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ORIGINAL RESEARCH

Associations of Dietary Live Microbes Intake and Prevalence of Prediabetes in US Adults: A Cross-Sectional Analysis

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Objective: A higher dietary intake of live microbes has been shown to be associated with a range of health benefits. We aimed to elucidate the associations between dietary intake of live microbes and the risk of prediabetes.

Methods: Adult participants from the 1999–2018 US National Health and Nutrition Examination Survey were included and categorized into the low, medium, and high live microbe intake groups based on the Sanders classification system. Associations between dietary consumption of live microbes and prevalence of prediabetes were explored using univariate and multivariate logistic regression, stratified analysis, and sensitivity analysis.

Results: Among the 28201 participants (mean age 45.83 years, 48.40% men, 32.78% with prediabetes) included, 9761 (31.80%), 12,076 (41.42%) and 6364 (26.78%) were classified into the low, medium, and high dietary live microbe intake groups, respectively. After adjusting for all potential covariates, the odds ratios and 95% confidence intervals for the medium and high dietary live microbe intake groups were 0.868 (0.803–0.937) and 0.891 (0.807–0.983), respectively (P for trend = 0.017), with the low dietary live microbes intake group as the reference. This association is robust and not affected by participant's age, sex, race, poverty–income ratio, education level, hypertension status and estimated glomerular filtration rate.

Conclusion: A higher consumption of dietary live microbes was found to be cross-sectionally linked to a lower prevalence of prediabetes in US adults.

Keywords: cross-sectional, live microbes, NHANES, prediabetes

Introduction

Prediabetes is considered to be an intermediary state of impaired glucose metabolism at risk of progressing to overt type 2 diabetes mellitus. The global prevalence of prediabetes was 9.1% and 5.8% in year 2021, which were expected to rise to 10.0% and 6.5% in 2045 based on criteria of impaired glucose tolerance and impaired fasting glucose, respectively, in people aged 20 to 79 years.[1] Currently, various criteria have been proposed for the diagnosis of prediabetes. Specifically, the World Health Organization proposed a fasting blood glucose of 6.1–6.9 mmol/L or a 2-hour post-load plasma glucose of 7.8–11.0 as criteria for prediabetes, while the International Expert Committee also advocated a glycated hemoglobin HbA1C of 6.0–6.4%.[2] The criteria proposed by the American Diabetes Association, on the other hand, were less stringent, with a fasting blood glucose of 5.6–6.9 mmol/L or a 2-hour post-load plasma glucose of 7.8–11.0 or a glycated hemoglobin HbA1C of 5.7–6.4% as fulfilling prediabetes.[2] Prediabetes represents an important public health concern that is both preventable and reversible. Early intervention has been shown to hold the promise of reversing prediabetes to normoglycemia.[3]

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Although the exact etiopathogenesis of prediabetes remains largely unknown, mounting evidence suggests that the gut microbiota may be implicated in the pathogenesis of prediabetes.[4] Contemporary researches revealed that, in addition to its function in digestion and nutrient absorption, the gut flora is also involved in the onset of various medical conditions. For instance, a large population-based study found that the insulin resistance in prediabetes and diabetes were predominantly responsible for the microbial variations observed.[5] Moreover, alterations in the composition or density of the gut microbiota, especially a reduction in the population of butyrate-producing bacteria, have been documented to promote impaired glucose tolerance and reduce insulin sensitivity, leading to prediabetes and subsequently overt type 2 diabetes.[6] A previous comparative study revealed that prediabetes is characterized by a significantly higher abundance of the genus from the family Pseudonocardiaceae as compared to healthy controls and type 2 diabetes.[7] Another study found a decreased abundance of the genus *Clostridium* and the mucin-degrading bacterium *A. muciniphila* in patients with prediabetes.[8] In light of these findings, intervention measures targeting the gut microbiota may represent a promising strategy for ameliorating prediabetes.

Dietary intervention represents a simple method for supplementing live microorganisms. Available studies in the literature have reported that dietary live microbes intake were beneficial for a variety of health conditions, such as depression, obesity, kidney stone, and even a lower mortality risk.[9–12] Thus, dietary live microbes may serve as an effective and convenient intervention strategy for prediabetes management. The relationships between overall dietary intake of live microbes and risk of prediabetes has not been fully elucidated. We hypothesized that higher intakes of dietary live microbes would be associated with a lower risk of prediabetes. To bridge this gap, the present study aimed to assess the associations between dietary intake of live microbes as assessed by the Sanders' method and the prevalence of prediabetes in US adults.

