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Association of long-term habitual dietary fiber intake since infancy with gut microbiota composition in young adulthood

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Abbreviations: ASV, amplicon sequence variant; PCoA, principal coordinate analysis; PERMANOVA, Permutational Analysis of Variation; SCFA, short-chained fatty acid; STRIP, Special Turku Coronary Risk Factor Intervention Project

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Abstract

Background: Dietary fiber is an important health-promoting component of the diet, which is fermented by the gut microbes that produce metabolites beneficial for the host's health.

Objective: We studied the associations of habitual long-term fiber intake from infancy with gut microbiota composition in young adulthood by leveraging data from the Special Turku Coronary Risk Factor Intervention Project (STRIP), an infancy-onset 20-year dietary counselling study.

Methods: Fiber intake was assessed annually using food diaries from infancy up to age 20 years. At age 26 years, the first post-intervention follow-up study was conducted including food diaries and fecal sample collection (N=357). Cumulative dietary fiber intake was assessed as the area under curve for energy-adjusted fiber intake throughout the study (age 0 – 26 years). Gut microbiota was profiled using 16S rRNA amplicon sequencing. The primary outcomes were 1) alpha diversity expressed as observed richness and Shannon index, 2) beta diversity using Bray-Curtis dissimilarity scores, and 3) differential abundance of each microbial taxa with respect to the cumulative energy-adjusted dietary fiber intake.

Results: Higher cumulative dietary fiber intake was associated with decreased Shannon index (β = -0.019 per unit change in cumulative fiber intake, p = 0.008). Overall microbial community composition was related to the amount of fiber consumed (permutational analysis of variation R^2 = 0.005, p = 0.024). The only genus that was increased with higher cumulative fiber intake was butyrate-producing *Butyrivibrio* ($\log_2$ fold-change per unit change in cumulative fiber intake 0.40, adjusted p = 0.023), while some other known butyrate producers such as *Faecalibacterium* and *Subdoligranulum* were decreased with higher cumulative fiber intake.

Conclusions: As early-life nutritional exposures may affect the lifetime microbiota composition and disease risk, this study adds novel information on the associations of long-term dietary fiber intake with the gut microbiota.

Clinical Trial Registration: NCT00223600, https://clinicaltrials.gov/study/NCT00223600

Keywords: dietary fiber; long-term habitual diet; gut microbiota; microbiota diversity; microbiota composition

Introduction

Dietary fiber is a carbohydrate in plant-based foods, such as whole grains, vegetables, fruit, and legumes, which has been a major component in the human diet throughout our history. Humans have co-evolved with gut microbes which have helped us to face the challenges of changing environmental exposures and to digest food sources that would otherwise be unusable to humans (1). For instance, dietary fibers contain plant-based carbohydrates that human enzymes are incapable of digesting, whereas they are fermented by gut microbes (2). As a result, gut microbes produce metabolites such as short-chained fatty acids (SCFA), the most abundant being butyrate, acetate, and propionate (2,3). The SCFAs not only play a role locally at the colon but they are released into the circulation, where they exert various beneficial effects throughout the body, contributing to glucose and fat metabolism and immune function, as well as affecting the host brain (4,5). Recent advances in microbiome research have highlighted the role of dietary fiber in human health and disease, and introducing more fiber into the diet could improve health outcomes.

The benefits of dietary fiber on gut microbiome profile have been studied mainly in adult populations exposed to short-term dietary intervention with a supplementation of a specific fiber type (2,3,6). The results of dietary intervention studies have been somewhat inconsistent both regarding the diversity of gut microbiota and the bacterial taxa changed after the intervention, but the majority of the studies administrating specific prebiotic fiber supplement have reported increased abundance of *Bifidobacterium* and *Lactobacillus* (2,3,6). While these short-term dietary studies have demonstrated that gut microbiota composition can change quickly, these alterations are transient and only persist a few days (7). On the other hand, cross-sectional studies comparing different types of diet (e.g. western-diet consumers and agricultural populations) have shown that dietary patterns are associated with microbial composition (7,8). A habitual diet, but not short-term dietary intake, is associated with

55 enterotypes, particularly a diet rich with protein and animal fat to *Bacteroides* and a diet rich
56 with carbohydrates to *Prevotella* enterotype (9), highlighting the role of habitual dietary
57 intake on long-term effects on the gut microbiota.

58 The gut microbiota changes dramatically after birth and for the second time when introducing
59 solid foods (10). By the age of three years, a more stable microbial composition is established,
60 and the microbial biodiversity seems to be at its greatest in six to twelve years old children
61 experimenting large variety of foods, eventually ending up to adulthood habitual dietary
62 patterns and a stable gut microbiota enterotype (8). While there are studies regarding the
63 beneficial effects of human milk oligosaccharides in infant gut microbiota (10), little is known
64 about the effects of fiber intake in childhood or adolescence on the gut microbiota
65 composition, although a few smaller dietary studies on prepuberty-aged children exist (11,12).
66 However, lifelong eating behaviors are established in childhood. For instance, dietary
67 exposure during the first thousand days of life is shown to affect lifetime obesity and disease
68 risk (13), highlighting the importance of early-life dietary patterns on the entire lifespan. How
69 the cumulative fiber intake from childhood through adolescence to young adulthood affects
70 gut microbiota profile in young adulthood is still unclear.

