to avoid differences in sequencing depth and limit sample size artifacts on low abundant genes. Rarefied gene counts were normalized using the FPKM strategy (normalization by gene size and total number of mapped reads).

Metagenomic Species Pangenome (MSP) determination. Metagenomic Species Pangenome (MSP)[6] were used to quantify species associated to the 9.9 million gene integrated reference catalog. MSP are clusters of co-abundant genes (min size \geq 500 genes) used as proxy for microbial species, reconstructed from the 9.9 million genes catalogue into 1776 MSP from 1267 individuals.[6, 7] MSP abundance profiles were calculated as the mean abundance of 50 marker genes, defined as the robust centroids of each MSP cluster. A threshold of 10% of the marker genes was applied as MSP detection limit. Taxonomical annotation was accomplished as described by Plaza Oñate *et al.*[6] During the analysis of data, in order to reduce individual microbial variability not directly impacted by diet, a closeup analysis was performed by focusing on MSPs with an occurrence threshold of 20%, resulting in an overall MSP count of 349.

Assessment of the functional potential. Functional potentials of the intestinal gut microbiota were determined by using the in-house FAnToMet pipeline (unpublished). First, genes of the 9.9 million genes catalogue were annotated using KEGG82 database and further clustered into functional pathway modules according to KEGG (Kyoto Encyclopaedia of Genes and Genomes) Orthology (KO) groups and Gut Metabolic Modules (GMM).[8-11] Second, KEGG and GMM modules were reconstructed in each MSP using their reaction pathways based on their detected annotated KO genes. GMM functional modules were further selected because they have been finely curated based on literature review and are specific to gut bacterial functions. For each pair of MSP/subject, we calculated the completeness of any given functional modules by considering the MSP completeness in the subject, determined by the presence of a collection of highly conserved genes in bacteria and archaea.[12] For a given MSP in a given subject, completeness of the modules was corrected by the completeness of the MSP as calculated above. After correction, functional modules of a detected MSP in a subject were considered as complete if at least 90% of the involved reactions were detected. Abundance of functional modules in each MSP corresponds to the abundance of the MSP

in the sample. Finally, abundance of functional modules in each sample was computed as the sum of module's abundances of the detected MSP.

Pangenome reconstruction of selected species. Pangenome reconstruction was carried out for selected species (*Prevotella copri*, *Faecalibacterium prausnitzii*, *Bacteroides uniformis* and *B. vulgatus*) by using PanPhlAn.[13] Pangenome databases were prepared using all the genomes available in NCBI (January 2019) for each species. An Euclidean distance matrix was built based on gene presence/absence pattern and a tree obtained using the R package *phytools*. The tree was visualized in iTOL (Interactive Tree of Life, <https://itol.embl.de>).

16S rRNA gene sequencing and data analysis

The V3-V4 region of the 16S rRNA gene was amplified by using primers and protocol previously described.[14] Library multiplexing, pooling, and sequencing were carried out according to the Illumina 16S metagenomic sequencing library preparation protocol on a MiSeq platform and using the MiSeq Reagent kit v2.

Reads were demultiplexed using FLASH[15] and quality filtered using Prinseq (Phreds score > 20 and length > 250 bp).[16] High-quality reads were then imported into QIIME1 v. 1.9. Operational taxonomic units (OTU) were picked using a *de novo* approach and the uclust method, and taxonomic assignments were obtained by using the RDP classifier[17] and the Human Intestinal Tract (HIT) database (<https://github.com/microbiome/HITdb.git>). Genera were clustered into 5 Co-Abundance Groups (CAGs) as previously reported.[18]

Metabolomics untargeted analysis

Chemicals and authentic standards. All solvents were of UPLC-MS grade and all aqueous solutions were prepared using ultrapure Millipore purified (MilliQ) water. The internal standards mixture (IS) for untargeted metabolomics included L-Arginine ¹³C₆ (Cambridge Isotope Laboratories Inc, Andover, MA), L-Tyrosine ¹³C₉ (Sigma Aldrich, St. Louis, MO, USA), Para-aminobenzoic acid (Sigma Aldrich), L-Tryptophan-(indole-d₅) (Sigma Aldrich), Hippuric Acid-[¹³C₆] (Biomol GmbH), Cortisone-d₈ (Sigma Aldrich), Glycocholic Acid-[²H₄] (Biomol GmbH, Hamburg, Germany) and Lysophosphatidylcholine (17:1d₇) (Avanti Polar Lipids, Birmingham, AL, USA).

Calibration standards for bile acid quantification were cholic acid (CA) (Calbiochem, San Diego, CA, USA), chenodeoxycholic acid (CDCA) (Fluka, St. Louis, MO, USA), deoxycholic acid (DCA) (Sigma Aldrich), glycochenodeoxycholic acid (GCDCA) (Calbiochem), lithocholic acid (LCA) (Sigma Aldrich), ursodeoxycholic acid (UDCA) (Calbiochem), glycodeoxycholic acid (GDCA) (Calbiochem), α-muricholic acid (αMCA) (Steraloids Inc, Newport, RI, USA), β-muricholic acid (βMCA) (Steraloids Inc), dehydrocholic acid (DHCA) (Fluka), glycocholic acid (GCA) (Calbiochem), glycolithocholic acid (GLCA) (Steraloids Inc), glycourosdeoxycholic acid (GUDCA) (Calbiochem), tauro-α-muricholic acid (TαMCA) (Steraloids Inc), tauro-β-muricholic acid (TβMCA) (Steraloids Inc), taurocholic acid (TCA) (Calbiochem), taurochenodeoxycholic acid (TCDCA) (Calbiochem), taurodeoxycholic acid (TDCA) (Calbiochem), tauroolithocholic acid

(TLCA) (Steraloids Inc), tauroursodeoxycholic acid (TUDCA) (Calbiochem) and hyodeoxycholic acid (HDCA) (Sigma Aldrich). Internal standards for bile acid quantification were cholic acid-d₄, glycocholic acid-d₄, taurocholic acid-d₅, taurochenodeoxycholic acid-d₅, chenodeoxycholic acid-d₄, glycochenodeoxycholic acid-d₄, ursodeoxycholic acid-d₄, glyoursodeoxycholic acid-d₄, deoxycholic acid-d₄, glycodeoxycholic acid-d₄, and lithocholic acid-d₄ and were obtained from Cambridge Isotope Laboratories. The standards for short-chain fatty acids (SCFA) quantification were acetic acid, propionic acid, butyric acid, butyric acid-d₇, isobutyric acid, 2-methylbutyric acid, isovaleric acid, valeric acid, caproic acid, 3-methylvaleric acid and isocaproic acid, and were all obtained from Sigma Aldrich. Other authentic standards used, which were not already in our in-house library, included urolithin A and tryptophan betaine, which were obtained from Sigma Aldrich. Finally, the following chemicals were used for SCFA derivatization; 3-nitrophenylhydrazine (3NPH) (Sigma Aldrich), N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide (EDC) (Sigma Aldrich) and ¹³C₆-3NPH (Isosciences, Ambler, PA, USA).

Urine preparation. After thawing at 4 °C, 60 µL of each urine sample was transferred to separate wells in a 96-well sample collection plate (Waters) and diluted with 240 µL internal standard (IS) mixture resulting in a final urine dilution of 1:5. In addition, 20 µL of each urine sample was pooled in a separate vial to create a pooled urine quality control (QC) sample. When preparing the urine samples, it was ensured that all urine samples of the same individual were placed on the same 96-well plate. The plates were sealed and stored at 4 °C until analysis (24 h max, otherwise stored at -80 °C). If the plate was frozen and thawed again before analysis, the plate was gently mixed by vortex stirring for 30 min immediately prior to analysis.

Serum preparation. Serum samples were thawed at 4 °C. A serum QC sample was prepared by pooling 20 µL of each serum sample in a separate vial. Each well in a 96-well pointed-bottom 1 mL Siroccotm plasma protein filtering plate (Waters, Manchester, UK) was washed three times with 180 µL 50:50 acetonitrile:methanol (solvent B), before the plate was dried by nitrogen gas. Upon drying, the filtering plate was placed on top of a 96-well sample collection plate (Waters). Then, 90 µL solvent B, 40 µL serum and 10 µL IS mixture was transferred to each well before another 90 µL solvent B was added as well. The plate was sealed and vortexed gently for 5 min. Subsequently, the plate was placed in the refrigerator at 4 °C for 10 min to promote further protein precipitation. Afterwards, the plate was placed in a manifold, left at room temperature for 5 min before vacuum was applied to the plate ensuring that the metabolites dripped into a 96-well sample collection plate. When the filtering plate was dry, 90 µL solvent B was added to each well to further extract metabolites from the precipitated protein and vacuum was continued until dryness. Finally, another 90 µL solvent B was added and the procedure was repeated. The eluted solvent was evaporated from the collection plate by using a cooled vacuum centrifuge. The dry plate was sealed and stored at -80 °C until analysis. When preparing the serum samples, it was ensured that all serum samples of the same individual were placed on the same 96-well plate. Before analysis, the dry samples were re-dissolved in 200 µL solvent A, resulting in a final serum dilution of 1:5, and gently vortexed.

Faeces preparation. Faecal samples were thawed at room temperature and homogenized 1:1 in MiliQ water. Approximately 50 mg±2mg (≈50 µL) of the homogenized faecal sample was upon vortexing weighed into a 2 mL Eppendorf tubes and 1362.5 µL 96% ethanol was added. Internal standard mixtures were added to the tube enabling bile acid quantification, SCFA quantification,

and untargeted metabolomics analyses, respectively. For the bile acid analysis, 20 μL of the internal bile acid mixture (Cholic acid- d_4 , glycocholic acid- d_4 , taurocholic acid- d_5 , chenodeoxycholic acid- d_4 , glycochenodeoxycholic acid- d_4 , ursodeoxycholic acid- d_4 , glycoursoxycholic acid- d_4 , deoxycholic acid- d_4 , glycodeoxycholic acid- d_4 , taurochenodeoxycholic acid- d_5 and lithocholic acid- d_4) was added (giving a final concentration of 8.65 μM of each). For the SCFA analysis, 7.5 μL of the internal standard for SCFA analysis (30 mM butyric acid- d_7) was added (giving a final concentration of 150 μM). Finally, 60 μL of the metabolomics IS mixture was added giving a final volume of 1500 μL (1:60 dilution of faecal sample). The mixture was vortexed two times 30 seconds and subsequently mixed at 60 °C for 2 min in a Thermomixer (Eppendorf, Hamburg, Germany) at 1400 rpm, before being centrifuged at 14000 rpm (Eppendorf centrifuge 5417R), 4 °C for 2 min. The supernatants were filtered through a 0.2 μm Q-Max Syringe Filter (Frisenette, Knebel, Denmark) into an Eppendorf tube and stored at -80 °C until plate preparation. Upon thawing, the tubes were gently mixed. For all analyses, it was ensured that all faecal samples of the same individual were placed on the same 96-well plate. For the bile acid quantification analysis and untargeted metabolomics analysis, respectively, 100 μL of each faecal suspension was transferred to a 96-well sample collection plate, evaporated using a cooled vacuum centrifuge, and re-dissolved in 200 μL solvent A prior to the UPLC-MS analysis resulting in a final faeces dilution of 1:120.

Untargeted metabolomics by UPLC-MS. The urine, serum and faecal samples were analysed separately. For each type of sample, samples were randomised and analysed by an ACQUITY ultra-performance liquid chromatography (UPLC) coupled with a Synapt G2 quadrupole-Time of Flight Mass Spectrometer (q-TOF-MS) equipped with an electrospray ionization (ESI) (Waters Corporation) in both positive and negative ionization mode with a pooled quality control (QC) sample injected for every 16th sample. A blank sample (0.1% formic acid) and a standard sample containing 40 different physiological compounds (metabolomics standard) was also injected regularly to evaluate LC-MS system stability, possible contamination and/or loss of metabolites in the subsequent filtering procedure. For each analysis, 5 μL was injected and the analytes were separated on a reversed-phase column (ACQUITY HSS T3 C18 column, 2.1x100 mm, 1.8 μm) coupled with a pre-column (ACQUITY VanGuard HSS T3 C18 column, 2.1x5 mm, 1.8 μm). The column was held at 50 °C and the sampler at 5 °C. The UPLC mobile phases consisted of 0.1% formic acid in water (phase A) and 0.1% formic acid in 70:30 acetonitrile:methanol (phase B). The mobile phase gradient during the 7 min run time was as follows: start condition (5% B), 1 min (8% B), 2 min (15% B), 3 min (40 % B), 4 min (70 % B), 4.5 min (100 % B), 6.6 min (5% B), 7 min (5% B). The flow rate gradient was as follows: start condition (0.5 mL/min), 1 min (0.5 mL/min), 2 min (0.6 mL/min), 3 min (0.7 mL/min), 4 min (0.8 mL/min), 4.5 min (1.0 mL/min), 6.4 min (1.1 mL/min), 6.6 min (1.0 mL/min), 6.8 min (0.5 mL/min), 7.0 min (0.5 mL/min). Mass spectrometry data were collected in full scan mode with a scan range of 50–1000 mass/charge (m/z), a scan time set as 0.08 s with 0.02 sec inter-scan time for both modes. A lock-mass calibration agent (leucine-enkephalin, 2000 ng/ml) was infused to calibrate the mass accuracy every 10 sec with 0.2 sec scan time. The following electrospray interphase settings were used: The cone voltage was 2.5 kV and 3.2 kV for negative and positive mode, respectively, the collision energy was 6.0 and 4.0 eV for negative and positive mode, respectively, the temperature of the ion source and desolvation nitrogen gas temperature was 120 °C and 400 °C, respectively, while the desolvation gas flow rate was 800 L/Hr.

The raw UPLC-MS data were converted to CDF files using the DataBridge software included in Masslynx (Waters Corporation) and were pre-processed using MZmine,[19] version 2.35. Data tables were generated comprising m/z , retention time (rt) and intensity (peak height) for each feature in every sample. The pre-processed data were subsequently filtered in Matlab R2014b (The MathWorksInc., Natick, MA) by removing features present in blanks, duplicates, potential isotopes, features detected in less than 50 % of samples, and early (rt < 0.3 min) and late eluting features (Urine positive, rt > 6.3 min; urine negative, rt > 6.4 min; serum positive, rt > 5.0 min; serum negative, rt > 6.3 min; faeces positive, rt > 6.43; faeces negative, rt > 6.47 min). Urine metabolome data were normalized by probabilistic quotient normalization (PQN),[20] while faeces and serum metabolome data were normalized by mean centring. Finally, the data were filtered using the pooled QC samples; unreproducible features showing high coefficient of variation (CV) in the QC samples were excluded (urine positive, CV > 0.4; urine negative, CV > 0.4; serum positive, CV > 0.5; serum negative, CV>0.5; faeces positive, CV>0.5; faeces negative, no exclusion).

The UPLC-MS features remaining after filtering had an average CV % of 45 % in faeces negative (360 features), 27 % in faeces positive (1840 features), 22 % in serum negative (176 features), 24 % in serum positive (3949 features), 27 % in urine negative (2744 features) and 26 % in urine positive (1901 features), respectively. The accurate masses of the discriminating features measured by UPLC-MS were searched for putative identities in the METLIN[21] and HMDB[22] databases. The metabolites were identified according to the four different levels described by the Metabolomics Standard Initiative;[23] metabolites confirmed by an authentic standard (Level I), metabolites confirmed based on a comparison of MS/MS fragmentation pattern compared with those found in databases and earlier literature (Level II), metabolites with similarities to published fragmentation patterns (Level III), and unknown compounds (Level IV).

Sulfation and glucuronidation of authentic compounds. Glycochenodeoxycholic acid, 3-methylpyrogallol, phenol sulfate and p-cresol were conjugated with sulfate using S9 human liver extract (Sigma Aldrich) in a TRIS buffer (pH = 7.5) with the presence of phosphoadenosine-5-phosphosulfate (PAPS) (1 mg/mL) as cofactor. Urolithin C and Urolithin A were conjugated with glucuronidate using S9 human liver extract (Sigma Aldrich), MgCl₂ (100 mM) and uridine-diphosphate-glucuronic acid (UDPGA, 7.73mM). The mixtures were incubated at 37 °C for 1 hour before cold methanol at a ratio 1:4 (v/v) was added to the mixtures. Subsequently, the mixtures were cooled in the freezer (-20 °C) for 10 min before being centrifuged at 10.000 g for 3 min at 5 °C. The supernatants were transferred to new tubes, evaporated to dryness with a vacuum centrifuge at 35 °C, and reconstituted in 10% acetonitrile. Finally, the conjugated compounds were analysed by UPLS-MS under the same conditions as previously outlined.

Faeces bile acid quantification by UPLC-MS. Concentrations of CA, CDCA, GCDCA, LCA, UDCA, DCA, GDCA and α MCA/ β MCA were determined in all faecal samples using labelled standards, except for α MCA/ β MCA, which were analysed without a labelled standard. DHCA, GCA, GLCA, GUDCA, TMCA, TCA, TCDCA, TDCA, TLCA, TUDCA and HDCA were also analysed, however concentrations in the faecal samples were below the detection limits. Standard curves were prepared for all bile acids for quantification and internal standards (listed above) were used to assess relative losses and ion suppression of each analyte. In addition, a pooled QC sample was analysed for every 16-17 faecal sample to assess reproducibility for each bile acid. The samples were analysed in negative ionization mode using the same UPLC-MS system as mentioned above,

however with a different LC gradient. The mobile phase gradient during the 7 min run time was as follows: start condition (5% B), 1.5 min (40% B), 3.5 min (60% B), 4.5 min (100 % B), 7.0 min (5 % B). The flow rate gradient was as follows: start condition (0.5 mL/min), 1.5 min (0.7 mL/min), 3.5 min (0.85 mL/min), 4.5 min (0.7 mL/min), 7 min (0.5 mL/min). The raw UPLC-MS data were converted to CDF files using the DataBridge software included in Masslynx (Waters Corporation) and were pre-processed using MZmine[19] version 2.35. Data tables were generated comprising m/z , rt and intensity (peak area) for all bile acids in every sample. The calibration curves were established by plotting the peak area ratios between the individual bile acid analytes and labelled internal bile acid standards against the concentrations of the calibration standards. Of notice, α MCA/ β MCA could not be separated and was given the name MCA. The calibration curve of MCA was established by plotting the MCA peak area against the concentrations of the calibration standards, as no internal standard of α MCA/ β MCA was available. The calibration curves were fitted to a linear regression. CV% of QC samples was 5% for CA, 19% for CDCA, 17% for GCDCA, 3% for GDCA, 31% for LCA, 11% for MCA and 30% for UDCA, respectively.

Faeces SCFA quantification by UPLC-MS. Quantification of SCFA in faecal samples was performed as previously published.[24] In brief, 100 μ L of the faecal extract containing internal SCFA standard butyric acid- d_7 was mixed with 20 μ L 200 mM 3-nitrophenylhydrazine (3NPH) in 50 % ethanol and 20 μ L 120 mM N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide (EDC) and 6% pyridine in 50 % ethanol in a 2 mL 96-wells plate. The solution was incubated at room temperature for 30 min while shaking. Subsequently, the derivatized mixture was diluted to 0.2 ml with 10 % ethanol. 100 μ L of the mixture was transferred to a 1 mL 96-wells plate and 100 μ L internal SCFA standard mixture was added (1:240 dilution of faecal sample). The remaining reaction mixture was further diluted 25x in 10% ethanol and 100 μ L was transferred to another 1 mL 96-wells plate where it was mixed with 100 μ L internal SCFA standard mixture (1:12000 dilution of faecal sample). Internal standard mixture was prepared by mixing 50 μ L of a solution of 20 mM acetic acid, 10 mM propionic acid, and 5 mM butyric acid, 5 mM isobutyric acid, 5 mM 2-methylbutyric acid, 5 mM isovaleric acid, 5 mM valeric acid, 5 mM caproic acid, 5 mM 3-methylvaleric acid, 5 mM isocaproic acid, 1 mg $^{13}C_6$ -3NPH in 50% ethanol and 25 μ l 120 mM EDC-6% pyridine solution and 25 μ l 50% ethanol. This mixture was left for derivatization at room temperature for 30 min while shaking. Hereafter, the mixture was transferred to a 100 mL volumetric flask and diluted 2000 times using 10% ethanol. This solution was the SCFA internal standard mix. The prepared 96-wells plates were sealed and stored at -80 °C until analysis. The plate was thawed before analysis and was gently mixed by vortex stirring for 30 min immediately prior to analysis. A dilution series of external standards was prepared of all SCFAs from 0.195 to 25 μ M together with an assay blank (96% ethanol). The dilution series and blank samples were initially injected, followed by the samples in random order with a 3.125 μ M bile acid mixture QC sample injected for every 15th sample, into a UPLC-QTOF-MS (Waters) and analysed in negative ionization mode. An ACQUITY BEH C18 guard column (2.1 x 5 mm, 1.7 μ m, Waters) was coupled to an ACQUITY BEH C18 column (2.1 x 100 mm, 1.7 μ m, Waters). The column was held at 50 °C and the sampler at 5 °C. The UPLC mobile phases consisted of 0.01% formic acid in water (phase A) and 0.01% formic acid in acetonitrile (phase B). The mobile phase gradient during the 10 min run time was as follows: start condition (20% B), 2 min (20% B), 7 min (40% B), 7.5 min (100 % B), 8 min (100 % B), 8.5 min (20 % B), 9.5 min (20% B). The flow rate was kept at 0.6 ml/min. The following electrospray interphase settings were used: The cone voltage was 3.0 kV, the collision energy was

5.0, the temperature of the ion source and desolvation nitrogen gas temperature was 120 °C and 400 °C, respectively, while the desolvation gas flow rate was 1000 L/Hr.

The raw UPLC-MS data were converted to CDF files using the DataBridge software included in Masslynx (Waters Corporation) and were pre-processed using MZmine[19] version 2.35. Data tables were generated comprising m/z , rt and intensity (peak area) for all SCFA in every sample. The calibration curves were established by plotting the peak area ratios between the individual SCFA analytes and labelled internal SCFA standards against the concentrations of the calibration standards. The calibration curves were fitted to a linear regression. Of notice, the calibration curve of butyrate was established by plotting the butyrate peak area against the concentrations of the calibration standards, as the internal standard of butyrate was unsuccessfully measured. The average CV for the bile acid mixture QCs ($n=7$ for each batch) in the three analytical batches was 0.16, and the average R^2 of all external standard calibration curves was 0.98. To adjust for batch differences, the SCFA concentrations were adjusted according to the mean concentrations across all plates.

Metabolomics targeted analysis by LC/MS/MS

Chemicals. All solvents were HPLC grade and were purchased from Merck (Darmstadt, Germany); formic acid and ammonium formate were from Sigma-Aldrich (St. Louis, MO). Standards of trimethylamine N-oxide (TMAO), choline chloride, creatinine, L-carnitine hydrochloride, betaine were purchased from Sigma-Aldrich (St. Louis, MO); trimethylamine N-oxide d9 standard was obtained from Cambridge Isotope Laboratories, Inc. (Andover, MA, USA). Urolithin B standard was from Sigma-Aldrich (St. Louis, MO).

Sample preparation. TMAO, choline, creatinine, carnitine and betaine extraction was performed according to Steuer *et al.*,[25] with slight modifications. Urine was diluted 5 times with water before extraction. A volume of 25 μ L of plasma and diluted urine was added with 225 μ L of CH_3CN containing internal standard TMAO-d9 25 μ M. The solution was vortexed for 30 s and then it was centrifuged at 14800 rpm for 5 min. Supernatants were analyzed by liquid chromatography coupled to electrospray tandem mass spectrometry (LC/MS/MS).

For the analysis of urolithins (Urolithin A, B, C and D) and derived metabolites (including the glucuronidated forms, Urolithin-A sulphate, Urolithin A sulfoglucuronide, and Urolithin D methyl ether glucuronide) urines were centrifuged at 14800 rpm for 10 min at 4 °C, filtered using a 0.22 μ m RC filter and used for LC/MS/MS analysis.

LC/MS/MS analysis. Chromatographic separation was performed using an HPLC apparatus equipped with two Micropumps Series 200 (Perkin Elmer, Shelton, CT, USA); mass spectrometry analysis was performed on an API 3000 triple quadrupole mass spectrometer (Applied Biosystems, Canada) equipped with a TurboIonSpray source.

LC/MS/MS analysis of TMAO, TMAO d9, choline, creatinine, betaine and carnitine was adapted by Steuer *et al.*[25]. A Luna 3 μ m HILIC 200 Å, 150x2 mm, (Phenomenex, USA) column thermostated at 30 °C was used. The mobile phase consisted of 10 mM ammonium formate in 90% CH_3CN (A) and 10 mM ammonium formate in water pH 3 (B).

The gradient program was as follows: 0% B (0-1,5 min), 0–70% B (1,5-7 min), 70% B (7-9 min); 70-0% B (9-10 min); 0 B% (10-17 min). The flow rate was set to 200 μ L/min and the injection

volume was 10 μ L. Mass spectrometry analysis was performed in the positive ion mode in MRM (Multiple Reaction Monitoring).

The MS characteristics of analysed compounds are reported in the Supplementary Table 2. A control plasma and urine sample were spiked with various concentrations of each analyte standard to prepare the calibration curves. They were generated by subtracting the endogenous amount of an analyte from the spiked amount (blank subtraction). The quantification of the analyte in the sample was based on the peak area ratio between the analyte and internal standard.

Calibration curves in urine were built in the linearity range: TMAO 0.5–50 μ M, carnitine 0.1–10 μ M, choline 0.2–50 μ M, creatinine 2.5–200 μ M, betaine 0.1–10 μ M.

Calibration curves in plasma were built in the linearity range: TMAO: 0.02–25 μ M, carnitine 0.2–50 μ M, choline 0.2–50 μ M, creatinine 0.2–50 μ M, betaine 0.2–25 μ M.

LC/MS/MS analysis of urolithins and urolithins derived metabolites was adapted by Tulipani *et al.*[26]. Chromatographic separation was carried out on a Gemini C18-110Å 5 μ m column (150 mm \times 2.0 mm) (Phenomenex, Torrance, CA) thermostated at 30 $^{\circ}$ C, the mobile phase consisted of 0.1% formic acid water (A) and 0.1% formic acid acetonitrile (B). Gradient elution was linearly programmed as follows: 10% B (1 min), 10–90% B (7 min), constant to 90% B (2 min), 90–10% B (2 min). The flow rate was set at 200 μ L/min and the injection volume was 20 μ L.

MS/MS analyses were performed in the negative ion mode in MRM (Multiple Reaction Monitoring).

The MS characteristics of analysed compounds are reported in the Supplementary Table 3.

Urolithin B calibration curve was built in the linearity range 10–5000 ng/mL and all the metabolites were expressed as urolithin B equivalents.

Analysis of urinary urolithins and derived metabolites by LC-HRMS

Urolithins (Urolithin A, B, C and D) and derived metabolites (including the glucuronidated forms, Urolithin-A sulphate, Urolithin A sulfoglucuronide, and Urolithin D methyl ether glucuronide) were determined in urines as previously described.[26] Briefly, 200 μ L of urine sample were added with 800 μ L of 0.2% acetic acid and after centrifuging at 16800xg for 5 min at 4 $^{\circ}$ C the sample was purified onto Oasis HLB 1 cc 30 mg cartridges. After washing with 1mL of H₂O and 1mL of 0.2% acetic acid, urolithins were eluted with 1mL of methanol 0.2% acetic acid. The eluate was dried under a stream of nitrogen, re-dissolved in 100 μ L MeOH/H₂O (70:30, v/v) and immediately used for High Resolution Mass Spectrometry (HRMS) analysis.

LC-MS data were acquired on an Accela U-HPLC system coupled to an Exactive mass spectrometer (Thermo Fisher Scientific, San Jose, CA). The Accela system consisted of a quaternary pump, a thermostated autosampler (10 $^{\circ}$ C) and a column oven.

Chromatographic separation was carried out on a Gemini C18-110Å 5 μ m column (150 mm \times 2.0 mm) (Phenomenex, Torrance, CA) thermostated at 30 $^{\circ}$ C, the mobile phase consisted of 0.1% formic acid water (A) and 0.1% formic acid acetonitrile (B). Gradient elution was linearly programmed as follows: 10% B (1 min), 10–90% B (7 min), constant to 90% B (2 min), 90–10% B (2 min). The flow rate was set at 200 μ L/min and the injection volume was 10 μ L. The U-HPLC was directly interfaced to an Exactive Orbitrap MS equipped with a heated electrospray interface (HESI). Acquisition was performed in negative ionization modes, in the mass range of m/z 100–1200.

The resolving power was set to 50,000 full width at half-maximum (FWHM, m/z 200) resulting in a scan time of 1 s. The automatic gain control was used in balanced mode (1×10^6 ions); maximum

injection time was 100 ms. The interface parameters were as follows: the spray voltage was at -3.2 kV, the capillary voltage was -50 V, the capillary temperature was at 275 °C, and a sheath and auxiliary gas flow of 30 and 15 arbitrary units were used.

The instrument was externally calibrated in the negative ion mode by infusion with a calibration solution consisted in sodium dodecyl sulfate, sodium taurocholate, and Ultramark 1621 in acetonitrile/methanol/water solution (2:1:1, v/v/v) containing 1% acetic acid.

Chromatographic data acquisition and peak integration were performed using Xcalibur software (Thermo Fisher Scientific, San Jose, USA).

Urolithins and their metabolites (Supplementary Table 3) were identified by comparison of retention times and MS data with those of reference compounds where standards were available, while in the absence of standards, the compounds were tentatively assigned using exact mass values up to the fifth decimal digit with mass tolerance ± 5 ppm.

The molecular formula and the selected ion for each compound are reported in Supplementary Table 3.

Analysis of markers of inflammation and metabolic disease

Serum high sensitivity C-Reactive Protein (hs-CRP), as biomarker of inflammation, was measured using a turbidimetric immunoassay (ADVIA 1800; Siemens Healthineers). According to the manufacturer, the detection limit was 0.01 mg/L, and the between-assay CVs were 5.4% and 1.4% at 0.5 and 4.5 mg/L CRP, respectively.

Other intermediate markers of metabolic disease were determined in 12.5 μ L plasma samples in duplicate by using the Bio-Plex Pro human diabetes immunoassays multiplex kit (Bio-Rad) and Luminex Technology (Bio-Plex; Bio-Rad), according to the manufacturer's instructions.

Blood samples were collected into EDTA-coated tubes and were immediately added with protease inhibitors, such as dipeptidylpeptidase IV inhibitor (Millipore) and phenylmethanesulfonyl fluoride (Sigma). They were centrifuged at 2400 g per 10 min at 4°C, and the supernatants were stored at -40°C before analysis.

The simultaneous quantification of C-peptide, ghrelin, glucose-dependent insulinotropic peptide (GIP), glucagon-like peptide-1 (GLP-1), glucagon, leptin, plasminogen activator inhibitor 1 (PAI-1), resistin, and visfatin was achieved by Bio-Plex Pro immunoassay kits. The sensitivity levels of the assay (in pg/mL) was for C-peptide 14.3, for ghrelin 1.2 for GIP 0.8, for GLP-1 5.3; for glucagon 4.8, for leptin 3.1, for PAI-1 2.2, for resistin 1.3, and for visfatin 37.1.

The interassay variation (% CV) was 4%, and the intra-assay variation (% CV) was 5%.

Blood glucose, insulin and HOMA

Glycaemia was measured in fasting subjects immediately before the blood collection by finger pricking and using a bedside glucometer (OneTouch Sure Step; Life Scan Inc.). Accuracy of the glucometer was evaluated by the manufacturer by using least squares linear regression analysis and it was found to be 97% "clinically accurate" compared with reference (YSI2700) results.

Insulin concentrations were measured in plasma samples by enzyme-linked immunosorbent assay (ELISA; DIAsource ImmunoAssays S.A., Nivelles, Belgium) on Triturus Analyzer (Diagnostics Grifols, S.A., Barcelona, Spain).

Fasting insulin resistance was evaluated by the Homeostatic Model Assessment for Insulin Resistance [HOMA-IR = (fasting glucose, mmol/L)*(fasting insulin, mU/L)/22.5].[27]

Determination of plasma lipids

Cholesterol and triglycerides were assayed in plasma and HDL by enzymatic colorimetric methods (ABX Diagnostics, Roche Molecular Biochemicals, and Wako Chemicals GmbH) on a Cobas Mira autoanalyzer (ABX Diagnostics). HDL was isolated from plasma by a precipitation method with a sodium phosphotungstate and magnesium chloride solution.

Determination of anthropometric measurements and body composition

Height of subjects was measured during the selection phase to the nearest 0.5 cm with a stadiometer (Model 213; Seca). Body weight was measured, after voiding, with subjects wearing light clothing to the nearest 0.1 kg on a digital scale (Model 703; Seca).

Waist circumference was measured on undressed subjects at the midpoint between the lower margin of the last palpable rib and the top of the iliac crest. Hip circumference was measured around the widest portion of the buttocks, with the tape parallel to the floor.

Body composition was determined by conventional bioelectrical impedance analysis with a single-frequency 50-kHz bioelectrical impedance analyzer (BIA 101 RJL; Akern Bioresearch) in the post-absorptive state, at an ambient temperature of 22–24°C, after voiding and after being in the supine position for 20 min. Body composition was calculated from bioelectrical measurements and anthropometric data by applying the software provided by the manufacturer by using validated predictive equations for total body water, fat mass, fat-free mass.

Statistical analysis

Sample size calculation. The sample size was calculated considering as primary endpoints fasting blood cholesterol and faecal SCFA. A sample size of 26 participants would be adequate to detect a 10% change in fasting total cholesterol by using variation in accordance with previous studies.[28, 1] The sample size needed to detect an effect of MedD on individual levels of faecal SCFA (acetate, propionate, butyrate) was calculated considering that in a previous study 6 subjects were sufficient to detect a difference of 20% between groups with a low vs high adherence of MedD.[29] Therefore 40 participants for each treatment group would be sufficient to detect a significant effect of MedD on selected biomarkers with an α error of 0.05, 80% power, and 2-sided testing.

Data analysis. Pairwise Spearman's rank correlations were calculated between microbiome, dietary variables, clinical markers and targeted metabolome datasets. The correlation plots were visualised using the *Hmisc* package, and the function *heatmap.2* or the *ComplexHeatmap* package.[30] Correction of p-values for multiple testing was performed when necessary (Benjamini-Hochberg method). In addition, single correlations were visualized as scatter plots (*ggscatter* function, *ggplot2* R package). Linear regression was performed using *lm* function (*stats* package) to predict the effect of one or more predictor variables on a quantitative outcome. In order to explore differences in metabolome profiles, a Partial Least Squares Discriminant Analysis (PLS-DA, *plsda* function) was applied (library *mixOmics*) on faecal, serum and urinary normalized datasets (*scale* function). The same R package was employed for the integration of targeted metabolomics and clinical variables, along with microbiota structure and functions using the DIABLO model (Data Integration Analysis for Biomarker discovery using Latent cOmponents). Moreover, statistical significance of the distance between MedD and ConD groups in the co-inertia analysis was computed using the Hotelling T₂ test (library *Hotelling*).

Classification. Machine learning-based classification analysis was done using the MetAML package.[31] A random forest classifier was used for all the experiments. An ensemble of 1,000 estimator trees and Shannon entropy were considered to evaluate the quality of a split at each node. Results were obtained through a five-fold cross-validation, stratified so that each fold contained a balanced proportion of positive and negative cases. The procedure of forming the folds and assessing the models was repeated 20 times. When classifying metabolomics data, the annotated and non-redundant profiles were considered as features.

SUPPLEMENTARY RESULTS

Pangenome analysis of selected microbial species. The pangenome of the HOMA- and serum hs-CRP-associated species (*P. copri*, *F. prausnitzii*, *B. uniformis*, *B. vulgatus*) was further investigated. No clear differences were found according to intervention, increase in dietary fibre consumption or decrease in HOMA indicating a high subject-specificity at the strain level (Supplementary Figure 36). We speculate that longer interventions or long-term diets may be required to detect diet-driven subspecies, as observed for *P. copri*.^[32] Indeed, high subject-specificity at the strain level was found. The phylogenetic tree obtained for *F. prausnitzii* is reported as an example, and similar results were obtained for the other species tested. No clustering of the samples according to the variables reported above was found, while samples from the same subject clearly grouped together.

