Supplementary 1: Microbiome analysis and statistical plan

To assess changes in microbial composition, functionality and diversity, faecal samples will be collected at baseline, and after 12, 26, and 52 weeks of intervention initiation. Samples will be analysed using metagenomic shotgun sequencing profiles. First, the different study groups will be analysed separately. A non-parametric statistical test will be used in which the variables prior to the intake of the dietary intervention will be compared with the outcomes after the intervention. This will be analysed with paired Wilcoxon signed-rank tests. For analysis between groups, Mann-Whitney *U*-tests will be used.

For the metagenomic sequencing: the extracted DNA will be sequenced with an Illumina HiSeq 2000 platform to generate approximately 8 Gb of genomic data per sample containing 150 bp paired-end reads. The Nextera XT Library preparation kit will be used for genomic library preparation, and Trimmomatic will be used to remove adapters and trim the ends of the metagenomic reads (1). Filtering of the cleaned metagenomic reads as well as the generation of taxonomic and metabolomic potential profiles will be performed using the BioBakery pipeline (2). For instance, the software tool MetaPhlAn3 will be used to profile the taxonomic composition, expressed as transformed relative abundances, and the composition of functional pathways will be annotated using HUMAnN3 and the multi-organism database MetaCyc (available at https://metacyc.org). Ecological measurements such as bacterial richness (Shannon index and bacterial diversity) and bacterial interindividual variations (Bray-Curtis distances, beta-diversity) will be calculated using the *vegan* R package. To test the proportion of explained variance in Bray-Curtis distances of clinical characteristics, permutational multivariate ANOVA (PERMANOVA, using 1,000 permutations) will be performed using the ADONIS function in the *vegan* R package. GLMMs

will be performed using MaAslin (2) to explore the associations between the relevant outcome parameters and the gut microbiota composition.

1. Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 2014;30:2114–20.
2. Beghini F, McIver LJ, Blanco-Míguez A, et al. Integrating taxonomic, functional, and strain-level profiling of diverse microbial communities with bioBakery 3 eLife 10:e65088. https://doi.org/10.7554/eLife.65088