71 The longitudinal Special Turku Coronary Risk Factor Intervention Project (STRIP),
72 established in 1989, was launched to reduce children's exposure to environmental
73 cardiovascular risk factors from infancy to early adulthood (14). The focus of the 20-year
74 intervention was to replace saturated fat with unsaturated fat in a child's diet and
75 concomitantly reduce the intake of cholesterol by dietary counselling. The intervention further
76 encouraged to favor whole grain products over more highly refined options, as well as to
77 increase the amount of fruit, vegetables, and berries in the diet. Our previous results have
78 shown that the STRIP intervention has successfully reached its targets by improving many
79 cardiometabolic health markers at the end of the intervention (15–17) and leading to higher

consumption of fiber-rich grain products, fruits and berries, as well as vegetables (18,19). Health benefits after 20 years of counselling were largely maintained into adulthood (20) but there were only subtle differences in the gut microbiota profiles between the intervention and control groups six years after the intervention had ended, possibly due to divergent adaption of the dietary counselling intervention by the participants (21). The only genus that was more abundant in the intervention group compared to the controls was *Veillonella* (21), which ferments lactates to short-chained fatty acids (22) but has no evident link with dietary components.

The present study leverages the extensive longitudinal data on the habitual dietary fiber intake from early childhood to young adulthood in a subset of the STRIP cohort, who gave a fecal sample at the first post-intervention follow-up study at the age of 26 years. As dietary fiber is one of the most potent food components affecting gut microbiota composition, we ranked the STRIP participants according to their cumulative dietary fiber intake, irrespective of belonging to the intervention or control group. This setting allows us to address the question, whether habitual dietary fiber intake throughout childhood and adolescence influences gut microbiota composition in young Finnish adults.

Materials and Methods

Study Design

The STRIP study is a prospective randomized trial that aims to prevent atherosclerosis beginning in infancy (14). In brief, families of 5-month-old infants born between July 1989 and December 1991 were recruited at well-baby clinics in Turku, Finland, by nurses. At the age of 7 months, 1062 infants (56.5% of the eligible age cohort) were randomly allocated to a dietary intervention (n = 540) or control (n = 522) group (**Figure 1**). The cohort additionally included two children with Down syndrome (both control), two with familial hypercholesterolemia (intervention and control), and five children who had been randomized to the intervention group, and who missed the first study visits prior to age 13 months and were later treated as controls. Furthermore, a group of 45 children born between March and July 1989 was similarly recruited and randomized (intervention n = 22, control n = 23) to first test the study protocols, and thus served as a 'pilot' group.

The intervention group received individualized dietary counselling at 1- to 3-month intervals until age 2 years, and biannually thereafter until the age of 20 years as described in detail previously (18). The control children were seen biannually until the age of seven years and annually thereafter. The children in the control group did not receive the counselling intervention, although similar measurements, including keeping food diaries, were performed for both study groups and they met with the same study personnel.

The first post-intervention follow-up with the participants was conducted between April 2015 and January 2018 at the age of 26 years (here defined as young adults), six years after the intervention had ended (20) (Figure 1). Of the cohort (n = 1116), 1072 were invited to participate (excluded n = 44; deceased n = 7; no information on place of residence n = 6; congenital physical impairment n = 5; lived abroad n = 26). Of these, 551 provided follow-up

120 data (51%; intervention n = 263 vs. control n = 288). Of the follow-up study participants, five
121 provided only questionnaire data. Reasons for non-participation (n = 521) were: no response
122 to invitation (n = 356); declined invitation (n = 153); and discontinuation of the study (n =
123 12).

124 Individuals included in the present study comprise those who successfully provided data on
125 gut microbiota composition at the 26-year follow-up (n = 357). The participants with
126 successful gut microbiota analyses consumed more vegetables, fruit, and berries and tended to
127 have higher total absolute daily fiber intake compared to individuals who had attended the 26-
128 year follow-up study clinic visit and either did not provide a fecal sample or provided a
129 sample that could not be successfully sequenced (n = 189) (21). At the age of 26 years,
130 energy-adjusted fiber intake as well as other dietary and anthropometric characteristics were
131 similar between the subgroup included in this study and those excluded (21). Long-term
132 cumulative fiber intake, both absolute and energy-adjusted, was similar between the subjects
133 included in this sub-study and those excluded (**Supplementary Table 1**). Moreover,
134 cumulative cardiometabolic risk markers and dietary fat intakes were similar between the
135 groups (**Supplementary Table 1**).

136 This study has been approved by the Joint Commission on Ethics of Turku University and
137 Turku University Central Hospital (Approval code: ETMK: 51/1801/2014. Approval date: 20
138 May 2014.). Written informed consent was obtained from parents at study entry and from the
139 participants at the ages of 15, 18, and 26 years.

