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ORIGINAL CONTRIBUTION

Association of the live microbe intake from foods with all-cause and cardiovascular disease-specific mortality: a prospective cohort study

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Abstract

Background Live dietary microbes have been hypothesized to promoting human health. However, there has been lacking perceptions to crystallize nexus between consumption of foods with live microbes and mortality.

Objective To investigate the association of consumption of foods with medium to high amounts of live microbes with all-cause, cancer-specific, and cardiovascular disease (CVD)-specific mortality.

Methods The data were obtained from the National Health and Nutrition Examination Survey 1999–2018 at baseline linked to the 2019 National Death Index records. Based on consumption of foods that were categorized as either having medium or high microbial content (MedHi foods), participants were classified into three groups. Kaplan–Meier survival curves and multivariable Cox regression models were used to estimate the association of consumption of MedHi foods with mortality. Population-attributable fractions (PAFs) of consumption of MedHi foods in relation to mortality risk were also estimated.

Results A total of 35,299 adults aged ≥ 20 years were included in this study. During a median follow-up of 9.67 years, compared with adults in G1, those in G3 had 16% (hazard ratio [HR], 0.84; 95% confidence interval [CI], 0.77–0.90) reduced risk of all-cause mortality, and 23% (HR, 0.77; 95% CI, 0.67–0.89) reduced risk of CVD-specific mortality. The PAF of high (G3) vs. intermediate or low consumption of MedHi foods (G1 + G2) with all-cause and CVD-specific mortality was 3.4% and 4.3%, respectively.

Conclusions Consumption of foods with higher microbial concentrations is associated with a reduced risk of all-cause and CVD-specific mortality in US adults.

Keywords Live dietary microbes · Health promotion · Population-attributable fractions · Mortality · Prospective study

Abbreviations

NHANES	National Health and Nutrition Examination Survey
CVD	Cardiovascular disease
PAFs	Population-attributable fractions
MedHi foods	Foods that were categorized as either having medium or high microbial content
BMI	Body mass index
HEI-2015	Healthy Eating Index-2015
PA	Physical activity
DM	Diabetes mellitus
TC	Total cholesterol
HDL-C	High density lipoprotein cholesterol
SE	Standard error
CIs	Confidence intervals
HRs	Hazard ratios

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Introduction

The potential positive contributions to human health from ingested microorganisms are being increasingly acknowledged [1]. The “old friends' hypothesis” states that exposure to commensal or non-harmful microbes in food serves as a pivotal and advantageous means of providing microbial stimuli for the immune system [2]. Notably, such immune regulatory activities may have an impact on current chronic immune, metabolic, and other “lifestyle” diseases associated with Western diets [3].

Previous studies have documented associations of live microorganisms and fermented foods with health outcomes [4, 5]. However, live microorganisms have been discovered to exist not only within fermented food, but also in a wide range of other foods, including raw, unpeeled fruits and vegetables [6, 7]. Recently, Sander et al. [8] developed a classification system that assigns each food recorded by National Health and Nutrition Examination Survey (NHANES) to one of three microbial categories (low, medium, and high), and estimated the number of live microbes in the diet of US population, finding that adults in US steadily increased their consumption of foods with live microbes between the earliest (2001–2002) and latest (2017–2018) survey cycles. Interestingly, based on this microbial classification method, previous studies have found that increased consumption of foods with medium to high amounts of live microbes is associated with a lower blood pressure, BMI, waist circumference, plasma glucose, C-reactive protein, insulin, triglyceride levels, and CVD risk [9, 10]. However, these studied failed to separate the contribution of foods with live microbes from the effects of those foods as a whole [11]. Moreover, the association between consumption of foods with live microbes and health benefits was based on cross-sectional study, findings based on prospective cohort study remain limited. From a public health perspective, investigation of the contributions of foods with live microbes to human survival promotes awareness to consumption of foods containing high levels of live microorganisms and provides new insights for healthy diet recommendations, thereby reducing the burden of mortality.

In present study, we investigated the association of consumption of foods with medium to high amounts of live microbes with all-cause, cancer-specific, and cardiovascular disease (CVD)-specific mortality among US adults. Moreover, we estimated population-attributable fractions (PAFs) of consumption of foods with medium to high amounts of live microbes in relation to mortality.

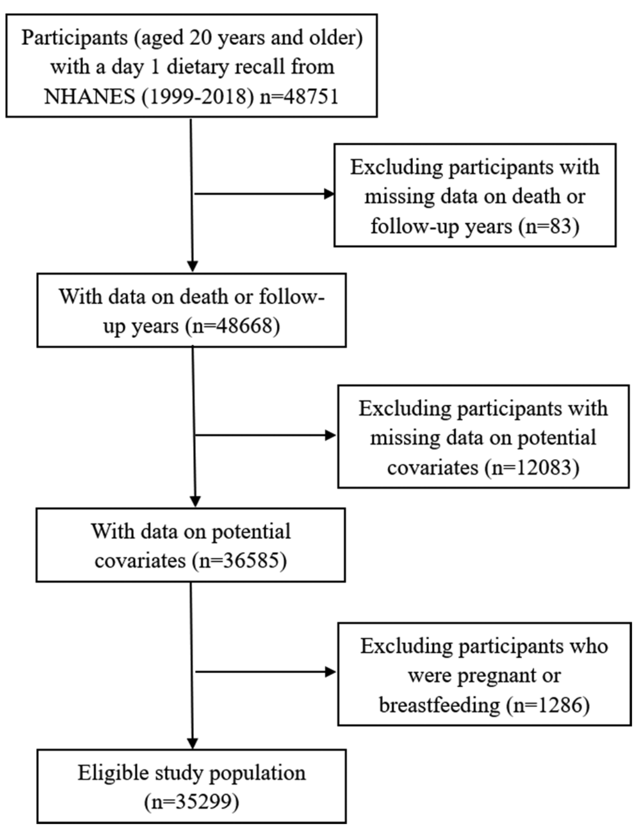

Fig. 1 Flow chart of inclusion/exclusion of participants

Methods

Study population

NHANES is a national survey program designed to assess health and nutritional status of people in the United States, obtained using a complex, stratified, clustered multi-staged probability sampling design [12]. The survey was approved by the Centers for Disease Control and Prevention Research Ethics Review Board. All participants have signed informed consent. This study was exempt from review because data used in this study was de-identified.

