**Usual dietary intake**

From each participant up to three 24-hour food lists (24HFL) and a food frequency questionnaire (FFQ) were available to estimate usual, that means long-term, intake by combining the results of these two instruments. Our approach follows the idea of the two-part statistical model of separation of consumption probability from consumption amount on consumption days, as proposed by the NCI and MSM methods(1; 2; 3; 4). Both models included the same covariates, thereby linking the two parts. The FFQ serves as a covariate for modeling consumption amounts and probabilities based on the 24HFL data. As the 24HFL does not assess consumption amounts, these were estimated based on the data of the Bavarian Food Consumption Survey II (BVS II) Like the KORA studies, the BVS II was conducted in Bavaria and is a cross-sectional population-based survey. We fitted mixed linear models to the BVS II consumption amounts, adjusting for age, sex, BMI, smoking, physical activity, and education level. These models were used to predict these amounts for our KORA participants. As distributions of amounts are skewed, the amounts have been transformed by Box-Cox transformations and predicted amounts have been back-transformed. The intake probability was modeled by fitting a logistic regression model, taking into account the intra-personal variation that arises from up to three measurements per participant by the 24HFL. The usual intake was then derived as the product of the probability of consuming a certain food and the usual amount consumed on a consumption day. See Mitry et al. for a detailed description.

P Mitry, N Wawro, J Six-Merker, D Zoller, C Jourdan, C Meisinger, S Thierry, U Nöthlings, S Knüppel, H Boeing, J Linseisen (2019). Usual dietary intake estimation based on a combination of repeated 24-hour food lists and a food frequency questionnaire in the KORA FF4 cross-sectional study. *(under revision)*

**Preprocessing and fecal sample lab measurement**

Frozen human stool samples in a weight range of 136 to 143 mg were weighed and were placed into 2 ml homogenization tubes containing ceramic beads with a diameter of 1.4 mm (Precellys Ceramic Kit 1.4 mm, 50x 2,0 ml tubes, Peqlab). Water with a ratio of 12.5µl/mg stool was added into the tubes. The samples were then homogenized in Precellys 24 homogenizer (PEQLAB Biotechnology GmbH, Germany) equipped with an integrated cooling unit for 3 times 20 s at 6,500 rpm, with 15 s intervals between the homogenization steps. After homogenization, 450 µl stool homogenate were transferred into 0.5 ml Eppendorf tube for stool dry mass determination, and 100 µl of the homogenate were pipetted onto a 2 ml 96- deep well plate for non-targeted metabolomics analysis.

In addition to samples from this study, a human reference plasma sample (Seralab, West Sussex, UK) and another reference of human stool (Seralab, West Sussex, UK) were pipetted into 1 and 6 wells of the 96- deep well plate, and were extracted as samples of the study. These samples served as technical replicates throughout the data set to assess process variability. LC-MS/MS based techniques are liable, to a greater or lesser extent, to degradation of instrument performance over time, e.g., columns become contaminated, the response of MS can decline over time for similar reason. Aliquots of a reference plasma sample were included across batches of different studies running on our non-targeted metabolomics platform, upon various matrices of sample of the studies. These samples were examined against a set of predefined criteria, including retention time, mass accuracy, fragmentation pattern, and resolution of some selected compounds in the reference samples. In addition to those samples, 100 μL of water was extracted the same way and placed in 6 wells of 96-well plate to serve as process blanks.

Protein was precipitated and the metabolites in the stool homogenates were extracted with 475 µL methanol, containing 4 recovery standards to monitor the extraction efficiency. The extraction efficiency was monitored by assessing the performance of 4 standard compounds which spanned wider range of metabolite classes. The assessment parameters include chromatogram peak shapes, area under the curves of the chromatograms, retention times, mass accuracies, and fragmentation pattern of the compounds. After centrifugation, the supernatant was split into 4 aliquots of 100 µL each onto two 96-well microplates. Two for analysis by 2 separate reverse phase (RP)/UPLC-MS/MS methods with positive ion mode electrospray ionization (ESI), 1 for analysis by (RP)/UPLC-MS/MS with negative ion mode ESI, and 1 for analysis by (HILIC)/UPLC-MS/MS with negative ion mode ESI. Sample extracts were dried on a TurboVap 96 (Zymark, Sotax, Lörrach, Germany). To minimize human error, liquid handling was performed on an automated MicroLab STAR® robot (Hamilton Bonaduz AG, Bonaduz, Switzerland).

