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Dietary convergence induces individual responses in faecal microbiome composition

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ABSTRACT

Background Dietary variation has been identified as a key contributor to microbiome diversification. However, assessing its true impact in a cross-sectional setting is complicated by biological confounders and methodological hurdles. We aimed to estimate the impact of a reduction of dietary variation (dietary convergence) on faecal microbiota composition among individuals consuming a Western-type diet.

Methods 18 healthy volunteers recruited in the region of Flanders (Belgium) were followed up for 21 days. Participants were allowed to consume their habitual diet during a baseline and follow-up period (7 and 8 days, respectively), intersected by a 6-day intervention during which dietary options were restricted to oat flakes, whole milk and still water. Faecal samples were collected on a daily basis. Quantitative microbiome profiles were constructed, combining 16S rRNA gene amplicon sequencing with flow cytometry cell counting. Blood samples were taken at the beginning and end of each study week.

Results While the intervention did not affect transit time (as assessed through the analysis of stool moisture), consumption of the restricted diet resulted in an increased prevalence of the *Bacteroides2* microbiome community type. Microbial load and *Faecalibacterium* abundance decreased markedly. Despite dietary restrictions, no convergence of microbial communities (reduction of interindividual and intraindividual variation) was observed. The effect size (ES) of the intervention on genus-level microbiome community differentiation was estimated as 3.4%, but substantial interindividual variation was observed (1.67%–16.42%).

Conclusion The impact of dietary variation on microbiome composition in a Western population is significant but limited in ES, with notable individual exceptions. Dietary convergence does not invariably translate into interindividual convergence of faecal microbial communities.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Changes in diet have been shown to induce swift and reversible changes in the gut microbiota. A first exploratory intervention study imposing a homogenous diet has indicated that converging food intake reduces interindividual variation in microbiome composition.

WHAT THIS STUDY ADDS

⇒ Following a 6-day dietary intervention during which participants consumed only oats, milk and water, intraindividual microbiome variation was shown to increase. The prevalence of *Bacteroides2* microbiome communities increased, notably accompanied by a decrease in the abundance of anti-inflammatory *Faecalibacterium* sp, a pattern typically associated with dysbiosis.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ The present study shows that restricted dietary options do not consistently induce convergence of the faecal microbiota across individuals, emphasising the personalised response of the microbiota to dietary modulation. We estimate the effect size of dietary variation in a Western population as 3.4%, with individual responses ranging between 1.67% and 16.42%. These estimates will aid in design and associated sample size calculations and interpretation of future intervention studies.

INTRODUCTION

While the human gut microbiota has been firmly linked to host health status,[1–3] current insights regarding community variation and potential modulation are still largely derived from cross-sectional monitoring efforts.[4–8] Such studies are inherently limited in their ability to characterise the impact of day-to-day variation in host and environmental factors on gut microbial communities. More specifically, they can be expected to underestimate the impact of diet on microbiome composition and metabolic activity.[9] Besides practical issues regarding accurate and timely collection of dietary data,[10] the extent of food options available combined with individual preferences generates a nearly unique dietary

fingerprint for every subject participating in a microbiome study. In a large-scale cross-sectional setting, this variety can be translated into associations between microbiome composition and widely consumed food groups or macronutrients. Yet, part of the dietary variation remains unassessed by such higher-level analyses and can be hypothesised to contribute to the inflation of unspecified interindividual microbiome differentiation.[4]

When conducting an intervention trial targeting the promotion of host health through microbiota modulation, background knowledge on expected diet-driven microbiome variation is crucial for the study design as well as interpretation of findings. In the present exploratory study, we aimed to quantify the impact of restricted food choices on microbiome variation among healthy individuals. Limiting dietary options to three food items during a 6-day intervention period, we assessed the impact of a converging diet on faecal microbiome composition.

