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Association of dietary live microbe intake with testosterone level in adult men: evidence from a national population-based study

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Testosterone deficiency (TD) is a prevalent disorder in men, associated with a range of health complications. Dietary live microbe intake has garnered attention for its potential to modulate gut microbiota and promote human health. This study aims to investigate the association between dietary live microbe intake and the risk of TD in a large, nationally representative sample. We analyzed data from the National Health and Nutrition Examination Survey (NHANES) for the years 2013–2016. Dietary live microbe intake was estimated using a 24-hour dietary recall, and participants were categorized into low, medium, and high intake groups. TD was defined as a serum testosterone level below 300 ng/dL. Multivariable weighted logistic regression models were used to assess the association between different dietary live microbe intake and TD risk, adjusting for potential confounders. A total of 4,034 male participants were included in the analysis. High dietary live microbe intake was associated with a significantly lower risk of TD in all models. In the fully adjusted model (Model 3), the odds ratio (OR) for TD in the high intake group was 0.71 (95% CI: 0.53 to 0.96; $p = 0.03$) compared to the low intake group. Subgroup analyses showed consistent results across different population characteristics, particularly among those without diabetes (OR: 0.64; 95% CI: 0.47 to 0.88; $p = 0.01$) and without cardiovascular disease (OR: 0.64; 95% CI: 0.47 to 0.88; $p = 0.02$). Restricted Cubic Spline analysis revealed a linear inverse relationship between food intake and TD risk in the medium and high intake groups. Our findings suggest that a higher intake of dietary live microbes is associated with a reduced risk of TD, highlighting the potential of dietary modifications as a preventive strategy for TD. Further research, including longitudinal studies and clinical trials, is necessary to confirm these results and explore the underlying mechanisms.

Keywords Dietary live microbe, Testosterone deficiency, Men's health, NHANES, Cross-sectional study

Testosterone, primarily produced by Leydig cells in the testes and, to a lesser extent, by the adrenal glands, is the key hormone regulating male physiology[1]. Low serum testosterone levels can lead to widespread organ dysfunction, manifesting as sexual issues like reduced libido and erectile dysfunction, reproductive challenges such as impaired spermatogenesis, and non-sexual problems including cognitive decline, cardiovascular impairment, depression, and osteoporosis[2–5]. Collectively, these symptoms, along with decreased serum testosterone levels, are recognized as testosterone deficiency (TD) syndrome or male hypogonadism[6,7]. Epidemiological studies have shown that TD predominantly affects middle-aged and older men, with a prevalence ranging from 2.2 to 5.1% in those aged 40–79 years[8]. With the anticipated increase in life expectancy over the next century, the disease burden associated with TD is expected to intensify, raising significant global public health concerns[3]. Therefore, identifying new strategies to prevent the onset and progression of TD is of paramount importance.

Dietary modifications are an easily adjustable factor that has garnered considerable attention in the context of testosterone regulation. Some studies have already demonstrated that certain dietary patterns and nutrients

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can influence the production of sex hormones[9,10]. For instance, Zhang et al. found that pro-inflammatory diets could increase the risk of TD[11]. However, these studies primarily focused on the role of food components while overlooking the potential impact of dietary live microbes. There is evidence suggesting that live microbes in the diet can influence the composition and diversity of the host's gut microbiota[12]. The gut microbiota interacts locally with the intestinal tract and communicates with distant organs like the testes[13]. Intriguingly, an animal study demonstrated that feeding elderly male mice with purified microbes could restore testosterone levels to the youthful level[14]. While direct gut microbiota transplantation in humans is still under development, the supplementation of dietary active microorganisms to improve health has already attracted significant attention, by modulating gut microbiota compositions and functions[15]. Recently, several studies have preliminarily explored the effects of dietary live microbe intake on human health, noting benefits such as improved mood, delayed aging, and reduced mortality[16–20]. The "Old Friends Hypothesis" offers an insightful perspective, suggesting that exposure to symbiotic or benign microbes in our diet is crucial for beneficial microbial stimulation of the immune system[21].

Despite these promising findings, no studies to date have explored the association between the intake of dietary live microbes and the risk of TD in men, which is the primary focus of our investigation. In this study, we utilize the extensive NHANES database, the largest of its kind, to objectively and systematically assess and categorize participants' intake of live microbes from their diet. Therefore, the objective of our research is to examine whether an increased intake of dietary live microbes can reduce the risk of TD in adult men, thereby promoting male health, reducing the disease burden, and offering a promising preventive measure against the progression of testosterone deficiency.

Materials and methods

Study design and population

The National Health and Nutrition Examination Survey (NHANES) is an ongoing project supervised by the U.S. Centers for Disease Control and Prevention (CDC). It aims to assess the health and nutritional status of the non-institutionalized population across the United States. NHANES plays a critical role in informing public health policies and adjustments. To ensure the diversity and representativeness of the sample, NHANES employs a complex, multistage probability sampling design. Data are collected through interviews, physical examinations, and laboratory tests, providing comprehensive information on participants, including demographic data, dietary intake, physical examinations, laboratory results, and questionnaire responses. All data are freely available to researchers worldwide through the NHANES database. The study design of NHANES has been reviewed and approved by the Research Ethics Review Board of the National Center for Health Statistics (NCHS), and all participants provided written informed consent before their inclusion in the study.

Given the availability of sex hormone data, participants were limited to those from the 2013–2014 and 2015–2016 NHANES cycles, totaling 20,146 individuals. The final analytical sample was restricted to adult males aged 20 years and older, with complete data on dietary live microbe intake, testosterone measurements, and potential covariates. A series of exclusions were applied to the initial sample. First, 10,251 females and 4,092 males under the age of 20 were excluded. Then, 407 participants lacking data on dietary live microbe intake and 541 participants without testosterone measurements were excluded. Additionally, 821 participants without complete covariate information were excluded. This left a final sample of 4,034 eligible participants for analysis, including 1,069 with a history of TD and 2,965 without. The detailed sample selecting process was shown in Fig. 1.