Methods

Study Participants

The participants of the current study were derived from the ten cycles (1999–2018) of the US National Health and Nutrition Examination Survey (NHANES), which is a nationwide cross-sectional survey designed to obtain data on the health and nutritional status of non-institutionalized US citizens. The detailed operational procedure has been previously outlined, and the health-related data are publicly and freely available at https://wwwn.cdc.gov/nchs/nhanes/Default.aspx. The cross-sectional design of NHANES could only be used to determine associations, not to establish causality between dietary intake of live microbes and risk of prediabetes. The NHANES protocol was approved by the NCHS Research Ethics Review Board, with informed consent obtained from all adult participants. According to the Item 1 and 2 of Article 32 of the Measures for Ethical Review of Life Science and Medical Research Involving Human Subjects dated February 18, 2023, China, this study is exempted from ethical approval from our institution since it used anonymized and publicly available data without incurring any potential dangers to the participants.

We restricted our study sample to adult participants with available data on glycemic status and dietary intake of live microbes. The exclusion criteria were participant age less than 20 years (n = 16,361), pregnancy (n = 1438), missing dietary intake of live microbes (n = 6988), and missing data of covariates (n = 7419). As presented in Figure 1, 28201 participants were eligible for inclusion into the final analysis.

Primary Exposure

Dietary live microbes intake, as assessed by the Sanders' method proposed by Marco et al, serves as the primary exposure of this study.[13] Dietary information was obtained from the first face-to-face 24-hour dietary recall. Four experts in the Marco's group evaluated the content of live microbes per gram for each of the 9388 food codes across 48 subgroups included in the NHANES dataset. In brief, live microbe level was broadly categorized as low ($<10^4$ colony-forming units per gram), medium (10^4–10^7 colony-forming units per gram), or high ($>10^7$ colony-forming units per gram), respectively, according to published references, expert opinions and also in consideration of the techniques of food processing. Any discrepancies were resolved through discussion within the team and consultation with expert microbiologist Fred Breidt from the US Department of Agriculture. This method has been used extensively in prior