METHODS

Study design

21 healthy volunteers were recruited in the region of Flanders (Belgium) to participate in an intervention study assessing the impact of restricted dietary options on faecal microbiome composition. The initial recruitment target was set at 20 participants based on the study of David *et al*.[9] Inclusion criteria were defined as follows: aged between 18 and 50 years and willingness to participate in the study. Given the nature of the intervention, relaxed exclusion criteria were handled: a body mass index (BMI) lower than 19 km/m^2 or higher than 35 km/m^2, the use of antibiotics less than 6 months prior to the start of the study, unwillingness or inability to follow the diet, inability to understand or sign the informed consent form, and pregnancy. Additionally, individuals with diagnosed gastrointestinal disorders such as gastrointestinal infection, inflammatory bowel disease, irritable bowel syndrome and colorectal cancer were excluded from participation. Over a 21-day study period, a reversal A–B–A design was applied, covering a baseline observation week, a 6-day intervention period during which participants only consumed food items provided by the research team and a follow-up period. Food items provided were oats (brand: Quaker), whole milk (Everyday) and still water (Chaudfontaine; nutritional value of food items as declared by manufacturers provided in online supplemental table 19). During baseline and follow-up, no dietary restrictions were imposed. At the moment of a first study visit, the participant's health was assessed by a medical doctor, and height, weight, waist and hip circumference and blood pressure were registered. A blood sample was taken for further analysis by an independent, certified clinical laboratory (Centrum voor Medische Analyse, Belgium; online supplemental table 1). Participants were asked to refrain from calorie intake for 8 hours before blood sample withdrawal (fasting status was recorded). During each weekly study visit, the study doctor followed up on participants' health and an additional blood sample was taken (four in total). Participants collected faecal samples on a daily basis, with a maximum of one sample per day following the procedures developed in the framework of the Flemish Gut Flora Project (FGFP).[4] Sampling kits including detailed instructions were handed over to the participants during their weekly visit to the research facility. After completion of the procedure, samples were stored immediately in participants' home freezers at −18°C. Within 1 week, participants were required to ensure cooled transport of samples to the research facility. There, samples were stored at −80°C until further analyses.

Faecal sample characterisation

Faecal cell counts were determined using flow cytometry as described previously.[11] Analyses were performed in duplicate; one sample was excluded from cell counting due to insufficient availability of faecal material. Stool moisture content was assessed by calculating the percentage of mass reduction after freeze-drying of non-homogenised faecal aliquots.[11]

DNA extraction, sequencing and data preprocessing

Faecal DNA extraction and microbiota profiling were performed as described previously.[11] Briefly, DNA was extracted from faecal material using the PowerMicrobiome RNA isolation kit (Mobio), with the addition of a 10 min incubation step at 90°C after initial vortexing. For amplicon sequencing, the V4 region of the 16S rRNA gene was amplified using the 515F/806R primer pair. Sequencing was performed on the Illumina MiSeq platform to generate paired-end reads of 250 bases in length in each direction. Amplicon data demultiplexing was performed using LotuS (V.1.565).[12]

Microbiome taxonomic profiling

Demultiplexed amplicon sequencing data were preprocessed and posterior taxonomical assignment was performed using the DADA2 pipeline (V.1.6.0)[13] with the Genome Taxonomy Database (GTDB) classifier (V.R86)[14] with default parameters. Briefly, the initial 30 base pairs were excluded, and the sequence length was adjusted to 130 base pairs for the forward strand and 200 base pairs for the reverse strand. The sequence error rate, dereplication, sample composition inference and chimeric sequence removal were performed using the default parameters of DADA2.[13] Sequences identified as *Runella* (added as control samples to each batch of faecal samples analysed), unclassified bacteria, unclassified Archaea, mitochondria or chloroplast were removed. Relative microbiome profiles were obtained by downsizing each sample matrix to 10 000 reads through random selection. Quantitative microbiome profiles (QMP) were constructed as described previously.[11] Eight samples (with low sampling depth) were excluded from QMP analyses to optimise the resulting final sampling depth (2.08e^{-8}

copy number variation-corrected reads per cell in a gram of faeces).

Analysis of food diaries

Throughout the study period, participants kept detailed food diaries, recording all foods and beverages consumed. They documented each item in grams or millilitres, specifying the quantities for every meal and snack. To ensure accuracy, participants received an instructional manual with guidelines on how to complete the diaries effectively. Once collected, the data were analysed by nutritionists. A systematic workflow was designed to process and analyse the food diaries. First, the list of non-composite products consumed during the study period was complemented with the ingredients constituting composite meals. The latter was based on data provided by participants for self-cooked meals or on external sources[15 16] for standard recipes. Second, portion sizes were quantified. When participants did not provide quantitative data regarding the food items consumed, portion sizes were determined using household measures (eg, a cup, a glass, a teaspoon)[17] or manufacturer-provided information. The third step concerned data linkage, allowing to determine intake based on region-specific and brand-specific databases.[15 18] Data linkages resulted in estimates of intake in terms of nutritional parameters such as energy content as well as quantities of macronutrients carbohydrates, proteins, fats, water, fibres and alcohol consumed. Fourth, food consumption data were categorised into 18 primary groups based on the widely used international GloboDiet food classification system.[19–21] Categories used for classification comprised alcoholic beverages, cakes and sweet biscuits, cereal (products), condiments and sauces, dairy (products), egg (products), fats and oils, fish and shellfish, fruits, nuts, and olives, legumes, meat (products), non-alcoholic beverages, potatoes, savoury snacks, soups and stocks, sugar and confectionery, and various vegetables,[22] in line with the approach applied for the Belgian food consumption survey.[22] The latter enabled a fifth and final validation step, during which the data obtained were analysed in function of age, gender, length and weight and subsequently compared with established regional population averages[22] in order to verify whether their distribution corresponded with the expected ranges.

