**Valentin PARTULA*, Mélanie DESCHASAUX-TANGUY*, et al. Associations between untargeted plasma metabolomic signatures and gut microbiota composition in the *Milieu Intérieur* population of healthy adults**

**SUPPLEMENTARY MATERIAL**

**Supplementary Material and Method 1.** Gut microbiota sequencing protocol, Milieu Intérieur study, France, 2012.

## DNA EXTRACTION

Bacterial DNA was extracted from stool samples using a Repeated Bead Beating Plus Column (RBB+C) method, as previously published (O'Cuiv et al. 2011; Yu et al. 2004). Cell lysis was achieved by bead beating in the presence of 4% (w/v) sodium dodecyl sulfate, 500mM sodium chloride, and 50mM ethylenediaminetetraacetic acid. Consequently to bead beating, the sodium dodecyl sulfate and most of the impurities were removed by precipitation with ammonium acetate. Nucleic acids were recovered by precipitation with isopropanol. Subsequent sequential digestions with RNase and proteinase K were used to remove RNA and proteins, respectively. Finally, genomic DNA was purified using QIAamp columns (Qiagen, Germantown, MD, USA); then recovered DNA was checked for quality and quantity using NanoDrop and Qubit together with Broad Range assay (ThermoFisher Scientific, Waltham, MA, USA).

## BARCODING POLYMERASE CHAIN REACTION (PCR)

Primers targeting the V3-V4 hypervariable regions of the 16S rRNA gene were used for the barcoding PCR (forward primer: CCTACGGRAGGCAGCAG; reverse primer: GGACTACHVGGGTWTCTAAT) (Kozich et al. 2013). PCR mix was made up of 18µL of AccuPrime™ Pfx SuperMix (Invitrogen-12344-040, ThermoFisher Scientific, Waltham, MA, USA), 0.5µL of both V3-340F and V4-806R primers (0.2µM), and 1µL of DNA (10ng). PCR cycle sequence was: 2min-95°C; 30 cycles of 20sec-95°C; 15sec-55°C; 5min-72°C; and 10min-72°C. PCR reaction was quantified using the Qubit BR DNA assay kit (ThermoFisher Scientific, Waltham, MA). Equal amounts of each PCR reaction were pooled and thoroughly mixed. The amplicon pool was then loaded on a 1.5%-agarose gel and electrophoresis was

run for 45min at 100V. The amplicon band corresponding to the 16S rDNA V3-V4 was extracted and purified using Qiaquick gel extraction kit (Qiagen-28704, Qiagen, Germantown, MD, USA). The amplicon quantity and integrity were checked on agarose gel electrophoresis and Qubit BR DNA assay kit (ThermoFisher Scientific, Waltham, MA, USA). Amplicon was stored at -20°C.

**SEQUENCING**

16S rDNA amplicon was sequenced at Institut Curie NGS platform on a MiSeq machine (Illumina, San Diego, CA, USA) using the 2*300 bp V3 kit.

*Bioinformatics pipeline and computation of microbial diversity indexes*

Pipelines sickle (Joshi et al. 2011), SPAdes (Bankevich et al. 2012), and PEAR (Zhang et al. 2014) were used to trim, error correct, and merge reads, respectively. vsearch (Rognes et al. 2016) was used to cluster reads at 97% of identity, in order to generate Operational Taxonomic Units (OTU). UCHIME (Edgar et al. 2011) was used to identify chimeric OTUs which were discarded from further analysis. RDP SeqMatch and reference database RDP Release 11.3 were used to determine taxonomy of representative OTU sequences. These were then aligned using ssu-align (Nawrocki 2009). Fasttree2 (Price et al. 2010) was used to infer the phylogenetic tree from the OTUs multiple alignments.

R package *vegan* was used to generate microbial diversity indicators – namely Simpson's index, observed richness, and Chao1 richness estimate (α-diversity) and Bray-Curtis dissimilarity (β-diversity) – from the non-rarefied OTU count table.

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**Supplementary Material and Method 2.** Metabolomic analysis protocol, Milieu Intérieur study, France, 2012.

**NMR SAMPLE PREPARATION**

350µL of plasma (thawed at room temperature) were mixed with 250µL of buffer solution (10mmol.$L^{-1}$ phosphate buffer saline/5mmol.$L^{-1}$ $NaN_3$ in $D_2O$). Mix was centrifuged (12,000g, 5min, 4°C) and supernatant placed into a NMR tube.

**ONE-DIMENSIONAL NMR SPECTRA ACQUISITION**

For each plasma sample, two complementary one-dimensional NMR sequences were acquired on a 500MHz-Bruker Avance III spectrometer (Bruker, Billerica, MA, USA) at 300K – namely the Nuclear Overhauser Effect SpectroscopY (NOESY) and the Carr-Purcell-Meiboom-Gill (CPMG) sequences. Both were used with 128 transients. The spectral window was 10 kHz. The water signal was suppressed by a presaturation sequence using low-power irradiation on the water-signal frequency during the relaxation delay.

**POST-ACQUISITION SPECTRA PRE-PROCESSING**

A Fourier transform was applied with an exponential window function to produce a 0.3 Hz broadening line. A zero-order phase and a manual baseline correction were applied to each spectrum using Bruker's TopSpin 4.0.3. Spectra were aligned using the icoshift algorithm (Savorani et al. 2010) and normalized using the probabilistic quotient normalization method (Dieterle et al. 2006). Finally, the grouped-batch profile calibration strategy (Fages et al. 2013) was used to remove variation presumably introduced by experimental procedures (i.e. batch-effect).

**INTELLIGENT BUCKETING OF SPECTRA AND OBTAINING OF NMR VARIABLES**

After processing and calibration, each spectrum was sliced into bins with pre-defined limits so as to maximize recovery of peak entities. This "intelligent bucketing" procedure (Sousa et al. 2013) mitigates spoiling of peak integrity compared to bucketing with steady 0.001 ppm-intervals. Then, the buckets were scaled to the total summed integrals for each spectrum,

and the integrals of peak entities were calculated to obtain continuous NMR variables that were used in further statistical analyses.

## NMR SIGNALS ASSIGNMENTS

NMR signals were assigned using ad hoc literature (Nicholson et al. 1995). CHENOMX software (CHENOMX, Edmonton, Alberta, Canada) and Human Metabolome Database (Wishart et al. 2013) were used to confirm the assignments using the medium spectrum (Nicholson et al. 1995). After the statistical analysis, the assignments of the buckets that had an association with the gut microbia were newly carefully looked and confirmed to be unambiguously assigned by gathering information from S-TOCSY, additional two-dimension NMR experiments (recorded on random samples) such as 1H–1H Total Correlated Spectroscopy (TOCSY) and J-resolved experiments.