140 ***Dietary data collection***

141 Before each study visit, a food diary on four consecutive days (until 2 years of age three
142 consecutive days), including 1–2 weekend days, was filled in to obtain meticulous data on the
143 participants' dietary intake, i.e. the foods/drinks and nutrients. Participants were instructed to

record regular days and avoid non-regular, e.g., holidays/sick days where food intake was atypical. In the beginning, the parents were carefully instructed to record their children's food intake. Parents and/or caregivers (e.g., nanny, grandparent) were responsible for filling out the food record during infancy. After the beginning of daycare or school, the establishment's personnel were asked to assist the child in completing the food records. As the children aged, they were given more responsibility in completing their food records, however, parents were still advised to check the records and assist the child. The food records were sent to the participants 3–4 weeks preceding each study visit with written instructions and a food portion estimation visual aid booklet to ensure accurate reporting. Portion sizes were estimated using household measures (e.g., spoons, cups), and details regarding the foods (e.g., brand and preparation method) were requested. During the study visit, the diary was reviewed for completeness and accuracy by a dietary technician, and missing details were added after discussion where necessary. The food diary data were entered into the Micro-Nutrica® food analysis software (developed at the Research and Development Centre of the Social Insurance Institution, Finland) to calculate food and nutrient intake. This software has been regularly updated throughout the study and can calculate 66 separate nutrient values from over 4000 foods and dishes.

Cumulative dietary fiber intake calculation

The energy-adjusted fiber intake was selected to represent the effects of long-term cumulative fiber intake in this study because the subjects were at growing age and their total energy intake changed during the study. Therefore, the energy-adjusted fiber intake is more comparable between the time points compared to the absolute fiber intake. To evaluate the long-term cumulative fiber intake throughout the study, subject-specific curves for the energy-adjusted fiber intake were estimated by mixed model regression splines (23). The covariance structure for the longitudinal setting was modeled by allowing for subject-specific regression

spline coefficients, which were incorporated as random effects into the model. We used three knots on the subject-specific and five knots on the fixed effects part. The mean profile was allowed to vary across sex in terms of possibly different fixed effects parts. Similar to the approach of Lai *et al* (24), we then evaluated the area under the curve (AUC) as a measure of the long-term effect of dietary fiber intake. The AUC variables were defined separately for early childhood (0 to 6 years), childhood (7 to 12 years), adolescence (13 to 18 years), young adulthood (19 to 26 years), and the entire follow-up period (0 to 26 years) (**Figure 2A**).

Anthropometrics and blood samples

Height, weight, and waist circumference were measured and BMI was calculated as weight (kg)/(height (m^2)) (20). Blood samples were drawn at the study visit annually throughout the intervention period and in the post-intervention follow-up study at age 26 years following overnight fasting, and serum samples were separated, aliquoted, and stored at −70 ◦C (20). The samples were thawed for the first time for the following analyses. Serum triglycerides, total cholesterol, HDL-cholesterol, and serum glucose were analyzed using an AU400 instrument (Olympus, Hamburg, Germany) and applicable system reagents (Beckman Coulter, Brea, CA, USA). LDL cholesterol concentration was estimated using the Friedewald formula. If triglyceride level was ≥4.5 mmol/L, LDL cholesterol was set to be missing. Serum insulin was determined using an ARCHITECT insulin assay (Abbott, Chicago, IL, USA) on an Architect ci8200 analyzer (Abbott, USA), and insulin resistance was estimated using the homeostatic model for assessing insulin resistance (HOMA-IR; (fasting insulin × fasting glucose)/22.5). Sitting blood pressure was measured using an oscillometric device with an average of three measurements used in the analyses. A similar AUC approach as for the dietary fiber intake was applied to define cumulative cardiovascular risk factor exposure and dietary components related to fat quality (Supplementary Tables 1-2).

Fecal Microbiota Composition

The gut microbiota of the STRIP participants was assessed for the first time in the 26-year follow-up study (21). Fecal samples were collected by the participants at their homes and sent to the study center by mail (n = 370). The protocol for the self-collection of approximately 500 mg of fecal material and the extraction of bacterial DNA from the fecal samples are described in detail previously (21). Three samples were omitted due to poor sample quality. Fecal microbiota profiles were analyzed by 16S rRNA gene sequencing; variable region V4 of the bacterial 16S rRNA gene was amplified with custom-designed dual-indexed primers and sequenced with an Illumina MiSeq system as previously described (25). Each sequencing run included a positive plasmid-mix control and a negative aqua control (25). The raw 16S rRNA gene sequencing data was demultiplexed and the sequence adapters, primers, and barcodes were clipped using the Illumina BaseSpace platform. Ten samples were excluded from further analyses due to unsuccessful 16S rRNA gene sequencing, resulting in the final sample cohort of 357 individuals. The raw sequence data was processed into an amplicon sequence variant (ASV) table as described in detail previously (21). The generated ASV table altogether comprised 6.3×10^7 trimmed and chimera-removed high-quality sequence reads. The acquired read counts from the 16S rRNA gene sequencing varied significantly within the study population (min: 11.8 k, max: 839 k, median: 160 k). Taxonomic classification of the sequences was performed using the NCBI RefSeq 16S rRNA database supplemented by the Ribosomal Database Project database (RefSeqRDP16S_v2_May2018). The generated unfiltered data was constructed into a TreeSummarizedExperiment object, which included 6591 unique ASVs that corresponded to 20 different bacterial phyla and 291 bacterial genera.