Definition of exposure variable: dietary live microbe intake

Dietary intake was estimated using a 24-hour dietary recall questionnaire conducted on the first day of the survey. Nutritional data from the United States Department of Agriculture (USDA) were utilized to accurately assess the energy and nutrient content of each food and beverage. The classification of live microbe levels in foods was derived from the research by Marco et al.[22]. To estimate the concentration of live microbes per gram of food, four experts in the field evaluated the 9,388 food codes contained in the 48 subgroups of the NHANES database. Based on this assessment, foods were categorized into three levels low, medium, or high, corresponding to microbial counts of $< 10^4$ CFU/g, 10^4–10^7 CFU/g, and $> 10^7$ CFU/g, respectively. These categories included pasteurized foods ($< 10^4$ CFU/g), unpeeled fresh fruits and vegetables (10^4–10^7 CFU/g), and unpasteurized fermented foods and microbe supplements ($> 10^7$ CFU/g)[22,23]. Next, we defined the overall dietary live microbe level. Based on microbial content, participants were divided into three groups: (1) low dietary live microbe intake group (consuming all food live microbes graded as low), (2) medium dietary live microbe intake group (consuming any foods categorized as medium levels of live microbe content, but not high), and (3) high dietary live microbe intake group (consuming any foods categorized as high levels of live microbe content)[24].

Definition of outcome variable: testosterone deficiency

After completing the dietary intake data collection, participants were asked to provide blood samples either on the same day or within a few days following the dietary interview. Considering the circadian rhythm of testosterone secretion, all participants were required to fast for 8.5 h and provide venous blood samples between 8:00 AM and 10:00 AM to minimize physiological variability. Testosterone levels were measured using isotope dilution liquid chromatography-tandem mass spectrometry (ID-LC-MS/MS), based on the National Institute for Standards and Technology's (NIST) reference method, with a lower limit of detection of 0.75 ng/dL. According to the guidelines of the Urological Association, TD was defined as a serum testosterone level below 300 ng/dL.

Selection for potential covariates

In light of previous research and clinical experience, we considered a comprehensive range of potential covariates that might influence the relationship between dietary live microbe intake and testosterone levels.

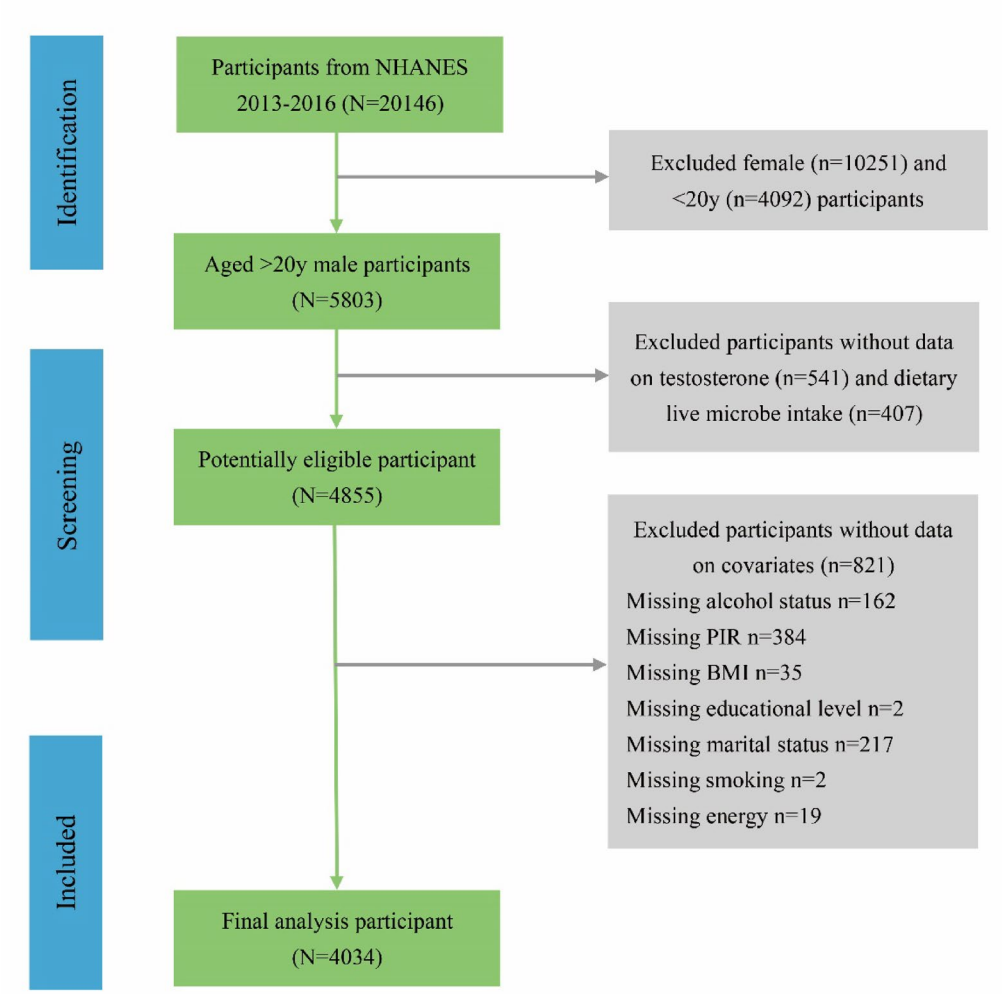

Fig. 1. Flowchart of participant selection from the NHANES 2013–2016 cycles. NHANES: National Health and Nutrition Examination Survey, PIR: Poverty Income Ratio, BMI: Body Mass Index.

