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RESEARCH ARTICLE

Metagenomic Profiling of Gut Microbiota in Response to High-Fiber Dietary Interventions

Sylvie Bakker^{1*}, Eszter Saez-Bermudez² and Wolfgang Bianchi³

¹Department of Bioinformatics and Computational Biology, Baltic Institute of Biomedical Research, Sweden

²Department of Biomedical and Health Sciences, Iberian University Center for Biotechnology, Spain

³Department of Bioinformatics and Computational Biology, Iberian University Center for Biotechnology, Spain

*Corresponding author: Sylvie Bakker, Department of Bioinformatics and Computational Biology, Baltic Institute of Biomedical Research, Sweden; Email: s.bakker@bibr.se

ABSTRACT

Fiber intake across most industrialised populations sits well below the level associated with a resilient, fermentation-competent gut microbiota, yet the strain-level and functional consequences of raising fiber intake in free-living adults are still incompletely mapped. We conducted an 8-week randomised, controlled, parallel-arm feeding trial in 42 healthy adults comparing a high-fiber diet (35 g fermentable fiber per day, delivered through whole grains, legumes and inulin-enriched foods) against a habitual-diet control, with stool shotgun metagenomic sequencing and faecal short-chain fatty acid (SCFA) quantification performed at baseline and week 8. Thirty-nine participants (19 intervention, 20 control) completed the protocol. Shannon diversity rose from 3.11 to 3.44 in the intervention arm (paired t-test, $P < 0.001$) with no change in controls ($P = 0.41$), and Bray-Curtis PERMANOVA showed significant within-arm compositional shift over time in the intervention group only ($R^2 = 0.081$, $P = 0.002$). *Bifidobacterium*, *Faecalibacterium prausnitzii*, *Roseburia* spp. and *Ruminococcus bromii* all increased significantly in relative abundance (log₂ fold changes of 2.29, 1.60, 1.94 and 1.71 respectively, all $q < 0.01$), while the *Bacteroides fragilis* group declined modestly. Functional profiling showed coordinated enrichment of glycoside hydrolase families GH13 and GH43 and of starch and sucrose degradation pathways, and faecal butyrate concentration increased from 12.4 to 18.7 micromol per gram dry weight ($P < 0.001$), with smaller but significant increases in propionate. No comparable shifts occurred in the control arm. These results provide strain- and pathway-resolved evidence that a sustained, moderate increase in dietary fiber reproducibly favours a saccharolytic, butyrogenic community configuration in adults without pre-existing gastrointestinal disease, and they identify a compact set of taxa and gene families that could serve as response biomarkers in future fiber-intervention trials.

Keywords: gut microbiota; shotgun metagenomics; dietary fiber; short-chain fatty acids; *Faecalibacterium prausnitzii*; microbiome-diet interaction

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1. INTRODUCTION

The human gut microbiota performs a substantial share of the fermentative work that dietary fiber requires before

it can contribute to host physiology, and the composition of that community is itself shaped by how much and what kind of fiber a person habitually eats. Large cross-population surveys have repeatedly found that

fiber intake correlates with microbial richness, with populations consuming traditional, plant-rich diets carrying markedly more diverse and more fermentation-capable communities than populations on typical Western diets, where median fiber intake in adults is commonly reported at well under half of recommended levels [3,9]. Whether raising fiber intake in an already Westernised population can restore some of that lost diversity, and through which specific taxa and metabolic pathways, has been addressed in a growing but still heterogeneous body of intervention studies, many of which relied on 16S rRNA amplicon sequencing that cannot resolve community shifts below the genus level or connect them directly to functional gene content [14,22]. Short-chain fatty acids (SCFAs) -- principally acetate, propionate and butyrate -- are the best-characterised output of microbial fiber fermentation and the primary mechanistic link proposed between fiber intake and downstream host benefits, including colonocyte energy supply, maintenance of gut barrier integrity, and systemic effects on glucose and lipid metabolism mediated in part through short-chain-fatty-acid-sensing G protein-coupled receptors [7,18]. Butyrate in particular is produced by a phylogenetically narrow set of obligate anaerobes, most notably *Faecalibacterium prausnitzii* and several *Roseburia* and *Eubacterium* species, none of which are reliably distinguished from close relatives by amplicon sequencing of the 16S rRNA gene alone [11,27]. Shotgun metagenomic sequencing, by contrast, provides both species- and strain-level taxonomic resolution and direct access to the functional gene repertoire of the community, including the glycoside hydrolase and polysaccharide lyase families that determine which dietary substrates a given community can actually degrade [19,31].