Statistical analyses

The primary outcomes of this study were 1) alpha diversity expressed as observed richness and Shannon index, 2) beta diversity using Bray-Curtis dissimilarity scores, and 3) differential

abundance of each microbial taxa with respect to the cumulative energy-adjusted dietary fiber intake, which was used as a continuous variable in all analyses. All analyses were performed for the whole study group adjusted for sex. The statistical analyses were performed using R (v. 4.2.1, R Foundation for Statistical Computing, Vienna, Austria; https://www.R-project.org/).

Gut microbiota alpha diversity, represented by the observed richness and Shannon index, was determined using *mia* (26). Observed richness describes the number of different ASVs present in a sample, while the Shannon index describes the bacterial diversity in a sample by counting the abundance and evenness of the ASVs present. The association between alpha diversity and cumulative fiber intake was assessed using linear regression. Microbiome beta diversity, which describes the dissimilarities in ecosystem-level community composition between samples, was performed for the ASV-level with a relative abundance transformation. Beta diversity was visualized with a principal coordinate analysis (PCoA) ordination with Bray-Curtis dissimilarity. Permutational Analysis of Variation (PERMANOVA) was performed using the *adonis2* function in *vegan* (27) with Bray-Curtis dissimilarity and 999 permutations. Differential abundance analysis of the microbiota was performed using *LinDA*, which fits linear regression models on the centered log-ratio (clr) transformed data and corrects the bias due to compositional effects (28). To reduce multiple comparisons, rare taxa were excluded before *LinDA* analysis by filtering out ASVs that were detected with <2% prevalence at <0.1% relative abundance threshold, resulting in 423 unique ASVs. We analyzed differential abundances at the phylum, family, and genus levels. We used the default parameters in *LinDA*, and *p*-values were adjusted for multiple comparisons using the Benjamini-Hochberg procedure. Adjusted *p*-values at the level of 0.05 were considered statistically significant.

Results

Participant characteristics

The study cohort consisted of 357 26-year-old participants, of whom 203 were females and 154 males. The participants were divided into quartiles according to their cumulative energy-adjusted fiber intake (AUC 0-26 years). The participants in the first quartile were considered as the low fiber intake group (mean energy-adjusted fiber intake 1.68 [SD 0.48] g/MJ at age 26 years), the participants in the second and third quartile as the medium fiber intake group (2.35 [SD 0.66] g/MJ), and the participants in the fourth quartile as the high fiber intake group (3.13 [SD 0.87] g/MJ) to highlight the difference between the high and low dietary fiber consumers. Characteristics of the participants at the age of 26 years are presented in **Table 1**. The percentage of females was highest in the high fiber intake group and decreased in the medium and the low fiber intake groups. In general, the cardiometabolic risk factor profile was slightly more favorable in the high fiber intake group especially compared to the low fiber intake group. For the cumulative cardiometabolic risk factors, only cumulative HDL cholesterol was increased in the high fiber intake group compared to the low fiber intake group (**Supplementary Table 2**).

Total energy intake, as well as protein intake, was higher in the low fiber intake group compared to the high fiber intake group, whereas the high fiber intake group consumed more carbohydrates. Total dietary fat intake was similar between the groups, but there were differences between the groups in types of fatty acids consumed both at the age of 26 years (Table 1) and cumulatively (Supplementary Table 2). Absolute fiber intake was decreased both in the medium and the low fiber intake group compared to the high fiber intake group, and the intake of fruits and berries followed the same trend. However, the consumption of whole grain bread was not different between the groups, and consumption of vegetables was

decreased in the high fiber intake group compared to the low fiber intake group (Table 1). Energy-adjusted fiber intake was higher in females compared to males both at age 26 years and cumulatively throughout the study (**Figure 2B**). The Nordic recommendation for energy-adjusted dietary fiber intake for adults is at least 3 g/MJ (29). At age 26 years, 31% of females and 9% of males reached the recommendation.

Patterns of habitual fiber intake

We first explored the patterns of habitual dietary fiber intake to evaluate whether the cumulative fiber intake expressed as AUC 0-26 years reflects the fiber intake throughout the study. When evaluated at each age period (early childhood, childhood, adolescence, and young adulthood), the dietary fiber intake status remained rather stable judging from a visual inspection of a Sankey flow chart (**Figure 2C**). Further, pairwise correlations between two consecutive age periods were high, and the correlation between cumulative fiber intake AUC 0-26 years correlated well with each age period (**Figure 2D**). Therefore, AUC 0-26 years appears to track the fiber intake during the whole study period. The correlation between cumulative fiber intake AUC 0-26 years and fiber intake at the age of 26 years was $r = 0.63$.

Association of cumulative dietary fiber intake with microbiota alpha and beta diversity

The mean observed richness in the study population was 249 (SD 79). There was no association between the continuous cumulative fiber intake and observed richness ($p = 0.21$, **Supplementary Figure 1A**). For the Shannon index, the mean was 3.23 (SD 0.95) and it was lower in males than in females ($\beta = -0.25$, $p = 0.020$). Shannon index decreased with increasing cumulative fiber intake ($\beta = -0.019$ per unit change in cumulative fiber intake, $p = 0.008$, **Supplementary Figure 1B**).

