Circulation Research

ORIGINAL RESEARCH

Gut Microbiota and Blood Metabolites Related to Fiber Intake and Type 2 Diabetes

Zheng Wang, Brandilyn A. Peters, Bing Yu, Megan L. Grove, Tao Wang, Xiaonan Xue, Bharat Thyagarajan, Martha L. Daviglus, Eric Boerwinkle, Gang Hu, Yasmin Mossavar-Rahmani, Carmen R. Isasi, Rob Knight, Robert D. Burk, Robert C. Kaplan, Qibin Qi

BACKGROUND: Consistent evidence suggests diabetes-protective effects of dietary fiber intake. However, the underlying mechanisms, particularly the role of gut microbiota and host circulating metabolites, are not fully understood. We aimed to investigate gut microbiota and circulating metabolites associated with dietary fiber intake and their relationships with type 2 diabetes (T2D).

METHODS: This study included up to 11 394 participants from the HCHS/SOL (Hispanic Community Health Study/Study of Latinos). Diet was assessed with two 24-hour dietary recalls at baseline. We examined associations of dietary fiber intake with gut microbiome measured by shotgun metagenomics (350 species/85 genera and 1958 enzymes; n=2992 at visit 2), serum metabolome measured by untargeted metabolomics (624 metabolites; n=6198 at baseline), and associations between fiber-related gut bacteria and metabolites (n=804 at visit 2). We examined prospective associations of serum microbial-associated metabolites (n=3579 at baseline) with incident T2D over 6 years.

RESULTS: We identified multiple bacterial genera, species, and related enzymes associated with fiber intake. Several bacteria (eg, *Butyrivibrio*, *Faecalibacterium*) and enzymes involved in fiber degradation (eg, xylanase EC3.2.1.156) were positively associated with fiber intake, inversely associated with prevalent T2D, and favorably associated with T2D-related metabolic traits. We identified 159 metabolites associated with fiber intake, 47 of which were associated with incident T2D. We identified 18 of these 47 metabolites associated with the identified fiber-related bacteria, including several microbial metabolites (eg, indolepropionate and 3-phenylpropionate) inversely associated with the risk of T2D. Both *Butyrivibrio* and *Faecalibacterium* were associated with these favorable metabolites. The associations of fiber-related bacteria, especially *Faecalibacterium* and *Butyrivibrio*, with T2D were attenuated after further adjustment for these microbial metabolites.

CONCLUSIONS: Among United States Hispanics/Latinos, dietary fiber intake was associated with favorable profiles of gut microbiota and circulating metabolites for T2D. These findings advance our understanding of the role of gut microbiota and microbial metabolites in the relationship between diet and T2D.

GRAPHIC ABSTRACT: A graphic abstract is available for this article.

Key Words: diabetes mellitus, type 2 ■ dietary fiber ■ gastrointestinal microbiome ■ genes, bacterial ■ metabolome

In This Issue, see p 833 | Meet the First Author, see p 834 | Editorial, see p 855

Consistent evidence suggests diabetes-protective effects of dietary fiber intake,[1,2] but the underlying mechanisms are not well elucidated. These mechanisms could potentially be related to gut microbiota and microbiota-derived metabolites that have been suggested to play important roles in human chronic diseases, including type 2 diabetes (T2D).[3,4] Dietary fibers, though not susceptible to hydrolysis by human digestive enzymes, can be metabolized by specific gut bacteria that produce a spectrum of metabolites through fiber fermentation and other metabolism pathways.[3]

Recent studies have indicated the potential influence of dietary fiber intake on the human gut microbiota composition. Higher fiber intake has been associated

Correspondence to: Qibin Qi, PhD, Department of Epidemiology and Population Health, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, NY 10461. Email qibin.qi@einsteinmed.edu

Supplemental Material is available at https://www.ahajournals.org/doi/suppl/10.1161/CIRCRESAHA.123.323634.
For Sources of Funding and Disclosures, see page 853.

Circulation Research is available at www.ahajournals.org/journal/res

Novelty and Significance

What Is Known?

- Higher dietary fiber intake is associated with a lower risk of type 2 diabetes (T2D); however, the underlying mechanisms are not well elucidated.
- Dietary fiber can be metabolized by specific gut microbes. However, to what extent the specific fiber-associated gut microbiota taxonomic features and functional capacities may affect host T2D, is not fully understood.

What New Information Does This Article Contribute?

- Our integrated multiomics analyses revealed the potential fiber-microbiota-metabolite-T2D route. We identified several bacterial genera (eg, *Butyrivibrio*, *Faecalibacterium*) and functional capacities involved in fiber degradation (eg, xylanase EC3.2.1.156) positively associated with fiber intake and linked to lower odds of T2D.
- *Butyrivibrio* and *Faecalibacterium* were positively associated with multiple beneficial serum metabolites, including indolepropionate, 3-phenylpropionate, and cinnamoylglycine, with the properties of anti-inflammation and antioxidant activity, and amelioration of glucose metabolism.
- The protective associations of *Butyrivibrio* and *Faecalibacterium* with T2D could be partially explained by these microbial metabolites.

In summary, by leveraging integrative omics data in United States Hispanics/Latinos, our study demonstrated that a higher intake of fiber was linked to beneficial patterns of gut microbiota taxonomic features, microbial functional enzymes, and circulating metabolites, all of which were associated with lower risk of T2D. Our analyses further revealed that for certain fiber-associated taxa such as *Butyrivibrio* and *Faecalibacterium*, the potentially beneficial effects on T2D risk could be explained by microbial-related metabolites, namely, indolepropionate, 3-phenylpropionate, and cinnamoylglycine. These findings contribute to our understanding of the complex relationships among dietary fiber intake, gut microbiota, and circulating metabolites, offering insights into their potential roles in the development of T2D. These insights facilitate the novel therapeutic strategies in precision nutrition and dietary intervention through modulating the gut microbiota and related microbial metabolites, potentially offering a more effective, precise way for T2D prevention.

Nonstandard Abbreviations and Acronyms

FDR	false discovery rate
HCHS/SOL	Hispanic Community Health Study/Study of Latinos
T2D	type 2 diabetes

with alterations in gut bacterial taxa (eg, higher levels of *Prevotella* or *Prevotella* to *Bacteroides* ratio,[5] *Roseburia*,[6,7] *Lachnospira*,[6] *Eubacterium*[7]) and in gut bacterial gene functions (eg, higher levels of genes encoding xylanase, β-glucanase, and other enzymes related to fiber degradation[8]). However, to what extent the specific fiber-associated gut microbiota taxonomic features and functional capacities may affect the host metabolic diseases, such as T2D, and the underlying mechanisms are not fully understood.

Microbial-derived metabolites can be absorbed into the host circulation and may affect host biological systems and, thus, have been suggested as functional mediators linking gut microbiota and host metabolic health and disease.[3] While the majority of existing research has focused on short-chain fatty acids, well-known microbial metabolites through fiber fermentation,[9] there is also evidence suggesting other microbial metabolites associated with fiber intake and T2D, such as indolepropionate.[10] However, few studies have integrated host metabolomics and gut metagenomics data to investigate the relationships among dietary fiber intake, gut microbiota, host circulating metabolites, and risk of T2D in population-based cohorts.[3] Data are even sparse in United States Hispanics/Latinos, with westernized diet (eg, reduced dietary intake of fiber-rich foods) and gut microbiome (eg, reduced bacterial diversity) during acculturation,[5,8] who are disproportionally affected by T2D.[10]

In this study, using fecal shotgun metagenomics data, we aimed to identify gut microbial taxa and functional capacities associated with dietary fiber intake in the HCHS/SOL (Hispanic Community Health Study/Study of Latinos) and then linked these identified gut microbial features with prevalent T2D. We also performed a metabolome-wide analysis to identify serum metabolites associated with dietary fiber intake and examined whether these fiber-associated metabolites at baseline

were associated with a risk of incident T2D over a 6-year follow-up in the HCHS/SOL. Moreover, through a late integration strategy,[11] we linked both fiber-associated gut bacteria and fiber-associated metabolites to help better understand the role of gut microbiota and microbial metabolites in the relationship between dietary fiber intake and T2D.

METHODS

Data Availability

The gut microbiome shotgun metagenomics sequencing data in this study are deposited in QIITA (https://qiita.ucsd.edu/), ID 11666. HCHS/SOL has established a process for the scientific community to apply for access to participant data and materials, with such requests reviewed by the project's steering committee. These policies are described at https://sites.cscc.unc.edu/hchs/. Please see the Major Resources Table in the Supplemental Material.

Study Population

The HCHS/SOL is a prospective, population-based cohort study of 16 415 Hispanic/Latino adults aged 18–74 years living in 4 United States metropolitan communities (Chicago, IL; Miami, FL; Bronx, NY; and San Diego, CA). A comprehensive battery of interviews and a clinical assessment with blood draws were conducted at in-person clinic visits from 2008 to 2011 (baseline) and 2014 to 2017 (visit 2).[5,12] In this study, we included up to 11 394 participants at baseline (including 6198 participants with serum metabolomics data) and 2992 participants in an ancillary gut microbiome study at visit 2. The specific sample size for each analysis is detailed in the respective sections. Information on demographics, behaviors, health status, medical histories, and medication use was collected using structured questionnaires.[12,13]

An expanded description of study populations, data assessments, and statistical analyses is provided in Supplemental Methods. The study was approved by the institutional review boards of corresponding site institutions. Written informed consent was obtained from all participants.