A total of 48,751 adults aged ≥ 20 years with a day 1 dietary recall participated in the 1999 to 2018 NHANES. After excluding participants with missing data on death or follow-up years ($n = 83$) or potential covariates ($n = 12{,}083$), and those who were pregnant or breastfeeding ($n = 1286$), a total of 35,299 adults aged ≥ 20 years were included in this study, of which 2861, 3748, 3448, 3365, 3951, 4142, 3618, 3889, 3525, and 2752 were in the survey cycles of 1999–2000, 2001–2002, 2003–2004, 2005–2006, 2007–2008, 2009–2010, 2011–2012, 2013–2014, 2015–2016, and 2017–2018, respectively (Fig. 1).

Determinations of the intakes of foods with microbes

According to Sanders' researches [8, 13], experts in the field relied on primary literature, manuscript reviews, professional knowledge, and microbial viability in relation to food processing to estimate the live microbial content for 9388 individual food codes within 48 NHANES subgroups, and then designated foods into three categories (low, medium [Med], and high [Hi]) based on the estimated live microbial content. These levels of low, Med, and hi were chosen to reflect the approximate numbers of viable microbes expected to be present in pasteurized foods ($<10^4$ CFU/g), fresh fruits and vegetables eaten unpeeled (10^4–10^7 CFU/g), and unpasteurized fermented foods ($>10^7$ CFU/g), respectively [14]. The differences were resolve through discussion and external consulting with Fred Breidt, USDA Agricultural research service microbiologist.

Considering that analysis of both Med and Hi foods included in the same model as separate terms showed that their coefficients were similar, and Hi category comprised primarily fermented dairy foods, we developed a fourth category (MedHi) as in previous study [8, 13], referring to foods that were categorized as either having medium or high microbial content. We then calculated the grams of four categories of food consumed for each participant, and used consumption of MedHi foods as a variable of interest. Consistent with previous study [13], participants were further classified into three groups (G1: Participants without MedHi foods intakes; G2: those with MedHi foods intakes greater than zero but less than the median intake for participants; and G3: those with MedHi foods intakes greater than the median intake for participants). Given the low consumption (2%), probiotic dietary supplements were not included in the current analyses.

Determination of mortality

The National Center for Health Statistics provides mortality data related to the National Death Index as of December 31, 2019. *The International Statistical Classification of Diseases and Related Health Problems, Tenth Revision* (ICD-10) is used to document the underlying causes of death. CVD-specific mortality was defined as ICD-10 codes I00-I09, I11, I13, I20-I51, or I60-I69; cancer-specific mortality was defined as ICD-10 codes C00-C97. The duration of follow-up was defined as the interval in months from the date of interview to the date of death or to December 31, 2019.

Assessment of covariates

Sociodemographic characteristics included age, sex (male or female), race and ethnicity (non-Hispanic Black, Hispanic [Mexican Americans/other Hispanic], non-Hispanic White, or others [American Indian/Native Alaskan/Pacific Islander, Asian, multiracial]), educational level (less than high school graduate, high school graduate or GED, and some college or above), and household poverty-to-income ratio (<1.30, 1.30–3.49, or ≥ 3.50) [15]. The body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. Alcohol use was classified as "never", "former" (a history of daily binge drinking), "current heavy" (≥ 3 drinks per day for females, ≥ 4 drinks per day for males, or binge drinking [≥ 4 drinks on same occasion for females, ≥ 5 drinks on same occasion for males] on 5 or more days per month), "current moderate" (≥ 2 drinks per day for females, ≥ 3 drinks per day for males, or binge drinking ≥ 2 days per month), or "current mild" (current drinking but not meeting the standard of moderate and heavy drinking) [16]. Smoking status was classified as "never" (smoked less than 100 cigarettes in life), "former" (smoked more than 100 cigarettes in life, but they were not currently smoking cigarettes.), or "current smokers" (smoked more than 100 cigarettes in life and smoke some days or every day) [17]. Healthy Eating Index-2015 (HEI-2015), consisting of thirteen dietary components scores, was calculated for each participant on the basis of data of 24 h dietary recalls, and a higher HEI-2015 score indicates a better dietary quality and a closer compliance with the intake level recommended by the 2015–2020 Dietary Guidelines for Americans [18]. Food calorie intake and total dietary fiber intake were considered continuous variables. Physical activity (PA) was assessed on the basis of data of self-reported Global Physical activity Questionnaire [19], which consists of work-related activity, transportation-related activity, and leisure time-related activity. Total PA was estimated as minutes of moderate-intensity activity plus twice the number of minutes of vigorous activity in work-related activity and leisure time-related activity, then plus the number of minutes of transportation-related activity. According to the 2018 Physical Activity Guidelines for Americans, participants with total PA less than 150 min/wk and those with PA of 150 min/wk or more were classified as inactive and active, respectively [20].

Hypertension was defined as self-reported physician's diagnosis, use of hypertensive drugs, or blood pressure of $\geq 140/90$ mmHg. Diabetes mellitus (DM) was defined as self-reported physician's diagnosis, a glycosylated hemoglobin A1c level of $\geq 6.5\%$, a fasting plasma glucose level of ≥ 7.0 mmol/l, random blood glucose level of ≥ 11.1 mmol/l, two-hour glucose tolerance test blood glucose level of ≥ 11.1 mmol/l, or use of diabetes medication or insulin [21]. Hyperlipidemia was defined as total triglyceride ≥ 200 mg/dL, total cholesterol (TC) ≥ 240 mg/dL, high density lipoprotein cholesterol (HDL-C) < 40 mg/dL, low density lipoprotein cholesterol ≥ 160 mg/dL, or TC /HDL-C ratio ≥ 5 [22]. CVD was defined as a composite of self-reported doctor diagnoses of coronary heart disease, myocardial infarction, angina pectoris, congestive heart failure, or stroke [23].

Statistical analysis

All analyses in this study were conducted in accordance with NHANES analysis guidelines and accounted for unequal selection probabilities, oversampling of certain subgroups, and nonresponse adjustment to ensure nationally representative estimates.

Continuous variables were expressed as weighted mean with standard error (SE), and categorical variables as weighted frequency distribution. Baseline characteristics were compared using the survey-weighted linear regression for continuous variables and the survey-weighted Chi-square test for categorical variables.

