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Impact of fibre supplementation on microbiome and resilience in healthy participants: A randomized, placebo-controlled clinical trial

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Abstract *Background and aims:* The gut microbiome exerts important roles in health, e.g., functions in metabolism and immunology. These functions are often exerted via short-chain fatty acid (SCFA) production by gut bacteria. Studies demonstrating causal relationships between interventions targeting the microbiome and clinical outcomes are limited. This study aimed to show a causal relationship between microbiome modulation through fibre intervention and health.

Methods and results: This randomized, double-blind, cross-over study included 65 healthy subjects, aged 45–70 years, with increased metabolic risk (i.e., body mass index [BMI] 25–30 kg/m^2, low to moderate daily dietary fibre intake, <30g/day). Subjects took daily a fibre mixture of Acacia gum and carrot powder or placebo for 12 weeks, with an 8-week wash-out period. Faecal samples for measurement of SCFAs and microbiome analysis were collected every 4 weeks. Before and after each intervention period subjects underwent the mixed-meal PhenFlex challenge Test (PFT). Health effects were expressed as resilience to the stressors of the PFT and as fasting metabolic and inflammatory state. The fibre mixture exerted microbiome modulation, with an increase in β-diversity ($p < 0.001$). α-diversity was lower during fibre mixture intake compared to placebo after 4, 8 and 12 weeks ($p = 0.002$; $p = 0.012$; $p = 0.031$). There was no effect observed on faecal SCFA concentrations, nor on any of the primary clinical outcomes (Inflammatory resilience: $p = 0.605$, Metabolic resilience: $p = 0.485$).

Conclusion: Although the intervention exerted effects on gut microbiome composition, no effects on SCFA production, on resilience or fasting metabolic and inflammatory state were observed in this cohort.

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1. Introduction

The human body is colonized by microorganisms: the microbiome. Most bacteria of the microbiome are located in the gut and these bacteria have been shown to affect human physiology profoundly [1]. Its composition is different for every individual and also changes over time within individuals [1–5]. The microbiome influences protective, metabolic and regulatory functions [1,2,6]. More specifically, an important metabolic activity of the gut microbiome is fermentation of non-digestible dietary fibres to short-chain fatty acids (SCFAs) [7]. SCFAs serve as fuel for colonocytes, but are also absorbed systemically and affect numerous physiological functions, including glucose and lipid metabolism and systemic inflammation [8,9]. Many intrinsic traits, e.g., production of SCFAs, are variable between bacterial species of the microbiome [10]. Interventions, such as prebiotics, target microbiome composition and may improve health by modulating metabolic activities of commensal gut bacteria [11]. Prebiotics are substrates that are selectively utilized by host bacteria [12], mostly indigestible food ingredients (e.g., dietary fibres) that specifically stimulate growth or functioning of beneficial bacterial species. In this study, a dietary fibre mixture of acacia gum (AG) and carrot powder was used as prebiotic intervention to modulate microbiome composition, thereby aiming to exert beneficial health effects.

AG contains complex soluble dietary fibres of galactan polymers and originates from Acacia trees [13,14]. *In vitro* and *in vivo* studies have shown that AG increases growth of beneficial bacteria in the gut microbiome, such as *Bifidobacterium* and *Lactobacillus species* [15–17], and its fermentation leads to increased SCFA production *in vitro* [18,19]. Clinically, dosages of 30g AG per day reduce blood pressure, serum cholesterol levels, body mass index (BMI) and postprandial satiety [20–23]. Carrot powder is a fibre mix from carrot peels, containing soluble and insoluble fibres. Main fibre components are pectin and cellulose. Pectin fibres have several beneficial effects, e.g., delay in gastric emptying and increase in postprandial satiety [24]. Like AG, it has been shown to reduce cholesterol and increase glucose tolerance [25,26]. Although many dietary fibres have been associated with beneficial health effects, randomized placebo-controlled studies showing this as causal relationships are limited [27].

In this study, health effects of the dietary fibre mixture were measured by determining changes in phenotypic flexibility, i.e., resilience: the ability of the human body to adapt to a stressor and return to homeostasis. In this case a metabolic stressor was used consisting of a standardized mixed-meal test, the previously described PhenFlex challenge test (PFT) [28]. Based on previous studies a set of biomarkers was selected which respond to the PFT and are used for a time-resolved analysis combining multiple markers, reflecting metabolic and inflammatory state [29–32].

Previous research has shown that dietary fibre interventions may increase microbiome diversity [33–35], which is generally considered as healthy [36], and that these interventions affect resilience [31]. This study aimed to demonstrate a causal relationship between microbiome modulation through dietary fibre intervention and resilience, i.e. the ability to return homeostasis as measured through selected biomarkers over time. To this end, three interdependent hypotheses were tested: dietary fibre intervention (AG and carrot powder) exerts microbiome modulation (1), this modulation results in increased SCFA production (2), and this increase leads to an altered metabolic and inflammatory state (under fasting conditions and in response to the PFT) (3).