Assessment of Dietary Intake

Dietary intake was collected using the multiple-pass methods of the Nutrition Data System for Research software (version 11) based on 2 24-hour dietary recalls assessed ≈6-week apart at baseline.[14] Dietary fiber intake assessment included the energy-adjusted total fiber intake (g/1000 kcal per day) and separate estimates for soluble and insoluble fiber intake. We created a new diet score that comprised 7 nonfiber dietary factors (long-chain omega-3 fatty acids, polyunsaturated fatty acids, alcohol, sugar-sweetened drinks, red and processed meat, trans fats, and sodium).

Metagenomics Sequencing, Taxonomic, and Functional Profiling

Shotgun metagenomics sequencing was performed on DNA extracted from fecal samples of 3035 participants at visit 2, using Illumina NovaSeq platforms.[5,8] A total of 2992 samples that passed quality control metrics were included in the analysis. Microbiome bioinformatics analyses, taxonomic assignment, and functional component identification were performed using the SHOGUN[15] pipeline.[8] A total of 350 gut microbial species (presented in >20% of samples and average relative abundance ≥0.001%) and 1958 annotated known enzymes were included in the analysis. We conducted a central log-ratio transformation on these gut microbial features.

Metabolomic Profiling

Serum metabolomic profiling was performed using an untargeted liquid chromatography-mass spectrometry–based protocol at Metabolon (Durham, NC) in 3972 randomly selected participants (at baseline) and 814 participants (at visit 2) who provided blood samples within 1 month of fecal sample collection.[16] Metabolomic profiling was performed in an additional 2282 participants (at baseline) as a replication data set. A total of 624 known metabolites with an undetectable rate of <20% were included in the analysis. Values of metabolites below detection were imputed by half of the minimum value. We performed inverse-normal transformation on levels of metabolites.

Ascertainment of T2D and Metabolic Traits

T2D cases were identified if participants met at least one of the following criteria[17]: fasting glucose ≥7.0 mmol/L (126 mg/dL); after 2-hour glucose ≥11.1 mmol/L (200 mg/dL); HbA1c ≥6.5%; or self-reported antidiabetic medication use. Participants free of diabetes at baseline who were identified as having T2D during the follow-up visits were deemed to be incident T2D. Metabolic traits, including body mass index, waist-hip ratio, systolic blood pressure, diastolic blood pressure, triglycerides, high-density lipoprotein cholesterol, fasting glucose, after 2-hour glucose, HbA1c, and homeostatic model assessment for insulin resistance, were measured following standardized methods and protocols.[17,18]

Statistical Analysis

Dietary Fiber Intake and Incident T2D

Multivariable Poisson regression was used to examine the association between baseline dietary fiber intake and incident T2D over 6 years of follow-up, among 8185 participants who were free of diabetes, cardiovascular disease or cancer at baseline, controlling for age, sex, study center, education, family income, physical activity, smoking, drinking, antihypertensive medication, and lipid-lowering medication.

Dietary Fiber Intake, Gut Microbial Taxa, and Prevalent T2D

Among 2992 participants at visit 2, we examined associations of dietary fiber intake with microbial taxonomic features using multivariate linear regressions, adjusting for age, sex, study center, education, family income, physical activity, smoking, drinking, use of antibiotics, probiotics, antihypertensive medication, antidiabetic medication, and lipid-lowering medication. We further adjusted for the diet score which comprised 7 nonfiber dietary factors to examine the potential influences of other dietary factors on the association results. We applied ANCOM2[19] to identify fiber-associated microbial taxa

in a compositionally coherent manner. We performed logistic regressions to examine multivariable-adjusted associations of gut microbial taxonomic features with prevalent T2D and prediabetes, controlling for aforementioned covariates except for antidiabetic medication use as this was used to define T2D. We constructed the integrated hierarchical phylogenetic tree using iTol.[20] Subsequently, we focused on those microbial taxa associated with both fiber intake and T2D and examined their associations with T2D-relaetd metabolic traits using multivariate linear regressions, adjusting for the aforementioned covariates.

Dietary Fiber Intake, Gut Microbial Functional Enzymes, and Prevalent T2D

We explored associations of dietary fiber intake with 1958 annotated microbial functional enzymes using multivariate linear regressions, adjusting for aforementioned covariates. An enrichment test was performed at enzyme category EC level II. We applied logistic regressions to examine multivariable-adjusted associations of the identified fiber-associated enzymes with prevalent T2D. We performed microbial genomic sequence–based-alignment analyses[8] to examine potential contributions of specific bacterial taxa to these fiber-associated functional enzymes.

Dietary Fiber Intake, Circulating Metabolites, and Incident T2D

We examined associations of serum metabolites with dietary fiber intake using multivariate linear regressions among 3916 participants at baseline, adjusting for aforementioned covariates except antibiotics and probiotics. These associations were further examined in an additional data set at baseline (n=2282). Then, we evaluated the prospective associations of fiber-associated metabolites with incident T2D over a 6-year follow-up. Among 3916 participants, we excluded those with prevalent diabetes, cardiovascular disease, or cancer at baseline and those who did not attend the second visit. The remaining 2010 participants were included in the multivariable Poisson regressions to estimate rate ratios of incident T2D per SD increment in metabolites. The same analysis was conducted in the replication data set (n=1569). Results from 2 data sets were combined using fixed-effects meta-analysis. We further examined associations of selected metabolites with metabolic traits using multivariate linear regressions.

Integrated Analyses of Gut Microbiota and Circulating Metabolites Associated With Fiber Intake and T2D

Among 804 participants with both omics data available at visit 2, we utilized partial Spearman correlation to assess correlations between the identified microbial taxa and metabolites, which were associated with both fiber intake and T2D. Associations of 9 fiber-associated microbial genera with prevalent T2D were estimated using multivariable logistic regressions, adjusted for aforementioned covariates (model 1), further adjusted for metabolites relevant to specific individual taxa (model 2), and further adjusted for all 18 microbial-related metabolites (model 3), to explore whether these metabolites could partially explain the observed associations. In addition, we conducted a proxy association analysis[21] to test potential prospective associations of these 9 bacterial genera with risk of T2D. The identified 18 microbial-related metabolites were used as proxies for these 9 bacterial genera. For each bacterial genus, we calculated a Spearman correlation coefficient between effect sizes (β coefficients) from the cross-sectional associations of this genus with 18 microbial-related metabolites and effects sizes (nature-log–transformed rate ratios) from the prospective associations of 18 microbial-related metabolites at baseline with risk of T2D. These effect sizes were standardized using *Z*-score transformation to ensure comparability. A significant correlation between these 2 sets of effect sizes was considered a significant proxy association.

Statistical analyses were performed using R, version 4.0.3. The Benjamini-Hochberg false discovery rate (FDR) method was used for multiple testing corrections.

RESULTS

Dietary Fiber Intake and Incident T2D

We first examined the prospective association between dietary fiber intake and incident T2D among 8185 participants who were free of diabetes at baseline (participant characteristics are shown in the Table). During the average 6 years of follow-up, 851 incident T2D cases were identified (Table S1). After adjustment for multiple covariates, higher dietary fiber intake was significantly associated with a lower risk of T2D (rate ratio, 0.95 [95% CI, 0.90–0.99] per g/1000 kcal per day; *P*=0.045). Compared with those in the lowest tertile of fiber intake (range, 3.4–8.4 g/1000 kcal per day), participants in the highest tertile (range, 10.8–22.1 g/1000 kcal per day) had a 29% (95% CI, 6%–47%) lower risk of T2D (*P*-trend=0.023; Figure S1).

Dietary Fiber Intake, Gut Microbial Taxa, and Prevalent T2D

We then examined associations of dietary fiber intake with individual gut microbial taxa at multiple taxonomic levels, among 2992 individuals (participant characteristics are shown in Table S2 and Figure S2). After controlling for multiple covariates, 24 of 85 predominant gut microbial genera were associated with fiber intake (FDR <0.05). As shown in the integrated phylogenetic tree (Figure 1A), 17 of these fiber-associated genera were under *Firmicutes* phylum, 4 under *Actinobacteria*, and 3 under *Bacteroidetes*. Associations between dietary fiber intake and these 24 gut microbial taxa remained significant after further adjustment for the diet score, which comprised 7 nonfiber dietary factors (Figure S3). Consistently, 23 of these 24 microbial genera were also identified to be associated with dietary fiber intake by ANCOM2 (Table S3). Correlations among these 24 genera were generally weak though a few moderate correlations were observed among 4 genera positively associated with fiber intake and among 9 genera inversely associated with fiber intake (Figure 1B). We identified 99 microbial species within these 24 fiber-associated genera, and the species-fiber associations were generally consistent with genus-level results (Figure 1C; Table S4). For example,

Table. Characteristics of Study Participants, Free of Diabetes at Baseline (n=8185)

	Fiber intake		
	Tertile 1	Tertile 2	Tertile 3
Age, y	39.1 (13.5)	44.8 (12.5)	49.5 (11.2)
Sex, %			
Female	50.2	62.9	75.6
Male	49.8	37.1	24.4
Field center, %			
Bronx	34.6	19.6	9.7
Chicago	13.9	25.9	39.1
Miami	40.7	26.5	9.2
San Diego	10.9	28	42
Smoking, %			
Never	58.7	64.1	71.2
Former	15.6	19.6	19.1
Current	13	9.2	6.5
Current heavy smoker	12.7	7.1	3.2
Alcohol consumption, %			
Never	17.7	19.7	21.1
Former	26.7	29.9	35.5
Current	48	46.4	40.4
Current heavy drinker	7.7	4	3
Education, %			
<High school	26.7	31	43.6
High school	30.6	27.5	22.6
>High school	42.7	41.5	33.7
Annually family income ($), %			
<30k	69.7	65.9	66
≥30k	30.3	34.1	34
Antihypertensive medication use, %			
No	92.4	91.5	88.3
Yes	7.6	8.5	11.7
Lipid-lowering medication use, %			
No	96.8	94.3	91.2
Yes	3.2	5.7	8.8
Obesity categories, %			
Normal	25.0	21.4	19.3
Overweight	36.9	41.2	42.7
Obese	38.1	37.4	38.0

Data are mean (SD) for continuous variables or percentage for categorical variables.

all *Roseburia*, *Butyrivibrio*, and *Faecalibacterium* species and 20 of 26 *Prevotella* species were positively associated with fiber intake.