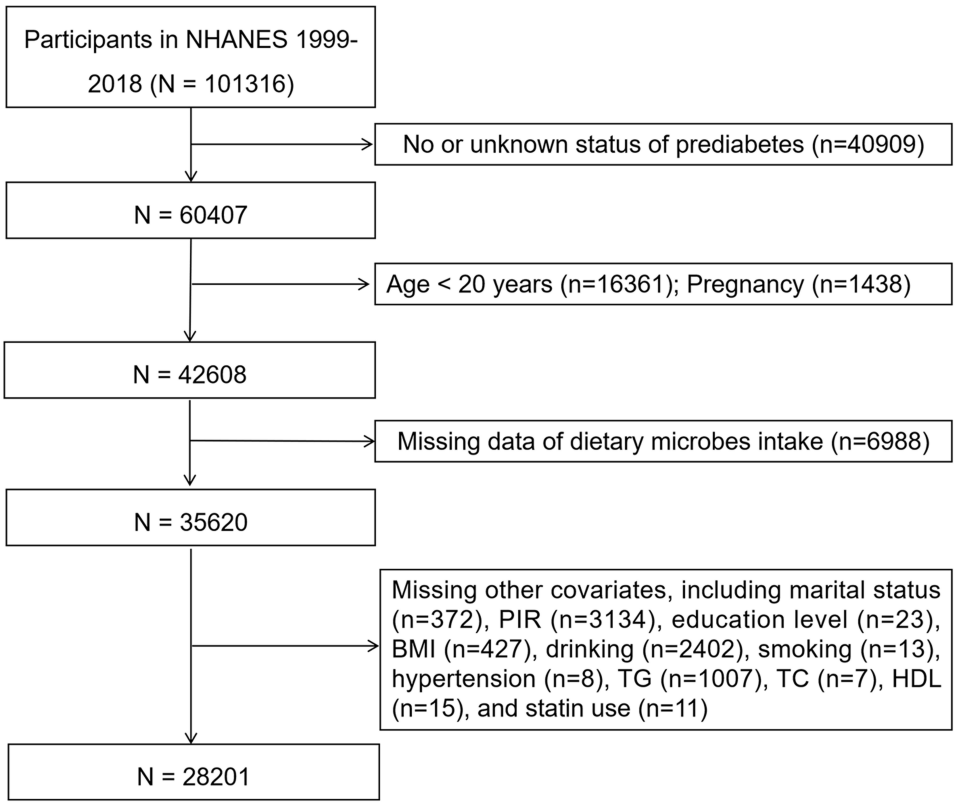

Figure 1 Flowchart of the inclusion and exclusion process of the participants.
Abbreviations: BMI, body mass index; HDL, high-density lipoprotein cholesterol; NHANES, national health and nutrition examination survey; PIR, poverty–income ratio; TC, total cholesterol; TG, triglyceride.

studies to estimate the relative abundance of live microbes.[9–12] Participants were grouped into the low, medium and high dietary live microbe intake group, as previously reported.[14]

Primary Outcome

Prediabetes status is the primary outcome of this study, defined as self-report, or 5.6 mmol/L ≤ fasting blood glucose < 7.0 mmol/L, or 5.7% ≤ HbA_{1C} < 6.5%, or a 2-hour post-load oral glucose tolerance test blood glucose of 7.8–11.0 mg/dL.[15]

Assessment of Covariates

Variables with the potential to be associated with dietary live microbes and prediabetes were collected and included. Specifically, the following parameters were included in the analysis: participant's age, sex, race/ethnicity, education level, poverty–income ratio, marital status, body mass index, alcohol consumption, cigarette use, hypertension, history of cardiovascular disease, physical activity, statin use, total dietary energy intake, and laboratory tests of triglyceride, total cholesterol, high-density lipoprotein cholesterol, uric acid, and estimated glomerular filtration rate. The definitions and categorization criteria for hypertension, diabetes, smoking, drinking, and physical activity were the same as reported previously.[16,17] The calculation of the estimated glomerular filtration was based on the 2009 serum creatinine-based CKD-EPI formula.[18] Physical activity included both work and leisure activities in a typical week that lasted at least 10 consecutive minutes and was categorized as none, moderate (requiring moderate physical effort and causing small increases in breathing or heart rate), and vigorous (requiring heavy physical effort and causing large increases in breathing or heart rate). Alcohol use was also categorized into never (<12 drinks in lifetime), former drinkers (≥12 drinks in lifetime but not in the last year), mild (≤1 drink per day for females or ≤2 drinks per day for males, on average over the past year), moderate (≤2 drinks per day for females, ≤3 drinks per day for males, or binge drinking on 2–5 days per month), and heavy (≥3 drinks per day for females, ≥4 drinks per day for males, or binge drinking ≥5 days per month).

Statistical Analysis

Statistical analysis was performed in accordance with the NHANES analytic recommendations, with appropriate weighting employed to obtain nationally representative estimates. The participants' characteristics were expressed as mean ± standard error or counts (weighted percentages), as appropriate, and compared weighted one-way analysis of variance or chi-squared test, respectively. The relationships between dietary intake of live microbes and the prevalence of prediabetes were explored using the logistic regression under 4 models in total. The Model 1 was unadjusted, while the Model 2 was adjusted for participant's age, sex, race, poverty–income ratio, marital status, and education level. The Model 3 was adjusted for Model 2 plus body mass index, smoking, alcohol consumption, hypertension status, physical activity, and history of cardiovascular disease. The Model 4 was further adjusted for triglyceride, total cholesterol, high-density lipoprotein cholesterol, uric acid, estimated glomerular filtration rate and dietary total energy intake. Stratified regression analyses were employed to account for differences as a function of participant's age, sex, race, poverty–income ratio, education level, hypertension status and estimated glomerular filtration rate. A two-side P value <0.05 was considered statistical significance.