2. Methods

2.1. Study design and population

A double-blind, randomized, placebo-controlled crossover study was conducted with two 12-week periods separated by 8 weeks wash-out period, in 65 healthy volunteers (Figure S1). In order to maximize effect of the intervention, participants with increased metabolic risk were selected, i.e. BMI 25–30 kg/m^2, age 45–70 years, average daily dietary fibre intake <30g. The study was conducted at the Centre for Human Drug Research (CHDR), Leiden, The Netherlands, between August 2020 and July 2021. All participants gave written informed consent according to Declaration of Helsinki recommendations prior to any study-related activity. Study protocol and informed consent process were approved by the Independent Ethics Committee of the Foundation 'Evaluation of Ethics in Biomedical Research' (Stichting Beoordeling Ethiek Biomedisch Onderzoek), Assen, The Netherlands. The study was performed according to Good Clinical Practice (GCP) and registered in Toetsingonline Registry (number NL71723.056.19) and in clinicaltrials.gov register (number NCT04829396).

2.2. Study protocol

Participants underwent medical screening, including medical history, physical examination, BMI, vital signs measurements, 12-lead electrocardiography (ECG), urine analysis, drug screening and chemistry and haematology blood sampling. During medical screening, baseline average daily fibre intake was measured by the Dietary Fibre Intake Short Food Frequency Questionnaire (DFI-FFQ) [37]. Key inclusion criteria were: no clinically significant abnormalities during screening, and aforementioned criteria for increased metabolic risk. Key exclusion criteria were: use of antibiotics, antacids, laxatives, statins, antidiarrheal, immunomodulatory or antidiabetic medication within 3 months before inclusion, use of any concomitant medication, vitamins or dietary supplements within 7 days (or 5 half-lives) before or during the study, excluding paracetamol and ibuprofen. Participants were instructed to take 13g powder (dietary fibre mixture or placebo) stirred into fluid daily for 12 weeks, followed by a wash-out period of 8 weeks, and to take the powder again daily for 12 weeks (Figure S1). They were instructed to keep

their diet as constant as possible and to avoid initiating new food habits or diets during participation. Randomization codes were generated by a study-independent statistician. Powders were dispensed by the Leiden University Medical Center (LUMC) pharmacy in identical jars and were matching in appearance. Study staff involved in dosing was not involved in performing assays or data analysis. The dietary fibre mixture was produced in a ratio ensuring that participants took 10g AG powder (Type 4880, A. Seyal, Willy Benecke, Germany) and 3g milled carrot powder (KaroPRO 1–26 SG, Food Solutions Team B.V., The Netherlands) with every dosing. Total fibre content was 10g fibre per 13g mixture. Placebo powder consisted of brown (Glucidex® 19) and white (Glucidex® 17) maltodextrin only (Roquette, France). On the first and last day of every study period of 12 weeks, participants visited the CHDR (4 times). During each visit, the PFT, anthropometric measurements, the Dutch Healthy Diet Index (DHDI) questionnaire [38,39] and blood sampling were performed. Faecal samples were collected every 4 weeks (Figure S1). To measure compliance, subjects were required to hand in empty dietary fibre intervention/placebo jars at the end of each intervention period. By weighing these jars, total intake was calculated. Intake of minimal 80% of total dosing was considered as compliant. Compliance to study restrictions (e.g., no use of concomitant medication) and adverse events were monitored during every visit to the CHDR, and during the treatment periods by regular phone calls.

2.3. Microbiome profiling

Microbial DNA was extracted from faecal samples, and 16S rRNA gene sequencing was performed as described previously [40].

2.4. DHDI questionnaire

The DHDI questionnaire consists of 40 questions, reflecting 16 components of the diet, and results in a score between 0 and 10 for each component. This results in a total score between 0 and 160, representing adherence to the Dutch guidelines for a healthy diet, with a higher score representing a better adherence [38,39].

2.5. PhenFlex challenge test

The PFT consisted of 500 mL mixed-meal challenge drink, composed as described previously (produced by 'Instituut voor Landbouw- Visserij-en Voedingsonderzoek, Eenheid Technologie & Voeding, Food Pilot', Melle, Belgium) [29]. Blood samples were taken at baseline and 30, 60, 120 and 240 min after intake of the drink.

2.6. SCFAs

Faecal SCFAs (iso-valeric acid, propionic acid, iso-butyric acid, butyric acid, valeric acid, acetic acid, 2-methylbutanoic acids) were measured by liquid chromatography-mass spectrometry (LC-MS) using high-resolution mass spectrometry (Q-Exactive, Thermo, USA) (Supplement 1).

2.7. Biochemical parameters and cytokines

Plasma levels of aspartate-amino-transferase (AST), alanine-amino-transferase (ALT), glucose, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, ratio HDL/LDL cholesterol, insulin, C-reactive protein (CRP), and gamma-glutamyltransferase (GGT) were measured in plasma according to standardized methods at the Leiden University Medical Center laboratory (Leiden, The Netherlands). Cytokines (interleukin [IL]-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12p70, IL-13, interferon [IFN]-γ, tumour necrosis factor [TNF]) were quantified by multiplexed immunoassays (Multiplex Panel Human Proinflammatory: Meso Scale Discovery). Plasma serum amyloid A (SAA) and Calprotectin S100A8/S100A9 heterodimer were measured by Enzyme-linked Immuno Sorbent Assay (ELISA) (both Duoset ELISA from R&D systems, Abingdon, United Kingdom) according to manufacturer's instructions. Non-esterified fatty acids (NEFAs) were quantified using the ASC-ACOD method (NEFA-HR assay, Fujifilm Wako Chemicals Europe GmbH, Neuss, Germany), after inhibiting plasma lipolytic activity with tetrahydrolipstatin (Orlistat; Sigma–Aldrich/Merck Life Science NV, Amsterdam, The Netherlands).