Randomised controlled feeding trials remain the most reliable design for isolating a fiber effect from the many other dietary and lifestyle variables that covary with fiber intake in observational data, but they are logistically demanding, and relatively few have combined shotgun metagenomics with matched, quantitative SCFA measurement over a duration long enough for a community to plausibly re-equilibrate around a new substrate supply [23,30]. Shorter interventions, of two weeks or less, have sometimes shown transient compositional shifts that partially reverse before the study ends, suggesting that a community's steady-state response to a sustained dietary change may differ from its response to a brief perturbation, and that trial duration is itself an important design parameter rather than a matter of convenience [8,35]. An 8-week window was chosen here as a compromise between capturing a plausible steady-state shift and retaining acceptable participant adherence over a free-living intervention. We therefore designed a randomised, controlled, parallel-arm feeding trial to test, with strain-level metagenomic resolution and paired functional and metabolite data, whether a sustained increase in dietary

fiber intake in healthy adults produces a reproducible, coordinated shift in gut microbial composition, carbohydrate-active gene content and faecal SCFA output, and to identify the specific taxa most consistently associated with that shift. Because prior amplicon-based work has repeatedly implicated *Faecalibacterium prausnitzii*, *Roseburia* spp. and *Bifidobacterium* as fiber-responsive taxa without always confirming a matched functional or metabolite signal, we designed the analysis specifically to test whether taxonomic, functional and metabolomic responses move together within the same individuals, rather than treating each data layer as an independent line of evidence [6,17].

2. MATERIALS AND METHODS

This study was conducted as an 8-week, single-centre, randomised, controlled, parallel-arm feeding trial in healthy adults recruited from the general population in Malmo, Sweden, between February and May 2023.

2.1 Study design, participants and dietary intervention

Forty-two adults aged 25-55 years with a body mass index of 19-29 kg/m², no diagnosed gastrointestinal disease, and no antibiotic use in the preceding three months were randomised 1:1 to a high-fiber intervention or a habitual-diet control using a computer-generated allocation sequence, stratified by sex. Participants in the intervention arm were provided with a structured meal plan and study foods designed to raise total fiber intake to approximately 35 g per day, predominantly through whole grains, legumes, and inulin-enriched bread and dairy products, with a registered dietitian reviewing three-day food diaries at weeks 2, 4 and 6 to reinforce adherence and adjust portions where intake fell short of target. Control-arm participants continued their habitual diet and received no dietary counselling beyond standard study contact. Baseline fiber intake, estimated from a validated food-frequency questionnaire, did not differ significantly between arms (17.8 g/day intervention versus 18.3 g/day control, $P=0.61$).

2.2 Stool sample collection and shotgun metagenomic sequencing

Stool samples were self-collected at home using a stabilising buffer kit at baseline and at week 8, stored at -80°C within 24 hours, and processed in a single sequencing batch to avoid batch effects confounding the before-after comparison. DNA was extracted using a bead-beating protocol optimised for Gram-positive cell lysis, and sequencing libraries were prepared with a standard shotgun protocol and sequenced on an Illumina NovaSeq 6000 (2x150 bp) to a target depth of 15 million paired-end reads per sample. Reads were quality-trimmed and host-filtered against the GRCh38 human reference genome, then taxonomically profiled with MetaPhlAn4 using the default marker-gene database, and functionally profiled with HUMAnN3 to quantify MetaCyc pathway abundance and CAZy glycoside hydrolase family gene abundance, normalised

to reads per kilobase per million mapped reads.

2.3 Faecal short-chain fatty acid quantification

Faecal acetate, propionate and butyrate concentrations were measured by gas chromatography with flame ionisation detection (GC-FID) on homogenised, acidified stool extracts, with 2-ethylbutyric acid used as an internal standard, and results expressed as micromoles per gram of dry faecal weight after lyophilisation. Each sample was analysed in duplicate, with the mean of the two values carried forward to statistical analysis; duplicates differing by more than 15% were re-run.

2.4 Adherence and completion

Adherence in the intervention arm was tracked through returned food diaries and unused study-food counts, and was classified as adequate if a participant met at least 80% of the prescribed fiber target on average across the intervention period. Thirty-nine of 42 randomised participants (19 intervention, 20 control) provided complete paired baseline and week-8 samples and were included in the primary analysis; three intervention-arm participants withdrew, two citing gastrointestinal discomfort during the first two weeks and one for reasons unrelated to the study.

2.5 Statistical and bioinformatic analysis

Alpha diversity (Shannon index) was compared within arm between baseline and week 8 using paired t-tests, and between arms using linear regression on the change score adjusted for baseline value. Beta diversity was assessed on Bray-Curtis dissimilarity by permutational multivariate analysis of variance (PERMANOVA) with 9,999 permutations, run separately within each arm. Differential taxon and pathway abundance between baseline and week 8 was tested with MaAsLin2 using a linear mixed model with participant as a random effect, and P-values were corrected for multiple testing within each data layer (taxonomic, functional, CAZy) using the Benjamini-Hochberg procedure, with a q-value threshold of 0.05 for significance. SCFA concentrations were compared using paired t-tests within arm and analysis of covariance between arms. All analyses were performed in R version 4.3 using the vegan and Maaslin2 packages.