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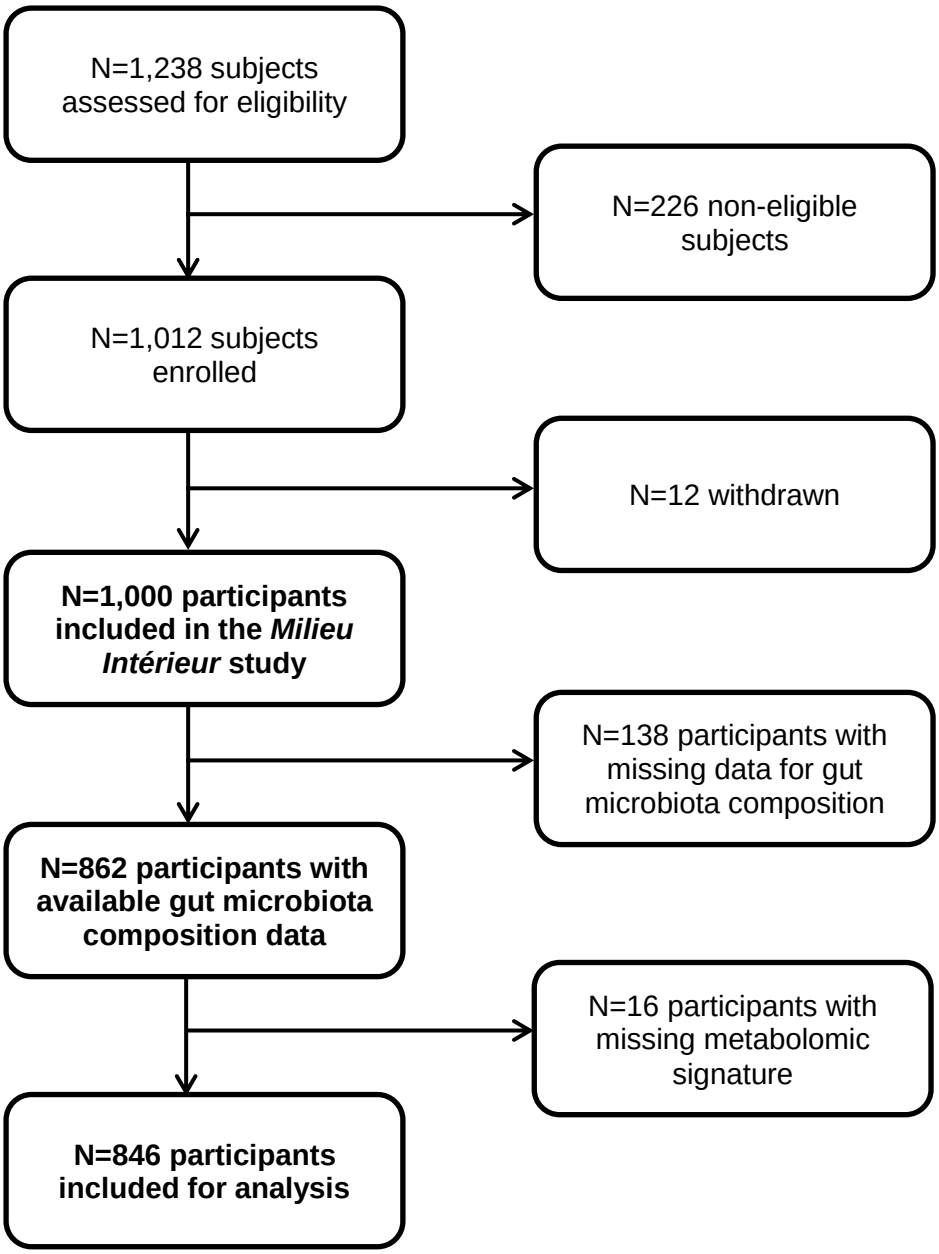
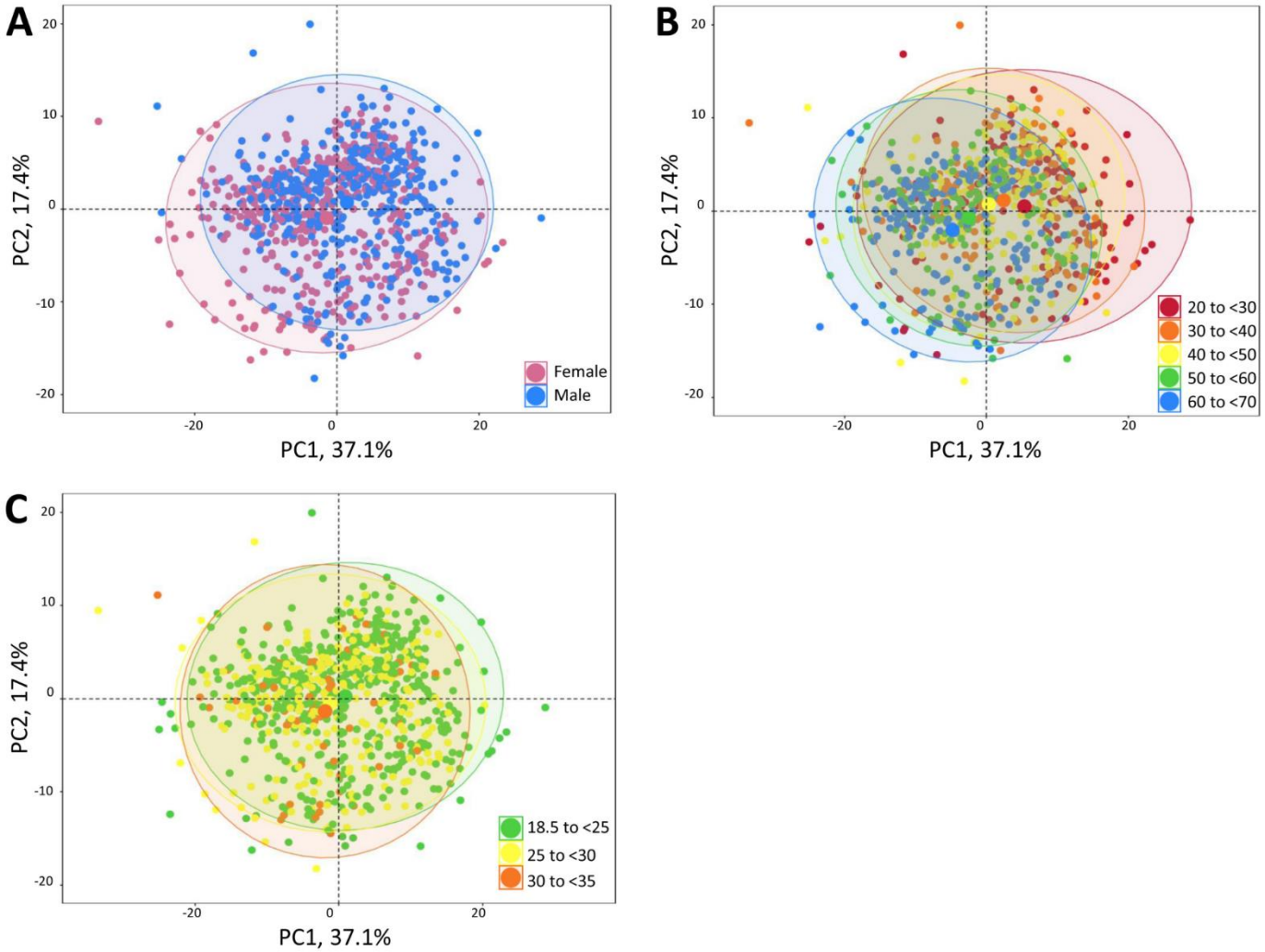
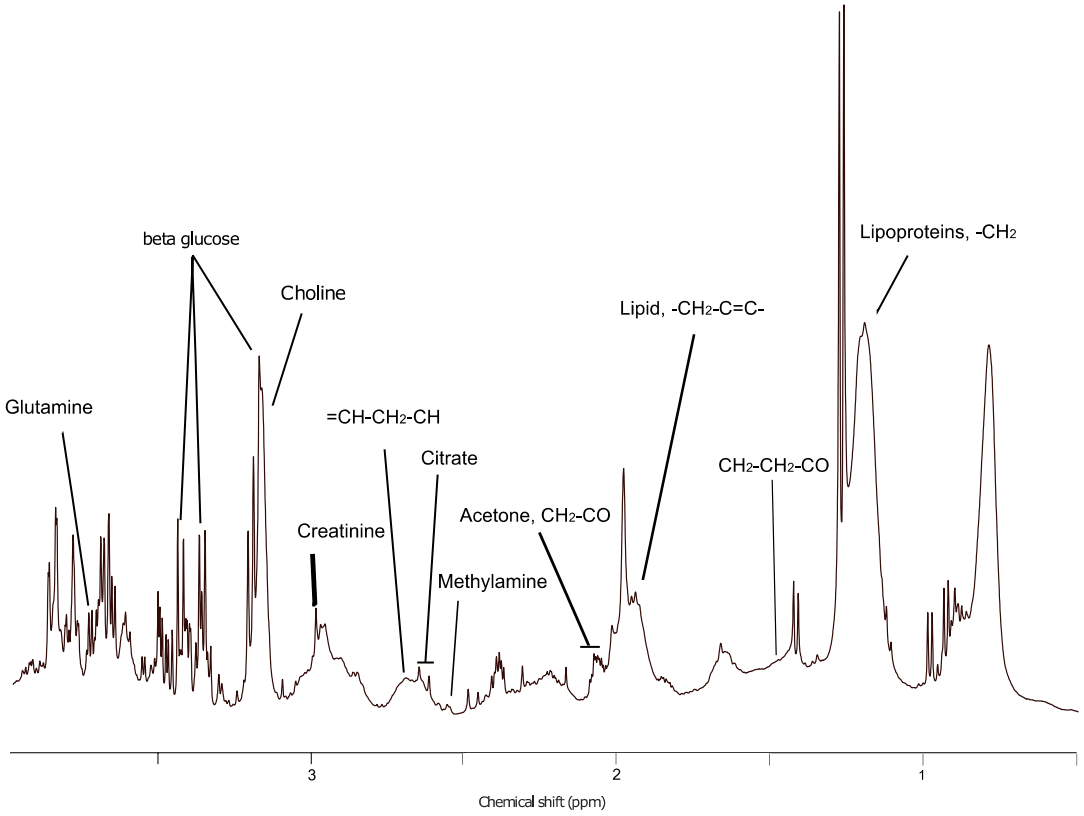
**Supplementary Figure 1.** Participant flow chart, *Milieu Intérieur* study, France, 2012.