2.8. Statistical analysis

Baseline characteristics for all participants and stratified by sex were described by standard statistical methods. Sample size was based on a previous study using the PFT, where the intervention resulted in 0.63 decrease in Inflammatory resilience. In the current study, estimated effect size was 65% and using the same standard deviation resulted in a sample size of minimal 52 subjects with power 80% and $\alpha = 0.05$ [31]. Results of the DHDI questionnaire were analysed by descriptive statistics and differences between visits were tested by two-way ANOVA.

Statistical analysis of the microbiome was performed using R version 4.1.2 (R Core Team 2020). All figures were composed using the *ggplot2* package version 3.3 [41]. Multivariate analysis and ordinations were performed using the *vegan* package, version 2.5–7 [42]. This package was also used to calculate the Shannon α-diversity (i.e., within-subject diversity) and β-diversity (i.e., between-subject diversity). The multivariate model fitted by Principal Response Curve analysis was tested by permutation analysis in order to produce Type III (marginal) p-values for the terms included in the model [43]. 10^3 permutations were used for all reported results. The 16S rRNA data were filtered (Supplement 2) to include only those Amplicon Sequence Variants (ASV) contributing to the first 95% of all counts in the data. Overall, 8199 taxa were determined in the microbiome samples. Of those taxa, 624 taxa represented 95% of the reads and were included in analyses (except for α-diversity, which included all taxa). Treatment effects (i.e., effects of the intervention) on α-diversity were

tested by ANOVA on a linear mixed effects (LME) model, with treatment and treatment*time as independent variables. Post-hoc tests were performed using the *emmeans* package [44]. Statistical differences in β-diversity were tested by repeated-measures permutational ANOVA.

All SCFA data were adjusted for the amount of bacterial 16S rRNA gene per sample (Supplement 2). Intervention effects on SCFAs were tested by the paired Wilcoxon signed-rank test for each timepoint in the study period.

Insulin resistance before and after each intervention period was calculated using previously published methods (Supplement 2). For every PFT, 2 scores were calculated: Inflammatory resilience and Metabolic resilience (Supplement 2). These scores were calculated by a regression equation incorporating the applicable biochemical parameters over time during the PFT. For these 2 different scores the ANOVA model residuals were checked for normality and homoscedasticity. The score for Inflammatory resilience was log-transformed. There were no carry-over effects (Inflammatory resilience: $p = 0.676$; Metabolic resilience: $p = 0.429$). Intervention effects on insulin resistance, biochemical parameters and cytokine levels at baseline (i.e., before PFT) and the Inflammatory and Metabolic resilience scores were calculated by means of a LME model, with period and treatment as fixed factors and subject as random factor. The interaction term treatment∗sex and sex were introduced in the model by forward selection based on the Akaike Information Criterion (AIC). Statistical methods of Table S3, investigating the effect of the PFT on individual parameters of the Inflammatory and Metabolic resilience scores are described in Supplement 2. For all analyses, a p-value <0.05 was considered as statistically significant.

3. Results

In total, 65 participants were enrolled in the study and 54 participants were included in the analyses (Figure S2). Baseline characteristics are shown in Table 1. Average fibre intake at baseline was 19g/day (median 17g/day) (Figure S3). The dietary fibre mixture was well-tolerated and safe: all adverse events possibly related to the intervention were mild. There was no effect of dietary fibres on BMI and other anthropometric parameters (Table S1). During the study, there was overall no change in diet of the participants as reflected in the total DHDI score ($p = 0.106$). Detailed analyses of the DHDI results are shown in Supplement 3.

3.1. Microbiome modulation

The effect of dietary fibre intervention compared to placebo on microbiome composition over time was determined by β-diversity at 0, 4, 8 and 12 weeks. The following factors had significant effect on β-diversity: intervention ($p \leq 0.001$), sex ($p \leq 0.001$), and time ($p \leq 0.001$). The absence of a significant intervention∗time interaction term on β-diversity indicates a stable effect of the intervention ($p = 0.736$). Principal Response Curve analysis presenting the percentage of total variation caused by the intervention over time (0.44%, $p \leq 0.001$; Fig. 1) demonstrated an increase in effect of the intervention on β-diversity during the first 4 weeks, remaining stable during the rest of the period. Fig. 2 shows the 10 taxa with the strongest increase and decrease over time associated with dietary fibre intervention. Bacteria showing the highest increase in abundance during dietary fibre intervention compared to placebo were *Bifidobacterium breve species*; bacteria showing the largest decrease *were* Erysipelotrichaceae *UCG-003* species. α-diversity (Shannon−index) after 0, 4, 8 and 12 weeks of dietary fibre intervention was compared to their respective sample during placebo use (Fig. 3). At week 4, 8 and 12, α-diversity differed significantly between participants during their placebo period and dietary fibre intervention period, with a lower diversity during the dietary fibre intervention period at each

Table 1 Baseline characteristics.