REFERENCES

1. Vitaglione P, Mennella I, Ferracane R, *et al.* Whole-grain wheat consumption reduces inflammation in a randomized controlled trial on overweight and obese subjects with unhealthy dietary and lifestyle behaviors: role of polyphenols bound to cereal dietary fiber. *Am J Clin Nutr* 2014;**101**:251–61.
2. Craig CL, Marshall AL, Sjöström M, *et al.* International physical activity questionnaire: 12-country reliability and validity. *Med Sci Sport Exerc* 2003;**35**:1381–95.
3. Li J, Jia H, Cai X, *et al.* An integrated catalog of reference genes in the human gut microbiome. *Nat Biotechnol* 2014;**32**:834.
4. Cotillard A, Kennedy SP, Kong LC, *et al.* Dietary intervention impact on gut microbial gene richness. *Nature* 2013;**500**:585.
5. Langmead B, Salzberg SL. Fast gapped-read alignment with Bowtie 2. *Nat Methods* 2012;**9**:357.
6. Plaza Oñate F, Le Chatelier E, Almeida M, *et al.* MSPminer: abundance-based reconstitution of microbial pan-genomes from shotgun metagenomic data. *Bioinformatics* 2018;**35**:1544–52.
7. Nielsen HB, Almeida M, Juncker AS, *et al.* Identification and assembly of genomes and genetic elements in complex metagenomic samples without using reference genomes. *Nat Biotechnol* 2014;**32**:822.
8. Kanehisa M, Goto S. KEGG: kyoto encyclopedia of genes and genomes. *Nucleic Acids Res* 2000;**28**:27–30.
9. Kanehisa M, Sato Y, Kawashima M, *et al.* KEGG as a reference resource for gene and protein annotation. *Nucleic Acids Res* 2015;**44**:D457–62.
10. Darzi Y, Falony G, Vieira-Silva S, *et al.* Towards biome-specific analysis of meta-omics data. *ISME J* 2016;**10**:1025.
11. Vieira-Silva S, Falony G, Darzi Y, *et al.* Species–function relationships shape ecological properties of the human gut microbiome. *Nat Microbiol* 2016;**1**:16088.
12. Rinke C, Schwientek P, Sczyrba A, *et al.* Insights into the phylogeny and coding potential of microbial dark matter. *Nature* 2013;**499**:431.
13. Scholz M, Ward D V, Pasolli E, *et al.* Strain-level microbial epidemiology and population genomics from shotgun metagenomics. *Nat Methods* 2016;**13**:435.
14. Klindworth A, Pruesse E, Schweer T, *et al.* Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. *Nucleic Acids Res* 2013;**41**:e1–e1.

15. Magoč T, Salzberg SL. FLASH: fast length adjustment of short reads to improve genome assemblies. *Bioinformatics* 2011;**27**:2957–63.
16. Schmieder R, Edwards R. Quality control and preprocessing of metagenomic datasets. *Bioinformatics* 2011;**27**:863–4.
17. Ritari J, Salojärvi J, Lahti L, *et al.* Improved taxonomic assignment of human intestinal 16S rRNA sequences by a dedicated reference database. *BMC Genomics* 2015;**16**:1056.
18. Claesson MJ, Jeffery IB, Conde S, *et al.* Gut microbiota composition correlates with diet and health in the elderly. *Nature* 2012;**488**:178.
19. Pluskal T, Castillo S, Villar-Briones A, *et al.* MZmine 2: modular framework for processing, visualizing, and analyzing mass spectrometry-based molecular profile data. *BMC Bioinformatics* 2010;**11**:395.
20. Dieterle F, Ross A, Schlotterbeck G, *et al.* Probabilistic quotient normalization as robust method to account for dilution of complex biological mixtures. Application in 1H NMR metabonomics. *Anal Chem* 2006;**78**:4281–90.
21. Smith CA, O’Maille G, Want EJ, *et al.* METLIN: a metabolite mass spectral database. *Ther Drug Monit* 2005;**27**:747–51.
22. Wishart DS, Jewison T, Guo AC, *et al.* HMDB 3.0—the human metabolome database in 2013. *Nucleic Acids Res* 2012;**41**:D801–7.
23. Sumner LW, Amberg A, Barrett D, *et al.* Proposed minimum reporting standards for chemical analysis. *Metabolomics* 2007;**3**:211–21.
24. Christiansen CB, Gabe MBN, Svendsen B, *et al.* The impact of short-chain fatty acids on GLP-1 and PYY secretion from the isolated perfused rat colon. *Am J Physiol Liver Physiol* 2018;**315**:G53–65.
25. Steuer C, Schütz P, Bernasconi L, *et al.* Simultaneous determination of phosphatidylcholine-derived quaternary ammonium compounds by a LC–MS/MS method in human blood plasma, serum and urine samples. *J Chromatogr B* 2016;**1008**:206–11.
26. Tulipani S, Urpi-Sarda M, García –Villalba R *et al.* Urolithins Are the Main Urinary Microbial-Derived Phenolic Metabolites Discriminating a Moderate Consumption of Nuts in Free-Living Subjects with Diagnosed Metabolic Syndrome. *J. Agric. Food Chem.* 2012; **60**(36):8930–40.
27. Matthews DR, Hosker JP, Rudenski AS, *et al.* Homeostasis model assessment: insulin resistance and β -cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985;**28**:412–9.
28. Annuzzi G, Bozzetto L, Costabile G, *et al.* Diets naturally rich in polyphenols improve fasting and postprandial dyslipidemia and reduce oxidative stress: a randomized controlled trial. *Am J Clin Nutr* 2013;**99**:463–71.

29. De Filippis F, Pellegrini N, Vannini L, *et al.* High-level adherence to a Mediterranean diet beneficially impacts the gut microbiota and associated metabolome. *Gut* 2016;**65**:1812–21.
30. Gu Z, Eils R, Schlesner M. Complex heatmaps reveal patterns and correlations in multidimensional genomic data. *Bioinformatics* 2016;**32**:2847–9.
31. Pasolli E, Truong DT, Malik F, *et al.* Machine learning meta-analysis of large metagenomic datasets: tools and biological insights. *PLoS Comput Biol* 2016;**12**:e1004977.
32. De Filippis F, Pasolli E, Tett A, *et al.* Distinct genetic and functional traits of human intestinal *Prevotella copri* strains are associated with different habitual diets. *Cell Host Microbe* 2019;**25**:444–53.

The DINAMIC (Diet- induced Arrangement of the gut Microbiome for Improvement of Cardiometabolic health) consortium

Jordana Bell¹, Tim Spector¹, Annette Peters², Stanislav Ehrlich³, Lars Ove Dragsted⁴, Max Nieuwdorp⁵, Danilo Ercolini⁶, Jean-Francois Brugère⁷, Paul W. O'Toole^{8 9}, Thomas Clavel^{10 11}, Dirk Haller¹¹

¹ The Department of Twin Research, Kings College London, 3-4th Floor South Wing Block D, St Thomas' Hospital, Westminster Bridge Road, SE1 7E

² Helmholtz Zentrum München, Ingolstädter Landstr 1, Germany

³ MetaGenoPolis, Rue du fond de la noue, 78350 Jouy-en-Josas, France

⁴ University of Copenhagen - Dept. Nutrition, Exercise and Sports, 30 Rolighedsvej, 1958 Frederiksberg C, Denmark

⁵ Amsterdam Diabetes Center AMC-VUmc, Meibergdreef 9, room F4-159.2, The Netherlands

⁶ University of Naples Federico II - Department of Agricultural Sciences, via Università 100 Portici, Italy

⁷ Université d'Auvergne Clermont 1, EA-CIDAM 5e CBRV - Facultés de médecine et pharmacie - Place Henri Dunant - Clermont-Ferrand, France

⁸ School of Microbiology School of Microbiology, University College Cork, Cork, Ireland

⁹ APC Microbiome Inst., Cork, IRELAND

¹⁰ Institute of Medical Microbiology, Functional Microbiome Research Group, University Hospital of RWTH Aachen, Pauwelsstrasse 30, 52074 Aachen, Germany

¹¹ TU Munich, ZIEL Institute for Food and Health, Gregor-Mendel-Strasse 2, 85354 Freising, Germany

SUPPLEMENTARY FIGURES

Supplementary Figure 1 Participant flow and study design.

Supplementary Figure 2 Spearman's rank-order correlation between MD index and Healthy Food Diversity index.

Supplementary Figure 3 Daily intake of proteins, dietary fibre, total lipids, saturated fatty acids, monounsaturated fatty acids and polyunsaturated fatty acids from the food categories recorded by subjects in ConD and MedD group at baseline, 4w and 8w.

Supplementary Figure 4 Linear regression analysis used to test if the MD index significantly predicted Total Cholesterol reduction, after adjusting for age, gender, BMI and energy intake.

Supplementary Figure 5 Retention times and measured features of acylcarnitines.

Supplementary Figure 6. Identification of acyl carnitines comparing retention time and m/z measured in urine with retention times and m/z of authentic standards.

Supplementary Figure 7. Identification of isovalerylcarnitine comparing retention time and m/z measured in urine with retention time and m/z of an authentic standard.

Supplementary Figure 8. Identification of branched-chain amino acids comparing retention time and m/z measured in urine with retention time and m/z of authentic standards.

Supplementary Figure 9. Identification of aromatic amino acids comparing retention time and m/z measured in urine with retention time and m/z of authentic standards.

Supplementary Figure 10. Identification of dihydroxybenzoic acids comparing retention time and m/z measured in urine with retention times and m/z of authentic standards.

Supplementary Figure 11. Identification of Urolithin A and Urolithin A-glucuronide comparing retention times and m/z measured in urine with retention times and m/z of authentic standards.

Supplementary Figure 12. Identification of Urolithin C-glucuronide and Urolithin C comparing retention times and m/z measured in urine and faeces, respectively, with retention times and m/z of authentic standards.

Supplementary Figure 13. Identification of Tryptophan betaine comparing retention time, m/z and spectrum measured in urine with retention time, m/z and spectrum of an authentic standard.

Supplementary Figure 14. Identification of Oxindole-3-acetic acid comparing retention time, m/z and spectrum measured in urine with retention time, m/z and spectrum of an authentic standard.

Supplementary Figure 15. Identification of Pipecolic acid betaine comparing retention time and m/z measured in urine with retention time and m/z of an authentic standard.

Supplementary Figure 16. Identification of Phenyllactic acid comparing retention time and m/z measured in urine with retention time and m/z of an authentic standard.

Supplementary Figure 17. Identification of 3-methylpyrogallol-sulfate comparing retention time and m/z measured in urine with retention time and m/z of an authentic standard.

Supplementary Figure 18. Identification of Phenylacetylglutamine comparing retention time, m/z and spectrum measured in urine with retention time, m/z and spectrum of an authentic standard.

Supplementary Figure 19. Identification of P-cresol sulfate comparing retention time, m/z and spectrum measured in urine with retention time, m/z and spectrum of an authentic standard.

Supplementary Figure 20. Identification of Indoxyl sulfate comparing retention time, m/z and spectrum measured in serum with retention time, m/z and spectrum of an authentic standard. We noted a shift in retention time (0.3 min) for indoxyl sulfate compared to the original data collected.

Supplementary Figure 21. Identification of Phenol sulfate comparing retention time, m/z and spectrum measured in urine with retention time, m/z and spectrum of an authentic standard.

Supplementary Figure 22. Identification of 3-carboxy-4-methyl-5-propanyl-2-furanpropionic acid (CMPF) comparing retention time, m/z and spectrum measured in urine with retention time, m/z and spectrum of an authentic standard.

Supplementary Figure 23. Identification of trimethylamine-N-oxide (TMAO) comparing retention time and m/z measured in urine with retention time and m/z of an authentic standard.

Supplementary Figure 24. Retention times of bile acid authentic standards.

Supplementary Figure 25. Identification of Glycochenodeoxycholic acid sulfate comparing retention time and m/z measured in urine with retention time and m/z of an authentic standard.

Supplementary Figure 26 Diverging bar charts showing Spearman's correlations between MD Index and annotated metabolites from faecal, serum and urine metabolome.

Supplementary Figure 27 Barcoding plots of the contrasted MSP species between ConD and MedD diets at baseline.

Supplementary Figure 28 Barcoding plots of the contrasted MSP species between ConD and MedD diets at 4w.

Supplementary Figure 29 Barcoding plots of the contrasted MSP species between ConD and MedD diets at 8w.

Supplementary Figure 30 Distinctive metabolic, dietary and microbial signatures between ConD and MedD diets after 4 weeks.

Supplementary Figure 31 N-integrative supervised analysis of different types of 'omics datasets.

Supplementary Figure 32 Correlation of microbiome-related metabolites with dietary and clinical variables.

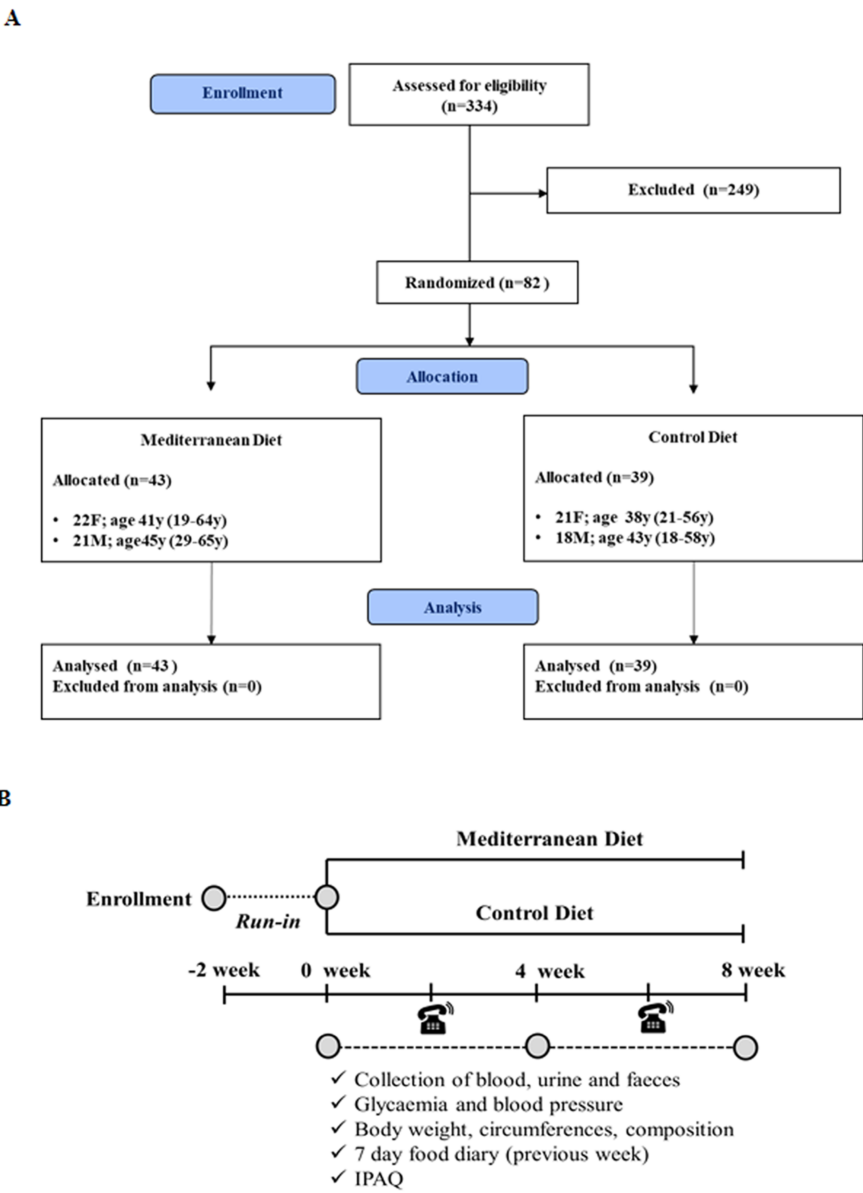
Supplementary Figure 33 Box plots showing faecal concentrations of several BAs measured throughout the intervention.

Supplementary Figure 34 Box plots showing differences in HOMA variation classifying the subjects in HOMA reducers and non-reducers after 4 weeks of intervention.

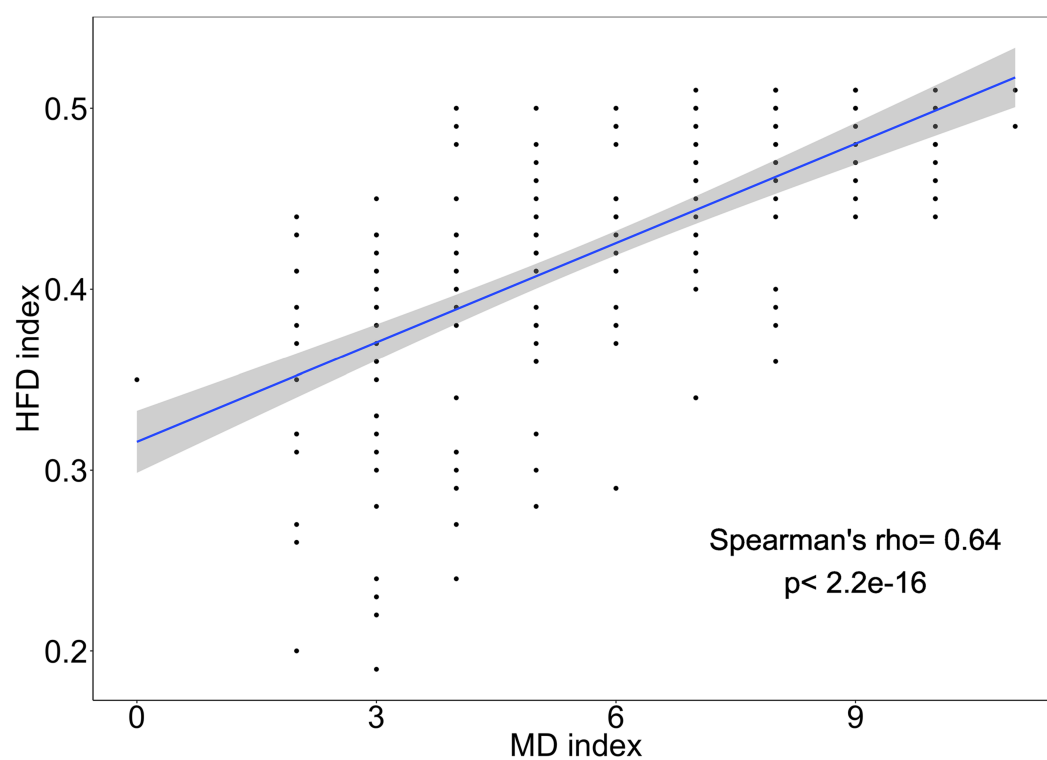
Supplementary Figure 35 Box plots showing differences in abundance of Co-Abundance Groups obtained from 16S rRNA gene sequences in subjects classified as HOMA reducers and non-reducers after 4 weeks of intervention.

Supplementary Figure 36 Circular tree showing clustering of the subjects based on *Faecalibacterium prausnitzii* pangenome.

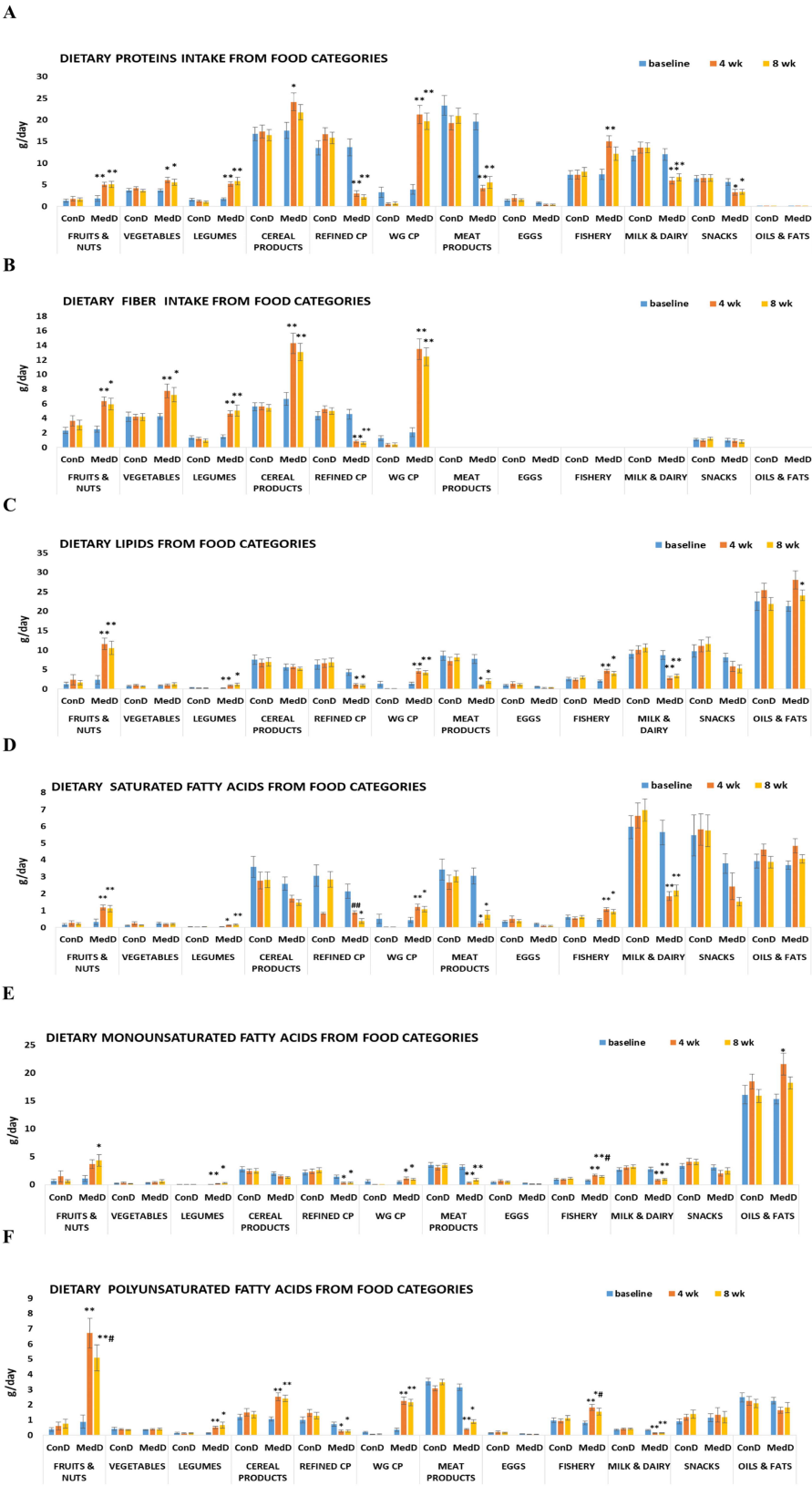
SUPPLEMENTARY FIGURES



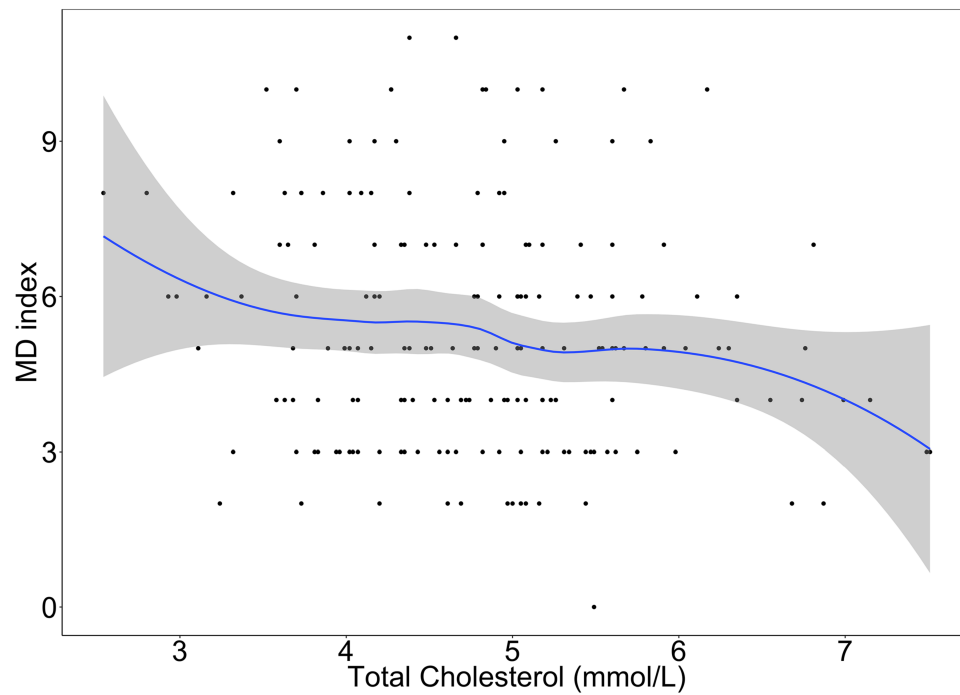
Supplementary Figure 1 (A) Participant flow and (B) study design.



Supplementary Figure 2 Spearman's rank-order correlation between MD index and Healthy Food Diversity (HFD) index.

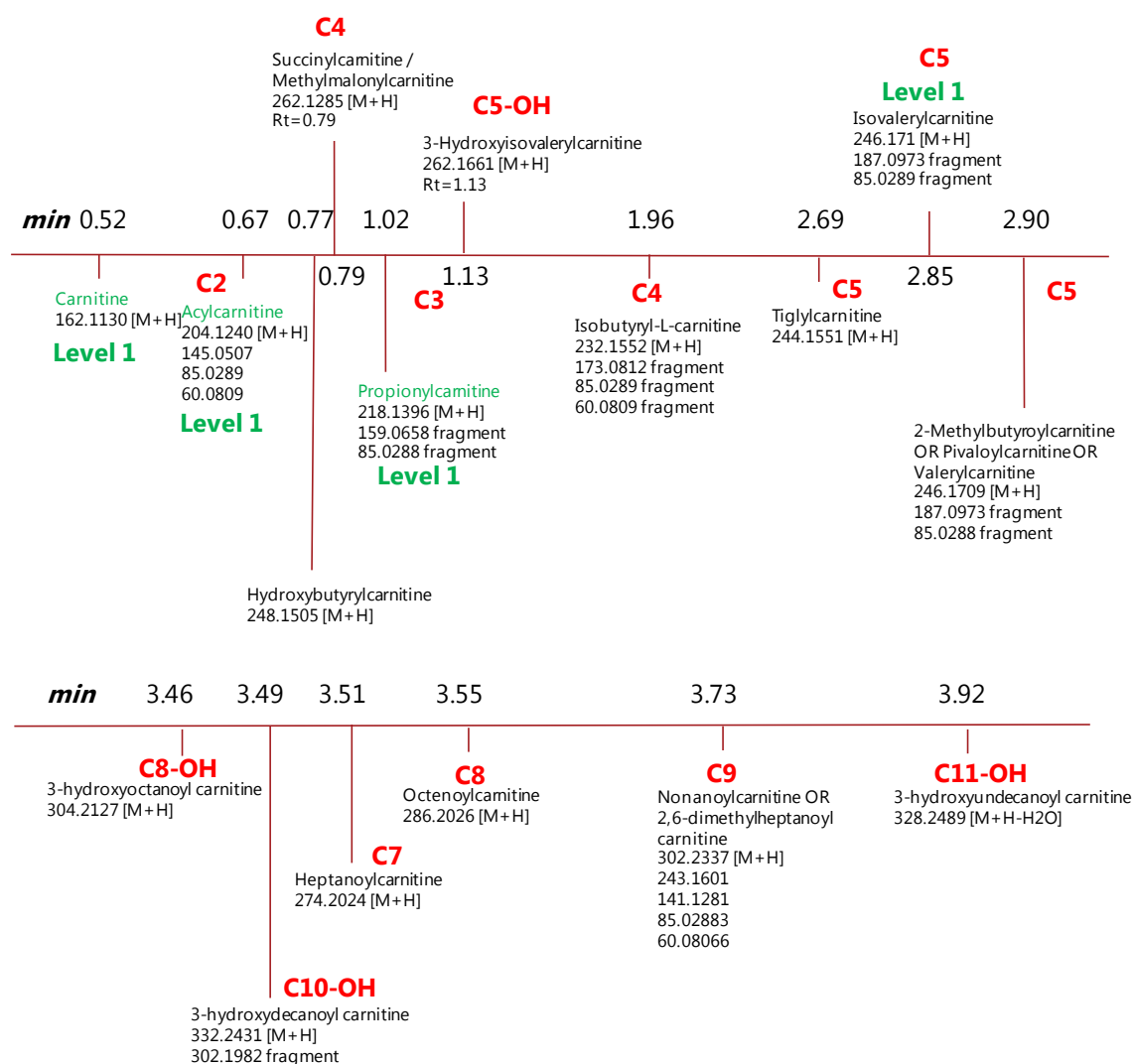


Supplementary Figure 3 Daily intake (g/day) of (A) proteins, (B) dietary fiber, (C) total lipids, (D) saturated fatty acids, (E) monounsaturated fatty acids and (F) polyunsaturated fatty acids from the food categories (fruits & nuts, vegetables, legumes, cereal products, refined cereal products, wholegrain-based cereal products, meat products, eggs, fishery, milk & dairy products, snacks, oil & fats) recorded by subjects in Control (ConD, n=32) and Mediterranean (MedD, n=30) diet group at baseline (week 0), 4w and 8w. Bars indicate the means \pm SEM. * indicates $p<0.05$ and ** indicates $p<0.001$ for MedD vs ConD at specific time point compared to baseline; # indicates $p<0.05$ and ## indicates $p<0.001$ for MedD vs ConD at 8w vs 4w; 2-way ANOVA with repeated measures and Tukey post hoc test.

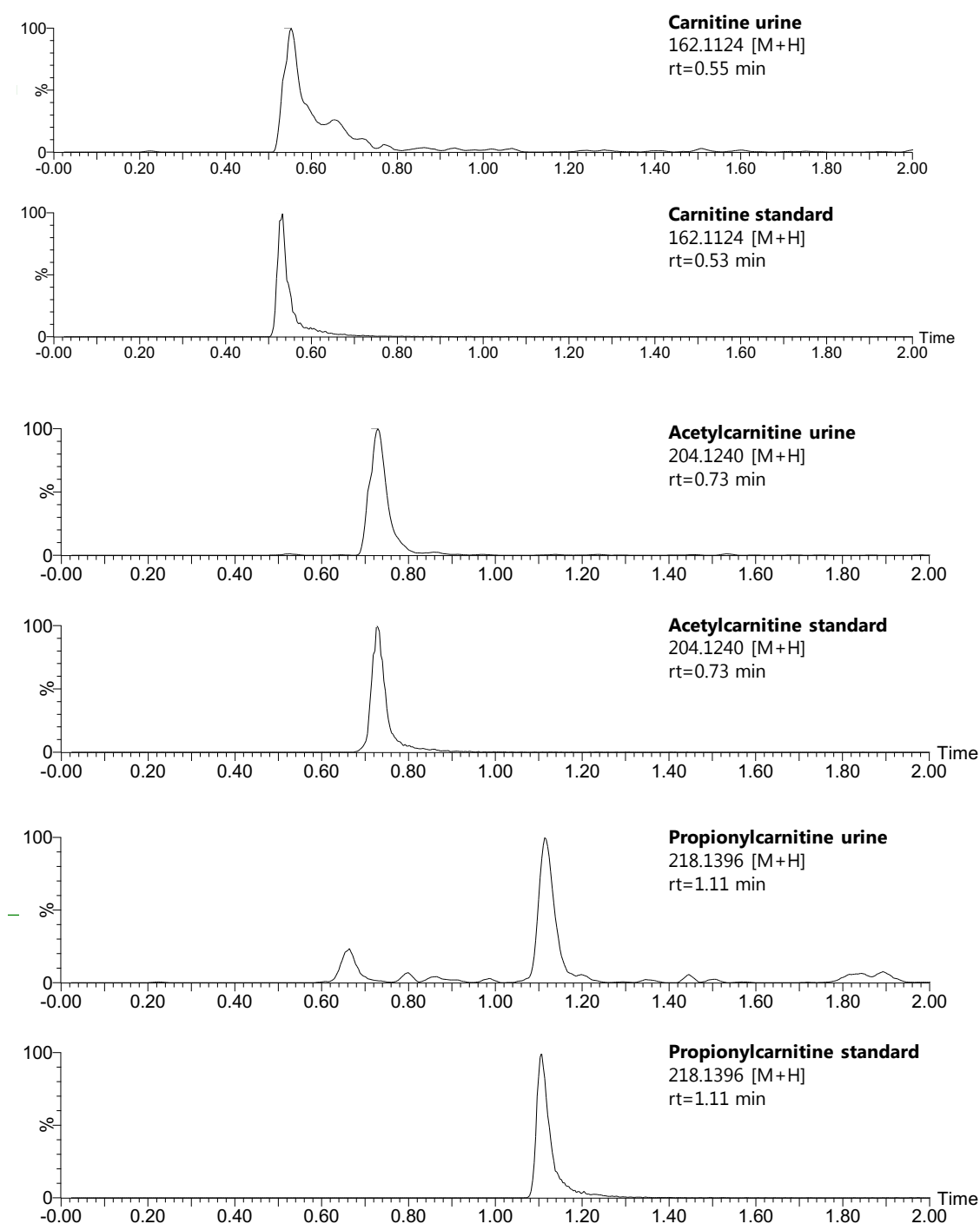


Supplementary Figure 4 Linear regression analysis used to test if the MD index significantly predicted Total Cholesterol reduction, after adjusting for age, gender, BMI and energy intake. The result of the regression indicated the predictors explained the 28% of the variance (Adjusted R-squared= 0.26, pvalue: 1.206e-11). Total Cholesterol (mmol/L) = $4.38 - 0.08$ MD index.

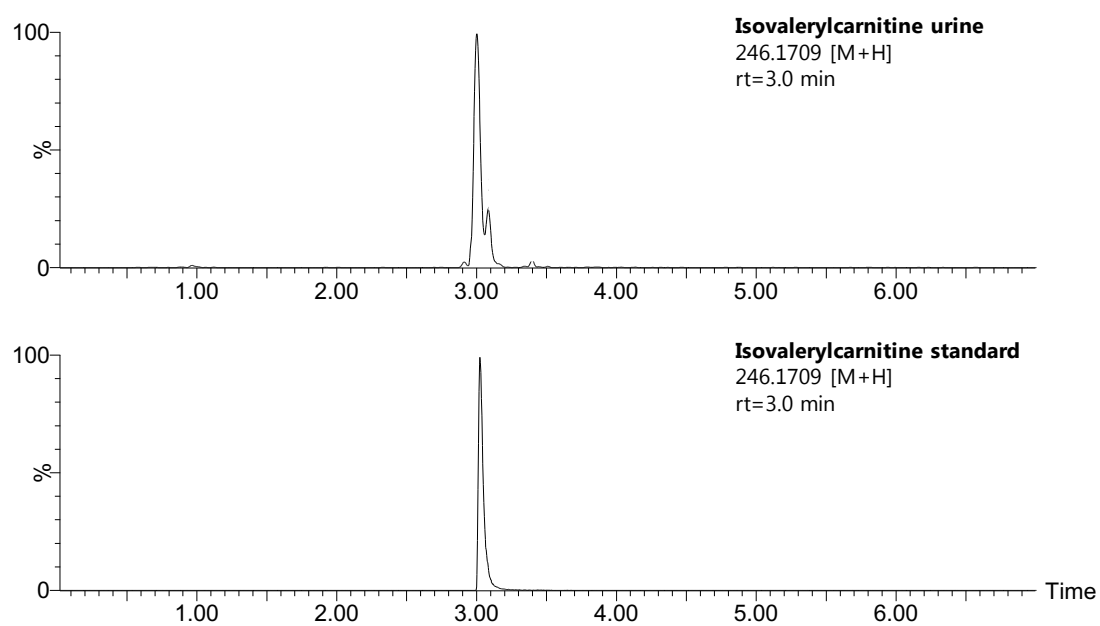
Acylcarnitines



Supplementary Figure 5. Retention times and measured features of acylcarnitines. The acyl chain length is marked with the red letters from C2 to C11. Carnitine, acylcarnitine, propionylcarnitine and isovaleryl carnitine were validated using authentic standards (level 1 identification). The other acylcarnitines were identified at level 3.



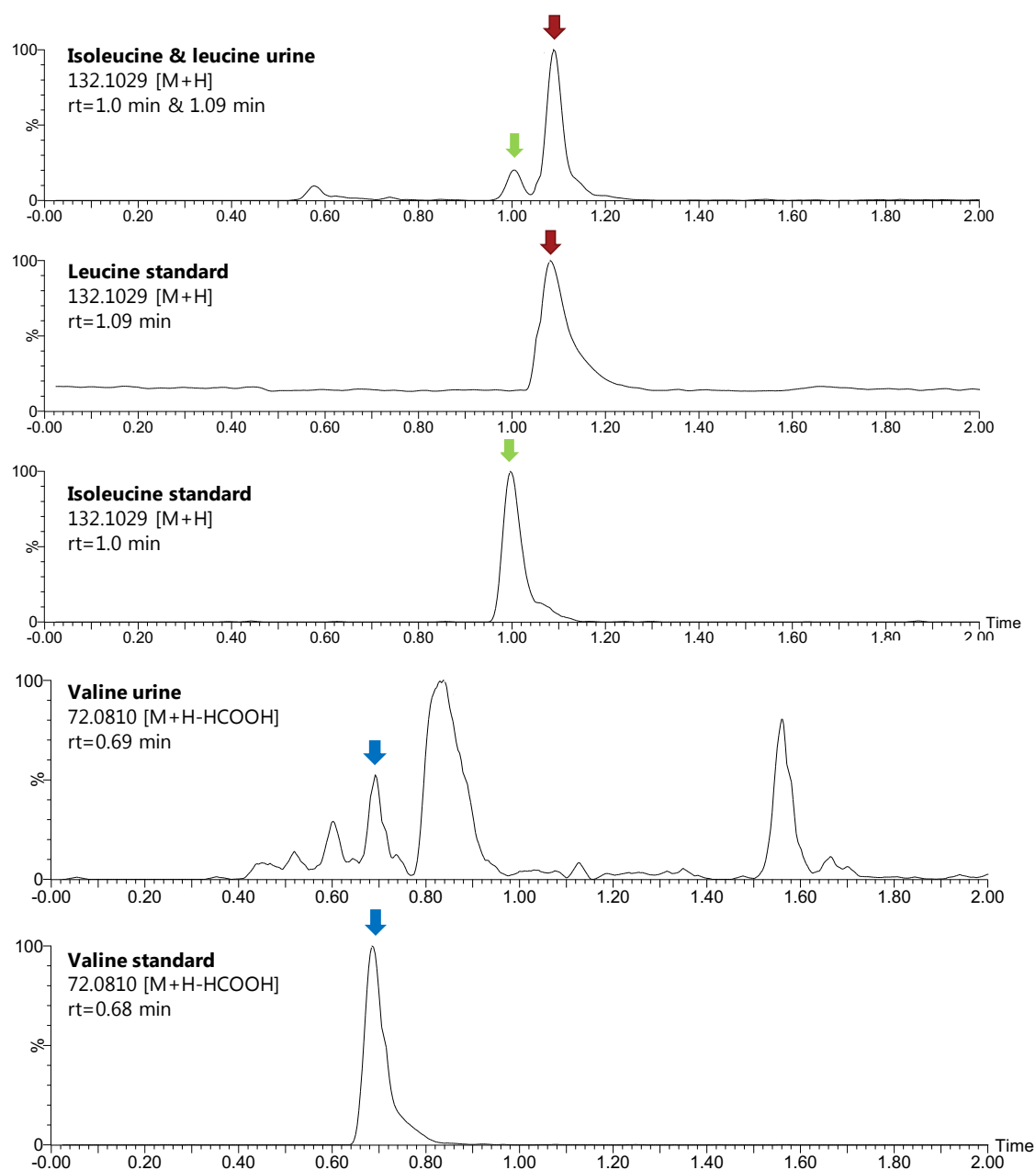
Supplementary Figure 6. Identification of acyl carnitines comparing retention time and m/z measured in urine with retention times and m/z of authentic standards.