We also examined associations of insoluble and soluble fiber intakes with these 24 identified microbial genera, and the results were similar (Figure S4). Among these 24 microbial genera, 21 and 15 genera were significantly associated with insoluble and soluble fiber intakes, respectively (14 genera associated with both), with consistent directions compared with results for the total fiber intake.

We, thus, focused on these 24 fiber-associated genera and examined their associations with prevalent T2D. After multivariable adjustment, 9 fiber-associated genera showed significant associations with T2D (Figure 1A and 1D). Higher levels of *Butyrivibrio*, *Faecalibacterium*, *Roseburia*, *Ruminococcus*, and *Marvinbryantia*, all of which were positively associated with fiber intake, were associated with lower odds of T2D. Higher levels of *Acidaminococcus*, *Erysipelatoclostridium*, *Hungatella*, and *Lachnoclostridium* were associated with lower fiber intake and higher odds of T2D (all $P<0.05$; Figure 1D). For most of the other genera, we observed the expected directions of the associations with fiber intake and T2D (ie, positively associated with fiber intake and inversely associated with T2D) though these associations were not significant (Figure 1E). After further adjustment of obesity, associations between 9 microbial genera and T2D remained significant (Table S5).

We observed generally consistent directions of associations of these 9 identified microbial genera with prediabetes and T2D (Figure S5). In addition, several fiber-associated beneficial taxa, notably *Butyrivibrio* and *Marvinbryantia*, were linked with a favorable profile of metabolic traits. On the contrary, the unbeneficial taxa associated with lower fiber intake, such as *Acidaminococcus* and *Lachnoclostridium*, exhibited unfavorable associations with metabolic traits (Figure 1F).

Dietary Fiber Intake, Gut Microbial Functional Enzymes, and Prevalent T2D

After controlling for multiple covariates, we identified 211 enzymes associated with fiber intake (all FDR <0.05). Our enrichment tests at EC enzyme category level II indicated that fiber intake was associated with the enrichment of enzymes belonging to specific categories (eg, EC3.2 glycosylases; EC2.5 transferring alkyl or aryl groups; Table S6).

In particular, we identified 17 enzymes under the glycosylases category associated with fiber intake (all FDR <0.05; Figure 2). The identified glycosylases formed 2 clusters, with enzymes within each cluster demonstrating high correlations with each other (Figure 2). The first cluster, which was positively associated with fiber intake and inversely associated with T2D, included several representative microbial fiber-degradation enzymes. For example, oligosaccharide reducing-end xylanase (EC3.2.1.156, K15531) is known as a high molecular mass xylanase, which can degrade xylan, a type of dietary fiber found in plant cell walls.[22] The second cluster was inversely associated with fiber intake and positively associated with T2D. This cluster comprised microbial encoding enzymes related to the metabolism of simple carbohydrates. In line with our results, one representative

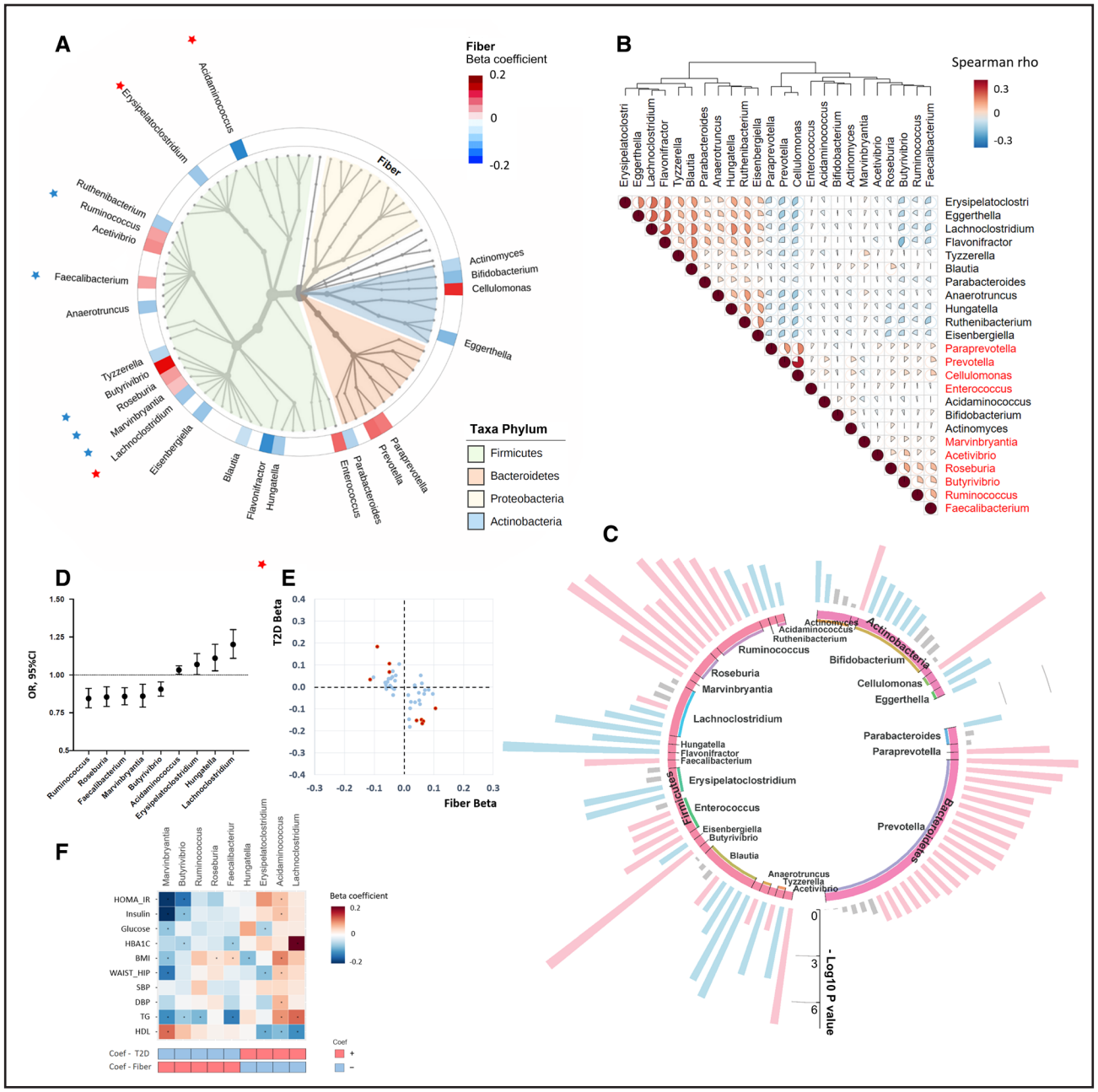

Figure 1. Dietary fiber intake, gut microbial taxa, and prevalent type 2 diabetes (T2D).
A, Integrated phylogenetic tree of gut microbial taxa associated with fiber intake (n=2992). Taxa from inner to outer circle represent bacteria kingdom to genus level. The branch widths reflect the relative abundance of each taxon. The red/blue colors of the ring depict the significant positive/inverse associations with fiber intake (false discovery rate [FDR] <0.05), and the gradient colors reflect the β coefficients estimated in linear regression models, after adjustment for age, sex, study center, education, family income, physical activity, smoking, drinking, use of antibiotics, probiotics, antihypertensive medication, antidiabetic medication, and lipid-lowering medication. Among the 24 fiber-associated genera, 9 were significantly associated with prevalent T2D in multivariable logistic models, after adjustment for the aforementioned covariates except for antidiabetic medication use. Red/blue stars depict the positive/inverse associations of genera with T2D (P<0.05). **B**, Partial Spearman correlation heatmap for the 24 identified fiber-associated microbial genera. The red font highlights those genera positively associated with fiber intake. The pie pieces reflect the strength of the correlation. Results were adjusted for the aforementioned covariates. **C**, Polar plot for associations of species-level microbial taxa with dietary fiber intake. The results for 99 predominant species (average relative abundance >0.001% and present in >20% samples) under the identified 24 fiber-associated genera are shown. The bar height represents the $-\log_{10}(P)$ value. Bacterial species significantly associated with fiber intake are highlighted in red/blue (positive/inverse associations; P<0.05). **D**, Associations of fiber-related genera with prevalent T2D. Data are odds ratios (ORs) and 95% CIs for T2D per increment of centered log ratio-transformed abundance of gut bacterial genera, adjusting for the aforementioned covariates. **E**, Associations of microbial genera with fiber intake and prevalent T2D. Data are β coefficients estimated in regressions (for fiber intake) and natural logarithms of ORs estimated in logistic regressions (for T2D), after adjustment for the aforementioned covariates in **A**. Each dot represents a bacterial genus. Red dots highlight the 9 genera significantly associated with both fiber intake and T2D. **F**, Associations of T2D-associated genera with metabolic traits. Data are β coefficients estimated in linear regression models after adjustment for the aforementioned covariates in **A** (*P<0.05). BMI indicates body mass index; DBP, diastolic blood pressure; HDL, high-density lipoprotein cholesterol; HOMA-IR, homeostatic model assessment for insulin resistance; SBP, systolic blood pressure; and TG, triglyceride.