Beta diversity was visualized in the PCoA ordination (**Figure 3**). Although no apparent patterns could be visually observed in the PCoA plot, PERMANOVA indicated that both

cumulative fiber intake and sex explained a significant proportion of the variation in the ecosystem-level microbiota composition (PERMANOVA $R^2 = 0.005$, $p = 0.024$ for cumulative fiber intake, $R^2 = 0.006$, $p = 0.007$ for sex).

Association of cumulative dietary fiber intake with the abundances of microbial taxa

After the removal of extremely rare taxa, nine different bacterial phyla, 30 bacterial families, and 77 bacterial genera were detected. *Bacteroidetes* was the most abundant phylum (mean relative abundance 52.0%), and its abundance tended to be associated with higher fiber intake ($\log_2$ fold-change 0.23 per unit change in cumulative fiber intake, adjusted $p = 0.076$). *Firmicutes* was the second most abundant phylum (mean relative abundance 40.6%), but its abundance was not related to fiber intake in *LinDA* analysis. *Firmicutes*/*Bacteroidetes* ratio was not associated with cumulative fiber intake. In *LinDA,* the only phylum that was differentially abundant was *Tenericutes* ($\log_2$ fold-change 0.44, adjusted $p = 0.008$), but its mean relative abundance was only 0.1% (**Supplementary Figure 2**).

At the family level, only the abundance of *Pasteurellaceae* was elevated with higher fiber intake ($\log_2$ fold-change 0.57, adjusted $p = 0.042$, **Supplementary Figure 3**). The differentially abundant bacterial genera with $|\log_2 \text{fold-change}| \geq 0.25$ are illustrated in **Figure 4**. A higher cumulative fiber intake was associated with an increased abundance of *Butyrivibrio* ($\log_2$ fold-change 0.40, adjusted $p = 0.023$). *Haemophilus*, a genus related to the *Pasteuerellacae* family, tended to be more abundant with higher fiber intake ($\log_2$ fold-change 0.46, adjusted $p = 0.081$). Genera that were statistically significantly decreased with higher fiber intake were *Intestinimonas, Subdoligranulum, Flavonifractor, Faecalibacterium,* and *Blautia*. The abundances of most of the differentially abundant genera found by *LinDA* were low (mean relative abundance < 0.3%), except for *Blautia* (1.2%) and *Faecalibacterium* (7.3%).

314 ***Additional analyses***

315 We have previously shown that the intervention improved children's diet quality compared to
316 the control group (18). Therefore, we repeated the analyses by including a categorical variable
317 describing the intervention/control group status in the models to study whether the overall diet
318 quality as induced by the intervention affects the results. However, after incorporating the
319 intervention/control group information into the models, the results remained qualitatively
320 similar (data not shown).

Discussion

The STRIP is a unique longitudinal study with two decades of dietary data from infancy to young adulthood. The present sub-study shows that long-term habitual fiber intake from early childhood to early adulthood associated with gut microbiota composition in young adulthood. Microbial diversity was decreased with higher cumulative fiber intake, and long-term fiber intake was related to the difference in the overall microbial community composition. The only genus that was increased with higher cumulative fiber intake was butyrate-producing *Butyrivibrio*, while some other known butyrate producers such as *Faecalibacterium* and *Subdoligranulum* were decreased with higher cumulative fiber intake in our study. As there are no other similar longitudinal studies beginning from infancy, the results of this study add novel information on the associations of long-term dietary fiber intake on gut microbiota in adulthood.

Generally, higher microbial richness and diversity are considered an indication of "a healthy gut microbiome", as a higher diversity of microbes can compensate for each other and provide a more robust ecosystem against environmental influences (30). In this study, richness was not affected by the cumulative fiber intake. Alpha diversity expressed as Shannon index decreased with higher cumulative fiber intake, though the decrease per unit change of cumulative fiber intake was small. Previous results from cross-sectional studies and randomized trials have been inconsistent regarding the effects of fiber intake on alpha diversity, inducing an increase (31), a decrease (3), or no effect on alpha diversity (6,32,33). While the increase in microbial diversity is generally considered to be a favorable change, it may be that higher fiber intake may increase the abundance of fiber-digesting bacterial taxa that are SCFA producers (34).

Previous studies regarding beta diversity have shown consistently that people with a fiber-rich diet (rural/unindustrialized diet, Mediterranean diet, or vegetarian diet) have distinctly different microbial community composition compared to people living in developed areas consuming a Western diet (2,3). In a large-scale Finnish adult population study, dietary fiber intake was also associated with a change in beta diversity (31). Furthermore, most intervention trials administrating high-fiber diets have reported a change in beta diversity (3). Our result is in line with previous studies, as habitual cumulative fiber intake was related to the difference in overall community composition.

Dietary fiber is known to improve the richness of bacterial taxa that are capable of digesting fiber and producing SCFA (35,36). Dietary fiber comes in various forms, and different bacterial species have the capacity to degrade specific fiber types (36). Moreover, a specific fiber type may require multiple steps in the catalytic pathway with multiple microbes contributing to the degradation of fiber and SCFA production. Therefore, intervention studies administrating different types of dietary fiber have been shown to lead to divergent changes in the microbial taxa (3,6,8). Moreover, habitual dietary patterns can lead to different core microbial populations, which can affect respondence or non-respondence to dietary intervention and thus, contribute to the heterogenous outcomes in previous studies (8). For instance, many of the short-term intervention studies have reported an increase in the abundance of *Bifidobacterium* and *Lactobacillus* (3,6). The growth of these bacterial taxa is related to the use of prebiotics which include dietary fiber types rich with inulin, fructo- and galactooligosaccharides, whereas fibers not classified as prebiotics have not affected the abundance of these species (8). In the present study, these genera were not related to cumulative fiber intake, which could be because cumulative fiber intake comprises the entire intake of dietary fibers from all sources.