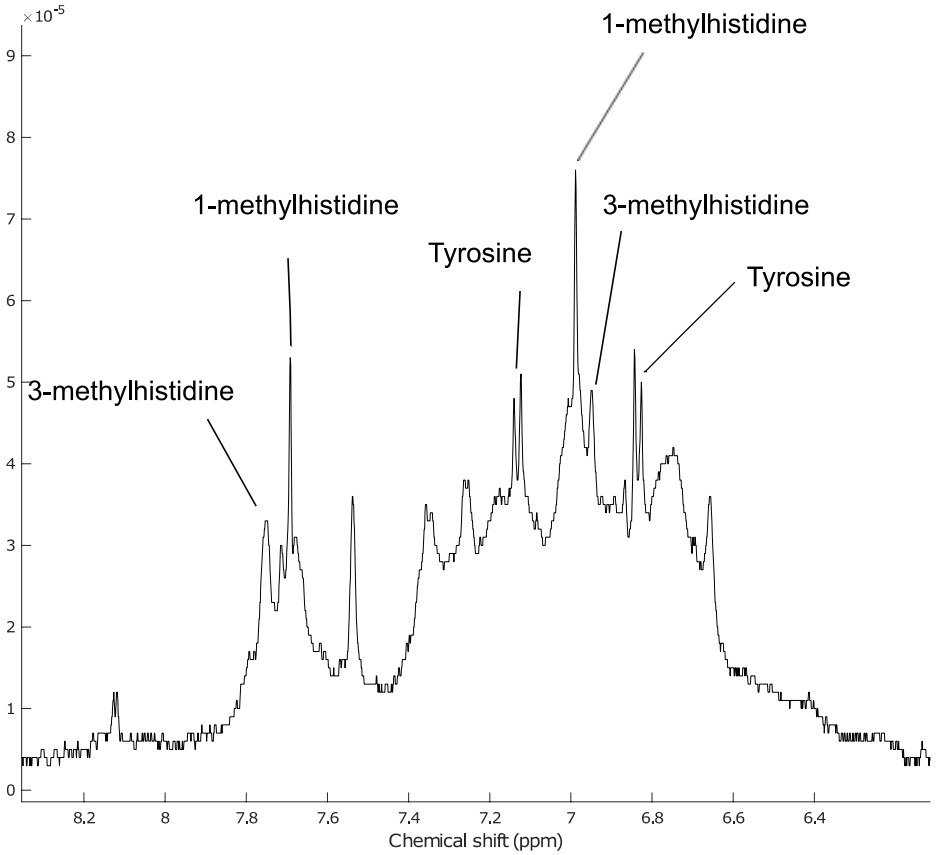


**Supplementary Figure 2.** Interindividual variation in metabolomic signatures represented by principal components (PCA) analysis of the NOESY1D metabolomic dataset, *Milieu Intérieur* study, France, 2012 (N=846). Each point represents an individual from the study sample. PCA was obtained via the *PCA* function (package *FactoMineR*), and plotted and color-coded based on sex (A), age (B), and BMI (C) via the *fviz_pca_ind* function (package *FactoExtra*). Concentration ellipses (95%) are shown. Percentages on the axes represent the proportion of variation explained by the two first components of the PCA.



**Supplementary Figure 3.** Medium NMR spectrum. The buckets that have been highlighted in the manuscript have been assigned on the spectrum.



**Supplementary Table 1.** "Core microbiota" (i.e. genera shared by at least 95% of the study population), *Milieu Intérieur* study, France, 2012 (N=846).

| Phylum\| Class\| Order\| Family\| | Genus[1] | Coverage (%)[2] |
|---|---|---|
| Firmicutes\| Clostridia\| Clostridiales\| Ruminococcaceae\| | Flavonifractor[3] | 100 |
| Firmicutes\| Clostridia\| Clostridiales\| Lachnospiraceae\| | Clostridium XlVa[2] | 100 |
| Firmicutes\| Clostridia\| Clostridiales\| Lachnospiraceae\| | Lachnospiracea incertae sedis[2] | 100 |
| Firmicutes\| Clostridia\| Clostridiales\| Lachnospiraceae\| | Blautia[2] | 100 |
| Firmicutes\| Clostridia\| Clostridiales\| Ruminococcaceae\| | Clostridium IV[2] | 100 |
| Bacteroidetes\| Bacteroidia\| Bacteroidales\| Bacteroidaceae\| | Bacteroides[2] | 99.9 |
| Firmicutes\| Clostridia\| Clostridiales\| Eubacteriaceae\| | Eubacterium | 99.9 |
| Firmicutes\| Clostridia\| Clostridiales\| Ruminococcaceae\| | Acetivibrio | 99.9 |
| Firmicutes\| Clostridia\| Clostridiales\| Ruminococcaceae\| | Oscillibacter[3] | 99.8 |
| Firmicutes\| Clostridia\| Clostridiales\| Lachnospiraceae\| | Coprococcus[2] | 99.8 |
| Firmicutes\| Clostridia\| Clostridiales\| Lachnospiraceae\| | Ruminococcus II | 99.8 |
| Firmicutes\| Clostridia\| Clostridiales\| Ruminococcaceae\| | Gemmiger[3] | 99.7 |
| Firmicutes\| Clostridia\| Clostridiales\| Lachnospiraceae\| | Roseburia[2] | 99.5 |
| Firmicutes\| Clostridia\| Clostridiales\| Ruminococcaceae\| | Faecalibacterium[2] | 99.4 |
| Firmicutes\| Clostridia\| Clostridiales\| Ruminococcaceae\| | Intestinimonas | 98.7 |
| Firmicutes\| Clostridia\| Clostridiales\| Lachnospiraceae\| | Fusicatenibacter | 98.7 |
| Bacteroidetes\| Bacteroidia\| Bacteroidales\| Rikenellaceae\| | Alistipes[2] | 98.5 |
| Bacteroidetes\| Bacteroidia\| Bacteroidales\| Porphyromonadaceae\| | Parabacteroides[2] | 98.2 |
| Firmicutes\| Clostridia\| Clostridiales\| Lachnospiraceae\| | Dorea[2] | 98.1 |
| Firmicutes\| Clostridia\| Clostridiales\| Ruminococcaceae\| | Clostridium III | 98.1 |
| Firmicutes\| Clostridia\| Clostridiales\| Ruminococcaceae\| | Ruminococcus[3] | 96.9 |
| Firmicutes\| Clostridia\| Clostridiales\| Lachnospiraceae\| | Eisenbergiella | 95.7 |

[1] Genera shared with the "Western" core described throughout 4,000 samples from 4,000 European or North American individuals (**) or found in the 95% core of at least one cohort (*), as reported by Falony et al. (Falony G, et al. Population-level analysis of gut microbiome variation. Science. 2016. 352 (6285): 560-564).