Differences of cumulative mortality between three consumption categories of MedHi foods (G1, G2, G3) were shown using survey-weighted Kaplan–Meier survival curves and were compared using the log-rank test. Multivariable Cox proportional hazards regression models were used to estimate hazard ratios (HRs) and 95% confidence intervals (CIs) for examining the associations of consumption of MedHi foods (low level as the reference) with all-cause, cancer-specific, and CVD-specific mortality. Model 1 was adjusted for age, sex, race and ethnicity; model 2 was additionally adjusted for educational level, family poverty income ratio, BMI, smoking status, alcohol use, total PA, CVD, hypertension, DM, and hyperlipidemia. To separate the contribution of MedHi foods from the effects of high-quality diet, model 3 was additionally adjusted for HEI-2015 score, food calorie intake, and total dietary fiber intake. We also applied the multivariable Cox regression based on restricted cubic splines to explore dose-response association. The adjusted PAFs of MedHi foods consumers (G2 + G3) vs. nonconsumers (G1) were estimated for the proportion of mortality that could be avoided if nonconsumers ingest MedHi foods. Furthermore, the adjusted PAFs of high (G3) vs. intermediate or low consumption of MedHi foods (G1 + G2) were also estimated, indicating that the proportion of mortality that could be avoided if each participant achieves a high consumption of MedHi foods (G3).

We conducted subgroup analyses by age (20–29 years, 40–59 years, and > = 60 years), sex, BMI (< 25, 25-29.9, and ≥ 30), race/ethnicity, educational level, ratio of family income to poverty, and PA level. We further examined the potential effect of interaction by adding an interaction term of each stratifying variable and consumption of MedHi foods in each model and assessing it via a likelihood ratio test. Sensitivity analysis was conducted by excluding participants with a history of CVD or death within the first 2 years of follow-up to lessen the probability of reverse causation.

A two-tailed *P* value < 0.05 was considered statistically significant. Statistical analyses were performed using R software version 4.2.1 and STATA version 16.0 (Stata Corporation, College Station, TX, USA).

Results

Characteristics of participants

The characteristics of participants across the three levels of consumption of MedHi foods (G1, G2, and G3) are shown in Table 1. The relative frequency of adults in G1 (nonconsumers), G2 (below median of consumers), and G3 (above median of consumers) was 35.22%, 32.43%, and 32.34%, respectively. Adults in G3 were prone to female, elderly people, non-Hispanic White, having a high education level and a high ratio of family income to poverty, mild drinking, having a low BMI, having an active PA, having a high HEI-2015 score, having high energy and dietary fiber intakes, and being without a history of CVD (All *P* < 0.001).

Association of microbes with mortality

During a median follow-up of 9.67 years (interquartile range: 5.42–14.25 years), participants who consumed more MedHi foods had a significantly lower cumulative incidence rate of all-cause and CVD-specific mortality (*P* < 0.001 for all log-rank tests, Fig. 2).

Multivariable Cox regression models showed that compared with adults in G1, those in G3 had 16% (HR, 0.84; 95% CI, 0.77–0.90) (*P* for trend = 0.03) reduced risk of all-cause mortality, and 23% (HR, 0.77; 95% CI, 0.67–0.89) (*P* for trend = 0.06) reduced risk of CVD-specific mortality after adjustment for all potential covariates (Table 2). However, no significant association between consumption of MedHi foods and cancer-specific mortality was observed. The adjusted PAFs of MedHi foods consumers (G2 + G3) vs. nonconsumers (G1) with all-cause and CVD-specific mortality were 3.6% and 3.6%, respectively. The adjusted PAFs of high (G3) vs. intermediate or low consumption of MedHi foods (G1 + G2) with all-cause and CVD-specific mortality were 3.4% and 4.3%, respectively (Table 2).

There were approximately linear dose–response associations of consumption of MedHi foods with all-cause (*P* for

Table 1 Characteristics of US adults by three levels of MedHi foods consumption

Characteristic	Levels of MedHi foods consumption			*P* value
	G1	G2	G3	
Overall, n (%)	12,434 (35.22)	11,448 (32.43)	11,417 (32.34)	
Age	45.77 (0.23)	47.35 (0.26)	49.49 (0.27)	**< 0.01**
HEI-2015	47.94 (0.18)	51.17 (0.21)	58.92 (0.20)	**< 0.01**
Calorie intake (kcal)	2066.58 (10.73)	2123.32 (9.74)	2203.20 (10.81)	**< 0.01**
Fiber intake (g)	13.94 (0.11)	15.51 (0.12)	20.24 (0.15)	**< 0.01**
BMI (kg/m^2)	29.37 (0.10)	28.90 (0.08)	28.39 (0.10)	**< 0.01**
Sex, n (%)				**< 0.01**
Male	6641 (53.44)	5566 (47.33)	5407 (46.13)	
Female	5793 (46.56)	5882 (52.67)	6010 (53.87)	
Race/ethnicity, n (%)				**< 0.01**
Hispanic	2697 (12.49)	2948 (12.71)	2818 (11.43)	
Non-Hispanic black	3551 (15.54)	2023 (9.02)	1611 (6.39)	
Non-Hispanic white	5257 (65.85)	5657 (72.77)	6018 (76.35)	
Others race	929 (6.11)	820 (5.50)	970 (5.83)	
Education, n (%)				**< 0.01**
Less than high school graduate	3659 (20.80)	2777 (14.79)	2252 (11.25)	
High school graduate or GED	3268 (28.44)	2717 (24.81)	2254 (19.25)	
Some college or above	5507 (50.76)	5954 (60.40)	6911 (69.50)	
Family income–to-poverty ratio, n (%)				**< 0.01**
< 1.30	4455 (26.75)	3174 (18.69)	2598 (14.17)	
1.30–3.49	4890 (38.67)	4504 (36.62)	4143 (32.45)	
≥ 3.50	3089 (34.58)	3770 (44.69)	4676 (53.38)	
PA, n (%)				**< 0.01**
Inactive	6886 (52.88)	6123 (50.11)	5556 (45.03)	
Active	5548 (47.12)	5325 (49.89)	5861 (54.97)	
Smoking, n (%)				**< 0.01**
Never	6150 (48.51)	6105 (53.99)	6540 (57.40)	
Former	2880 (22.42)	2964 (24.91)	3337 (29.46)	
Now	3404 (29.07)	2379 (21.11)	1540 (13.14)	
Alcohol, n (%)				**< 0.01**
Never	1764 (11.46)	1500 (10.85)	1538 (10.39)	
Former	2534 (17.09)	1912 (13.50)	1870 (13.47)	
Current mild	3691 (31.30)	3903 (36.28)	4448 (42.35)	
Current moderate	1826 (16.49)	1767 (17.67)	1736 (17.55)	
Current heavy	2619 (23.66)	2366 (21.70)	1825 (16.24)	
Hypertension, n (%)				0.07
No	6871 (61.42)	6511 (61.93)	6572 (63.20)	
Yes	5563 (38.58)	4937 (38.07)	4845 (36.80)	
DM, n (%)				0.94
No	10,238 (87.18)	9481 (87.22)	9475 (87.36)	
Yes	2196 (12.82)	1967 (12.78)	1942 (12.64)	
Hyperlipidemia, n (%)				0.50
No	3694 (29.73)	3239 (29.48)	3298 (30.38)	
Yes	8740 (70.27)	8209 (70.52)	8119 (69.62)	
CVD, n (%)				**< 0.01**
No	10,877 (90.39)	10,179 (91.32)	10,206 (91.86)	
Yes	1557 (9.61)	1269 (8.68)	1211 (8.14)	