368 The most abundant bacterial genera in this study were *Bacteroides* and *Prevotella*. The
369 abundance of *Bacteroides* tended to increase with lower cumulative fiber intake. While
370 *Prevotella* was elevated with higher fiber intake, its variance was so large that the change
371 remained non-significant. These two genera seem to be the main drivers differentiating the gut
372 microbiota profiles between long-term diets based on carbohydrates (*Prevotella*) and protein
373 and animal fat (*Bacteroides*) (9). *Prevotella* can ferment complex carbohydrates and many
374 different fiber types (9), and it is often reported to be increased in unindustrialized or rural
375 populations or Western populations in individuals on vegan or vegetarian diets (2), but not in
376 short-term single-fiber interventions (3). *Bacteroides* is a bile-tolerant genus associated with
377 increased colorectal cancer risk (37). It is one of the bacterial taxa whose increase is
378 associated with a microbial signature of disease and poor diet quality along with
379 *Flavonifractor* (38,39), which was also increased with lower fiber intake in our study.

380 The mean abundance of those bacterial genera that were differently abundant with respect to
381 the cumulative fiber intake in our study was mostly present in relatively low abundances. The
382 only genus that was increased with higher cumulative fiber intake was *Butyrivibrio*, a fiber-
383 degrading bacterial taxa producing butyrate (40,41) that has been associated with fiber intake
384 and healthy dietary patterns (31,39,42). In addition, *Haemophilus* tended to increase with
385 higher fiber intake, and in accordance, the family *Pasteurellaceae* was also elevated. Large-
386 scale population studies with deep shotgun sequencing have revealed *Haemophilus*
387 *parainfluenza* and *Butyrivibrio crossotus* as novel microbiome-health associations
388 (38,39,42,43). The results of our study support the growing evidence on the potential health-
389 promoting capacity of these bacterial taxa and encourage to further explore their function and
390 mechanisms to study their possible therapeutic modulation.

391 Surprisingly, *Faecalibacterium* and *Subdoligranulum* were associated with lower cumulative
392 fiber intake in our study. Both of these taxa are related to butyrate production (41) and a

393 decrease in their abundances has been connected to the microbial signature of disease in a
394 large-scale Dutch population study (38). *Faecalibacterium prausnitzii* is one of the most
395 abundant core taxa in healthy adult population and a major butyrate producer, and change in
396 its abundance has been linked to dysbiosis in several disorders, including inflammatory bowel
397 disease, irritable bowel syndrome, and type 2 diabetes mellitus (44,45). So and colleagues
398 included 13 studies in their meta-analysis regarding the abundance of *F. prausnitzii* in dietary
399 fiber and placebo/low-fiber groups, resulting in no difference between the groups, though the
400 variation between individual studies was considerable (6). In a Finnish adult population-based
401 study, *F. prausnitzii* was associated with higher healthy food choices scores (31). Interestingly,
402 the abundance of *F. prausnitzii* was increased in a large-scale population study in those
403 individuals who consumed more than 30 plant types a week compared to those consuming
404 less than 10 plant types (46), highlighting that it may not be only the amount of fiber that is
405 important but also the variation in types of dietary fiber. In our study population, the
406 abundance of *Faecalibacterium* was relatively high (mean relative abundance 7.3%) and the
407 fold-change between higher and lower cumulative fiber intake was relatively small. It may be
408 that *Faecalibacterium* is related to a healthy diet with variation, which is not captured by
409 assessing only the cumulative dietary fiber intake.

410 Abundances of *Intestinimonas* and *Blautia* were decreased with higher cumulative fiber
411 intake in our study. The relationship between these bacterial genera and dietary fiber intake is
412 studied less. For instance, while *Intestinimonas* is capable of producing butyrate and acetate,
413 it may use lysine rather than plant-based carbohydrates as its energy substrate (47). Reduced
414 abundance of *Intestinimonas* has been linked to obesity in previous studies (48,49). Similarly,
415 *Blautia* was inversely correlated with visceral fat accumulation (50). We tested whether the
416 differential abundances of these genera were related to obesity by adding cumulative body
417 mass index (0 – 26 years) into the model used in *LinDA.* While the other results remained the

same, *Blautia* was no longer associated with cumulative fiber intake (data not shown), suggesting that obesity may have a role in its abundance. *Blautia* is a bacterial genus with numerous different species and its potential as a probiotic has been investigated, but the evidence is inconsistent and whether or not it is beneficial for the health may differ by species (51).

We have previously shown in the STRIP cohort that at the age of 26 years, gut microbiota profiles had only subtle differences between the intervention and control group six years after the intervention had ended, with genus *Veillonella* being more abundant in the intervention group (21). While the abundance of *Veillonella* was somewhat increased with higher cumulative fiber intake in the present study, the change was not statistically significant. On the other hand, the present study with specific cumulative exposure revealed more differences in the microbial taxa compared to the overall effect of the intervention.