Results

Baseline Characteristics of Study Participants

Table 1 presents the comparison of baseline characteristics of the study participants stratified by dietary live microbe intake. Among the 28201 participants (mean age 45.83 years, 48.40% men) included, 9761 (31.80%), 12,076 (41.42%), and 6364 (26.78%) were classified into the low, medium, and high dietary live microbe intake groups, respectively. Compared to the low dietary live microbe intake group, the high dietary live microbe intake group were significantly older, more likely to be women and non-Hispanic white, less likely to be single, more educated with higher poverty–income ratio, associated with lower body mass index, less likely to be current smokers and heavy drinkers, less likely to have hypertension and cardiovascular diseases, and less likely to be physically inactive. In terms of laboratory findings, the high dietary live microbe intake group were associated with significantly lower uric acid, triglyceride, estimated glomerular filtration rate, and more likely to have higher total cholesterol, high-density lipoprotein cholesterol, and statin

Table 1 Comparison of Participant's Characteristics Based Upon Different Dietary Consumption of Live Microbes

	Total (n=28201)	Low (n=9761)	Medium (n=12076)	High (n=6364)	P value
N (weighted)	**132,732,719**	**42,208,316**	**54,979,993**	**35,544,410**	
Age, years	45.83 ± 0.21	43.89 ± 0.25	47.32 ± 0.27	45.82 ± 0.30	**< 0.001**
Sex (n, %)					**< 0.001**
Male	13900 (48.40)	5185 (52.98)	5875 (47.36)	2840 (44.57)	
Female	14301 (51.60)	4576 (47.02)	6201 (52.64)	3524 (55.43)	
Race/Ethnicity (n, %)					**< 0.001**
Non-Hispanic White	14101 (72.90)	4308 (67.06)	6030 (72.65)	3763 (80.21)	
Non-Hispanic Black	5261 (9.28)	2614 (14.32)	1920 (8.15)	727 (5.04)	
Mexican American	4556 (7.18)	1355 (6.84)	2342 (8.62)	859 (5.34)	
Other Hispanic	2074 (4.99)	732 (5.71)	861 (4.82)	481 (4.38)	
Other race	2209 (5.66)	752 (6.07)	923 (5.75)	534 (5.03)	
Marital status (n, %)					**< 0.001**
Non-single	17365 (65.32)	5516 (60.34)	7707 (66.98)	4142 (68.69)	
Single	10836 (34.68)	4245 (39.66)	4369 (33.02)	2222 (31.31)	

(*Continued*)

Table 1 (Continued).

	Total (n=28201)	Low (n=9761)	Medium (n=12076)	High (n=6364)	P value
N (weighted)	**132,732,719**	**42,208,316**	**54,979,993**	**35,544,410**	
Education (n, %)					**< 0.001**
< High school	2516 (4.20)	988 (5.44)	1198 (4.61)	330 (2.08)	
High school	10323 (33.58)	4257 (42.34)	4271 (32.18)	1795 (25.36)	
> High school	15362 (62.22)	4516 (52.22)	6607 (63.21)	4239 (72.56)	
Poverty-income ratio	3.12 ± 0.03	2.73 ± 0.04	3.19 ± 0.03	3.49 ± 0.04	**< 0.001**
BMI (kg/m^2)	28.25 ± 0.07	28.78 ± 0.10	28.09 ± 0.08	27.85 ± 0.10	**< 0.001**
Drinking (n, %)					**< 0.001**
Never	3600 (10.27)	1297 (10.86)	1621 (11.03)	682 (8.41)	
Former	4414 (13.17)	1758 (15.43)	1833 (12.69)	823 (11.21)	
Mild	9780 (37.01)	2949 (31.59)	4329 (38.36)	2502 (41.34)	
Moderate	4569 (18.08)	1552 (17.28)	1871 (17.55)	1146 (19.86)	
Heavy	5838 (21.47)	2205 (24.83)	2422 (20.38)	1211 (19.19)	
Smoking (n, %)					**< 0.001**
Never	15357 (54.47)	4903 (49.19)	6749 (55.64)	3705 (58.93)	
Former	6818 (24.29)	2098 (21.26)	3131 (26.11)	1589 (25.07)	
Current	6026 (21.24)	2760 (29.55)	2196 (18.25)	1070 (16.00)	
Hypertension (n, %)	10,407 (32.68)	3713 (33.41)	4589 (33.83)	2105 (30.02)	**< 0.001**
CVD (n, %)	2345 (6.47)	894 (7.05)	1041 (6.85)	410 (5.19)	**< 0.001**
Physical activity (n, %)					**< 0.001**
None	13788 (43.90)	5038 (46.34)	5786 (42.85)	2964 (42.63)	
Moderate	7105 (27.10)	2251 (24.97)	3159 (28.13)	1695 (28.05)	
Vigorous	7305 (29.00)	2470 (28.69)	3130 (29.02)	1705 (29.32)	
Energy intake, kcal/day	2166.62 ± 7.11	2101.79 ± 11.48	2170.30 ± 9.39	2237.92 ± 13.80	**< 0.001**
Uric acid, μmol/L	320.12 ± 0.72	326.60 ± 1.15	319.92 ± 1.14	312.72 ± 1.24	**< 0.001**
Triglyceride, mmol/L	1.62 ± 0.01	1.67 ± 0.02	1.62 ± 0.02	1.54 ± 0.02	**< 0.001**
TC, mmol/L	5.12 ± 0.01	5.08 ± 0.02	5.14 ± 0.01	5.13 ± 0.02	**< 0.001**
HDL, mmol/L	1.39 ± 0.01	1.33 ± 0.01	1.41 ± 0.01	1.45 ± 0.01	**< 0.001**
eGFR, mL/min/1.73m^2	94.77 ± 0.28	96.67 ± 0.32	93.50 ± 0.34	94.50 ± 0.41	**< 0.001**
Statin use (n, %)	3422 (11.25)	1047 (9.50)	1609 (12.32)	766 (11.66)	**< 0.001**
Prediabetes (n, %)	10,549 (32.78)	3805 (33.97)	4588 (33.04)	2156 (30.96)	**<0.001**