	Entire cohort (N = 54)	Males (N = 29)	Females (N = 25)
Sex (N, %)	N/A	29, 53.7%	25, 46.3%
Age, years (mean ± standard deviation [SD])	57.8 ± 5.8	60.0 ± 6.1	55.4 ± 4.4
BMI, kg/m^2 (mean ± SD)	27.3 ± 1.4	27.3 ± 1.4	27.3 ± 1.4
Body weight, kg (mean ± SD)	84.0 ± 10.1	90.2 ± 8.3	76.7 ± 6.5
Systolic blood pressure, mmHg (mean ± SD)	133 ± 15	140 ± 14	125 ± 13
Diastolic blood pressure, mmHg (mean ± SD)	82 ± 9	85 ± 8	78 ± 8
Heart rate, beats per minute (mean ± SD)	63 ± 8	63 ± 8	63 ± 7
Hip circumference, cm (mean ± SD)	100.8 ± 6	100.4 ± 5	101.3 ± 7
Waist circumference, cm (mean ± SD)	94.3 ± 8	98.8 ± 6	89.2 ± 6
CRP, mg/L (mean ± SD)	1.8 ± 1.9	1.6 ± 1.7	2.1 ± 2.1
AST, U/L (mean ± SD)	23 ± 7	23 ± 5	23 ± 9
ALT, U/L (mean ± SD)	26 ± 10	27 ± 9	25 ± 12
GGT, U/L (mean ± SD)	24 ± 11	28 ± 12	20 ± 8
Fasted glucose, mmol/L (mean ± SD)	5.2 ± 0.7	5.4 ± 0.6	4.9 ± 0.7
Sodium, mmol/L (mean ± SD)	142 ± 2	142 ± 2	142 ± 2
Potassium, mmol/L (mean ± SD)	4.3 ± 0.3	4.3 ± 0.3	4.3 ± 0.4
Triglycerides, mmol/L (mean ± SD)	1.6 ± 0.9	1.7 ± 0.9	1.5 ± 0.8
Daily fibre intake, g/day (mean ± SD)	18.7 ± 5.9	19.0 ± 6.4	18.5 ± 5.3

ALT: alanine-amino-transferase, AST: aspartate-amino-transferase, BMI: Body Mass Index, CRP: C-reactive protein, GGT; gamma-glutamyltransferase, SD: standard deviation.

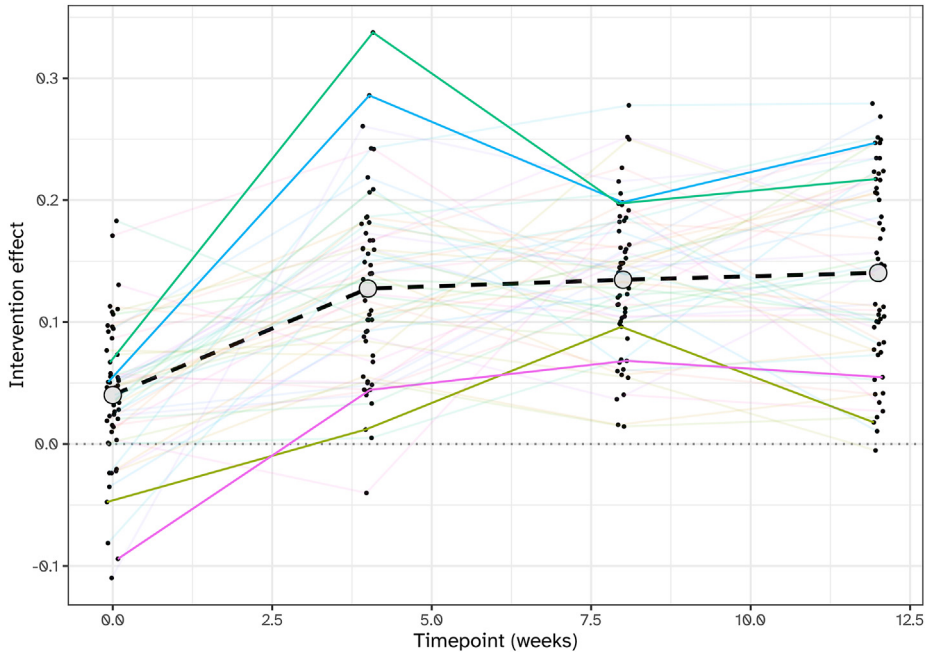

Figure 1 Effect of dietary fibre intervention on β-diversity (between-subject variation) over time. Principal Response Curve analysis presenting effect of the intervention isolated from all other factors that affect microbiome composition (% of total variation caused by the intervention) over time. The y-axis represents the amount of variation that the intervention has induced per sample amongst the total microbiome variation of all participants at that specific timepoint. Coloured lines represent the two participants with the highest (blue, dark green) and lowest (light green, pink) median treatment effects highlighted. The variation that was induced by the intervention in these specific subjects contributed the most/least to the total variation induced by the intervention. Total fibre intervention effect was 0.44%, $p \leq 0.001$ without significant intervention∗time interaction. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

timepoint (week 4: $p < 0.001$; week 8: $p = 0.003$; week 12: $p = 0.042$).

3.2. SCFAs

There was a significantly higher concentration of faecal SCFAs during the dietary fibre intervention period compared to the placebo period at some timepoints: 2-methyl butanoic acid at 12 weeks ($p = 0.025$), acetic acid at 2 weeks ($p = 0.038$), butyric acid at 4 weeks ($p = 0.045$), iso-butyric acid at 12 weeks ($p = 0.040$), iso-valeric acid at 12 weeks ($p = 0.045$) and propionic acid at 4 weeks ($p = 0.034$). However, overall there was no consistent significant difference in faecal SCFA concentrations between the dietary fibre intervention and placebo periods (Fig. 4).