Statistical analyses

Statistical analyses were performed in R (V.4.1.0) with RStudio (V. 2022.07.2) using the packages vegan (V.2.6-2),[23] phyloseq (V.1.38.0),[24] DirichletMultinomial (V.1.28.0)[25] and Survey (V.4.1-1).[26] Non-parametric statistical tests were used, as data did not follow normality or equal variance assumptions. All p values were corrected for multiple testing using the Benjamini-Hochberg method (reported as adjusted p value (adjP)) unless specified otherwise. The significance threshold was set at adjP<0.05.

Multivariate analysis of microbiome and dietary composition

Unconstrained ordination techniques, namely principal component analysis and its extension to non-Euclidean space, principal coordinates analysis, were used for visualisation of dietary and microbiome composition, respectively. Constrained ordination was applied to determine the explanatory power of metadata variables (notably classification into baseline, intervention and follow-up period) on microbiome composition variation, in single (distance-based redundancy analysis (dbRDA), capscale function) or multivariable models (stepwise dbRDA, ordiR2step). Multivariate analysis of dispersion (betadisper function (vegan)) was used to assess the impact of the dietary intervention on the variance within microbiome and dietary data. The function is an extension of Levene's test for homogeneity of variances, measuring distances of samples to the centroids of their respective groups in a multivariate space, thereby assessing changes in dispersion. The statistical significance of observed differences was determined using a post hoc Wilcoxon rank-sum test.

Analysis of community types

Community typing (or enterotyping) based on the Dirichlet-multinomial mixtures approach[25] was performed on the complete set of 381 stool samples collected merged with 2998 samples from the FGFP cohort,[4] all downsized to 10 000 reads. The optimal number of Dirichlet components based on the Bayesian information criterion was four. The clusters were labelled *Prevotella*, *Bacteroides1* (Bact1), *Bacteroides2* (Bact2) and *Ruminococcaceae* community types as described in Vandeputte *et al*.[27] When comparing cohort community-type prevalence between study phases, a weighted approach was implemented to account for variation in number of samples collected per participant. Each sample was attributed a weight calculated as the inverse of the number of the total number of samples collected by a specific participant in the study period analysed. Additionally, community-type stability was quantified for each subject during baseline by dividing the number of faecal samples stratified into the dominant fraction by the total amount collected in that specific phase. A baseline community type was determined based on an individual's most prevalent enterotype observed in that study period.

Determination of lag time

To determine the transit-time associated lag between ingestion and egestion, for each individual, food group and genus-level microbiome matrices were correlated using a Procrustes test, assessing maximal similarity between multivariate datasets on rotation. Using an iterative approach, time offsets of 0,+1, +2, and +3 days between food (rotated matrix) and microbiome matrices (target matrix) were subjected to Procrustes analysis. The offset resulting in maximum correlation of the rotation was selected for each individual, and the offset was used as an estimate of lag time.

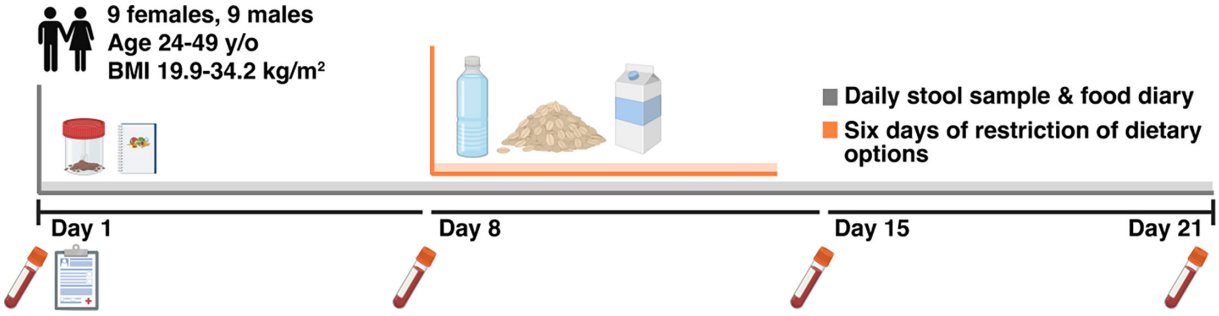

Figure 1 Design of The Oatmeal Study. The 21-day study period consisted of a baseline, an intervention and a follow-up phase following an A–B–A reversal design. During the intervention period, participants consumed only oatmeal, whole milk and still water. A food diary was kept throughout the entire study period, and a daily faecal sample was collected. An initial health assessment was performed by a medical practitioner and blood samples were taken on a weekly basis. BMI, body mass index.