The main limitation of this study is that fecal samples were collected only at the age of 26 years. While the dietary information starting from infancy is unique, it cannot be fully linked to changes in gut microbiota composition during the early lifespan to young adulthood without fecal samples at multiple time points. In addition, the fecal samples were analyzed using 16S rRNA gene sequencing, allowing genus-level microbial profiles, whereas species-level identification is not reliable with this technique. This method allows the study of abundances of bacterial taxa, but it cannot be utilized to reliably estimate strain-level profiles and gut bacterial metabolic activity in complex communities (52). Deep shotgun sequencing could provide more information on the changes in metabolic activities of the bacterial taxa as a response to long-term fiber intake. Actions induced by dietary fiber on the gut microbiota depend on fiber's physiochemical properties such as its solubility, viscosity, and fermentability, and for instance, soluble and insoluble fiber may affect microbiota differently (2). In this context, it would be interesting to study in more detail the effects of different types

443 of dietary fiber on gut microbes and their metabolic activities. Also, as we have no data on
444 fecal SCFAs, we were not able to confirm whether SCFAs were related to increased
445 cumulative fiber intake or the change in those bacterial taxa found in this study. Information
446 about the diet was collected using food diaries on four consecutive days before each study
447 visit. Participants may report dietary intake inaccurately, for example, due to inaccurate
448 estimation of food/drink amount, biased reporting, or change in typical diet so that the four
449 recorded days do not represent the actual habitual diet. Also, there are limitations in food
450 composition database applied to convert the reported food consumption to energy and nutrient
451 intakes. Another limitation is related to the study population. While the sample size was
452 relatively large (n = 357), it may be that even a larger population would have been needed to
453 detect the changes in more abundant bacterial taxa with larger variation. The models used in
454 the analyses were adjusted for sex, and the overall diet quality as induced by the intervention
455 did not change the qualitative results. Nevertheless, other dietary components may modulate
456 the interaction between dietary fiber and gut microbiota. However, adding multiple covariates
457 to the models could reduce the statistical power of the analyses. Finally, the study population
458 may not reflect the general population of the same-aged individuals. The individuals who
459 provided a fecal sample were different compared to those individuals who did not provide a
460 fecal sample. Furthermore, the participants of the STRIP cohort, both those in the intervention
461 and the control group, have been followed at least annually since infancy and received
462 information about their health regularly, which could have affected their dietary patterns
463 differently compared to the general population of the same age. Nevertheless, the major
464 strength of the STRIP cohort is the extensive cumulative data on dietary intake and
465 cardiometabolic risk factors from infancy to early adulthood, which are not available in any
466 other study. The current evidence on the associations of dietary fiber intake on gut
467 microbiome composition is mostly based on short-term dietary interventions without follow-

up or cross-sectional studies across populations, and long-term interventions are mostly lacking (8).

Conclusions

We investigated whether long-term cumulative dietary fiber intake from infancy is associated with gut microbiota composition in young adulthood. Alpha diversity was decreased with higher cumulative fiber intake, and the overall microbial community composition was affected by the amount of fiber consumed. While *Butyrivibrio*, a butyrate-producing bacterial genus, was increased with higher cumulative fiber intake in our study, other butyrate producers such as *Faecalibacterium* and *Subdoligranulum* were decreased. Further studies using metagenomic shotgun sequencing will be needed to study the function and metabolic activities of different microbial taxa at the species level. Nevertheless, as the majority of the current evidence is based on either short-term dietary intervention or cross-sectional studies, our unique longitudinal study adds novel information about the long-term habitual fiber intake on gut microbiota, which has mostly been lacking.

Author contributions

MAH, AA, PHa, HN, JV, TR, HL, AJ, OR, SPR, and KP designed research; HN, JV, TR, OR, SPR, and KP conducted research; MAH, AA, PHa, and LL analyzed the data and performed statistical analysis; MAH, LL, SPR, and KP wrote the original draft; AA, PHa, NK, EM, AK, PHu, HN, JV, TR, HL, AJ, and OR reviewed and edited the draft; MAH prepared figures; HN, OR, and KP had primary responsibility for the final content. All authors have read and approved the final manuscript.

Data Availability Statement

The dataset supporting the conclusions of this article was obtained from the STRIP study. The STRIP dataset comprises health-related participant data, and its use is therefore restricted

under the regulations on professional secrecy (Act on the Openness of Government Activities, 612/1999) and on sensitive personal data (Personal Data Act, 523/1999, implementing the EU data protection directive 95/46/EC). Due to these legal restrictions, the data from this study cannot be stored in public repositories or otherwise made publicly available. However, data access may be permitted on a case-by-case basis upon request only. Data sharing outside the group is carried out in collaboration with the STRIP group and requires a data-sharing agreement. Investigators can submit an expression of interest to the chairman of the STRIP steering group (Prof Olli Raitakari, University of Turku, Turku, Finland).

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Conflict of interests

Eveliina Munukka is a Medical Advisor at Biocodex Nordics. The other authors declare no conflict of interest.

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Table 1. Cardiometabolic risk markers and dietary measures of the participants at the first follow-up at the age of 26 years (n = 357).