These variables include demographic factors such as age, race, marital status, and educational level, as well as economic status assessed using the poverty income ratio (PIR). Additionally, we incorporated body mass index (BMI) from physical examination data as a key covariate. We also included participants' smoking and alcohol consumption habits, gathered through questionnaire data. Lastly, the health status regarding hypertension, diabetes, chronic kidney disease (CKD), and cardiovascular disease (CVD) was evaluated using a combination of objective examinations and questionnaire results. The grouping of demographic characteristics can be found in our Table 1. Smoking status was categorized based on whether participants had smoked ≥ 100 cigarettes in their lifetime and their current smoking status, dividing them into three groups: never (no to both), current (yes to both), and former (those who previously smoked but no longer do). Alcohol consumption was categorized as follows: (1) Never: Consumed fewer than 12 drinks in their lifetime; (2) Former: Consumed at least 12 drinks in one year but did not drink in the past year; (3) Mild: Consumed 1–2 drinks per week; (4) Moderate: Consumed 2–3 drinks per week, or 2–4 drinks per binge; (5) Heavy: Consumed 3–4 drinks per week, or 5 + drinks per binge[25].

For comorbid clinical conditions, both objective and subjective measures were used. Hypertension and diabetes were defined by a history of diagnosis and current use of corresponding medications. Objective criteria for hypertension were consecutive blood pressure measurements ≥ 140/90 mmHg. For diabetes, objective criteria included a fasting glucose level ≥ 126 mg/dL or a plasma glucose level ≥ 200 mg/dL at 2 h after an oral glucose tolerance test (OGTT). Participants with abnormal blood glucose levels not meeting the criteria for diabetes were classified as having prediabetes. CKD was evaluated based on a prior diagnosis or an estimated glomerular filtration rate (eGFR) of < 60 ml/min/1.73 m^2. CVD was entirely assessed through self-reported diagnoses, including a history of myocardial infarction, angina, coronary artery disease, or heart failure.

Statistical analysis

Given the sampling design and the need to ensure the representativeness of the results, appropriate sample weights were applied in all analyses. Participants were grouped based on the presence or absence of TD and categorized

Characteristics	Total participants	History of TD		*P* value
		No	Yes	
Participants number	4034	2965	1069	
Age, years	46.94 ± 0.36	45.95 ± 0.44	49.77 ± 0.76	< 0.001
BMI, kg/m^2	29.11 ± 0.15	27.92 ± 0.15	32.51 ± 0.35	< 0.0001
Energy, Kcal	2493.07 ± 20.02	2523.29 ± 26.82	2406.38 ± 30.30	0.01
Total testosterone, ng/dl	417.97 ± 4.16	484.39 ± 3.49	227.47 ± 2.08	< 0.0001
Age group, %				0.002
20-40y	37.37	39.80	30.40	
40-60y	37.06	36.16	39.63	
> 60y	25.57	24.04	29.97	
Race, %				0.05
Mexican American	9.02	8.86	9.48	
Non-Hispanic White	68.46	67.99	69.81	
Non-Hispanic Black	9.32	10.00	7.39	
Other Race	13.20	13.15	13.32	
Education, %				0.92
Less than high school	13.99	14.09	13.72	
High school	22.80	22.64	23.26	
More than high school	63.21	63.27	63.02	
BMI, %				< 0.0001
Normal (< 25 kg/m^2)	25.03	30.00	10.75	
Overweight (25–30 kg/m^2)	37.69	39.94	31.24	
Obese (> 30 kg/m^2)	37.28	30.05	58.00	
PIR, %				0.90
<1	12.87	12.83	13.00	
≥1	87.13	87.17	87.00	
Marital status, %				< 0.0001
Solitude	31.81	34.06	25.39	
Cohabitation	68.19	65.94	74.61	
Smoke, %				< 0.0001
Never	50.30	51.59	46.61	
Former	29.71	26.77	38.16	
Current	19.99	21.64	15.23	
Alcohol, %				0.002
Never	7.90	7.40	9.32	
Former	92.10	92.60	90.68	
Mild	41.31	41.59	40.51	
Moderate	12.67	13.01	11.71	
Heavy	23.91	25.32	19.87	
Hypertension, %				< 0.0001
No	60.82	64.33	50.74	
Yes	39.18	35.67	49.26	
Diabetes, %				< 0.0001
No	74.14	76.74	66.65	
Prediabetes	9.56	9.93	8.48	
Yes	16.31	13.32	24.87	
CKD, %				< 0.0001
No	86.48	88.73	82.73	
Yes	12.71	11.27	17.27	
CVD, %				< 0.0001
No	90.56	91.89	86.75	
Yes	9.44	8.11	13.25	
Dietary live microbe group, %				0.02
Intake of foods low in live microbes (< 10^4 CFU/g)	38.65	37.65	41.53	
Continued				

Characteristics	Total participants	History of TD		*P* value
		No	Yes	
Intake of foods Medium in live microbes (10^4–10^7 CFU/g)	32.82	31.99	35.21	
Intake of foods High in live microbes (> 10^7 CFU/g)	28.52	30.36	23.26	

Table 1. Baseline characteristics of the study population by testosterone deficiency status, weighted. Statistical methods: Continuous variables are presented as mean ± SE and were compared using weighted linear regression. Categorical variables are presented as percentages and were compared using weighted chi-square tests. A P-value of less than 0.05 was considered statistically significant. *TD* testosterone deficiency, *BMI* body mass index, *PIR* poverty income ratio, *CKD* chronic kidney disease, *CVD* cardiovascular disease, *SE* standard error.

into Low, Medium, and High dietary live microbe intake groups. For descriptive statistics, continuous variables were expressed as weighted means with standard errors, while categorical variables were presented as weighted percentages. Group comparisons were performed using weighted chi-square tests for categorical variables and weighted linear regression for continuous variables. Multivariable weighted regression analyses were conducted to explore the association between dietary live microbe intake and the risk of TD, with results expressed as odds ratios (OR) and 95% confidence intervals (CI). Three models were constructed: Model 1 included only the dietary live microbe intake categories; Model 2 was adjusted for age, race, marital status, education level, and poverty income ratio (PIR); and Model 3 further adjusted for BMI, total dietary energy intake, smoking status, alcohol consumption, and histories of hypertension, diabetes, CKD, and CVD. The Low dietary live microbe intake group served as the reference in all regression analyses, and trend tests were performed to assess dose-response relationships.