RESULTS

In the present study, 21 healthy volunteers were recruited to participate in an intervention study (The Oatmeal Study; figure 1). Briefly, following a reversal study design, participants were allowed to consume their habitual diet during a baseline and follow-up period (7 and 8 days, respectively), intersected by a 6-day intervention during which dietary options were restricted to oat flakes, whole milk and still water provided by the research team. No constraints regarding cooking methods or portion sizes (*ad libitum*) were imposed. While the food products selected did provide all essential micronutrients, their combination could be expected to represent a significant decrease in dietary variation for all participants. Given the monotonous nature of the diet imposed, additional recommendations to maintain caloric intake at baseline levels were deemed futile. Instead, to keep track of actual food consumption, participants kept detailed dietary records during the entire study period. A maximum of one faecal sample was taken daily. At the start of the study, participants were screened by a medical practitioner; blood samples were taken on days 1, 8, 15 and 21. 18 participants (female/male ratio=1; median age=28 (24–49) years; median BMI=23.6 (19.9–34.2) kg/m^2; average energy intake baseline=2300 (1549–3177) kcal; stool moisture online supplemental table 1) completed the study (three volunteers dropped out before completion of the intervention; online supplemental figure 1).

The analysis of the records kept by participants clearly demonstrated the converging effect of the restricted diet on weight-based daily food intake (dispersion test on Euclidean distances at food group level, n=378, variance 380 pre/post intervention vs 266 during the intervention, p<0.001; figure 2A,B and online supplemental table 2). Overall, the intervention explained 9.49% of dietary variation observed at the food group level (Adonis, n=378, p=0.01), with intraindividual effect sizes (ESs) ranging between 24.9% and 47.3% (online supplemental table 3).

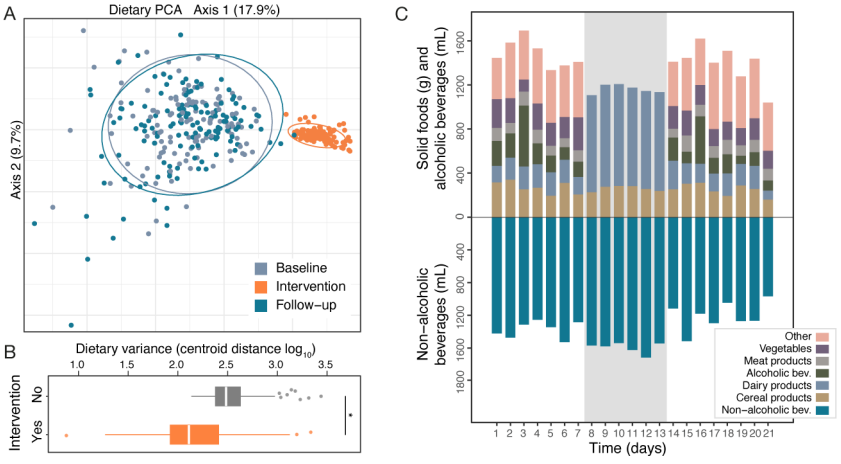

Figure 2 Restriction of dietary variation as observed during the intervention period. (A) Principal component analysis of weight-based, food-group-level dietary variation over the study period (Euclidean distance; n=378). Each dot represents the dietary consumption of an individual participant during a day. Ellipses represent the 95% CI of the relevant group. (B) Boxplot presenting dietary variance observed before/after (baseline and follow-up combined) and during the intervention period. Variance significantly decreased during the intervention (n=378; dispersion test on Euclidean distances at food group level). The body of the boxplot represents the first and third quartiles of the distribution, with the median line, and the whiskers extend from the quartiles to the last data point within 1.5 times IQR, with outliers beyond. *Adjusted p value<0.001. (C) Distribution of weight-based average daily consumption of food groups (n=378). CI, confidence interval; IQR, interquartile range; PCA, Principal Component Analysis.

The quantity of food consumed on a daily basis decreased significantly during the intervention period (macronutrient and fibre intake; Wilcoxon rank sum, n=378, ES=−0.35, adjP<0.001; figure 2C and online supplemental figure 2A and online supplemental tables 4 and 5), which was also reflected by a reduction of caloric intake of 31.5% (Wilcoxon rank sum, n=378, ES=−0.45, adjP<0.001; online supplemental figure 2B and online supplemental tables 6 and 7). At macronutrient level, a significant weight-based as well as caloric decrease in intake of fats (Wilcoxon rank sum, n=378, ES=−0.49, adjP<0.001 and ES=−0.49, adjP<0.001, respectively), carbohydrates (ES=−0.30, adjP<0.001; ES=−0.30, adjP<0.001) and proteins (ES=−0.24, adjP<0.001; ES=−0.24, adjP<0.001) was reported. In contrast, fibre intake increased both quantitatively (ES=0.18, adjP<0.001) and in terms of energy intake (ES=0.18, adjP<0.001) during the intervention. No significant changes in water consumption were observed (Wilcoxon rank sum, n=378, ES=0.095, adjP=0.06).