Continuous variables were presented as mean ± SE. Categorical variables were presented as n (%). The bold values mean statistical significance

Abbreviations HEI-2015, Healthy Eating Index-2015; PA, physical activity; BMI, body mass index; DM, diabetes mellitus; CVD, cardiovascular disease; SE, standard error

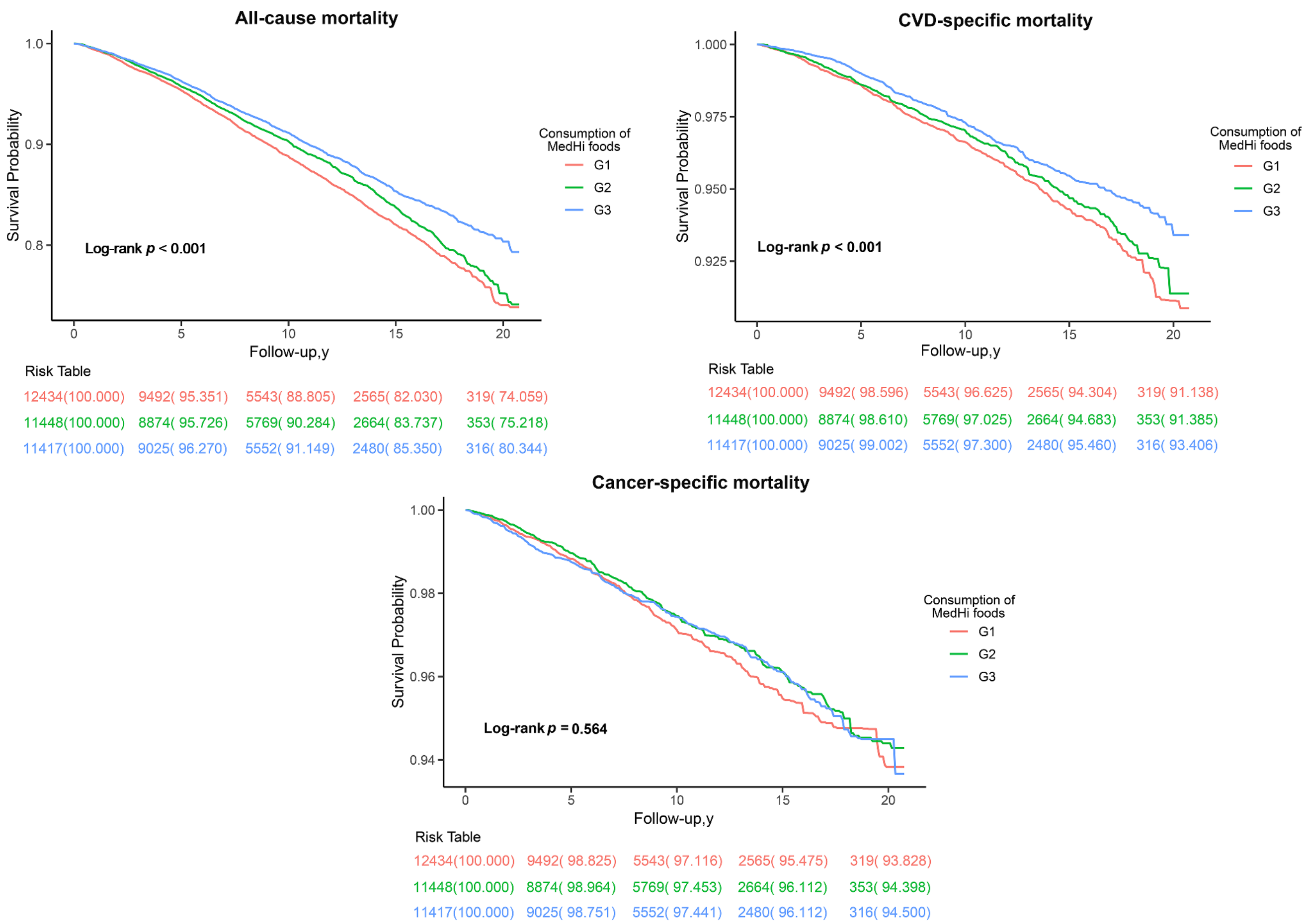

Fig. 2 Survey-weighted Kaplan–Meier survival curves shows differences of cumulative all-cause, CVD-specific, and cancer-specific mortality between three levels of MedHi foods consumption (G1, G2, G3)

non-linear association = 0.59) and CVD-specific (*P* for non-linear association = 0.75) mortality, and the risk of all-cause and CVD-specific mortality decreased linearly as consumption of MedHi foods (per 100 g) increased (Fig. 3).

Subgroup analysis

Subgroup analyses showed that the patterns of high consumption of MedHi foods in relation to reduced risk of all-cause and CVD-specific mortality remained consistent across subgroups of age, sex, BMI, race/ethnicity, educational level, family income-to-poverty ratio, and PA level (Fig. 4) (Supplementary Table 1). Interestingly, we observed a significant interaction between age and consumption of MedHi foods both on all-cause and CVD-specific mortality.

