

Gut Microbiota Associated with Type 2 Diabetes and Dietary Balance in Older Adults: A Longitudinal Community-Based Cohort in China

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Purpose: This prospective cohort study with a nested case–control analysis aimed to identify gut microbial taxa associated with type 2 diabetes in older Chinese adults and to examine whether dietary balance influences diabetes-related microbial features.

Patients and Methods: This study included 507 community-dwelling adults aged 65 years and older from the Surveillance and Management of Disability and Cognitive Impairment in Older Adults (SUM-DCI) cohort. Fecal samples were collected at baseline and analyzed using 16S ribosomal RNA gene sequencing to characterize gut microbial composition. Diabetes status was defined by fasting plasma glucose concentration ≥ 7.0 mmol/L or a self-reported physician diagnosis. A nested case–control study of 93 individuals (31 cases) was conducted within the baseline non-diabetic population to evaluate prospective associations. Metastats and Multivariate Association with Linear Models (MaAsLin) were used to identify diabetes-associated microbial taxa. Dietary intake was assessed using a self-reporting food frequency questionnaire, dietary balance was quantified using the Chinese Dietary Balance Index-22. Multivariable logistic regression was used to evaluate associations between dietary balance and gut microbial abundance.

Results: Roseburia abundance was significantly lower in individuals with type 2 diabetes (FDR -adjusted $P < 0.05$). Higher baseline Roseburia abundance was associated with a lower risk of incident diabetes (OR: 0.225, 95% CI: 0.115–0.818, $P < 0.05$). Low soybean intake was independently associated with reduced Roseburia abundance (OR: 0.61, 95% CI: 0.39–0.94, $P < 0.05$), whereas overall dietary imbalance scores were not significantly related.

Conclusion: Reduced Roseburia abundance is linked to both prevalent and future incident type 2 diabetes among older adults. Low soybean intake may contribute to this microbial depletion, suggesting a potential mechanism by which dietary habits influence diabetes risk through modulation of the gut microbiota.

Keywords: gut microbiota, Roseburia, type 2 diabetes, dietary balance, older adults

Introduction

Type 2 diabetes is a highly prevalent chronic metabolic disorder worldwide.^{1,2} In 2021, an estimated 536 million people were affected globally; this figure is expected to rise to 783 million by 2045.³ The prevalence of diabetes in adults in China is 15.88%,⁴ that includes 30% of adults aged 60 years and older.⁵ If not well controlled, diabetes can lead to serious complications—particularly in older adults—that substantially reduce lifespan and quality of life, imposing a considerable disease burden.⁶ Effective approaches to preventing and managing type 2 diabetes in older adults are essential to protect health in later life.

Gut microbiota are increasingly recognized as a critical factor in the pathogenesis and progression of type 2 diabetes.^{7,8} Alterations in the intestinal microbial community can modulate insulin sensitivity, glucose metabolism, and low-grade



chronic inflammation—critical pathways in diabetes development.^{9–11} In particular, depletion of bacteria producing short-chain fatty acids (SCFAs) and enrichment of pro-inflammatory taxa have been closely linked to hyperglycemia and insulin resistance.^{12–16} Accumulating evidence from multiple cohort and clinical studies supports a link between reduced abundance of butyrate-producing and other SCFA-producing microbes and increased risk of type 2 diabetes.¹⁷ However, some studies have reported inconsistent or conflicting findings. One study found no systematic changes in the abundance of SCFA-producing bacteria, thereby failing to support a clear association between their levels and type 2 diabetes status.¹⁸ Another study even reported an opposite trend, with increased levels of certain SCFA-producing taxa in individuals with type 2 diabetes.¹⁹ These discrepancies highlight the need for further longitudinal research to clarify causal relationships, especially in older adults, among whom age-related shifts in the gut microbiota—including reductions in beneficial SCFA-producing taxa—are common.²⁰ Notably, most existing research has focused on younger or general adult populations, and among the few studies involving older adults, at least one has used a cross-sectional design.²¹ Longitudinal studies targeting aging populations are needed to better elucidate the gut microbiota–diabetes relationship in later life.

The composition and function of SCFA-producing gut microbes are strongly influenced by dietary factors,²² such as high-fiber²³ or plant-based patterns.²⁴ Dietary balance reflects adherence to guideline-recommended intakes across major food groups, that is, the extent of both insufficiency and excess for certain food group, thereby capturing the availability and diversity of fermentable substrates that may be particularly relevant to SCFA-producing microbial communities.²⁵ So SCFA-producing gut microbes may be affected by dietary balance, however, the role of dietary balance has received less attention. The Chinese Dietary Balance Index (DBI) was developed based on the Dietary Guidelines for Chinese residents and the Food Guide Pagoda to operationalize this concept of balance by assigning component scores according to deviations from recommended intake ranges: negative scores indicate insufficient intake, positive scores indicate excessive intake, and a score of 0 indicates meeting recommendations.²⁶ Therefore, DBI provides a guideline-based, quantitative framework to characterize dietary balance at both the whole-diet and food-group levels,²⁷ facilitating the evaluation of diet–microbiota associations relevant to diabetes in this study.