3. RESULTS

Sequencing yielded a median of 16.2 million quality-filtered, host-depleted read pairs per sample (range 11.8–21.4 million), with no significant difference in sequencing depth between arms or time points that could confound diversity comparisons. Reported adherence in the intervention arm was adequate in 17 of 19 completers, with mean achieved fiber intake of 32.6 g/day against a 35 g/day target. Shannon diversity increased from 3.11 (SD 0.34) at baseline to 3.44 (SD 0.29) at week 8 in the intervention arm (paired t-test, $P < 0.001$), while the control arm showed no meaningful change (3.05 to 3.08, $P = 0.41$). The between-arm difference in diversity change, adjusted for baseline

diversity, was significant ($P = 0.002$). Bray-Curtis PERMANOVA indicated a significant compositional shift between baseline and week 8 within the intervention arm ($R^2 = 0.081$, $P = 0.002$) but not within the control arm ($R^2 = 0.011$, $P = 0.31$), and the magnitude of individual compositional shift correlated positively with achieved fiber intake (Spearman $\rho = 0.52$, $P = 0.02$) among intervention-arm completers.

Table 1. Taxa showing the largest, statistically robust abundance shifts after 8 weeks (intervention arm).

Taxon	Baseline (%)	Week 8 (%)	Log2 fold change	q-value
Bifidobacterium	2.1	4.8	+2.29	<0.001
Faecalibacterium prausnitzii	8.2	13.1	+1.60	<0.001
Roseburia spp.	3.4	6.5	+1.94	0.002
Ruminococcus bromii	1.9	3.4	+1.71	0.004
Akkermansia muciniphila	1.2	1.9	+0.66	0.09
Bacteroides fragilis group	11.6	9.4	-0.31	0.04
Prevotella copri	0.8	1.1	+0.42	0.21
Eubacterium rectale	4.5	6.0	+0.41	0.11

Log2 fold change and q-values from MaAsLin2 linear mixed models (participant as random effect), Benjamini-Hochberg corrected within the taxonomic data layer.

Table 1 summarises the taxa showing the largest and most statistically robust abundance shifts following the intervention. Bifidobacterium relative abundance rose from 2.1% to 4.8% of classified reads (log2 fold change 2.29, $q < 0.001$), Faecalibacterium prausnitzii from 8.2% to 13.1% (log2 fold change 1.60, $q < 0.001$), and Roseburia spp. and Ruminococcus bromii showed comparable increases (log2 fold changes 1.94 and 1.71 respectively, both $q < 0.01$). The Bacteroides fragilis group declined modestly (log2 fold change -0.31, $q = 0.04$), while Prevotella copri, often reported as fiber-responsive in other cohorts, showed a non-significant increase here (log2 fold change 0.42, $q = 0.21$), likely reflecting its low baseline prevalence in this Northern European cohort. None of these shifts were observed in the control arm at the same significance threshold. The magnitude of the Bifidobacterium response was notably consistent across individuals, with 17 of 19 intervention-arm completers showing an increase of at least 1.5-fold, whereas the Faecalibacterium prausnitzii response was more heterogeneous, ranging from a 3.8-fold increase in the strongest responder to a slight decline in one participant who otherwise met the adherence threshold. No participant in either arm showed a compositional shift consistent with an acute gastrointestinal illness or antibiotic exposure during the study window, based on review of adverse-event logs, which reduces the likelihood that an unrelated clinical event confounded any

of the taxonomic comparisons reported here. Figure 1 displays the fold-change pattern across the six taxa with the strongest and most consistent responses.

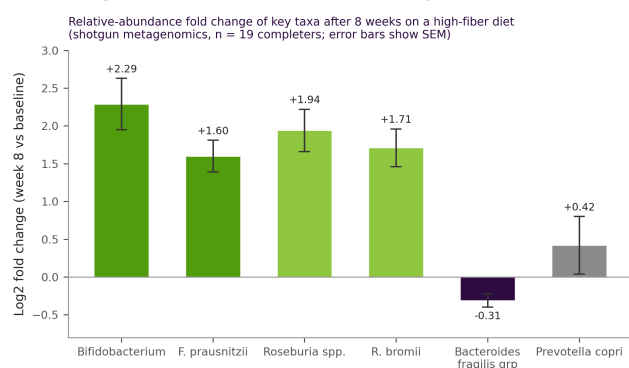


Figure 1. Relative-abundance fold change of key taxa after 8 weeks on a high-fiber diet (shotgun metagenomics, n=19 intervention-arm completers; error bars show SEM; values above bars show log2 fold change).