To evaluate the robustness of our findings, subgroup analyses and interaction tests were conducted across key demographic and clinical characteristics, including age, BMI, smoking status, hypertension, diabetes, and CKD. Finally, within each Low, Medium, and High dietary live microbe intake group, restricted cubic spline (RCS) analyses were performed in Model 3 to investigate the relationship between the quantity of food consumed (in grams) and the risk of TD. All statistical analyses were performed using R software (http://www.R-project.org, The R Foundation) and EmpowerStats (www.empowerstats.com; X&Y Solutions, Inc., Boston MA). Statistical significance was defined as a two-sided p-value of less than 0.05.

Results

Baseline characteristics of study population

The study population was first stratified based on the presence or absence of TD. Participants with TD had a mean age of 49.77 ± 0.76 years and a significantly lower mean testosterone level of 227.47 ± 2.08 ng/dL, which is markedly lower than that of the non-TD group. Additionally, the TD group exhibited higher BMI, as well as a greater prevalence of hypertension, diabetes, CKD, and CVD. Notably, the proportion of participants with high dietary live microbe intake in the TD group was significantly lower than in the non-TD group (23.26% vs. 30.36%). Detailed comparisons of these characteristics can be found in Table 1. Subsequently, the population was divided into groups based on low, medium, and high dietary live microbe intake. Compared to the low intake group, which had a TD prevalence of 27.78%, the high intake group demonstrated a lower TD prevalence of 21.08%. Other baseline characteristics also showed statistically significant differences across the groups, as detailed in Table 2.

Logistic associations between dietary live microbe intake and risk of TD

In the regression analysis, using the low dietary live microbe intake group as the reference, participants in the high intake group demonstrated a significantly reduced risk of TD in the unadjusted Model 1, with an OR (95% CI) of 0.69 (0.54, 0.90) and a trend test P-value of 0.016. When adjusting for additional covariates in Model 2, the high intake group continued to show a lower risk of TD, with an OR (95% CI) of 0.64 (0.50, 0.82) and a trend test P-value of 0.002. In the fully adjusted Model 3, the high intake group still exhibited a significantly reduced risk of TD, with an OR (95% CI) of 0.72 (0.52, 0.98) and a trend test P-value of 0.04. In summary, a significantly reduced risk of TD was observed in the high dietary live microbe intake group, as demonstrated across all models (Table 3).

Subgroup analysis and RCS analysis

To assess the robustness of our findings, we conducted subgroup analyses stratified by key demographic and clinical characteristics, including age, BMI, smoking status, hypertension, DM, and CKD. Across these subgroups, the association between high dietary live microbe intake and reduced risk of TD remained consistent, with no significant interactions detected. For participants without DM and without CVD, high dietary live microbe intake was associated with a lower risk of TD, with the OR (95% CI) for those without DM being 0.65 (0.47 to 0.90) and a P-value of 0.02, and for those without CKD, the OR (95% CI) was also 0.64 (0.46 to 0.89) with a P-value of 0.02. This suggests that the protective effect of high dietary live microbe intake on TD risk is generally applicable across different population subgroups. Detailed results of the subgroup analyses can be found in Table 4; Fig. 2.

Characteristics	Total participants	Dietary live microbe group			*P* value
		Low	Medium	High	
Participants number	4034	1691	1418	925	
Age, years	46.94 ± 0.36	45.40 ± 0.59	49.32 ± 0.64	46.29 ± 0.67	< 0.001
BMI, kg/m^2	29.11 ± 0.15	29.51 ± 0.33	29.06 ± 0.19	28.61 ± 0.26	0.17
Energy, Kcal	2493.07 ± 20.02	2393.90 ± 28.82	2493.25 ± 33.71	2627.24 ± 39.45	< 0.001
Dietary intake in the low group, g	-	3834.87 ± 83.57	3684.77 ± 63.65	3791.51 ± 72.25	0.24
Dietary intake in the medium group, g	-	-	175.60 ± 5.40	131.67 ± 7.34	< 0.0001
Dietary intake in the high group, g	-	-	-	73.82 ± 2.81	-
Total testosterone, ng/dl	417.97 ± 4.16	417.24 ± 6.65	412.35 ± 6.02	425.42 ± 6.98	0.32
Age group, %					0.01
20-40y	37.37	40.82	32.31	38.52	
40-60y	37.06	37.03	36.50	37.73	
> 60y	25.57	22.15	31.19	23.75	
Race, %					< 0.0001
Mexican American	9.02	8.77	12.41	5.45	
Non-Hispanic White	68.46	64.27	64.56	78.63	
Non-Hispanic Black	9.32	13.54	8.30	4.79	
Other Race	13.20	13.42	14.73	11.13	
Education, %					< 0.0001
Less than high school	13.99	18.86	14.33	7.01	
High school	22.80	28.05	20.83	17.95	
More than high school	63.21	53.09	64.84	75.04	
BMI, %					0.02
Normal (< 25 kg/m^2)	25.03	24.54	23.49	27.46	
Overweight (25–30 kg/m^2)	37.69	35.08	37.80	41.11	
Obese (> 30 kg/m^2)	37.28	40.38	38.71	31.43	
PIR, %					< 0.0001
<1	12.87	15.91	12.86	8.76	
≥1	87.13	84.09	87.14	91.24	
Marital status, %					< 0.0001
Solitude	31.81	38.38	28.87	26.31	
Cohabitation	68.19	61.62	71.13	73.69	
Smoke, %					< 0.0001
Never	50.30	45.47	48.39	59.04	
Former	29.71	28.64	34.49	25.68	
Current	19.99	25.89	17.12	15.28	
Alcohol, %					< 0.001
Never	7.90	6.41	8.60	9.11	
Former	14.20	16.03	14.52	11.35	
Mild	41.31	36.82	42.95	45.51	
Moderate	12.67	12.59	12.07	13.48	
Heavy	23.91	28.15	21.85	20.54	
Hypertension, %					0.19
No	60.82	59.64	59.85	63.53	
Yes	39.18	40.36	40.15	36.47	
Diabetes, %					0.12
No	74.14	73.82	71.18	77.96	
Prediabetes	9.56	9.11	10.84	8.69	
Yes	16.31	17.07	17.99	13.34	
CKD, %					0.01
No	86.48	86.93	85.12	89.91	
Yes	12.71	13.07	14.88	10.09	
CVD, %					0.06
No	90.56	89.62	89.84	92.67	
Yes	9.44	10.38	10.16	7.33	
TD, %					0.02
Continued					