Using the data of Surveillance and Management of Disability and Cognitive Impairment in Older Adults (SUM-DCI) cohort, the primary aim of our study was to identify gut microbial signatures associated with type 2 diabetes in older adults, and explore the relationship between dietary balance and microbial markers.

Materials and Methods

Study Design

For this study, participants from the SUM-DCI cohort, established between August 2018 and January 2019, included 1972 adults aged 65+ from Yuanbao District in Liaoning Province and Korla City in Xinjiang Uygur Autonomous Region. In April 2019, a gut microbiota sub-cohort was established with 690 randomly selected individuals.²⁸ Inclusion criteria for the sub-cohort were older adults who completed the baseline survey. Exclusion criteria included: individuals with neurological disorders (such as dementia or psychiatric illnesses) or other severe diseases; a history of gastrointestinal surgery within the past five years; a history of bowel resection, inflammatory bowel disease, irritable bowel syndrome, persistent gastroenteritis, chronic diarrhea, colitis, *Clostridioides difficile* infection, or chronic constipation; and those who had used systemic antibiotics or high-dose probiotics within the past three months. The selection process of study participants is illustrated in [Figure 1](#).

This study was approved by the Ethics Committee of the Chinese Center for Disease Control and Prevention. Written informed consent was obtained from all participants (or their proxies).

Assessment of Diabetes at Baseline and Follow-up

Diabetes was defined as fasting plasma glucose (FPG) ≥ 7.0 mmol/L and/or a self-reported in-hospital diagnosis. FPG was measured from venous blood samples collected at baseline and follow-up. Incident diabetes was defined as the occurrence of diabetes meeting the above criteria among participants without diabetes at baseline.

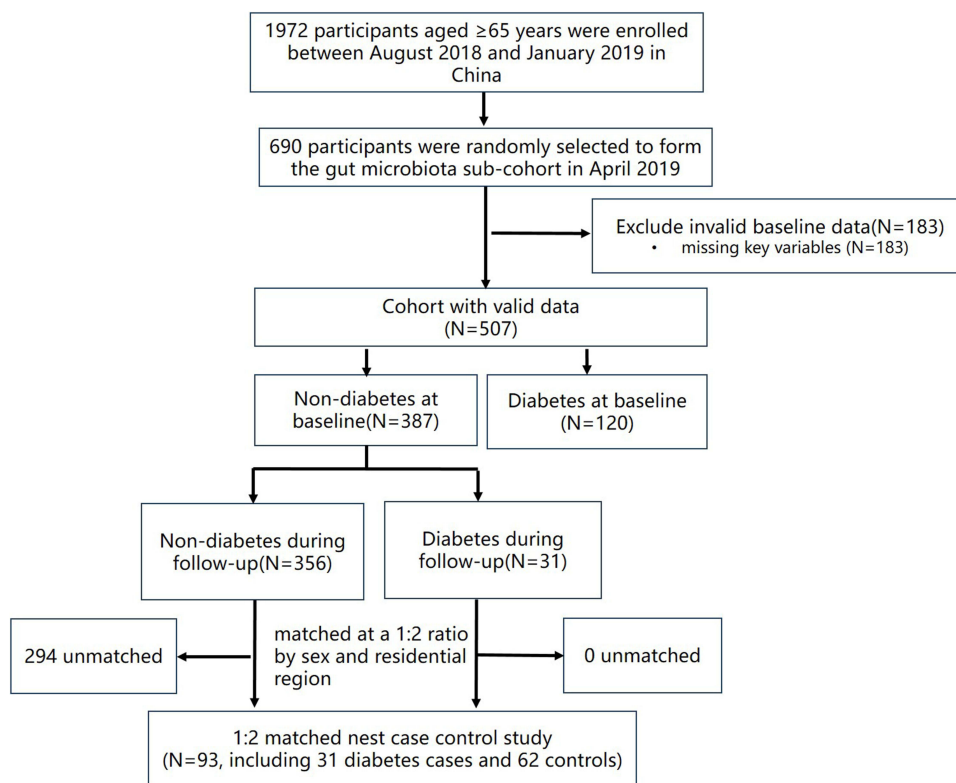


Figure 1 Flow chart of participants selection.