[2] Prevalence of genus in the study population.

**Supplementary Table 2.** Relative abundance, taxonomical assignment and coverage of the detected genera and species. Milieu Intérieur study, France, 2012 (N=846)

*Provided as an Excel file*

**Supplementary Table 3.** Associations between covariates and Bray-Curtis dissimilarity, using univariate PERMANOVAs (999 permutations), *Milieu Intérieur* study, France, 2012 (N=846).

| | Bray-Curtis dissimilarity | |
|---|---|---|
| Covariate | $R^2$ | p-value[1] |
| Age | 0.005 | 0.001 |
| Sex | 0.006 | 0.001 |
| BMI | 0.001 | 0.4 |
| Smoking status | 0.004 | 0.008 |
| Physical activity | 0.001 | 0.5 |
| Sequencing depth | 0.07 | 0.001 |

[1] p-value for univariate PERMANOVA, 999 permutations, computed using the *adonis* function of the package *vegan*.

**Supplementary Table 4.** Description of the 202 CPMG continuous variables used in statistical analyses, *Milieu Intérieur* study, France, 2012 (N=846).

| Spectral region (ppm) | Metabolite assignment | Mean | SD |
|---|---|---|---|
| 0.527-0.567 | | 0.00100 | 0.00007 |
| 0.567-0.607 | | 0.00133 | 0.00008 |
| 0.607-0.647 | | 0.00166 | 0.00007 |
| 0.647-0.713 | $CH_3$ cholesterol | 0.00344 | 0.00017 |
| 0.713-0.727 | $CH_3$ cholesterol | 0.00112 | 0.00006 |
| 0.727-0.767 | | 0.00754 | 0.00044 |
| 0.767-0.807 | $CH_3$ lipoproteins | 0.03093 | 0.00287 |
| 0.807-0.911 | $CH_3$ lipoproteins | 0.04315 | 0.00242 |
| 0.911-0.937 | Leucine, valine, isoleucine | 0.00461 | 0.00024 |
| 0.937-0.983 | Leucine, valine, isoleucine | 0.00537 | 0.00028 |
| 0.983-1.019 | Leucine, valine, isoleucine | 0.00339 | 0.00017 |
| 1.019-1.053 | Valine | 0.00214 | 0.00011 |
| 0.911-0.97 | Leucine | 0.00801 | 0.00040 |
| 0.97-1.0 | Valine | 0.00391 | 0.00021 |
| 1.0-1.021 | Isoleucine | 0.00141 | 0.00008 |
| 1.021-1.051 | | 0.00188 | 0.00010 |
| 1.051-1.053 | | 0.00019 | 0.00001 |
| 1.053-1.081 | Isobutyrate | 0.00265 | 0.00010 |
| 1.081-1.088 | | 0.00116 | 0.00004 |
| 1.088-1.127 | | 0.10672 | 0.00504 |
| 1.127-1.167 | Propylene glycol | 0.06318 | 0.00253 |
| 1.127-1.145 | Propylene glycol | 0.05066 | 0.00237 |
| 1.145-1.167 | | 0.01345 | 0.00074 |
| 1.167-1.207 | $CH_2$ lipoproteins | 0.04104 | 0.00335 |
| 1.207-1.311 | $CH_2$ lipoproteins | 0.13277 | 0.01211 |
| 1.311-1.351 | Lactate threonine/$CH_2$ lipoproteins | 0.00684 | 0.00040 |
| 1.351-1.391 | $CH_2$ lipoproteins | 0.00396 | 0.00019 |
| 1.391-1.431 | Lysine | 0.00677 | 0.00036 |
| 1.431-1.437 | | 0.00110 | 0.00006 |
| 1.437-1.447 | | 0.00129 | 0.00007 |
| 1.447-1.521 | Alanine | 0.01054 | 0.00063 |
| 1.521-1.527 | $CH_2CH_2COOC$ | 0.00148 | 0.00015 |
| 1.527-1.631 | $CH_2CH_2COOC$ | 0.01103 | 0.00049 |
| 1.631-1.636 | | 0.00063 | 0.00004 |
| 1.636-1.647 | | 0.00133 | 0.00009 |
| 1.647-1.751 | Arginine lysine | 0.00845 | 0.00060 |
| 1.647-1.707 | Arginine | 0.00604 | 0.00043 |
| 1.707-1.759 | Lysine | 0.00288 | 0.00021 |
| 1.759-1.791 | | 0.00156 | 0.00011 |
| 1.751-1.791 | | 0.00197 | 0.00014 |
| 1.791-1.821 | | 0.00152 | 0.00010 |
| 1.821-1.849 | | 0.00191 | 0.00013 |
| 1.849-1.89 | Lysine acetate | 0.00550 | 0.00020 |
| 1.89-1.927 | Lysine acetate | 0.00589 | 0.00032 |
| 1.849-1.899 | Lysine | 0.00648 | 0.00024 |

| | | | |
|---|---|---|---|
| 1.899-1.927 | Acetate | 0.00495 | 0.00028 |
| 1.927-1.967 | Glycoproteins N-acetyl, $CH_2CH$ | 0.01230 | 0.00078 |
| 1.967-2.063 | Glycoproteins N-acetyl, $CH_2CH$ | 0.02268 | 0.00102 |
| 1.927-2.017 | $CH_2CH$ | 0.02872 | 0.00128 |
| 2.017-2.063 | Glycoproteins N-acetyl | 0.00614 | 0.00033 |
| 2.063-2.094 | Methionine | 0.00360 | 0.00023 |
| 2.094-2.151 | Methionine | 0.00354 | 0.00017 |
| 2.151-2.166 | Lipid -$CH_2$=C=C- | 0.00198 | 0.00022 |
| 2.166-2.167 | Lipid -$CH_2$=C=C- | 0.00036 | 0.00004 |
| 2.167-2.264 | Acetone $CH_2CO$ | 0.01194 | 0.00068 |
| 2.264-2.311 | | 0.00339 | 0.00022 |
| 2.264-2.277 | Acetoacetate | 0.00103 | 0.00006 |
| 2.277-2.311 | | 0.00243 | 0.00017 |
| 2.311-2.339 | | 0.00195 | 0.00013 |
| 2.311-2.315 | | 0.00052 | 0.00004 |
| 2.315-2.354 | Glutamate | 0.00243 | 0.00016 |
| 2.354-2.372 | Pyruvate | 0.00124 | 0.00008 |
| 2.372-2.411 | | 0.00387 | 0.00031 |
| 2.339-2.386 | Pyruvate | 0.00353 | 0.00025 |
| 2.386-2.471 | Glutamine | 0.00518 | 0.00040 |
| 2.411-2.471 | Glutamine | 0.00272 | 0.00021 |
| 2.471-2.501 | | 0.00111 | 0.00008 |
| 2.501-2.551 | Citrate | 0.00111 | 0.00007 |
| 2.551-2.571 | Methylamine | 0.00060 | 0.00005 |
| 2.571-2.612 | | 0.00170 | 0.00011 |
| 2.612-2.647 | | 0.00285 | 0.00020 |
| 2.647-2.687 | Citrate | 0.00424 | 0.00031 |
| 2.647-2.705 | Citrate | 0.00641 | 0.00046 |
| 2.705-2.715 | | 0.00133 | 0.00010 |
| 2.715-2.848 | =CH-$CH_2$-CH= | 0.00849 | 0.00040 |
| 2.687-2.791 | Citrate/=CH-CH2-CH= | 0.00909 | 0.00063 |
| 2.791-2.831 | Citrate/=CH-CH2-CH= | 0.00164 | 0.00011 |
| 2.831-2.848 | Citrate/=CH-CH2-CH= | 0.00123 | 0.00010 |
| 2.848-2.887 | | 0.00325 | 0.00026 |
| 2.848-2.873 | | 0.00211 | 0.00017 |
| 2.873-2.927 | Albumine | 0.00521 | 0.00042 |
| 2.887-2.927 | | 0.00408 | 0.00033 |
| 2.927-2.967 | | 0.00548 | 0.00043 |
| 2.967-3.071 | Creatine/albumin lysyl | 0.01003 | 0.00077 |
| 2.967-2.98 | | 0.00218 | 0.00017 |
| 2.98-3.028 | Lysine | 0.00539 | 0.00042 |
| 3.028-3.044 | Creatine | 0.00121 | 0.00010 |
| 3.044-3.071 | | 0.00158 | 0.00012 |
| 3.071-3.111 | Creatinine | 0.00233 | 0.00013 |
| 3.111-3.129 | | 0.00111 | 0.00007 |
| 3.129-3.167 | | 0.01657 | 0.00141 |
| 3.167-3.269 | Choline, glucose | 0.02303 | 0.00174 |
| 3.269-3.311 | Glucose | 0.00337 | 0.00020 |
| 3.311-3.312 | Glucose | 0.00007 | 0.00001 |