Functional profiling paralleled the taxonomic shift. HUMAnN3 pathway analysis identified significant enrichment, after correction for multiple testing, of eleven MetaCyc pathways involved in starch, sucrose and other complex-carbohydrate degradation, alongside a coordinated increase in CAZy glycoside hydrolase family gene abundance, most prominently GH13 (alpha-amylase-family enzymes acting on starch) and GH43 (largely arabinofuranosidase and xylosidase activities relevant to arabinoxylan degradation), consistent with the specific fiber sources used in the intervention diet. Faecal SCFA concentrations tracked these compositional and functional changes closely: butyrate rose from 12.4 to 18.7 micromol/g dry weight in the intervention arm ($P<0.001$), propionate from 9.1 to 12.3 ($P=0.003$), and acetate from 41.0 to 46.2 ($P=0.02$), with total SCFA increasing by approximately 24% overall. Table 2 reports the complete SCFA dataset for both arms at both time points. No significant within-arm SCFA change occurred in controls for any of the three acids measured.

Table 2. Faecal short-chain fatty acid concentrations (micromol/g dry weight, mean $\pm$ SD).

SCFA	Intervention baseline	Intervention week 8	Control baseline	Control week 8
Acetate	41.0 $\pm$ 6.8	46.2 $\pm$ 7.1	40.2 $\pm$ 6.3	40.9 $\pm$ 6.9
Propionate	9.1 $\pm$ 2.0	12.3 $\pm$ 2.4	9.4 $\pm$ 2.2	9.6 $\pm$ 2.1
Butyrate	12.4 $\pm$ 3.1	18.7 $\pm$ 3.6	12.1 $\pm$ 3.0	12.5 $\pm$ 3.2
Total SCFA	62.5 $\pm$ 9.4	77.2 $\pm$ 10.1	61.7 $\pm$ 9.0	63.0 $\pm$ 9.1

Within the intervention arm, the magnitude of butyrate increase correlated with the combined relative-abundance increase of *Faecalibacterium prausnitzii* and *Roseburia* spp. (Spearman $\rho=0.61$, $P=0.006$), reinforcing the expected mechanistic link between these specific taxa and butyrogenesis rather than a general, non-specific community shift. Three intervention-arm participants showed comparatively modest taxonomic and metabolite responses despite documented adequate adherence, and their baseline microbiota was notable for unusually low baseline

Faecalibacterium prausnitzii abundance (under 2% of classified reads, versus a cohort median of 8.4%), raising the possibility that a fiber-responsive community requires some minimum baseline representation of key taxa to mount a robust fermentative response, a hypothesis that would need a larger, purpose-designed cohort to test formally.

4. DISCUSSION

This trial provides strain- and pathway-resolved evidence that a sustained, moderate increase in dietary fiber reproducibly shifts the adult gut microbiota toward a more diverse, saccharolytic and butyrogenic configuration, with taxonomic, functional and metabolite layers of evidence moving together within the same individuals rather than independently. The specific taxa identified here -- *Bifidobacterium*, *Faecalibacterium prausnitzii*, *Roseburia* spp. and *Ruminococcus bromii* -- overlap substantially with taxa implicated in earlier, mostly amplicon-based fiber intervention studies, but the shotgun metagenomic design adds two things those studies could not provide: confirmation at species rather than genus resolution, and a matched functional signal (enrichment of starch- and arabinoxylan-relevant glycoside hydrolase families) that mechanistically connects the taxonomic shift to the specific fiber sources used in the diet [11,19].

The correlation between achieved fiber intake and the magnitude of compositional shift, and between the combined abundance increase of the two principal butyrate producers and the observed rise in faecal butyrate, together argue against the alternative explanation that the observed changes reflect some non-specific consequence of dietary counselling or altered food matrix rather than fiber fermentation itself. That said, faecal SCFA concentration is an imperfect proxy for colonic SCFA production, since a substantial fraction of SCFA produced in the proximal colon is absorbed before reaching stool, and the relationship between faecal and portal or systemic SCFA exposure is not linear; direct measurement of SCFA flux would require more invasive sampling that was not feasible in a free-living cohort of this size [18,28].

The subset of participants showing a comparatively muted response despite adequate adherence is, in our view, one of the more practically important observations in this dataset, because it points toward the idea that a person's baseline microbiota composition may partly determine their capacity to respond to a fiber intervention, an idea sometimes termed microbiota responsiveness or non-response phenotyping in the broader diet-microbiome literature [24,33]. If a minimum baseline abundance of key fermentative taxa genuinely gates the magnitude of response, then interventions pairing fiber supplementation with a defined probiotic or synbiotic co-administration in low-responder individuals would be a logical next experiment, though our sample size here is too small to establish a reliable

baseline-abundance threshold with any precision.