Characteristics	Total participants	Dietary live microbe group			*P* value
		Low	Medium	High	
No	74.14	72.22	72.26	78.92	
Yes	25.86	27.78	27.74	21.08	

Table 2. Baseline characteristics of the study population by dietary live microbe intake, weighted. Statistical methods: Continuous variables are presented as mean ± SE and were compared using weighted linear regression. Categorical variables are presented as percentages and were compared using weighted chi-square tests. A P-value of less than 0.05 was considered statistically significant. *TD* testosterone deficiency, *BMI* body mass index, *PIR* poverty income ratio, *CKD* chronic kidney disease, *CVD* cardiovascular disease, *SE* standard error.

Exposure variable	Model 1		Model 2		Model 3	
	OR (95%CI)	*P* value	OR (95%CI)	*P* value	OR (95%CI)	*P* value
Dietary live microbe intake group						
Low	Reference		Reference		Reference	
Medium	1.00(0.75,1.33)	0.99	0.90(0.68,1.20)	0.45	0.93(0.63,1.38)	0.67
High	0.69(0.54,0.90)	0.01	0.64(0.50,0.82)	0.001	0.72(0.52,0.98)	0.04
P for trend	0.016		0.002		0.048	

Table 3. Logistic regression analysis of the association between dietary live microbe intake and the risk of testosterone deficiency, weighted. Statistical methods: Model 1: Unadjusted. Model 2: Minimally adjusted for age, ethnicity, education level, marital status, and PIR. Model 3: Fully adjusted for age, ethnicity, education level, marital status, PIR, BMI, total dietary energy intake, smoking status, alcohol consumption, hypertension, DM, CKD, and CVD. A P-value of less than 0.05 was considered statistically significant. *OR* odds ratio, *CI* confidence interval, *PIR* poverty income ratio, *DM* diabetes mellitus, *CKD* chronic kidney disease, *CVD* cardiovascular disease.

RCS analysis was employed to explore the dose-response relationship between total food intake at three different levels of live microbes and the risk of TD in different dietary live microbe intake groups. The findings indicated that in the low live microbe intake group, there was no significant association between food intake and TD risk. However, in the medium and high intake groups, food intake was inversely associated with TD risk, displaying a linear trend with P-values of less than 0.01. Detailed results of the RCS analysis are presented in Fig. 3, where the black bars at the bottom illustrate the density distribution of dietary live microbe intake, and the dotted lines indicate the 95% CI. Additionally, the x-axis represents total food intake, while the y-axis represents the adjusted OR for TD.

Discussion

Testosterone, a hormone critical for male health, supports key functions such as maintaining muscle mass, bone density, and reproductive health, while TD is a prevalent condition among middle-aged and elderly men, often associated with a range of adverse health outcomes[26,27]. In this American NHANES survey, a higher intake of dietary live microbes was found to effectively reduce the risk of TD, suggesting that it could be an important preventive measure against the onset and progression of TD. Subgroup analyses demonstrated the generalizability of our findings across different population characteristics, particularly among participants without DM or CKD, with no significant interactions observed in subgroup analyses. Furthermore, the RCS analysis revealed a linear relationship between increased food intake and reduced TD risk in the medium and high dietary live microbe intake groups. In conclusion, a higher intake of dietary live microbes may reduce the risk of TD, enhance testosterone levels, and thereby promote overall male health.

The quantity and diversity of microorganisms (including bacteria, yeasts, and molds) in food depend on the type of food, its source, and the degree of processing. Fresh fruits and vegetables contain a wide variety of microorganisms, typically less than 10^6 CFU/g[28], while fermented foods, such as yogurt, may contain between 10^8 and 10^{11} CFU/g[29]. In 2012, the Food and Agriculture Organization (FAO)/World Health Organization (WHO) defined microbes as "live microorganisms which, when administered in adequate amounts, confer a health benefit on the host," a definition that has undergone revisions and is now widely accepted[30]. Bacterial genera such as Bifidobacterium, Bacillus, Enterococcus, Escherichia, Lactobacillus, Lactococcus, Candida albicans, Lactococcus, Propionibacterium, and Streptococcus, along with yeast (Saccharomyces) species, are recognized microbes[31]. Several studies have preliminarily demonstrated the health benefits of dietary intake of live microbes, citing improvements in mood, delayed aging, and reduced mortality[16–20]. The results of our study are consistent with early animal experiments. Research by Poutahidis et al. demonstrated that supplementation of purified microbes in aging male mice was able to restore their testosterone levels[14]. Additionally, other studies investigating the effects of diet on sex hormones suggest that dietary habits may influence male testosterone levels. However, previous research has primarily focused on how specific dietary components, such as high-protein or