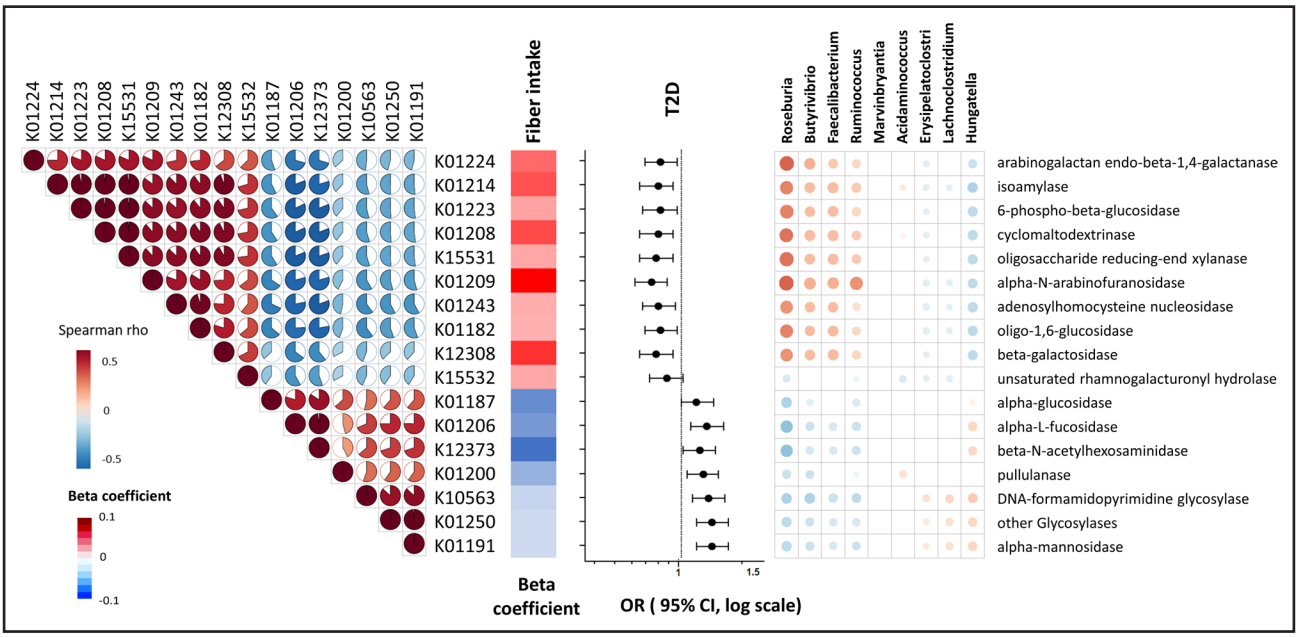

Figure 2. Fiber-associated gut microbial functional enzymes and prevalent type 2 diabetes (T2D).
The partial Spearman correlation heatmap (**left**) includes 17 microbial functional enzymes under the glycosylases category, which were significantly associated with fiber intake (all false discovery rate [FDR] <0.05; n=2992). For the associations of microbial functional enzymes with fiber intake (**middle left**), the gradient colors reflect the ranks of β coefficients estimated in multivariate linear regressions, after adjustment for age, sex, study center, education, family income, physical activity, smoking, drinking, use of antibiotics, probiotics, antihypertensive medication, antidiabetic medication, and lipid-lowering medication. For the associations of microbial functional enzymes with prevalent T2D (**middle right**), data are odds ratios (ORs) and 95% CIs, estimated in multivariable logistic regressions, after adjustment for the aforementioned covariates except for antidiabetic medication use. The partial Spearman correlation heatmap (**right**) indicates correlations between these 17 microbial functional glycosylases and the 9 identified fiber-associated genera.

enzyme in this cluster, alpha-mannosidase (EC3.2.1.24, K01191), has been linked with insulin resistance.[23]

Besides the EC 3.2 glycosylases, our results indicated that fiber intake was also associated with the potential enrichment of enzymes in EC2.5, transferring alkyl, or aryl groups (Figure S6). We identified 11 fiber-associated enzymes under this transferases category, all of which were associated with T2D in the expected directions (Figure S6). These 11 transferases showed weak-to-moderate correlations with the 17 enzymes under the glycosylases category (Figure S7).

We then explored the potential contributions of the 9 selected fiber-associated bacterial genera to these enzymes (Figure 2; Figure S6). Our genomic analyses provided further evidence supporting the presence of enzyme-encoding genes on the specific bacterial genomes (Table S7). For example, we confirmed the presence of the xylanase gene on representative *Roseburia* and *Butyrivibrio* genomes. These results expanded our previous findings[8] and, also consistent with other studies, indicated that *Roseburia* species from the human gut displayed high xylanolytic activity.[22]

Dietary Fiber Intake, Circulating Metabolites, and Incident T2D

After adjustment for multiple covariates, 164 of 624 metabolites were significantly associated with fiber intake (FDR <0.05) in 3916 HCHS/SOL participants (Tables S8 and S9), and associations of 159 metabolites with fiber intake were validated in an additional data set in HCHS/SOL (n=2282; Table S9; Figure 3A).

We then focused on these 159 fiber-associated metabolites and examined prospective associations of these metabolites with incident T2D among 2010 participants who were free of diabetes at baseline, with 224 incident T2D cases identified after 6 years of follow-up (Table S10). We found that 69 fiber-associated metabolites were also associated with incident T2D (Table S11). After further adjustment for obesity, the associations between metabolites and risk of T2D only changed slightly, and 43 of the 69 remained significant (Figure S8). In addition, we conducted stratified analyses based on baseline prediabetes status, and results among participants with prediabetes were highly consistent with those observed in the overall sample (Figure S9). This was expected as the majority of incident T2D cases were identified among participants with prediabetes at baseline (Table S12).

We further conducted replication analyses in the additional HCHS/SOL data set (1569 participants free of diabetes at baseline with 204 incident T2D cases; Table S10) and found that 44 of 69 metabolites showed significant associations with risk of T2D (Table S12). We then combined results from both data sets by meta-analysis,