Fecal Sample Collection, DNA Extraction, and 16S rRNA Gene Sequencing

Participants were instructed to collect a stool sample immediately following defecation, and to deliver the specimen to the designated center within 30 minutes. Upon receipt, samples were promptly logged, sealed in sterile containers, and stored at -80°C as the preferred long-term storage condition. Microbial genomic DNA was extracted using the cetyltrimethylammonium bromide (CTAB)/sodium dodecyl sulfate (SDS) method,²⁹ followed by PCR amplification of the 16S rRNA gene and library preparation. Raw reads were quality-filtered, clustered into operational taxonomic units (OTUs) at 97% similarity, and taxonomically annotated against the SILVA database.³⁰ Data were normalized for downstream phylogenetic and compositional analyses. Detailed information regarding DNA extraction, gut microbiota 16S rRNA gene sequencing, and fecal metabolic profiles is provided in [Supplementary File 1: Supplementary Method S1](#). Diabetes-related taxa were evaluated using relative abundance, with classification based on the median split.

Assessment of Diet Balance

The food frequency questionnaire (FFQ) was used to collect information on the dietary consumption of 43 different food categories over the prior 12 months at baseline. Participants were asked to report the consumption frequency of each food item or food group, followed by a question on the amount of consumption (grams for solid or milliliter for liquid food items). Consumption frequency was ascertained with the following questions: (1) how many times per day; (2) how many times per week; (3) how many times per month; or (4) how many times per year.

Diet balance was evaluated according to the revised Chinese Dietary Balance Index-22 (DBI-22) standards.²⁶ Seven food-group indicators—cereals, vegetables and fruits, dairy and soy products, animal-source foods, alcohol, and overall food variety—were assigned component scores based on each participant's reported daily consumption. Component scores were summed to derive three summary metrics: the High-Bound Score (DBI-HBS), representing the degree of excessive intake, calculated by summing the absolute values of positive scores across dietary components; the Low-Bound Score (DBI-LBS), indicating insufficient intake, calculated by summing the absolute values of negative scores;

and the Diet Quality Distance (DBI-DQD) that quantifies the overall dietary imbalance by summing the absolute values of both positive and negative scores across all dietary components.

Assessment of Covariates

Covariates included sociodemographic characteristics (age, sex, geographic region, and educational attainment), lifestyle behaviors (smoking status, alcohol use, tea consumption, physical activity, and body mass index [BMI]), and health status indicators (hypertension and sleep disturbances). Physical exercise was categorized as “yes” if a participant exercised at least 30 minutes per day, three days or more per week. Sleep disturbances were defined as experiencing one or more of the following within the past month: taking longer than 30 minutes to fall asleep; awakening two or more times during the night; or waking up too early and having difficulty returning to sleep. Hypertension status was defined by a self-reported in-hospital diagnosis.

Statistical Analysis

Baseline characteristics were compared between diabetes and non-diabetes. We conducted *t*-tests for continuous variables and chi-square tests for categorized variables.

To pinpoint OTUs at baseline that were significantly associated with type 2 diabetes, we applied two complementary approaches, Metastats analysis and Multivariate Association with Linear Models (MaAsLin), that were used jointly to identify baseline gut microbial taxa associated with type 2 diabetes status. Metastats was applied to detect differentially abundant taxa between the type 2 diabetes and non-type 2 diabetes groups.³¹ Relative abundances of taxa were analyzed using MaAsLin to evaluate their associations with type 2 diabetes while adjusting for potential confounders.³² Multiple testing correction was performed using the Benjamini–Hochberg procedure, with a false discovery rate (FDR) -adjusted $P < 0.05$ considered statistically significant.

To validate the predictive value of the identified baseline taxa, a nested case–control analysis was conducted within the final cleaned cohort over a three-year follow-up period. Among those who were non-diabetic at baseline, individuals who developed incident type 2 diabetes were identified as cases. Controls were selected from participants who remained free of diabetes throughout the follow-up, resulting in a nested case–control sample. Cases and controls were matched in a 1:2 ratio based on sex and residential region to enhance comparability across key demographic variables. Within this nested sample, conditional logistic regression models were applied to estimate odds ratios (ORs) and 95% confidence intervals (CIs) for incident type 2 diabetes associated with per-unit changes in the relative abundance of each diabetes-associated taxon. Three models were constructed: Model 1 included only diabetes-associated taxon; Model 2 additionally adjusted for demographic covariates (age, education and BMI); and Model 3 further accounted for lifestyle factors, including smoking, alcohol drinking, tea drinking, hypertension and sleep disorder.