Sensitivity analysis

Similar patterns of high consumption of MedHi foods in relation to reduced risk of all-cause and CVD-specific mortality were observed after excluding adults with CVD history or death occurred during the first 2-year follow-up (Supplementary Tables 2 and 3).

Discussion

To our knowledge, this study is the first to directly provide evidence of an association between dietary intake of live microbes and risk of all-cause and CVD-specific mortality. Based on a large nationally representative sample of US adults, we found that adults with a high level of MedHi food intake had a reduced risk of all-cause and CVD-specific mortality. These association remained consistent in sensitivity analyses and in most subgroups. There was an approximately linear dose–response association of increased MedHi food intake with reduced risk of all-cause and CVD-specific mortality. Interestingly, we observed that the increase at any level of the MedHi food intake has a benefit based on the spline plots, and adults with the lower MedHi food intake seem to benefit more from a small change. In addition, we documented that 3.4–3.6% of all-cause mortality and

Table 2 Adjusted hazard ratios of microbes with risk of all-cause mortality and CVD mortality, NHANES 2005–2018

	Levels of MedHi foods consumption				*P* for trend	PAFs	
	Continuous	G1	G2	G3		(G2 + G3) vs. (G1)	(G3) vs. (G1 + G2)
All-cause mortality (cases/n)	5737/35,299	2167/12,434	1901/11,448	1669/11,417			
Model 1	**0.87 (0.85, 0.89)**	1.00 (reference)	**0.80 (0.74, 0.86)**	**0.62 (0.57, 0.66)**			
Model 2	**0.93 (0.91, 0.96)**	1.00 (reference)	**0.92 (0.86, 0.99)**	**0.79 (0.73, 0.86)**			
Model 3	**0.95 (0.92, 0.97)**	1.00 (reference)	**0.93 (0.87, 0.99)**	**0.84 (0.77, 0.90)**	**0.03**	3.6	3.4
CVD-specific mortality (cases/n)	1792/35,299	693/12,434	587/11,448	512/11,417			
Model 1	**0.85 (0.81, 0.89)**	1.00 (reference)	**0.80 (0.70, 0.91)**	**0.59 (0.51, 0.68)**			
Model 2	**0.91 (0.87, 0.95)**	1.00 (reference)	0.90 (0.79, 1.02)	**0.74 (0.64, 0.85)**			
Model 3	**0.92 (0.88, 0.97)**	1.00 (reference)	0.91 (0.80, 1.03)	**0.77 (0.67, 0.89)**	0.06	3.6	4.3
Cancer-specific mortality (cases/n)	1339/35,299	494/12,434	421/11,448	424/11,417			
Model 1	**0.94 (0.90, 0.99)**	1.00 (reference)	**0.84 (0.71, 0.99)**	**0.79 (0.67, 0.93)**			
Model 2	0.99 (0.95, 1.04)	1.00 (reference)	0.96 (0.80, 1.14)	1.00 (0.84, 1.19)			
Model 3	1.01 (0.96, 1.06)	1.00 (reference)	0.97 (0.81, 1.15)	1.05 (0.88, 1.26)	0.37		

The bold values mean statistical significance

Abbreviations CVD, cardiovascular disease; PAFs, population-attributable fractions

Model 1: Adjusted for age, sex, race and race/ethnicity. Model 2: Adjusted for educational level, family poverty income ratio, BMI, smoking status, alcohol use, total PA, CVD, hypertension, DM, and hyperlipidemia plus covariates in Model 1. Model 3: Adjusted for HEI-2015 score, food calorie intake, and total dietary fiber intake plus covariates in Model 2

3.6–4.3% of CVD-specific mortality could be avoided if all of adults were able to obtain a high level of MedHi food intake.

Several concepts formed the basis for the hypothesis that the ingestion of live microbes could have positive effects on human health [24]. First, improved hygiene and sanitation, coupled with modern food production and processing, have led to a decrease in the amounts of live microbes ingested by modern humans compared to our ancestors [25]. Although improved food and environmental hygiene can bring about significant public health benefits, reductions in microbial exposures could also lead to unexpected negative health outcomes. Previous studies have documented the contribution of diet to the rise over the past century of many modern chronic immune, metabolic, and other "lifestyle" diseases such as asthma, eczema, lupus, type 1 diabetes, and CVD [10, 26, 27]. Second, studies have shown that fruits, vegetables and fermented dairy products are the top three food groups that provide live microorganisms, and that in addition to providing live microbes, these foods also provide a significant amount of key nutrients that are lacking in the diets of children and adults, including calcium, fiber, and potassium [8]. Third, immune regulatory activities of live microorganisms can potentially improve health by reducing excessive inflammation caused by Western diets lacking in live microbes [28]. For example, eating fermented foods can reduce levels of inflammatory markers [13]. Fourth, it has been demonstrated that the ingestion of live microbes in the form of probiotics can lead to enhanced health outcomes such as prevention of necrotizing enterocolitis, functional bowel disorders, and antibiotic-associated diarrhea, treatment of ulcerative colitis, and the reduction of both the incidence and duration of common upper respiratory and gastrointestinal infections [29].

Interestingly, a cross-sectional study demonstrated that dietary intakes of live microbes are associated with lower blood pressure, BMI, and a range of other indicators related to population health [9]. Although cause and effect relationships cannot be determined, these findings do provide additional evidence supporting a link between dietary intakes of live microbes and decreased mortality. Indeed, we observed that participants with high dietary intakes of live microbes were prone to having high HEI-2015 score and dietary fiber intakes, which suggested that MedHi foods are more