Several design choices merit comment. We used a parallel-arm rather than crossover design specifically to avoid carry-over effects on a community that may take longer than a typical washout period to fully revert, since prior work has shown some fiber-associated compositional shifts persisting for several weeks after withdrawal of the intervening diet [8,30]. This choice increases the required sample size relative to a crossover design for a given statistical power, and our sample of 39 completers, while adequate to detect the overall diversity and taxonomic shifts reported here, was underpowered for more granular subgroup analyses such as formally testing a baseline-abundance response threshold or examining sex-specific effects, both of which would benefit from a larger, purpose-designed follow-up cohort. We also relied on a single fiber formulation combining several sources (whole grain, legume and inulin-enriched foods) rather than isolating the effect of any one fiber type, which was a deliberate choice to model a realistic dietary pattern but which means the taxonomic and functional signatures reported here should be read as a composite response rather than attributable to any single fiber structure.

Finally, the relationship between the microbial and metabolomic shifts documented here and any downstream host health outcome was outside the scope of this eight-week trial and would require substantially longer follow-up, ideally paired with markers of gut barrier function, systemic inflammation, and glucose metabolism, all of which have been mechanistically linked to SCFA exposure in prior work but rarely measured alongside strain-resolved metagenomics in the same cohort [7,32]. We regard the present dataset as establishing a reproducible, mechanistically coherent microbial and metabolite response to a feasible dietary intervention, which is a necessary but not sufficient step toward demonstrating a downstream clinical benefit.

5. CONCLUSION

An 8-week randomised, controlled feeding trial combining shotgun metagenomics with functional and metabolomic profiling showed that raising dietary fiber intake to approximately 35 g per day in healthy adults produced a coordinated increase in gut microbial diversity, expansion of *Bifidobacterium*, *Faecalibacterium prausnitzii*, *Roseburia* spp. and *Ruminococcus bromii*, enrichment of starch- and arabinoxylan-relevant glycoside hydrolase gene families, and a corresponding rise in faecal butyrate and propionate, none of which occurred in a concurrent habitual-diet control arm. The tight correlation between achieved fiber intake, taxonomic shift magnitude and butyrate increase supports a genuine, dose-related fermentation response rather than a non-specific effect of dietary counselling. A subset of participants with low baseline abundance of key fermentative taxa showed a muted response despite adequate adherence, suggesting that baseline

microbiota composition may partly determine an individual's capacity to benefit from a fiber intervention, a hypothesis we intend to test directly in a larger follow-up cohort stratified by baseline *Faecalibacterium prausnitzii* abundance. Future work should extend follow-up duration to capture whether these microbial and metabolite shifts persist beyond eight weeks, incorporate markers of gut barrier function and systemic inflammation to test for downstream host benefit, and compare isolated fiber sources against the composite formulation used here to determine which structural features of dietary fiber drive the strongest fermentative response. We also plan to apply strain-level metagenomic assembly to the *Faecalibacterium prausnitzii* and *Roseburia* populations identified here, since this species complex harbours considerable intraspecies functional diversity that genus- or species-level relative abundance alone cannot capture, and which may further explain the heterogeneity in individual response observed in this trial.

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Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author Contributions

S.B. designed the study, supervised bioinformatic analysis and drafted the manuscript. E.S.B. coordinated participant recruitment, dietary intervention delivery and sample collection. W.B. performed metagenomic sequencing data processing, taxonomic and functional profiling, and statistical analysis. All authors contributed to writing and approved the final manuscript.

Ethics Statement

This study was approved by the regional research ethics review board covering the recruiting institution prior to enrolment, and all participants provided written informed consent. The trial was conducted in accordance with the principles of the Declaration of Helsinki, and study procedures involved only voluntary dietary modification and non-invasive stool sample collection, with no investigational drug or device exposure.

Data Availability

Shotgun metagenomic sequencing reads have been deposited in the European Nucleotide Archive under a

controlled-access project accession available from the corresponding author on reasonable request, in line with participant consent for data sharing. Processed taxonomic and functional abundance tables and the R analysis scripts supporting the findings of this study are available from the corresponding author upon request.