Diabetes-associated taxon that demonstrated robust inverse associations with incident type 2 diabetes were selected for diet evaluation. The relationship between dietary quality and these selected microbial taxa was then assessed using multivariable logistic regression analyses with baseline dietary data. Three logistic regression models were constructed: Model 1 included either the total DBI score or individual DBI components as the primary exposure variables; Model 2 additionally adjusted for demographic covariates (age, sex, education, and region); and Model 3 further accounted for lifestyle factors, including physical activity, smoking, alcohol use, and BMI. ORs and CIs were estimated to quantify the associations between each DBI indicator and relative abundance of diabetes-associated taxa.

Due to substantial imbalances in data distribution within DBI-defined categories—primarily reflecting low adherence to recommendations for added sugar intake and dietary variety—these two components were excluded from subsequent analyses to avoid biased or unstable statistical estimates.

All statistical analyses were conducted using R version 4.4.2, with a significance level of $P < 0.05$ unless otherwise specified.

Results

Demographic Characteristic

After data cleaning and exclusion of participants with missing key variables or invalid stool or dietary data, a final analytic sample of 507 older adults was included in the study. The baseline characteristics of the participants are shown

according to diabetes status (Table 1). Among the 507 participants, 120 had diabetes, and 387 were non-diabetic at baseline. The mean age of the participants was 71.74 years, and males accounted for 45.17%. Participants with diabetes were more likely to be male and have a higher prevalence of hypertension compared to those without diabetes ($P < 0.05$). Between the two groups, there was no significant difference in lifestyle factors, including smoking, alcohol consumption, tea drinking, exercise habits, or sleep disorders.

Baseline Gut Microbial Composition in Type 2 Diabetes and Non-Type 2 Diabetes Groups

A total of 58,031,495 reads were sequenced from 507 samples, and 57,236,330 clean tags were generated after splicing and filtering, averaging 165,662 clean tags per sample. A total of 11,019 OTUs were obtained from all samples at the 97% similarity level. The relative abundance of the top 10 gut microbial phyla in the type 2 diabetes and non-type 2 diabetes groups is shown in [Supplementary Figure S1](#). Firmicutes and Bacteroidetes dominated the gut microbiota in both groups. These patterns are consistent with previous studies,^{33,34} supporting the reliability and validity of the microbiota data used in this analysis.

Associations of Gut Microbiota with Prevalence of Diabetes

As shown in [Figure 2](#), the Metastats analysis revealed notable differences in gut microbial composition between the type 2 diabetes and non-type 2 diabetes groups. While Roseburia was significantly more abundant in the non-type 2 diabetes group ($P < 0.01$), Collinsella was significantly more abundant in the type 2 diabetes group ($P < 0.05$).

The results of the MaAsLin analysis examining associations between gut microbial OTUs and type 2 diabetes status are shown in [Table 2](#). Among individuals with type 2 diabetes, the significantly associated taxa, Roseburia (OTU_4870) was significantly less abundant ($Q < 0.05$), whereas both Bacteroides (OTU_11002) and Bifidobacterium bifidum (OTU_3633) were significantly more abundant (*False Discovery Rate -adjusted* $P < 0.05$). These findings are further illustrated in [Figure 3](#), which presents boxplots of the relative abundances for the key taxa identified in the MaAsLin analysis.

Table 1 Baseline Characteristics of the Participants by Diabetes Status (n=507)

Characteristic	Overall (n=507)	Diabetes Status		P
		Diabetes (n=120)	Non-Diabetes (n=387)	
Age (years)	71.74 (4.51)	71.57 (4.34)	71.79 (4.56)	0.637
Sex				0.040*
Male	229 (45.17%)	64 (53.33%)	165 (42.64%)	
Female	278 (54.83%)	56 (46.67%)	222 (57.36%)	
Education				0.532
0	100 (19.72%)	21 (17.50%)	79 (20.41%)	
1–6 years	159 (31.36%)	35 (29.17%)	124 (32.04%)	
>6 years	248 (48.92%)	64 (53.33%)	184 (47.55%)	
Smoking status				0.113
Smokers	440 (86.79%)	99 (82.50%)	341 (88.11%)	
Nonsmokers	67 (13.22%)	21 (17.50%)	46 (11.89%)	
Alcohol drinking	407 (80.28%)	101 (84.17%)	306 (79.07%)	0.220
Tea drinking	74 (14.50%)	21 (17.50%)	53 (13.70%)	0.377
Exercise	136 (26.82%)	35 (29.18%)	101 (26.10%)	0.507
Sleep disorder	226 (44.58%)	50 (41.67%)	176 (45.48%)	0.463
Hypertension	212 (41.81%)	60 (50.00%)	152 (39.28%)	0.048*
Region				0.147
Yuanbao	179 (35.31%)	49 (40.83%)	130 (33.59%)	
Korla	328 (64.69%)	71 (59.17%)	257 (66.41%)	
BMI (kg/m ²)	28.85 (3.43)	25.72 (3.45)	26.26 (3.31)	0.135

Notes: Categorical variables are shown as n (%) and continuous variables are shown as mean (SD). * $P < 0.05$.