While intervention-associated changes in clinical blood parameters remained limited to a temporary increase in total bilirubin levels (characteristic for caloric restriction[28]; Kruskal-Wallis, n=51, adjP=0.001; online supplemental figure 3 and online supplemental tables 8 and 9), far larger fluctuations were anticipated to occur at the level of the faecal microbiome (online supplemental figure 4). To identify stool samples truly resulting from digestion and subsequent microbial fermentation of the food components making up the restricted diet, a transit-time-dependent lag phase between intake and egestion was determined. For each participant, weight-based food group and quantitative genus-level microbiome matrices were correlated with an offset of 0, 1, 2 and 3 days in order to establish an optimal fit (Procrustes; online supplemental table 10). Median estimated lag time was 2 days, in accordance with literature on average transit times among adult Western populations,[29] with individual lag times ranging between 0 and 3 days (online supplemental figure 5). Excluding lag samples from the dataset (online supplemental figure 6), a marked decrease in faecal microbial load was observed to occur during the intervention, with median cell counts being respectively 40.2 and 30.2% lower than measured during baseline and follow-up (Kruskal-Wallis, n=266, adjP<0.001; figure 3A and online supplemental table 11). No significant associated changes in stool moisture content were detected (Kruskal-Wallis, n=266, adjP=0.87; online supplemental figure 7). Across the full study period, we did observe a positive (r=0.24) correlation between cell counts and energy intake (corrected for lag time; linear mixed-effects model, n=331, F=15.39, p<0.001; online supplemental figure 8). In terms of microbiome community types or enterotypes,[30 31] the imposed restriction of dietary options resulted in a transient shift in community prevalence (weighted contingency test, n=266, χ^2=3.74, p=0.001, figure 3B). More specifically, an increase in the prevalence of the aberrant community type previously labelled Bact2[32–34] was observed during the intervention (weighted pairwise contingency test, n=174, χ^2=15.69, adjP<0.001) at the expense of Bact1 detection (χ^2=16.56, adjP<0.001; online supplemental figure 9 and online supplemental table 12). When resuming habitual diet post intervention, Bact2 prevalence decreased again (n=152, χ^2=5.05, adjP=0.02), compensated by a re-emergence of Bact1 (χ^2=5.25, adjP=0.04). Across the study period, no

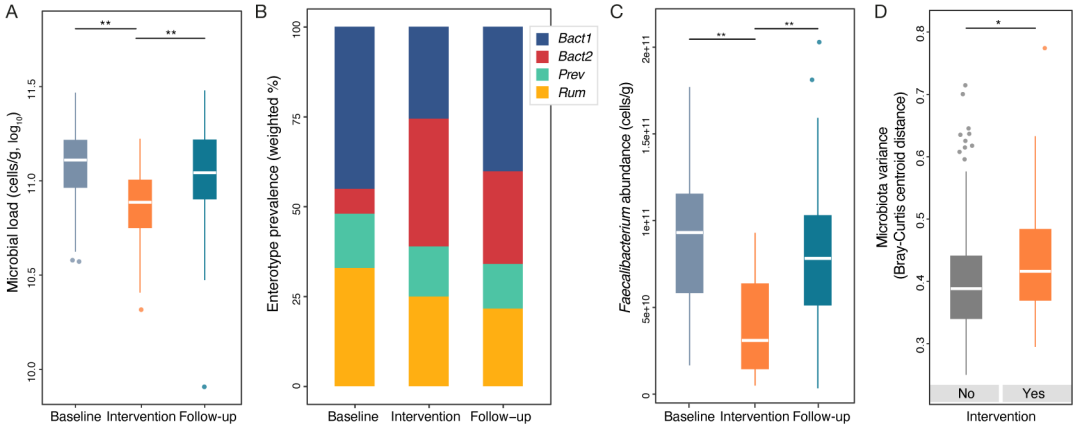

Figure 3 Microbiome variation associated with dietary convergence. (A) Boxplot comparing faecal microbial load variation over study phases. Load decrease during the intervention period (n=266; Kruskal-Wallis with post hoc Dunn test). (B) Weighted distribution of community-type prevalence over study phases. Bact2 prevalence increased significantly on dietary convergence (n=266; online supplemental figure 9). (C) Boxplot representing *Faecalibacterium* abundance distribution over study phases. A decrease in the abundance was observed during the intervention period (n=266; Kruskal-Wallis with post hoc Dunn test). (D) Boxplot presenting quantitative microbiome variance observed before/after (baseline and follow-up combined) and during the intervention period. Variance significantly increased during the intervention (n=266; dispersion test on Bray-Curtis dissimilarities at genus level). (A–D) Analyses performed excluding lag samples. (A,C,D) The body of the boxplot represents the first and third quartiles of the distribution, with the median line and the whiskers extend from the quartiles to the last data point within 1.5 times IQR, with outliers beyond. *adjP<0.05, **adjP<0.001. adjP, adjusted p value; Bact1, *Bacteroides1;* Bact2, *Bacteroides2;* IQR, interquartile range; Prev, *Prevotella;* Rum, *Ruminococcaceae*.

significant association between caloric intake and faecal enterotypes could be established (Kruskal-Wallis, n=296, p=0.05).