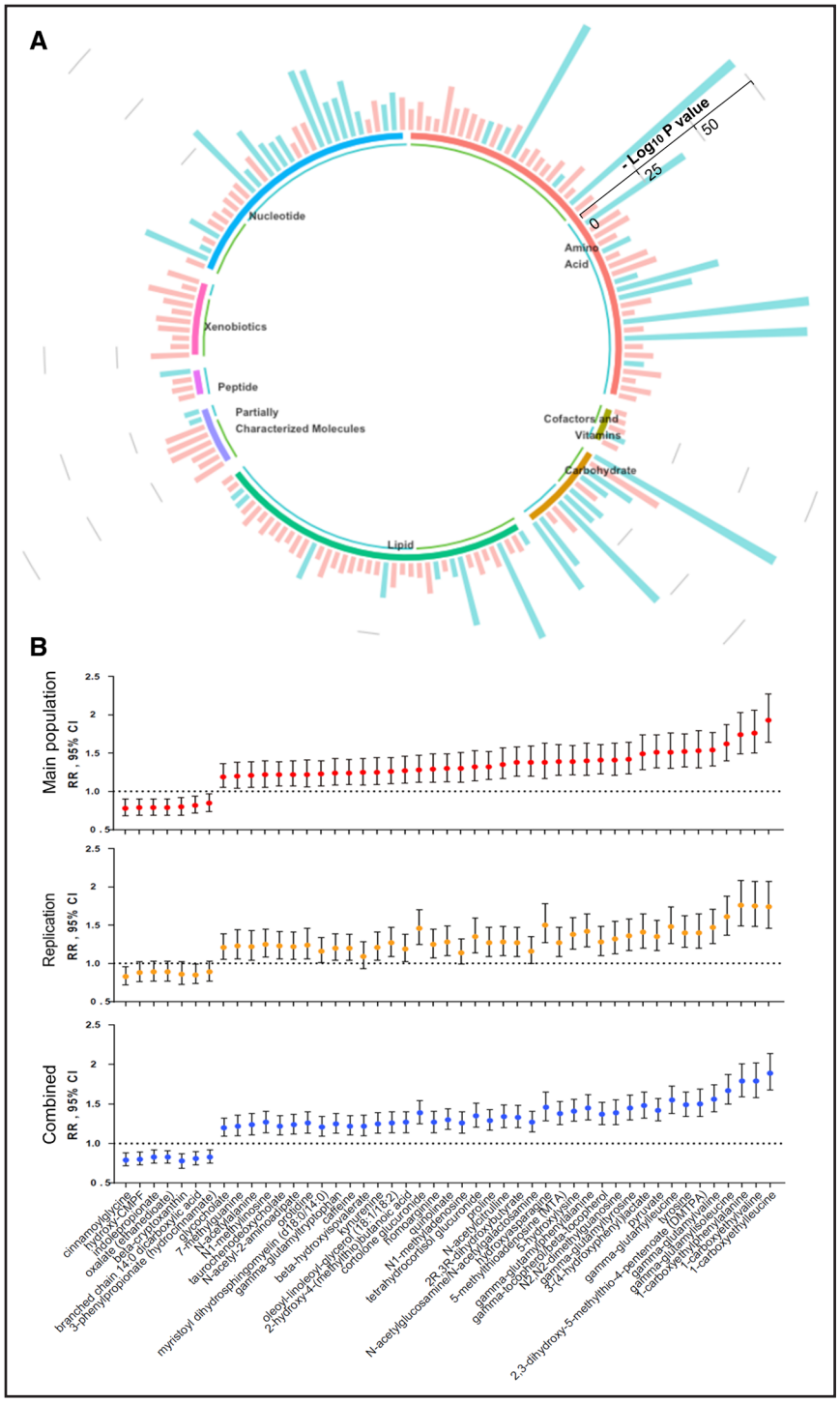

Figure 3. Dietary fiber intake, circulating metabolites, and incident type 2 diabetes (T2D).
A, Polar plot for associations of serum metabolites with fiber intake. Data are $-\text{Log}_{10}(P)$ values for 159 metabolites, which were significantly associated with fiber intake (false discovery rate [FDR] <0.05; n=3916) and validated in an additional data set (n=2282), from multivariate linear regressions, with adjustment for age, sex, study center, education, family income, physical activity, smoking, drinking, use of antibiotics, probiotics, antihypertensive medication, antidiabetic medication, and lipid-lowering medication. Red/blue: positive/inverse associations (FDR <0.05). **B**, Prospective associations between fiber-related metabolites and incident T2D. Data are rate ratios and 95% CIs, estimated by multivariable Poisson regressions, with adjustment for the aforementioned covariates except for antidiabetic medication use. Results were from the discovery data (**top**) including 2010 participants free of diabetes at baseline, with 224 incident T2D cases over 6 y; from the replication analyses (**middle**) including 1569 participants free of diabetes at baseline with 204 incident T2D cases over 6 y; and combined (**bottom**) from both data sets using fixed-effects meta-analysis.

and 47 metabolites were associated with the risk of T2D (Table S11; Figure 3B).

To explore potential relationships among these 47 metabolites, which were associated with both fiber intake and T2D, we examined their correlations (Figure S10) and performed network analysis (Figure S11). Notably, 3-phenylpropionate, indolepropionate, and cinnamoylglycine, which were associated with higher fiber intake and lower risk of T2D, were clustered into the same module. This module also showed close relationships with β-cryptoxanthin and oxalate. We also observed several modules that comprised metabolites positively associated with the risk of T2D. As expected, the host kynurenine metabolites (kynurenine, kynurenate, and quinolinate) clustered into the same module. Another representative unfavorable metabolite module comprised gamma-glutamyl amino acids.

As shown in Figure S12, among the 47 identified metabolites, those beneficial metabolites, which were associated with a lower risk of T2D, were generally linked

with a favorable profile of metabolic traits, whereas unfavorable associations with metabolic traits were observed for metabolites positively associated with T2D, such as N-acetylglucosamine and hydroxyasparagine.

Integrated Analyses of Gut Microbiota and Circulating Metabolites Associated With Fiber Intake and T2D

We next examined associations between the microbial taxa and serum metabolites, among 804 participants at visit 2. After controlling for multiple covariates, we identified 18 potential microbial-related metabolites of the 47 metabolites that were associated with both fiber intake and T2D (Figure 4A). Many of the fiber-associated genera were significantly correlated with multiple metabolites. For example, the beneficial taxa, *Butyrivibrio* and *Faecalibacterium*, were correlated with 16 and 4 metabolites, respectively. Both of them demonstrated positive correlations with the recognized microbial metabolites, indolepropionate[10] and 3-phenylpropionate,[24] suggesting that *Butyrivibrio* and *Faecalibacterium* could be potential contributors of these metabolites. In contrast, *Lachnoclostridium*, which was associated with lower fiber intake and higher odds of T2D, showed negative correlations with indolepropionate and 3-phenylpropionate, and was positively linked with several unfavorable metabolites, including N-acetylglucosamine, a metabolite associated with insulin resistance and weight gain,[24] and hydroxyasparagine, a marker of the mild obesity-related diabetes.[25] Because fecal samples were collected after a blood draw, with a median of 10 (interquartile range, 6–14) days, we conducted stratified analyses on the correlations between gut bacteria and serum metabolites by the sample collection time difference (≤10 versus 10–30 days), and correlations between genera and metabolites were highly consistent (Figure S13A and S13B).

To examine whether the identified microbial-related metabolites could partially explain the associations between microbial genera and T2D, we included these microbial-related metabolites as covariates in the regression models. Associations between 4 genera (*Faecalibacterium*, *Butyrivibrio*, *Acidaminococcus*, and *Lachnoclostridium*) and T2D were attenuated or abolished after further adjusting for their corresponding taxa-related metabolites, and a similar or more pronounced attenuation was observed when including all these 18 microbial-related metabolites in the regression models (Figure 4B). In contrast, the association between *Ruminococcus* and T2D did not change materially after further adjusting for metabolites.

We also conducted a proxy association analysis[21] to estimate potential prospective associations between these 9 gut bacteria and the risk of T2D using these 18 microbial-related metabolites measured at baseline as proxies for gut microbiota. Our analysis suggested 4 bacterial genera potentially associated with the risk of T2D (all Spearman $|r|>0.5$ and $P<0.05$, represented by *Butyrivibrio* and *Lachnoclostridium*; Figure 4D; Figure S14).

DISCUSSION

Our integrative analyses shed light on the complex relationships among dietary fiber intake, gut microbiota, and circulating metabolites, offering insights into their potential roles in the development of T2D. We identified 9 gut microbial genera associated with both fiber intake and T2D in a United States Hispanic/Latino population. Further functional analysis highlighted specific microbial enzymes, particularly glycosylases involved in fiber degradation, which were enriched in individuals with higher fiber intake and exhibited inverse associations with T2D. Enhanced by longitudinal metabolomics data, we identified multiple microbial-related metabolites that could help explain the beneficial associations between specific fiber-associated bacterial genera and T2D.

Our study revealed a potential pathway/route through the fiber-*Faecalibacterium*-metabolite-T2D axis. *Faecalibacterium* is a Gram-positive anaerobe, which is deemed a symbiotic microorganism in human gastrointestinal tracts.[26] *Faecalibacterium prausnitzii* was the only predominant *Faecalibacterium* species identified in our study. Consistent with our results, *Faecalibacterium* has been associated with the high fiber diet[27] and possesses the ability to metabolize various types of fibers and plant polysaccharides.[26] Although *Faecalibacterium* is known as a butyrate producer, its anti-inflammatory and other beneficial effects could not be explained by butyrate alone.[28] Our study revealed that *Faecalibacterium* was associated with multiple potentially beneficial metabolites in serum, including indolepropionate, 3-phenylpropionate, and cinnamoylglycine, all linked to higher fiber intake and lower risk of T2D. Our findings are consistent with the reported beneficial role of indolepropionate in anti-inflammation, antioxidant activity, and amelioration of glucose metabolism.[10,29] The antimicrobial properties of 3-phenylpropionate may result in low production of inflammatory lipopolysaccharide, and its antioxidant activities may contribute to insulin sensitivity.[24] Cinnamoylglycine is a marker of a healthy gut microbiome, which inhibits the growth of pathogenic microorganisms and has potential metabolic health benefits in vitro.[30] Notably, the association between *Faecalibacterium* and T2D was greatly attenuated after adjusting for these *Faecalibacterium*-related metabolites, suggesting that the potentially protective effect of *Faecalibacterium* on T2D could be partially explained by these microbial metabolites.

Another representative route identified in our study was the fiber-*Butyrivibrio*-metabolite-T2D axis. Members of *Butyrivibrio* are known as important degraders of hemicelluloses and plant polysaccharides.[31]