| | | | |
|---|---|---|---|
| 3.312-3.367 | Glucose | 0.01065 | 0.00080 |
| 3.367-3.437 | Glucose | 0.01468 | 0.00112 |
| 3.437-3.496 | Glucose | 0.00896 | 0.00057 |
| 3.496-3.587 | Glucose | 0.02650 | 0.00102 |
| 3.587-3.616 | Ethanol | 0.06577 | 0.00304 |
| 3.616-3.711 | Glutamine | 0.03258 | 0.00145 |
| 3.711-3.713 | | 0.00061 | 0.00005 |
| 3.713-3.791 | Glucose | 0.01459 | 0.00102 |
| 3.791-3.795 | Glucose | 0.00077 | 0.00006 |
| 3.795-3.86 | Glucose | 0.01559 | 0.00107 |
| 3.86-3.951 | Creatine | 0.00972 | 0.00067 |
| 3.86-3.919 | | 0.00668 | 0.00047 |
| 3.919-3.932 | Creatine | 0.00147 | 0.00011 |
| 3.932-3.951 | | 0.00179 | 0.00013 |
| 3.951-3.978 | | 0.00177 | 0.00012 |
| 3.978-4.021 | | 0.00330 | 0.00018 |
| 4.021-4.081 | Creatine glyceryl | 0.00871 | 0.00051 |
| 4.021-4.034 | | 0.00149 | 0.00009 |
| 4.034-4.055 | Creatinine | 0.00337 | 0.00018 |
| 4.055-4.081 | Choline | 0.00408 | 0.00026 |
| 4.081-4.151 | Lactate | 0.00480 | 0.00037 |
| 4.151-4.184 | | 0.00193 | 0.00013 |
| 4.184-4.198 | | 0.00096 | 0.00005 |
| 4.198-4.208 | | 0.00077 | 0.00004 |
| 4.208-4.311 | Threonine | 0.00702 | 0.00037 |
| 5.007-5.081 | | 0.00454 | 0.00016 |
| 5.081-5.087 | | 0.00039 | 0.00001 |
| 5.087-5.127 | | 0.00227 | 0.00008 |
| 5.127-5.167 | | 0.00260 | 0.00013 |
| 5.167-5.207 | CH glyceryl | 0.00726 | 0.00039 |
| 5.207-5.247 | Glucose | 0.00901 | 0.00080 |
| 5.247-5.351 | Unsaturation | 0.00959 | 0.00091 |
| 5.351-5.389 | Unsaturation | 0.00109 | 0.00004 |
| 5.389-5.431 | | 0.00102 | 0.00004 |
| 5.431-5.471 | | 0.00085 | 0.00003 |
| 5.471-5.51 | | 0.00075 | 0.00003 |
| 5.51-5.528 | | 0.00034 | 0.00001 |
| 5.528-5.63 | | 0.00165 | 0.00006 |
| 5.63-5.634 | | 0.00008 | 0.00000 |
| 5.634-5.647 | | 0.00022 | 0.00001 |
| 5.647-5.687 | | 0.00072 | 0.00005 |
| 5.687-5.727 | Urea | 0.00104 | 0.00011 |
| 5.727-5.831 | Urea | 0.00157 | 0.00011 |
| 5.831-5.864 | | 0.00039 | 0.00002 |
| 5.864-5.889 | | 0.00028 | 0.00001 |
| 5.889-5.969 | | 0.00079 | 0.00003 |
| 5.969-6.07 | | 0.00086 | 0.00004 |
| 6.07-6.1 | | 0.00024 | 0.00001 |
| 6.1-6.133 | | 0.00026 | 0.00001 |

| | | | |
|---|---|---|---|
| 6.133-6.17 | | 0.00028 | 0.00001 |
| 6.17-6.207 | | 0.00028 | 0.00001 |
| 6.207-6.248 | | 0.00032 | 0.00002 |
| 6.248-6.34 | | 0.00064 | 0.00003 |
| 6.34-6.367 | | 0.00019 | 0.00001 |
| 6.367-6.407 | | 0.00029 | 0.00002 |
| 6.407-6.447 | | 0.00029 | 0.00002 |
| 6.447-6.492 | | 0.00032 | 0.00002 |
| 6.492-6.527 | | 0.00026 | 0.00002 |
| 6.527-6.567 | | 0.00032 | 0.00002 |
| 6.567-6.613 | | 0.00040 | 0.00003 |
| 6.613-6.648 | | 0.00038 | 0.00003 |
| 6.648-6.687 | | 0.00067 | 0.00006 |
| 6.687-6.728 | | 0.00070 | 0.00006 |
| 6.728-6.767 | Protein | 0.00080 | 0.00007 |
| 6.767-6.857 | | 0.00202 | 0.00015 |
| 6.857-6.943 | Tyrosine | 0.00171 | 0.00013 |
| 6.857-6.912 | Tyrosine | 0.00107 | 0.00009 |
| 6.912-6.943 | Protein | 0.00066 | 0.00005 |
| 6.943-6.968 | Protein | 0.00059 | 0.00005 |
| 6.968-7.023 | 1-methylhistidine | 0.00163 | 0.00013 |
| 7.023-7.054 | Histidine | 0.00081 | 0.00006 |
| 7.054-7.071 | | 0.00036 | 0.00003 |
| 6.968-7.071 | Histidine | 0.00276 | 0.00021 |
| 7.071-7.108 | | 0.00076 | 0.00006 |
| 7.108-7.12 | Protein | 0.00026 | 0.00002 |
| 7.12-7.127 | | 0.00024 | 0.00002 |
| 7.127-7.211 | Tyrosine | 0.00171 | 0.00013 |
| 7.128-7.159 | | 0.00071 | 0.00006 |
| 7.159-7.211 | Tyrosine | 0.00099 | 0.00008 |
| 7.211-7.27 | | 0.00106 | 0.00009 |
| 7.27-7.279 | | 0.00018 | 0.00001 |
| 7.279-7.344 | Phenylalanine | 0.00110 | 0.00009 |
| 7.344-7.375 | | 0.00056 | 0.00005 |
| 7.375-7.431 | Phenylalanine | 0.00061 | 0.00005 |
| 7.431-7.471 | | 0.00033 | 0.00003 |
| 7.471-7.508 | | 0.00031 | 0.00002 |
| 7.508-7.539 | 3-methylhistidine | 0.00052 | 0.00003 |
| 7.539-7.629 | 3-methylhistidine | 0.00123 | 0.00007 |
| 7.629-7.647 | 3-methylhistidine | 0.00025 | 0.00002 |
| 7.647-7.687 | 1-methylhistidine | 0.00073 | 0.00005 |
| 7.687-7.772 | Histidine | 0.00162 | 0.00010 |
| 7.772-7.831 | 1-methylhistidine | 0.00066 | 0.00005 |
| 7.831-7.871 | | 0.00025 | 0.00002 |
| 7.871-7.911 | | 0.00023 | 0.00002 |
| 7.911-7.921 | | 0.00006 | 0.00001 |
| 7.921-7.984 | | 0.00036 | 0.00003 |
| 7.984-8.018 | | 0.00015 | 0.00001 |
| 8.018-8.047 | | 0.00012 | 0.00001 |