Community-level changes in enterotype prevalence reflected in genus-level variation in microbiome composition (online supplemental table 13). Among the taxa decreasing in the abundance included butyrate-producing *Faecalibacterium* (Kruskal-Wallis, n=266, adjP<0.001; figure 3C and online supplemental table 13), previously described as low abundant in Bact2-typed faecal samples. In contrast, GTDB-defined[14 35] genus *Blautia_A* (comprising, eg, the formate-consuming acetogen *Blautia hydrogenotrophica*)[36] significantly increased during the intervention phase (Kruskal-Wallis, n=266, adjP<0.001; online supplemental figure 10 and online supplemental table 13). Overall community diversity was lower during the intervention period (Shannon index; Kruskal-Wallis, n=266, adjP=0.03; online supplemental table 14). Notwithstanding dietary convergence, consumption of a restricted diet resulted in an increase of microbiome variation (Dispersion test on Bray-Curtis dissimilarities at genus level, n=266, variance 0.406 pre/post intervention vs 0.433 during the intervention, p=0.02; figure 3D). When restricting analyses to intraindividual variation, a similar decrease in variance was observed following dietary restriction (Friedman, n=54, adjP<0.001; online supplemental table 15).

Next, using the full dataset, the ES of the restricted diet on genus-level microbiome composition was weighted against the impact of established microbiome covariates as well as the potentially confounding effects of auxiliary metadata. For this purpose, and in line with the physiological hypothesis behind the concept, lag samples were added to the study period preceding the time of sampling. While non-specified interindividual differences emerged as the main covariate of microbiome variation (ES of 55.8%; Bray-Curtis dissimilarity dbRDA, n=262, p<0.001; figure 4A,B and online supplemental table 16), study period (classifying samples as taken during baseline, intervention or follow-up) added 3.4% of non-redundant explanatory power to the model generated. As such, the effect of the intervention outweighed the impact of stool moisture (1.2%), previously identified as the key contributor to variation in faecal microbiota composition in cross-sectional population studies.[27 29] The multivariate model did not identify a significant, non-redundant association between energy intake and community variation. Substantial interindividual differences in microbiota response to the intervention were noted, with participant-specific ES ranging between 1.67% and 16.42% (online supplemental table 17). The intraindividual impact of the intervention on dietary and faecal microbiome composition, as evaluated by their respective ES, was not significantly correlated (Spearman's rank correlation, n=18, ρ=0.048, p=0.85), nor was there a significant association between reduction in average caloric intake and magnitude of community shift (Spearman's rank correlation, n=18, ρ=−0.15, p=0.55). Screening for baseline conditions (days 1–7) that could potentially predict persistence or susceptibility of the microbiome toward dietary modulation, ES were analysed in function of age, gender, BMI, median faecal microbial load and moisture, median richness, microbiota variance, community type and community-type stability (online supplemental table 18). Although no longer statistically significant after correction for multiple testing, associations were identified between the intervention ES and both gender (higher impact in male participants; Wilcoxon rank sum, n=18, ES=0.51, p=0.03)