	High fiber intake (n = 89)	***Medium fiber intake (n = 178)***	***Low fiber intake (n = 90)***
Female, %	79.8 *†	60.1 ‡	27.8
Body mass index, kg/m^2	23.5 (3.3) †	24.3 (4.1)	25.1 (4.8)
Waist circumference, cm	76.8 (10.3) †	80.1 (10.8) ‡	84.7 (12.6)
Systolic blood pressure, mmHg	117.6 (9.0) *†	121.2 (11.7)	123.3 (12.1)
Diastolic blood pressure, mmHg	70.0 (6.8) *	72.5 (7.8)	72.6 (8.0)
Serum biomarkers			
Total cholesterol, mmol/L	4.64 (0.94)	4.59 (0.9)	4.41 (0.86)
LDL cholesterol, mmol/L	2.79 (0.76)	2.78 (0.76)	2.70 (0.71)
HDL cholesterol, mmol/L	1.44 (0.37) †	1.36 (0.32) ‡	1.23 (0.33)
Triglycerides, mmol/L	0.80 [0.50]	0.90 [0.50]	0.90 [0.60]
Glucose, mmol/L	5.0 (0.4)	5.0 (0.5) ‡	5.2 (0.9)
Insulin, mU/L	6.2 [3.0]	6.8 [3.8]	6.8 [4.9]
HOMA-IR	1.39 [0.74]	1.54 [0.95]	1.58 [1.11]
Dietary intakes			
Energy, kcal/day	1872 (437) †	2002 (581) ‡	2255 (640)
Protein, E%	18.3 (4.6) †	19.7 (5.2)	20.6 (5.3)
Carbohydrates, E%	43.3 (7.2) †	40.9 (8.1)	38.6 (7.8)
Fat, E%	37.1 (6.0)	37.7 (7.8)	37.7 (6.9)
SAFA, E%	12.5 (3.7) *†	13.9 (3.5)	13.9 (3.3)

MUFA, E%	13.4 (4.2)	13.2 (4.0)	12.8 (3.1)
PUFA, E%	6.8 (2.0)	6.5 (2.4)	6.7 (1.8)
(P + M)/S	1.77 (0.73) *†	1.48 (0.50)	1.44 (0.30)
P/S	0.61 (0.34) *†	0.50 (0.22)	0.50 (0.16)
Cholesterol, mg/day	256 (154) †	313 (172) ‡	377 (240)
Fiber, g/day	24.6 (9.1) *†	19.4 (7.2) ‡	15.7 (5.9)
Whole grain bread, g/day	53.0 (40.8)	55.2 (42.9)	43.9 (34.8)
Vegetables, g/day	118 (65) †	132 (93)	158 (112)
Fruits and berries, g/day	242 [259] *†	177 [188] ‡	94 [201]
Sodium, mg/day	2644 (887) †	2925 (891) ‡	3417 (1162)

Participants are divided into quartiles according to their cumulative energy-adjusted fiber intake AUC 0-26 years. The medium fiber intake group includes 50% and high and low fiber intake groups include 25% of the participants. The presented values are mean (SD), except for triglycerides, insulin, HOMA-IR, and fruit and berries, for which median [IQR] are shown. The statistical significances between the groups were analyzed using one-way analysis of variance (Kruskal-Wallis test for non-normally distributed variables and pairwise chi-squared tests for categorical variables). The reported *p* values are not corrected for multiple testing. E%, percentage of energy intake; SAFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; (P + M)/S, polyunsaturated and monounsaturated fat to saturated fat ratio; P/S, polyunsaturated fat to saturated fat ratio.

* $p \leq 0.05$ between high and medium fiber intake groups.

† $p \leq 0.05$ between high and low fiber intake groups.

‡ $p \leq 0.05$ between medium and low fiber intake groups.

Figure legends

Figure 1. Flow chart of the STRIP study (FH; familial hypercholesterolemia).

Figure 2. A. Timepoints when information about energy-adjusted fiber intake and fecal samples are collected, and calculation of area under curve (AUC) variables. **B**. Cumulative fiber intake AUC 0-26 years for females and males, *** $p < 0.001$. **C.** Sankey diagram illustrating the patterns of habitual fiber intake. For each age group, quartiles are calculated to categorize the participants in the highest (25%), middle (50%), and lowest (25%) dietary fiber intake groups **D.** Pairwise Pearson correlations of cumulative fiber intake between age groups. The shade of the color indicates the strength of the correlation. All correlations are statistically significant ($p < 0.001$).

Figure 3. ASV-level PCoA ordination with Bray-Curtis dissimilarity for gut microbiota beta diversity. The color indicates cumulative fiber intake AUC 0-26 years and the shape indicates sex according to the legend.

Figure 4. Differential abundance analysis on genus level performed with *LinDA* presenting $\log_2$ fold-change per unit change in cumulative fiber intake. Only the genera with $|\log_2$ fold-change$| \geq 0.25$ are shown. The colors of the circles show the phyla that each genus is related to, and the size of the circles indicates the mean relative abundance of the genera according to the legend. The error bars present the upper and lower 95% confidence limit for each genus separately, and the color indicates the level of statistical significance after adjusting for multiple comparisons according to the legend.