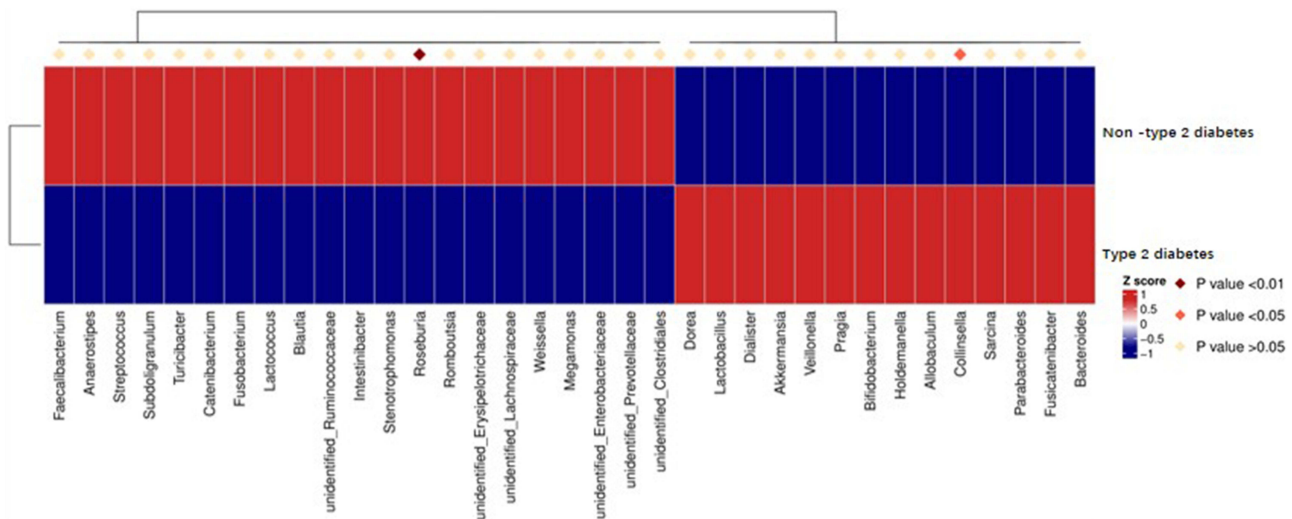


Figure 2 Metastats analysis between type 2 diabetes and non-type 2 diabetes group. Genera with significant differences between groups are indicated by color-coded Z-scores, with red representing higher abundance and blue representing lower abundance.

Associations of Gut Microbiota with Incident Diabetes

Among the 387 non-diabetic individuals at baseline, 31 developed type 2 diabetes during follow-up and were matched to 62 controls, resulting in a total analytic sample of 93 individuals.

As shown in Table 3, conditional logistic regression models demonstrated that participants with higher baseline Roseburia abundance (OTU_4870) had a significantly lower risk of developing type 2 diabetes, with the OR of 0.306 (95% CI: 0.115–0.818, $P < 0.05$) in the unadjusted model. This association remained robust after adjusting for demographic and lifestyle factors in subsequent models, with the OR of 0.225 (95% CI: 0.115–0.818, $P < 0.05$) in the final model. No statistically significant associations were observed for either OTU_11002 or OTU_3633.

Association Between Roseburia Abundance and DBI Index

Overall, study participants exhibited moderate dietary insufficiency (DBI_LBS: 26.92±10.15) and low levels of excessive intake (DBI_HBS: 3.48±4.84)—the latter likely underestimated due to the omission of edible oil and salt assessments—resulting in a moderate overall dietary imbalance (DBI_DQD: 30.40±9.05).

Neither the dietary imbalance-low (insufficiency of intake) balance score (DBI_LBS) nor the dietary imbalance-high (excessive intake) balance score (DBI_HBS) showed a statistically significant association with Roseburia abundance (Table 4). However, among the DBI components, low soybean intake was consistently associated with lower odds of higher Roseburia abundance in both adjusted models (Model 2: OR=0.60, 95% CI: 0.39–0.92; Model 3: OR=0.61, 95% CI: 0.39–0.94; both $P < 0.05$).