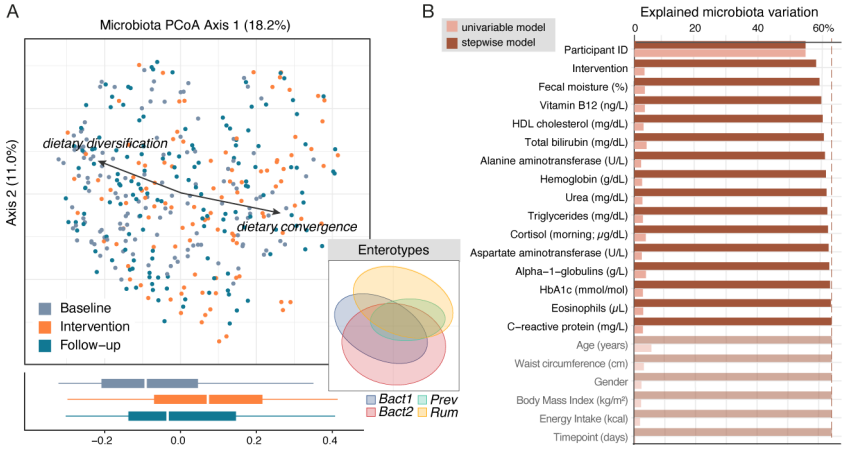

Figure 4 Impact of dietary convergence on microbiome diversification. (A) Principal coordinate analysis representing quantitative genus-level microbiome variation (n=381; Bray-Curtis dissimilarity). Arrows on the plot indicate direction and relative magnitude of shifts observed between baseline and intervention (dietary convergence) vs intervention and follow-up (dietary divergence). Microbiome community-type distribution is shown on the insert. Boxplots below axis 1 show the distribution of microbiomes belonging to the different study phases along the principal axis of variation. (B) Distance-based redundancy analysis of the association of potential covariates with quantitative genus-level microbiome variation (n=294). Transparent bars correspond with covariates that, while displaying a significant association in a univariable model, did not contribute significantly to microbiome variation in the stepwise variant. Bact1, *Bacteroides1;* Bact2, *Bacteroides2;* HbA1c, haemoglobin A1C; HDL, high-density lipoprotein; Prev, *Prevotella;* Rum, *Ruminococcaceae*.

and baseline intraindividual microbiota variance (Spearman's rank correlation, n=18, ρ=0.49, p=0.04).

DISCUSSION

The exploration of associations between microbiota composition and variation is an area of intensive research.[37] This study stands out from previous investigations by specifically examining the impact of dietary restriction, using a high sampling frequency combined with extensive phenotyping, implementing quantitative microbiome profiling and introducing the concept of lag time between food intake and its anticipated effect on the microbiota. Although the cohort size is limited (hampering extrapolation of results to a broad population), this approach allowed for both significant and meaningful observations. Based on a cohort of n=18 individuals (331 samples) and using a multivariate model, we estimated the impact of dietary convergence on the microbiome of healthy individuals consuming a Western-type diet as 3.4% of the variation observed—an estimate that can be interpreted reversely as the ES of dietary preferences on the faecal microbiota. Overall, a decrease in faecal microbiota diversity was observed during the intervention period, in line with previous reports on associations between dietary variation and microbiome composition.[38 39] In terms of community-type variation, the effect of the intervention translated in a transient increase in the prevalence of the putatively dysbiotic Bact2 enterotype characterised by an accompanying decrease in the abundance of butyrate-producing *Faecalibacterium*, in line with a microbiome-rooted definition of dysbiosis that would extend beyond the context of disease associations.[32] These findings largely fit within the concept of successional ecosystem maturation we postulated previously.[40] This concept describes the faecal microbiome as the outcome of a community maturation process starting the moment the chyme passes the ileocaecal valve, characterised by a gradual increase in community richness and ending abruptly on egestion. The driving force behind this process has been hypothesised to be nutrient depletion alongside passage through the large intestinal tract,[29 40 41] with readily fermentable substrates such as oligosaccharides and polysaccharides gradually being replaced by proteins as main energy sources of the community colonising the digested food matrix.

In healthy individuals on an average Western diet, substrate depletion is paced by transit time, with immature, low-richness (Bact2) communities dominated by fast-growing, saccharolytic taxa reflecting short colonic retention times.[41 42] In the present study, following a dramatic reduction of dietary substrate variation, transit time no longer emerged as the determining factor driving nutrient availability and the associated ecosystem maturation and diversification in the colon. Indeed, the observed changes in microbiome diversity and community-type prevalence were not associated with an increase in stool moisture (a proxy of colonic transit time).[29] Instead, succession appears hampered or delayed by the changes in composition of the chyme imposed by the dietary restriction. Hampered, as the resulting reduction of available substrate variation might not contain the full spectrum of (micro)nutrients required to sustain the growth of secondary colonisers and move further along the expected gradient of ecosystem maturation. Alternatively, the lowered caloric intake might not have been sufficient to sustain a critical bacterial load required to initiate, for example, cross-feeding interactions with secondary colonisers and facilitate a next step in successional development. Delayed, as the increase in readily fermentable substrates reaching the colon (fibres, but also lactose, depending on participants' digestive capacities) might prolong the initial colonisation phase and postpone the development of more proteolytic communities (an effect that has been proposed as a potential target of prebiotic interventions).[43] These scenarios, which are not mutually exclusive, would result in an overall reduction of community diversity and increased prevalence of Bact2 communities, which have been shown to be intrinsically more variable than more mature configurations.[44] Variation among resulting communities obfuscates the impact of the dietary intervention, contributing to the observed lack of convergence across individuals.