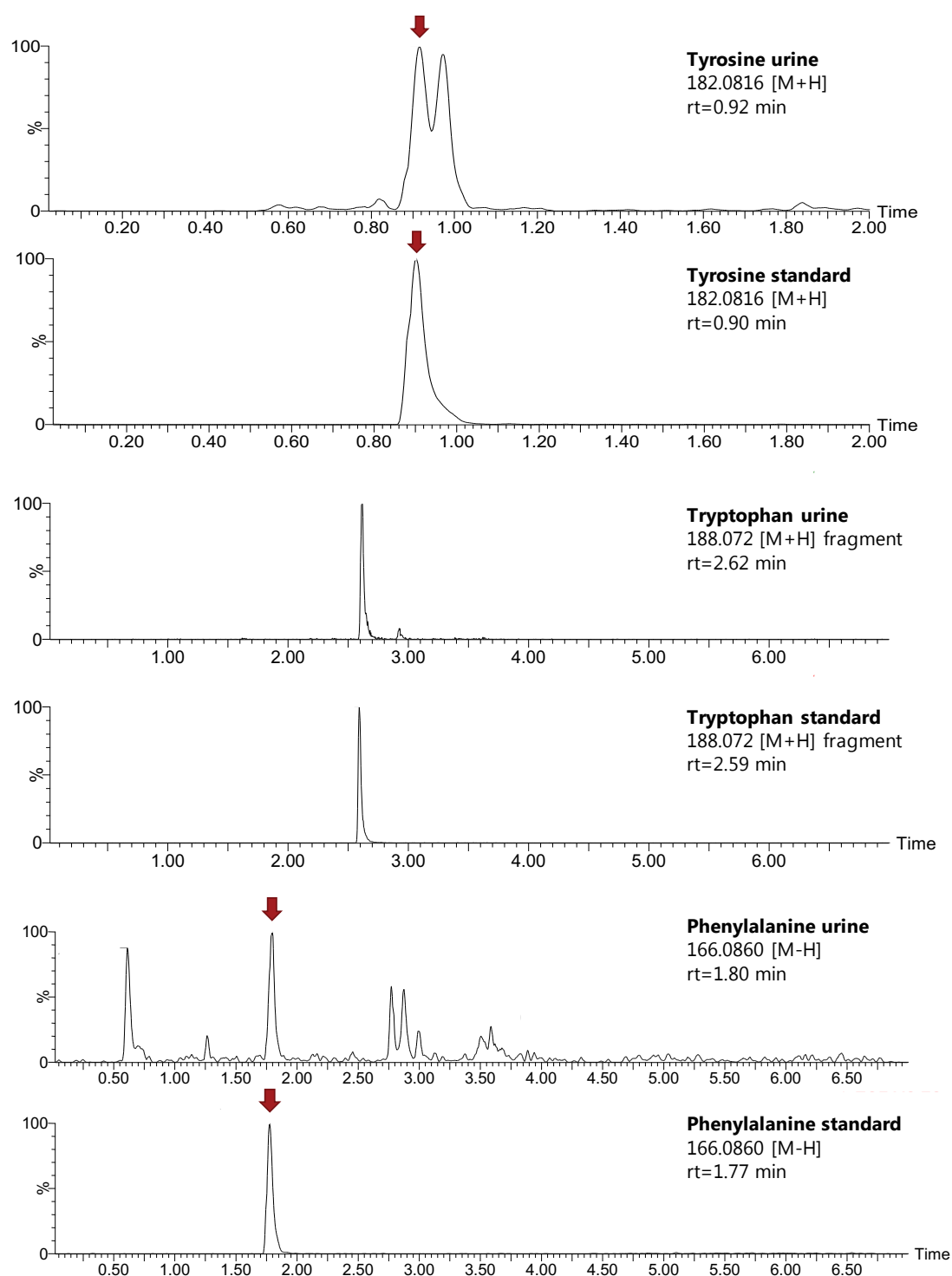
Supplementary Figure 7. Identification of isovalerylcarnitine comparing retention time and m/z measured in urine with retention time and m/z of an authentic standard.

Amino acids

Isoleucine and leucine

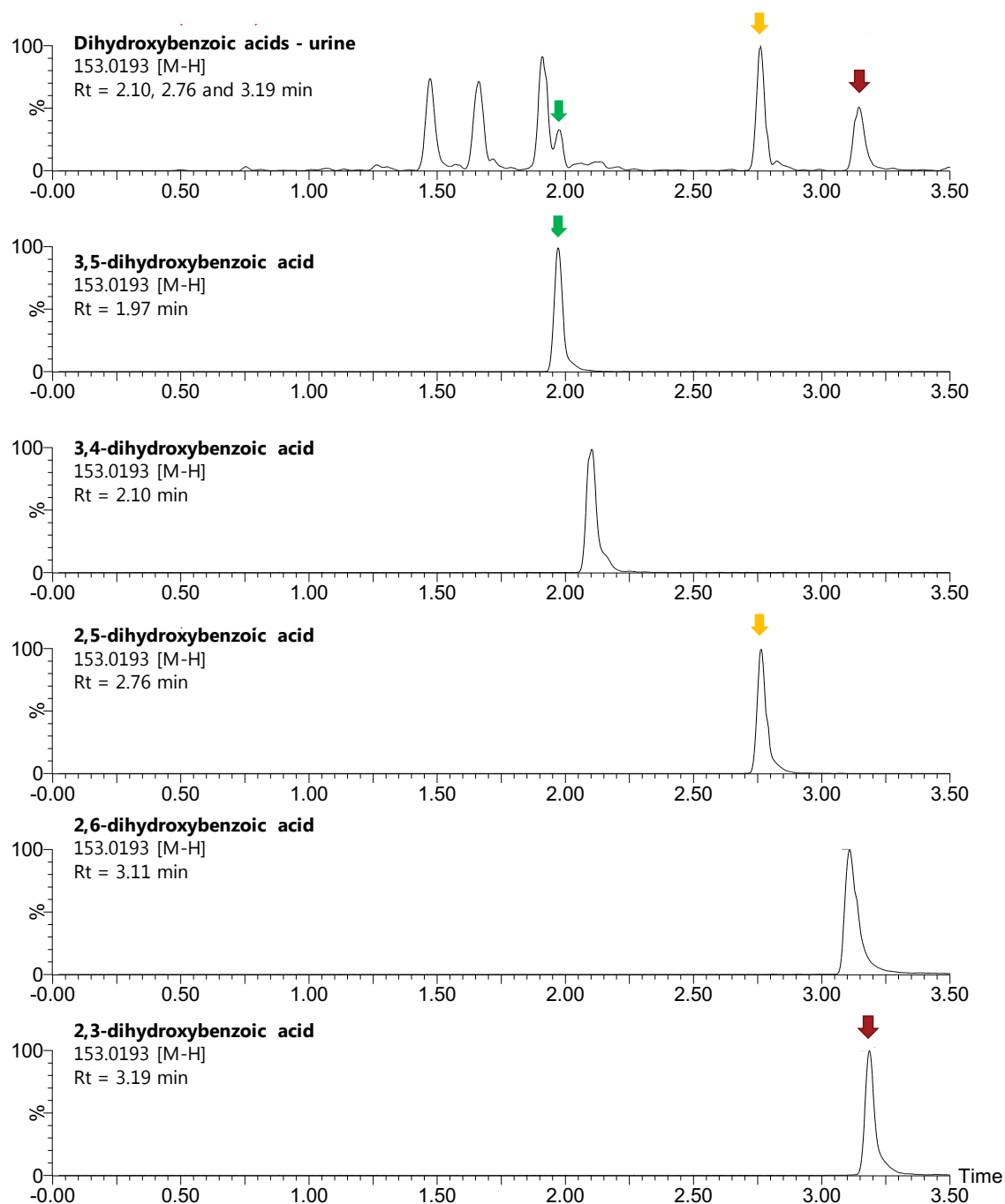


Supplementary Figure 8. Identification of branched-chain amino acids comparing retention time and m/z measured in urine with retention time and m/z of authentic standards.

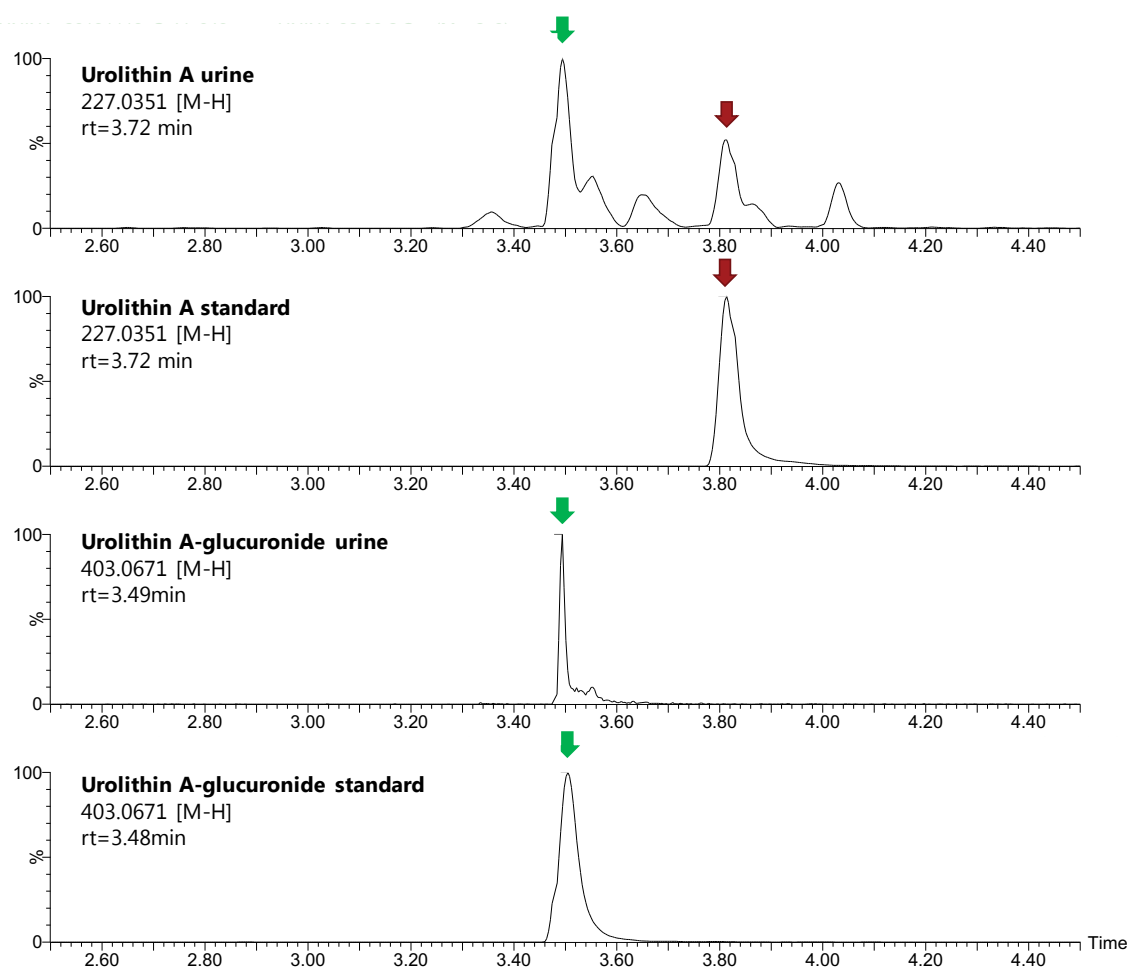


Supplementary Figure 9. Identification of aromatic amino acids comparing retention time and m/z measured in urine with retention time and m/z of authentic standards.

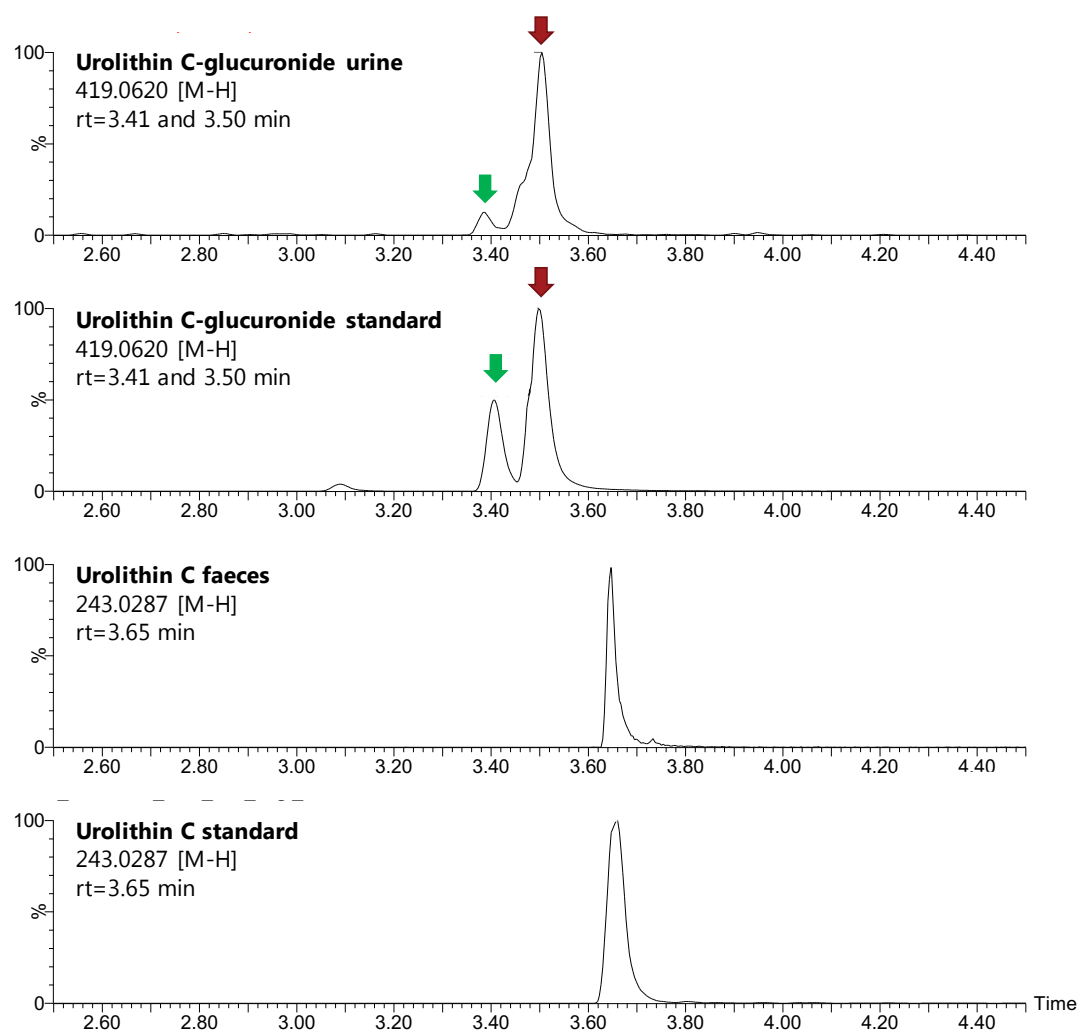
Plant biomarkers



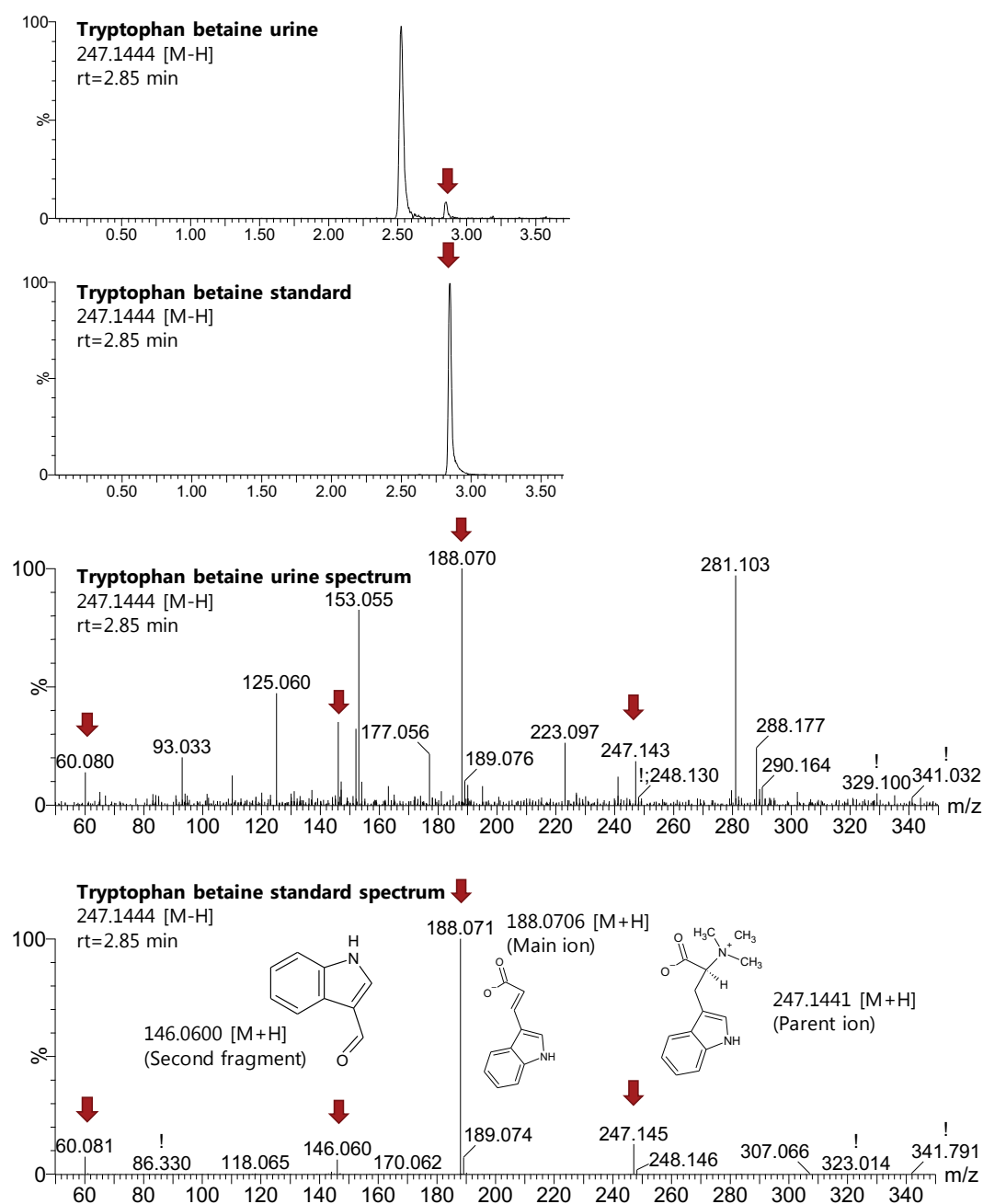
Supplementary Figure 10. Identification of dihydroxybenzoic acids comparing retention time and m/z measured in urine with retention times and m/z of authentic standards.



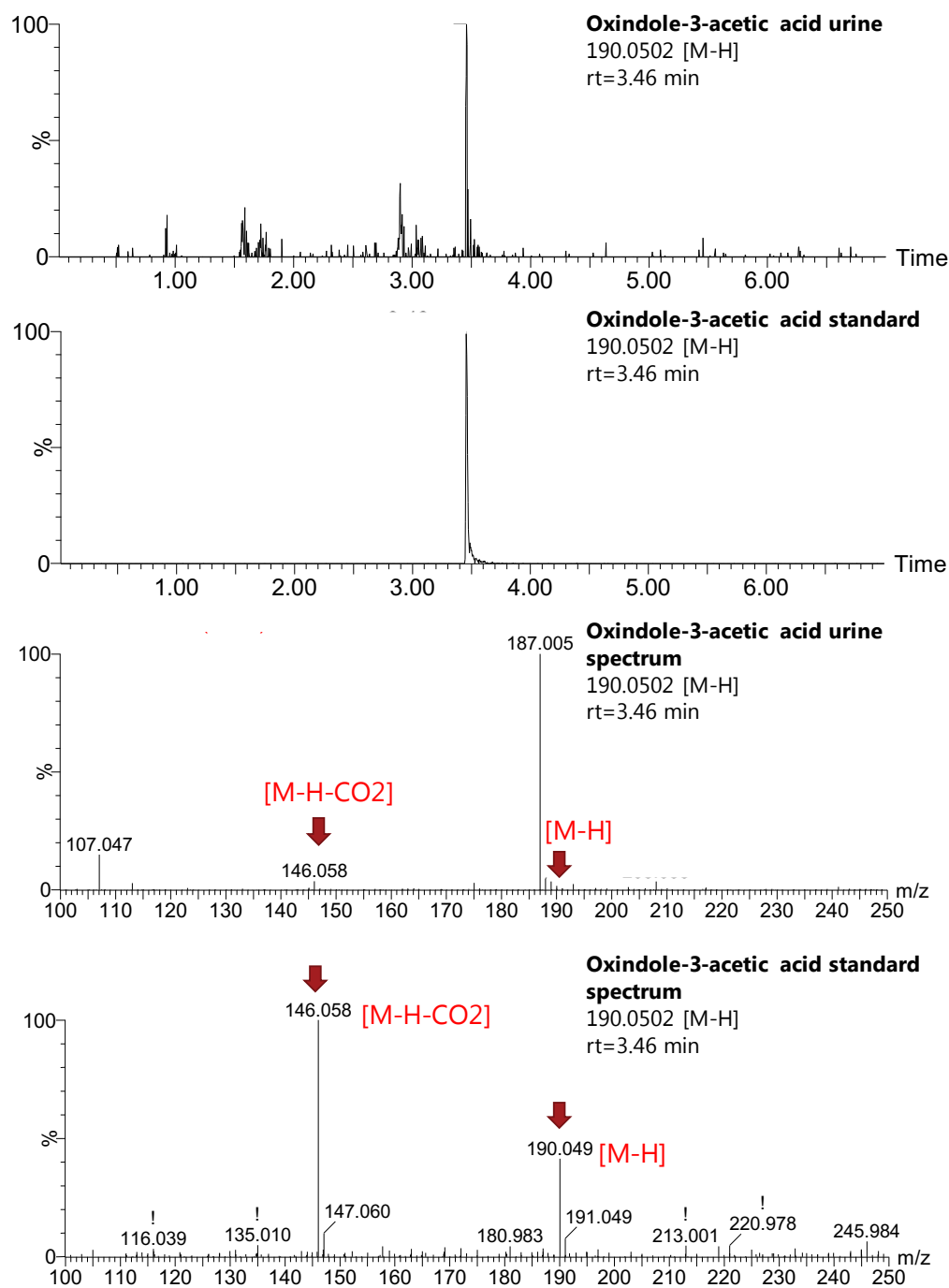
Supplementary Figure 11. Identification of Urolithin A and Urolithin A-glucuronide comparing retention times and m/z measured in urine with retention times and m/z of authentic standards.



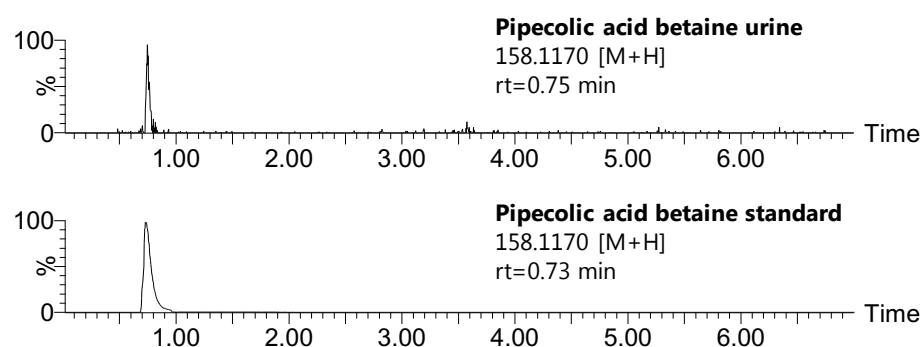
Supplementary Figure 12. Identification of Urolithin C-glucuronide and Urolithin C comparing retention times and m/z measured in urine and faeces, respectively, with retention times and m/z of authentic standards.



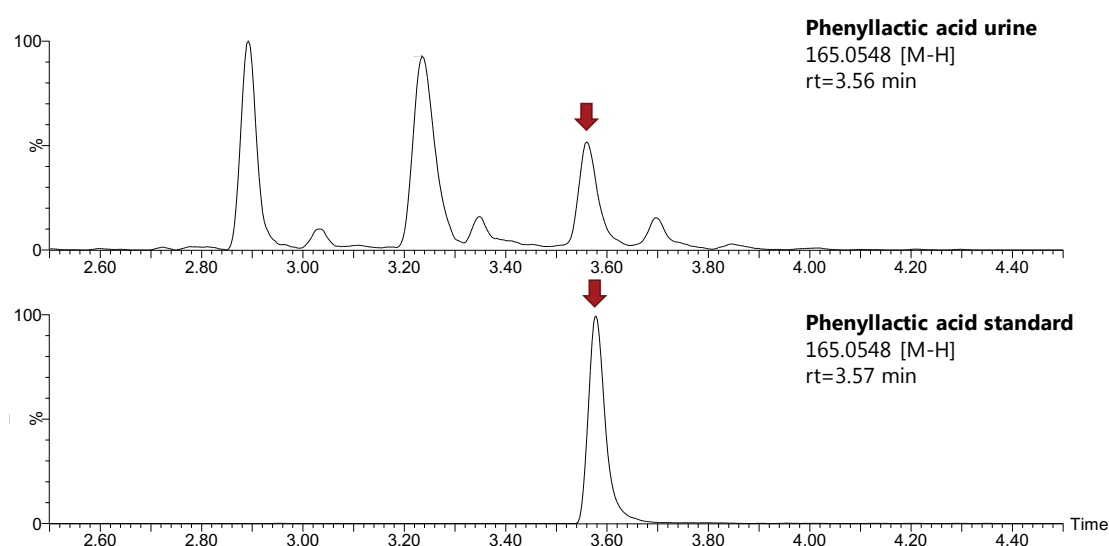
Supplementary Figure 13. Identification of Tryptophan betaine comparing retention time, m/z and spectrum measured in urine with retention time, m/z and spectrum of an authentic standard.



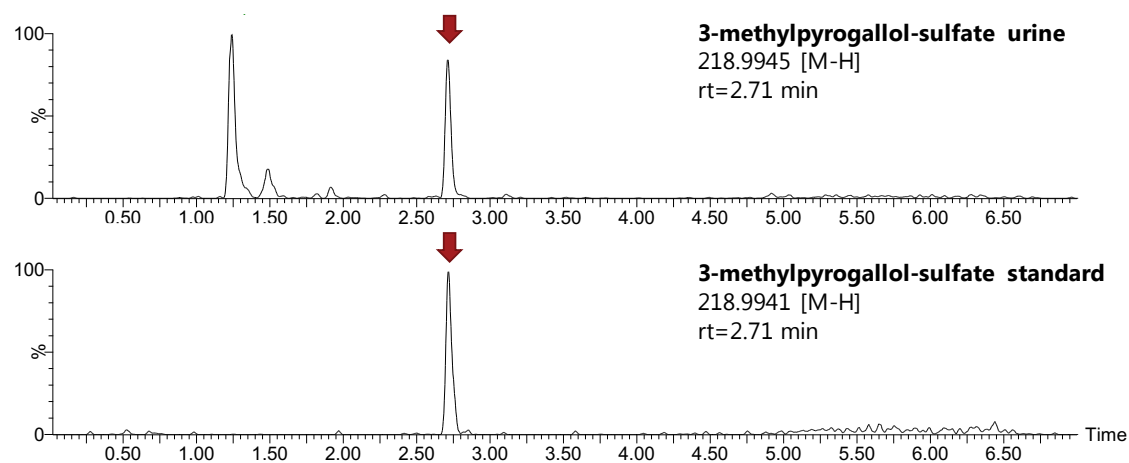
Supplementary Figure 14. Identification of Oxindole-3-acetic acid comparing retention time, m/z and spectrum measured in urine with retention time, m/z and spectrum of an authentic standard.



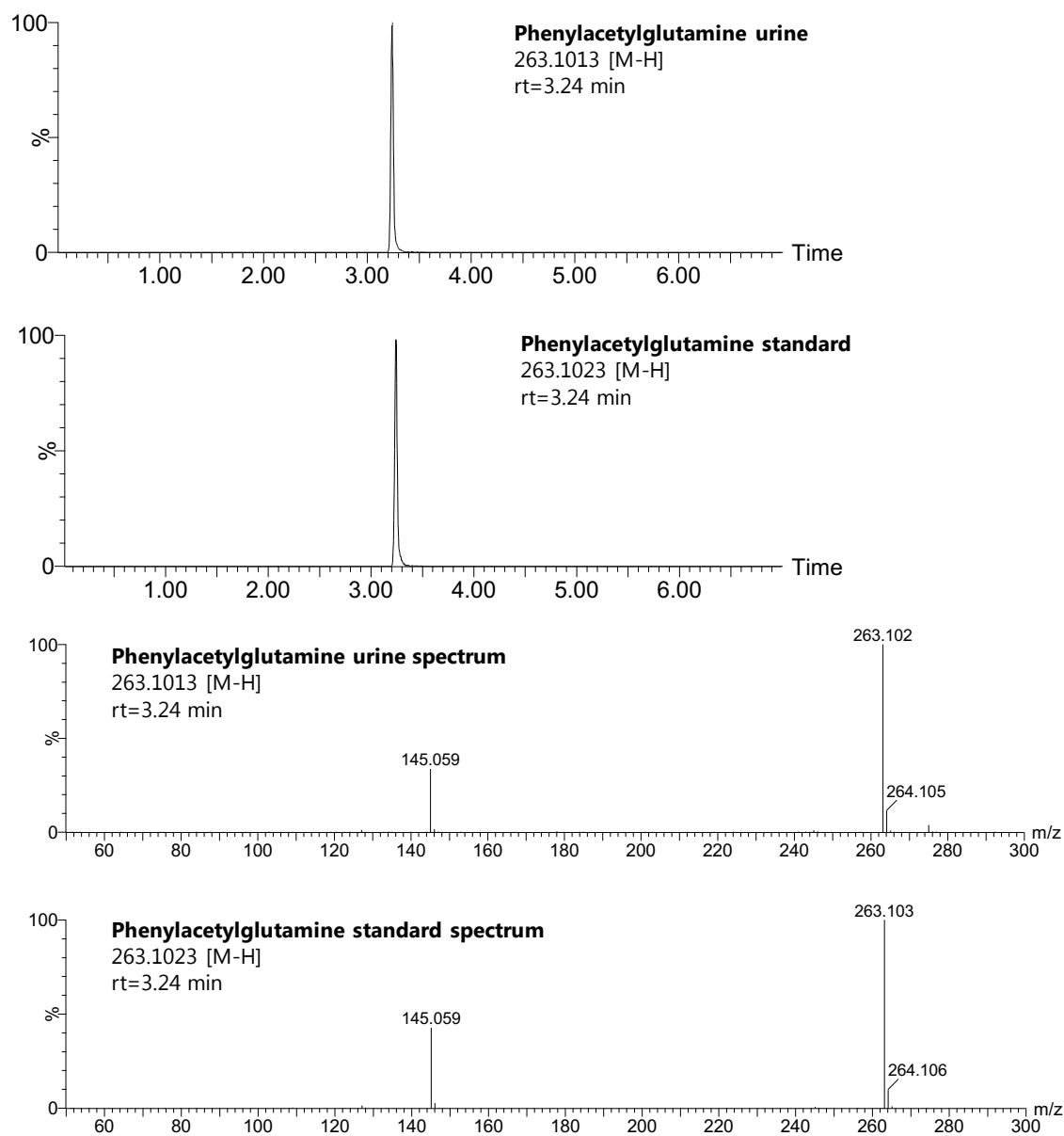
Supplementary Figure 15. Identification of Pipecolic acid betaine comparing retention time and m/z measured in urine with retention time and m/z of an authentic standard.



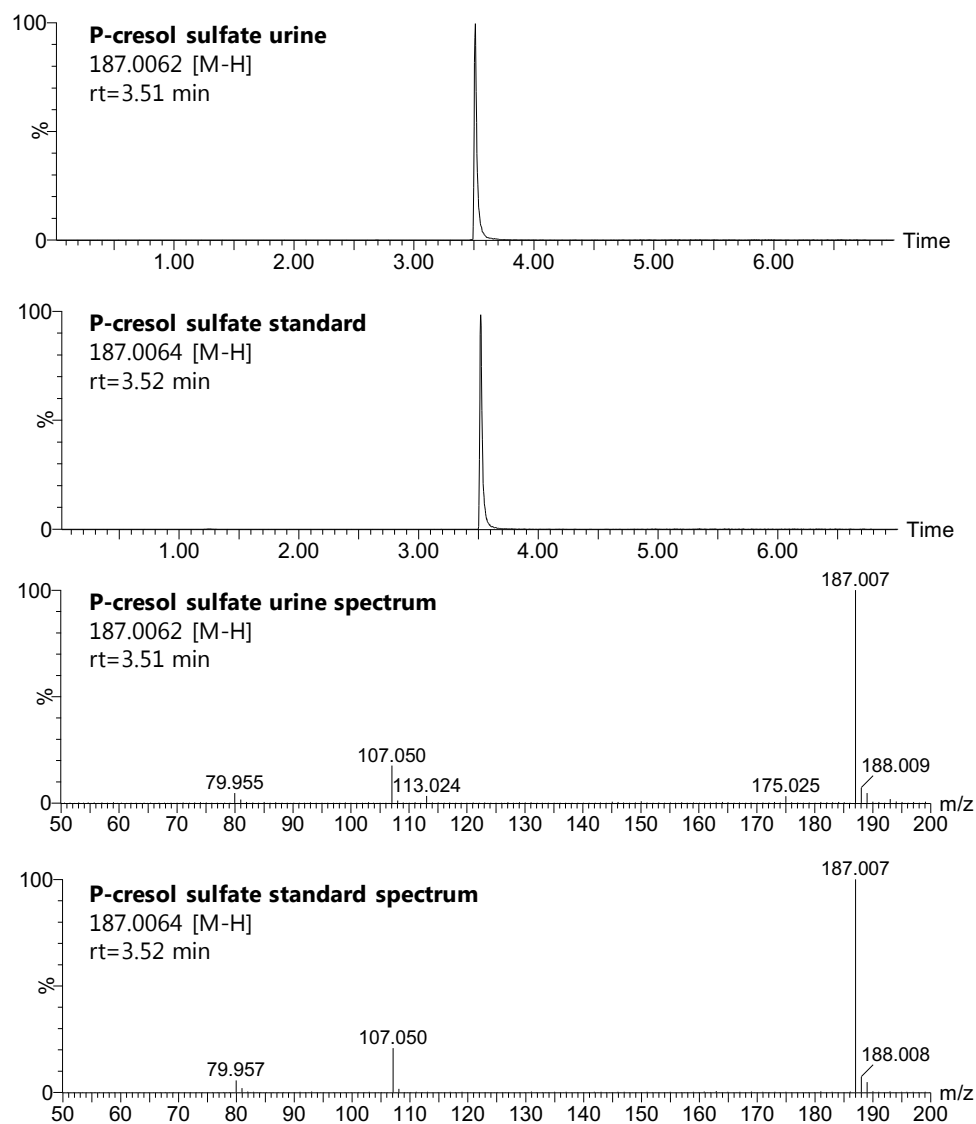
Supplementary Figure 16. Identification of Phenyllactic acid comparing retention time and m/z measured in urine with retention time and m/z of an authentic standard.