3.3. Inflammatory and metabolic state

There was no effect of dietary fibre intervention on fasting biochemical parameters and cytokines, except for IFN-γ ($p = 0.040$), with higher levels after 12 weeks of placebo compared to 12 weeks of dietary fibre intervention (Table S1). These results show no effect of dietary fibre intervention on metabolic and inflammatory state under fasting conditions.

3.4. Resilience

There was no significant effect of the dietary fibre intervention compared to placebo on the PFT outcome measures (Inflammatory resilience: $p = 0.605$, Metabolic resilience: $p = 0.485$) (Fig. 5, Table S2). There was also no significant effect on Disposition Index ($p = 0.864$), Matsuda Index ($p = 0.104$) and Muscle Insulin Resistance ($p = 0.806$), while there was a significant effect on Hepatic Insulin Resistance Index [HIRI] ($p = 0.027$).

3.5. Fibre intake

To further explore whether aforementioned observations were attributable to baseline daily fibre intake, analysis stratified on median baseline fibre intake according to the DFI-FFQ (17g/day) was performed. This analysis showed significant differences in microbiome changes in amount and direction induced by dietary fibre intervention between subjects with baseline fibre intake below and above 17g/day (interaction term baseline dietary fibre intake∗intervention $p \leq 0.001$). This was demonstrated by redundancy analysis (RDA), as shown in Figure S4. However, there were no differences in clinical outcomes between subjects with a baseline daily fibre intake below or above 17 g/day.

4. Discussion

In this study, effects of daily dietary fibre supplementation (AG/carrot powder mixture) on gut microbiome composition and Metabolic and Inflammatory resilience were studied. A significant effect of dietary fibre intervention on microbiome composition was observed in almost all

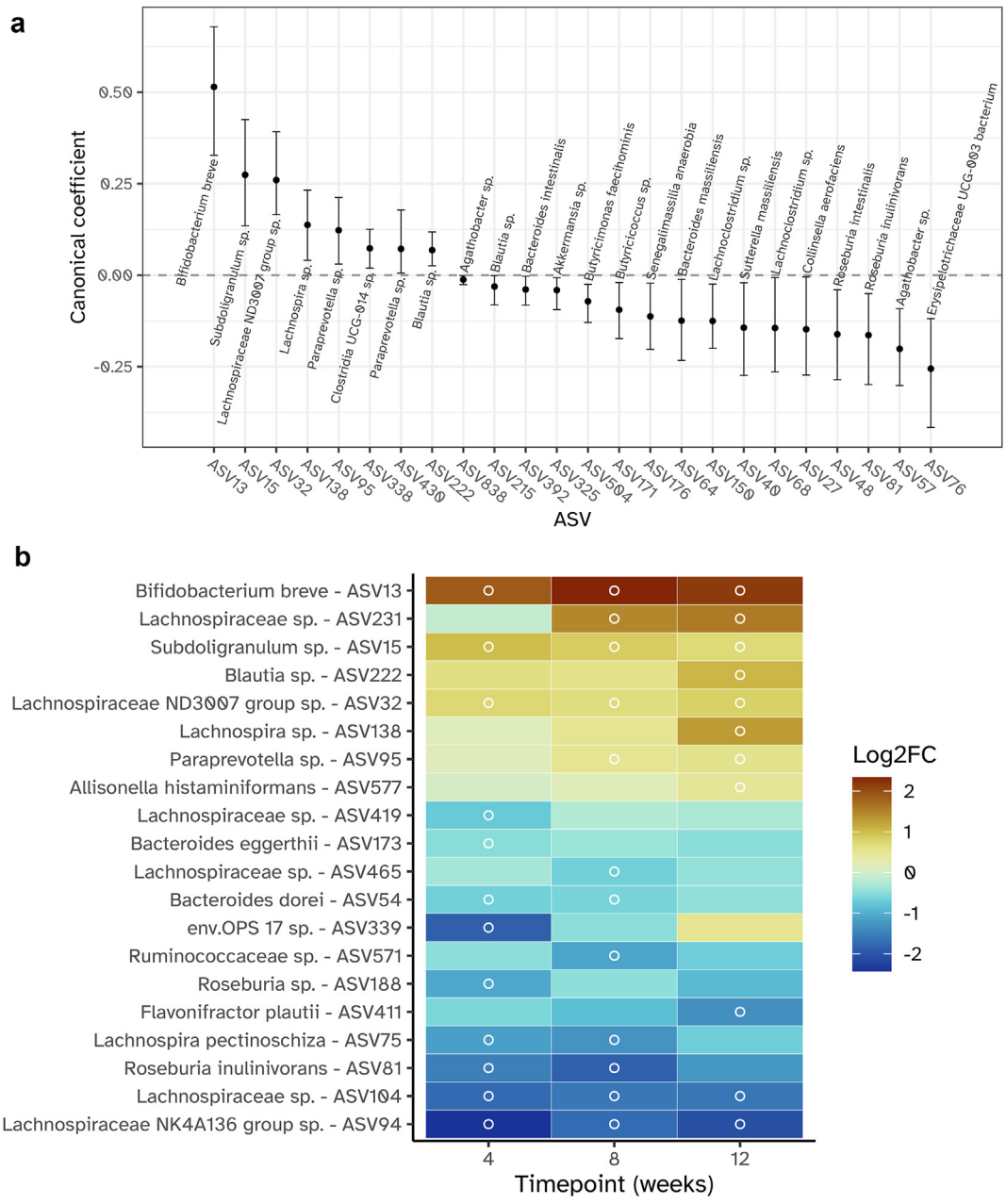

Figure 2 Taxa with strongest increase and decrease over time associated with dietary fibre intervention. A. Canonical coefficients from Principal Response Curve analysis of 10 bacterial taxa (amplicon sequence variants) with the highest and lowest associations with the fibre intervention. Error bars show bootstrapped 99% confidence intervals. Showing only taxa with bootstrapped p-value $\leq$0.01. B. Heatmap visualising the difference in Log_2 fold-change (Log_2FC) per bacterial taxis between subjects in their fibre and their placebo period, when comparing week 0 to a later week. Taxa with a significant ($p \leq 0.01$) intervention:time interaction are marked.