| | | |
|---|---|---|
| 8.047-8.144 | 0.00051 | 0.00004 |
| 8.144-8.231 | 0.00036 | 0.00003 |
| 8.231-8.263 | 0.00010 | 0.00001 |
| 8.263-8.306 | 0.00013 | 0.00001 |
| 8.306-8.344 | 0.00011 | 0.00001 |
| 8.344-8.364 | 0.00006 | 0.00001 |
| 8.364-8.427 | 0.00021 | 0.00001 |
| 8.427-8.468 | 0.00009 | 0.00001 |
| 8.468-8.503 | 0.00008 | 0.00001 |
| 8.503-8.551 | 0.00010 | 0.00001 |

**Supplementary Table 5.** Description of the 215 NOESY continuous variables used in statistical analyses, *Milieu Intérieur* study, France, 2012 (N=846).

| Spectral region (ppm) | Metabolite assignment | Mean | SD |
|---|---|---|---|
| 0.487-0.527 | | 0.00383 | 0.00006 |
| 0.527-0.567 | | 0.00451 | 0.00007 |
| 0.567-0.607 | | 0.00581 | 0.00017 |
| 0.607-0.647 | $CH_3$ cholesterol | 0.00667 | 0.00019 |
| 0.647-0.687 | $CH_3$ cholesterol | 0.00724 | 0.00013 |
| 0.687-0.727 | $CH_3$ cholesterol | 0.00861 | 0.00013 |
| 0.727-0.767 | | 0.01317 | 0.00037 |
| 0.767-0.807 | $CH_3$ lipoproteins | 0.02595 | 0.00239 |
| 0.807-0.911 | $CH_3$ lipoproteins | 0.04896 | 0.00336 |
| 0.911-0.95 | Leucine valine | 0.01381 | 0.00042 |
| 0.95-0.984 | Leucine valine | 0.01024 | 0.00032 |
| 0.984-1.021 | Leucine valine | 0.01050 | 0.00033 |
| 1.021-1.067 | Leucine valine | 0.01267 | 0.00052 |
| 0.97-1 | Valine | 0.00897 | 0.00026 |
| 1-1.021 | Isoleucine | 0.00592 | 0.00020 |
| 1.021-1.051 | Valine | 0.00830 | 0.00033 |
| 1.051-1.067 | | 0.00464 | 0.00020 |
| 1.067-1.089 | | 0.00647 | 0.00027 |
| 1.09-1.124 | | 0.01154 | 0.00535 |
| 1.089-1.127 | | 0.01296 | 0.00582 |
| 1.127-1.167 | Isobutyrate | 0.02197 | 0.00281 |
| 1.167-1.207 | $CH_2$ lipoproteins | 0.03355 | 0.00469 |
| 1.207-1.311 | $CH_2$ lipoproteins | 0.06990 | 0.01291 |
| 1.311-1.351 | Lactate threonine | 0.01167 | 0.00059 |
| 1.351-1.391 | $CH_2$ lipoproteins | 0.01139 | 0.00032 |
| 1.391-1.429 | Lysine | 0.01244 | 0.00037 |
| 1.429-1.447 | | 0.00611 | 0.00020 |
| 1.447-1.522 | Alanine | 0.02360 | 0.00115 |
| 1.522-1.591 | $CH_2CH_2COOC$ | 0.01775 | 0.00063 |
| 1.591-1.631 | $CH_2CH_2COOC$ | 0.01008 | 0.00013 |
| 1.631-1.636 | $CH_2CH_2COOC$ | 0.00156 | 0.00002 |
| 1.636-1.647 | $CH_2CH_2COOC$ | 0.00317 | 0.00004 |
| 1.647-1.677 | | 0.00818 | 0.00010 |
| 1.677-1.707 | Arginine | 0.00751 | 0.00009 |
| 1.707-1.751 | Lysine | 0.01026 | 0.00012 |
| 1.751-1.791 | | 0.00921 | 0.00012 |
| 1.791-1.831 | | 0.00906 | 0.00012 |
| 1.831-1.851 | | 0.00479 | 0.00006 |
| 1.851-1.887 | | 0.00888 | 0.00014 |
| 1.887-1.927 | Acetate | 0.01233 | 0.00048 |
| 1.927-1.967 | Glycoproteins N-acetyl, $CH_2CH$ | 0.01538 | 0.00116 |
| 1.927-2.018 | $CH_2CH_2COOC$ | 0.03397 | 0.00208 |
| 2.018-2.057 | Glycoproteins N-acetyl, $CH_2CH$ | 0.00990 | 0.00020 |
| 2.057-2.151 | Glutamine | 0.02009 | 0.00043 |
| 2.064-2.11 | | 0.01025 | 0.00016 |

| | | | | |
|---|---|---|---|---|
| 2.11-2.151 | | | 0.00838 | 0.00029 |
| 2.151-2.174 | | | 0.00544 | 0.00045 |
| 2.174-2.264 | Acetone, $CH_2CO$ | | 0.01879 | 0.00070 |
| 2.264-2.311 | | | 0.00825 | 0.00011 |
| 2.311-2.351 | | | 0.00623 | 0.00008 |
| 2.351-2.388 | Pyruvate | | 0.00584 | 0.00011 |
| 2.351-2.374 | Pyruvate | | 0.00357 | 0.00005 |
| 2.374-2.414 | | | 0.00640 | 0.00015 |
| 2.388-2.414 | | | 0.00414 | 0.00009 |
| 2.414-2.471 | Glutamine | | 0.00704 | 0.00011 |
| 2.471-2.502 | | 0 | 0.00337 | 0.00005 |
| 2.502-2.551 | Citrate | | 0.00454 | 0.00006 |
| 2.551-2.58 | Methylamine | | 0.00272 | 0.00006 |
| 2.58-2.627 | | | 0.00502 | 0.00009 |
| 2.627-2.647 | | | 0.00263 | 0.00011 |
| 2.647-2.687 | Citrate/$=CH-CH_2-CH=$ | | 0.00536 | 0.00034 |
| 2.687-2.791 | Citrate/$=CH-CH_2-CH=$ | | 0.01113 | 0.00062 |
| 2.791-2.831 | Citrate/$=CH-CH_2-CH=$ | | 0.00402 | 0.00007 |
| 2.831-2.84 | Citrate/$=CH-CH_2-CH=$ | | 0.00113 | 0.00003 |
| 2.84-2.847 | Citrate/$=CH-CH_2-CH=$ | | 0.00095 | 0.00003 |
| 2.647-2.708 | Citrate | | 0.00811 | 0.00055 |
| 2.708-2.847 | $=CH-CH_2-CH=$ | | 0.01415 | 0.00040 |
| 2.847-2.887 | | | 0.00510 | 0.00010 |
| 2.887-2.927 | | | 0.00573 | 0.00012 |
| 2.927-2.978 | | | 0.00821 | 0.00016 |
| 2.978-3.071 | Creatine /albumin lysyl | | 0.01079 | 0.00020 |
| 3.071-3.111 | Creatinine | | 0.00354 | 0.00005 |
| 3.111-3.13 | | | 0.00195 | 0.00004 |
| 3.13-3.167 | | | 0.00967 | 0.00102 |
| 3.167-3.27 | Choline, glucose | | 0.01351 | 0.00070 |
| 3.27-3.311 | Glucose | | 0.00306 | 0.00010 |
| 3.311-3.314 | Glucose | | 0.00026 | 0.00001 |
| 3.314-3.366 | Glucose | | 0.00686 | 0.00040 |
| 3.366-3.367 | Glucose | | 0.00021 | 0.00001 |
| 3.367-3.438 | Glucose | | 0.00941 | 0.00057 |
| 3.438-3.497 | Glucose | | 0.00667 | 0.00027 |
| 3.497-3.532 | Glucose | | 0.00421 | 0.00017 |
| 3.532-3.588 | Glucose | | 0.00633 | 0.00107 |
| 3.588-3.611 | Glucose | | 0.00403 | 0.00347 |
| 3.611-3.707 | | | 0.01605 | 0.00092 |
| 3.707-3.791 | Glucose | | 0.01341 | 0.00049 |
| 3.791-3.793 | Glucose | | 0.00046 | 0.00002 |
| 3.793-3.807 | Glucose | | 0.00243 | 0.00008 |
| 3.807-3.86 | Glucose | | 0.01081 | 0.00041 |
| 3.86-3.951 | Creatine | | 0.01395 | 0.00025 |
| 3.861-3.918 | | | 0.00884 | 0.00019 |
| 3.918-3.95 | Creatine | | 0.00497 | 0.00009 |
| 3.951-3.952 | | | 0.00028 | 0.00000 |
| 3.952-4.03 | | | 0.01085 | 0.00025 |