Notes: P denotes comparison between the low-, medium-, and high dietary live microbe intake group. P values that are statistically significant are highlighted in bold.

Abbreviations: BMI, body mass index; CVD, cardiovascular disease; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein cholesterol; TC, total cholesterol.

use, in comparison to the low dietary live microbe intake group. Notably, the high dietary live microbe intake group exhibited the lowest prevalence of prediabetes at 30.96%, followed by the medium intake group at 33.04%, in comparison to the highest prevalence of prediabetes of 33.97% in the low dietary microbe intake group.

Association Between Dietary Live Microbe Intake and Prediabetes Risk

Table 2 shows the associations between dietary live microbe intake and the likelihood of prediabetes with and without covariate adjustment. In the crude analysis, medium and high dietary live microbe intake was associated with 17.4% and 20.6% decreased risk of prediabetes, respectively, as compared to the low dietary live microbe intake group. After adjustment for all covariates, the Model 4 showed the odds ratios and 95% confidence intervals for the medium and high dietary live microbe intake were 0.868 (0.803–0.937) and 0.891 (0.807–0.983), respectively (P for trend = 0.017), with the low dietary live microbe intake group as the reference.

Subgroup Analysis

The stratified analysis, shown in Table 3, indicated that participant's age, sex, race, poverty–income ratio, education level, hypertension status and estimated glomerular filtration rate did not significantly modify the relationship between dietary microbes intake and risk of prediabetes.

Sensitivity Analysis

We performed the sensitivity analysis by also incorporating prebiotic or probiotic intake from the supplement. There were 496 and 409 participants who took prebiotics and probiotics, respectively, and 14060 and 14147 participants who did not take prebiotics and probiotics, respectively. Similar to the main results, sensitivity analysis after adjusting for all covariates also showed decreased risk for prediabetes in participants with higher dietary live microbes intake (Table 4).

Discussion

To the best of our knowledge, this study represents the inaugural investigation into the relationship between dietary intake of live microbes and the prevalence of prediabetes in a nationally representative sample. The results showed that a higher dietary intake of live microbes was associated with a reduced prevalence of prediabetes in US adults even after adjusting for a range of confounding variables. In addition, this association was found to be robust and did not appear to be modified by participant's age, sex, race, poverty–income ratio, education level, hypertension status, or kidney function. The preliminary results of this study imply that a diet rich in live microbes may potentially serve as a protective factor against the development of prediabetes.