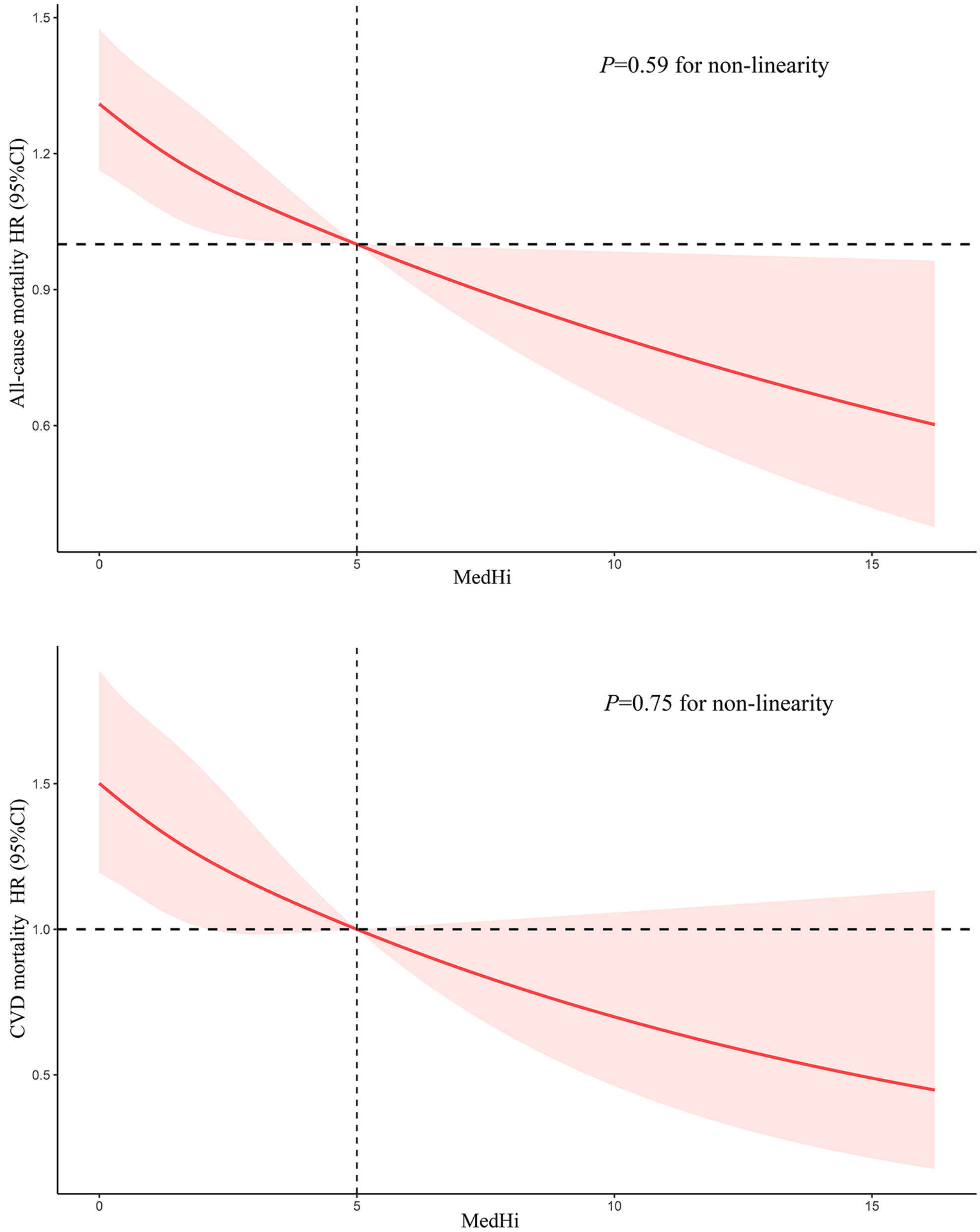

Fig. 3 Dose–response relationships of consumption of MedHi foods (per 100 g) with all-cause, and CVD-specific mortality. The reference is set at 5 points for consumption of MedHi foods. Models were adjusted by age, sex, race and race/ethnicity, educational level, family poverty income ratio, BMI, smoking status, alcohol use, total PA, CVD, hypertension, DM, hyperlipidemia, HEI-2015 score, food calorie intake and total dietary fiber intake

likely to contain higher levels of dietary fiber and better meet human needs. To separate the contribution of MedHi foods from the effects of high-quality diet, we additionally adjusted HEI-2015 score, food calorie intake and total dietary fiber intake in model 3. Interestingly, there were attenuations of associations of MedHi foods with all-cause and CVD-specific mortality, while the associations remain significant, indicating that consumption of MedHi foods is independently associated with decreased mortality.

It is noteworthy that there existed interaction effects of age and consumption of MedHi foods on all-cause and CVD-specific mortality. With advancing age, an individual's dietary habits will undergo changes due to decreased tooth and saliva function, modifications in their lifestyle, and reduced digestion and absorption capabilities [30]. Middle-aged and elderly individuals can benefit greatly from consumption of foods with medium to high amounts of live microbes, especially in light of the reduced variety in their diets and the rise in inflammatory cytokines [31].

We broke through the limitations of previous studies. First, this is the first large-scale, nationally representative cohort study to examine the association of consumption of foods with live microbes with mortality. Second, to separate the contribution of foods with live microbes from the effects of dietary patterns, we adjusted for various possible confounding dietary factors, including HEI-2015, food calorie intake, and total dietary fiber intake in models. We acknowledge the potential limitations of this study. First, there may