Table 2 Associations Between Gut Microbial OTU Relative Abundance and Type 2 Diabetes Status: MaAsLin Analysis (n=120 for Type 2 Diabetes and N= 387 for Non-Type 2 Diabetes)

OTUs	Coefficient	Correlation Direction	FDR-Adjusted P-value	Phylum	Family	Genus	Species
OTU_4870	-0.58777	Negative	0.011415	Firmicutes	Lachnospiraceae	Roseburia	
OTU_11002	0.312979	Positive	0.025174	Bacteroidetes	Bacteroidaceae	Bacteroides	
OTU_3633	0.58294	Positive	0.040846	Actinobacteria	Bifidobacterium		Bifidobacterium_bifidum

Notes: The gut microbiota biomarkers were determined by MaAsLin adjusted by age, gender, region, education, BMI, smoking status, drinking status, exercise status, sleep status. Negative indicates lower in type 2 diabetes vs non-type 2 diabetes; Positive indicates higher in type 2 diabetes vs non-type 2 diabetes. The false discovery rate (FDR)-adjusted P-value, calculated using the Benjamini-Hochberg procedure.

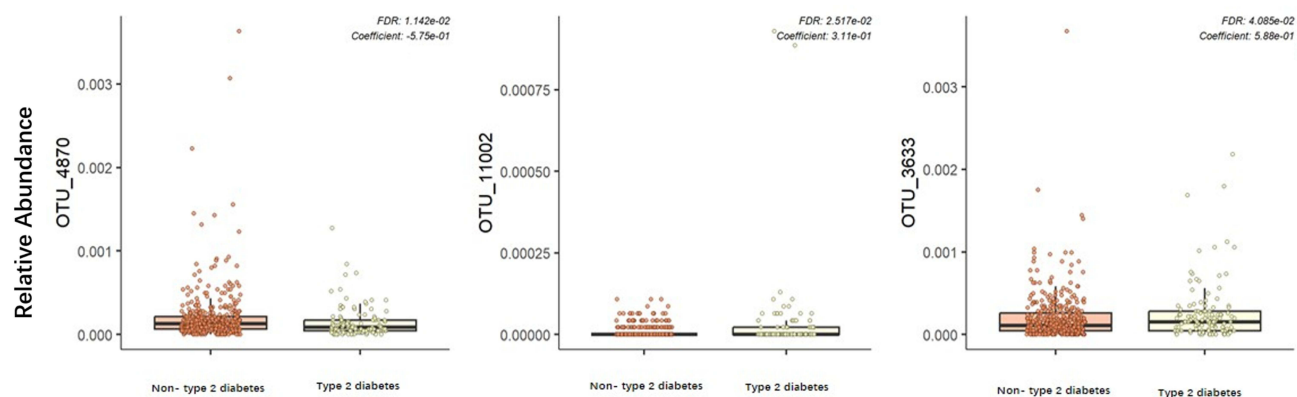


Figure 3 Relative abundance of gut microbial OTUs between type 2 diabetes and non-type 2 diabetes groups. Boxplots display the relative abundance distributions of three representative OTUs significantly associated with type 2 diabetes status as identified by MaAsLin. Coefficient indicates the strength and direction of association between OTUs abundance and type 2 diabetes status. FDR denotes the Benjamini–Hochberg–adjusted p-value. Coef indicates the MaAsLin regression coefficient (β) for type 2 diabetes status; negative β indicates lower abundance in the type 2 diabetes group and positive β indicates higher abundance.

Discussion

This study demonstrated a significant inverse association between the relative abundance of Roseburia and both prevalent and incident type 2 diabetes in an older adult Chinese cohort. Low soybean intake was independently associated with reduced Roseburia abundance.

These findings are consistent with previous studies demonstrating that Roseburia abundance is inversely associated with type 2 diabetes.^{12,33,35} Several reports have proposed that Roseburia play a role in glucose regulation and the progression from normoglycemia to diabetes.^{36,37} Several mechanistic explanations have been proposed. First, Roseburia spp. are prominent butyrate producers in the gut, utilizing the acetyl-CoA transferase pathway to convert dietary fibers into SCFAs, predominantly butyrate,³⁸ which functions as a key signaling molecule that modulates host metabolism. It promotes the secretion of GLP-1 and PYY through activation of FFAR2 and FFAR3 receptors on enteroendocrine cells, thereby enhancing insulin secretion and satiety.^{39,40} Additionally, butyrate activates PPAR- γ and inhibits histone deacetylases, promoting fatty acid oxidation and improving insulin sensitivity.⁴¹ It also serves as a primary energy

Table 3 Associations of Baseline Diabetes-Associated OTU Relative Abundance with Incident Type 2 Diabetes: Conditional Logistic Regression in a 1:2 Matched Nested Case–Control Sample (31 Cases, 62 Controls)

Relative Abundance	Model 1	Model 2	Model 3
OUT_4870			
Low	1.00 (ref)		
High	0.306 (0.115,0.818)*	0.190 (0.056,0.643)**	0.225 (0.052,0.968)*
OUT_11002			
Low	1.00 (ref)		
High	1.784 (0.626,5.082)	2.255 (0.737,6.895)	2.302 (0.646,8.198)
OUT_3633			
Low	1.00 (ref)		
High	1.000 (0.404,2.478)	1.116 (0.377,3.297)	1.007 (0.304,3339)

Notes: Gut microbiota grouped based on the median relative abundance. Data are shown as (OR, 95% CI). * $P < 0.05$, ** $P < 0.01$. Model 1: not adjusted for covariates; Model 2: adjusted for age, BMI, education level, income; Model 3: further adjusted for smoking, alcohol drinking, tea drinking, exercise, hypertension and sleep disorder.