participants, but this did not translate into effects on faecal SCFAs, fasting metabolic and inflammatory state and phenotypic flexibility.

Comparing the microbiome of participants after 12 weeks of dietary fibre intervention with placebo, a significant change in microbiome composition emerged, mostly during the first 4 weeks of dietary fibre intervention. These changes, induced during the first 4 weeks, remained stable during the rest of the intervention period. The rate at which effects were emerging varied between participants, which can be explained by the inherent wide variation in gut microbiome composition. The finding that almost all participants responded to the intervention is a notable finding, as many studies investigating interventions that target the microbiome show variable results [45]. The high response rate may have been a beneficial effect of the two different fibres in the intervention used in this study, since the response of microbial taxa to different fibres varies [46,47]. A subject was considered to be compliant in case of an intake of at least 80% of the total dose, which is a common cut-off value for comparable studies. Only 1

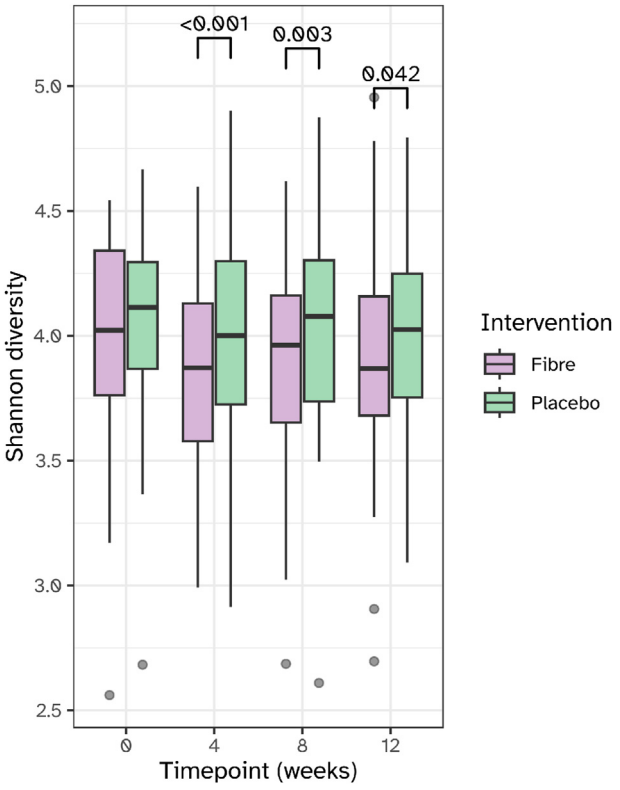

Figure 3 α-diversity (within-subject variation). α-diversity (Shannon diversity index) of participants during dietary fibre intervention period and placebo period, measured at 0, 4, 8 and 12 weeks. Boxplots show median, first and third quartile; error bars show maximum and minimum. Outliers are shown as grey dots.

subject was excluded from analysis for not meeting this compliance rule.

Although an increase in α-diversity is generally considered as characteristic of a 'healthier' microbiome [36], the decreased α-diversity after dietary fibre intervention compared to placebo may be a result of the isolated increase of specific bacterial species by the dietary fibre intervention. Indeed, we found an increase of *Bifidobacteria,* Lachnospiraceae and *Subdoligranulum* during 12 weeks of dietary fibre intervention. Particularly, the increase in *Bifidobacteria* is in line with findings of earlier *in vitro* and *in vivo* studies, demonstrating that AG increases mostly *Bifidobacteria* and *Lactobacilli* [15,17–19,48]. Pectins are associated with increased abundance of *Lactobacilli, Bacteroides* and *Prevotella species* [49], which was not observed in the current study, possibly due to the small share of pectins as compared to AG in the dietary fibre mixture. However, the observed increased abundance of *Paraprevotella species*, belonging to the same family as *Prevotella*, may be attributed to pectins administered in this study. *Bifidobacteria* are already used widely as probiotic strains, as they have numerous beneficial properties such as regulation of the intestinal immune homeostasis and suppression of pro-inflammatory cytokines [50]. Although the specific role of Lachnospiraceae has not been established, they are considered SCFA producers [51,52]. *Subdoligranulum species* are strongly associated with health benefits, such as high HDL-cholesterol levels and low insulin, CRP and IL-6 levels [53]. The decrease in Erysipelotrichaceae *species* may be a favourable effect of the intervention, as they are associated with negative effects on metabolism and increased inflammation of the gut [54]. Thus, the decreased α-diversity over time during dietary fibre intervention compared to placebo may have been caused by an increased share of bacteria associated with beneficial properties, such as *Bifidobacteria*, at expense of bacteria associated with less favourable outcomes.