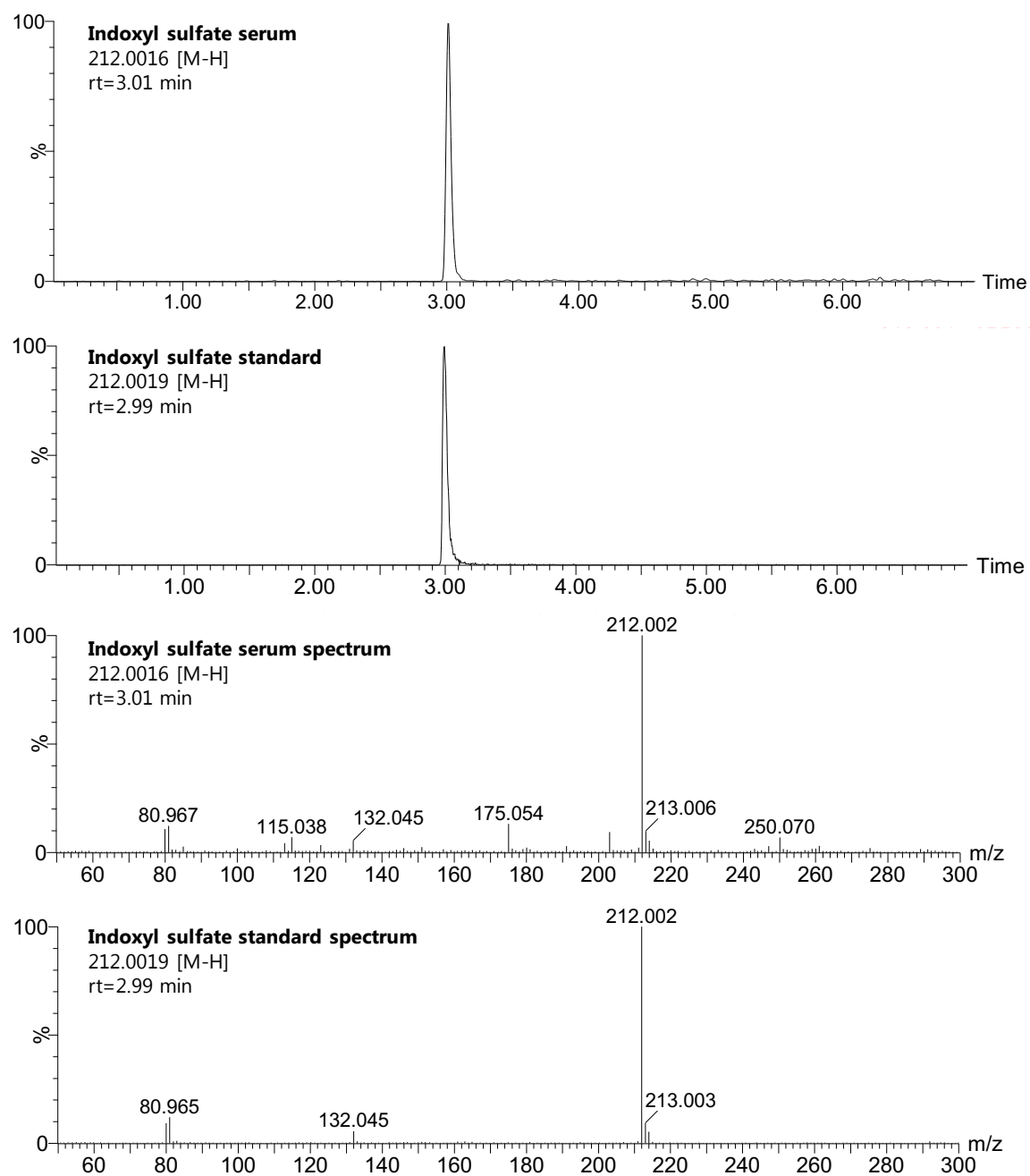
Supplementary Figure 17. Identification of 3-methylpyrogallol-sulfate comparing retention time and m/z measured in urine with retention time and m/z of an authentic standard.

Proteolytic degradation products

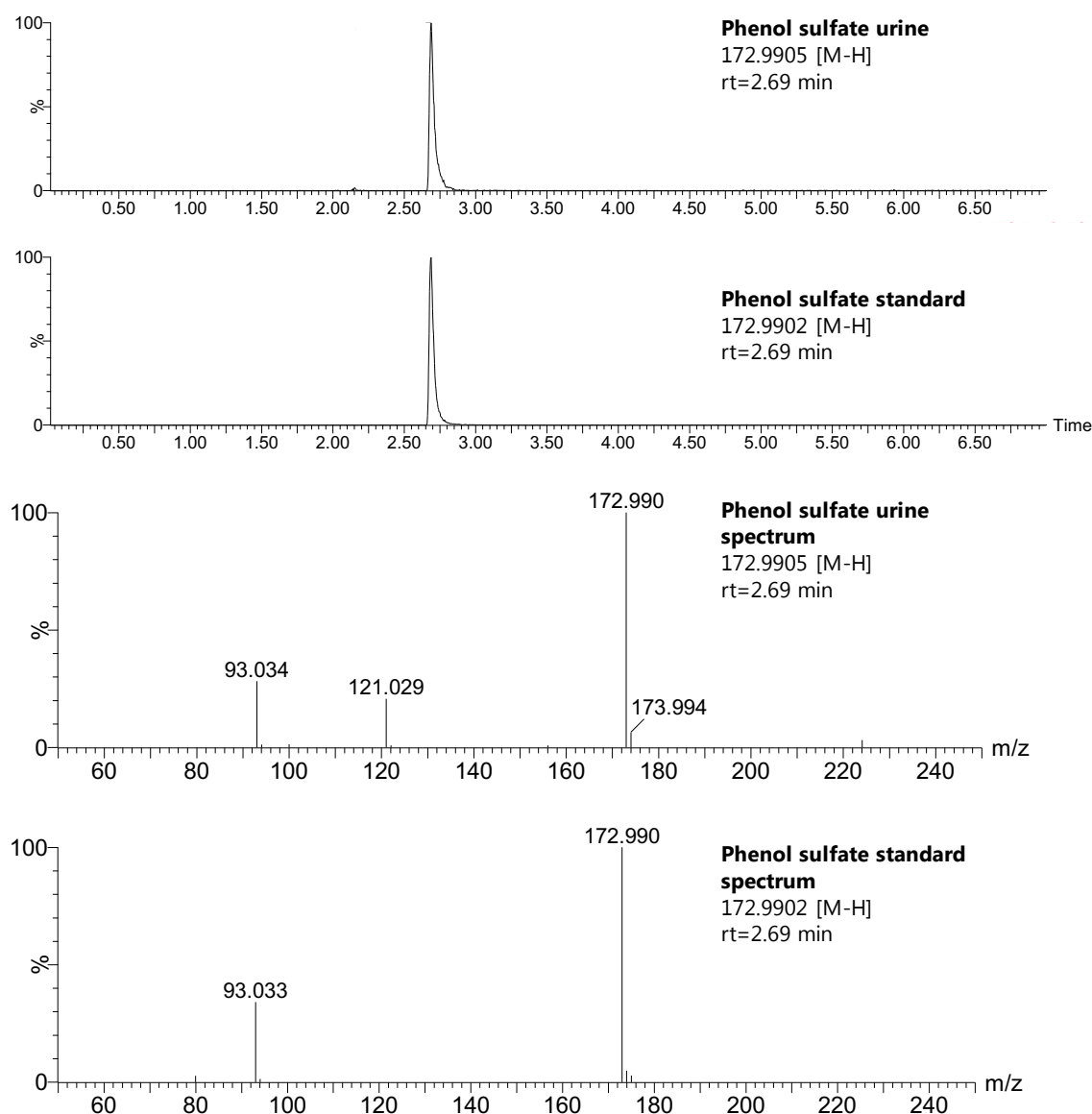
Supplementary Figure 18. Identification of Phenylacetylglutamine comparing retention time, m/z and spectrum measured in urine with retention time, m/z and spectrum of an authentic standard.



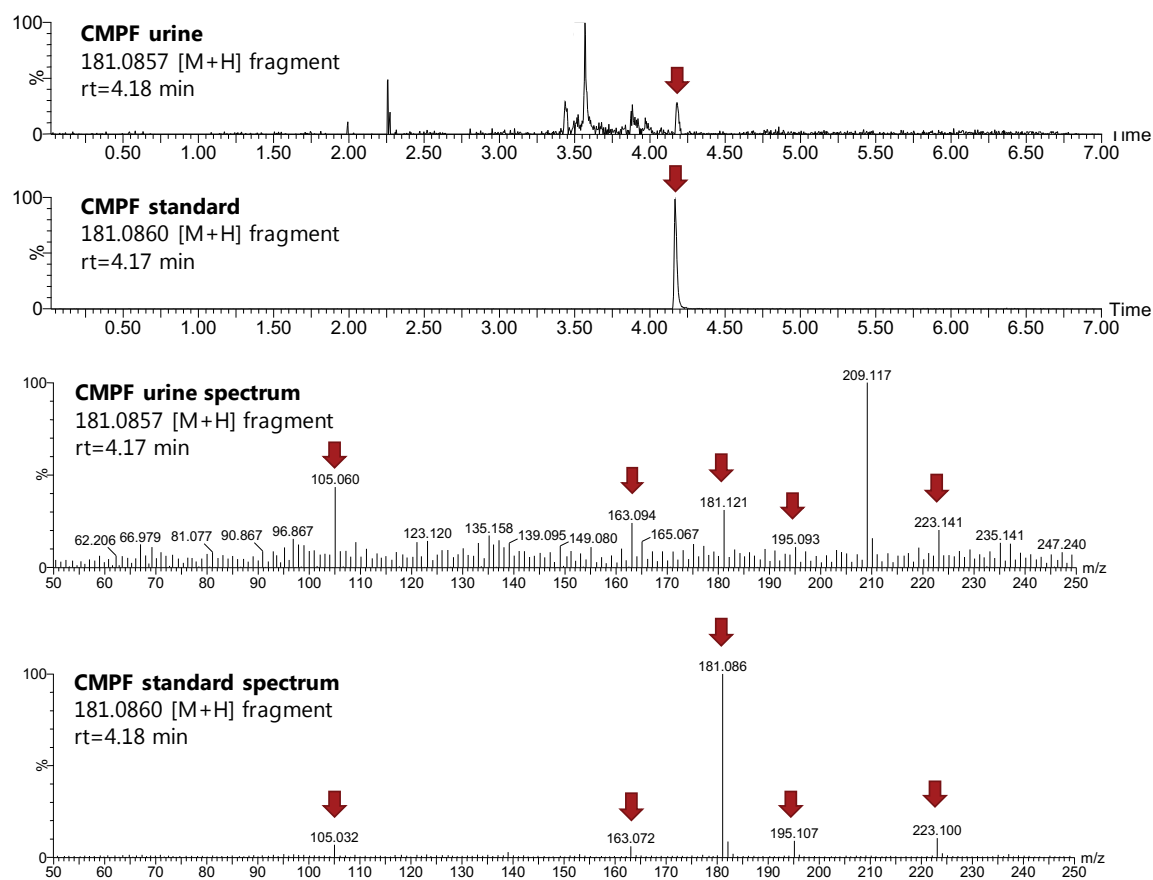
Supplementary Figure 19. Identification of P-cresol sulfate comparing retention time, m/z and spectrum measured in urine with retention time, m/z and spectrum of an authentic standard.



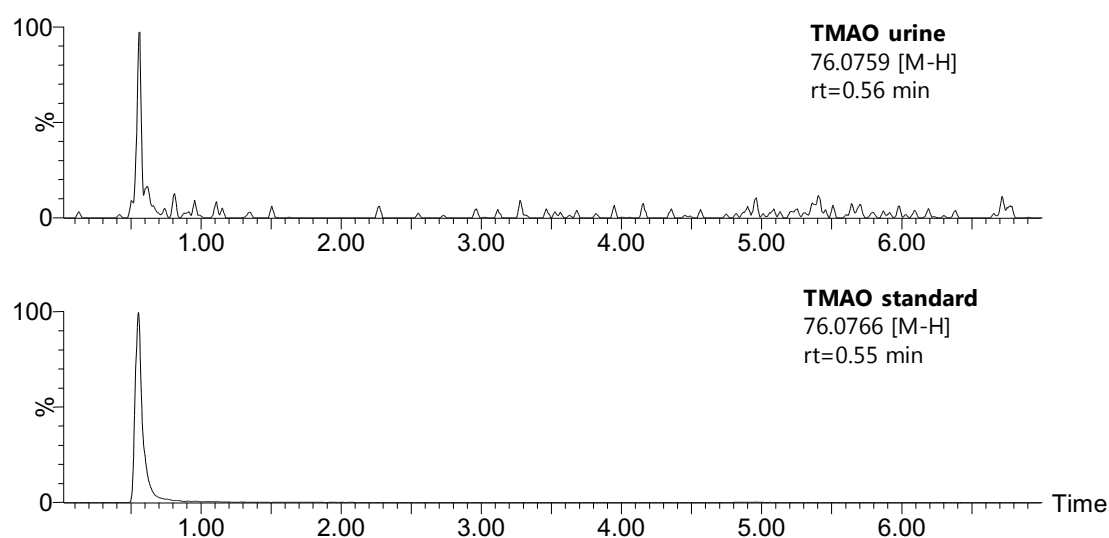
Supplementary Figure 20. Identification of Indoxyl sulfate comparing retention time, m/z and spectrum measured in serum with retention time, m/z and spectrum of an authentic standard. We noted a shift in retention time (0.3 min) for indoxyl sulfate compared to the original data collected.



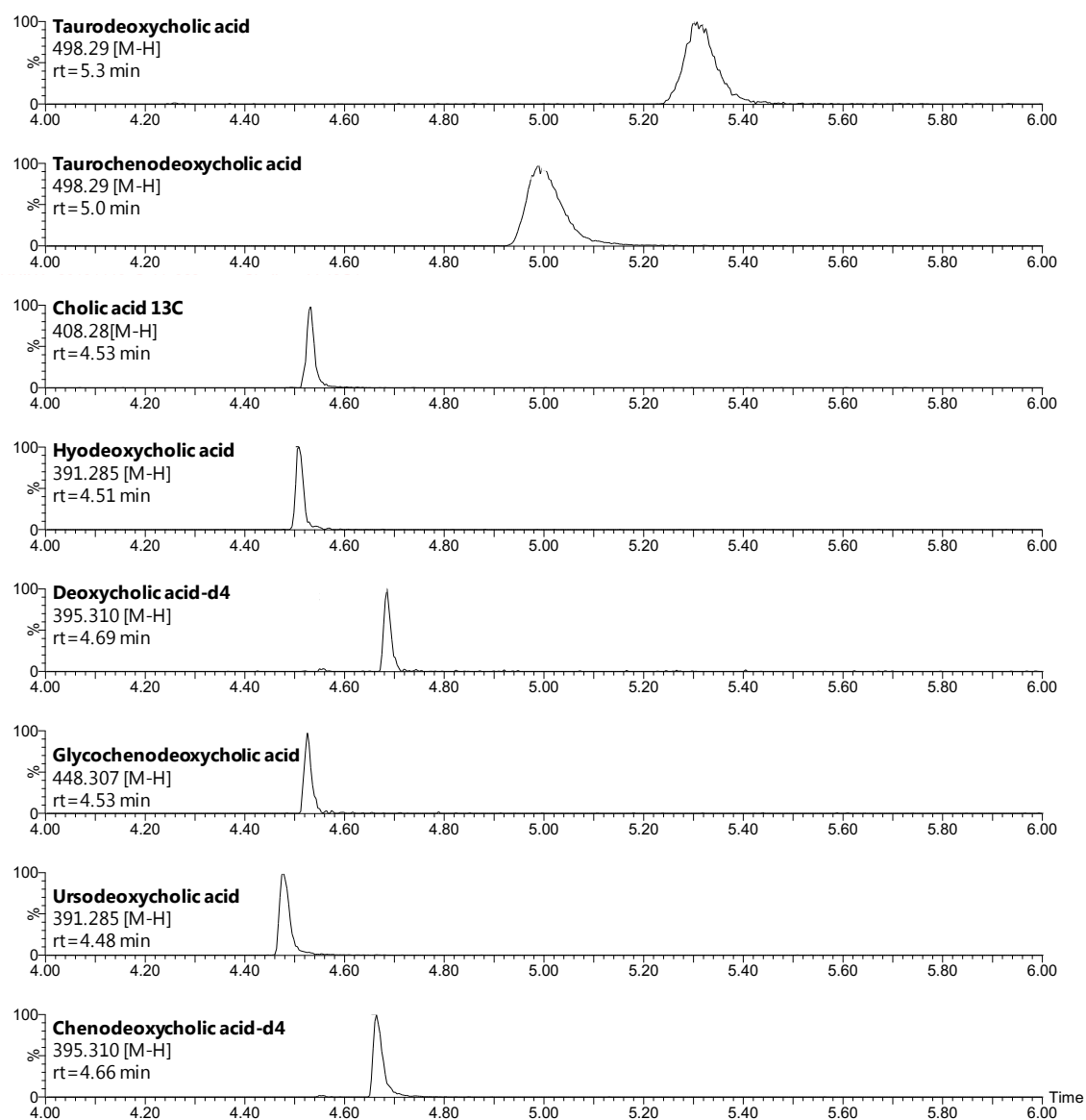
Supplementary Figure 21. Identification of Phenol sulfate comparing retention time, m/z and spectrum measured in urine with retention time, m/z and spectrum of an authentic standard.

Biomarkers of fish

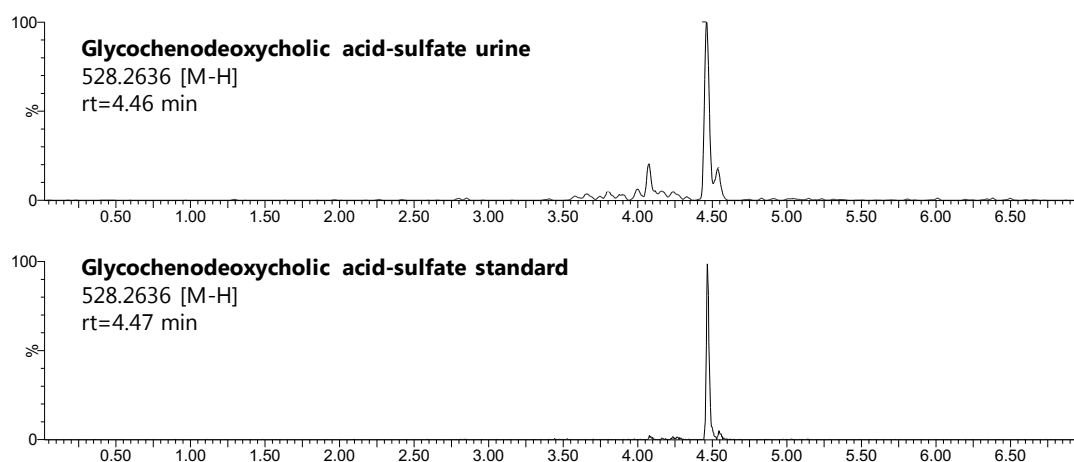
Supplementary Figure 22. Identification of 3-carboxy-4-methyl-5-propenyl-2-furanpropionic acid (CMPF) comparing retention time, m/z and spectrum measured in urine with retention time, m/z and spectrum of an authentic standard.



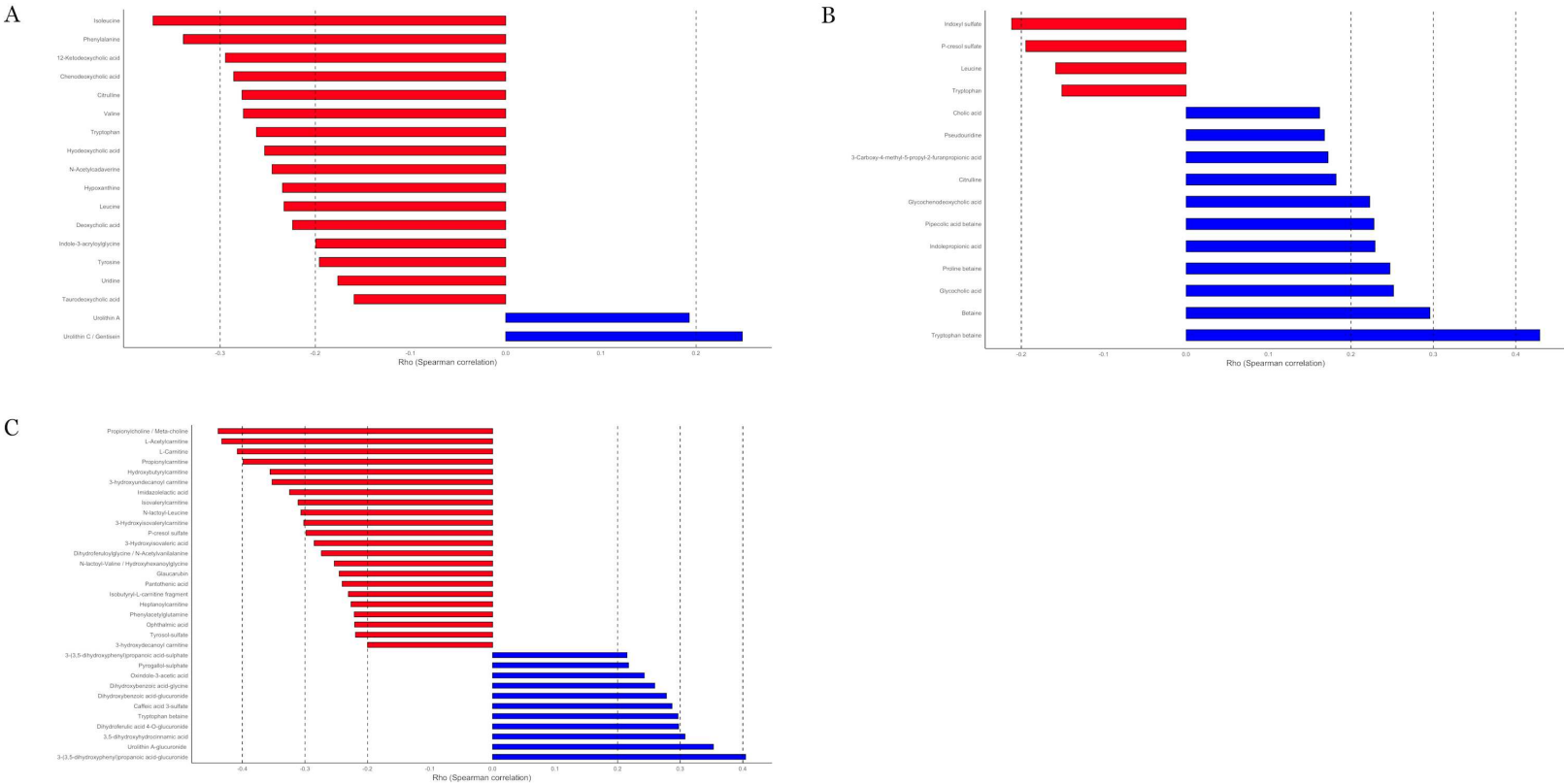
Supplementary Figure 23. Identification of trimethylamine-N-oxide (TMAO) comparing retention time and m/z measured in urine with retention time and m/z of an authentic standard.

Bile acids

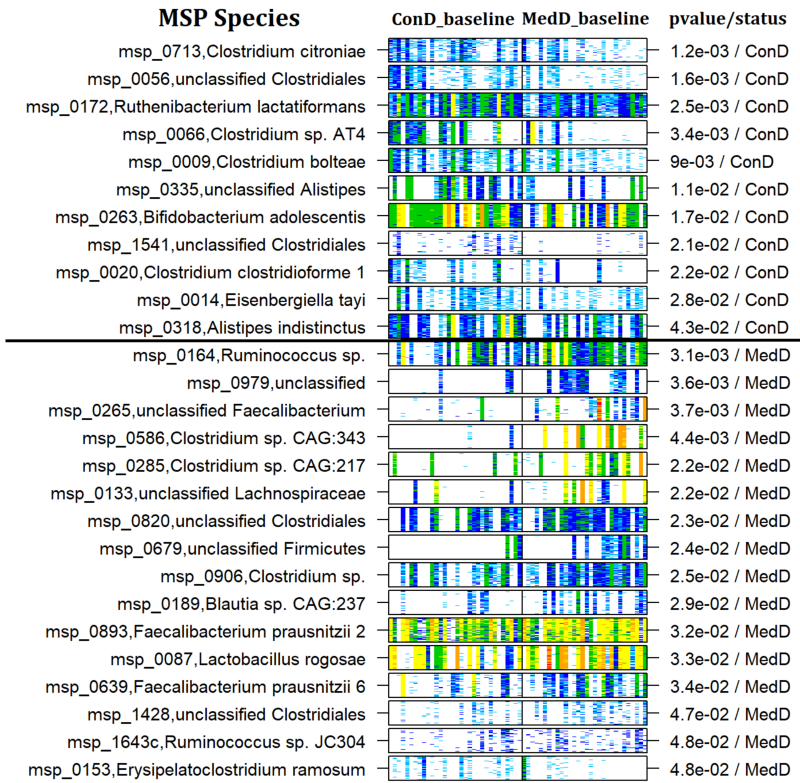
Supplementary Figure 24. Retention times of bile acid authentic standards.



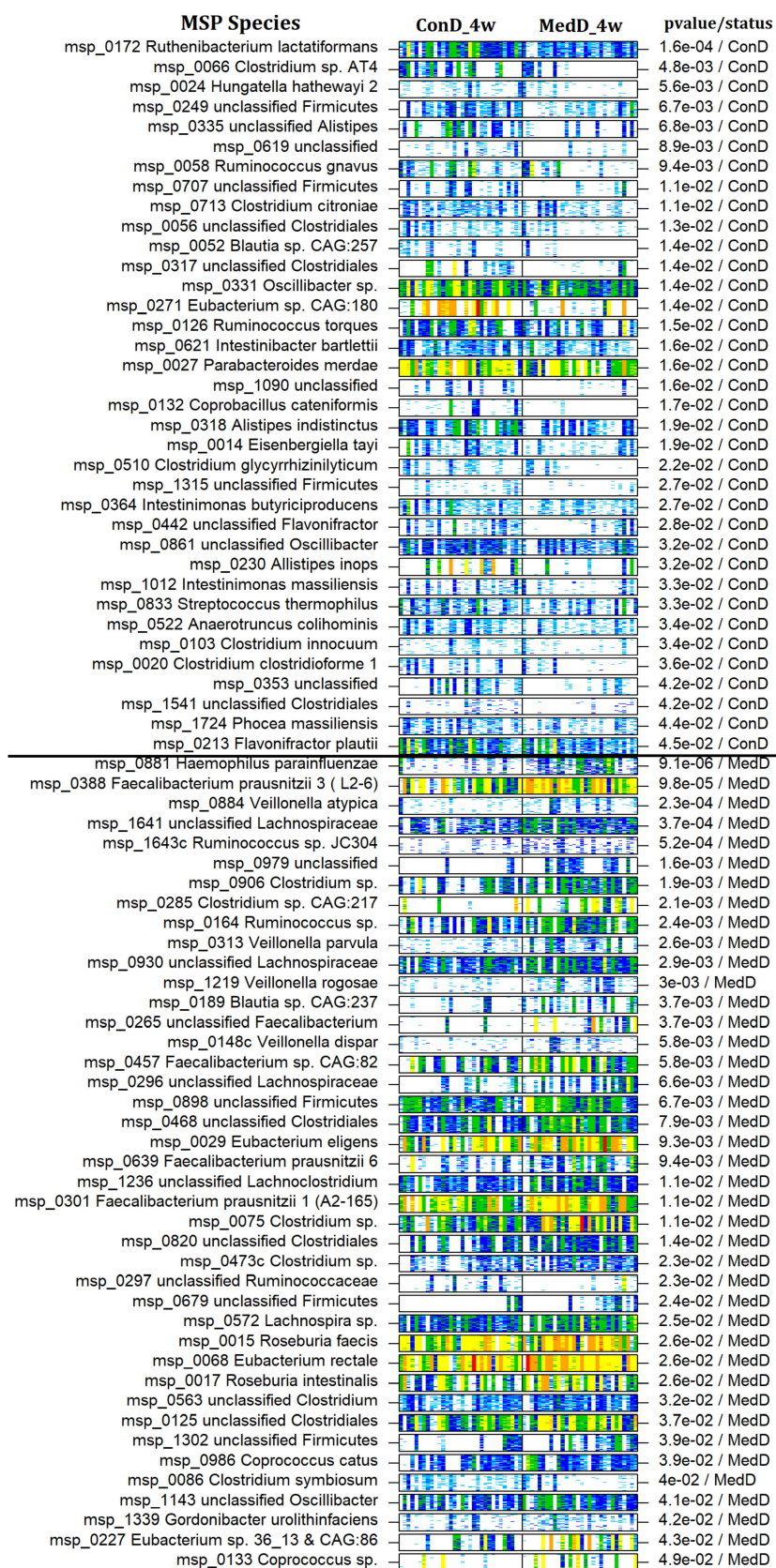
Supplementary Figure 25. Identification of Glycochenodeoxycholic acid sulfate comparing retention time and m/z measured in urine with retention time and m/z of an authentic standard.



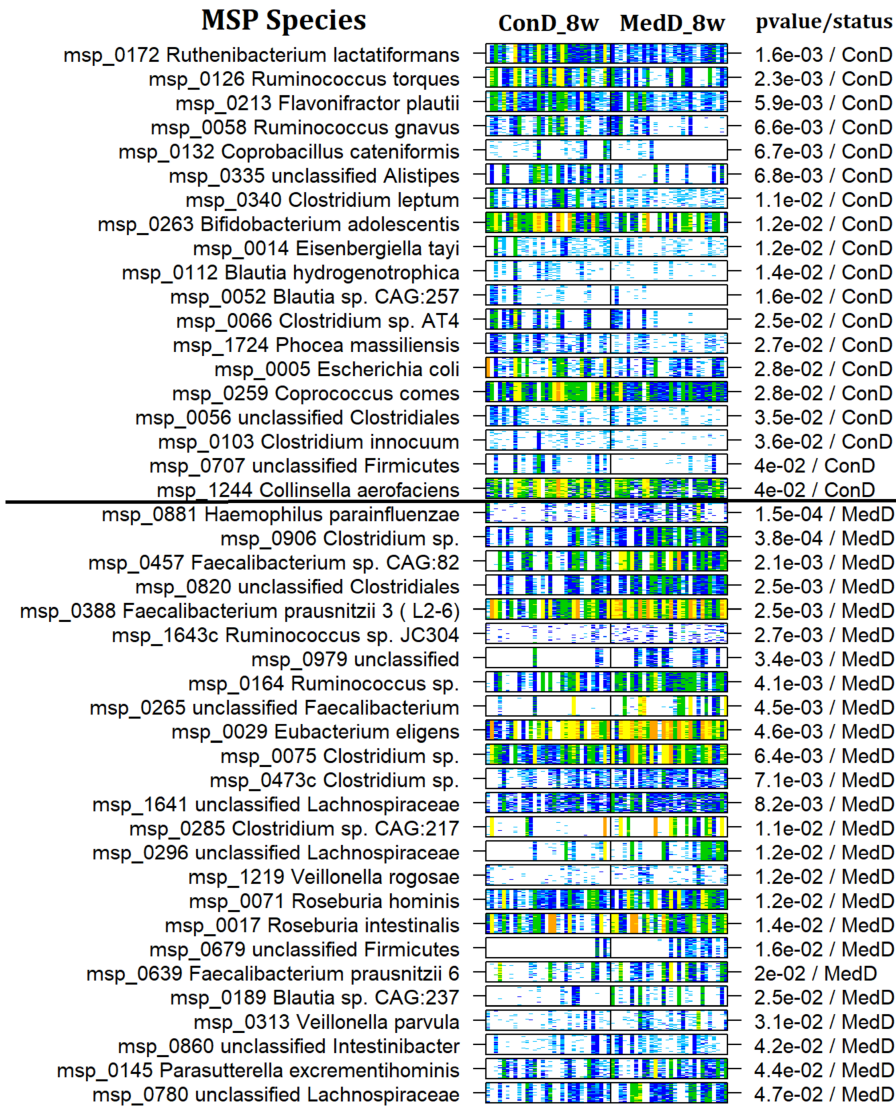
Supplementary Figure 26 Diverging bar charts showing Spearman’s correlations between MD Index and annotated metabolites from (A) faecal, (B) serum and (C) urine metabolome. Red and blue horizontal bars indicate negative and positive correlations, respectively. Spearman’s rho coefficients are displayed on the x-axis. (FDR<0.05).



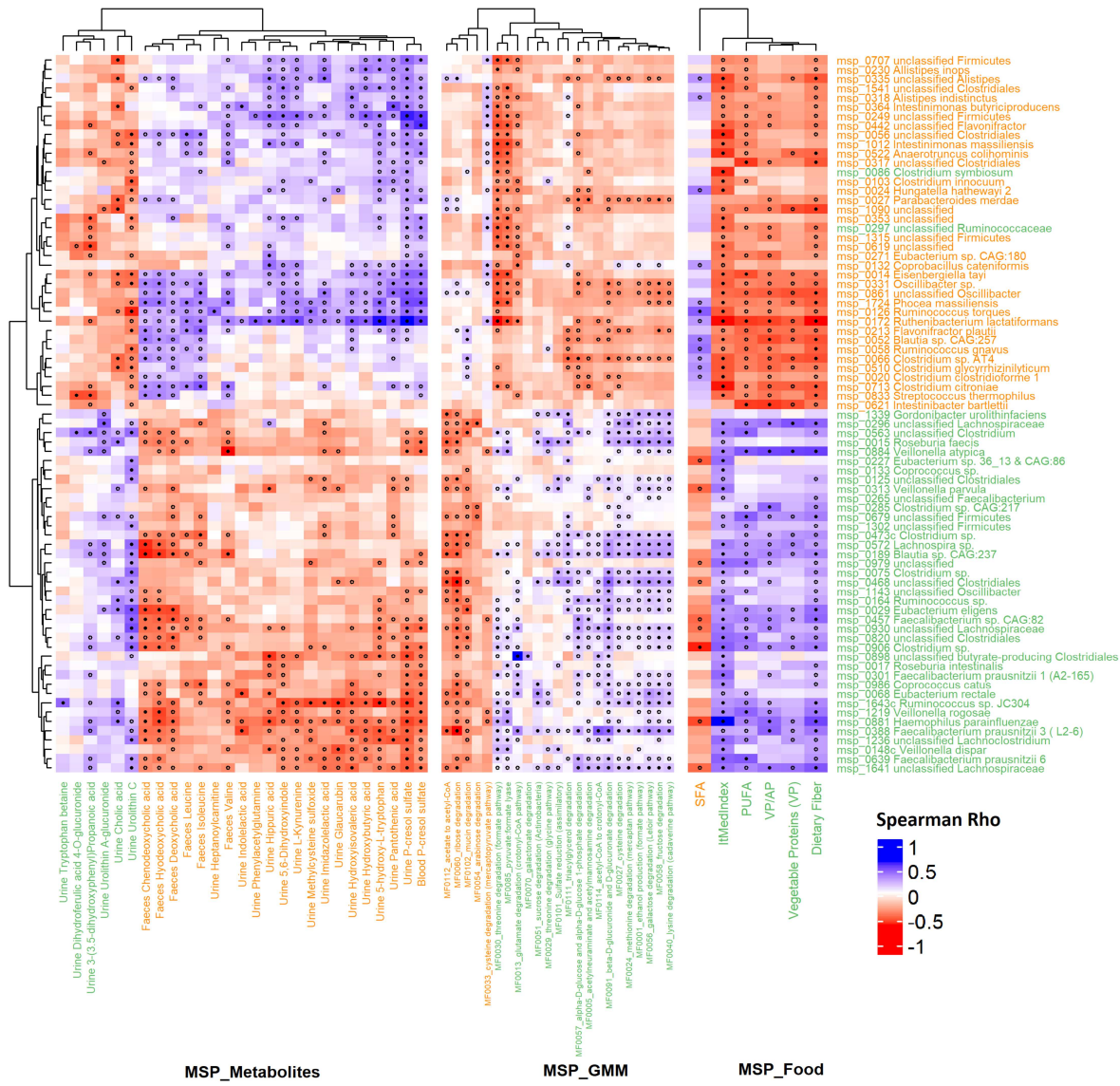
Supplementary Figure 27 Barcoding plots of the contrasted MSP species between ConD and MedD diets at baseline. Contrasted MSP species were computed using unpaired Wilcoxon rank sum tests on the MSP matrix filtered with a 20% occurrence threshold across samples. Contrasted MSP were organized in rows by blocks of their 50 marker genes and ranked by enrichment status (ConD or MedD) and by pvalues. Within each block, barcoding plots (heatmaps) of the frequency abundances of the marker genes were represented (white, absent; light blue to red, low to high abundance). Individuals were represented in columns by diet-time groups and ordered by their MSP richness at baseline.



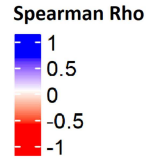
Supplementary Figure 28 Barcoding plots of the contrasted MSP species between ConD and MedD diets at 4w. Contrasted MSP species were computed using unpaired Wilcoxon rank sum tests on the MSP matrix filtered with a 20% occurrence threshold across samples. Contrasted MSP were organized in rows by blocks of their 50 marker genes and ranked by enrichment status (ConD or MedD) and by pvalues. Within each block, barcoding plots (heatmaps) of the frequency abundances of the marker genes were represented (white, absent; light blue to red, low to high abundance). Individuals were represented in columns by diet-time groups and ordered by their MSP richness at baseline.