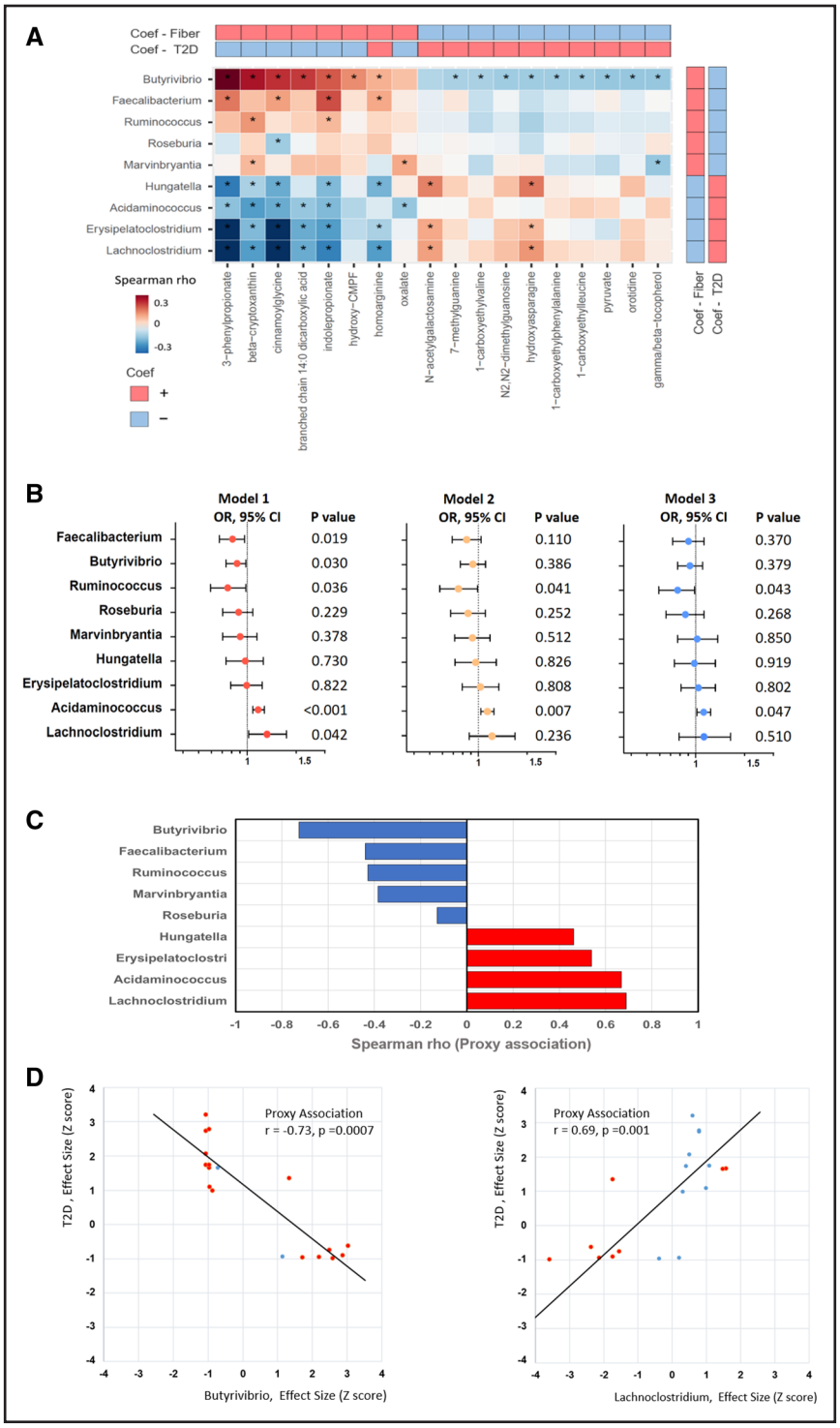

Figure 4. Integrated analyses of gut microbiota and circulating metabolites associated with fiber intake and type 2 diabetes (T2D).

A, Correlation heatmap for the identified microbial taxa and serum metabolites associated with both fiber intake and T2D. Data are partial Spearman correlation coefficients among 804 participants after adjustment for age, sex, study center, education, family income, physical activity, smoking, drinking, use of antibiotics, probiotics, antihypertensive medication, antidiabetic medication, and lipid-lowering medication. (*P<0.05). **B**, Associations of fiber-related microbial genera with prevalent T2D with and without adjustment for microbial-related metabolites. Data are odds ratios (ORs) and 95% CIs for T2D per increment of centered log ratio-transformed abundance of gut bacterial genera, estimated in logistic regression models after adjustment for the aforementioned covariates (model 1), further adjustment for metabolites relevant to specific individual taxa (model 2), and further adjustment for all 18 microbial-related metabolites (model 3). **C**, The proxy associations between fiber-related microbial genera and risk of T2D. The identified 18 microbial-related metabolites were used as proxies for these 9 bacterial genera. For each bacterial genus, we calculated a Spearman correlation coefficient between effect sizes (β coefficients) from the associations of this (*Continued*)

Our metagenomics data indicated that among United States Hispanics/Latinos, *Butyrivibrio fibrisolvens* was the most predominant species under the *Butyrivibrio* genus. Our microbial genomic analysis detected the presence of several glycosylases on the representative *Butyrivibrio fibrisolvens* genome, including high molecular mass xylanases (K15531; EC3.2.1.156). In line with our results, *Butyrivibrio* species have been reported to grow on xylan,[32] a type of dietary fiber found in plant cell walls.[22,33] In addition, 2 recent pilot studies also observed decreased *Butyrivibrio* abundance in patients with diabetes using 16S amplicon sequencing.[34,35] Our integrative analysis linked *Butyrivibrio* with several potentially beneficial metabolites, including 3 aforementioned metabolites (ie, indolepropionate, 3-phenylpropionate, and cinnamoylglycine) and β-cryptoxanthin, an antioxidant, and provitamin A carotenoid associated with a reduced risk of T2D.[36] The protective association between *Butyrivibrio* and T2D might be partially explained by these microbial metabolites. In addition, we observed an inverse association between *Butyrivibrio* and circulating pyruvate, which might be related to its role in butyrate production via pyruvate fermentation.[37] Further studies are warranted to clarify the relationship between circulating and fecal pyruvate and their associations with gut butyrate producers, such as *Butyrivibrio*.

Our study also identified several potentially pathogenic bacteria, including *Lachnoclostridium* and *Acidaminococcus*, associated with lower fiber intake and higher risk of T2D. Moreover, by integrating data on serum metabolomics, we found that *Lachnoclostridium* was positively associated with circulating metabolites implicated in T2D development, such as N-acetylglucosamine and hydroxyasparagine. Of note, N-acetylglucosamine is a recognized contributor to insulin resistance[38] and has been linked with insulin resistance and weight gain in mouse models [38,39] High levels of hydroxyasparagine were reported to play a role in obesity.[25] In addition, both *Lachnoclostridium* and *Acidaminococcus* were inversely associated with serum levels of 3 beneficial metabolites: indolepropionate, 3-phenylpropionate, and cinnamoylglycine. The associations between these 2 potentially pathogenic bacteria and T2D could be partially explained by their related microbial metabolites. Moreover, using metabolite signatures as proxies for gut microbiota, our results also support potential prospective associations of these 2 microbial taxa with increased risk of T2D.

The observed relationships between some fiber-associated genera (eg, *Roseburia* and *Ruminococcus*) and T2D might not be related to fiber-associated metabolites identified in this study. This may reflect the complexity of the microbiota-host crosstalk and suggests that these bacteria may contribute to host metabolic health and disease through other microbial metabolites or metabolite-independent pathways. For example, *Roseburia* species, known butyrate producers, have been reported to affect host metabolism through butyrate inhibiting NF-κB (nuclear factor-κB) activation or influence T-cell proliferation.[40,41] Both our findings and previous results support the protective association between *Roseburia* and T2D.[42] In addition, our study found enriched *Hungatella* in individuals with T2D, and consistently, a decreased relative abundance of *Hungatella* in response to T2D treatment was observed in mouse model.[43]

Our results indicate that both higher insoluble and soluble fiber intakes were associated with a favorable gut microbiota profile. Our findings align with recent in vitro studies showing that beneficial butyrogenic bacteria in *Firmicutes* preferentially use insoluble substrates to support their energy needs.[44,45] Additionally, insoluble fiber may affect the gut microbiota composition via other mechanisms, such as fecal bulking effect,[46] which can reduce the amount of time available for gut bacterial fermentation of nondigested foodstuff and stimulate bacterial growth.[46] On the other hand, soluble fiber can be metabolized by gut bacteria efficiently, which produces many beneficial metabolites and, thus, offers health benefits.[47]

Our study also pinpointed certain circulating metabolites that were associated with both fiber intake and T2D but not correlated with the identified T2D-related gut bacteria. Some of these associations could potentially be attributed to host factors. For instance, we found a set of gamma-glutamyl dipeptides associated with an increased risk of T2D. These dipeptides are recognized as bioactive peptides, and, in particular, gamma-glutamyl-leucine has been extensively documented for its association with inflammation, oxidative stress, and T2D risk.[48] A recent genome-wide study also identified genetic variants in the host genome that may regulate levels of serum gamma-glutamyl-leucine.[48]

This study has several limitations. The ascertainment of dietary fiber intake was based on self-report data at baseline, which could potentially inject bias into our findings. The association between gut microbiota and T2D

Figure 4 Continued. genus with the microbial-related metabolites and effects sizes (nature-log–transformed rate ratios [RRs]) from the associations of the microbial-related metabolites with risk of T2D. A significant correlation between these 2 sets of effect sizes was considered a significant proxy association. **D**, The representative proxy associations of *Butyrivibrio* (**left**) and *Lachnoclostridium* (**right**) with risk of T2D. Each dot represents a microbial-related metabolite. The *x* axis shows effect sizes (β coefficients) from the cross-sectional associations of *Butyrivibrio* or *Lachnoclostridium* with 18 metabolites, and the *y* axis shows the effect sizes (nature-log–transformed RRs) from prospective associations of 18 metabolites at baseline with risk of T2D. These effect sizes were standardized using *Z*-score transformation to ensure comparability. Metabolites significantly associated with *Butyrivibrio* and *Lachnoclostridium*, respectively, are highlighted in red.

was examined in a cross-sectional data set. However, using longitudinal metabolomics data, we identified specific gut microbial-related serum metabolites associated with incident T2D over 6 years. Our further analyses using microbial metabolites as proxies also supported a potential prospective relationship between gut microbiota and the risk of T2D. Finally, given the observational nature of this study, our results should be interpreted with caution, and causal inference could not be established without further evidence.

In summary, in this study of United States Hispanics/Latinos, we demonstrated that higher dietary fiber intake was linked to favorable gut microbiota and circulating metabolite profiles for T2D. The relationships between some fiber-related gut bacteria and T2D could be partially explained by circulating microbial-related metabolites. Our findings provide new information that helps to better understand the relationships of dietary fiber intake with gut microbiota and circulating metabolites and their roles in the development of T2D.

ARTICLE INFORMATION

Received September 5, 2023; revision received February 9, 2024; accepted February 14, 2024.