The only observed systemic significant effects of the intervention were on fasting levels of IFN-γ and the HIRI. IFN-γ increased more during placebo use compared to intervention, probably due to unnoticed infections. For the HIRI, considering the large spread in this index and the small difference between groups, this does not appear to be an effect of clinical significance. No effect of the intervention was observed on phenotypic flexibility, expressed as the Metabolic and Inflammatory resilience.

The absence of clinical effects of the intervention as opposed to the effects on α- and β-diversity is contradictory. It should be considered that the effect of the intervention was, although significant, very small, explaining only 0.44% of the total microbiome variation in the study. Possibly, bacteria increasing in abundance after the dietary fibre intervention are correlated with beneficial properties, but are not causally related with physiological effects [27,50–54]. As SCFAs are mediators in the causal relationship between gut microbiome composition and both metabolic traits and systemic inflammation [8,55–57], it is in line with the absence of physiological effects that little change in faecal SCFA levels was observed. Although bacterial species that increased in abundance are associated with SCFA production, there was also a decrease in SCFA-producing *Roseburia* species [58]. Possibly, there was no net effect on SCFA production because of a balance between in- and decrease of SCFA producers. It should also be considered that faecal SCFAs are not always well correlated with the amount of produced SCFAs in the gut, as they are metabolized by colonocytes and absorbed into the blood [9,59]. Given the absence of any treatment effect on faecal SCFAs and clinical outcomes, circulating SCFAs were not measured, although circulating SCFAs are correlated more with metabolic outcomes (e.g., insulin sensitivity) than faecal SCFA concentrations [60]. Measuring additional mediators between microbiome and systemic outcomes may provide more insight into the reason for absence of clinical effects.

Strengths of this study include the cross-over design, long-term follow-up with considerable wash-out period and large sample size. Factors that may have affected the results are, firstly, the used placebo (maltodextrin) potentially impacting microbiome composition, resulting in smaller differences between dietary fibre and placebo effects [61]. However, RDA analysis (Figure S4) showed that the effect of maltodextrin on microbiome variation was much smaller than the effect of the dietary fibre intervention. Therefore, we believe that maltodextrin was

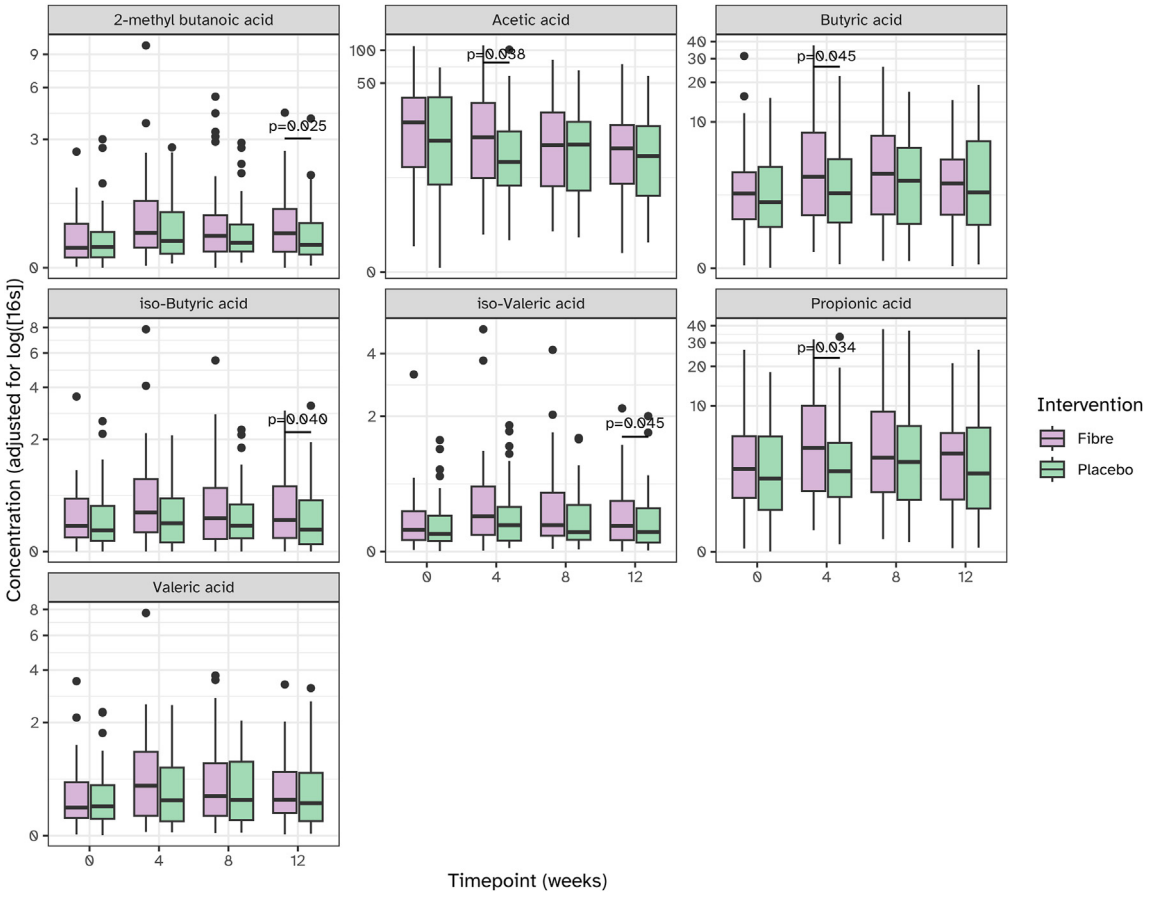

Figure 4 Faecal SCFA concentrations. Concentration (μM) of various SCFA in faecal samples, adjusted for amount (ng/mL) of 16S bacterial RNA gene in the specific sample, measured at different timepoints during dietary fibre intervention period and placebo period.