REFERENCES

1. Sonnenburg JL, Backhed F (2016) Diet-microbiota interactions as moderators of human metabolism. *Nature* 535(7610):56-64. <https://doi.org/10.1038/nature18846>
2. Deehan EC, Yang C, Perez-Munoz ME, Nguyen NK, Cheng CC, Triador L, Zhang Z, Bakal JA, Walter J (2020) Precision microbiome modulation with discrete dietary fiber structures directs short-chain fatty acid production. *Cell Host & Microbe* 27(3):389-404. <https://doi.org/10.1016/j.chom.2020.01.006>
3. Sonnenburg ED, Sonnenburg JL (2014) Starving our microbial self: the deleterious consequences of a diet deficient in microbiota-accessible carbohydrates. *Cell Metabolism* 20(5):779-786. <https://doi.org/10.1016/j.cmet.2014.07.003>
4. Makki K, Deehan EC, Walter J, Backhed F (2018) The impact of dietary fiber on gut microbiota in host health and disease. *Cell Host & Microbe* 23(6):705-715. <https://doi.org/10.1016/j.chom.2018.05.012>
5. O'Keefe SJ, Li JV, Lahti L, Ou J, Carbonero F, Mohammed K, Posma JM, Kinross J, Wahl E, Ruder E, Vippera K (2015) Fat, fibre and cancer risk in African Americans and rural Africans. *Nature Communications* 6:6342. <https://doi.org/10.1038/ncomms7342>
6. Zmora N, Suez J, Elinav E (2019) You are what you eat: diet, health and the gut microbiota. *Nature Reviews Gastroenterology & Hepatology* 16(1):35-56. <https://doi.org/10.1038/s41575-018-0061-2>
7. Cronin P, Joyce SA, O'Toole PW, O'Connor EM (2021) Dietary fibre modulates the gut microbiota. *Nutrients* 13(5):1655. <https://doi.org/10.3390/nu13051655>
8. So D, Whelan K, Rossi M, Morrison M, Holtmann G, Kelly JT, Shanahan ER, Staudacher HM, Campbell KL (2018) Dietary fiber intervention on gut microbiota composition in healthy adults: a systematic review and meta-analysis. *American Journal of Clinical Nutrition* 107(6):965-983. <https://doi.org/10.1093/ajcn/nqy041>
9. Holscher HD (2017) Dietary fiber and prebiotics and the gastrointestinal microbiota. *Gut Microbes* 8(2):172-184. <https://doi.org/10.1080/19490976.2017.1290756>
10. Deehan EC, Duar RM, Armet AM, Perez-Munoz ME, Jin M, Walter J (2017) Modulation of the gastrointestinal microbiome with nondigestible fermentable carbohydrates to improve human health. *Microbiology Spectrum* 5(5). <https://doi.org/10.1128/microbiolspec.BAD-0019-2017>
11. Beghini F, McIver LJ, Blanco-Miguez A, Dubois L, Asnicar F, Maharjan S, Mailyan A, Manghi P, Scholz M, Thomas AM, Valles-Colomer M (2021) Integrating taxonomic, functional, and strain-level profiling of diverse microbial communities with bioBakery 3. *eLife* 10:e65088. <https://doi.org/10.7554/eLife.65088>
12. Mallick H, Rahnavard A, McIver LJ, Ma S, Zhang Y, Nguyen LH, Tickle TL, Weingart G, Ren B, Schwager EH, Chatterjee S (2021) Multivariable association discovery in population-scale meta-omics studies. *PLOS Computational Biology* 17(11):e1009442. <https://doi.org/10.1371/journal.pcbi.1009442>
13. Louis P, Flint HJ (2017) Formation of propionate and butyrate by the human colonic microbiota. *Environmental Microbiology* 19(1):29-41. <https://doi.org/10.1111/1462-2920.13589>