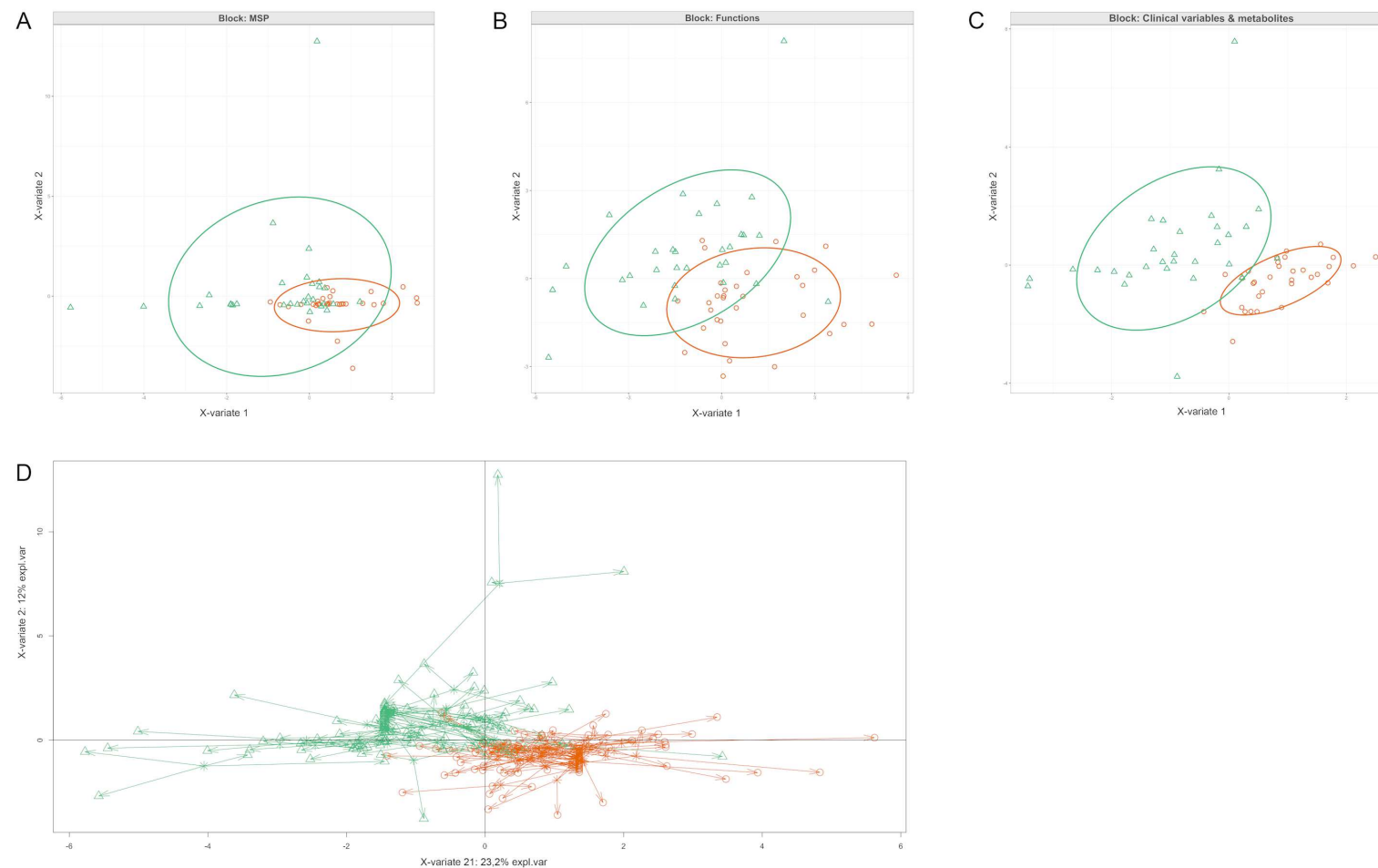
Supplementary Figure 29 Barcoding plots of the contrasted MSP species between ConD and MedD diets at 8w. Contrasted MSP species were computed using unpaired Wilcoxon rank sum tests on the MSP matrix filtered with a 20% occurrence threshold across samples. Contrasted MSP were organized in rows by blocks of their 50 marker genes and ranked by enrichment status (ConD or MedD) and by pvalues. Within each block, barcoding plots (heatmaps) of the frequency abundances of the marker genes were represented (white, absent; light blue to red, low to high abundance). Individuals were represented in columns by diet-time groups and ordered by their MSP richness at baseline.



- msp_0707 unclassified Firmicutes
- msp_0230 Alistipes indistinctus
- msp_0335 unclassified Alistipes
- msp_1541 unclassified Clostridiales
- msp_0318 Alistipes indistinctus
- msp_0364 Intestinimonas butyriciproducens
- msp_0249 unclassified Firmicutes
- msp_0442 unclassified Flavonifractor
- msp_0056 unclassified Clostridiales
- msp_1012 Intestinimonas massiliensis
- msp_0522 Anaerotruncus colihominis
- msp_0317 unclassified Clostridiales
- msp_0086 Clostridium symbiosum
- msp_0103 Clostridium innocuum
- msp_0024 Hungateia pathway 2
- msp_0027 Parabacteroides merdae
- msp_1090 unclassified
- msp_0355 unclassified
- msp_0297 unclassified Ruminococcaceae
- msp_1545 unclassified Firmicutes
- msp_0819 unclassified
- msp_0271 Eubacterium sp. CAG-180
- msp_0130 Coprobacillus cateniformis
- msp_0014 Eisenbergella layi
- msp_0331 Oscillibacter sp.
- msp_0881 unclassified Oscillibacter
- msp_1724 Phocaea massiliensis
- msp_0126 Ruminococcus torques
- msp_0172 Ruminococcus lactatiformans
- msp_0213 Flavonifractor plautii
- msp_0052 Blautia sp. CAG-257
- msp_0058 Ruminococcus gnavus
- msp_0066 Clostridium sp. A14
- msp_0510 Clostridium glycyrrhizinioliticum
- msp_0020 Clostridium clostridioforme 1
- msp_0171 Clostridium citroniae
- msp_0933 Streptococcus thermophilus
- msp_0621 Intestinibacter bartlettii
- msp_1339 Gordonibacter urothinfaciens
- msp_0296 unclassified Lachnospiraceae
- msp_0563 unclassified Clostridium
- msp_0015 Roseburia faecis
- msp_0884 Veillonella atypica
- msp_0227 Eubacterium sp. 36_13 & CAG-86
- msp_0135 Coprococcus sp.
- msp_0125 unclassified Clostridiales
- msp_0313 Veillonella parvula
- msp_0265 unclassified Faecalibacterium
- msp_0285 Clostridium sp. CAG-217
- msp_0679 unclassified Firmicutes
- msp_1302 unclassified Firmicutes
- msp_0473 Clostridium sp.
- msp_0572 Lachnospira sp.
- msp_0189 Blautia sp. CAG-237
- msp_0979 unclassified
- msp_0075 Clostridium sp.
- msp_0468 unclassified Clostridiales
- msp_1143 unclassified Oscillibacter
- msp_0164 Ruminococcus sp.
- msp_0029 Eubacterium eligens
- msp_0457 Faecalibacterium sp. CAG-82
- msp_0930 unclassified Lachnospiraceae
- msp_0820 unclassified Clostridiales
- msp_0906 Clostridium sp.
- msp_0898 unclassified Butyrate-producing Clostridiales
- msp_0017 Roseburia intestinalis
- msp_0301 Faecalibacterium prausnitzii 1 (A2-165)
- msp_0586 Coprococcus catus
- msp_0068 Eubacterium rectale
- msp_1643 Ruminococcus sp. JC304
- msp_1219 Veillonella rogosae
- msp_0881 Haemophilus parainfluenzae
- msp_0388 Faecalibacterium prausnitzii 3 (L2-6)
- msp_1236 unclassified Lachnospiraceae
- msp_0148 Veillonella dispar
- msp_0639 Faecalibacterium prausnitzii 6
- msp_1641 unclassified Lachnospiraceae

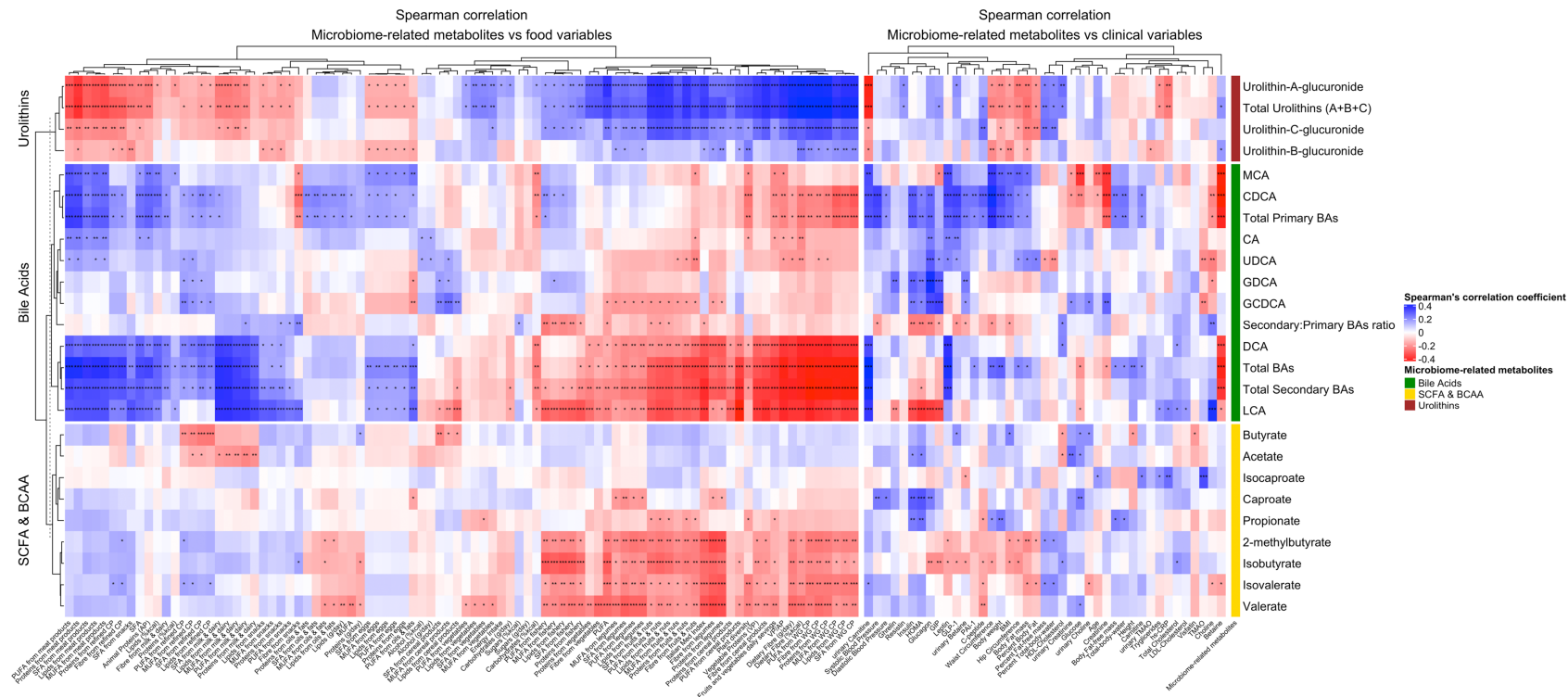


Supplementary Figure 30 Distinctive metabolites, dietary and microbial signatures between ConD and MedD diets at 4 weeks. Spearman correlations of contrasted MSP species with metabolites, GMM modules and nutrients at 4w. Rows, complete list of contrasted MSP at 4w between ConD and MedD diets ($p \leq 0.05$); Columns; contrasted annotated metabolites, contrasted GMM functional modules and nutrients data. Coloured-text referred to enrichment status (orange; ConD, green; MedD). Adjustments were performed using the Benjamini-Hochberg procedure and Spearman rho values were filtered by keeping correlations with at least one $FDR \leq 0.05$. Solid dot, $FDR \leq 0.05$; Open dot, $FDR \leq 0.2$.

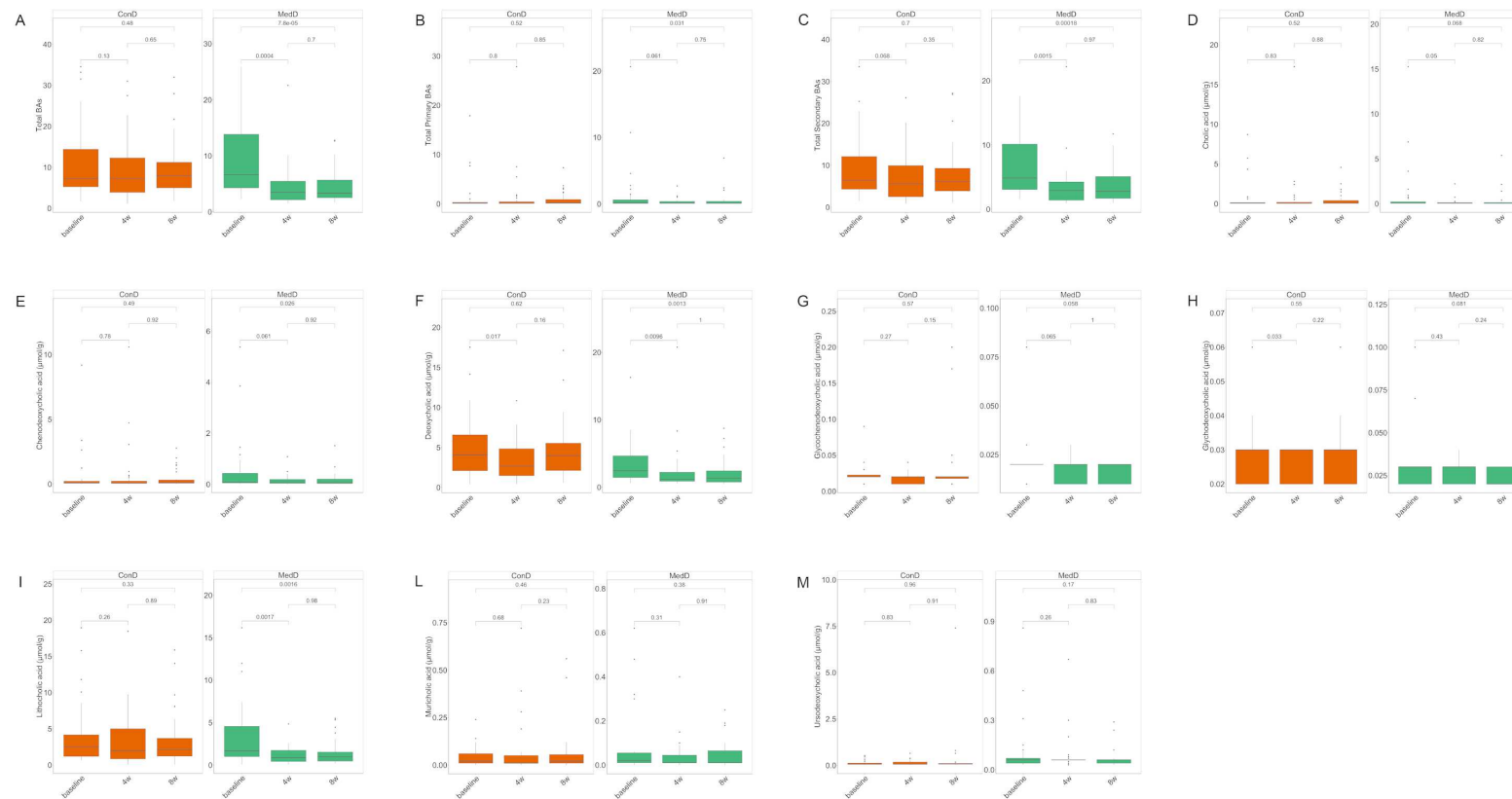


Supplementary Figure 31 N-integrative supervised analysis of different types of 'omics datasets. The DIABLO model for the discrimination of ConD and MedD groups is displayed as sample plot per single 'omic level. (A) Overall microbiota composition, (B) gut metabolic modules, (C)

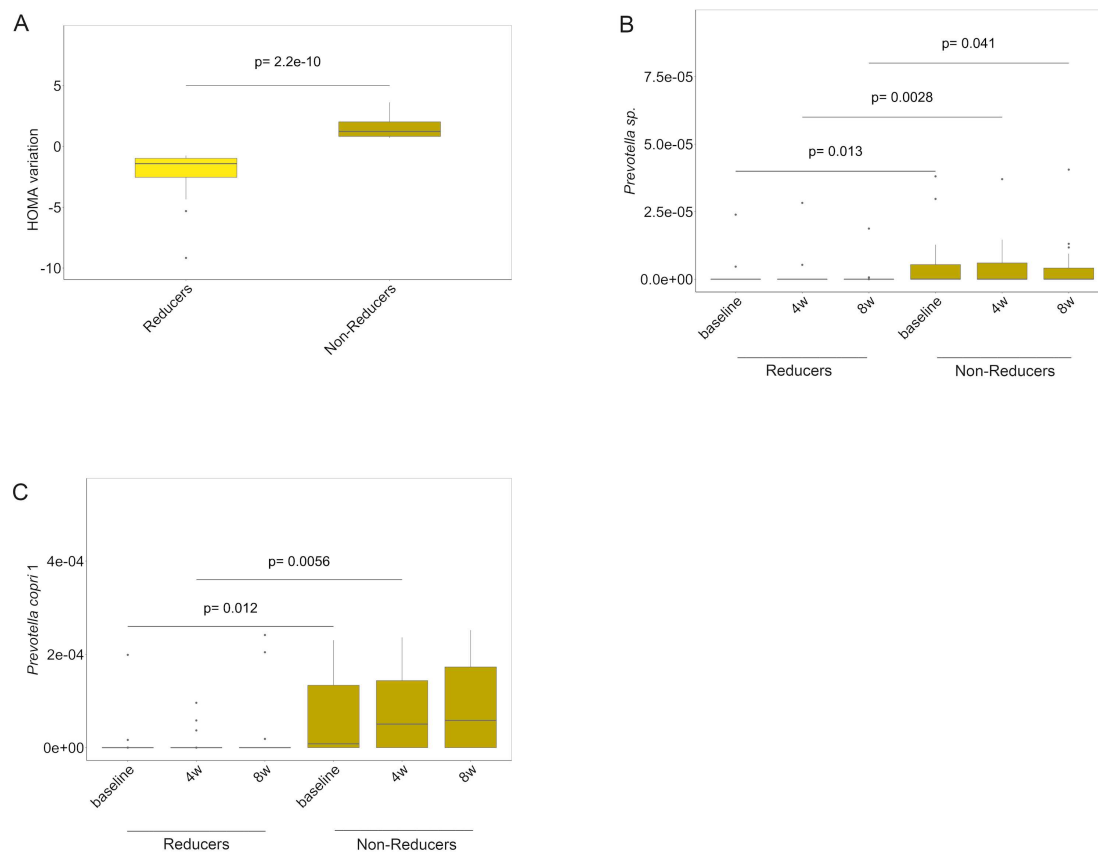
clinical variables and metabolome single-omic levels are reported along with (D) co-inertia analysis quantifying the co-variability between the three multi-omics datasets. Shapes represent the projected coordinates of each subject. The centroid for a given sample between all 'omics datasets is indicated by the start of the arrow and the location of the same sample in each dataset by the tips of the arrows. The length of the arrow is proportional to the divergence between data from different blocks. The percentage of total explained variance describing the separation of the groups on the first two components are displayed on the x and y axis, respectively. Green triangles, MedD subjects. Orange circles, ConD subjects. The integration of meta-omics products was associated with an increase in classification accuracies in discriminating between the ConD and MedD groups with respect to the cases in which a single data type was used. The AUC increased to 0.92 (from 0.88) when using metabolomics data only and to 0.90 (from 0.87) when comparing the ConD and MedD groups at 4 weeks and 8 weeks, respectively.



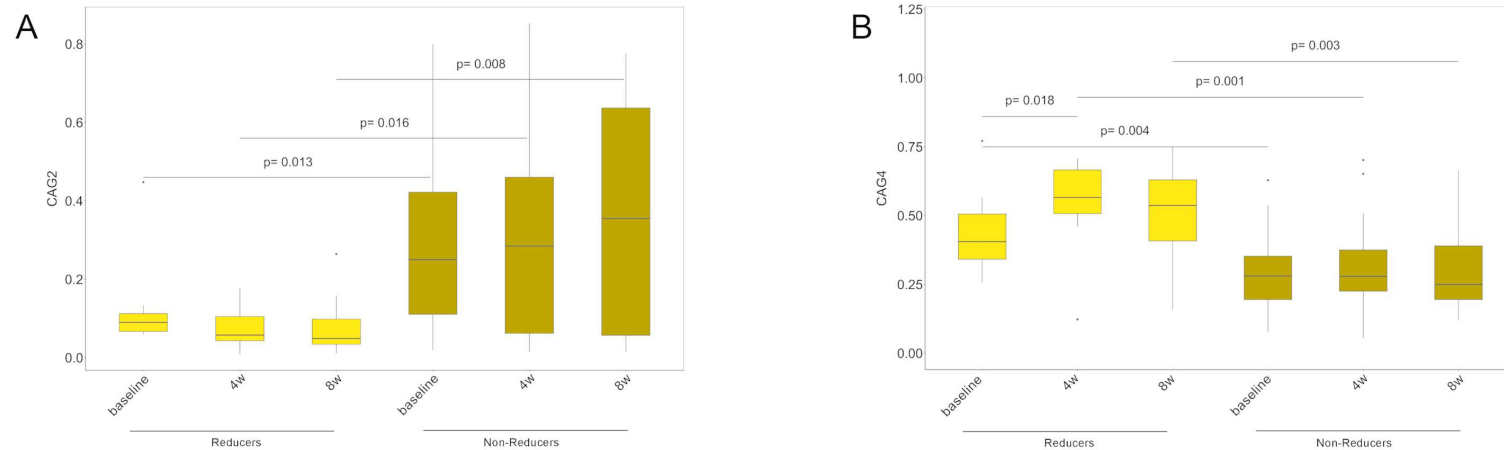
Supplementary Figure 32 Correlation of microbiome-related metabolites with dietary and clinical variables. Heatmap showing hierarchical Ward-linkage clustering of microbiome-related metabolites based on Spearman’s correlation with dietary variables and clinical parameters. The colour scale represents the scaled version of Spearman’s rho coefficients, with red indicating negative and blue indicating positive correlations. BAs, bile acids; CDCA, Chenodeoxycholic acid; MCA, Muricholic acid; DCA, Deoxycholic acid; GCDCA, Glycochenodeoxycholic acid; GDCA, Glychodeoxycholic acid; LCA, Lithocholic acid; UDCA, Ursodeoxycholic acid; SCFA, short-chain fatty acids; BCAA, branched-chain amino acids. TMAO, trimethylamine oxide; GLP-1, glucagon-like peptide-1; GIP, glucose-dependent insulinotropic peptide. VP/AP, Vegetable Protein/Animal Protein; PUFA, polyunsaturated fatty acid; MUFA, monounsaturated fatty acids; SFA, saturated fatty acids; WG, whole grain; CP, cereal products. Adjustments were performed using the the Benjamini-Hochberg procedure and Spearman rho values were filtered by keeping correlations with at least one $FDR \leq 0.05$. (* $FDR < 0.05$, ** $FDR < 0.01$ and *** $FDR < 0.001$).



Supplementary Figure 33 Box plots showing faecal concentrations of several BAs measured throughout the intervention. (A) Total BAs, (B) Total primary BAs, (C) Total secondary BAs, (D) Cholic acid, (E) Chenodeoxycholic acid, (F) Deoxycholic acid, (G) Glycochenodeoxycholic acid, (H) Glycodeoxycholic acid, (I) Lithocholic acid and, (L) Muricholic acid, (M) Ursodeoxycholic acid. Orange indicates ConD while green colour refers to MedD subjects, respectively. P values indicate paired Wilcoxon rank-sum tests within each group. Baseline, 0 weeks; 4w, 4 weeks; 8w, 8 weeks of intervention.



Supplementary Figure 34 Box plots showing differences in (A) HOMA variation classifying the subjects in HOMA reducers (Yellow) and non-reducers (Dark gold) after 4 weeks of intervention. Differences in levels of (B) *Prevotella* sp. and (C) *Prevotella copri* 1 in HOMA reducers and non-reducers. Statistical differences between groups were determined using unpaired Wilcoxon rank-sum tests. Baseline, 0 weeks; 4w, 4 weeks; 8w, 8 weeks of intervention.

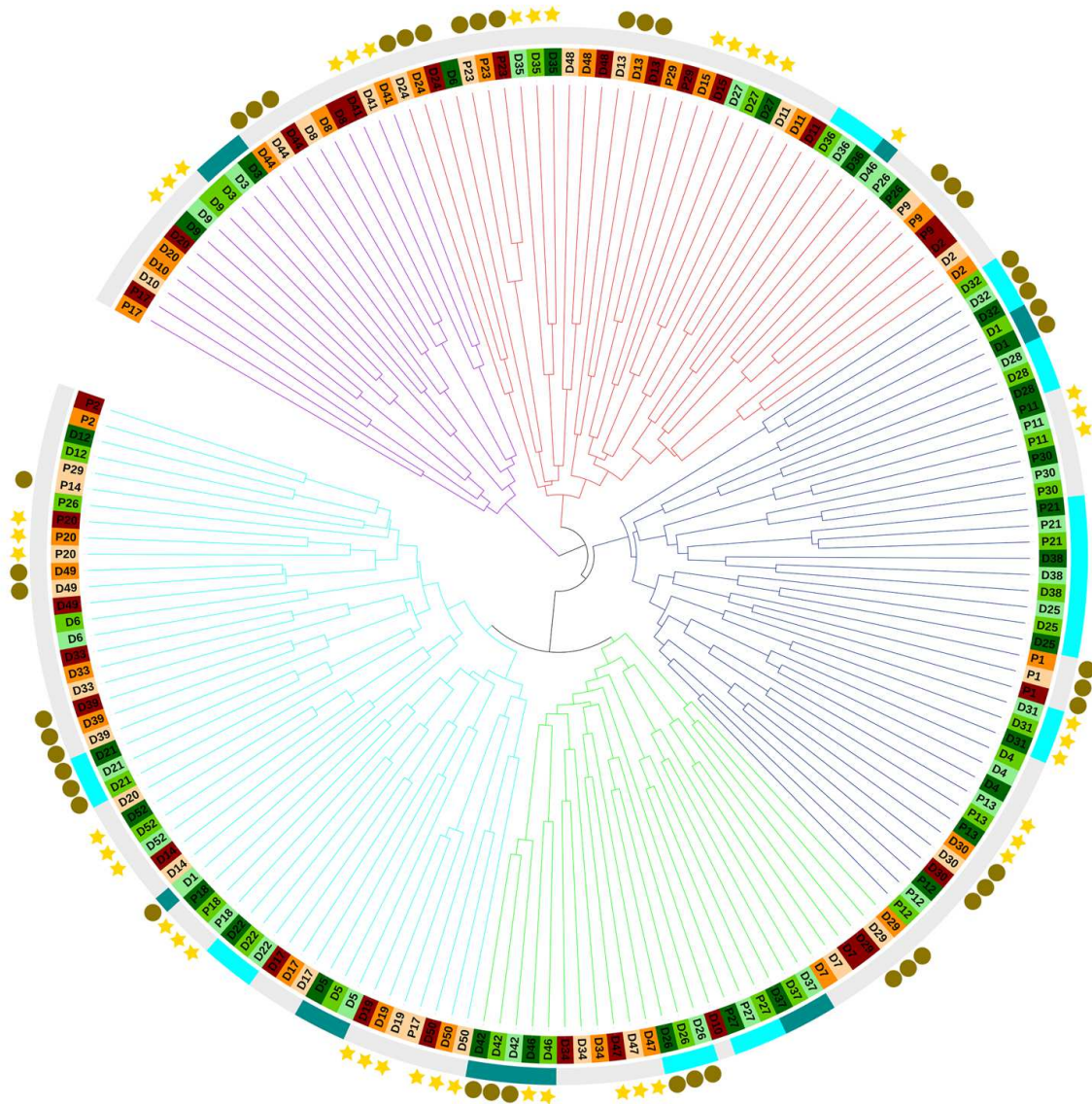


Supplementary Figure 35 Box plots showing differences in abundance of Co-Abundance Groups (CAGs) obtained from 16S rRNA gene sequences in subjects classified as HOMA reducers (Yellow) and non-reducers (Dark gold) after 4 weeks of intervention. Statistical differences between groups were determined using unpaired Wilcoxon rank-sum tests. Baseline, 0 weeks; 4w, 4 weeks; 8w, 8 weeks of intervention. Only (A) CAG2 and (B) CAG4 are reported, since no significant variation was found for the others.

- Diet**
- ConD_baseline
 - ConD_4w
 - ConD_8w
 - MedD_baseline
 - MedD_4w
 - MedD_8w

- Fibre intake**
- Highest intake
 - Lowest intake

- HOMA reduction**
- Non-reducers
 - Reducers



Supplementary Figure 36 Circular tree showing clustering of the subjects based on *Faecalibacterium prausnitzii* pangenome. Subjects are coloured according to dietary treatment and time-points. Coloured ring indicates the quartile of highest (green) versus lowest (cyan) increase of dietary fibre intake. Subjects not falling in the highest or lowest quartile were coloured in grey. Yellow stars or dark gold dots indicate HOMA reducers and non-reducers

Supplementary Table 1 General characteristics and main parameters measured in the body, blood, urines and diet of all participants over the study period. Data are expressed as mean \pm standard deviation (SD). * indicates $p < 0.05$ and ** indicates $p < 0.001$ for variation at the specific timepoint compared to baseline in MedD vs ConD by 2-way ANOVA with repeated measures and Tukey post hoc test; § indicates $p < 0.05$ for variation at 8w compared to 4w in MedD vs ConD by 2-way ANOVA with repeated measures and Tukey post hoc test.

	MedD (n=43)									ConD (n=39)									P values		
	baseline			4w			8w			baseline			4w			8w			$\Delta(4w - baseline)$	$\Delta(8w - baseline)$	$\Delta(8w - 4w)$
	mean	\pm	SD	mean	\pm	SD	mean	\pm	SD	mean	\pm	SD	mean	\pm	SD	mean	\pm	SD			
Gender (n, F/M)	22/21									21/18											
Age (y)	43	\pm	13							42	\pm	12									
Height (m)	1,68	\pm	0,08							1,67	\pm	0,07									
Body weight (kg)	87,1	\pm	12,4	85,8	\pm	11,9	86,0	\pm	12,2	87,9	\pm	16,0	87,4	\pm	16,4	87,1	\pm	16,4	0.18	0.42	0.07
BMI (kg/m ²)	30,9	\pm	3,8	30,4	\pm	3,6	30,5	\pm	3,6	31,2	\pm	5,3	31,2	\pm	5,5	30,9	\pm	5,5	0.16	0.4	0.07
Waist Circumference (cm)	105,8	\pm	12,1	104,9	\pm	11,2	104,8	\pm	10,8	107,8	\pm	13,4	108,1	\pm	13,4	107,2	\pm	13,0	0.25	0.75	0.4
Hip Circumference (cm)	113,4	\pm	9,1	112,7	\pm	8,7	112,7	\pm	8,2	114,5	\pm	9,8	113,9	\pm	9,5	112,7	\pm	9,4	0.77	0.82	0.99
Body Fat mass (kg)	26,1	\pm	7,0	25,3	\pm	6,5	25,6	\pm	6,1	27,9	\pm	9,8	25,8	\pm	7,6	25,2	\pm	8,1	0.79	0.53	0.56
Percent Body Fat (%)	30,0	\pm	6,8	29,5	\pm	6,3	29,9	\pm	5,7	31,1	\pm	7,4	30,0	\pm	6,7	29,6	\pm	7,6	0.67	0.4	0.61
Body Fat-free mass (kg)	61,1	\pm	10,3	60,5	\pm	10,0	60,2	\pm	10,0	60,6	\pm	10,6	59,8	\pm	10,8	58,4	\pm	11,7	0.28	0.89	0.34
Percent Fat-free mass (%)	70,1	\pm	6,7	70,2	\pm	6,9	70,2	\pm	5,7	68,3	\pm	8,2	69,6	\pm	7,2	69,7	\pm	9,0	0.36	0.41	0.54
Total-body-water (kg)	45,0	\pm	8,2	44,5	\pm	7,6	44,3	\pm	7,6	44,8	\pm	8,4	44,0	\pm	8,6	43,6	\pm	7,5	0.36	0.38	0.92
Percent Total-body-water (%)	51,6	\pm	5,3	51,7	\pm	4,9	51,6	\pm	4,9	50,8	\pm	5,3	51,0	\pm	5,1	5,7	\pm	5,5	0.98	0.4	0.81
Systolic Blood Pressure (mmHg)	123,2	\pm	21,9	120,6	\pm	16,2	120,6	\pm	17,1	119,9	\pm	17,3	120,3	\pm	17,1	122,0	\pm	16,4	0.49	0.15	0.43
Diastolic Blood Pressure (mmHg)	74,5	\pm	12,1	75,3	\pm	9,8	76,4	\pm	10,5	74,7	\pm	11,1	75,6	\pm	11,8	76,4	\pm	12,1	0.82	0.91	0.99
Physical Activity (MET min/wk)	322.1	\pm	21.3	323.0	\pm	19.8	321.9	\pm	22.9	323.9	\pm	18.7	324.3	\pm	21.2	322.9	\pm	21.8	0.71	0.5	0.62
HOMA	4,5	\pm	3,0	3,96	\pm	1,92	4,02	\pm	2,16	3,9	\pm	1,9	3,9	\pm	2,4	3,7	\pm	1,8	0.45	0.92	0.46
Italian Med Index	6,2	\pm	2,8	9,0	\pm	2,6 **	8,7	\pm	2,8 **	5,3	\pm	2,2	4,7	\pm	1,5	4,4	\pm	1,5	0.001	0.001	0.79

BLOOD	MedD (n=43)									ConD (n=39)									P values		
	baseline			4w			8w			baseline			4w			8w			$\Delta(4w - baseline)$	$\Delta(8w - baseline)$	$\Delta(8w-4w)$
	mean	±	SD	mean	±	SD	mean	±	SD	mean	±	SD	mean	±	SD	mean	±	SD			
Total Cholesterol (mmol/L plasma)	4,85	±	1,01	4,60	±	1,02 *	4,64	±	1,00	4,93	±	0,95	4,96	±	0,94	4,86	±	0,92	0.022	0.23	0.27
HDL-Cholesterol (mmol/L plasma)	1,24	±	0,26	1,17	±	0,25 *	1,19	±	0,23	1,23	±	0,30	1,26	±	0,26	1,20	±	0,29	0.004	0.35	0.07
LDL-Cholesterol (mmol/L plasma)	3,03	±	0,86	2,88	±	0,89	2,91	±	0,84	3,12	±	0,79	3,13	±	0,77	3,08	±	0,78	0.14	0.4	0.51
Tryglicerides (mmol/L plasma)	1,25	±	0,56	1,19	±	0,46	1,17	±	0,54	1,26	±	0,61	1,26	±	0,53	1,24	±	0,50	0.44	0.52	0.86
Glucose (mmol/L blood)	5,20	±	0,58	5,17	±	0,52	5,31	±	0,47	5,17	±	0,62	5,07	±	0,54	5,20	±	0,56	0.43	0.36	0.51
Insulin (mU/L plasma)	18,53	±	10,69	17,50	±	8,76	17,08	±	8,57	17,98	±	8,60	17,74	±	9,30	16,67	±	8,42	0.65	0.81	0.54
hs-CRP (mg/L serum)	2,66	±	2,99	1,96	±	2,28	2,74	±	3,83	2,68	±	3,41	2,71	±	3,41	3,29	±	4,48	0.41	0.62	0.71
TMAO (µmol/L plasma)	4,54	±	11,81	5,90	±	11,62	4,63	±	5,69	4,57	±	8,40	4,70	±	7,63	2,70	±	2,60	0.7	0.41	0.75
Betaine (µmol/L plasma)	50,99	±	31,25	57,90	±	35,30	50,67	±	29,35	48,94	±	30,35	54,18	±	39,59	58,28	±	35,14	0.35	0.17	0.83
Carnitine (µmol/L plasma)	34,89	±	12,12	37,85	±	19,9 *	36,94	±	15,60 **	37,86	±	13,52	36,30	±	12,99	38,11	±	16,38	0.05	0.001	0.903
Choline (µmol/L plasma)	18,90	±	8,87	17,38	±	8,74	17,91	±	8,41	22,65	±	16,50	22,21	±	16,34	21,63	±	15,59	0.49	0.61	0.76
Creatinine (µmol/L plasma)	100,36	±	40,72	99,43	±	49,13	102,41	±	44,47	83,09	±	36,66	82,65	±	40,20	77,41	±	34,56	0.46	0.53	0.113
Ghrelin (pg/mL plasma)	288,4	±	200,3	294,7	±	196,3	268,5	±	160,3	264,4	±	208,6	257,5	±	163,7	278,2	±	224,3	0.69	0.3	0.13
GLP-1 (pg/mL plasma)	155,0	±	120,7	157,2	±	101,0	156,8	±	99,9	151,6	±	88,0	171,4	±	140,6	168,2	±	130,1	0.49	0.54	0.91
GIP (pg/mL plasma)	216,5	±	173,5	229,5	±	190,3	215,6	±	194,7	189,8	±	158,4	197,5	±	209,8	232,1	±	259,1	0.86	0.25	0.19
Glucagon (pg/mL plasma)	495,5	±	383,0	499,1	±	369,0	470,3	±	365,6	454,2	±	340,3	455,5	±	346,8	459,5	±	355,6	0.77	0.27	0.39
C-peptide (pg/mL plasma)	1698,9	±	1399,7	1632,1	±	1169,7	1957,2	±	2558,1	1458,1	±	1372,4	1606,7	±	1431,6	1595,6	±	1651,7	0.35	0.78	0.42
Leptin (pg/mL plasma)	35365,8	±	36561,9	26351,8	±	29577,9	27078,9	±	34440,4	36309,1	±	46047,1	34175,6	±	44952,3	37923,1	±	44252,7	0.21	0.12	0.49
Resistin (pg/mL plasma)	20655,9	±	27416,2	26835,9	±	38118,5	20510,1	±	31075,3	18537,6	±	25602,4	21791,8	±	34767,7	16034,6	±	31208,5	0.64	0.61	0.97
Visfatin (pg/mL plasma)	8374,3	±	6585,3	6836,3	±	5992,6	6954,0	±	6265,0	8319,9	±	11100,8	7022,0	±	5849,5	6434,8	±	7352,4	0.94	0.81	0.72
PAI-1 (pg/mL plasma)	58661,0	±	95724,4	52610,7	±	100084,3	42519,5	±	60183,5	43960,6	±	122184,7	35623,0	±	57545,8	41475,5	±	78448,3	0.89	0.26	0.12