The results of the present study both contradict and confirm different aspects of microbiome-diet associations identified by previous intervention studies. On one hand, our findings contrast markedly with those of a recently published intervention study during which participants were requested to consume a homogenous diet recapitulating nutritional ranges of the average adult US inhabitant for 7 days.[45] Based on a more limited number of faecal samples analysed, intraindividual microbiome variation was observed to decrease during the dietary intervention. In line with the concept of successional maturation,[27] these conflicting findings can be hypothesised to result from the nature of the diet imposed by Guthrie *et al*,[45] that is, targeting a nutritionally more balanced consumption pattern and comprising substantially less fibre than the one applied in the present study (1.1 g per 100 g of the homogenous diet administered by Guthrie *et al* vs an estimated 3.0 g per 100 g of oatmeal in the present study). Notwithstanding differences in study design and outcomes, the authors estimated the ES of their intervention on microbiome variation as 1%, smaller but comparable in magnitude to the estimate we reported. On the other hand, our study does recapitulate the reduction in faecal bacterial load following caloric restriction reported by von Schwartzenberg *et al*.[46] Analysing faecal microbiomes of 40 overweight/obese postmenopausal women on an 800 kcal/day liquid diet, the authors observed a significant decrease in qPCR-assessed 16S rRNA gene amplicon copies per gram of wet stool weight compared with baseline.

STUDY LIMITATIONS

Beyond the limited cohort size, the present study has several limitations. These include the somewhat arbitrary BMI ranges used as exclusion criteria, the reliance on self-reported dietary intake and the use of amplicon sequencing data for microbiota analysis. Regarding BMI, a narrower inclusion range would be preferable to mitigate potential confounding effects of obesity. In terms of dietary data collection, food diaries are inherently subject to reporting bias. Although standardised instruction manuals were provided to minimise this bias, no specific training sessions were conducted for participants. Despite these limitations, this pilot study provides valuable insights for future research. Subsequent studies examining the impact of specific food components on the microbiota should account for these limitations, incorporate metagenomic sequencing to assess functional microbiota variations and consider metabolomic analyses to enhance the mechanistic understanding of microbiome shifts.

CONCLUSIONS

Based on the present, explorative dietary intervention study, we qualify the impact of dietary variation on microbiome diversification in a culturally homogeneous population to be significant but, while outweighing established covariates such as stool moisture and notwithstanding substantial interindividual variation, limited in ES. Our analyses demonstrate that dietary convergence does not invariably result in a convergence of microbiome community profiles and advocate for the interpretation of microbiome studies based on the concept of successional maturation.

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Contributors JR and GF conceived and designed the study. Cohort recruitment, data collection and analyses of faecal samples were performed by GK. JS and SV supervised the intervention study as medical doctor and JS performed blood sampling. Preprocessing of microbiome data was handled by S-VS, JFV-C and RYT. AV and EB analysed the food data collected, CM assisted in the interpretation. Statistical analyses were designed by SV-S, SP and GF. Data analysis was performed by AV, EB, SV-S and SP. AV, SV-S, JR and GF interpreted the results. The manuscript was drafted by AV, S-VS, JR and GF and critically revised by all authors. JR is the guarantor of this manuscript. All authors approved the final version for publication.

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Competing interests SV-S, JS, JR and GF are listed as inventors on patent WO2019115755A1 'A new inflammation-associated, low cell count enterotype', in the name of VIB VZW, Katholieke Universiteit Leuven, KU Leuven R&D and Vrije Universiteit Brussel, covering the features of the microbiome associated with inflammation. SV-S, SP, JR and GF are credited as inventors on WO2022073973A1 'Means and methods to diagnose gut flora dysbiosis and inflammation', in the name of VIB VZW, Katholieke Universiteit Leuven, KU Leuven R&D and University of Bristol, covering methods to diagnose and treat or reduce the severity of gut microbiota dysbiosis as well as of gastrointestinal inflammation and inflammation-associated disorders or conditions in a subject in need thereof. RYT and JR are included as inventors on the patent application WO2017109059A1, in the name of VIB VZW, Katholieke Universiteit Leuven, KU Leuven R&D and Universiteit Gent covering methods for detecting the presence or assessing the risk of development of inflammatory arthritis disease. SV received financial support for research from AbbVie, J&J, Pfizer, Takeda and Galapagos and speakers' and/or consultancy fees from AbbVie, Abivax, AbolerIS Pharma, AgomAb, Alimentiv, Arena Pharmaceuticals, AstraZeneca, BMS, Boehringer Ingelheim, Celgene, Cytoki Pharma, Dr Falk Pharma, Ferring, Galapagos, Genentech-Roche, Gilead, GSK, Hospira, IMIDomics, Janssen, J&J, Lilly, Materia Prima, Mestag Therapeutics, MiroBio, Morphic, MRM Health, Mundipharma, MSD, Pfizer, Prodigest, Progenity, Prometheus, Robarts Clinical Trials, Surrozen, Takeda, Theravance, Tillotts Pharma AG, VectivBio, Ventyx, Zealand Pharma. All other authors declare no competing interests.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

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Ethics approval This study involves human participants. All experimental protocols were approved by the ethical commission of UZ Leuven (Ethische Commissie Onderzoek, UZ/KU Leuven, reference numbers: S58005). Study design complied with all relevant ethical regulations, aligning with the Declaration of Helsinki and in accordance with Belgian privacy legislation. Participants gave informed consent to participate in the study before taking part.

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