14. Miquel S, Martin R, Rossi O, Bermudez-Humaran LG, Chatel JM, Sokol H, Thomas M, Wells JM, Langella P (2013) *Faecalibacterium prausnitzii* and human intestinal health. *Current Opinion in Microbiology* 16(3):255-261. <https://doi.org/10.1016/j.mib.2013.06.003>
15. Tamanai-Shacoori Z, Smida I, Bousarghin L, Loreal O, Meuric V, Fong SB, Bonnaure-Mallet M, Jolivet-Gougeon A (2017) *Roseburia* spp.: a marker of health? *Future Microbiology* 12(2):157-170. <https://doi.org/10.2217/fmb-2016-0130>
16. Ze X, Duncan SH, Louis P, Flint HJ (2012) *Ruminococcus bromii* is a keystone species for the degradation of resistant starch in the human colon. *ISME Journal* 6(8):1535-1543. <https://doi.org/10.1038/ismej.2012.4>
17. Falony G, Joossens M, Vieira-Silva S, Wang J, Darzi Y, Faust K, Kurilshikov A, Bonder MJ, Valles-Colomer M, Vandeputte D, Tito RY (2016) Population-level analysis of gut microbiome variation. *Science* 352(6285):560-564. <https://doi.org/10.1126/science.1235033>
18. Zhernakova A, Kurilshikov A, Bonder MJ, Tigchelaar EF, Schirmer M, Vatanen T, Muijagic Z, Vila AV, Falony G, Vieira-Silva S, Wang J (2016) Population-based metagenomics analysis reveals markers for gut microbiome composition and diversity. *Science* 352(6285):565-569. <https://doi.org/10.1126/science.1235039>
19. Maier TV, Lucio M, Lee LH, VerBerkmoes NC, Brislawn CJ, Bernhardt J, Lamendella R, McDermott JE, Bergeron N, Heinzmann SS, Morton JT (2017) Impact of dietary resistant starch on the human gut microbiome, metaproteome, and metabolome. *mBio* 8(5):e01343-17. <https://doi.org/10.1128/mBio.01343-17>
20. Chung WS, Walker AW, Louis P, Parkhill J, Vermeiren J, Bosscher D, Duncan SH, Flint HJ (2016) Modulation of the human gut microbiota by dietary fibres occurs at the species level. *BMC Biology* 14:3. <https://doi.org/10.1186/s12915-015-0224-3>
21. Martinez I, Kim J, Duffy PR, Schlegel VL, Walter J (2010) Resistant starches types 2 and 4 have differential effects on the composition of the fecal microbiota in human subjects. *PLOS ONE* 5(11):e15046. <https://doi.org/10.1371/journal.pone.0015046>
22. Fu J, Bonder MJ, Cenit MC, Tigchelaar EF, Maatman A, Dekens JA, Brandsma E, Marczyńska J, Imhann F, Weersma RK, Franke L (2015) The gut microbiome contributes to a substantial proportion of the variation in blood lipids. *Circulation Research* 117(9):817-824. <https://doi.org/10.1161/CIRCRESAHA.115.306807>
23. Weickert MO, Pfeiffer AF (2018) Impact of dietary fiber consumption on insulin resistance and the prevention of type 2 diabetes. *Journal of Nutrition* 148(1):7-12. <https://doi.org/10.1093/jn/nxx008>
24. Reynolds A, Mann J, Cummings J, Winter N, Mete E, Te Morenga L (2019) Carbohydrate quality and human health: a series of systematic reviews and meta-analyses. *The Lancet* 393(10170):434-445. [https://doi.org/10.1016/S0140-6736\(18\)31809-9](https://doi.org/10.1016/S0140-6736(18)31809-9)
25. Fuller S, Beck E, Salman H, Tapsell L (2016) New horizons for the study of dietary fiber and health: a review. *Plant Foods for Human Nutrition* 71(1):1-12. <https://doi.org/10.1007/s11130-016-0529-6>