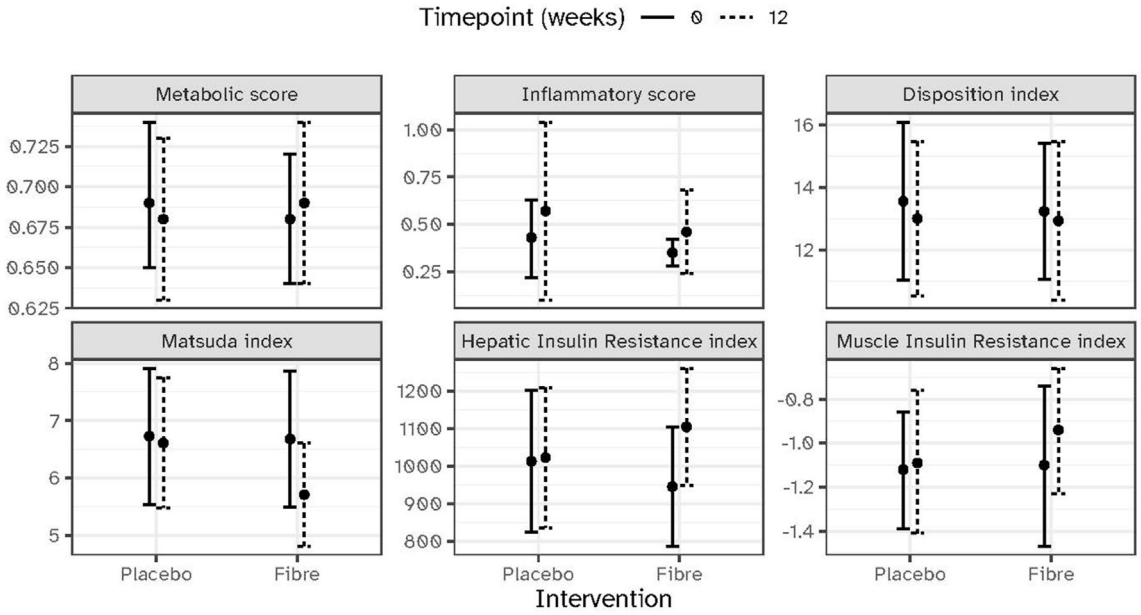

Figure 5 Measures of phenotypic flexibility. Metabolic resilience (metabolic score), Inflammatory resilience (inflammatory score), Disposition index (β-cell function), Matsuda index (insulin sensitivity), and hepatic and muscle insulin resistance index, before and after 12 weeks of dietary fibre or placebo intervention. Dots show mean, error bars indicate 95%CI.

a suitable placebo for our study. Secondly, the cut-off of <30g baseline average daily dietary fibre intake of participants may have been too high. According to limits of the DFI-FFQ, low average daily dietary fibre intake is defined as <18g/day for females and <22 g/day for males (Table S4) [37]. Possibly, selected participants were already 'too healthy' or consumed too much fibre to gain beneficial effects of the intervention. However, considering the relatively low mean dietary fibre intake at baseline (Figure S3, females: 18.5 g/day, males 19 g/day), an addition of 10g dietary fibres per day is a considerable increase in dietary fibre intake for the majority of the participants. This is especially the case considering the DFI-FFQ guidelines, that would classify most participants as having a low to moderate dietary fibre intake at baseline, but as having a high dietary fibre intake (females: ≥25 g/day, males: ≥30 g/day) with the addition of 10g dietary fibre per day (Table S4). Although it is difficult to establish dose–response relationships of nutritional interventions, systematic reviews have shown that doses of minimal 5g dietary fibre per day can induce clinically beneficial effects in cardiovascular patients [62], and that a dose of 7.6–8.3g per day is associated with improved glycaemic control in patients with type 2 diabetes [63]. Additionally, it was shown that there is a dose–response relationship between dietary fibre intake and mortality in patients with diabetes [64], and in patients with cardiovascular disease [62]. Based on these data, it cannot be concluded that the used dose of 10g dietary fibre per day was insufficient to induce a clinical effect in our population of participants with increased metabolic risk. Lastly, although parameters of the scores expressing phenotypic flexibility (i.e. Metabolic and Inflammatory resilience) were selected based on their response in previous research [31], TNF did not show a response to the PFT, possibly due to differences in study population (Table S3). Otherwise, it is possible that the intervention had effects on targets that were not included in the scores, although parameters were selected to measure subtle changes in various pathways [29].

Concluding, this study investigated the effect of dietary fibre intervention in a randomized, double-blind, cross-over trial, and related microbiome changes to physiological effects. Although microbiome changes were observed in almost all participants, and the observed changes are mostly associated with favourable health effects, there was no translation to effects on phenotypic flexibility and other clinical outcomes in this population.

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Declaration of competing interest

All authors declare that they have no relevant financial interests or disclosures to report. This study was part of the collaboration project No Guts No Glory.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.numecd.2024.01.028.

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