Indeed, compositional shifts of the gut microbiota in individuals with type 2 diabetes have been extensively characterized previously, with relatively less attention devoted to prediabetes. Chang et al identified a reduction in the abundance of nine bacterial genera and an increase in the abundance of 14 in individuals with prediabetes in comparison to healthy controls.[19] A recent comprehensive review indicated a decreased abundance of genus *Clostridium*,

Table 2 Associations Between Dietary Live Microbes Intake and Prevalence of Prediabetes

Dietary live microbes intake	Model 1		Model 2		Model 3		Model 4	
	OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value
Low	1.000 (Reference)	/	1.000 (Reference)	/	1.000 (Reference)	/	1.000 (Reference)	/
Medium	0.826 (0.767–0.889)	**<0.001**	0.864 (0.800–0.933)	**<0.001**	0.875 (0.810–0.946)	**<0.001**	0.868 (0.803–0.937)	**<0.001**
High	0.794 (0.722–0.873)	**<0.001**	0.887 (0.805–0.977)	**0.016**	0.903 (0.818–0.998)	**0.046**	0.891 (0.807–0.983)	**0.022**
P for trend	**<0.001**		**0.012**		**0.036**		**0.017**	

Notes: Model 1 was crude analysis without adjustment; Model 2 was adjusted for participant's age, sex, race, poverty–income ratio, marital status, and education level. The Model 3 was adjusted for Model 2 plus body mass index, smoking, alcohol consumption, hypertension status, physical activity, and history of cardiovascular disease. The Model 4 was further adjusted for triglyceride, total cholesterol, high-density lipoprotein cholesterol, uric acid, estimated glomerular filtration rate and dietary total energy intake. P values that are statistically significant are highlighted in bold.
Abbreviations: CI, confidence interval; OR, odds ratio.

Table 3 Subgroup Analysis for the Associations Between Dietary Live Microbes Intake and Prevalence of Prediabetes

Dietary live microbes intake	Low	Medium	P	High	P	P for trend	P for interaction
Age, years							0.661
< 45	Reference	0.866 (0.764–0.981)	0.024	0.934 (0.814–1.070)	0.322	0.237	
≥ 45, < 60	Reference	0.834 (0.725–0.960)	0.012	0.858 (0.705–1.045)	0.127	0.113	
≥ 60	Reference	0.931 (0.803–1.080)	0.341	0.872 (0.713–1.066)	0.179	0.180	
Sex							0.694
Male	Reference	0.884 (0.787–0.993)	0.038	0.949 (0.825–1.091)	0.457	0.348	
Female	Reference	0.891 (0.802–0.990)	0.033	0.901 (0.789–1.030)	0.125	0.130	
Race							0.459
Non-Hispanic White	Reference	0.849 (0.765–0.943)	0.002	0.882 (0.782–0.996)	0.042	0.048	
Non-Hispanic Black	Reference	0.833 (0.715–0.972)	0.021	1.032 (0.851–1.252)	0.745	0.511	
Mexican American	Reference	0.996 (0.842–1.179)	0.963	0.929 (0.738–1.169)	0.524	0.561	
Other Hispanic	Reference	1.012 (0.738–1.387)	0.941	0.835 (0.597–1.167)	0.286	0.331	
Other race	Reference	0.886 (0.668–1.176)	0.399	0.858 (0.638–1.154)	0.308	0.286	
Poverty-income ratio							0.565
< 1.0	Reference	1.002 (0.827–1.215)	0.980	0.991 (0.794–1.236)	0.935	0.954	
1.0–3.0	Reference	0.829 (0.735–0.936)	0.003	0.884 (0.772–1.011)	0.072	0.027	
> 3.0	Reference	0.867 (0.761–0.988)	0.032	0.876 (0.746–1.030)	0.108	0.132	
Education							0.296
< High school	Reference	0.996 (0.794–1.248)	0.969	0.723 (0.507–1.032)	0.074	0.158	
High school	Reference	0.917 (0.808–1.040)	0.177	0.915 (0.780–1.074)	0.274	0.201	
> High school	Reference	0.822 (0.735–0.920)	<0.001	0.870 (0.768–0.986)	0.029	0.045	
Hypertension							0.592
No	Reference	0.885(0.791,0.991)	0.034	0.927 (0.792–1.085)	0.342	0.283	
Yes	Reference	0.863(0.778,0.956)	0.005	0.869 (0.772–0.978)	0.02	0.019	
eGFR, mL/min/1.73m^2							0.214
< 30	Reference	0.403(0.145, 1.121)	0.079	0.322 (0.078–1.325)	0.111	0.302	
≥ 30, < 60	Reference	1.153(0.861,1.545)	0.337	1.055 (0.736–1.513)	0.77	0.735	
≥ 60	Reference	0.847(0.781,0.919)	<0.001	0.867 (0.785–0.957)	0.005	0.004	