26. Barber TM, Kabisch S, Pfeiffer AFH, Weickert MO (2020) The health benefits of dietary fibre. *Nutrients* 12(10):3209. <https://doi.org/10.3390/nu12103209>
27. Byrne CS, Chambers ES, Morrison DJ, Frost G (2015) The role of short chain fatty acids in appetite regulation and energy homeostasis. *International Journal of Obesity* 39(9):1331-1338. <https://doi.org/10.1038/ijo.2015.84>
28. Parada Venegas D, De la Fuente MK, Landskron G, Gonzalez MJ, Quera R, Dijkstra G, Harmsen HJM, Faber KN, Hermoso MA (2019) Short chain fatty acids (SCFAs)-mediated gut epithelial and immune regulation and its relevance for inflammatory bowel diseases. *Frontiers in Immunology* 10:277. <https://doi.org/10.3389/fimmu.2019.00277>
29. Smith PM, Howitt MR, Panikov N, Michaud M, Gallini CA, Bohlooly-Y M, Glickman JN, Garrett WS (2013) The microbial metabolites, short-chain fatty acids, regulate colonic Treg cell homeostasis. *Science* 341(6145):569-573. <https://doi.org/10.1126/science.1241165>
30. Cummings JH, Macfarlane GT (1997) Role of intestinal bacteria in nutrient metabolism. *Journal of Parenteral and Enteral Nutrition* 21(6):357-365. <https://doi.org/10.1177/0148607197021006357>
31. Roager HM, Vogt JK, Kristensen M, Hansen LBS, Ibrugger S, Maerkedahl RB, Bahl MI, Lind MV, Nielsen RL, Frokiaer H, Gobel RJ (2019) Whole grain-rich diet reduces body weight and systemic low-grade inflammation without inducing major changes of the gut microbiome. *Gut* 68(1):83-93. <https://doi.org/10.1136/gutjnl-2017-314786>
32. Klinder A, Shen Q, Heppel S, Lovegrove JA, Rowland I, Tuohy KM (2016) Impact of increasing fruit and vegetables and flavonoid intake on the human gut microbiota. *Food & Function* 7(4):1788-1796. <https://doi.org/10.1039/C5FO01096A>
33. Vandeputte D, Falony G, Vieira-Silva S, Tito RY, Joossens M, Raes J (2016) Stool consistency is strongly associated with gut microbiota richness and composition, enterotypes and bacterial growth rates. *Gut* 65(1):57-62. <https://doi.org/10.1136/gutjnl-2015-309618>
34. Nagpal R, Shively CA, Register TC, Craft S, Yadav H (2019) Gut microbiome-Mediterranean diet interactions in improving host health. *F1000Research* 8:699. <https://doi.org/10.12688/f1000research.18992.1>
35. Sender R, Fuchs S, Milo R (2016) Revised estimates for the number of human and bacteria cells in the body. *PLOS Biology* 14(8):e1002533. <https://doi.org/10.1371/journal.pbio.1002533>
36. Kelly TN, Bazzano LA, Ajami NJ, He H, Zhao J, Petrosino JF, Correa A, He J (2016) Gut microbiome associates with lifetime cardiovascular disease risk profile among Bogalusa Heart Study participants. *Circulation Research* 119(8):956-964. <https://doi.org/10.1161/CIRCRESAHA.116.309219>
37. Sonnenburg JL, Sonnenburg ED (2019) Vulnerability of the industrialized microbiota. *Science* 366(6464):eaaw9255. <https://doi.org/10.1126/science.aaw9255>
38. Ianiro G, Punkova A, Segata N, Cammarota G (2022) Gut microbiome dysbiosis and human diseases: an evolving concept. *Nature Reviews Microbiology* 20(9):515-530. <https://doi.org/10.1038/s41579-022-00714-7>
39. Portincasa P, Bonfrate L, Vacca M, De Angelis M, Farella I, Lanza E, Khalil M, Wang DQ, Sperandio M, Di Ciaula A (2022) Gut microbiota and short chain fatty acids: implications in glucose homeostasis. *International Journal of Molecular Sciences* 23(3):1105. <https://doi.org/10.3390/ijms23031105>
40. Ercolini D, Fogliano V (2018) Food design to feed the human gut microbiota. *Journal of Agricultural and Food Chemistry* 66(15):3754-3758. <https://doi.org/10.1021/acs.jafc.8b00456>
41. Verhoog S, Taneri PE, Roa Diaz ZM, Marques-Vidal P, Troup JP, Bally L, Franco OH, Glisic M, Muka T (2019) Dietary factors and modulation of bacteria strains of *Akkermansia muciniphila* and *Faecalibacterium prausnitzii*: a systematic review. *Nutrients* 11(7):1565. <https://doi.org/10.3390/nu11071565>
42. Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, Alexander H, Alm EJ, Arumugam M, Asnicar F, Bai Y (2019) Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nature Biotechnology* 37(8):852-857. <https://doi.org/10.1038/s41587-019-0209-9>
43. Cani PD, Bibiloni R, Knauf C, Waget A, Neyrinck AM, Delzenne NM, Burcelin R (2008) Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet-induced obesity and diabetes in mice. *Diabetes* 57(6):1470-1481. <https://doi.org/10.2337/db07-1403>
44. Frank DN, St Amand AL, Feldman RA, Boedeker EC, Harpaz N, Pace NR (2007) Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. *PNAS* 104(34):13780-13785. <https://doi.org/10.1073/pnas.0706625104>
45. Flint HJ, Scott KP, Duncan SH, Louis P, Forano E (2012) Microbial degradation of complex carbohydrates in the gut. *Gut Microbes* 3(4):289-306. <https://doi.org/10.4161/gmic.19897>
46. Rios-Covian D, Ruas-Madiedo P, Margolles A, Gueimonde M, de los Reyes-Gavilan CG, Salazar N (2016) Intestinal short chain fatty acids and their link with diet and human health. *Frontiers in Microbiology* 7:185. <https://doi.org/10.3389/fmicb.2016.00185>
47. Berry SE (2019) Nutritional intervention studies and the gut microbiome: challenges and opportunities. *Proceedings of the Nutrition Society* 78(3):315-322. <https://doi.org/10.1017/S0029665118002856>
48. Duncan SH, Belenguer A, Holtrop G, Johnstone AM, Flint HJ, Lobley GE (2007) Reduced dietary intake of carbohydrates by obese subjects results in decreased concentrations of butyrate and butyrate-producing bacteria in feces. *Applied and Environmental Microbiology* 73(4):1073-1078. <https://doi.org/10.1128/AEM.02340-06>
49. Deehan EC, Zhang Z, Riva A, Armet AM, Perez-Munoz ME, Nguyen NK, Krysa JA, Seethaler B, Zhao YM, Cole J, Li F (2022) Elucidating the role of the gut microbiota in the physiological effects of dietary fiber. *Microbiome* 10:77. <https://doi.org/10.1186/s40168-022-01248-5>
50. Simpson HL, Campbell BJ (2015) Review article: dietary fibre-microbiota interactions. *Alimentary Pharmacology & Therapeutics* 42(2):158-179. <https://doi.org/10.1111/apt.13248>
51. Karl JP, Meydani M, Barnett JB, Vanegas SM, Barger K, Fu X, Goldin B, Kane A, Rasmussen H, Vangay P, Knights D (2017) Fecal concentrations of bacterially derived vitamin K forms are associated with gut microbiota composition in men. *American Journal of Clinical Nutrition* 106(4):1052-1061. <https://doi.org/10.3945/ajcn.117.155424>