Table 4 Associations of DBI Scores with Roseburia (OTU_4870) Relative Abundance: Multivariable Logistic Regression

	Model 1	Model 2	Model 3
DBI scores			
DBI_LBS	0.99 (0.97–1.01)	0.98 (0.96–1.00)	0.99 (0.97–1.01)
DBI_HBS	0.99 (0.95–1.03)	0.98 (0.94–1.03)	0.98 (0.94–1.03)
DBI components			
Cereal			
Recommended	1.00 (ref)		
Low	1.24 (0.73–2.11)	0.97 (0.56–1.70)	1.00 (0.56–1.77)
Excess	0.95 (0.55–1.64)	0.85 (0.48–1.50)	0.87 (0.49–1.56)
Vegetable			
Recommended	1.00 (ref)		
Low	1.79 (0.89–3.61)	1.53 (0.75–3.12)	1.58 (0.76–3.29)
Fruit			
Recommended	1.00 (ref)		
Low	0.89 (0.34–2.31)	0.86 (0.32–2.29)	0.92 (0.34–2.48)
Diary			
Recommended	1.00 (ref)		
Low	2.33 (0.81–6.71)	2.50 (0.84–7.47)	2.53 (0.83–7.75)
Red meat and poultry			
Recommended	1.00 (ref)		
Low	1.09 (0.57–2.10)	0.95 (0.48–1.87)	0.90 (0.45–1.79)
Excess	0.74 (0.48–1.13)	0.74 (0.47–1.15)	0.72 (0.45–1.14)
Soybean			
Recommended	1.00 (ref)		
Low	0.69 (0.46–1.03)	0.60 (0.39–0.92) *	0.61 (0.39–0.94) *
Fish			
Recommended	1.00 (ref)		
Low	1.20 (0.43–3.35)	1.65 (0.57–4.75)	1.41 (0.48–4.12)
Egg			
Recommended	1.00 (ref)		
Low	1.02 (0.64–1.62)	0.89 (0.55–1.45)	0.94 (0.57–1.55)
Excess	2.24 (1.31–3.85) **	1.30 (0.69–2.44)	1.28 (0.67–2.45)

(Continued)

Table 4 (Continued).

	Model 1	Model 2	Model 3
Alcoholic beverage			
Recommended	1.00 (ref)		
Excess	3.13 (0.54–18.12)	3.61 (0.59–22.15)	2.96 (0.44–19.88)

Notes: The outcome variable is gut microbiota grouping based on the median relative abundance of Roseburia. Data are shown as OR (95% CI). * $P < 0.05$, ** $P < 0.01$. Model 1: not adjusted for covariates; Model 2: adjusted for age, BMI, education level; Model 3: further adjusted for smoking, alcohol drinking, tea drinking, exercise, hypertension and sleep disorder.

Abbreviations: DBI_LBS, dietary imbalance-low (insufficiency of intake) balance score; DBI_HBS, dietary imbalance-high (excessive intake) balance score.

source for colonocytes and supports gut barrier integrity.⁴² Secondly, Roseburia contributes to immune homeostasis by modulating inflammation.¹⁷ Its metabolites promote IL-10 and IL-22 production and regulatory T cell differentiation,^{43,44} while suppressing pro-inflammatory cytokines via inhibition of the NF- κ B pathway.⁴⁵ Disruption of this balance can shift the immune landscape toward a chronic pro-inflammatory state, thereby exacerbating insulin resistance.⁴⁶ Third, Roseburia spp. produce a range of bioactive compounds, including AI-2-like molecules involved in quorum sensing,⁴⁷ nuclease-like proteins capable of degrading extracellular DNA or RNA via reactive oxygen species,⁴⁸ and bacteriocin-like substances with antimicrobial activity.⁴⁹ These functional products may contribute to microbial community stability, host–microbe communication, and maintenance of gut ecological balance.