Subgroup	Dietary Live Microbe Intake group			*P* for trend	*P* for trend
	Low	Medium	High		
Age group					0.06
20-40y	Ref	0.87(0.43, 1.77)	0.64(0.35, 1.20)	0.16	
40-60y	Ref	1.30(0.80, 2.10)	0.65(0.39, 1.07)	0.10	
> 60y	Ref	0.66(0.41, 1.06)	0.85(0.47, 1.53)	0.45	
BMI,					0.84
Normal (< 25 kg/m^2)	Ref	1.05(0.55, 2.00)	0.64(0.32, 1.29)	0.19	
Overweight (25–30 kg/m^2)	Ref	0.81(0.54, 1.22)	0.73(0.45, 1.17)	0.15	
Obese (> 30 kg/m^2)	Ref	1.00(0.60, 1.68)	0.74(0.47, 1.16)	0.22	
Smoke status					0.84
Never	Ref	0.97(0.61, 1.53)	0.68(0.41, 1.14)	0.13	
Former	Ref	0.85(0.46, 1.58)	0.78(0.48, 1.28)	0.30	
Current	Ref	1.05(0.53, 2.09)	0.70(0.28, 1.75)	0.48	
Hypertension					0.85
No	Ref	1.00(0.61, 1.65)	0.70(0.48, 1.01)	0.07	
Yes	Ref	0.88(0.56, 1.41)	0.75(0.46, 1.22)	0.21	
DM					0.58
No	Ref	0.93(0.64, 1.36)	0.65(0.47, 0.90)	0.02	
Borderline	Ref	0.90(0.24, 3.38)	1.16(0.39, 3.44)	0.79	
Yes	Ref	0.96(0.53, 1.72)	0.93(0.51, 1.68)	0.76	
CKD					0.09
No	Ref	0.93(0.59, 1.47)	0.64(0.46, 0.89)	0.02	
Yes	Ref	0.94(0.50, 1.77)	1.27(0.67, 2.43)	0.51	

Table 4. Subgroup analysis of the association between dietary live microbe intake and the risk of testosterone deficiency, weighted. Statistical methods: Subgroup analyses were performed to assess the consistency of the association between dietary live microbe intake and TD risk across various population characteristics, including age, BMI, smoking status, hypertension, DM, and CKD. All regression analyses were adjusted for all variables in Model 3, except for the grouping variable itself, and used the low dietary live microbe intake group as the reference. P-values for trend were calculated within each subgroup to evaluate the linear trend of TD risk across the different levels of dietary live microbe intake. Additionally, P-values for interactions were calculated to assess the presence of heterogeneity across subgroups. A P-value of less than 0.05 was considered statistically significant. *OR* odds ratio, *CI* confidence interval, *BMI* body mass index, *DM* diabetes mellitus, *CKD* chronic kidney disease, *CVD* cardiovascular disease.

plant-based diets, affect sex hormones[9,10], while limited attention has been given to how live microorganisms in food can influence sex hormones by modulating the gut microbiota. Our study is the first to demonstrate a direct association between dietary live microbe intake and TD in a large-scale human population.

The impact of dietary live microbe intake on male testosterone levels may be mediated through several biological mechanisms. First, live microbes can significantly improve the composition and diversity of the gut microbiota, promoting the proliferation of beneficial bacteria while inhibiting the growth of pathogenic bacteria[32]. This balanced gut microbiota not only enhances gut barrier function but also reduces the translocation of endotoxins, such as lipopolysaccharides (LPS), which can lower systemic inflammation levels[33]. Reduced systemic inflammation is conducive to a favorable environment for testosterone synthesis, as chronic inflammation has been shown to suppress testosterone production[6]. Second, the gut microbiota is intricately linked to host metabolic functions[34]. Certain beneficial microbes can metabolize dietary fibers to produce short-chain fatty acids (SCFAs), such as butyrate, acetate, and propionate, which not only provide energy for intestinal epithelial cells but also modulate systemic energy metabolism and immune responses[35]. SCFAs may influence endocrine function, particularly by regulating the hypothalamic-pituitary-gonadal (HPG) axis, thereby impacting testosterone synthesis and secretion[13,36]. Third, live microbes may exert their effects through the gut-testis axis[13]. For example, a study demonstrated that the gut bacterium Mycobacterium neoaurum, isolated from feces of male patients with depression, degraded testosterone and caused reduced testosterone levels in rats, along with depressive-like behaviors[37]. Alterations in gut microbiota composition have also been associated with decreased sperm quality. For instance, a study found that dietary fiber supplementation improved gut microbiota and promoted short-chain fatty acid production, enhancing spermatogenesis and semen quality in a boar model[38]. By maintaining a healthy gut microbiome, live microbes may improve the local testicular microenvironment, thereby promoting normal testosterone secretion. Additionally, there is a bidirectional relationship between obesity and low testosterone levels, and live microbes have been shown to modulate lipid metabolism and energy balance, reducing fat accumulation and insulin resistance, thereby aiding in weight management[39].

Our findings suggest a link between dietary live microbes and testosterone regulation in men, mediated through the gut-testis axis. However, this study did not include female participants. In women, testosterone is