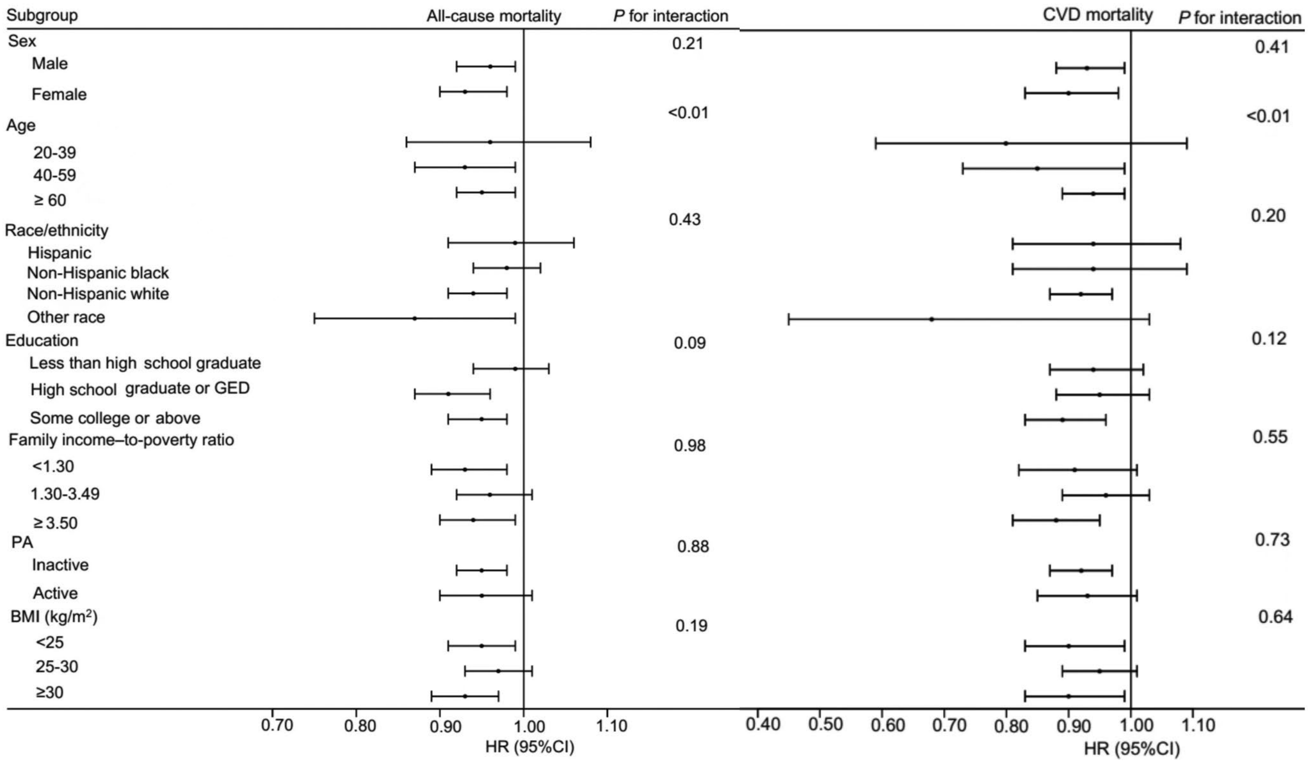

Fig. 4 Association between consumption of foods with medium to high amounts of live microbes and all-cause and CVD-specific mortality in subgroups. Models were adjusted for age, sex, race and race/ethnicity, educational level, family poverty income ratio, BMI, smoking status, alcohol use, total PA, CVD, hypertension, DM, hyperlipidemia, HEI-2015 score, food calorie intake and total dietary fiber intake

be recall bias in self-reported dietary intake and PA. Second, additional confounding by measured or unmeasured covariates may affect the observed associations. Third, our analysis utilized a classification system that did not have precise measurements of microbial types as well as may not have captured more subtle differences. Further studies are needed to measure more accurately the species and content of consumed microorganisms and to get the risk model for estimation of all-type-death of CVD development based on analyzing the missing diversity of microbial food components.

Conclusion

Consumption of foods with higher microbial concentrations is associated with a reduced risk of all-cause and CVD-specific mortality in US adults. Middle-aged and elderly individuals can benefit greatly from consumption of foods with medium to high amounts of live microbes. Given the potential for small dietary adjustments to yield substantial health advantages, it is crucial to enhance public understanding regarding the consumption of foods with high microbial concentrations. This includes incorporating the intake of live microbes into dietary evaluations and establishing suggested values for live microbes within healthy dietary regimens, similar to the current recommended values for fiber.

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Data availability The datasets supporting the conclusions of this article are available in the National Health and Nutrition Examination Survey [https://www.cdc.gov/nchs/nhanes/index.htm].

Declarations

Ethical standards The survey was approved by the Centers for Disease Control and Prevention Research Ethics Review Board. All participants have signed informed consent. This study was exempt from review because data used in this study was de-identified.

Consent for publication Not applicable.

Competing interests The authors declare they have no conflicts of interest.

