

Figure S1: Principal coordinate analysis based on species-level Bray-Curtis dissimilarity. Long-term fiber intake was represented by a cumulative average of dietary intake values based on seven validated, semi-quantitative food frequency questionnaires from 1986 through 2010. In 2012-2013, 307 participants provided up to four stool samples over a six-month study period with concurrent blood samples and assessments of recent dietary intake using 7-day dietary records. Shotgun metagenomic and metatranscriptomic sequencing and profiling were conducted, with bacteria abundance determined using MetaPhIAn 2. A total of 139 microbial species were included in the analysis after filtering by minimum prevalence (>10%) and relative abundance (>0.01%). Dietary fiber did not explain overall microbial communities.

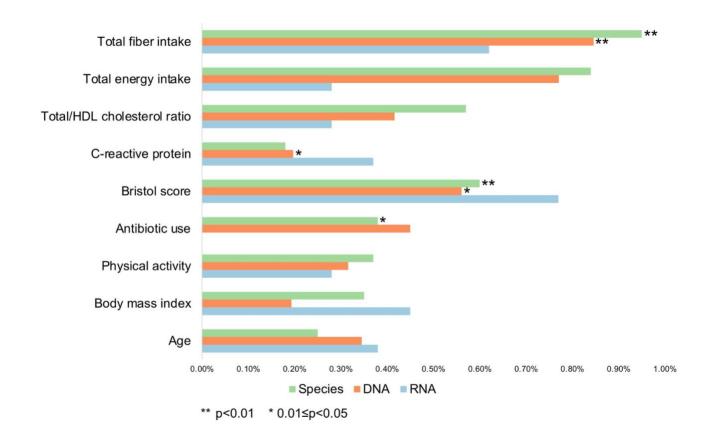


Figure S2: Omnibus testing with permutational analysis of variance testing (PERMANOVA) of Bray-Curtis dissimilarities (999 permutations). In our study, 307 generally healthy men (mean age: 70.6±4.3 years) provided up to four stool samples over a six-month study period with concurrent blood samples. They also provided assessments of recent dietary intake using 7-day dietary records and lifestyle information via questionnaires. A total of 925 metagenomes and 372 metatranscriptomes were included in the analysis. Bacteria abundance was determined using MetaPhIAn 2, with 139 microbial species retained after filtering by minimum prevalence (>10%) and relative abundance (>0.01%). Functional profiles of DNA and RNA reads were generated using HUMAnN 2. Individual factors including age, lifestyle, diet and clinical biomarkers only explained a minimal amount of the variation of the gut microbiome profile (all R²<0.01; Additional file 2: Table S1). Among them, fiber intake was the leading factor explaining small but significant variance in taxonomic composition (R²=0.0095, p=0.005) and functional potential (R²=0.0085, p=0.001).

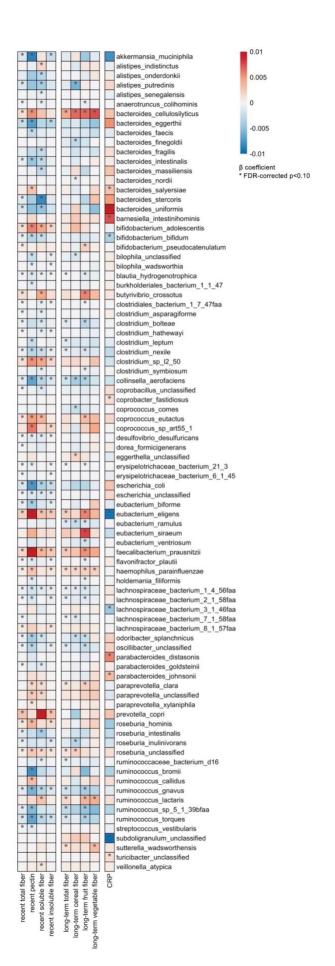


Figure S3: Species abundances significantly associated with C-reactive protein and dietary fiber intake (FDR-corrected p<0.25). We included 925 metagenomic samples from 307 participants in this analysis. Comparisons used log-transformed CRP and fiber assessed as recent intake using both 7-day dietary records and long-term cumulative averages from food frequency questionnaires over 1986-2010. Significant associations between recent and long-term dietary fiber and CRP and metagenomic microbial species abundances were obtained using multivariate linear association testing (Methods). Models were adjusted for age, recent antibiotics, and total calorie intake; models for CRP were additionally adjusted for body mass index. Both recent and long-term higher dietary fiber were associated with shifts in individual microbial species such as Clostridiales. Greater microbial differences were observed in association with intake of pectin and fiber from fruits and to a lesser extent, cereals, compared to vegetable fiber. Higher CRP levels were associated with an inflammation-associated gut microbial configuration. Data are also shown in Additional file 2: Table S2.