All analytical methods utilized a Waters ACQUITY ultra-performance liquid chromatography (UPLC) and a Thermo Scientific Q-Exactive high resolution/accurate mass spectrometer interfaced with a heated electrospray ionization (HESI-II) source and Orbitrap mass analyzer operated at 35,000 mass resolution. Prior to the UPLC-MS/MS runs, the dried extract samples were reconstituted with 80 µL of solvents compatible with each of the 4 methods Each reconstitution solvent contained a series of labeled standard compounds at fixed concentrations to monitor the performance of the metabolomics analysis. One aliquot was analyzed using acidic positive ion conditions, chromatographically optimized for more hydrophilic compounds. In this method, the extract was gradient eluted from a C18 column (Waters UPLC BEH C18-2.1 x 100 mm, 1.7 µm) using water and methanol, containing 0.05% perfluoropentanoic acid (PFPA) and 0.1% formic acid (FA). Another aliquot was also analyzed using acidic positive ion conditions; however, it was chromatographically optimized for more hydrophobic compounds. In this method, the extract was gradient eluted from the same afore mentioned C18 column using methanol, acetonitrile, water, 0.05% PFPA and 0.01% FA and was operated at an overall higher organic content. Another aliquot was analyzed using basic negative ion optimized conditions using a separate, dedicated C18 column. The basic extracts were gradient eluted from the column using methanol and water, but with 6.5 mM Ammonium Bicarbonate at a pH of 8. The fourth aliquot was analyzed via negative ionization following elution from a HILIC column (Waters UPLC BEH Amide 2.1x150 mm, 1.7 µm) using a gradient consisting of water and acetonitrile with 10 mM ammonium formate, pH 10.8. The MS analysis alternated between MS and data-dependent MSn scans using dynamic exclusion. The scan range varied slightly between methods but covered 70-1000 m/z.

Raw data was extracted, peak-identified and QC processed using Metabolon’s hardware and software (Metabolon, Inc., North Carolina, USA). Compounds were identified by comparison to library entries of purified standards or recurrent unknown entities based on 3 criteria: retention index within a narrow RI window of the proposed identification, accurate mass match to the library +/- 10 ppm, and the MS/MS forward and reverse scores between the experimental data and authentic standards. The MS/MS scores are based on a comparison of the ions present in the experimental spectrum to the ions present in the library spectrum. While there may be similarities between these molecules based on one of these factors, the use of all three data points can be utilized to distinguish and differentiate biochemicals.

Overall,1262 metabolites were measured in samples from 1415 participants, whereof 1140 metabolites were in the reference data set provided by Metabolon. For every metabolite in the reference data set, we computed the coefficient of variation (CV) of measurements by run day. The median CV over run days was used as a measure of the variability of the measurement process. A median CV greater than 0.25 was used as cut-off for reliable measurements for a given metabolite, leading to the exclusion of 248 metabolites (5). We also excluded a metabolite unless the CV could be computed for at least two run days. In particular, metabolites that had only missing values in the reference data were excluded. Overall, 85 metabolites were excluded due to missing CV values. Two samples were excluded as they were classified as outliers. An outlier is a metabolite-sample pair where the distance of the log10-transformed metabolite measurement from the mean of the metabolite is greater than 4 times the standard deviation of the metabolite.

Sample weight correction was achieved by dividing the measurement by the sample weight. No further technical adjustment was performed, as inspection of data showed consistent performance across all rundays.

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