URINE	MedD (n=43)									ConD (n=39)									P values		
	baseline			4w			8w			baseline			4w			8w			Δ(4w - baseline)	Δ(8w - baseline)	Δ(8w-4w)
	mean	±	SD	mean	±	SD	mean	±	SD	mean	±	SD	mean	±	SD	mean	±	SD			
Creatinine (mmol/L)	10,15	±	7,40	9,58	±	6,66	9,25	±	5,44	10,68	±	8,29	10,17	±	6,59	10,57	±	6,73	0.98	0.86	0.77
TMAO (mmol/mol creatinine)	38,31	±	59,56	90,28	±	150,51	70,33	±	90,99	60,97	±	83,12	62,72	±	91,17	53,77	±	70,84	0.11	0.21	0.64
Betaine (mmol/mol creatinine)	5,79	±	5,77	6,78	±	6,64 *	8,22	±	9,19	7,00	±	5,77	5,73	±	3,59	7,88	±	7,09	0.04	0.17	0.7
Choline (mmol/mol creatinine)	1,24	±	0,98	1,41	±	1,43	1,38	±	1,31	1,73	±	2,10	1,35	±	1,52	1,67	±	1,96	0.07	0.3	0.2
Carnitine (mmol/mol creatinine)	13,37	±	18,74	4,64	±	7,09 *	6,81	±	8,99 *	13,24	±	15,00	12,40	±	13,83	14,78	±	13,93	0.04	0.003	0.8
DIET																					
Energy Intake (kcal)	1780,2	±	579,8	1915,2	±	505,7	1849,5	±	588,4	1883,3	±	732,4	1865,0	±	660,9	1839,9	±	602,6	0.14	0.31	0.68
Carbohydrates (% kcal)	49,1	±	7,7	50,3	±	5,9	51,2	±	7,1	49,3	±	7,5	49,2	±	5,9	49,6	±	7,7	0.16	0.26	0.78
Carbohydrates (g/day)	221,1	±	83,7	242,8	±	75,7	236,0	±	79,0	231,7	±	93,1	230,7	±	90,3	227,9	±	80,6	0.49	0.32	0.78
Sugars (% kcal)	14,7	±	5,0	15,2	±	3,7	15,9	±	4,8	16,1	±	4,4	16,7	±	4,7	16,3	±	5,2	0.33	0.07	0.29
Sugars (g/day)	64,4	±	26,1	71,7	±	23,2	74,4	±	29,2	76,4	±	37,2	77,6	±	35,8	73,4	±	31,4	0.93	0.37	0.36
Dietary Fiber (% kcal)	2,1	±	0,8	3,7	±	0,7 **	3,6	±	0,8 **	1,7	±	0,6	1,8	±	0,8	1,6	±	0,5	0.001	0.001	0.63
Dietary Fiber (g/day)	18,5	±	7,3	35,1	±	11,8 **	34,0	±	15,5 **	16,4	±	9,6	16,9	±	9,5	15,1	±	6,9	0.001	0.001	0.71
Proteins (% kcal)	16,0	±	2,9	14,8	±	1,9	14,7	±	2,3	16,3	±	3,2	15,9	±	2,2	16,1	±	2,8	0.27	0.17	0.61
Proteins (g/day)	70,8	±	25,5	70,5	±	18,1	67,9	±	23,2	74,6	±	28,6	72,5	±	22,3	72,7	±	23,8	0.71	0.82	0.5
Lipids (% kcal)	30,7	±	6,6	29,9	±	6,1	29,4	±	6,1	31,4	±	6,8	32,1	±	5,7	32,0	±	6,9	0.42	0.22	0.82
Lipids (g/day)	59,1	±	19,4	62,6	±	17,6	60,0	±	20,6	66,9	±	33,1	66,8	±	26,6	65,9	±	26,3	0.49	0.69	0.71
SFA (g/day)	20,2	±	8,4	13,8	±	6,1 *	13,1	±	5,2 *	25,2	±	15,7	23,6	±	9,6	23,5	±	10,4	0.005	0.005	0.7
MUFA (g/day)	29,8	±	9,3	33,4	±	12,0	32,6	±	11,9	32,9	±	16,3	34,4	±	14,7	32,6	±	12,4	0.49	0.21	0.71
PUFA (g/day)	9,1	±	4,1	15,4	±	5,0 **	14,3	±	6,2 **	8,8	±	4,2	8,8	±	3,9	9,1	±	3,8	0.001	0.001	0.19
Vegetable Proteins (VP) (g/day)	26,5	±	11,4	41,6	±	13,3 *	39,5	±	16,2 **	24,1	±	11,5	24,5	±	10,7	23,2	±	9,7	0.001	0.001	0.74
Animal Proteins (AP) (g/day)	39,0	±	17,9	25,5	±	9,4 *	24,5	±	10,9 *	43,4	±	19,1	42,0	±	14,3	43,1	±	17,7	0.002	0.001	0.46
VP/AP	0,8	±	0,4	1,9	±	1,1 **	1,9	±	1,1 **	0,6	±	0,4	0,6	±	0,3	0,6	±	0,4	0.001	0.001	0.97

BMI, body mass index; HOMA, Homeostatic Model Assessment for Insulin Resistance; HDL, High Density Lipoprotein; LDL, Low density Lipoprotein; hs-CRP, high sensitivity C-reactive protein; TMAO, trimethylamine oxide; GLP-1, glucagon-like peptide-1; GIP, glucose-dependent insulinotropic peptide, PAI-1, plasminogen activator inhibitor 1; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

Supplementary Table 2 Mass Spectrometry characteristics of compounds monitored by targeted analysis through Liquid Chromatography - Tandem Mass Spectrometry (LC/MS/MS) in blood and urine samples collected over the study period.

Compound	Precursor ion	Product ions
	[M+H] ⁺ <i>m/z</i>	[M+H] ⁺ <i>m/z</i>
TMAO	76	58
TMAO-d9	85	66
Betaine	118	59
Carnitine	162	103; 85; 60
Choline	104	60
Creatinine	114	44

TMAO: trimethylamine n-oxide; TMAO-d9: deuterium-labeled methyl d9-TMAO. *m/z*, mass-to-charge.

Supplementary Table 3 Mass Spectrometry characteristics of urolithins and their metabolites determined in urine samples by Liquid Chromatography – Tandem Mass Spectrometry (LC/MS/MS).

Compound	Precursor ion	Product ions
	[M-H] ⁻ <i>m/z</i>	[M-H] ⁻ <i>m/z</i>
Urolithin-A glucuronide	403	227; 175
Urolithin-B glucuronide	387	211, 175
Urolithin-C glucuronide	419	243; 175
Urolithin-C methyl ether glucuronide	433	257; 243;175
Urolithin-D glucuronide	435	259; 175
Urolithin-A	227	198
Urolithin-B	211	167
Urolithin-C	243	199
Urolithin D	259	215
Urolithin-A sulfate	307	227
Urolithin A sulfoglucuronide	483	307; 175
Urolithin D methyl ether glucuronide	449	273; 175

m/z, mass-to-charge

Supplementary Table 4 Identified metabolites in urine, serum and faeces by untargeted metabolomics

#	Tentative annotation	Sample	Level of identification ₁	MS mode	Adduct	rt* (min)	Experimental m/z	Authentic standard m/z	Database m/z	Mass error (mDa)
1	Allantoic acid	Serum	1	ESI+	[M+H]	0.46	177.0608	177.0630		2.2
2	Trimethylamine N-oxide	Serum; Urine	1	ESI+	[M+H]	0.50	76.0759	76.0759		0.0
3	Carnitine	Serum; Urine	1	ESI+	[M+H]	0.52	162.1124	162.1134		1.0
4	Betaine	Serum	1	ESI+	[M+H]	0.52	118.0865	118.0867		0.2
5	Citrulline	Faeces; Serum	1	ESI-	[M-H]	0.54	174.0872	174.0879		0.7
6	2-aminoisobutyric acid	Faeces	1	ESI-	[M-H]	0.54	102.0546	102.0554		0.6
7	Imidazolelactic acid	Urine	3	ESI-	[M-H]	0.54	155.0458	-	155.0462	0.4
8	Imidazolepropionic acid	Urine	1	ESI+	[M+H]	0.56	141.0670	141.0658		1.2
9	Proline	Faeces; Serum	1	ESI+	[M+H]	0.56	116.0707	116.0711		0.4
10	Proline betaine	Serum; Urine	1	ESI+	[M+H]	0.58	144.1023	144.1019		0.4
11	Propionylcholine / Meta-choline	Urine	3	ESI+	[M+H]	0.62	160.1337	-	160.1332	0.5
12	Dopamine	Faeces	1	ESI+	[M+H]	0.62	154.0847	154.0853		0.6
13	Nicotinamide	Faeces	1	ESI+	[M+H]	0.62	123.0561	123.0566		0.5
14	Citric acid	Serum; Urine	1	ESI-	[M-H]	0.64	191.0190	191.0192		0.2
15	Valine	Faeces; Serum	1	ESI+	[M+H-HCOOH]	0.66	72.0810	72.0814		0.4
16	Acetylcarnitine	Serum; Urine	1	ESI+	[M+H]	0.67	204.1240	204.1240		0.0
				ESI+	[M+H] fragment	0.67	145.0507	145.0510		0.3
				ESI+	[M+H] fragment	0.67	85.0289	85.0290		0.1
				ESI+	[M+H] fragment	0.68	60.0809	60.0830		2.1

17	Pipecolic acid betaine	Serum	1	ESI+	[M+H]	0.71	158.1170	158.1174	0.4	
18	N-Acetylcadaverine	Faeces	3	ESI+	[M+H]	0.73	145.1325	-	145.1335	1.0
19	Hypoxanthine	Faeces; Serum	1	ESI+	[M+H]	0.76	137.0462	137.0468	0.6	
20	Hydroxybutyrylcarnitine	Urine	3	ESI+	[M+H]	0.77	248.1505	-	248.1492	1.3
21	Uridine	Faeces; Serum	1	ESI-	[M-H]	0.83	243.0616	243.0606	1.0	
22	N-Acetylglutamic acid	Serum	1	ESI+	[M+H-C2H4O2]	0.79	130.0502	130.0497	0.5	
23	Succinylcarnitine / Methylmalonylcarnitine	Faeces	3	ESI+	[M+H]	0.79	262.1285		262.1285	0.0
24	Tyrosine	Faeces; Serum; Urine	1	ESI+	[M+H]	0.82	182.0816	182.0819	0.3	
				ESI-	[M-H]	0.82	180.0647	180.0666	1.9	
25	3-Methylthiohexyl acetate / 3-Mercaptoheptyl acetate	Urine	3	ESI-	[M-H]	0.83	189.0962		189.0955	0.7
26	Methylcysteine sulfoxide	Urine	3	ESI-	[M+Cl]	0.88	185.9978		185.9997	1.9
27	1-(1-Pyrrolidinyl)-2- butanone	Urine	3	ESI+	[M+H]	0.89	142.1235		142.1226	0.9
				ESI+	[M+NH4]	0.89	159.1500		159.1492	0.8
28	Methoxy-Pyrogallol	Urine	3	ESI-	[M-H]	0.93	139.0400		139.0401	0.1
29	Isoleucine	Faeces; Serum; Urine	1	ESI+	[M+H]	0.94	132.1029	132.1024	0.5	
				ESI+	[M+H] fragment	0.94	86.0969	86.0972	0.3	
30	Inosine	Serum	1	ESI-	[M-H]	0.94	267.0720	267.0730	1.0	
31	Dihydroxybenzoic acid- glucuronide	Urine	3	ESI-	[M-H]	1.01	329.0492		329.0514	2.2
	Leucine	Faeces; Serum; Urine	1	ESI-	[M-H]	1.06	130.0863	130.0868	0.5	

				ESI+	[M+H] fragment	1.03	86.0967	86.0972	0.5
32	Propionylcarnitine	Urine	1	ESI+	[M+H]	1.02	218.1396	218.1400	0.4
				ESI+	[M+H] fragment	1.02	159.0658	159.0660	0.2
				ESI+	[M+H] fragment	1.02	85.0288	85.0290	0.2
33	5-hydroxytryptophan	Urine	1	ESI+	[M+H-HCOOH]	1.11	175.0875	175.0876	0.1
			1	ESI-	[M-H]	1.13	219.0769	219.0759	1.0
34	3-Hydroxyisovaleryl carnitine	Urine	3	ESI+	[M+H]	1.13	262.1661	262.1649	1.2
35	2-hydroxybutyric acid	Urine	1	ESI-	[M-H]	1.17	103.0395	103.0400	0.5
36	Ophthalmic acid	Urine	3	ESI-	[M-H]	1.28	288.1186	288.1201	1.5
				ESI-	[M-H-H ₂ O]	1.28	270.1087	270.1090	0.3
				ESI-	[M-H] fragment	1.28	253.0822	-	-
37	Casimiroedine	Serum	3	ESI+	[M+H]	1.29	418.1933	418.1973	4.0
38	Pyrogallol-sulphate-glucuronide	Urine	3	ESI-	[M-H]	1.43	381.0119	381.0133	1.4
				ESI-	[M-H-glucuronide]	1.42	204.9812	204.9812	0.0
39	Kynurenine	Urine	1	ESI+	[M+H-NH ₃ -COCH ₂]	1.45	150.0557	150.0542	1.5
40	Gammaglutamylvaline	Urine	1	ESI+	[M+H]	1.47	247.1289	247.1300	1.1
				ESI+	[M+H] fragment	1.48	230.1032	230.1030	0.2
				ESI+	[M+H] fragment	1.48	184.0973	184.0980	0.7
				ESI+	[M+H] fragment	1.47	118.0869	118.0870	0.1
				ESI+	[M+H] fragment	1.48	72.0810	72.0810	0.0
41	Pyrogallol-sulphate	Urine	3	ESI-	[M-H]	1.50	204.9807	204.9812	0.5

42	Dihydroxybenzoic acid-glycine	Urine	3	ESI-	[M-H]	1.60	210.0406	210.0408	0.2
43	Phenylalanine	Faeces; Serum; Urine	1	ESI+	[M+H-HCOOH]	1.61	120.0815	120.0815	0.0
				ESI-	[M-H]	1.62	164.0702	164.0700	0.2
				ESI+	[M+H]	1.63	166.0860	168.0854	0.6
44	3-Hydroxyisovaleric acid	Urine	1	ESI-	[M-H]	1.66	117.0551	117.0560	0.9
45	Methoxy-Pyrogallol-sulphate	Urine	3	ESI-	[M-H]	1.91	218.9963	218.9969	0.6
				ESI-	[M-H-CH ₂]	1.91	204.9807	204.9812	0.5
				ESI-	[M-H-sulfate]	1.91	139.0397	139.0401	0.4
46	Isobutyryl-L-carnitine	Urine	3	ESI+	[M+H]	1.96	232.1552	232.1543	0.9
				ESI+	[M+H] fragment	1.96	173.0812	-	-
				ESI+	[M+H] fragment	1.96	85.0289	-	-
				ESI+	[M+H] fragment	1.96	60.0809	-	-
47	3-(3,5-dihydroxyphenyl)propanoic acid-glucuronide	Urine	3	ESI-	[M-H]	1.98	357.0810	357.0827	1.7
				ESI+	[M+H-glucuronide]	1.98	183.0654	183.0652	0.2
				ESI+	[M+H-H ₂ O-glucuronide]	1.98	165.0559	165.0546	1.3
48	3,5-dihydroxybenzoic acid	Urine	1	ESI-	[M-H]	1.99	153.0192	153.0181	1.1
49	Pantothenic acid	Urine	1	ESI+	[M+H]	2.01	220.1187	220.1187	0.0
				ESI-	[M-H]	2.02	218.1028	218.1031	0.3
50	Theobromine	Serum	1	ESI+	[M+H]	2.06	181.0717	181.0707	1.0
				ESI+	[M+H-H ₂ O]	2.06	163.0608	163.0618	1.0

51	3-hydroxyanthranilic acid	Urine	1	ESI+	[M+H-H ₂ O]	2.11	136.0405	136.0392	1.3
52	3-(3,5-dihydroxyphenyl)propanoic acid-sulphate	Urine	3	ESI-	[M-H]	2.17	261.0068	261.0074	0.6
				ESI-	[M-H-sulfate]	2.18	181.0501	181.0506	0.5
				ESI-	[M-H-CO ₂ -sulfate]	2.18	137.0616	137.0608	0.8
53	Tryptophan	Faeces; Serum; Urine	1	ESI+	[M+H]	2.40	205.0979	205.0981	0.2
				ESI-	[M-H]	2.40	203.0822	203.0821	0.1
54	1,7-dimethylxanthine	Serum	3	ESI+	[M+H]	2.53	181.0718	181.0720	-
				ESI+	[M+H] fragment	2.53	124.0508	-	-
55	5,6-Dihydroxyindole	Urine	3	ESI+	[M+H]	2.53	150.0556	150.0550	0.6
				ESI+	[M+H-H ₂ O]	2.53	132.0454	132.0444	1.0
56	Phenol sulfate	Urine	1	ESI-	[M-H]	2.57	172.9907	172.9902	0.5
				ESI-	[M-H-sulfate]	2.57	93.0338	-	-
				ESI-	[M-H-phenol]	2.58	79.9564	-	-
57	Xanthurenic acid	Urine	1	ESI-	[M-H]	2.57	204.0295	204.0302	0.7
				ESI+	[M+H]	2.58	206.0456	206.0460	0.4
				ESI+	[M+H-H ₂ O]	2.58	188.0345	188.0352	0.7
				ESI+	[M+H-HCOOH]	2.58	160.0400	160.0405	0.5
58	Caffeic acid-sulfate	Urine	3	ESI-	[M-H]	2.60	258.9911	258.9918	0.7
59	Caffeic acid-sulfate	Urine	3	ESI-	[M-H]	2.65	258.9913	258.9918	0.5
60	3,5-dihydroxyhydrocinnamic acid	Urine	3	ESI+	[M+H-HCOOH]	2.66	137.0606	137.0597	0.9

61	Tryptamine	Urine	1	ESI+	[M+H-NH3]	2.66	144.0815	144.0820	0.5
62	Tiglylcarnitine	Urine	3	ESI+	[M+H]	2.69	244.1551	244.1543	0.8
				ESI+	[M+H] fragment	2.69	185.0813	-	-
				ESI+	[M+H] fragment	2.68	181.0615	-	-
				ESI+	[M+H] fragment	2.68	135.1373	-	-
				ESI+	[M+H] fragment	2.68	135.0562	-	-
				ESI+	[M+H] fragment	2.68	106.0293	-	-
				ESI+	[M+H] fragment	2.69	85.0289	-	-
				ESI+	[M+H] fragment	2.68	78.0344	-	-
63	Indoxyl sulfate	Serum	1	ESI-	[M-H]	2.70	212.0008	212.0037	2.9
64	Isovalerylglycine	Urine	1	ESI-	[M-H]	2.71	158.0817	158.0807	1.0
65	Dihydroferulic acid 4-O-glucuronide	Urine	3	ESI-	[M-H]	2.71	371.0974	371.0984	1.0
66	Methylpyrogallol-sulfate	Urine	3	ESI-	[M-H]	2.72	218.9965	218.9969	0.4
				ESI-	[M-H-sulfate]	2.72	139.0397	139.0401	0.4
67	Dihydroxycinnamic acid-sulfate	Urine	3	ESI-	[M-H]	2.75	258.9914	258.9918	0.6
				ESI-	[M-H-sulfate]	2.75	179.0348	179.0350	0.2
68	3-methylpyrogallol-sulfate	Urine	1	ESI-	[M-H]	2.77	218.9965	218.9941	2.4
				ESI-	[M-H-sulfate]	2.77	139.0399	139.0401	0.2
69	Tryptophan betaine	Serum; Urine	1	ESI+	[M+H]	2.78	247.1444	247.1450	0.6
				ESI+	[M+H-C3H9N]	2.78	188.0705	188.0707	0.2
				ESI+	[M+H] fragment	2.78	146.0602	-	-
				ESI+	[M+H] fragment	2.78	60.0810	-	-

				ESI-	[M-H] fragment	2.83	142.0655	-	-
70	5-Hydroxyindole-3-acetic acid	Urine	1	ESI-	[M-H-HCOOH]	2.78	144.0449	144.0454	0.5
71	Dihydroxycinnamic acid-sulfate	Urine	3	ESI-	[M-H]	2.82	258.9909	258.9918	0.9
				ESI-	[M-H-sulfate]	2.82	179.0342	179.0350	0.8
72	Isovalerylcarnitine	Urine	1	ESI+	[M+H]	2.85	246.1710	246.1709	0.1
				ESI+	[M-H] fragment	2.85	187.0973	-	-
				ESI+	[M-H] fragment	2.85	85.0289	-	-
73	2-Methylbutyrylcarnitine / Pivaloylcarnitine / Valerylcarnitine	Urine	3	ESI+	[M+H]	2.90	246.1709	246.1700	0.9
				ESI+	[M-H] fragment	2.90	187.0973	-	-
				ESI+	[M-H] fragment	2.89	85.0288	-	-
74	Dihydroferuloylglycine / N-Acetylvaniilalanine	Urine	3	ESI-	[M-H]	2.92	252.0870	252.0877	0.7
75	Dihydroxycinnamic acid-sulfate	Urine	3	ESI-	[M-H]	2.95	258.9911	258.9918	0.7
				ESI-	[M-H-sulfate]	2.95	179.0343	179.0350	0.7
76	Dihydroxycinnamic acid	Faeces	3	ESI-	[M-H]	2.99	181.0502	181.0506	0.4
77	Dihydroxycinnamic acid-sulfate	Urine	3	ESI-	[M-H]	3.00	258.9913	258.9918	0.5
				ESI-	[M-H-sulfate]	3.00	179.0342	179.0350	0.8
78	Dihydrocaffeic acid-sulfate	Urine	3	ESI-	[M-H]	3.02	261.0044	261.0074	3.0
				ESI-	[M-H-sulfate]	3.02	181.0493	181.0506	1.3

79	2,4-dihydroxycinnamic acid	Faeces	3	ESI-	[M-H]	3.06	181.0497	181.0506	0.9
80	Hippuric acid	Serum; Urine	1	ESI-	[M-H]	3.06	178.0503	178.0504	0.1
				ESI+	[M+H]	3.07	180.0660	180.0660	0.0
81	Caffeine	Serum	1	ESI+	[M+H]	3.12	195.0876	195.0882	0.6
82	N-lactoyl-Valine / Hydroxyhexanoylglycine	Urine	3	ESI-	[M-H]	3.12	188.0923	188.0928	0.5
83	Phenylacetylglutamine	Urine	1	ESI-	[M-H]	3.15	263.1030	263.1020	1.0
				ESI+	[M+H-H ₂ O]	3.16	247.1084	-	-
84	Benzeneacetamide-sulphate	Urine	3	ESI-	[M-H]	3.30	230.0120	230.0129	0.9
				ESI-	[M-H-sulfate]	3.30	150.0557	150.0561	0.4
85	Indole-3-carboxylic acid-glucuronide	Urine	1	ESI-	[M-H]	3.40	336.0712	336.0704	0.8
				ESI-	[M-H] fragment	3.40	193.0351	193.0341	1.0
86	P-cresol sulfate	Urine; Serum	1	ESI-	[M-H]	3.44	187.0051	187.0053	0.2
				ESI-	[M-H-SO ₃]	3.44	107.0495	107.0505	1.0
				ESI-	[M-H-cresol]	3.44	79.9563	79.9589	3.6
				ESI-	[2M-H]	3.44	375.0201	375.0194	0.7
				ESI-	[2M-2H+Na]	3.44	397.0048	397.0044	0.4
87	Oxindole-3-acetic acid	Faeces; Urine	1	ESI-	[M-H]	3.42	190.0502	190.0480	2.2
				ESI-	[M-H-CO ₂]	3.42	146.0609	146.0590	1.9
				ESI+	[M+H-CO ₂]	3.42	146.0610	-	-
88	Urolithin C-glucuronide	Urine	1	ESI-	[M-H-glucuronide]	3.42	243.0313	243.0294	1.9
89	O-methoxycatechol-O-sulphate	Urine	3	ESI-	[M-H]	3.44	203.0014	203.0020	0.6

90	Urolithin A-glucuronide	Urine	1	ESI+	[M+H]	3.45	405.0826	405.0870	4.4
				ESI+	[M+H-glucuronide]	3.44	229.0507	229.0520	1.3
				ESI-	[M-H-glucuronide]	3.46	227.0341	-	-
91	Kiwiionoside	Urine	3	ESI-	[M-H]	3.44	405.2123	405.2130	0.7
92	Indole-3-acetic acid-glucuronide	Urine	1	ESI-	[M-H]	3.46	350.0868	350.0861	0.7
				ESI-	[M-H-NH3-CO-COCH2-C4H6O]	3.46	193.0351	193.0334	0.7
				ESI-	[M-H] fragment	3.46	174.0553	-	-
				ESI+	[M+NH4]	3.45	369.1286	-	-
				ESI+	[M+H-glucuronide]	3.45	176.0709	-	-
93	3-hydroxyoctanoyl carnitine	Urine	3	ESI+	[M+H]	3.47	304.2127	304.2119	0.8
94	Phenylalanylphenylalanine	Serum	3	ESI+	[M+H]	3.47	313.1550	313.1547	0.3
				ESI+	[M+H-phenylalanine]	3.47	166.0860	166.0863	0.3
				ESI+	[M+H] fragment (C8H9N)	3.47	120.0810	120.0808	0.2
95	Methylhippuric acid	Urine	1	ESI-	[M-H]	3.48	192.0662	192.0657	0.5
				ESI-	[M-H-CO2]	3.49	148.0764	148.0767	0.3
96	3-hydroxydecanoyl carnitine	Urine	3	ESI+	[M+H]	3.49	332.2431	332.2431	0.0
				ESI+	[M+H] fragment	3.49	302.1982	-	-
97	Tyrosol-sulfate	Urine	3	ESI-	[M-H]	3.49	217.0172	217.0176	0.4
				ESI-	[M-H-sulfate]	3.50	137.0606	-	-

98	3-(3-hydroxyphenyl)propanoic acid	Urine	1	ESI-	[M-H]	3.50	165.0550	165.0530	2.0
99	N-lactoyl-Leucine	Urine	3	ESI-	[M-H]	3.50	202.1081	202.1085	0.4
100	Heptanoylcarnitine	Urine	3	ESI+	[M+H]	3.51	274.2024	274.2013	1.1
101	Phenyllactic acid	Faeces	1	ESI-	[M-H]	3.52	165.0548	165.0552	0.4
				ESI-	[M-H-CO ₂]	3.51	121.0653	-	-
				ESI-	[M-H-HCOOH]	3.52	119.0494	119.0497	0.3
102	5-Butyltetrahydro-2-oxo-3-furancarboxylic acid	Urine	3	ESI-	[M-H]	3.53	185.0813	185.0819	0.6
				ESI-	[M-H] fragment (C ₈ H ₁₂ O)	3.53	123.0810	123.0815	0.5
103	Octenoylcarnitine	Urine	3	ESI+	[M+H]	3.55	286.2026	286.2013	1.3
				ESI+	[M+H] fragment	3.55	227.1287	-	-
				ESI+	[M+H] fragment	3.56	85.0289	-	-
104	Indolelactic acid	Urine	1	ESI-	[M-H]	3.56	204.0662	204.0649	1.3
				ESI+	[M+H-H ₂ O-C ₂ H ₂ O ₂]	3.57	130.0659	130.0663	0.4
105	Indoleacrylic glycine	Faeces; Urine	1	ESI-	[M-H]	3.56	243.0771	243.0764	0.7
				ESI-	[M-H-CO ₂]	3.59	199.0964	199.0892	7.2
				ESI-	[M-H] fragment	3.57	168.0445	168.0448	0.3
				ESI-	[M-H] fragment	3.56	142.0658	142.0652	0.6
				ESI-	[M-H] fragment	3.56	100.0034	100.0020	1.4
				ESI+	[M+H] fragment	3.56	170.0549	170.0565	1.6
106	Indole-3-methyl acetate	Urine	3	ESI+	[M+H]	3.57	190.0874	190.0863	1.1
107	Gentisein	Faeces	3	ESI-	[M-H]	3.59	243.0301	243.0299	0.2

108	Indoleacetic acid	Serum	1	ESI+	[M-H-CO ₂]	3.63	130.0654	130.0650	0.4
109	Azelaic acid	Faeces	1	ESI-	[M-H]	3.66	187.0971	187.0971	0.0
110	Urolithin C	Faeces	1	ESI-	[M-H]	3.67	243.0287	243.0289	0.2
111	Nonanoylcarnitine	Urine	3	ESI+	[M+H]	3.73	302.2337	302.2326	1.1
				ESI+	[M+H] fragment	3.73	243.1601	-	-
				ESI+	[M+H] fragment	3.74	141.1281	-	-
				ESI+	[M+H] fragment	3.73	85.0288	-	-
				ESI+	[M+H] fragment	3.74	60.0807	-	-
112	Urolithin A	Faeces	1	ESI-	[M-H]	3.74	227.0351	227.0340	- 1.1
113	Deoxypyridinoline	Urine	3	ESI-	[M+Cl]	3.74	447.1690	447.1652	3.8
114	Urobilin / Urobilinogen	Faeces	3	ESI-	[M-H]	3.75	589.3033	589.3032	0.1
115	Stercobilin	Faeces	3	ESI-	[M-H]	3.77	593.3343	593.3345	0.2
116	Indolepropionic acid	Serum	1	ESI+	[M+H-C ₂ H ₄ O ₂]	3.77	130.0656	130.0644	1.2
117	3-Carboxy-4-methyl-5-propyl-2-furanpropionic acid-glucuronide	Urine	3	ESI-	[M-H]	3.77	415.1252	415.1246	0.6
118	3-hydroxyundecanoyl carnitine	Urine	3	ESI+	[M+H-H ₂ O]	3.92	328.2489	328.2488	0.1
				ESI+	[M+H] fragment	3.93	269.1754	-	-
119	Dehydroisoandrosterone 3-sulfate	Serum	1	ESI-	[M-H]	4.04	367.1572	367.1570	0.2
120	Glaucarubin	Urine	3	ESI-	[M-H]	4.05	495.2225	495.2235	1.0
121	3-Carboxy-4-methyl-5-propyl-2-furanpropionic acid	Serum; Urine	1	ESI-	[M-H]	4.05	239.0911	239.0901	1.0

				ESI+	[M+H] fragment	4.05	181.0857	181.0869	1.2
122	Dodecanedioic acid	Urine	3	ESI-	[M-H]	4.08	229.1446	229.1445	0.1
123	Glycocholic acid	Faeces; Serum; Urine	1	ESI-	[M-H]	4.27	464.2991	464.3012	2.1
				ESI+	[M+H-H ₂ O]	4.31	448.3066	448.3060	0.6
				ESI+	[M+H-2H ₂ O]	4.25	430.2948	430.2959	1.1
				ESI+	[M+H] fragment	4.22	373.2695	373.2710	1.5
124	Muricholic acid	Faeces	3	ESI-	[M-H]	4.23	407.2802	407.2803	0.1
125	7-Ketodeoxycholic acid / 3-Oxocholeic acid	Faeces	3	ESI-	[M-H]	4.25	405.2640	405.2646	0.6
126	Glycochenodeoxycholic acid 3-sulfate	Urine	1	ESI-	[M-H]	4.44	528.2621	528.2635	1.4
127	Ursodeoxycholic acid	Faeces	1	ESI-	[M-H]	4.45	391.2851	391.2849 391.2854	0.2
128	Glycochenodeoxycholic acid	Serum	1	ESI+	[M+H-2H ₂ O]	4.46	414.3013	414.3004	0.9
129	Cholic acid	Faeces; Serum; Urine	1	ESI-	[M-H]	4.46	407.2793	407.2798	0.5
130	Hyodeoxycholic acid	Faeces	1	ESI-	[M-H]	4.56	391.2838	391.2849	1.1
131	Chenodeoxycholic acid	Faeces	1	ESI-	[M-H]	4.63	391.2849	391.2849	0.0
132	12-Ketodeoxycholic acid or similar	Faeces	4	ESI-	[M-H]	4.71	389.2697	389.2697	0.0
133	Deoxycholic acid	Faeces	1	ESI-	[M-H]	4.73	391.2846	391.2849	0.3

* We noted a modest retention time shift (<0.1 min) when comparing the retention times of the initial analyses with the retention times obtained when analysing authentic standards. However, for a few compounds (Phenol sulfate and Indoxyl-sulfate) this shift was more pronounced (rt shift = 0.2-0.3 min).

†Identification levels by Metabolomics Standard Initiative.[1] ESI, electrospray ionization; rt, retention time; *m/z*, mass-to-charge.

References

1. Sumner LW, Amberg A, Barrett D, *et al.* Proposed minimum reporting standards for chemical analysis. *Metabolomics* 2007;**3**:211–21.

Supplementary Table 5 List of contrasted MSP species and Spearman correlations with MD index by time point. Reporting of contrasted MSP between ConD and MedD groups at each timepoint (after 20% occurrence filter) with their enrichment status, pvalues from Wilcoxon rank sum tests (pvaluew) and after adjustment for multiple comparisons using the Benjamini-Hochberg procedure (qvaluew), Spearman Rho and pvalues_p calculated with MD index score and the respective status based on Venn diagram results (status VD).