| | | | |
|---|---|---|---|
| 4.03-4.094 | Creatinine glyceryl lactate | 0.01032 | 0.00034 |
| 4.094-4.151 | Creatinine glyceryl lactate | 0.00693 | 0.00012 |
| 4.031-4.075 | Creatinine glyceryl | 0.00761 | 0.00031 |
| 4.075-4.151 | Lactate | 0.00953 | 0.00016 |
| 4.151-4.191 | | 0.00458 | 0.00006 |
| 4.191-4.214 | | 0.00282 | 0.00011 |
| 4.214-4.311 | Threonine | 0.00959 | 0.00018 |
| 5.031-5.042 | | 0.00018 | 0.00001 |
| 5.042-5.047 | | 0.00009 | 0.00001 |
| 5.047-5.087 | | 0.00065 | 0.00004 |
| 5.087-5.127 | | 0.00081 | 0.00006 |
| 5.127-5.167 | | 0.00124 | 0.00015 |
| 5.167-5.207 | CH glyceryl | 0.00418 | 0.00038 |
| 5.207-5.247 | Glucose | 0.00576 | 0.00099 |
| 5.247-5.351 | Unsaturation | 0.00452 | 0.00098 |
| 5.351-5.391 | Unsaturation | 0.00052 | 0.00004 |
| 5.391-5.431 | | 0.00042 | 0.00003 |
| 5.431-5.471 | | 0.00037 | 0.00003 |
| 5.471-5.511 | | 0.00035 | 0.00002 |
| 5.511-5.551 | | 0.00034 | 0.00002 |
| 5.551-5.591 | | 0.00032 | 0.00002 |
| 5.591-5.631 | | 0.00033 | 0.00002 |
| 5.631-5.639 | | 0.00008 | 0.00001 |
| 5.639-5.647 | | 0.00008 | 0.00001 |
| 5.647-5.687 | | 0.00039 | 0.00004 |
| 5.687-5.727 | | 0.00051 | 0.00009 |
| 5.727-5.83 | Urea | 0.00095 | 0.00009 |
| 5.83-5.835 | | 0.00006 | 0.00001 |
| 5.835-5.861 | | 0.00026 | 0.00002 |
| 5.861-5.887 | | 0.00026 | 0.00002 |
| 5.887-5.965 | | 0.00071 | 0.00005 |
| 5.965-5.966 | | 0.00002 | 0.00000 |
| 5.966-6.07 | | 0.00102 | 0.00004 |
| 6.07-6.11 | | 0.00038 | 0.00001 |
| 6.11-6.118 | | 0.00009 | 0.00000 |
| 6.118-6.126 | | 0.00009 | 0.00000 |
| 6.126-6.166 | | 0.00042 | 0.00001 |
| 6.166-6.208 | | 0.00047 | 0.00002 |
| 6.208-6.246 | | 0.00054 | 0.00002 |
| 6.246-6.286 | | 0.00060 | 0.00002 |
| 6.286-6.327 | | 0.00066 | 0.00002 |
| 6.327-6.366 | | 0.00073 | 0.00002 |
| 6.366-6.406 | | 0.00091 | 0.00002 |
| 6.406-6.446 | | 0.00102 | 0.00002 |
| 6.446-6.486 | | 0.00103 | 0.00002 |
| 6.486-6.526 | | 0.00114 | 0.00003 |
| 6.526-6.566 | | 0.00129 | 0.00003 |
| 6.566-6.606 | | 0.00137 | 0.00004 |
| 6.606-6.646 | | 0.00150 | 0.00005 |

| | | | |
|---|---|---|---|
| 6.646-6.686 | | 0.00191 | 0.00006 |
| 6.686-6.726 | | 0.00213 | 0.00006 |
| 6.726-6.766 | Protein | 0.00222 | 0.00007 |
| 6.766-6.863 | | 0.00554 | 0.00014 |
| 6.863-6.905 | Tyrosine | 0.00258 | 0.00007 |
| 6.905-6.926 | | 0.00138 | 0.00004 |
| 6.926-6.966 | | 0.00274 | 0.00007 |
| 6.966-7.007 | | 0.00315 | 0.00009 |
| 7.007-7.11 | Histidine | 0.00701 | 0.00019 |
| 7.11-7.15 | | 0.00254 | 0.00005 |
| 7.15-7.183 | Tyrosine | 0.00207 | 0.00004 |
| 7.183-7.209 | Tyrosine | 0.00157 | 0.00004 |
| 7.209-7.27 | | 0.00350 | 0.00007 |
| 7.27-7.276 | | 0.00037 | 0.00001 |
| 7.276-7.35 | Phenylalanine | 0.00369 | 0.00009 |
| 7.35-7.367 | | 0.00087 | 0.00002 |
| 7.367-7.43 | Phenylalanine | 0.00264 | 0.00008 |
| 7.43-7.47 | | 0.00152 | 0.00006 |
| 7.47-7.51 | | 0.00147 | 0.00005 |
| 7.51-7.543 | | 0.00126 | 0.00004 |
| 7.543-7.566 | 3-methylhistidine | 0.00094 | 0.00003 |
| 7.566-7.606 | 3-methylhistidine | 0.00162 | 0.00006 |
| 7.606-7.646 | 3-methylhistidine | 0.00168 | 0.00005 |
| 7.646-7.686 | 1-methylhistidine | 0.00168 | 0.00005 |
| 7.686-7.772 | Histidine | 0.00366 | 0.00011 |
| 7.772-7.83 | 1-methylhistidine | 0.00214 | 0.00006 |
| 7.83-7.87 | | 0.00138 | 0.00004 |
| 7.87-7.908 | | 0.00134 | 0.00004 |
| 7.908-7.917 | | 0.00035 | 0.00001 |
| 7.917-7.926 | | 0.00035 | 0.00001 |
| 7.926-7.966 | | 0.00150 | 0.00007 |
| 7.966-8.006 | | 0.00155 | 0.00007 |
| 8.006-8.046 | | 0.00161 | 0.00006 |
| 8.046-8.086 | | 0.00165 | 0.00006 |
| 8.086-8.157 | | 0.00293 | 0.00010 |
| 8.157-8.23 | | 0.00289 | 0.00010 |
| 8.23-8.27 | | 0.00151 | 0.00005 |
| 8.27-8.31 | | 0.00145 | 0.00005 |
| 8.31-8.35 | | 0.00140 | 0.00004 |
| 8.35-8.39 | | 0.00135 | 0.00004 |
| 8.39-8.431 | | 0.00133 | 0.00004 |
| 8.431-8.471 | Formic acid | 0.00120 | 0.00003 |
| 8.471-8.511 | | 0.00120 | 0.00003 |
| 8.511-8.551 | | 0.00108 | 0.00003 |
| 8.551-8.591 | | 0.00105 | 0.00003 |
| 8.591-8.631 | | 0.00095 | 0.00003 |
| 8.631-8.671 | | 0.00099 | 0.00003 |
| 8.671-8.71 | | 0.00093 | 0.00003 |
| 8.71-8.751 | | 0.00094 | 0.00004 |