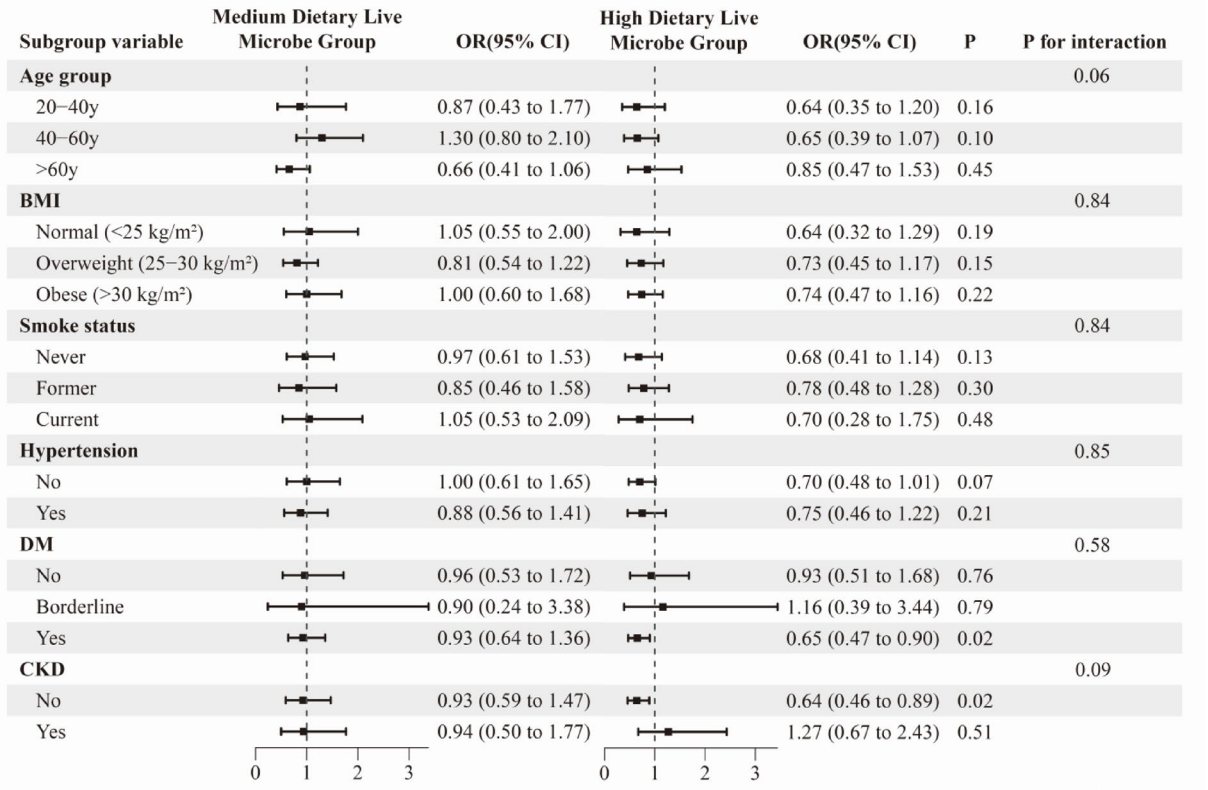

Fig. 2. Subgroup analysis of the association between dietary live microbe intake and the risk of testosterone deficiency. The odds ratios (OR) with 95% confidence intervals (CI) are presented for the medium and high dietary live microbe intake groups compared to the low intake group, which serves as the reference. Subgroup analyses were performed by adjusting for all covariates in Model 3, except for the subgroup variable itself. The P-values for interaction were calculated to evaluate potential heterogeneity in the association between dietary live microbe intake and TD risk across the different subgroups. OR: Odds Ratio, CI: Confidence Interval, BMI: Body Mass Index, DM: Diabetes Mellitus, CKD: Chronic Kidney Disease, TD: Testosterone Deficiency.

primarily produced by the ovaries and adrenal glands, and hyperandrogenism can result in conditions such as polycystic ovary syndrome (PCOS), a significant health burden. Future research should explore whether dietary live microbes influence testosterone levels in women through similar mechanisms, particularly focusing on their potential role in ovarian or adrenal function and PCOS pathophysiology[40]. Investigating these associations would provide a more comprehensive understanding of the gut-microbiota-endocrine axis across genders.

One of the key limitations of our study is its cross-sectional design, which precludes the establishment of causal relationships between dietary live microbe intake and the risk of TD. Although our findings suggest a strong association, the lack of temporal sequencing inherent to cross-sectional studies limits our ability to determine whether higher dietary live microbe intake directly reduces the risk of TD or if individuals with lower testosterone levels are less likely to consume live microbe-rich foods. To address this limitation, we accounted for a comprehensive set of potential confounders. Despite this, unmeasured confounders such as physical activity levels, stress, or additional dietary components could still influence the observed associations, highlighting the need for cautious interpretation. Furthermore, sensitivity analyses, such as instrumental variable approaches or propensity score adjustments, could potentially strengthen causal arguments. Unfortunately, the NHANES database's cross-sectional nature and its limitations regarding temporal data precluded such analyses in our study. Nevertheless, we recommend that future longitudinal studies and randomized controlled trials explore these associations to establish causality and further validate our findings. Another limitation of our study is the definition of TD, which was based solely on biochemical testosterone levels (serum testosterone < 300 ng/dL) without considering clinical symptoms[41]. This approach was necessitated by the NHANES database, which does not include detailed clinical assessments of TD-related symptoms such as reduced libido, fatigue, or erectile dysfunction. The exclusion of clinical symptoms could potentially lead to an underestimation or overestimation of the true prevalence of TD. For instance, some individuals with low testosterone levels may not exhibit clinical symptoms and might not meet the full diagnostic criteria for TD, whereas others with normal testosterone levels might still experience symptoms due to androgen resistance or other factors[42]. This limitation underscores the need for future research that combines biochemical and clinical criteria to improve the accuracy and applicability of TD diagnoses.

One notable limitation of this study lies in the use of a single 24-hour dietary recall to estimate live microbe intake. While this method is widely employed in large-scale epidemiological studies, including NHANES, it