We did not observe significant associations between the overall DBI summary scores (DBI_LBS and DBI_HBS) and Roseburia abundance. One possible explanation is that the DBI_LBS and DBI_HBS includes multiple food-group components and may therefore obscure food-group-specific associations with microbial composition. Notably, at the food group-specific level, lower soybean intake was associated with lower Roseburia abundance, independent of age, BMI, education, and lifestyle factors. This finding is consistent with previous studies demonstrating a positive correlation between legume intake and the abundance of SCFA-producing bacteria, particularly Roseburia.^{33,50} There are several potential mechanistic explanations for this. First, soybeans are rich in dietary fiber and non-digestible oligosaccharides, such as raffinose and stachyose, which serve as preferential substrates for SCFA-producing bacteria like Roseburia.⁵⁰ These fermentable components support the microbial production of butyrate, thereby creating a gut environment conducive to the proliferation of butyrate-producing taxa.^{51,52} Second, soy-derived bioactive compounds, including isoflavones,⁵³ modulate gut microbial composition by stimulating beneficial taxa and suppressing pro-inflammatory bacteria, thereby contributing to a more favorable microbial configuration.⁵⁴ Third, the effects of fiber fermentation may enhance gut barrier integrity¹⁴ and immune homeostasis⁵⁵—conditions under which Roseburia is more likely to thrive⁵⁶—thereby reinforcing the link between habitual soybean intake and the maintenance of beneficial microbial populations.

This study has several notable strengths. First, it integrates both cross-sectional and prospective nested case–control analyses, allowing for a comprehensive evaluation of associations with both prevalent and incident type 2 diabetes. Second, the investigation was conducted within a well-characterized, community-based cohort of older Chinese adults, providing a robust population framework and enabling reliable identification of incident type 2 diabetes over a three-year follow-up period. Third, the use of both Metastats and MaAsLin ensured robust detection of differentially abundant taxa, while conditional logistic regression in the matched case–control framework effectively controlled for key demographic confounders. Furthermore, by integrating detailed dietary data with microbiome profiles, the study was able to explore potential diet–microbiota–diabetes pathways, offering valuable insights for developing microbiome-informed nutritional interventions in older populations.

Despite its strengths, this study has several limitations. First, the dietary intake data were based on self-reported information, which may introduce misclassification or recall bias. Second, matching on region and sex may lead to overmatching, thereby diminishing the ability to detect true associations. Third, 16S rRNA sequencing provides taxonomic resolution primarily at the genus or OTU level, which hinders the identification of microbes at finer taxonomic

levels, such as species. Last, given the small number of incident cases, the study may have been underpowered to detect weaker associations, and additional diabetes-related taxa may have remained undetected. Although our findings suggest a link between lower Roseburia abundance and an increased risk of diabetes, the strength and generalizability of the conclusions should be further validated by large-scale trial studies, due to the study's limited incident cases and the observational design.

Conclusion

This study demonstrated a significant association between the relative abundance of Roseburia and both prevalent and incident type 2 diabetes in an older adult Chinese cohort, and low soybean intake was independently associated with reduced Roseburia abundance, suggesting that increased legume consumption or Roseburia-riched supplement could be recommended by the general practitioners to prevent type 2 diabetes if these findings are validated. Future studies incorporating multi-omics are needed to precisely define the microbial taxa at the species level and the causal pathways linking diet, gut microbiota and type 2 diabetes.

Abbreviations

BMI, Body Mass Index; CTAB, Cetyltrimethylammonium Bromide; DBI, Dietary Balance Index; FDR, False Discovery Rate; FFQ, Food Frequency Questionnaire; FPG, Fasting Plasma Glucose; MaAsLin, Multivariate Association with Linear Models; OTUs, Operational taxonomic units; SCFAs, Short-Chain Fatty Acids; SDS, Sodium Dodecyl Sulfate; SUM-DCI, Surveillance and Management of Disability and Cognitive Impairment.

Data Sharing Statement

Data supporting the results from this study are available from the corresponding author on reasonable request.

Ethics Approval

This study was approved by the Institutional Review Board of the Chinese Center for Disease Control and Prevention (Approval No. 201830), with ethical clearance granted on November 29, 2018. The study was conducted in accordance with the Declaration of Helsinki.

Acknowledgments

We would like to express our gratitude to all field survey staff of SUM-DCI.

Author Contributions

YL: Methodology; Data curation; Formal analysis; Visualization; Writing – original draft.

BX: Investigation; Writing – review & editing.

VBK: Validation; Writing – review & editing.

HZ: Investigation; Writing – review & editing.

XG: Investigation; Writing – review & editing.

XQ: Methodology; Writing – review & editing.

ZY: Conceptualization; Investigation; Methodology; Validation; Supervision; Writing – review and editing
Supervision.

All authors gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Funding

National Key R&D Program of China (2020YFC2003000).

Disclosure

The author(s) report no conflicts of interest in this work.

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