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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≥ age≤65 years;
- BMI≥24 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 dieticians, 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 Adaptaters 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 Alientrimmer 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 ± 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 ≥ 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 markers 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 $^{13}C_6$ (Cambridge Isotope Laboratories Inc, Andover, MA), L-Tyrosine $^{13}C_9$ (Sigma Aldrich, St. Louis, MO, USA), Para-aminobenzoic acid (Sigma Aldrich), L-Tryptophan-(indole-d_5) (Sigma Aldrich), Hippuric Acid-[$^{13}C_6$] (Biomol GmbH), Cortisone-d8 (Sigma Aldrich), Glycocholic Acid-[$^{2}H_4$] (Biomol GmbH, Hamburg, Germany) and Lysophosphatidylcholine (17:1d_7) (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), taurolithocholic acid

(TLCA) (Steraloids Inc), tauroursodeoxycholic acid (TUDCA) (Calbiochem) and hyodeoxycholic acid (HDCA) (Sigma Aldrich). Internal standards for bile acid quantification were cholic acid-d_4, glycocholic acid-d_4, taurocholic acid-d_5, taurochenodeoxycholic acid-d_5, chenodeoxycholic acid-d_4, glycochenodeoxycholic acid-d_4, ursodeoxycholic acid-d_4, glycoursodeoxycholic acid-d_4, deoxycholic acid-d_4, glycodeoxycholic acid-d_4, and lithocholic acid-d_4 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-d_7, 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 $^{13}C_6$-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 Sirocco™ 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, glycoursodeoxycholic 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 16_{th} 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 uridirine-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 αMCA/βMCA were determined in all faecal samples using labelled standards, except for αMCA/βMCA, 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, αMCA/βMCA 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 αMCA/βMCA 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 µL of the faecal extract containing internal SCFA standard butyric acid-d_7 was mixed with 20 µL 200 mM 3-nitrophenylhydrazine (3NPH) in 50 % ethanol and 20 µL 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 µL of the mixture was transferred to a 1 mL 96-wells plate and 100 µL 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 µL was transferred to another 1 mL 96-wells plate where it was mixed with 100 µL internal SCFA standard mixture (1:12000 dilution of faecal sample). Internal standard mixture was prepared by mixing 50 µL 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 µl 120 mM EDC-6% pyridine solution and 25 µl 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 µM 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 µM 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 µm, Waters) was coupled to an ACQUITY BEH C18 column (2.1 x 100 mm, 1.7 µm, 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 µL of plasma and diluted urine was added with 225 µL of CH_3CN containing internal standard TMAO-d9 25 µM. 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 µm 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, Shellton, 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 µm 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 µL/min and the injection

volume was 10 µL. 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 µM, carnitine 0.1-10 µM, choline 0.2-50 µM, creatinine 2.5-200 µM, betaine 0.1-10 µM.
Calibration curves in plasma were built in the linearity range: TMAO: 0.02-25 µM, carnitine 0.2-50 µM, choline 0.2-50 µM, creatinine 0.2-50 µM, betaine 0.2-25 µM.
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 µm column (150 mm × 2.0 mm) (Phenomenex, Torrance, CA) thermostated at 30 °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 µL/min and the injection volume was 20 µL.
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 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 µL of urine sample were added with 800 µL of 0.2% acetic acid and after centrifuging at 16800xg for 5 min at 4°C the sample was purified onto Oasis HLB 1 cc 30 mg cartridges. After washing with 1mL of H_2O 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 µL MeOH/H_2O (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 °C) and a column oven.
Chromatographic separation was carried out on a Gemini C18-110Å 5 µm column (150 mm × 2.0 mm) (Phenomenex, Torrance, CA) thermostated at 30 °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 µL/min and the injection volume was 10 µL. 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 ($1 \times 10_6$ 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 ±5ppm.
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 µL 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 3 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 22–248C, 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 T^2 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.

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

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