Affiliations

Department of Epidemiology and Population Health (Z.W., B.A.P., T.W., X.X., Y.M.-R., C.R.I., R.D.B., R.C.K., Q.Q.), Department of Pediatrics (R.D.B.), Department of Obstetrics and Gynecology and Women's Health (R.D.B.), and Department of Microbiology and Immunology (R.D.B.), Albert Einstein College of Medicine, Bronx, NY. Department of Epidemiology, Human Genetics, and Environmental Sciences, School of Public Health, The University of Texas Health Science Center at Houston, Houston (B.Y., M.L.G., E.B.). Division of Molecular Pathology and Genomics, University of Minnesota, Minneapolis, MN (B.T.). Institute for Minority Health Research, University of Illinois at Chicago College of Medicine, Chicago, IL (M.L.D.). Human Genome Sequencing Center, Baylor College of Medicine, Houston, TX (E.B.). Chronic Disease Epidemiology Laboratory, Pennington Biomedical Research Center, Baton Rouge, LA (G.H.). Department of Bioengineering (R.K.), Department of Computer Science and Engineering (R.K.), Center for Microbiome Innovation (R.K.), and Department of Pediatrics (R.K.), University of California, San Diego, La Jolla. Public Health Sciences Division, Fred Hutchinson Cancer Research Center, Seattle, WA (R.C.K.). Department of Nutrition, Harvard T.H. Chan School of Public Health, Boston, MA (Q.Q.).

Acknowledgments

The authors thank all investigators and participants of this study for their valuable contributions.

Sources of Funding

The Hispanic Community Health Study/Study of Latinos (HCHS/SOL) is a collaborative study supported by contracts from the National Heart, Lung, and Blood Institute (NHLBI) to the University of North Carolina (HHSN268201300001I/N01-HC-65233), the University of Miami (HHSN268201300004I/N01-HC-65234), the Albert Einstein College of Medicine (HHSN268201300002I/N01-HC-65235), the University of Illinois at Chicago (HHSN268201300003I/N01-HC-65236, Northwestern University), and San Diego State University (HHSN268201300005I/N01-HC-65237). The following institutes/centers/offices have contributed to the HCHS/SOL through a transfer of funds to the NHLBI: the National Institute on Minority Health and Health Disparities, the National Institute on Deafness and Other Communication Disorders, the National Institute of Dental and Craniofacial Research, the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), the National Institute of Neurological Disorders and Stroke, and the National Institutes of Health (NIH) Institution-Office of Dietary Supplements. This work was supported by grants R01-DK119268 and R01-DK126698 from NIDDK and grant R01-MD011389 from the National Institute on Minority Health and Health Disparities. Other funding sources for this study include grant UM1-HG008898 from the National Human Genome Research Institute; grants R01-HL060712, R01-HL170904, K01-HL169019, R01-HL140976, and R01-HL136266 from NHLBI; and grant R01-DK120870 and the New York Regional Center for Diabetes Translation Research (grant P30DK111022) from NIDDK.

Disclosures

None.

Supplemental Material

Expanded Materials and Methods
Figures S1–S14
Tables S1–S12
References 49–51

REFERENCES

1. McRae MP. Dietary fiber intake and type 2 diabetes mellitus: an umbrella review of meta-analyses. *J Chiropr Med*. 2018;17:44–53. doi: 10.1016/j.jcm.2017.11.002
2. Jenkins DJA, Kendall CWC, McKeown-Eyssen G, Josse RG, Silverberg J, Booth GL, Vidgen E, Josse AR, Nguyen TH, Corrigan S, et al. Effect of a low-glycemic index or a high-cereal fiber diet on type 2 diabetes: a randomized trial. *JAMA*. 2008;300:2742–2753. doi: 10.1001/jama.2008.808
3. Myhrstad MCW, Tunsjø H, Charnock C, Telle-Hansen VH. Dietary fiber, gut microbiota, and metabolic regulation—current status in human randomized trials. *Nutrients*. 2020;12:859. doi: 10.3390/nu12030859
4. Zhao L, Zhang F, Ding X, Wu G, Lam YY, Wang X, Fu H, Xue X, Lu C, Ma J, et al. Gut bacteria selectively promoted by dietary fibers alleviate type 2 diabetes. *Science*. 2018;359:1151–1156. doi: 10.1126/science.aao5774
5. Kaplan RC, Wang Z, Usyk M, Sotres-Alvarez D, Daviglus ML, Schneiderman N, Talavera GA, Gellman MD, Thyagarajan B, et al. Gut microbiome composition in the Hispanic Community Health Study/Study of Latinos is shaped by geographic relocation, environmental factors, and obesity. *Genome Biol*. 2019;20:2–19-z. doi: 10.1186/s13059-019-1831-z
6. Tuomainen M, Lindström J, Lehtonen M, Auriola S, Pihlajamäki J, Peltonen M, Tuomilehto J, Uusitupa M, De Mello VD, Hanhineva K. Associations of serum indolepropionic acid, a gut microbiota metabolite, with type 2 diabetes and low-grade inflammation in high-risk individuals. *Nutrition and Diabetes*. 2018;8:35. doi: 10.1038/s41387-018-0046-9
7. Ma W, Nguyen LH, Song M, Wang DD, Franzosa EA, Cao Y, Joshi A, Drew DA, Mehta R, Ivey KL, et al. Dietary fiber intake, the gut microbiome, and chronic systemic inflammation in a cohort of adult men. *Genome Med*. 2021;13:102. doi: 10.1186/s13073-021-00921-y
8. Wang Z, Usyk M, Vázquez-Baeza Y, Chen G-, Isasi CR, Williams-Nguyen JS, Hua S, McDonald D, Thyagarajan B, Daviglus ML, et al. Microbial co-occurrence complicates associations of gut microbiome with US immigration, dietary intake and obesity. *Genome Biol*. 2021;22:336. doi: 10.1186/s13059-021-02559-w
9. Koh A, De Vadder F, Kovatcheva-Datchary P, Bäckhed F. From dietary fiber to host physiology: short-chain fatty acids as key bacterial metabolites. *Cell*. 2016;165:1332–1345. doi: 10.1016/j.cell.2016.05.041
10. Qi Q, Li J, Yu B, Moon JY, Chai JC, Merino J, Hu J, Ruiz-Canela M, Rebholz C, Wang Z, et al. Host and gut microbial tryptophan metabolism and type 2 diabetes: an integrative analysis of host genetics, diet, gut microbiome and circulating metabolites in cohort studies. *Gut*. 2022;71:1095–1105. doi: 10.1136/gutjnl-2021-324053
11. Cai Z, Poulos RC, Liu J, Zhong Q. Machine learning for multi-omics data integration in cancer. *iScience*. 2022;25:103798. doi: 10.1016/j.isci.2022.103798
12. Sorlie PD, Aviles-Santa LM, Wassertheil-Smoller S, Kaplan RC, Daviglus ML, Giachello AL, Schneiderman N, Raij L, Talavera G, Allison M, et al. Design and implementation of the Hispanic Community Health Study/Study of Latinos. *Ann Epidemiol*. 2010;20:629–641. doi: 10.1016/j.annepidem.2010.03.015
13. Arredondo EM, Sotres-Alvarez D, Stoutenberg M, Davis SM, Crespo NC, Carnethon MR, Castañeda SF, Isasi CR, Espinoza RA, Daviglus ML, et al. Physical activity levels in U.S. Latino/Hispanic adults: results from the Hispanic Community Health Study/Study of Latinos. *Am J Prev Med*. 2016;50:500–508. doi: 10.1016/j.amepre.2015.08.029
14. Siega-Riz AM, Sotres-Alvarez D, Ayala GX, Ginsberg M, Himes JH, Liu K, Loria CM, Mossavar-Rahmani Y, Rock CL, Rodriguez B, et al. Food-group and nutrient-density intakes by Hispanic and Latino backgrounds in the Hispanic Community Health Study/Study of Latinos. *Am J Clin Nutr*. 2014;99:1487–1498. doi: 10.3945/ajcn.113.082685