MSP	Phylum	Species annotation	Baseline					
			Enriched in	pvaluew	qvaluew	Rho	pvalues _p	Status VD
msp_0713	Firmicutes	<i>Clostridium citroniae</i>	ConD	0,001	0,19	-0,007	0,96	baseline and 4w
msp_0056	Firmicutes	<i>unclassified Clostridiales</i>	ConD	0,002	0,19	0,015	0,90	baseline, 4w and 8w
msp_0172	Firmicutes	<i>Ruthenibacterium lactatiformans</i>	ConD	0,002	0,19	0,138	0,29	baseline, 4w and 8w
msp_0066	Firmicutes	<i>Clostridium</i> sp. AT4	ConD	0,003	0,19	-0,183	0,15	baseline, 4w and 8w
msp_0009	Firmicutes	<i>Clostridium bolteae</i>	ConD	0,009	0,35	0,015	0,91	baseline
msp_0335	Bacteroidetes	<i>unclassified Alistipes</i>	ConD	0,011	0,39	-0,262	0,04	baseline, 4w and 8w
msp_0263	Actinobacteria	<i>Bifidobacterium adolescentis</i>	ConD	0,017	0,49	-0,066	0,61	baseline and 8w
msp_1541	Firmicutes	<i>unclassified Clostridiales</i>	ConD	0,021	0,49	0,016	0,90	baseline and 4w
msp_0020	Firmicutes	<i>Clostridium clostridioforme</i> 1	ConD	0,022	0,49	-0,174	0,18	baseline and 4w
msp_0014	Firmicutes	<i>Eisenbergiella tayi</i>	ConD	0,028	0,51	0,153	0,24	baseline, 4w and 8w
msp_0318	Bacteroidetes	<i>Alistipes indistinctus</i>	ConD	0,043	0,61	-0,027	0,84	baseline and 4w
msp_0164	Firmicutes	<i>Ruminococcus</i> sp.	MedD	0,003	0,19	0,140	0,28	baseline, 4w and 8w
msp_0979	NA	NA	MedD	0,004	0,19	0,244	0,06	baseline, 4w and 8w
msp_0265	Firmicutes	<i>unclassified Faecalibacterium</i>	MedD	0,004	0,19	-0,004	0,97	baseline, 4w and 8w
msp_0586	Firmicutes	<i>Clostridium</i> sp. CAG:343	MedD	0,004	0,19	0,306	0,02	baseline
msp_0285	Firmicutes	<i>Clostridium</i> sp. CAG:217	MedD	0,022	0,49	0,009	0,95	baseline, 4w and 8w
msp_0133	Firmicutes	<i>Coprococcus</i> sp.	MedD	0,022	0,49	0,149	0,25	baseline and 4w
msp_0820	Firmicutes	<i>unclassified Clostridiales</i>	MedD	0,023	0,49	0,277	0,03	baseline, 4w and 8w
msp_0679	Firmicutes	<i>unclassified Firmicutes</i>	MedD	0,024	0,49	0,040	0,76	baseline, 4w and 8w
msp_0906	Firmicutes	<i>Clostridium</i> sp.	MedD	0,025	0,49	0,041	0,75	baseline, 4w and 8w
msp_0189	Firmicutes	<i>Blautia</i> sp. CAG:237	MedD	0,029	0,51	0,166	0,20	baseline, 4w and 8w
msp_0893	Firmicutes	<i>Faecalibacterium prausnitzii</i> 2	MedD	0,032	0,51	0,034	0,79	baseline
msp_0087	Firmicutes	<i>Lactobacillus rogosae</i>	MedD	0,033	0,51	0,264	0,04	baseline

msp_0639	Firmicutes	<i>Faecalibacterium prausnitzii</i> 6	MedD	0,034	0,51	0,096	0,46	baseline, 4w and 8w
msp_1428	Firmicutes	<i>unclassified Clostridiales</i>	MedD	0,047	0,61	0,155	0,23	baseline
msp_1643c	Firmicutes	<i>Ruminococcus</i> sp. JC304	MedD	0,048	0,61	0,258	0,04	baseline, 4w and 8w
msp_0153	Firmicutes	<i>Erysipelatoclostridium ramosum</i>	MedD	0,048	0,61	0,146	0,26	baseline

Total Number MSP species at baseline= 1065

4w

MSP	Phylum	Species annotation	Enriched in	pvaluew	qvaluew	Rho	pvaluesp	Status VD
msp_0172	Firmicutes	<i>Ruthenibacterium lactatiformans</i>	ConD	0,00016	0,019	-0,44	0,0004	baseline, 4w and 8w
msp_0066	Firmicutes	<i>Clostridium</i> sp. AT4	ConD	0,00481	0,10	-0,35	0,0058	baseline, 4w and 8w
msp_0024	Firmicutes	<i>Hungatella hathewayi</i> 2	ConD	0,00559	0,10	-0,34	0,0066	4w
msp_0249	Firmicutes	<i>unclassified Firmicutes</i>	ConD	0,00665	0,10	-0,23	0,0695	4w
msp_0335	Bacteroidetes	<i>unclassified Alistipes</i>	ConD	0,00681	0,10	-0,38	0,0026	baseline, 4w and 8w
msp_0619	NA	NA	ConD	0,00895	0,12	-0,31	0,0155	4w
msp_0058	Firmicutes	<i>Ruminococcus gnavus</i>	ConD	0,00939	0,12	-0,23	0,0734	4w and 8w
msp_0707	Firmicutes	<i>unclassified Firmicutes</i>	ConD	0,01057	0,12	-0,24	0,0614	4w and 8w
msp_0713	Firmicutes	<i>Clostridium citroniae</i>	ConD	0,01088	0,12	-0,43	0,0005	baseline and 4w
msp_0056	Firmicutes	<i>unclassified Clostridiales</i>	ConD	0,01270	0,13	-0,42	0,0008	baseline, 4w and 8w
msp_0052	Firmicutes	<i>Blautia</i> sp. CAG:257	ConD	0,01368	0,13	-0,28	0,0296	4w and 8w
msp_0317	Firmicutes	<i>unclassified Clostridiales</i>	ConD	0,01368	0,13	-0,13	0,3082	4w
msp_0331	Firmicutes	<i>Oscillibacter</i> sp.	ConD	0,01379	0,13	-0,33	0,0085	4w
msp_0271	Firmicutes	<i>Eubacterium</i> sp. CAG:180	ConD	0,01420	0,13	-0,28	0,0299	4w
msp_0126	Firmicutes	<i>Ruminococcus torques</i>	ConD	0,01453	0,13	-0,34	0,0063	4w and 8w
msp_0621	Firmicutes	<i>Intestinibacter bartlettii</i>	ConD	0,01563	0,13	-0,14	0,2883	4w
msp_0027	Bacteroidetes	<i>Parabacteroides merdae</i>	ConD	0,01625	0,13	-0,17	0,1937	4w
msp_1090	NA	NA	ConD	0,01640	0,13	-0,25	0,0528	4w
msp_0132	Firmicutes	<i>Coprobacillus cateniformis</i>	ConD	0,01734	0,14	-0,26	0,0425	4w and 8w
msp_0318	Bacteroidetes	<i>Alistipes indistinctus</i>	ConD	0,01862	0,14	-0,23	0,0765	baseline and 4w
msp_0014	Firmicutes	<i>Eisenbergiella tayi</i>	ConD	0,01883	0,14	-0,32	0,0115	baseline, 4w and 8w
msp_0510	Firmicutes	<i>Clostridium glycyrrhizinilyticum</i>	ConD	0,02199	0,16	-0,35	0,0056	4w
msp_1315	Firmicutes	<i>unclassified Firmicutes</i>	ConD	0,02687	0,17	-0,21	0,0981	4w
msp_0364	Firmicutes	<i>Intestinimonas butyriciproducens</i>	ConD	0,02694	0,17	-0,23	0,0675	4w
msp_0442	Firmicutes	<i>unclassified Flavonifractor</i>	ConD	0,02787	0,17	-0,33	0,0089	4w
msp_0861	Firmicutes	<i>unclassified Oscillibacter</i>	ConD	0,03166	0,19	-0,30	0,0164	4w
msp_0230	Bacteroidetes	<i>Alistipes inops</i>	ConD	0,03206	0,19	-0,18	0,1559	4w
msp_1012	Firmicutes	<i>Intestinimonas massiliensis</i>	ConD	0,03310	0,19	-0,32	0,0109	4w
msp_0833	Firmicutes	<i>Streptococcus thermophilus</i>	ConD	0,03320	0,19	-0,30	0,0166	4w

msp_0522	Firmicutes	<i>Anaerotruncus colihominis</i>	ConD	0,03393	0,19	-0,40	0,0012	4w
msp_0103	Firmicutes	<i>Clostridium innocuum</i>	ConD	0,03433	0,19	-0,30	0,0190	4w and 8w
msp_0020	Firmicutes	<i>Clostridium clostridioforme</i> 1	ConD	0,03648	0,20	-0,33	0,0080	baseline and 4w
msp_0353	NA	NA	ConD	0,04238	0,20	-0,31	0,0157	4w
msp_1541	Firmicutes	<i>unclassified Clostridiales</i>	ConD	0,04247	0,20	-0,34	0,0074	baseline and 4w
msp_1724	Firmicutes	<i>Phoceia massiliensis</i>	ConD	0,04435	0,21	-0,27	0,0327	4w and 8w
msp_0213	Firmicutes	<i>Flavonifractor plautii</i>	ConD	0,04472	0,21	-0,23	0,0679	4w and 8w
msp_0881	Proteobacteria	<i>Haemophilus parainfluenzae</i>	MedD	0,00001	0,003	0,55	4,0E-06	4w and 8w
msp_0388	Firmicutes	<i>Faecalibacterium prausnitzii</i> 3 (L2-6)	MedD	0,00010	0,017	0,24	0,0614	4w and 8w
msp_0884	Firmicutes	<i>Veillonella atypica</i>	MedD	0,00023	0,02	0,32	0,0104	4w
msp_1641	Firmicutes	<i>unclassified Lachnospiraceae</i>	MedD	0,00037	0,03	0,35	0,0049	4w and 8w
msp_1643c	Firmicutes	<i>Ruminococcus</i> sp. JC304	MedD	0,00052	0,03	0,28	0,0277	baseline, 4w and 8w
msp_0979	NA	NA	MedD	0,00156	0,08	0,37	0,0030	baseline, 4w and 8w
msp_0906	Firmicutes	<i>Clostridium</i> sp.	MedD	0,00188	0,08	0,40	0,0013	baseline, 4w and 8w
msp_0285	Firmicutes	<i>Clostridium</i> sp. CAG:217	MedD	0,00207	0,08	0,10	0,4188	baseline, 4w and 8w
msp_0164	Firmicutes	<i>Ruminococcus</i> sp.	MedD	0,00238	0,08	0,34	0,0077	baseline, 4w and 8w
msp_0313	Firmicutes	<i>Veillonella parvula</i>	MedD	0,00264	0,08	0,35	0,0060	4w and 8w
msp_0930	Firmicutes	<i>unclassified Lachnospiraceae</i>	MedD	0,00294	0,08	0,34	0,0066	4w
msp_1219	Firmicutes	<i>Veillonella rogosae</i>	MedD	0,00302	0,08	0,32	0,0115	4w and 8w
msp_0189	Firmicutes	<i>Blautia</i> sp. CAG:237	MedD	0,00366	0,09	0,29	0,0245	baseline, 4w and 8w
msp_0265	Firmicutes	<i>unclassified Faecalibacterium</i>	MedD	0,00373	0,09	0,20	0,1173	baseline, 4w and 8w
msp_0148c	Firmicutes	<i>Veillonella dispar</i>	MedD	0,00581	0,10	0,31	0,0128	4w
msp_0457	Firmicutes	<i>Faecalibacterium</i> sp. CAG:82	MedD	0,00581	0,10	0,30	0,0193	4w and 8w
msp_0296	Firmicutes	<i>unclassified Lachnospiraceae</i>	MedD	0,00665	0,10	0,32	0,0112	4w and 8w
msp_0898	Firmicutes	<i>unclassified butyrate-producing Clostridiales</i>	MedD	0,00670	0,10	0,27	0,0315	4w
msp_0468	Firmicutes	<i>unclassified Clostridiales</i>	MedD	0,00788	0,11	0,18	0,1566	4w
msp_0029	Firmicutes	<i>Eubacterium eligens</i>	MedD	0,00928	0,12	0,27	0,0317	4w and 8w
msp_0639	Firmicutes	<i>Faecalibacterium prausnitzii</i> 6	MedD	0,00944	0,12	0,39	0,0015	baseline, 4w and 8w
msp_1236	Firmicutes	<i>unclassified Lachnoclostridium</i>	MedD	0,01079	0,12	0,37	0,0027	4w
msp_0301	Firmicutes	<i>Faecalibacterium prausnitzii</i> 1 (A2-165)	MedD	0,01144	0,12	0,38	0,0022	4w
msp_0075	Firmicutes	<i>Clostridium</i> sp.	MedD	0,01145	0,12	0,28	0,0300	4w and 8w
msp_0820	Firmicutes	<i>unclassified Clostridiales</i>	MedD	0,01424	0,13	0,37	0,0031	baseline, 4w and 8w

msp_0473c	Firmicutes	<i>Clostridium sp.</i>	MedD	0,02327	0,17	0,16	0,2225	4w and 8w
msp_0297	Firmicutes	<i>unclassified Ruminococcaceae</i>	MedD	0,02327	0,17	-0,31	0,0136	4w
msp_0679	Firmicutes	<i>unclassified Firmicutes</i>	MedD	0,02439	0,17	0,25	0,0525	baseline, 4w and 8w
msp_0572	Firmicutes	<i>Lachnospira sp.</i>	MedD	0,02460	0,17	0,33	0,0091	4w
msp_0015	Firmicutes	<i>Roseburia faecis</i>	MedD	0,02557	0,17	0,22	0,0888	4w
msp_0068	Firmicutes	<i>Eubacterium rectale</i>	MedD	0,02557	0,17	0,24	0,0658	4w
msp_0017	Firmicutes	<i>Roseburia intestinalis</i>	MedD	0,02610	0,17	0,31	0,0150	4w and 8w
msp_0563	Firmicutes	<i>unclassified Clostridium</i>	MedD	0,03159	0,19	0,34	0,0077	4w
msp_0125	Firmicutes	<i>unclassified Clostridiales</i>	MedD	0,03709	0,20	0,27	0,0373	4w
msp_1302	Firmicutes	<i>unclassified Firmicutes</i>	MedD	0,03912	0,20	0,24	0,0562	4w
msp_0986	Firmicutes	<i>Coprococcus catus</i>	MedD	0,03927	0,20	0,31	0,0137	4w
msp_0086	Firmicutes	<i>Clostridium symbiosum</i>	MedD	0,03984	0,20	-0,41	0,0009	4w
msp_1143	Firmicutes	<i>unclassified Oscillibacter</i>	MedD	0,04124	0,20	0,23	0,0680	4w
msp_1339	Actinobacteria	<i>Gordonibacter urolithinfaciens</i>	MedD	0,04209	0,20	0,16	0,2228	4w
msp_0227	Firmicutes	<i>Eubacterium sp. 36_13 & CAG:86</i>	MedD	0,04285	0,20	0,37	0,0029	4w
msp_0133	Firmicutes	<i>Coprococcus sp.</i>	MedD	0,04861	0,22	0,28	0,0276	baseline and 4w

Total Number MSP species at 4w = 1051

8w								
MSP	Phylum	Species annotation	Enriched in	pvaluew	qvaluew	Rho	pvaluesp	Status VD
msp_0172	Firmicutes	<i>Ruthenibacterium lactatiformans</i>	ConD	0,002	0,12	-0,27	0,033	baseline, 4w and 8w
msp_0126	Firmicutes	<i>Ruminococcus torques</i>	ConD	0,002	0,12	-0,33	0,009	4w and 8w
msp_0213	Firmicutes	<i>Flavonifractor plautii</i>	ConD	0,006	0,14	-0,28	0,026	4w and 8w
msp_0058	Firmicutes	<i>Ruminococcus gnavus</i>	ConD	0,007	0,14	-0,21	0,094	4w and 8w
msp_0132	Firmicutes	<i>Coprobacillus cateniformis</i>	ConD	0,007	0,14	-0,22	0,082	4w and 8w
msp_0335	Bacteroidetes	<i>unclassified Alistipes</i>	ConD	0,007	0,14	-0,37	0,003	baseline, 4w and 8w
msp_0340	Firmicutes	<i>Clostridium leptum</i>	ConD	0,011	0,17	-0,20	0,110	8w
msp_0263	Actinobacteria	<i>Bifidobacterium adolescentis</i>	ConD	0,012	0,17	-0,28	0,029	baseline and 8w
msp_0014	Firmicutes	<i>Eisenbergiella tayi</i>	ConD	0,012	0,17	-0,27	0,034	baseline, 4w and 8w
msp_0112	Firmicutes	<i>Blautia hydrogenotrophica</i>	ConD	0,014	0,18	-0,27	0,037	8w
msp_0052	Firmicutes	<i>Blautia</i> sp. CAG:257	ConD	0,016	0,19	-0,34	0,007	4w and 8w
msp_0066	Firmicutes	<i>Clostridium</i> sp. AT4	ConD	0,025	0,27	-0,28	0,027	baseline, 4w and 8w
msp_1724	Firmicutes	<i>Phocaea massiliensis</i>	ConD	0,027	0,27	-0,33	0,009	4w and 8w
msp_0005	Proteobacteria	<i>Escherichia coli</i>	ConD	0,028	0,27	-0,24	0,058	8w
msp_0259	Firmicutes	<i>Coprococcus comes</i>	ConD	0,028	0,27	-0,20	0,111	8w
msp_0056	Firmicutes	<i>unclassified Clostridiales</i>	ConD	0,035	0,32	-0,23	0,070	baseline, 4w and 8w
msp_0103	Firmicutes	<i>Clostridium innocuum</i>	ConD	0,036	0,32	-0,22	0,090	4w and 8w
msp_0707	Firmicutes	<i>unclassified Firmicutes</i>	ConD	0,040	0,34	-0,02	0,879	4w and 8w
msp_1244	Actinobacteria	<i>Collinsella aerofaciens</i>	ConD	0,040	0,34	-0,19	0,138	8w
msp_0881	Proteobacteria	<i>Haemophilus parainfluenzae</i>	MedD	0,000	0,05	0,40	0,001	4w and 8w
msp_0906	Firmicutes	<i>Clostridium</i> sp.	MedD	0,000	0,07	0,36	0,004	baseline, 4w and 8w
msp_0457	Firmicutes	<i>Faecalibacterium</i> sp. CAG:82	MedD	0,002	0,12	0,33	0,008	4w and 8w
msp_0820	Firmicutes	<i>unclassified Clostridiales</i>	MedD	0,003	0,12	0,25	0,049	baseline, 4w and 8w
msp_0388	Firmicutes	<i>Faecalibacterium prausnitzii</i> 3 (L2-6)	MedD	0,003	0,12	0,24	0,056	4w and 8w
msp_1643c	Firmicutes	<i>Ruminococcus</i> sp. JC304	MedD	0,003	0,12	0,30	0,017	baseline, 4w and 8w
msp_0979	NA	NA	MedD	0,003	0,13	0,38	0,002	baseline, 4w and 8w
msp_0164	Firmicutes	<i>Ruminococcus</i> sp.	MedD	0,004	0,13	0,23	0,074	baseline, 4w and 8w
msp_0265	Firmicutes	<i>unclassified Faecalibacterium</i>	MedD	0,005	0,13	0,17	0,176	baseline, 4w and 8w
msp_0029	Firmicutes	<i>Eubacterium eligens</i>	MedD	0,005	0,13	0,37	0,003	4w and 8w

msp_0075	Firmicutes	<i>Clostridium</i> sp.	MedD	0,006	0,14	0,27	0,031	4w and 8w
msp_0473c	Firmicutes	<i>Clostridium</i> sp.	MedD	0,007	0,14	0,27	0,034	4w and 8w
msp_1641	Firmicutes	<i>unclassified Lachnospiraceae</i>	MedD	0,008	0,15	0,38	0,002	4w and 8w
msp_0285	Firmicutes	<i>Clostridium</i> sp. CAG:217	MedD	0,011	0,17	0,05	0,717	baseline, 4w and 8w
msp_0296	Firmicutes	<i>unclassified Lachnospiraceae</i>	MedD	0,012	0,17	0,26	0,040	4w and 8w
msp_1219	Firmicutes	<i>Veillonella rogosae</i>	MedD	0,012	0,17	0,20	0,121	4w and 8w
msp_0071	Firmicutes	<i>Roseburia hominis</i>	MedD	0,012	0,17	0,18	0,173	8w
msp_0017	Firmicutes	<i>Roseburia intestinalis</i>	MedD	0,014	0,18	0,24	0,065	4w and 8w
msp_0679	Firmicutes	<i>unclassified Firmicutes</i>	MedD	0,016	0,19	0,26	0,041	baseline, 4w and 8w
msp_0639	Firmicutes	<i>Faecalibacterium prausnitzii</i> 6	MedD	0,020	0,22	0,28	0,027	baseline, 4w and 8w
msp_0189	Firmicutes	<i>Blautia</i> sp. CAG:237	MedD	0,025	0,27	0,27	0,036	baseline, 4w and 8w
msp_0313	Firmicutes	<i>Veillonella parvula</i>	MedD	0,031	0,29	0,21	0,099	4w and 8w
msp_0860	Firmicutes	<i>unclassified Intestinibacter</i>	MedD	0,042	0,35	0,25	0,052	8w
msp_0145	Proteobacteria	<i>Parasutterella excrementihominis</i>	MedD	0,044	0,36	0,01	0,927	8w
msp_0780	Firmicutes	<i>unclassified Lachnospiraceae</i>	MedD	0,047	0,38	0,22	0,081	8w

Total Number MSP species at 8w = 1037

Supplementary Table 6 List of contrasted MSP within diets. List of contrasted MSP within ConD and MedD groups for the 4w-baseline and 8w-4w periods (after 20% occurrence filter) with their enrichment status and pvalues from paired Wilcoxon rank sum tests (pvaluew) and after adjustment for multiple comparisons using the Benjamini-Hochberg procedure (qvaluew).

ConD_baseline vs 4w					
MSP	phylum	species	pvaluew	qvaluew	Enriched at
msp_1349	Firmicutes	<i>unclassified Ruminococcaceae</i>	0,0046	0,79	baseline
msp_0215	Firmicutes	<i>Dialister</i> sp. CAG:357	0,0068	0,79	baseline
msp_1302	Firmicutes	<i>unclassified Firmicutes</i>	0,0113	0,80	baseline
msp_0554	Firmicutes	<i>Anaerostipes hadrus</i> 2	0,0147	0,86	baseline
msp_0324	Firmicutes	<i>Faecalibacterium prausnitzii</i> 7	0,0238	1,00	baseline
msp_0335	Bacteroidetes	<i>unclassified Alistipes</i>	0,0263	1,00	baseline
msp_1556	Firmicutes	<i>Holdemania filiformis</i>	0,0274	1,00	baseline
msp_1403	Firmicutes	<i>Blautia</i> sp.	0,0333	1,00	baseline
msp_0129	Firmicutes	<i>Clostridium</i> sp. CAG:58	0,0383	1,00	baseline
msp_0221	Firmicutes	<i>Acidaminococcus intestini</i>	0,0465	1,00	baseline
msp_0331	Firmicutes	<i>Oscillibacter</i> sp.	0,0475	1,00	baseline
msp_0440	Firmicutes	<i>unclassified Eubacterium</i>	0,0042	0,79	4w
msp_0893	Firmicutes	<i>Faecalibacterium prausnitzii</i> 2	0,0114	0,80	4w
msp_0035	Bacteroidetes	<i>Bacteroides dorei</i>	0,0349	1,00	4w

Total Number MSP species ConD baseline vs 4w = 975

ConD_4w vs 8w

MSP	phylum	species	pvaluew	qvaluew	Enriched at
msp_1362	Firmicutes	<i>Holdemania massiliensis</i>	0,014	0,701	4w
msp_0225	Bacteroidetes	<i>Alistipes obesi</i>	0,015	0,701	4w
msp_0432	unclassified	<i>unclassified</i>	0,017	0,701	4w
msp_0440	Firmicutes	<i>unclassified Eubacterium</i>	0,017	0,701	4w
msp_0046	Bacteroidetes	<i>Bacteroides uniformis</i>	0,018	0,701	4w
msp_0069	Bacteroidetes	<i>Bacteroides vulgatus</i>	0,034	0,793	4w
msp_0906	Firmicutes	<i>Clostridium sp.</i>	0,041	0,793	4w
msp_0259	Firmicutes	<i>Coprococcus comes</i>	0,008	0,701	8w
msp_1381	Firmicutes	<i>Butyricicoccus sp.</i>	0,009	0,701	8w
msp_1244	Actinobacteria	<i>Collinsella aerofaciens</i>	0,009	0,701	8w
msp_1349	Firmicutes	<i>unclassified Ruminococcaceae</i>	0,015	0,701	8w
msp_1339	Actinobacteria	<i>Gordonibacter urolithinfaciens</i>	0,024	0,701	8w
msp_0126	Firmicutes	<i>Ruminococcus torques</i>	0,025	0,701	8w
msp_0215	Firmicutes	<i>Dialister sp. CAG:357</i>	0,025	0,701	8w
msp_0467	Firmicutes	<i>Firmicutes bacterium CAG:94</i>	0,026	0,701	8w
msp_0045	Firmicutes	<i>Ruminococcus bromii 2</i>	0,036	0,793	8w
msp_0385	Firmicutes	<i>Ruminococcaceae bacterium D16</i>	0,041	0,793	8w
msp_0419	Actinobacteria	<i>Bifidobacterium bifidum</i>	0,042	0,793	8w
msp_0874	Firmicutes	<i>unclassified Firmicutes</i>	0,045	0,793	8w

Total Number MSP species ConD 4w vs 8w = 960

MedD_baseline vs 4w

MSP	phylum	species	pvaluew	qvaluew	Enriched at
msp_0213	Firmicutes	<i>Flavonifractor plautii</i>	0,0001	0,0240	baseline
msp_0331	Firmicutes	<i>Oscillibacter</i> sp.	0,001	0,0815	baseline
msp_0342	Firmicutes	<i>Flavonifractor</i> sp.	0,001	0,0859	baseline
msp_0613	Firmicutes	<i>unclassified Clostridiales</i>	0,003	0,1030	baseline
msp_0565	Firmicutes	<i>unclassified Ruminococcaceae</i>	0,003	0,1030	baseline
msp_0024	Firmicutes	<i>Hungatella hathewayi</i> 2	0,005	0,1560	baseline
msp_0172	Firmicutes	<i>Ruthenibacterium lactatiformans</i>	0,006	0,1560	baseline
msp_1060c	Firmicutes	<i>unclassified Flavonifractor</i>	0,007	0,1560	baseline
msp_0781	Firmicutes	<i>unclassified Clostridiales</i>	0,008	0,1560	baseline
msp_0833	Firmicutes	<i>Streptococcus thermophilus</i>	0,009	0,1560	baseline
msp_0312	Firmicutes	<i>Firmicutes bacterium</i> CAG:110	0,012	0,1560	baseline
msp_0152	Firmicutes	<i>Ruminococcus faecis</i>	0,012	0,1560	baseline
msp_0467	Firmicutes	<i>Firmicutes bacterium</i> CAG:94	0,012	0,1560	baseline
msp_0500	Firmicutes	<i>unclassified Firmicutes</i>	0,012	0,1560	baseline
msp_0621	Firmicutes	<i>Intestinibacter bartlettii</i>	0,013	0,1560	baseline
msp_1349	Firmicutes	<i>unclassified Ruminococcaceae</i>	0,013	0,1560	baseline
msp_0364	Firmicutes	<i>Intestinimonas butyriciproducens</i>	0,013	0,1560	baseline
msp_0357	Firmicutes	<i>Clostridium</i> sp. CAG:169	0,013	0,1560	baseline
msp_0121	Firmicutes	<i>unclassified Oscillibacter</i>	0,014	0,1560	baseline
msp_0335	Bacteroidetes	<i>unclassified Alistipes</i>	0,014	0,1560	baseline
msp_1090	unclassified	<i>unclassified</i>	0,014	0,1560	baseline
msp_0308	Firmicutes	<i>Clostridium saccharolyticum</i> 2	0,014	0,1560	baseline
msp_0510	Firmicutes	<i>Clostridium glycyrrhizinilyticum</i>	0,017	0,1678	baseline
msp_0545	Firmicutes	<i>Pseudoflavonifractor</i> sp. An184	0,018	0,1678	baseline
msp_0314	Firmicutes	<i>unclassified Clostridiales</i>	0,019	0,1678	baseline
msp_0132	Firmicutes	<i>Coprobacillus cateniformis</i>	0,019	0,1678	baseline
msp_0462	Firmicutes	<i>unclassified Clostridiales</i>	0,020	0,1678	baseline
msp_0126	Firmicutes	<i>Ruminococcus torques</i>	0,020	0,1678	baseline
msp_0777	Firmicutes	<i>Firmicutes bacterium</i> CAG:129	0,021	0,1678	baseline
msp_0040	Bacteroidetes	<i>Bacteroides massiliensis</i>	0,022	0,1690	baseline
msp_0249	Firmicutes	<i>unclassified Firmicutes</i>	0,023	0,1717	baseline
msp_0592	Firmicutes	<i>unclassified Clostridiales</i>	0,024	0,1732	baseline
msp_0861	Firmicutes	<i>unclassified Oscillibacter</i>	0,025	0,1758	baseline
msp_1013	Firmicutes	<i>unclassified Firmicutes</i>	0,025	0,1771	baseline
msp_0027	Bacteroidetes	<i>Parabacteroides merdae</i>	0,027	0,1835	baseline
msp_0435	Firmicutes	<i>unclassified Oscillibacter</i>	0,029	0,1864	baseline
msp_1315	Firmicutes	<i>unclassified Firmicutes</i>	0,037	0,2189	baseline
msp_0205	Firmicutes	<i>Firmicutes bacterium</i> CAG:124	0,037	0,2189	baseline
msp_0317	Firmicutes	<i>unclassified Clostridiales</i>	0,038	0,2189	baseline
msp_1356	Firmicutes	<i>Anaeromassilibacillus</i> sp. An250	0,041	0,2291	baseline
msp_0144	Firmicutes	<i>Clostridium</i> sp. CAG:138	0,041	0,2291	baseline
msp_0471	Firmicutes	<i>unclassified Clostridiales</i>	0,044	0,2291	baseline
msp_0287	Bacteroidetes	<i>Alistipes ihumii</i>	0,044	0,2291	baseline
msp_1724	Firmicutes	<i>Phoceia massiliensis</i>	0,044	0,2291	baseline

msp_0046	Bacteroidetes	<i>Bacteroides uniformis</i>	0,047	0,2426	baseline
msp_0388	Firmicutes	<i>Faecalibacterium prausnitzii</i> 3 (L2-6)	4,7E-08	0,00002	4w
msp_0906	Firmicutes	<i>Clostridium</i> sp.	0,001	0,0815	4w
msp_0756	Firmicutes	<i>unclassified Blautia</i>	0,002	0,0987	4w
msp_0296	Firmicutes	<i>unclassified Lachnospiraceae</i>	0,003	0,1030	4w
msp_0473c	Firmicutes	<i>Clostridium</i> sp.	0,007	0,1560	4w
msp_0015	Firmicutes	<i>Roseburia faecis</i>	0,008	0,1560	4w
msp_0881	Proteobacteria	<i>Haemophilus parainfluenzae</i>	0,008	0,1560	4w
msp_0047	Firmicutes	<i>Eubacterium</i> sp. CAG:115	0,008	0,1560	4w
msp_0572	Firmicutes	<i>Lachnospira</i> sp.	0,011	0,1560	4w
msp_1219	Firmicutes	<i>Veillonella rogosae</i>	0,012	0,1560	4w
msp_0071	Firmicutes	<i>Roseburia hominis</i>	0,015	0,1560	4w
msp_0075	Firmicutes	<i>Clostridium</i> sp.	0,017	0,1678	4w
msp_0772	Firmicutes	<i>Clostridiales</i> sp.	0,019	0,1678	4w
msp_0820	Firmicutes	<i>unclassified Clostridiales</i>	0,021	0,1678	4w
msp_0903	Firmicutes	<i>Oscillibacter</i> sp. 57_20	0,022	0,1690	4w
msp_1062	Firmicutes	<i>unclassified Oscillibacter</i>	0,022	0,1707	4w
msp_0654	Firmicutes	<i>Firmicutes bacterium</i> CAG:103	0,028	0,1864	4w
msp_1236	Firmicutes	<i>unclassified Lachnoclostridium</i>	0,029	0,1864	4w
msp_1533	Firmicutes	<i>unclassified Clostridiales</i>	0,029	0,1864	4w
msp_0175	Firmicutes	<i>unclassified Lachnospiraceae</i>	0,030	0,1887	4w
msp_0930	Firmicutes	<i>unclassified Lachnospiraceae</i>	0,036	0,2176	4w
msp_0139	Bacteroidetes	<i>Coprobacter fastidiosus</i>	0,041	0,2291	4w
msp_1492	Firmicutes	<i>unclassified Lachnospiraceae</i>	0,043	0,2291	4w
msp_0107	Firmicutes	<i>Anaerostipes hadrus</i> 1	0,049	0,2456	4w

Total Number MSP species MedD baseline vs 4w = 928

MedD_4w vs 8w					
MSP	phylum	species	pvaluew	qvaluew	Enriched at
msp_0065	Firmicutes	<i>Blautia sp.</i>	0,0054	0,9540	4w
msp_0530	Firmicutes	<i>Dorea longicatena 2</i>	0,0111	0,9540	4w
msp_0930	Firmicutes	<i>unclassified Lachnospiraceae</i>	0,0129	0,9540	4w
msp_1622	Firmicutes	<i>Clostridium sp.</i>	0,0175	0,9540	4w
msp_0188	Firmicutes	<i>Coprobacillus sp.</i>	0,0179	0,9540	4w
msp_0005	Proteobacteria	<i>Escherichia coli</i>	0,0249	0,9540	4w
msp_0388	Firmicutes	<i>Faecalibacterium prausnitzii 3 (L2-6)</i>	0,0262	0,9540	4w
msp_0906	Firmicutes	<i>Clostridium sp.</i>	0,0314	0,9540	4w
msp_0018	Firmicutes	<i>Clostridium sp.</i>	0,0328	0,9540	4w
msp_0107	Firmicutes	<i>Anaerostipes hadrus 1</i>	0,0345	0,9540	4w
msp_0356	Firmicutes	<i>unclassified Clostridiales</i>	0,0352	0,9540	4w
msp_0572	Firmicutes	<i>Lachnospira sp.</i>	0,0355	0,9540	4w
msp_0340	Firmicutes	<i>Clostridium leptum</i>	0,0415	0,9580	4w
msp_0467	Firmicutes	<i>Firmicutes bacterium CAG:94</i>	0,0464	0,9580	4w
msp_0898	Firmicutes	<i>Clostridium sp.</i>	0,0467	0,9580	4w
msp_0342	Firmicutes	<i>Flavonifractor sp.</i>	0,0234	0,9540	8w
msp_0031	Bacteroidetes	<i>Bacteroides stercoris</i>	0,0422	0,9580	8w
Total Number MSP species MedD 4w vs 8w = 913					

Supplementary Table 7 List of contrasted GMM functional modules between ConD and MedD groups at each timepoint, their enrichment status and pvalues from Wilcoxon rank sum tests (pvaluew) and after adjustment for multiple comparisons using the Benjamini-Hochberg procedure (qvaluew).

baseline					
GMM	Module definition	HIER1	Enriched in	pvaluew	qvaluew
MF0108	glycerol degradation (dihydroxyacetone pathway)	lipid degradation	ConD	0,018	0,963
MF0068	glucarate degradation	carbohydrate degradation	ConD	0,044	0,963
MF0024	methionine degradation (mercaptan pathway)	amino acid degradation	MedD	0,036	0,963
MF0085	pyruvate:formate lyase	central metabolism	MedD	0,044	0,963
MF0005	acetylneuraminate and acetylmannosamine degradation	amines and polyamines degradation	MedD	0,049	0,963

4w

GMM	Module definition	HIER1	Enriched in	pvaluew	qvaluew
MF0112	acetate to acetyl-CoA	organic acid metabolism	ConD	0,0017	0,0182
MF0060	ribose degradation	carbohydrate degradation	ConD	0,0046	0,0382
MF0054	arabinose degradation	carbohydrate degradation	ConD	0,0067	0,0518
MF0033	cysteine degradation (mercaptopyruvate pathway)	amino acid degradation	ConD	0,0076	0,0559
MF0102	mucin degradation	glycoprotein degradation	ConD	0,0205	0,1290
MF0040	lysine degradation (cadaverine pathway)	amino acid degradation	MedD	0,0002	0,0125
MF0056	galactose degradation (Leloir pathway)	carbohydrate degradation	MedD	0,0003	0,0125
MF0114	acetyl-CoA to crotonyl-CoA	organic acid metabolism	MedD	0,0003	0,0125
MF0024	methionine degradation (mercaptan pathway)	amino acid degradation	MedD	0,0005	0,0125
MF0101	Sulfate reduction (assimilatory)	gas metabolism	MedD	0,0005	0,0125
MF0057	alpha-D-glucose and alpha-D-glucose 1-phosphate degradation	carbohydrate degradation	MedD	0,0006	0,0125
MF0058	fructose degradation	carbohydrate degradation	MedD	0,0007	0,0125
MF0001	ethanol production (formate pathway)	alcohol metabolism	MedD	0,0009	0,0145
MF0091	beta-D-glucuronide and D-glucuronate degradation	glycoprotein degradation	MedD	0,0011	0,0145
MF0043	arginine degradation (agmatinase pathway)	amino acid degradation	MedD	0,0011	0,0145
MF0085	pyruvate:formate lyase	central metabolism	MedD	0,0012	0,0145
MF0027	cysteine degradation	amino acid degradation	MedD	0,0020	0,0206
MF0111	triacylglycerol degradation	lipid degradation	MedD	0,0033	0,0314
MF0005	acetylneuraminate and acetylmannosamine degradation	amines and polyamines degradation	MedD	0,0040	0,0354
MF0013	glutamate degradation (crotonyl-CoA pathway)	amino acid degradation	MedD	0,0156	0,1082
MF0051	sucrose degradation (Actinobacteria)	carbohydrate degradation	MedD	0,0183	0,1205
MF0070	galactonate degradation	carbohydrate degradation	MedD	0,0322	0,1901
MF0030	threonine degradation (formate pathway)	amino acid degradation	MedD	0,0333	0,1901
MF0029	threonine degradation (glycine pathway)	amino acid degradation	MedD	0,0346	0,1901

8w

GMM	Module definition	HIER1	Enriched in	pvaluew	qvaluew
MF0019	proline degradation (aminopentanoate pathway)	amino acid degradation	ConD	0,008	0,584
MF0004	putrescine degradation	amines and polyamines degradation	ConD	0,024	0,584
MF0106	anaerobic fatty acid beta-oxidation	lipid degradation	ConD	0,026	0,584
MF0083	pyruvate dehydrogenase complex	central metabolism	ConD	0,027	0,584
MF0047	lactose and galactose degradation (PTS)	carbohydrate degradation	ConD	0,036	0,584
MF0043	arginine degradation (agmatinase pathway)	amino acid degradation	MedD	0,021	0,584
MF0113	acetyl-CoA to acetate	organic acid metabolism	MedD	0,023	0,584
MF0013	glutamate degradation (crotonyl-CoA pathway)	amino acid degradation	MedD	0,038	0,584
MF0005	acetylneuraminate and acetylmannosamine degradation	amines and polyamines degradation	MedD	0,040	0,584

Supplementary Table 8 Spearman's correlations between targeted urinary urolithins and microbiota.