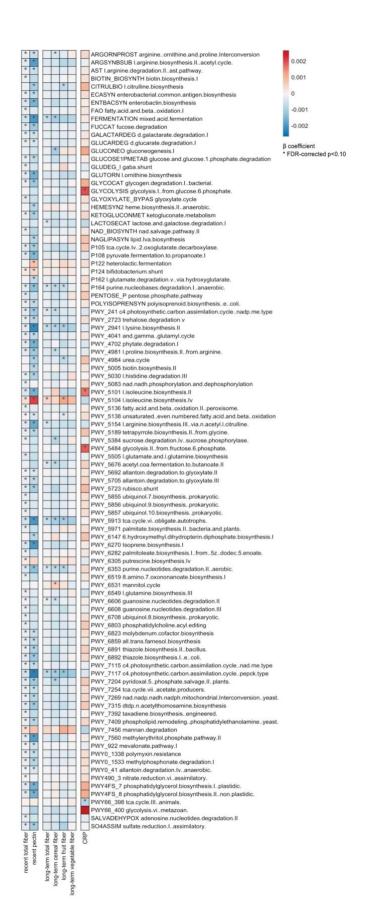


Figure S4. Metagenomic pathways significantly associated with C-reactive protein and dietary fiber intake. We included 925 metagenomic samples from 307 participants in this analysis. Comparisons used log-transformed CRP and fiber assessed as recent intake using both 7-day dietary records and long-term cumulative averages from food frequency questionnaires over 1986-2010. Significant associations between recent and long-term dietary fiber and CRP and metagenomic pathways were obtained using multivariate linear association testing (Methods). Models were adjusted for age, recent antibiotics, and total calorie intake; models for CRP were additionally adjusted for body mass index. Dietary fiber intake in particular recent intake from pectin was significantly associated with a large number of metagenomic functional pathways involved in the metabolism of carbohydrates and amino acids. Data are also shown in Additional file 2: Table S3.

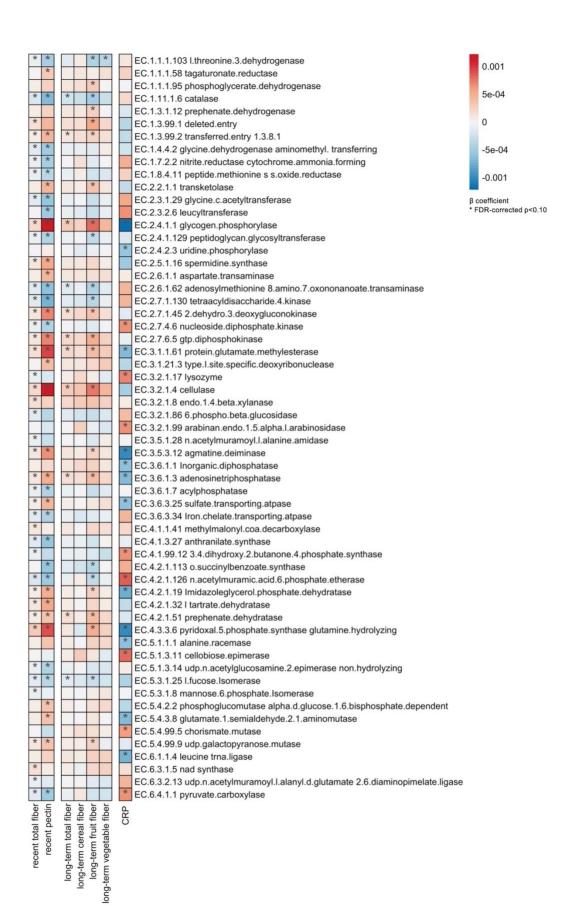


Figure S5. Microbial functional potential abundances significantly associated with C-reactive protein and dietary fiber intake. We included 925 metagenomic samples from 307 participants in this analysis. Comparisons used log-transformed CRP and fiber assessed as recent intake using both 7-day dietary records and long-term cumulative averages from food frequency questionnaires over 1986-2010. Significant associations between recent and long-term dietary fiber and CRP and abundances of 100 most abundant, uncorrelated metagenomic enzyme commissions were obtained using multivariate linear association testing (Methods). Models were adjusted for age, recent antibiotics, and total calorie intake; models for CRP were additionally adjusted for body mass index. Both recent and long-term dietary fiber were significantly associated with a large number of metagenomic functional features involved in metabolism of carbohydrates, amino acids, vitamins, and other compounds. Greater differences were observed associated with pectin and fruit fiber. Data are also shown in Additional file 2: Table S4.

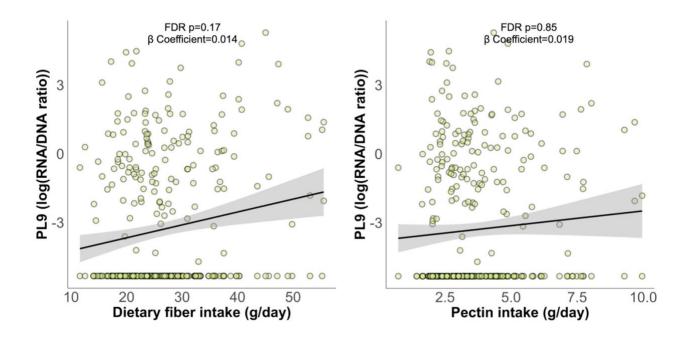


Figure S6: Functional activity of CAZy polysaccharide lyase family 9 (PL9) associated with recent dietary fiber/pectin intake. We included 372 samples with both metatranscriptomes and metagenomes from a subset of 96 participants selected because they provided stool at both sampling periods and did not report antibiotic use during the past year. Multivariate linear mixed models were adjusted for age, recent antibiotics, and total calorie intake. Functional activity of PL9 was represented by RNA/DNA ratio and log2 transformed. Positive associations were observed between functional activity of PL9 and dietary intake of fiber or pectin. All results for multivariable-adjusted associations between fiber and CAZy RNA/DNA ratios are shown in Additional file 2: Table S6.