A. Muciniphila, phylum Firmicutes, *Lactobacillus plantarum* HAC01, *Faecalibacterium prausnitzii*, and an increased abundance of *Ruminococcus*, *Streptococcus*, *Chloracidobacterium* and *Pseudonocardiaceae* in patients with prediabetes as compared with normoglycemic individuals.[20] In addition to these cross-sectional observations, Takeuchi's group provided evidence of a causal relationship between specific gut bacteria and insulin resistance, which is an early feature of prediabetes.[21]

The human diet provides a rich and convenient source of living microorganisms that have the potential to significantly influence the composition, function and diversity of gut microbiome. The content of bacteria, yeasts and other fungi in the diet varies significantly according to the specific food types, preparation and processing techniques, and duration of

Table 4 Sensitivity Analysis for the Associations Between Dietary Live Microbes Intake and Prevalence of Prediabetes

Dietary live microbes intake	Model 1		Model 2		Model 3		Model 4	
	OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value
Low	1.000 (Reference)	/	1.000 (Reference)	/	1.000 (Reference)	/	1.000 (Reference)	/
Medium	0.793 (0.711–0.883)	**<0.001**	0.832 (0.746–0.929)	**0.001**	0.849 (0.759–0.949)	**0.004**	0.843 (0.755–0.942)	**0.003**
High	0.751 (0.662–0.852)	**<0.001**	0.825 (0.726–0.937)	**0.003**	0.849 (0.745–0.969)	**0.015**	0.837 (0.735–0.953)	**0.008**
P for trend	**<0.001**		**0.004**		**0.016**		**0.009**	

Notes: Model 1 was crude analysis without adjustment; Model 2 was adjusted for participant's age, sex, race, poverty–income ratio, marital status, and education level. The Model 3 was adjusted for Model 2 plus body mass index, smoking, alcohol consumption, hypertension status, physical activity, and history of cardiovascular disease. The Model 4 was further adjusted for triglyceride, total cholesterol, high-density lipoprotein cholesterol, uric acid, estimated glomerular filtration rate, dietary total energy intake, prebiotics and probiotics. P values that are statistically significant are highlighted in bold.
Abbreviations: CI, confidence interval; OR, odds ratio.

food storage. Earlier studies have revealed associations between certain dietary patterns and microbial composition, highlighting that habitual intake plays a critical role in modulating the gut microbial profile. For instance, Wastyk's 17-week randomized trial showed that compared with a high-fiber diet, a fermented-food diet could increase microbiome diversity and decrease inflammatory markers.[22] Animal studies also showed that an energy-restricted diet including yogurt, fruits and vegetables could ameliorate metabolic syndrome by increasing the Akkermansia bacteria by 3.5-fold.[23] Although the exact mechanisms underlying the beneficial health effects of dietary live microbes have not been completely understood, previous studies employing Sander's classification method have also documented a range of positive health effects for conditions like frailty, depression, diabetic kidney disease, and chronic obstructive pulmonary disease.[24–27]

In light of the intimate association between gut flora and diabetes, there is a growing interest in the scientific community to manipulate gut microbiota for the prevention and treatment of prediabetes. We are aware that various techniques have been employed to manipulate gut microbiota for the amelioration of diabetes, such as fecal microbiota transplantation, increased dietary fiber intake, and probiotics supplementation.[28] Of note, the current study demonstrated that a high dietary intake of live microbes was more strongly associated with a reduced risk of prediabetes than a medium intake of live microbes, which is compatible with several prior reports but contradicts others. The report from Liu et al showed a negative correlation between dietary live microbes intake and risk of rheumatoid arthritis.[29] However, Tang et al and Han et al reported that diets with medium live microbes are more strongly associated with cognitive function and cardiovascular disease, respectively.[30,31]