| | | |
|---|---|---|
| 8.751-8.791 | 0.00088 | 0.00004 |
| 8.791-8.831 | 0.00084 | 0.00004 |
| 8.831-8.871 | 0.00079 | 0.00003 |
| 8.871-8.911 | 0.00073 | 0.00003 |
| 8.911-8.951 | 0.00067 | 0.00003 |
| 8.951-8.991 | 0.00060 | 0.00002 |
| 8.991-9.031 | 0.00054 | 0.00002 |
| 9.031-9.071 | 0.00049 | 0.00002 |
| 9.071-9.111 | 0.00045 | 0.00002 |
| 9.111-9.151 | 0.00041 | 0.00002 |
| 9.151-9.191 | 0.00038 | 0.00002 |
| 9.191-9.231 | 0.00036 | 0.00002 |
| 9.231-9.271 | 0.00034 | 0.00002 |
| 9.271-9.311 | 0.00031 | 0.00002 |
| 9.311-9.351 | 0.00028 | 0.00002 |
| 9.351-9.391 | 0.00026 | 0.00001 |
| 9.391-9.431 | 0.00024 | 0.00001 |
| 9.431-9.471 | 0.00022 | 0.00001 |
| 9.471-9.511 | 0.00021 | 0.00001 |

**Supplementary Table 6.** Associations between covariates and metabolomic spectra (CPMG and NOESY sequences), using Principal Component Partial R-square (PC-PR2), *Milieu Intérieur* study, France, 2012 (N=846).

| | CPMG sequence | | NOESY sequence | |
|---|---|---|---|---|
| Covariate | $R^2$ | p-value[1] | $R^2$ | p-value[1] |
| Age | 0.02 | <0.0001 | 0.05 | <0.0001 |
| Sex | 0.05 | <0.0001 | 0.03 | <0.0001 |
| BMI | 0.03 | <0.0001 | 0.02 | <0.0001 |
| Smoking status | 0.01 | 0.5 | 0.03 | 0.02 |
| Physical activity | 0.002 | 0.8 | 0.001 | 0.4 |

[1] p-value for PC-PR2 analysis (Fages A, et al. Batch profiling calibration for robust NMR metabonomic data analysis. Anal. Bioanal. Chem. 405 (27): 8819-8827).

**Supplementary Table 7.** Associations between NMR variables and α-diversity indexes with a Q-value ≤ 0.1 after multiple-testing correction, using Spearman partial correlations, *Milieu Intérieur* study, France, 2012 (N=846).

| | | | Observed richness | | | Chao1 richness estimate | | |
|---|---|---|---|---|---|---|---|---|
| Sequence | Shift (ppm) | Assignment | ρ | p-value[1] | Q-value[2] | ρ | p-value[1] | Q-value[2] |
| CPMG | 0.567-0.607 | Unassigned signal | 0.090 | 0.009 | 0.09 | 0.083 | 0.02 | 0.12 |
| | 1.207-1.311 | Lipoproteins, $-CH_2$ | -0.105 | 0.002 | 0.05 | -0.103 | 0.003 | 0.08 |
| | 1.521-1.527 | $CH_2CH_2COOC$ | -0.101 | 0.003 | 0.05 | -0.097 | 0.005 | 0.08 |
| | 2.151-2.166 | Lipid, $-CH_2=C=C-$ | -0.097 | 0.005 | 0.06 | -0.099 | 0.004 | 0.08 |
| | 2.166-2.167 | | -0.101 | 0.003 | 0.05 | -0.103 | 0.003 | 0.08 |
| | 2.167-2.264 | Acetone, $CH_2CO$ | -0.108 | 0.002 | 0.05 | -0.092 | 0.008 | 0.09 |
| | 2.551-2.571 | Methylamine | 0.118 | 0.001 | 0.04 | 0.105 | 0.002 | 0.08 |
| | 2.831-2.848 | Citrate/$=CH-CH_2-CH=$ | 0.088 | 0.01 | 0.09 | 0.077 | 0.03 | 0.12 |
| | 3.071-3.111 | Creatinine | 0.109 | 0.002 | 0.05 | 0.095 | 0.006 | 0.08 |
| | 3.167-3.269 | Choline and glucose | 0.095 | 0.006 | 0.06 | 0.073 | 0.03 | 0.14 |
| | 3.311-3.312 | Glucose | 0.095 | 0.006 | 0.06 | 0.081 | 0.02 | 0.12 |
| | 3.616-3.711 | Glutamine | 0.087 | 0.012 | 0.1 | 0.094 | 0.007 | 0.08 |
| | 5.127-5.167 | Unassigned signal | -0.105 | 0.002 | 0.05 | -0.104 | 0.003 | 0.08 |
| | 5.864-5.889 | | 0.088 | 0.01 | 0.09 | 0.077 | 0.03 | 0.12 |
| | 5.889-5.969 | | 0.100 | 0.004 | 0.05 | 0.079 | 0.02 | 0.12 |
| | 6.492-6.527 | | 0.099 | 0.004 | 0.05 | 0.091 | 0.008 | 0.09 |
| | 6.728-6.767 | Proteins | 0.108 | 0.002 | 0.05 | 0.100 | 0.004 | 0.08 |
| | 6.857-6.943 | Tyrosine | 0.093 | 0.007 | 0.07 | 0.091 | 0.008 | 0.09 |
| | 6.912-6.943 | Proteins | 0.103 | 0.003 | 0.05 | 0.095 | 0.006 | 0.08 |
| | 6.943-6.968 | | 0.121 | 0.0005 | 0.04 | 0.109 | 0.002 | 0.08 |
| | 7.508-7.539 | 3-methylhistidine | 0.099 | 0.004 | 0.05 | 0.084 | 0.01 | 0.12 |
| | 7.629-7.647 | | 0.102 | 0.003 | 0.05 | 0.095 | 0.006 | 0.08 |
| | 7.647-7.687 | 1-methylhistidine | 0.126 | 0.0002 | 0.04 | 0.105 | 0.002 | 0.08 |
| | 7.687-7.772 | Histidine | 0.110 | 0.001 | 0.05 | 0.107 | 0.002 | 0.08 |
| NOESY | 4.191-4.214 | Unassigned signal | -0.133 | 0.0001 | 0.02 | -0.118 | 0.0006 | 0.04 |
| | 5.830-5.835 | | -0.112 | 0.001 | 0.05 | -0.100 | 0.004 | 0.1 |
| | 5.861-5.887 | | 0.114 | 0.001 | 0.05 | 0.109 | 0.002 | 0.05 |
| | 5.887-5.965 | | 0.126 | 0.0003 | 0.03 | 0.117 | 0.0007 | 0.04 |
| | 5.965-5.966 | | 0.110 | 0.001 | 0.05 | 0.117 | 0.0007 | 0.04 |
| | 6.486-6.526 | | 0.104 | 0.003 | 0.08 | 0.096 | 0.005 | 0.12 |
| | 6.726-6.766 | Proteins | 0.122 | 0.0004 | 0.03 | 0.119 | 0.001 | 0.04 |
| | 7.646-7.686 | 1-methylhistidine | 0.101 | 0.003 | 0.09 | 0.086 | 0.01 | 0.18 |

[1] p-value for non-parametric Spearman partial correlations (adjusted for age, sex, BMI, smoking status, physical activity, and sequencing depth) before Benjamini-Hochberg correction.

[2] Corrected p-value (Benjamini-Hochberg method, 10%-FDR). Only associations with Q-value ≤ 0.1 for observed richness, as well as subsequent associations with Chao1 richness are presented.