	Urolithin-A-glucuronide	Urolithin-B-glucuronide	Urolithin-C-glucuronide	Total Urolithins (A+B+C)	Family level	Taxonomy
Bifidobacterium adolescentis_msp_0263	-0.18	-0.09	-0.16	-0.18	<i>Bifidobacteriaceae</i>	Actinobacteria; Actinobacteria; Bifidobacteriales; Bifidobacteriaceae; Bifidobacterium; Bifidobacterium adolescentis
Bifidobacterium bifidum_msp_0419	-0.12	-0.2	-0.08	-0.13	<i>Bifidobacteriaceae</i>	Actinobacteria; Actinobacteria; Bifidobacteriales; Bifidobacteriaceae; Bifidobacterium; Bifidobacterium bifidum
Collinsella aerofaciens_msp_1244	-0.15	-0.06	-0.04	-0.18	<i>Coriobacteriaceae</i>	Actinobacteria; Coriobacteriia; Coriobacteriales; Coriobacteriaceae; Collinsella; Collinsella aerofaciens
unclassified Adlercreutzia_msp_0396	0.09	0.01	0.16	0.12	<i>Eggerthellaceae</i>	Actinobacteria; Coriobacteriia; Eggerthellales; Eggerthellaceae; Adlercreutzia; unclassified Adlercreutzia
Eggerthella lenta_msp_0573	0.03	-0.15	0.08	0.03	<i>Eggerthellaceae</i>	Actinobacteria; Coriobacteriia; Eggerthellales; Eggerthellaceae; Eggerthella; Eggerthella lenta
Gordonibacter urolithinifaciens_msp_1339	0.19	-0.05	0.18	0.19	<i>Eggerthellaceae</i>	Actinobacteria; Coriobacteriia; Eggerthellales; Eggerthellaceae; Gordonibacter; Gordonibacter urolithinifaciens
Bacteroides cellulosilyticus_msp_0003	0.12	0.11	0.15	0.15	<i>Bacteroidaceae</i>	Bacteroidetes; Bacteroidia; Bacteroidales; Bacteroidaceae; Bacteroides; Bacteroides cellulosilyticus
Bacteroides dorei_msp_0035	0.12	0.23	0.14	0.19	<i>Bacteroidaceae</i>	Bacteroidetes; Bacteroidia; Bacteroidales; Bacteroidaceae; Bacteroides; Bacteroides dorei
Bacteroides faecis_msp_0019	0.09	-0.11	0.15	0.07	<i>Bacteroidaceae</i>	Bacteroidetes; Bacteroidia; Bacteroidales; Bacteroidaceae; Bacteroides; Bacteroides faecis
Bacteroides salyersiae_msp_0037	0.17	0.11	0.05	0.17	<i>Bacteroidaceae</i>	Bacteroidetes; Bacteroidia; Bacteroidales; Bacteroidaceae; Bacteroides; Bacteroides salyersiae
Bacteroides sp. CAG:144_msp_0412	0.2	0.26	0.15	0.24	<i>Bacteroidaceae</i>	Bacteroidetes; Bacteroidia; Bacteroidales; Bacteroidaceae; Bacteroides; Bacteroides sp. CAG:144
Coproacter secundus == Gabonia massiliensis_msp_0198	0.2	0.13	0.14	0.2	<i>Barnesiellaceae</i>	Bacteroidetes; Bacteroidia; Bacteroidales; Barnesiellaceae; Coproacter; Coproacter secundus == Gabonia massiliensis
Alistipes inops == Tidjanibacter massiliensis_msp_0230	0.05	-0.17	0	-0.02	<i>Rikenellaceae</i>	Bacteroidetes; Bacteroidia; Bacteroidales; Rikenellaceae; Alistipes; Alistipes inops == Tidjanibacter massiliensis
Alistipes obesi_msp_0225	0.1	0.23	0.08	0.16	<i>Rikenellaceae</i>	Bacteroidetes; Bacteroidia; Bacteroidales; Rikenellaceae; Alistipes; Alistipes obesi
Alistipes senegalensis_msp_0381	0.11	0.16	0.02	0.11	<i>Rikenellaceae</i>	Bacteroidetes; Bacteroidia; Bacteroidales; Rikenellaceae; Alistipes; Alistipes senegalensis
Parabacteroides goldsteinii_msp_0028	0.2	0.06	0.14	0.17	<i>Tannerellaceae</i>	Bacteroidetes; Bacteroidia; Bacteroidales; Tannerellaceae; Parabacteroides; Parabacteroides goldsteinii
Parabacteroides merdae_msp_0027	-0.02	-0.15	-0.05	-0.09	<i>Tannerellaceae</i>	Bacteroidetes; Bacteroidia; Bacteroidales; Tannerellaceae; Parabacteroides; Parabacteroides merdae
Streptococcus thermophilus_msp_0833	-0.16	-0.06	-0.23	-0.16	<i>Streptococcaceae</i>	Firmicutes; Bacilli; Lactobacillales; Streptococcaceae; Streptococcus; Streptococcus thermophilus
Butyricicoccus sp. 2789STDY5834927 / Clostridia bacterium UC5.1-1D1_msp_1381	-0.06	-0.17	-0.03	-0.05	<i>Clostridiaceae</i>	Firmicutes; Clostridia; Clostridiales; Clostridiaceae; Butyricicoccus; Butyricicoccus sp. 2789STDY5834927 / Clostridia bacterium UC5.1-1D1
Clostridium saudense_msp_0362	-0.15	-0.17	-0.07	-0.17	<i>Clostridiaceae</i>	Firmicutes; Clostridia; Clostridiales; Clostridiaceae; Clostridium; Clostridium saudense
Clostridium sp. 2789STDY5608831_msp_1608c	-0.11	0.16	-0.03	-0.02	<i>Clostridiaceae</i>	Firmicutes; Clostridia; Clostridiales; Clostridiaceae; Clostridium; Clostridium sp. 2789STDY5608831
Clostridium sp. AT4_msp_0066	-0.14	-0.14	-0.14	-0.17	<i>Clostridiaceae</i>	Firmicutes; Clostridia; Clostridiales; Clostridiaceae; Clostridium; Clostridium sp. AT4
unclassified Clostridium_msp_0563	0.19	-0.02	0.12	0.13	<i>Clostridiaceae</i>	Firmicutes; Clostridia; Clostridiales; Clostridiaceae; Clostridium; unclassified Clostridium
Eubacterium eligens_msp_0029	0.18	0.2	0.25	0.26	<i>Eubacteriaceae</i>	Firmicutes; Clostridia; Clostridiales; Eubacteriaceae; Eubacterium; Eubacterium eligens

Eubacterium sp. 36_13 & CAG:86_msp_0227	0.19	0.14	0.24	0.24	<i>Eubacteriaceae</i>	Firmicutes; Clostridia; Clostridiales; Eubacteriaceae; Eubacterium; Eubacterium sp. 36_13 & CAG:86
Eubacterium sp. CAG:115_msp_0047	0.21	0.06	0.16	0.22	<i>Eubacteriaceae</i>	Firmicutes; Clostridia; Clostridiales; Eubacteriaceae; Eubacterium; Eubacterium sp. CAG:115
Eubacterium sp. CAG:180_msp_0271	-0.19	-0.05	-0.13	-0.18	<i>Eubacteriaceae</i>	Firmicutes; Clostridia; Clostridiales; Eubacteriaceae; Eubacterium; Eubacterium sp. CAG:180
Eubacterium sp. CAG:248_msp_0161	-0.02	-0.01	-0.15	-0.05	<i>Eubacteriaceae</i>	Firmicutes; Clostridia; Clostridiales; Eubacteriaceae; Eubacterium; Eubacterium sp. CAG:248
Blautia massiliensis_msp_0141	-0.18	-0.2	-0.13	-0.21	<i>Lachnospiraceae</i>	Firmicutes; Clostridia; Clostridiales; Lachnospiraceae; Blautia; Blautia massiliensis
Blautia obeum_msp_0436	0.02	0.15	0.04	0.06	<i>Lachnospiraceae</i>	Firmicutes; Clostridia; Clostridiales; Lachnospiraceae; Blautia; Blautia obeum
Blautia sp. CAG:237_msp_0189	0.21	-0.03	0.19	0.19	<i>Lachnospiraceae</i>	Firmicutes; Clostridia; Clostridiales; Lachnospiraceae; Blautia; Blautia sp. CAG:237
Blautia sp. CAG:257_msp_0052	-0.19	-0.15	-0.15	-0.24	<i>Lachnospiraceae</i>	Firmicutes; Clostridia; Clostridiales; Lachnospiraceae; Blautia; Blautia sp. CAG:257
Blautia sp. Marseille-P3087 / Ruminococcus sp. 2789STDY560882_msp_0722	-0.16	-0.08	-0.01	-0.14	<i>Lachnospiraceae</i>	Firmicutes; Clostridia; Clostridiales; Lachnospiraceae; Blautia; Blautia sp. Marseille-P3087 / Ruminococcus sp. 2789STDY560882
Blautia wexlerae_msp_0076	-0.17	-0.25	-0.1	-0.21	<i>Lachnospiraceae</i>	Firmicutes; Clostridia; Clostridiales; Lachnospiraceae; Blautia; Blautia wexlerae
Ruminococcus gnavus_msp_0058	-0.2	-0.21	-0.24	-0.26	<i>Lachnospiraceae</i>	Firmicutes; Clostridia; Clostridiales; Lachnospiraceae; Blautia; Ruminococcus gnavus
Ruminococcus sp. CAG:60 / Blautia sp. 2789STDY5608836_msp_0244	0.23	0.09	0.15	0.19	<i>Lachnospiraceae</i>	Firmicutes; Clostridia; Clostridiales; Lachnospiraceae; Blautia; Ruminococcus sp. CAG:60 / Blautia sp. 2789STDY5608836
Ruminococcus torques_msp_0126	-0.13	-0.17	-0.15	-0.15	<i>Lachnospiraceae</i>	Firmicutes; Clostridia; Clostridiales; Lachnospiraceae; Blautia; Ruminococcus torques
unclassified Blautia_msp_0756	0.14	0.23	0.15	0.16	<i>Lachnospiraceae</i>	Firmicutes; Clostridia; Clostridiales; Lachnospiraceae; Blautia; unclassified Blautia
unclassified Blautia_msp_1385	0	0.15	-0.04	0.04	<i>Lachnospiraceae</i>	Firmicutes; Clostridia; Clostridiales; Lachnospiraceae; Blautia; unclassified Blautia
Coprococcus catus_msp_0986	-0.01	0.01	0.19	0	<i>Lachnospiraceae</i>	Firmicutes; Clostridia; Clostridiales; Lachnospiraceae; Coprococcus; Coprococcus catus
Coprococcus comes_msp_0259	-0.2	-0.03	-0.12	-0.15	<i>Lachnospiraceae</i>	Firmicutes; Clostridia; Clostridiales; Lachnospiraceae; Coprococcus; Coprococcus comes
Coprococcus eutactus 2_msp_0042	0.13	0.16	0.03	0.14	<i>Lachnospiraceae</i>	Firmicutes; Clostridia; Clostridiales; Lachnospiraceae; Coprococcus; Coprococcus eutactus 2
Dorea formicigenerans_msp_0506	-0.22	-0.15	-0.04	-0.22	<i>Lachnospiraceae</i>	Firmicutes; Clostridia; Clostridiales; Lachnospiraceae; Dorea; Dorea formicigenerans
Dorea longicatena 1_msp_0307	-0.24	-0.03	-0.06	-0.19	<i>Lachnospiraceae</i>	Firmicutes; Clostridia; Clostridiales; Lachnospiraceae; Dorea; Dorea longicatena 1
Eisenbergiella tayi_msp_0014	-0.2	-0.09	0	-0.16	<i>Lachnospiraceae</i>	Firmicutes; Clostridia; Clostridiales; Lachnospiraceae; Eisenbergiella; Eisenbergiella tayi
Clostridium asparagiforme == lavalense_msp_0360	0.09	-0.24	0.04	0.04	<i>Lachnospiraceae</i>	Firmicutes; Clostridia; Clostridiales; Lachnospiraceae; Lachnoclostridium; Clostridium asparagiforme == lavalense
Clostridium bolteae_msp_0009	-0.04	-0.26	-0.02	-0.07	<i>Lachnospiraceae</i>	Firmicutes; Clostridia; Clostridiales; Lachnospiraceae; Lachnoclostridium; Clostridium bolteae
Clostridium clostridioforme 1_msp_0020	-0.09	-0.09	-0.15	-0.08	<i>Lachnospiraceae</i>	Firmicutes; Clostridia; Clostridiales; Lachnospiraceae; Lachnoclostridium; Clostridium clostridioforme 1
Clostridium glycyrrhizinilyticum_msp_0510	-0.16	-0.14	-0.12	-0.2	<i>Lachnospiraceae</i>	Firmicutes; Clostridia; Clostridiales; Lachnospiraceae; Lachnoclostridium; Clostridium glycyrrhizinilyticum
Clostridium symbiosum_msp_0086	-0.01	-0.17	-0.08	-0.05	<i>Lachnospiraceae</i>	Firmicutes; Clostridia; Clostridiales; Lachnospiraceae; Lachnoclostridium; Clostridium symbiosum
unclassified Lachnoclostridium_msp_0049	0.22	-0.06	0.03	0.18	<i>Lachnospiraceae</i>	Firmicutes; Clostridia; Clostridiales; Lachnospiraceae; Lachnoclostridium; unclassified Lachnoclostridium

Lachnospira sp. 2789STDY5834967 / Clostridiales bacterium KLE1615 & 41_12_two_minus_msp_0572	0.15	0.09	0.23	0.19	<i>Lachnospiraceae</i>	Firmicutes; Clostridia; Clostridiales; Lachnospiraceae; Lachnospira; Lachnospira sp. 2789STDY5834967 / Clostridiales bacterium KLE1615 & 41_12_two_minus
Roseburia faecis_msp_0015	0.15	-0.03	0.11	0.17	<i>Lachnospiraceae</i>	Firmicutes; Clostridia; Clostridiales; Lachnospiraceae; Roseburia; Roseburia faecis
Roseburia hominis_msp_0071	0.19	0.12	0.27	0.21	<i>Lachnospiraceae</i>	Firmicutes; Clostridia; Clostridiales; Lachnospiraceae; Roseburia; Roseburia hominis
Roseburia intestinalis_msp_0017	0.19	-0.06	0.13	0.19	<i>Lachnospiraceae</i>	Firmicutes; Clostridia; Clostridiales; Lachnospiraceae; Roseburia; Roseburia intestinalis
Roseburia sp. CAG:45 & sp. 2789STDY5608886_msp_0057	0.24	-0.02	0.16	0.23	<i>Lachnospiraceae</i>	Firmicutes; Clostridia; Clostridiales; Lachnospiraceae; Roseburia; Roseburia sp. CAG:45 & sp. 2789STDY5608886
Coprococcus sp. 2789STDY5608819 / Clostridium sp. CAG:264_msp_0133	0.11	0.16	0.05	0.14	<i>Lachnospiraceae</i>	Firmicutes; Clostridia; Clostridiales; Lachnospiraceae; unclassified Lachnospiraceae; Coprococcus sp. 2789STDY5608819 / Clostridium sp. CAG:264
Lachnospiraceae bacterium TF01-11 / Clostridium sp. CAG:122_msp_0175	0.14	0.16	0.14	0.17	<i>Lachnospiraceae</i>	Firmicutes; Clostridia; Clostridiales; Lachnospiraceae; unclassified Lachnospiraceae; Lachnospiraceae bacterium TF01-11 / Clostridium sp. CAG:122
unclassified Lachnospiraceae_msp_0254	-0.17	-0.14	-0.14	-0.21	<i>Lachnospiraceae</i>	Firmicutes; Clostridia; Clostridiales; Lachnospiraceae; unclassified Lachnospiraceae; unclassified Lachnospiraceae
unclassified Lachnospiraceae_msp_0296	0.32	0.2	0.26	0.35	<i>Lachnospiraceae</i>	Firmicutes; Clostridia; Clostridiales; Lachnospiraceae; unclassified Lachnospiraceae; unclassified Lachnospiraceae
unclassified Lachnospiraceae_msp_0780	0.1	0.14	0.16	0.12	<i>Lachnospiraceae</i>	Firmicutes; Clostridia; Clostridiales; Lachnospiraceae; unclassified Lachnospiraceae; unclassified Lachnospiraceae
unclassified Lachnospiraceae_msp_0930	0.13	0.02	0.2	0.11	<i>Lachnospiraceae</i>	Firmicutes; Clostridia; Clostridiales; Lachnospiraceae; unclassified Lachnospiraceae; unclassified Lachnospiraceae
unclassified Lachnospiraceae_msp_1641	0.18	0.03	0.12	0.15	<i>Lachnospiraceae</i>	Firmicutes; Clostridia; Clostridiales; Lachnospiraceae; unclassified Lachnospiraceae; unclassified Lachnospiraceae
Oscillibacter sp. 57_20_msp_0903	0.07	0.19	0.21	0.12	<i>Oscillospiraceae</i>	Firmicutes; Clostridia; Clostridiales; Oscillospiraceae; Oscillibacter; Oscillibacter sp. 57_20
Oscillibacter sp. ER4 / Firmicutes bacterium CAG:129_59_24_msp_0763	0.07	0.17	0.16	0.08	<i>Oscillospiraceae</i>	Firmicutes; Clostridia; Clostridiales; Oscillospiraceae; Oscillibacter; Oscillibacter sp. ER4 / Firmicutes bacterium CAG:129_59_24
unclassified Oscillibacter_msp_1062	-0.02	0.31	-0.02	0.02	<i>Oscillospiraceae</i>	Firmicutes; Clostridia; Clostridiales; Oscillospiraceae; Oscillibacter; unclassified Oscillibacter
unclassified Oscillibacter_msp_1143	0.05	0.18	0.05	0.11	<i>Oscillospiraceae</i>	Firmicutes; Clostridia; Clostridiales; Oscillospiraceae; Oscillibacter; unclassified Oscillibacter
Firmicutes bacterium CAG:129_msp_0777	0.18	0.09	0.14	0.18	<i>Oscillospiraceae</i>	Firmicutes; Clostridia; Clostridiales; Oscillospiraceae; unclassified Oscillospiraceae; Firmicutes bacterium CAG:129
Intestinibacter bartlettii_msp_0621	-0.24	-0.04	-0.1	-0.23	<i>Peptostreptococcaceae</i>	Firmicutes; Clostridia; Clostridiales; Peptostreptococcaceae; Intestinibacter; Intestinibacter bartlettii
Faecalibacterium prausnitzii 3 (L2-6)_msp_0388	0.1	0.08	0.21	0.15	<i>Ruminococcaceae</i>	Firmicutes; Clostridia; Clostridiales; Ruminococcaceae; Faecalibacterium; Faecalibacterium prausnitzii 3 (L2-6)
Faecalibacterium prausnitzii 4 (cf. KLE1255)_msp_0389	0.02	-0.16	-0.05	-0.01	<i>Ruminococcaceae</i>	Firmicutes; Clostridia; Clostridiales; Ruminococcaceae; Faecalibacterium; Faecalibacterium prausnitzii 4 (cf. KLE1255)
Faecalibacterium prausnitzii 5_msp_0399	0.09	0.34	-0.06	0.11	<i>Ruminococcaceae</i>	Firmicutes; Clostridia; Clostridiales; Ruminococcaceae; Faecalibacterium; Faecalibacterium prausnitzii 5
Faecalibacterium prausnitzii 6_msp_0639	0.18	0.1	0.09	0.15	<i>Ruminococcaceae</i>	Firmicutes; Clostridia; Clostridiales; Ruminococcaceae; Faecalibacterium; Faecalibacterium prausnitzii 6
Faecalibacterium prausnitzii 7_msp_0324	-0.14	-0.1	0	-0.15	<i>Ruminococcaceae</i>	Firmicutes; Clostridia; Clostridiales; Ruminococcaceae; Faecalibacterium; Faecalibacterium prausnitzii 7
Faecalibacterium sp. CAG:74_msp_0034	0.28	0.21	0.18	0.29	<i>Ruminococcaceae</i>	Firmicutes; Clostridia; Clostridiales; Ruminococcaceae; Faecalibacterium; Faecalibacterium sp. CAG:74
Faecalibacterium sp. CAG:82_msp_0457	0.09	0.08	0.2	0.09	<i>Ruminococcaceae</i>	Firmicutes; Clostridia; Clostridiales; Ruminococcaceae; Faecalibacterium; Faecalibacterium sp. CAG:82
unclassified Faecalibacterium_msp_0265	0.13	0.18	-0.03	0.14	<i>Ruminococcaceae</i>	Firmicutes; Clostridia; Clostridiales; Ruminococcaceae; Faecalibacterium; unclassified Faecalibacterium

Gemmiger formicilis_msp_0374	-0.07	-0.19	-0.09	-0.12	<i>Ruminococcaceae</i>	Firmicutes; Clostridia; Clostridiales; Ruminococcaceae; Gemmiger; Gemmiger formicilis
unclassified Gemmiger_msp_0456	0.16	0.01	0.1	0.15	<i>Ruminococcaceae</i>	Firmicutes; Clostridia; Clostridiales; Ruminococcaceae; Gemmiger; unclassified Gemmiger
Phoceia massiliensis_msp_1724	-0.16	-0.13	-0.12	-0.19	<i>Ruminococcaceae</i>	Firmicutes; Clostridia; Clostridiales; Ruminococcaceae; Phoceia; Phoceia massiliensis
Eubacterium siraeum_msp_0053	0.06	0.07	0.14	0.15	<i>Ruminococcaceae</i>	Firmicutes; Clostridia; Clostridiales; Ruminococcaceae; Ruminiclostridium; Eubacterium siraeum
Ruminococcus bicirculans_msp_0013	0.2	0.1	0.12	0.2	<i>Ruminococcaceae</i>	Firmicutes; Clostridia; Clostridiales; Ruminococcaceae; Ruminococcus; Ruminococcus bicirculans
Ruminococcus bromii_2_msp_0045	0.09	-0.19	0.04	0.03	<i>Ruminococcaceae</i>	Firmicutes; Clostridia; Clostridiales; Ruminococcaceae; Ruminococcus; Ruminococcus bromii_2
Ruminococcus faecis_msp_0152	-0.15	-0.02	-0.06	-0.13	<i>Ruminococcaceae</i>	Firmicutes; Clostridia; Clostridiales; Ruminococcaceae; Ruminococcus; Ruminococcus faecis
Ruminococcus lactaris_msp_0250	0.06	0.25	0.03	0.1	<i>Ruminococcaceae</i>	Firmicutes; Clostridia; Clostridiales; Ruminococcaceae; Ruminococcus; Ruminococcus lactaris
Ruminococcus sp. 2789STDY5608794 & sp. 2789STDY5834890 / Firmicutes bacterium CAG:56_msp_0164	0.1	0.2	0.17	0.16	<i>Ruminococcaceae</i>	Firmicutes; Clostridia; Clostridiales; Ruminococcaceae; Ruminococcus; Ruminococcus sp. 2789STDY5608794 & sp. 2789STDY5834890 / Firmicutes bacterium CAG:56
Ruminococcus sp. JC304_msp_1643c	0.15	0.15	0.19	0.16	<i>Ruminococcaceae</i>	Firmicutes; Clostridia; Clostridiales; Ruminococcaceae; Ruminococcus; Ruminococcus sp. JC304
unclassified Ruminococcaceae_msp_0124	0.09	-0.04	0.19	0.08	<i>Ruminococcaceae</i>	Firmicutes; Clostridia; Clostridiales; Ruminococcaceae; unclassified Ruminococcaceae; unclassified Ruminococcaceae
Flavonifractor plautii_msp_0213	-0.2	-0.15	-0.18	-0.22	<i>unclassified Clostridiales</i>	Firmicutes; Clostridia; Clostridiales; unclassified Clostridiales; Flavonifractor; Flavonifractor plautii
unclassified Flavonifractor_msp_1323	0	0.15	0.01	0.05	<i>unclassified Clostridiales</i>	Firmicutes; Clostridia; Clostridiales; unclassified Clostridiales; Flavonifractor; unclassified Flavonifractor
Clostridium sp. 2789STDY5608793_msp_1622	0.09	0.3	-0.02	0.09	<i>unclassified Clostridiales</i>	Firmicutes; Clostridia; Clostridiales; unclassified Clostridiales; unclassified Clostridiales; Clostridium sp. 2789STDY5608793
Clostridium sp. 2789STDY5608884_msp_0373	0.11	0.22	0.03	0.12	<i>unclassified Clostridiales</i>	Firmicutes; Clostridia; Clostridiales; unclassified Clostridiales; unclassified Clostridiales; Clostridium sp. 2789STDY5608884
Clostridium sp. 2789STDY5834874 & sp. 2789STDY5608885_msp_0473c	0.09	0.06	0.14	0.12	<i>unclassified Clostridiales</i>	Firmicutes; Clostridia; Clostridiales; unclassified Clostridiales; unclassified Clostridiales; Clostridium sp. 2789STDY5834874 & sp. 2789STDY5608885
Clostridium sp. 2789STDY5834924_msp_0906	0.23	0.18	0.33	0.27	<i>unclassified Clostridiales</i>	Firmicutes; Clostridia; Clostridiales; unclassified Clostridiales; unclassified Clostridiales; Clostridium sp. 2789STDY5834924
Clostridium sp. 42_12 & CAG:75_msp_0194	0.14	0.01	0.18	0.14	<i>unclassified Clostridiales</i>	Firmicutes; Clostridia; Clostridiales; unclassified Clostridiales; unclassified Clostridiales; Clostridium sp. 42_12 & CAG:75
Clostridium sp. CAG:138_msp_0144	0.22	0.01	0.14	0.17	<i>unclassified Clostridiales</i>	Firmicutes; Clostridia; Clostridiales; unclassified Clostridiales; unclassified Clostridiales; Clostridium sp. CAG:138
Clostridium sp. CAG:169_msp_0357	-0.18	-0.17	-0.01	-0.17	<i>unclassified Clostridiales</i>	Firmicutes; Clostridia; Clostridiales; unclassified Clostridiales; unclassified Clostridiales; Clostridium sp. CAG:169
Clostridium sp. CAG:217_msp_0285	0.11	0.02	0.27	0.16	<i>unclassified Clostridiales</i>	Firmicutes; Clostridia; Clostridiales; unclassified Clostridiales; unclassified Clostridiales; Clostridium sp. CAG:217
Clostridium sp. CAG:245_msp_0546	0.05	0.23	0.14	0.18	<i>unclassified Clostridiales</i>	Firmicutes; Clostridia; Clostridiales; unclassified Clostridiales; unclassified Clostridiales; Clostridium sp. CAG:245
Clostridium sp. CAG:343_msp_0586	0.21	0.02	0.17	0.25	<i>unclassified Clostridiales</i>	Firmicutes; Clostridia; Clostridiales; unclassified Clostridiales; unclassified Clostridiales; Clostridium sp. CAG:343
Clostridium sp. CAG:58_msp_0129	-0.15	-0.23	-0.06	-0.18	<i>unclassified Clostridiales</i>	Firmicutes; Clostridia; Clostridiales; unclassified Clostridiales; unclassified Clostridiales; Clostridium sp. CAG:58
Clostridium sp. CAG:62_msp_0093	0.28	0.22	0.34	0.33	<i>unclassified Clostridiales</i>	Firmicutes; Clostridia; Clostridiales; unclassified Clostridiales; unclassified Clostridiales; Clostridium sp. CAG:62
Clostridium sp. CAG:91 & sp. 2789STDY5834873_msp_0075	0.21	0.06	0.17	0.23	<i>unclassified Clostridiales</i>	Firmicutes; Clostridia; Clostridiales; unclassified Clostridiales; unclassified Clostridiales; Clostridium sp. CAG:91 & sp. 2789STDY5834873
Clostridium sp. KLE 1755 & Clostridiales bacterium VE202-27_msp_0018	0.12	-0.05	0.18	0.13	<i>unclassified Clostridiales</i>	Firmicutes; Clostridia; Clostridiales; unclassified Clostridiales; unclassified Clostridiales; Clostridium sp. KLE 1755 & Clostridiales bacterium VE202-27

unclassified Clostridiales_msp_0056	-0.13	-0.16	-0.16	-0.15	<i>unclassified Clostridiales</i>	Firmicutes; Clostridia; Clostridiales; unclassified Clostridiales; unclassified Clostridiales; unclassified Clostridiales
unclassified Clostridiales_msp_0317	-0.15	-0.07	-0.07	-0.13	<i>unclassified Clostridiales</i>	Firmicutes; Clostridia; Clostridiales; unclassified Clostridiales; unclassified Clostridiales; unclassified Clostridiales
unclassified Clostridiales_msp_0424	0.24	0.14	0.24	0.23	<i>unclassified Clostridiales</i>	Firmicutes; Clostridia; Clostridiales; unclassified Clostridiales; unclassified Clostridiales; unclassified Clostridiales
unclassified Clostridiales_msp_0480	0.19	0.08	0.16	0.18	<i>unclassified Clostridiales</i>	Firmicutes; Clostridia; Clostridiales; unclassified Clostridiales; unclassified Clostridiales; unclassified Clostridiales
unclassified Clostridiales_msp_0591	-0.15	-0.13	-0.16	-0.17	<i>unclassified Clostridiales</i>	Firmicutes; Clostridia; Clostridiales; unclassified Clostridiales; unclassified Clostridiales; unclassified Clostridiales
unclassified Clostridiales_msp_0665	0.11	0.21	0.17	0.18	<i>unclassified Clostridiales</i>	Firmicutes; Clostridia; Clostridiales; unclassified Clostridiales; unclassified Clostridiales; unclassified Clostridiales
unclassified Clostridiales_msp_0761	0.22	0.18	0.06	0.19	<i>unclassified Clostridiales</i>	Firmicutes; Clostridia; Clostridiales; unclassified Clostridiales; unclassified Clostridiales; unclassified Clostridiales
unclassified Clostridiales_msp_0820	0.14	0.16	0.22	0.18	<i>unclassified Clostridiales</i>	Firmicutes; Clostridia; Clostridiales; unclassified Clostridiales; unclassified Clostridiales; unclassified Clostridiales
unclassified Clostridiales_msp_0931	0.11	0.18	-0.1	0.08	<i>unclassified Clostridiales</i>	Firmicutes; Clostridia; Clostridiales; unclassified Clostridiales; unclassified Clostridiales; unclassified Clostridiales
unclassified Clostridiales_msp_1428	0.18	0.17	0.13	0.21	<i>unclassified Clostridiales</i>	Firmicutes; Clostridia; Clostridiales; unclassified Clostridiales; unclassified Clostridiales; unclassified Clostridiales
unclassified Clostridiales_msp_1533	0.06	0.21	-0.03	0.05	<i>unclassified Clostridiales</i>	Firmicutes; Clostridia; Clostridiales; unclassified Clostridiales; unclassified Clostridiales; unclassified Clostridiales
Clostridia bacterium UC5.1-2E3_msp_0647	0.15	-0.06	0.2	0.18	<i>unclassified Clostridia</i>	Firmicutes; Clostridia; unclassified Clostridia; unclassified Clostridia; unclassified Clostridia; Clostridia bacterium UC5.1-2E3
Clostridium innocuum_msp_0103	-0.06	-0.16	-0.21	-0.11	<i>Erysipelotrichaceae</i>	Firmicutes; Erysipelotrichia; Erysipelotrichales; Erysipelotrichaceae; Erysipelatoclostridium; Clostridium innocuum
Erysipelatoclostridium ramosum_msp_0153	0.02	-0.17	-0.06	-0.05	<i>Erysipelotrichaceae</i>	Firmicutes; Erysipelotrichia; Erysipelotrichales; Erysipelotrichaceae; Erysipelatoclostridium; Erysipelatoclostridium ramosum
Turicibacter sanguinis 2_msp_1308	-0.13	-0.04	-0.12	-0.18	<i>Erysipelotrichaceae</i>	Firmicutes; Erysipelotrichia; Erysipelotrichales; Erysipelotrichaceae; Turicibacter; Turicibacter sanguinis 2
Phascolarctobacterium sp. CAG:207_msp_0131	0.12	-0.16	0.02	0.04	<i>Acidaminococcaceae</i>	Firmicutes; Negativicutes; Acidaminococcales; Acidaminococcaceae; Phascolarctobacterium; Phascolarctobacterium sp. CAG:207
Dialister invisus_msp_0212	-0.06	-0.15	-0.03	-0.08	<i>Veillonellaceae</i>	Firmicutes; Negativicutes; Veillonellales; Veillonellaceae; Dialister; Dialister invisus
Dialister succinatiphilus_msp_0383	0.07	0.18	-0.09	0.05	<i>Veillonellaceae</i>	Firmicutes; Negativicutes; Veillonellales; Veillonellaceae; Dialister; Dialister succinatiphilus
Veillonella atypica_msp_0884	0.15	-0.06	0.06	0.13	<i>Veillonellaceae</i>	Firmicutes; Negativicutes; Veillonellales; Veillonellaceae; Veillonella; Veillonella atypica
Veillonella rogosae_msp_1219	0.18	0.01	0.17	0.13	<i>Veillonellaceae</i>	Firmicutes; Negativicutes; Veillonellales; Veillonellaceae; Veillonella; Veillonella rogosae
Firmicutes bacterium CAG:103_msp_0654	-0.02	0.03	0.15	-0.02	<i>unclassified Firmicutes</i>	Firmicutes; unclassified Firmicutes; unclassified Firmicutes; unclassified Firmicutes; unclassified Firmicutes; Firmicutes bacterium CAG:103
Firmicutes bacterium CAG:124_msp_0205	0.12	0.1	0.22	0.13	<i>unclassified Firmicutes</i>	Firmicutes; unclassified Firmicutes; unclassified Firmicutes; unclassified Firmicutes; unclassified Firmicutes; unclassified Firmicutes; Firmicutes bacterium CAG:124
Firmicutes bacterium CAG:212 / Clostridium sp. 2789STDY5834871_msp_0581	-0.16	0.01	0.02	-0.09	<i>unclassified Firmicutes</i>	Firmicutes; unclassified Firmicutes; unclassified Firmicutes; unclassified Firmicutes; unclassified Firmicutes; unclassified Firmicutes; Firmicutes bacterium CAG:212 / Clostridium sp. 2789STDY5834871
Firmicutes bacterium CAG:41 / Clostridium sp. 2789STDY5834935 & sp. 2789STDY5608853_msp_0468	0.17	0.04	0.23	0.2	<i>unclassified Firmicutes</i>	Firmicutes; unclassified Firmicutes; unclassified Firmicutes; unclassified Firmicutes; unclassified Firmicutes; Firmicutes bacterium CAG:41 / Clostridium sp. 2789STDY5834935 & sp. 2789STDY5608853
Firmicutes bacterium CAG:95_msp_0060	0.23	0.19	0.05	0.21	<i>unclassified Firmicutes</i>	Firmicutes; unclassified Firmicutes; unclassified Firmicutes; unclassified Firmicutes; unclassified Firmicutes; Firmicutes bacterium CAG:95
unclassified Firmicutes_msp_0404	0.16	0.16	0.05	0.16	<i>unclassified Firmicutes</i>	Firmicutes; unclassified Firmicutes; unclassified Firmicutes; unclassified Firmicutes; unclassified Firmicutes; unclassified Firmicutes

unclassified Firmicutes_msp_0425	0.09	-0.16	0.07	0.04	<i>unclassified Firmicutes</i>	Firmicutes; unclassified Firmicutes; unclassified Firmicutes; unclassified Firmicutes; unclassified Firmicutes; unclassified Firmicutes
unclassified Firmicutes_msp_0596	0.16	0.13	0.12	0.16	<i>unclassified Firmicutes</i>	Firmicutes; unclassified Firmicutes; unclassified Firmicutes; unclassified Firmicutes; unclassified Firmicutes; unclassified Firmicutes
unclassified Firmicutes_msp_0622	0.13	0.09	0.25	0.17	<i>unclassified Firmicutes</i>	Firmicutes; unclassified Firmicutes; unclassified Firmicutes; unclassified Firmicutes; unclassified Firmicutes; unclassified Firmicutes
unclassified Firmicutes_msp_0679	0.21	0.15	0.1	0.21	<i>unclassified Firmicutes</i>	Firmicutes; unclassified Firmicutes; unclassified Firmicutes; unclassified Firmicutes; unclassified Firmicutes; unclassified Firmicutes
unclassified Firmicutes_msp_0723	0.07	0.16	-0.02	0.05	<i>unclassified Firmicutes</i>	Firmicutes; unclassified Firmicutes; unclassified Firmicutes; unclassified Firmicutes; unclassified Firmicutes; unclassified Firmicutes
unclassified Firmicutes_msp_0874	0.17	0.18	0.05	0.2	<i>unclassified Firmicutes</i>	Firmicutes; unclassified Firmicutes; unclassified Firmicutes; unclassified Firmicutes; unclassified Firmicutes; unclassified Firmicutes
unclassified Firmicutes_msp_1302	0.17	0.22	0.19	0.21	<i>unclassified Firmicutes</i>	Firmicutes; unclassified Firmicutes; unclassified Firmicutes; unclassified Firmicutes; unclassified Firmicutes; unclassified Firmicutes
unclassified Firmicutes_msp_1342	0.09	0.23	-0.03	0.12	<i>unclassified Firmicutes</i>	Firmicutes; unclassified Firmicutes; unclassified Firmicutes; unclassified Firmicutes; unclassified Firmicutes; unclassified Firmicutes
Parasutterella excrementihominis_msp_0145	0.23	0.11	0.17	0.24	<i>Sutterellaceae</i>	Proteobacteria; Betaproteobacteria; Burkholderiales; Sutterellaceae; Parasutterella; Parasutterella excrementihominis
Bilophila wadsworthia_msp_0110	-0.16	-0.02	-0.1	-0.13	<i>Desulfovibrionaceae</i>	Proteobacteria; Deltaproteobacteria; Desulfovibrionales; Desulfovibrionaceae; Bilophila; Bilophila wadsworthia
Acinetobacter sp. N54.MGS-139 / Proteobacteria bacterium CAG:139_msp_0202	0.15	0.03	-0.04	0.13	<i>Moraxellaceae</i>	Proteobacteria; Gammaproteobacteria; Pseudomonadales; Moraxellaceae; Acinetobacter; Acinetobacter sp. N54.MGS-139 / Proteobacteria bacterium CAG:139
Akkermansia muciniphila_msp_0025	0.19	0.05	0.22	0.18	<i>Akkermansiaceae</i>	Verrucomicrobia; Verrucomicrobiae; Verrucomicrobiales; Akkermansiaceae; Akkermansia; Akkermansia muciniphila

Numbers in bold indicate significance at the FDR<0.05 level.