Although the precise mechanisms underlying the beneficial effects of live microbes for prediabetes remain elusive, we hypothesize that the following mechanisms may be potentially involved in this association. First, dietary living microorganisms may reduce the concentrations of branched-chain amino acids, thereby alleviating insulin resistance.[32] The branched-chained amino acids have been found to promote insulin resistance by continuously activating mammalian rapamycin complex 1 via insulin receptor substrate 1 phosphorylation.[33] Second, the dietary live microbes could boost immune response and reduce inflammatory cytokine production, which in turn would decrease pancreatic β cell injury from external pathogenic microorganisms.[34] Ultimately, the protective effect of dietary live microbes for prediabetes may be related to its ability to reduce oxidative stress,[35] a critical pathophysiologic process involved in pancreatic β cell injury and exhaustion.

In the sensitivity analysis, we also excluded the confounding effects of probiotics and prebiotics, given that both have been shown to exert health effects. Prebiotics are selectively fermented ingredients degraded by gut microbiota to stimulate the growth and activity of beneficial bacteria in the gut. Probiotics, on the other hand, are living microorganisms, such as Lactobacillus, Lactococcus, Leuconostoc, and other typically found in kefir and yogurt. A recent meta-analysis of randomized controlled trials in prediabetes demonstrated that probiotics could decrease glycated hemoglobin and improve post-load glycemic levels.[36] Although the use of prebiotics did not appear to improve glycemic control, it has been shown to alter gut microbiota composition.[37] Therefore, the results of the sensitivity analysis provide further evidence in support of the hypothesis that dietary live microbes exert a beneficial effect on prediabetes.

Our study may have some practical implications for dietary interventions. The findings may potentially suggest that the amount of live microbes should also be incorporated into dietary recommendations to promote health. For instance, foods rich in live microorganisms, such as fruits, vegetables, yogurt, and other fermented foods, may be recommended for individuals at high risk of developing prediabetes. Future studies should apply alternative methods to accurately quantify dietary live microbes and prospectively determine their potential utility in preventing prediabetes.

The strength of the present study includes the use of nationally representative data, thereby enabling the external applicability of study results across broader contexts. Moreover, we also considered a range of sociodemographic characteristics, health-related variables, and prebiotics and probiotics that might introduce potential confounding influences. This study also suffers from several limitations that warrant further consideration. First, the cross-sectional design of NHANES made it difficult to establish a causal relationship between dietary intake of live microbes and prevalence of prediabetes. Second, although the Sanders' classification method has been successfully applied for the categorization of dietary microbial content, it is imprecise and subject to bias. Thus, methods for the precise calculation of dietary viable microorganisms and the optimal dose of live microorganisms needs to be further explored in the future. Third, the calculation of dietary live microorganisms is derived from self-report dietary intake, which would be subject to recall bias and may not be representative of habitual dietary habits. In addition, this study included individuals with self-reported prediabetes, which is another source of recall bias. Fourth, the participant selection and exclusion process may introduce selection bias and limit the generalizability of the study findings to a wider population. Finally, despite the adjustment for a range of potential confounding variables, the possibility of residual confounding factors, such as the use of antibiotics or other medications on gut microbiota composition, cannot be completely excluded.

Conclusion

In conclusion, this cross-sectional study based on national survey data showed a negative association between dietary live microbe intake and prevalence of prediabetes in US adults. The results suggest that dietary counseling to increase intake of foods rich in live microbes may be helpful in reducing the risk of prediabetes. Given the cross-sectional study design and the imprecision of the Sanders' classification system, prospective large-scale randomized clinical trials are needed in the future to definitively establish a causal relationship between dietary live microorganism consumption and risk of prediabetes.

Data Sharing Statement

This study was carried out using publicly available data from the US National Health and Nutrition Examination Survey at https://www.cdc.gov/nchs/nhanes/.

Ethics Approval and Consent to Participate

The NHANES protocol was approved by the NCHS Research Ethics Review Board, with informed consent obtained from all adult participants.

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Disclosure

Xiaoxu Ge, Juan Du and Jiajia Wang are co-first authors. The authors report no conflicts of interest in this work.

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