Supplementary Materials and Methods.

Metabolomics Data Pretreatment.

Batch Feature Finding: MassHunter Profinder. Feature extraction was carried out on Agilent Profinder B.06.00 (Agilent Technologies, Waldbronn, Germany), a stand-alone feature extraction program for LC/MS-based profiling analyses. Feature extraction reduces acquired data size and complexity by removing redundant and non-specific information by identifying the critical variables (features) associated with the data. Proper feature extraction yields a smaller data set that is more easily processed without compromising the information quality. Profinder is optimized to extract features from large data sets and provides you with an intuitive user interface to inspect and review each feature across the files associated with your data set. Extracted ion chromatograms and mass spectral data related to each feature are revised and compared simultaneously and scored by the software.

Once selected the data files, the recursive feature extraction workflow algorithm was selected, and then the method for the feature extraction algorithm was edited and reviewed. Find compounds by Molecular Features (MFs) was carried out using a prefilter to take peaks with a height greater or equal to 5000 counts, allowing only –H and +HCOO as negative ion species and +H as positive ions, a peak spacing tolerance of 0.0025 *m/z* plus 7.0 ppm, and charge states limited to a maximum of 2. The minimum ion count threshold was two or more ions to filter out compounds with only one ion, which may be a noise spike and not a real compound. The absolute height of compound filters and score were greater or equal to 10000 counts and 80.00, respectively. Moreover, for a compound to be included in the Compound Groups window, it must pass each of the Molecular Feature Extraction (MFE) filters in a certain number of data files. There were two files in at least one sample group, limiting the number of compound groups included in the Compound Groups window to 2000. In the 'find by ion' mode, the retention time score was 90. An Agilent integrator was chosen for peak integration, and any peak filter was applied to integrate all data. In post-processing filters, the absolute height of ion filters was greater or equal to 10000 counts, and two files in at least one sample group were the value for minimum filter matches. The number of compound groups that are included in the compound groups window was limited to 2000.

The file obtained with this pre-processing was created for each sample in format CEF. The definitive files were exported into the Mass Profiler Professional (MPP) software package (version 2.0, Agilent Technologies, Santa Clara, CA, USA) for statistical analysis.

A new experiment typed as unidentified was created in MPP, and a significance test and fold change were done. Select data source and organism selection were the first steps of the experiment creation. The data source used for the experiment was MassHunter Qual, and the organism was Homo Sapiens. In the second step, data was imported from files. The next steps were sample reordering and experiment grouping. After data was imported, several filtering options were applied, such as a minimum absolute abundance of 5000 counts, minimum mass filtering of 70 and maximum mass filtering of 1200, the number of ions required greater or equal to 2, and the option to all charge states forbidden.

Alignment. The next step was the alignment parameters, where unidentified compounds were aligned across the different samples based on their retention times', tolerance, and mass spectral similarity. Compounds from different samples with the same *m/z* (mass tolerance window: 5 ppm, 2mDa) and retention time (tolerance window: 0.15 min) were considered the same.

Normalization and baseline transformation. The next step was to select the pre-processing baseline options. The goal of normalization is to limit systematic non-biological variation to reveal the true biological variation. Sources of systematic variation may be due to the instrument's technical variation, sample preparation, or differing starting material. Normalization steps are performed 'within samples' whereas baselining is 'per entity.' Using baseline transformation can improve visualization of abundance pattern similarities and improve the grouping of masses with similar abundance patterns in clustering analysis.

Data were transformed to the log 2 scale and centered to the median across samples set to lower the relatively large differences in the respective MFs abundance. All compounds were treated equally regardless of their abundance.

Quality control on samples and entities. Quality control can be performed at both the sample level and the mass level to eliminate low-quality samples and entities with unreliable measurements. Carrying over low-quality samples and masses can limit statistically significant findings or informative clustering results. An unsupervised technique was used, principal component analysis (PCA), to visualize similarities or differences between the different samples. An n-dimensional vector approach was applied to visualize sample distribution by the

cumulative correlation of all metabolite data. PCA was calculated according to its values for the first four principal components. To reduce the dimensionality of the data and to select the most representative compounds, ‘‘filter on frequency, on flags, and by abundance’’ was applied to filter out entities that are rarely detected (therefore not very reliable) and to filter out entities that do not have reproducible measurements within a condition. Only the MFs present in at least 80 % of the replicates in at least one condition were considered. This entity filtering allowed creating a higher quality data set so that the following multivariate analysis should be more significant.

Multivariate and statistical analysis. In a second step, statistical comparisons were performed between control and each treatment. Student’s t-test unpaired analyses were applied with a level of significance of $p < 0.05$ with Bonferonni Holm Family-wise Error Rate (FWER) multiple testing corrections and a fold-change cut-off of 2.0. The Student’s t-test was mainly focused on searching for the statistical significance of the null hypothesis between control and each treatment. A list of compounds that significantly differed between groups was generated. Tools such as PCA-3D, Profile Plot, Heat Map, Box Whisker, and Volcano plot were used to visualize some statistical results, using the final list of statistically significant MFs.

Compound identification. Molecular structure correlator. The MassHunter MSC (Molecular Structure Correlator) program correlates accurate mass MS/MS fragment ions for a compound of interest with one or more proposed molecular structures for that compound. The MSC then uses the selected formula, retrieves one or multiple possible structures from a .mol file, an .sdf file, a MassHunter compound database (PCDL) or METLIN/ChemSpider/KEGG/Lipid Maps (on-line databases and libraries), and scores how well each candidate structure correlates with the MS/MS spectrum.