References

1. Roselli M, Natella F, Zinno P, Guantario B, Canali R, Schifano E, De Angelis M, Nikoloudaki O, Gobbetti M, Perozzi G, Devirgiliis C (2021) Colonization ability and impact on human gut microbiota of Foodborne microbes from traditional or probiotic-added Fermented foods: a systematic review. Front Nutr 8:689084. https://doi.org/10.3389/fnut.2021.689084
2. Rook GA (2010) 99th Dahlem conference on infection, inflammation and chronic inflammatory disorders: darwinian medicine and the 'hygiene' or 'old friends' hypothesis. Clin Exp Immunol 160(1):70–79. https://doi.org/10.1111/j.1365-2249.2010.04133.x
3. Margină D, Ungurianu A, Purdel C, Tsoukalas D, Sarandi E, Thanasoula M, Tekos F, Mesnage R, Kouretas D, Tsatsakis A (2020) Chronic inflammation in the context of Everyday Life: dietary changes as mitigating factors. Int J Environ Res Public Health 17(11). https://doi.org/10.3390/ijerph17114135
4. Mathur H, Beresford TP, Cotter PD (2020) Health benefits of lactic acid Bacteria (LAB) fermentates. Nutrients 12(6). https://doi.org/10.3390/nu12061679
5. Sánchez B, Delgado S, Blanco-Míguez A, Lourenço A, Gueimonde M, Margolles A (2017) Probiotics, gut microbiota, and their influence on host health and disease. Mol Nutr Food Res 61(1). https://doi.org/10.1002/mnfr.201600240
6. Jeddi MZ, Yunesian M, Gorji ME, Noori N, Pourmand MR, Khaniki GR (2014) Microbial evaluation of fresh, minimally-processed vegetables and bagged sprouts from chain supermarkets. J Health Popul Nutr 32(3):391–399
7. Valentin-Bon I, Jacobson A, Monday SR, Feng PC (2008) Microbiological quality of bagged cut spinach and lettuce mixes. Appl Environ Microbiol 74(4):1240–1242. https://doi.org/10.1128/aem.02258-07
8. Marco ML, Hutkins R, Hill C, Fulgoni VL, Cifelli CJ, Gahche J, Slavin JL, Merenstein D, Tancredi DJ, Sanders ME (2022) A classification system for defining and estimating Dietary Intake of Live microbes in US adults and children. J Nutr 152(7):1729–1736. https://doi.org/10.1093/jn/nxac074
9. Hill C, Tancredi DJ, Cifelli CJ, Slavin JL, Gahche J, Marco ML, Hutkins R, Fulgoni VL 3rd, Merenstein D, Sanders ME (2023) Positive Health Outcomes Associated with Live Microbe Intake from foods, including Fermented Foods, assessed using the NHANES database. J Nutr 153(4):1143–1149. https://doi.org/10.1016/j.tjnut.2023.02.019
10. Han L, Wang Q (2022) Association of Dietary Live Microbe Intake with Cardiovascular Disease in US adults: a cross-sectional study of NHANES 2007–2018. Nutrients 14(22). https://doi.org/10.3390/nu14224908
11. Marco ML, Sanders ME, Gänzle M, Arrieta MC, Cotter PD, De Vuyst L, Hill C, Holzapfel W, Lebeer S, Merenstein D, Reid G, Wolfe BE, Hutkins R (2021) The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on fermented foods. Nat Reviews Gastroenterol Hepatol 18(3):196–208. https://doi.org/10.1038/s41575-020-00390-5
12. Chen T-C, Clark J, Riddles MK, Mohadjer LK, Fakhouri TH (2020) National Health and Nutrition Examination Survey. sample design and estimation procedures, 2015 – 2018
13. Hill C, Tancredi DJ, Cifelli CJ, Slavin JL, Gahche J, Marco ML, Hutkins R, Fulgoni VL 3rd, Merenstein D, Sanders ME (2023) Positive Health Outcomes Associated with Live Microbe Intake from foods, including Fermented Foods, assessed using the NHANES database. J Nutr. https://doi.org/10.1016/j.tjnut.2023.02.019
14. Lv JJ, Li XY, Wang JB, Yang XT, Yin MY, Yang CH (2024) Association of dietary live microbe intake with various cognitive domains in US adults aged 60 years or older. Sci Rep 14(1):5714. https://doi.org/10.1038/s41598-024-51520-x
15. Vilar-Gomez E, Nephew LD, Vuppalanchi R, Gawrieh S, Mladenovic A, Pike F, Samala N, Chalasani N (2022) High-quality diet, physical activity, and college education are associated with low risk of NAFLD among the US population. Hepatology (Baltimore MD) 75(6):1491–1506. https://doi.org/10.1002/hep.32207
16. Rattan P, Penrice DD, Ahn JC, Ferrer A, Patnaik M, Shah VH, Kamath PS, Mangaonkar AA, Simonetto DA (2022) Inverse Association of Telomere length with Liver Disease and Mortality in the US Population. Hepatol Commun 6(2):399–410. https://doi.org/10.1002/hep4.1803
17. Natto SSYAL, Midle ZS, Gyurko JB, O'Neill R, Steffensen R B (2019) Association between time since quitting smoking and periodontitis in former smokers in the National Health and Nutrition Examination Surveys (NHANES) 2009 to 2012. J Periodontol 90(1):16–25. https://doi.org/10.1002/jper.18-0183
18. Krebs-Smith SM, Pannucci TE, Subar AF, Kirkpatrick SI, Lerman JL, Tooze JA, Wilson MM, Reedy J (2018) Update of the healthy eating index: HEI-2015. J Acad Nutr Dietetics 118(9):1591–1602. https://doi.org/10.1016/j.jand.2018.05.021
19. Bull FC, Maslin TS, Armstrong T (2009) Global physical activity questionnaire (GPAQ): nine country reliability and validity study. J Phys Act Health 6(6):790–804. https://doi.org/10.1123/jpah.6.6.790
20. Piercy KL, Troiano RP, Ballard RM, Carlson SA, Fulton JE, Galuska DA, George SM, Olson RD (2018) The physical activity guidelines for americans. JAMA 320(19):2020–2028. https://doi.org/10.1001/jama.2018.14854
21. Chen C, Chen Q, Nie B, Zhang H, Zhai H, Zhao L, Xia P, Lu Y, Wang N (2020) Trends in Bone Mineral density, osteoporosis, and Osteopenia among U.S. adults with Prediabetes, 2005–2014. Diabetes Care 43(5):1008–1015. https://doi.org/10.2337/dc19-1807
22. Lin P, Chang CC, Yuan KC, Yeh HJ, Fang SU, Cheng T, Teng KT, Chao KC, Tang JH, Kao WY, Lin PY, Liu JS, Chang JS (2018) Red Blood Cell Aggregation-Associated Dietary Pattern predicts hyperlipidemia and metabolic syndrome. Nutrients 10(8). https://doi.org/10.3390/nu10081127
23. Xue Q, Pan A, Wen Y, Huang Y, Chen D, Yang CX, Hy Wu J, Yang J, Pan J, Pan XF (2021) Association between pyrethroid exposure and cardiovascular disease: a national population-based cross-sectional study in the US. Environ Int 153:106545. https://doi.org/10.1016/j.envint.2021.106545
24. Marco ML, Hill C, Hutkins R, Slavin J, Tancredi DJ, Merenstein D, Sanders ME (2020) Should there be a recommended Daily Intake of microbes? J Nutr 150(12):3061–3067. https://doi.org/10.1093/jn/nxaa323
25. Sonnenburg JL, Sonnenburg ED (2019) Vulnerability of the industrialized microbiota. Sci (New York NY) 366(6464). https://doi.org/10.1126/science.aaw9255
26. Lynch SV, Pedersen O (2016) The human intestinal microbiome in Health and Disease. N Engl J Med 375(24):2369–2379. https://doi.org/10.1056/NEJMra1600266

27. Muscogiuri G, Balercia G, Barrea L, Cignarelli A, Giorgino F, Holst JJ, Laudisio D, Orio F, Tirabassi G, Colao A (2018) Gut: a key player in the pathogenesis of type 2 diabetes? Crit Rev Food Sci Nutr 58(8):1294–1309. https://doi.org/10.1080/10408398.2016.1252712
28. Sonnenburg ED, Sonnenburg JL (2019) The ancestral and industrialized gut microbiota and implications for human health. Nat Rev Microbiol 17(6):383–390. https://doi.org/10.1038/s41579-019-0191-8
29. Merenstein DJ, Sanders ME, Tancredi DJ (2020) Probiotics as a tx resource in primary care. J Fam Pract 69(3):E1–e10
30. Biagi E, Rampelli S, Turroni S, Quercia S, Candela M, Brigidi P (2017) The gut microbiota of centenarians: signatures of longevity in the gut microbiota profile. Mech Ageing Dev 165 (Pt B) 180–184. https://doi.org/10.1016/j.mad.2016.12.013
31. Gomaa EZ (2020) Human gut microbiota/microbiome in health and diseases: a review. Antonie Van Leeuwenhoek 113(12):2019–2040. https://doi.org/10.1007/s10482-020-01474-7