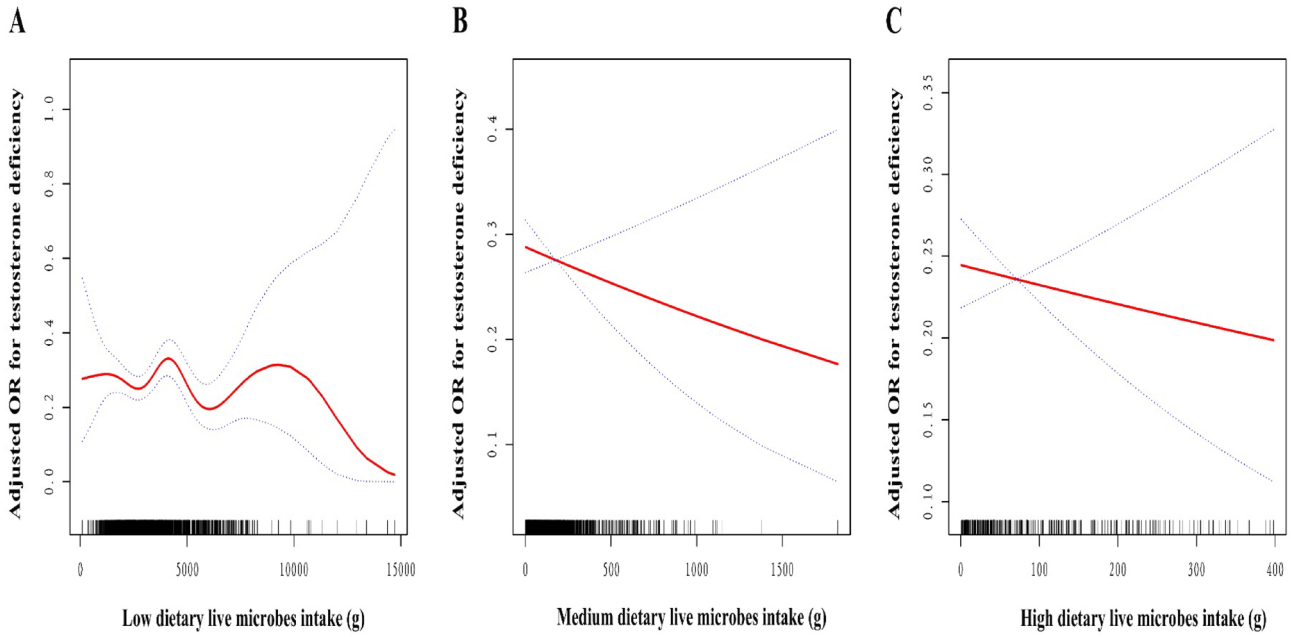

Fig. 3. Restricted cubic spline analysis of the association between different dietary live microbe and the risk of TD in different population group. (**A**) Association between dietary low live microbe intake and TD in participants intaking of foods low in live microbes, (**B**) Association between dietary medium live microbe intake and TD in participants intaking of foods medium in live microbes, and (**C**) Association between dietary high live microbe intake and TD in participants intaking of foods high in live microbes. The red line represents the adjusted OR for TD, and the dotted lines indicate the 95% confidence intervals. The black bars at the bottom illustrate the total food take total food intake at three different levels of live microbes. RCS analysis was adjusted for age, ethnicity, education level, marital status, PIR, BMI, total dietary energy intake, smoking status, alcohol consumption, hypertension, DM, CKD, and CVD. OR: Odds Ratio, CI: Confidence Interval, TD: Testosterone Deficiency, RCS: Restricted Cubic Spline, PIR: Poverty Income Ratio, BMI: Body Mass Index, DM: Diabetes Mellitus, CKD: Chronic Kidney Disease, CVD: Cardiovascular Disease.

may not adequately reflect individuals' habitual dietary patterns due to substantial day-to-day variability and contextual factors such as weekends and holidays. This issue is particularly relevant when assessing short-lived exposures like dietary microbes, whose intake may fluctuate considerably over time. Consequently, the use of a single-day recall could introduce exposure misclassification. However, this misclassification is most likely non-differential with respect to the outcome, meaning that it is unrelated to the disease status. In such cases, the expected effect is a bias toward the null, potentially leading to attenuation of the true association rather than inflation or reversal of direction. Therefore, the observed associations in our study may in fact underestimate the strength of the relationship. From a practical standpoint, limiting the analysis to participants with complete multi-day recalls would have substantially reduced the sample size and statistical power, and could have introduced selection bias. To preserve the representativeness and generalizability of the NHANES data, we opted to use the first-day recall, which is available for the full sample. This approach is consistent with prior studies utilizing NHANES dietary data, where a single recall has been shown to provide acceptable estimates of group-level intake distributions[43,44]. While repeated dietary assessments would be ideal for capturing habitual intake, they are not always feasible in secondary data analysis. Our study thus represents an initial step in characterizing the association between estimated live microbe intake and health outcomes at the population level. Further research using repeated recalls, dietary biomarkers, or prospective cohort designs is needed to validate and extend these findings.

Although our study is the first large-scale investigation into the relationship between dietary live microbe intake and TD risk, several additional limitations, beyond those discussed above, must be acknowledged when interpreting the results. First, our findings are based on a U.S. population, which may limit the generalizability of the results to other populations. Future research in diverse ethnic groups and populations is necessary to confirm the universality of our findings. Second, dietary data were obtained through a single 24-hour dietary recall interview, which may not accurately reflect participants' habitual intake, potentially leading to misclassification due to factors such as weekends or holidays. In summary, while our preliminary results suggest that higher dietary live microbe intake may reduce the risk of TD, better-designed large-scale studies are essential to further substantiate our findings. Additionally, animal studies are needed to explore the underlying mechanisms in more detail.

Conclusion

In conclusion, our study provides the first large-scale evidence that higher dietary live microbe intake is associated with a reduced risk of TD in a U.S. population. This finding highlights the potential role of dietary live microbes as a preventive measure against TD, suggesting that dietary modifications to include more live microbes could be a promising strategy to enhance male reproductive health. Despite the limitations of our study, the observed associations underscore the need for future research, including longitudinal studies and randomized controlled trials, to confirm these findings and explore the underlying biological mechanisms. Additionally, extending this research to diverse populations and clinical settings, and examining the specific content of live microbes in various foods, will be crucial to fully understand the broader implications of our findings for public health.

Data availability

The data utilized in this study are publicly available from the National Health and Nutrition Examination Survey (NHANES) database, which can be accessed at https://www.cdc.gov/nchs/nhanes/index.htm. All materials and data supporting the findings of this study are included in the published article and its supplementary files. More detailed data and the code used for analysis can be obtained from the corresponding author upon reasonable request.

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Author contributions
Yiming Chen: Conceptualization, Methodology, Investigation, Data curation, Writing—original draft, Writing—review & editing. Qianfeng Zhuang: Data curation, Writing—review & editing. Wei Xia: Conceptualization, Data curation, Writing—review & editing. Naiyuan Shao: Conceptualization, Methodology, Supervision, Writing—review & editing. Bo Zhang: Conceptualization, Methodology, Supervision, Writing—review & editing. Xingliang Feng: Conceptualization, Methodology, Investigation, Supervision, Writing—review & editing.

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Declarations

Competing interests
The authors declare no competing interests.

Ethics approval and informed consent
The NHANES protocol was reviewed and approved by the Research Ethics Review Board of the National Center for Health Statistics (NCHS). All participants provided written informed consent prior to their participation in the survey. This study was conducted in accordance with the ethical standards of the Declaration of Helsinki.

Additional information

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