15. Hillmann B, Al-Ghalith GA, Shields-Cutler RR, Zhu Q, Gohl DM, Beckman KB, Knight R, Knights D. Evaluating the information content of shallow shotgun metagenomics. *mSystems*. 2018;3:e00069–e00018. doi: 10.1128/mSystems.00069-18
16. Wang TJ, Larson MG, Vasan RS, Cheng S, Rhee EP, McCabe E, Lewis GD, Fox CS, Jacques PF, Fernandez C, et al. Metabolite profiles and the risk of developing diabetes. *Nat Med*. 2011;17:448–453. doi: 10.1038/nm.2307
17. Chai JC, Chen GC, Yu B, Xing J, Li J, Khambaty T, Perreira KM, Perera MJ, Vidot DC, Castaneda SF, et al. Serum metabolomics of incident diabetes and glycemic changes in a population with high diabetes burden: the Hispanic Community Health Study/Study of Latinos. *Diabetes*. 2022;71:1338–1349. doi: 10.2337/db21-1056
18. Qi Q, Strizich G, Merchant G, Sotres-Alvarez D, Buelna C, Castaneda SF, Gallo LC, Cai J, Gellman MD, Isasi CR, et al. Objectively measured sedentary time and cardiometabolic biomarkers in US Hispanic/Latino adults: the Hispanic Community Health Study/Study of Latinos (HCHS/SOL). *Circulation*. 2015;132:1560–1569. doi: 10.1161/CIRCULATIONAHA.115.016938
19. Mandal S, Van Treuren W, White RA, Eggesbø M, Knight R, Peddada SD. Analysis of composition of microbiomes: a novel method for studying microbial composition. *Microb Ecol Health Dis*. 2015;26:27663. doi: 10.3402/mehd.v26.27663
20. Letunic I, Bork P. Interactive Tree Of Life (iTOL) v4: recent updates and new developments. *Nucleic Acids Res*. 2019;47:W256–W259. doi: 10.1093/nar/gkz239
21. Amin N, Liu J, Bonnechere B, MahmoudianDehkordi S, Arnold M, Batra R, Chiou Y, Fernandes M, Ikram MA, Kraaij R, et al. Interplay of metabolome and gut microbiome in individuals with major depressive disorder vs control individuals. *JAMA Psychiatry*. 2023;80:597–609. doi: 10.1001/jamapsychiatry.2023.0685
22. Mirande C, Kadlecikova E, Matulova M, Capek P, Bernalier-Donadille A, Forano E, Bera-Maillet C. Dietary fibre degradation and fermentation by two xylanolytic bacteria Bacteroides xylanisolvens XB1A and Roseburia intestinalis XB6B4 from the human intestine. *J Appl Microbiol*. 2010;109:451–460. doi: 10.1111/j.1365-2672.2010.04671.x
23. Lee S, Zhang C, Kilicarslan M, Piening BD, Bjornson E, Hallström BM, Groen AK, Ferrannini E, Laakso M, Snyder M, et al. Integrated network analysis reveals an association between plasma mannose levels and insulin resistance. *Cell Metab*. 2016;24:172–184. doi: 10.1016/j.cmet.2016.05.026
24. McMurray F, Patten DA, Harper ME. Reactive oxygen species and oxidative stress in obesity-recent findings and empirical approaches. *Obesity (Silver Spring.)*. 2016;24:2301–2310. doi: 10.1002/oby.21654
25. Zaghlool SB, Halama A, Stephan N, Gudmundsdottir V, Gudnason V, Jennings LL, Thangam M, Ahlqvist E, Malik RA, Albagha OME, et al. Metabolic and proteomic signatures of type 2 diabetes subtypes in an Arab population. *Nat Commun*. 2022;13:7121. doi: 10.1038/s41467-022-34754-z
26. He X, Zhao S, Li Y. Faecalibacterium prausnitzii: a next-generation probiotic in gut disease improvement. *Can J Infect Dis Med Microbiol*. 2021;2021:6666114. doi: 10.1155/2021/6666114
27. Benno Y, Endo K, Miyoshi H, Okuda T, Koishi H, Mitsuoka T. Effect of rice fiber on human fecal microflora. *Microbiol Immunol*. 1989;33:435–440. doi: 10.1111/j.1348-0421.1989.tb01992.x
28. Heinken A, Khan MT, Paglia G, Rodionov DA, Harmsen HJM, Thiele I. Functional metabolic map of Faecalibacterium prausnitzii, a beneficial human gut microbe. *J Bacteriol*. 2014;196:3289–3302. doi: 10.1128/JB.01780-14
29. Dodd D, Spitzer MH, Van Treuren W, Merrill BD, Hryckowian AJ, Higginbottom SK, Le A, Cowan TM, Nolan GP, Fischbach MA, et al. A gut bacterial pathway metabolizes aromatic amino acids into nine circulating metabolites. *Nature*. 2017;551:648–652. doi: 10.1038/nature24661
30. Adisakwattana S. Cinnamic acid and its derivatives: mechanisms for prevention and management of diabetes and its complications. *Nutrients*. 2017;9:163. doi: 10.3390/nu9020163
31. Kelly WJ, Leahy SC, Altermann E, Yeoman CJ, Dunne JC, Kong Z, Pacheco DM, Li D, Noel SJ, Moon CD, et al. The glycobiome of the rumen bacterium butyrivibrio proteoclasticus B316T highlights adaptation to a polysaccharide-rich environment. *PLoS One*. 2010;5:e11942. doi: 10.1371/journal.pone.0011942
32. Palevich N, Kelly WJ, Ganesh S, Rakonjac J, Attwood GT. Butyrivibrio hungatei MB2003 competes effectively for soluble sugars released by Butyrivibrio proteoclasticus B316^T during growth on xylan or pectin. *Appl Environ Microbiol*. 2019;85:e02056–e02018. doi: 10.1128/AEM.02056-18
33. Zhang T, Yang Y, Liang Y, Jiao X, Zhao C. Beneficial effect of intestinal fermentation of natural polysaccharides. *Nutrients*. 2018;10:1055. doi: 10.3390/nu10081055
34. Kulkarni P, Devkumar P, Chattopadhyay I. Could dysbiosis of inflammatory and anti-inflammatory gut bacteria have an implications in the development of type 2 diabetes? A pilot investigation. *BMC Res Notes*. 2021;14:52. doi: 10.1186/s13104-021-05466-2
35. Talukdar R, Sarkar P, Jakkampudi A, Sarkar S, Aslam M, Jandhyala M, Deepika G, Unnisa M, Reddy DN. The gut microbiome in pancreatogenic diabetes differs from that of type 1 and type 2 diabetes. *Sci Rep*. 2021;11:10978. doi: 10.1038/s41598-021-90024-w
36. Montonen J, Knekt P, Järvinen R, Reunanen A. Dietary antioxidant intake and risk of type 2 diabetes. *Diabetes Care*. 2004;27:362–366. doi: 10.2337/diacare.27.2.362
37. Anand S, Kaur H, Mande SS. Comparative in silico analysis of butyrate production pathways in gut commensals and pathogens. *Front Microbiol*. 2016;7:1945. doi: 10.3389/fmicb.2016.01945
38. Pedersen HK, Gudmundsdottir V, Nielsen HB, Hyotylainen T, Nielsen T, Jensen BA, Forslund K, Hildebrand F, Prifti E, Falony G, et al; MetaHIT Consortium. Human gut microbes impact host serum metabolome and insulin sensitivity. *Nature*. 2016;535:376–381. doi: 10.1038/nature18646
39. Hesketh GG, Dennis JW. N-acetylglucosamine: more than a silent partner in insulin resistance. *Glycobiology*. 2017;27:595–598. doi: 10.1093/glycob/cwx035
40. Tamanai-Shacoori Z, Smida I, Bousarghin L, Loreal O, Meuric V, Fong SB, Bonnaure-Mallet M, Jolivet-Gougeon A. Roseburia spp.: a marker of health? *Future Microbiol*. 2017;12:157–170. doi: 10.2217/fmb-2016-0130
41. Inan MS, Rasoulpour RJ, Yin L, Hubbard AK, Rosenberg DW, Giardina C. The luminal short-chain fatty acid butyrate modulates NF-κB activity in a human colonic epithelial cell line. *Gastroenterology*. 2000;118:724–734. doi: 10.1016/s0016-5085(00)70142-9
42. Bakir-Gungor B, Bulut O, Jabeer A, Nalbantoglu OU, Yousef M. Discovering potential taxonomic biomarkers of type 2 diabetes from human gut microbiota via different feature selection methods. *Front Microbiol*. 2021;12:628426. doi: 10.3389/fmicb.2021.628426
43. Silamiķele L, Silamiķelis I, Ustinova M, Kalniņa Z, Elbere I, Petrovska R, Kalniņa I, Kloviņš J. Metformin strongly affects gut microbiome composition in high-fat diet-induced type 2 diabetes mouse model of both sexes. *Front Endocrinol*. 2021;12:626359. doi: 10.3389/fendo.2021.626359
44. Lamothe LM, Cantu-Jungles TM, Chen T, Green S, Naqib A, Srichuwong S, Hamaker BR. Boosting the value of insoluble dietary fiber to increase gut fermentability through food processing. *Food Funct*. 2021;12:10658–10666. doi: 10.1039/d1fo02146j
45. Baky MH, Salah M, Ezzelarab N, Shao P, Elshahed MS, Farag MA. Insoluble dietary fibers: structure, metabolism, interactions with human microbiome, and role in gut homeostasis. *Crit Rev Food Sci Nutr*. 2022;0:1–15. doi: 10.1080/10408398.2022.2119931
46. Abreu-Y-Abreu AT, Milke-García MP, Argüello-Arévalo GA, Calderón-de-la-Barca AM, Carmona-Sánchez RI, Consuelo-Sánchez A, Coss-Adame E, García-Cedillo MF, et al. Dietary fiber and the microbiota: a narrative review by a group of experts from the Asociación Mexicana de Gastroenterología. *Rev Gastroenterol Mex (Engl Ed)*. 2021;86:287–304. doi: 10.1016/j.rgmxen.2021.02.002
47. Guan Z, Yu E, Feng Q. Soluble dietary fiber, one of the most important nutrients for the gut microbiota. *Molecules*. 2021;26:6802. doi: 10.3390/molecules26226802
48. Wu Q, Li J, Zhu J, Sun X, He D, Li J, Cheng Z, Zhang X, Xu Y, Chen Q, et al. Gamma-glutamyl-leucine levels are causally associated with elevated cardio-metabolic risks. *Front Nutr*. 2022;9:936220. doi: 10.3389/fnut.2022.936220
49. Chiuve SE, Fung TT, Rimm EB, Hu FB, McCullough ML, Wang M, Stampfer MJ, Willett WC. Alternative dietary indices both strongly predict risk of chronic disease. *J Nutr*. 2012;142:1009–1018. doi: 10.3945/jn.111.157222
50. Langfelder P, Horvath S. WGCNA: an R package for weighted correlation network analysis. *BMC Bioinf*. 2008;9:559. doi: 10.1186/1471-2105-9-559
51. Otasek D, Morris JH, Bouças J, Pico AR, Demchak B. Cytoscape Automation: empowering workflow-based network analysis. *Genome Biol*. 2019;20:185–184. doi: 10.1186/s13059-019-1758-4