Nutrition Journal

RESEARCH

Open Access

Association between sulfur microbial diet and the risk of esophageal cancer: a prospective cohort study in 101,752 American adults

Xiaorui Ren[1†], Li Xin[1†], Linglong Peng[1], Yi Xiao[1], Zhihang Zhou[2], Haoyun Luo[1], Zhiyong Zhu[1], Qi Wei[1], Yahui Jiang[1], Hongmei He[1], Ling Xiang[3], Yaxu Wang[1], Yunhao Tang[1*] and Haitao Gu[1*]

Abstract

Background Sulfur microbial diet (SMD) is a dietary pattern closely related to the intestinal load of sulfur-metabolizing microbes in humans. Diet and microbes may play an important role in the carcinogenesis of esophagus. However, epidemiological studies on SMD and esophageal cancer (EC) risk are scarce. Here, we evaluated this association based on a large American cohort.

Methods In the cohort of the Prostate, Lung, Colorectal, and Ovarian (PLCO) Cancer Screening Trial, a SMD score was calculated to evaluate participants' compliance of SMD pattern, with higher scores presenting greater adherence. Cox hazards regression model was used to explore the association between the SMD score and the incidence of EC, esophageal squamous cell carcinoma (ESCC), and esophageal adenocarcinoma (EA). Subgroup analyses were conducted to figure out potential modifiers interacting with SMD on EC. Sensitivity analyses were used to testify the robustness of our main result.

Results Among 101,752 participants, 154 EC cases, consisted of 41 ESCC cases and 97 EA cases, were identified with mean follow-up of 8.9 years. In the fully adjusted model, the highest versus the lowest quartiles of the SMD score were found to be associated with an increased risk of EC and ESCC (EC: $HR_{Q4\ vs.\ Q1}$: 1.64; 95% CI: 1.05, 2.56; $P=0.016$ for trend; ESCC: $HR_{Q4\ vs.\ Q1}$: 2.37; 95% CI: 1.02, 5.47; $P=0.031$ for trend), while not significantly associated with increases risk of EA ($HR_{Q4\ vs.\ Q1}$: 1.41; $P=0.144$ for trend). The main result remained through a series of sensitivity analyses. Subgroup analyses showed a stronger association between SMD and EC in participants with no regular consumption of aspirin ($HR_{Q4\ vs.\ Q1}$: 1.90; 95% CI: 1.04, 3.47) than in those using aspirin regularly ($HR_{Q4\ vs.\ Q1}$: 1.37; 95% CI: 0.71, 2.66) ($P=0.008$ for interaction).

†Xiaorui Ren and Li Xin contributed equally to this work.

*Correspondence:
Yunhao Tang
303790@hospital.cqmu.edu.cn
Haitao Gu
ght302211@cqmu.edu.cn

Full list of author information is available at the end of the article

Conclusion Adherence to the SMD pattern may be associated with increased risks of EC and ESCC, particularly for EC in individuals who do not regularly consume aspirin.

Keywords Sulfur microbial diet, Esophageal cancer, Sulfur-metabolizing microbes, PLCO cancer screening trial, Cox hazards regression analysis

Introduction

Esophageal cancer (EC) is reported to be the 7th most common and 6th most lethal cancer in the world, contributing to 18,440 new cases and 16,170 cancer-related deaths in America in 2020 [1, 2]. Although the incidence of EC shows a downward trend, its 5-year survival rate (20%) is the second lowest, only after pancreatic cancer [3, 4]. Therefore, it is imperative to explore some strategies to prevent the onset and progression of EC. Esophagus serves as the entrance into the gastrointestinal tract, its malignant transformation has been proved to be related to dietary habits and specific food items [5–7]. Epidemiologic studies have reported high intake of fruits, vegetables, and dietary fiber were associated with a decreased risk of EC [8, 9]. In contract, a significantly higher risk of EC was linked with higher intake of red and processed meat and higher alcohol consumption [8–10]. Recently, Nguyen et al. constructed and derived a particular pattern of food intake closely associated with the enrichment of sulfur-metabolizing microbes in human gut, namely sulfur microbial diet (SMD) [11], which is characterized by higher intake of processed meat, liquor, and low-calorie drinks, and lower intake of beer, legumes, vegetables, fruit juice, and sweets/desserts [11]. Among them, processed meat, liquor, and low-calorie drinks were found to be positively associated with the enrichment of sulfur-metabolizing microbes, whereas the remaining five components showed an inverse association [11]. Indeed, sulfur-metabolizing microbes can produce a large amount of genotoxic hydrogen sulfide gas (H_2S), which plays a vital role in tumor pathogenicity [12, 13]. Previous prospective studies have demonstrated that the SMD pattern could significantly increase the risk of digestive system neoplasms, such as colorectal cancer and early-onset colorectal adenoma [11, 14, 15]. However, the relationship between the SMD and the incidence of EC remains uncertain. Given the features of SMD and the existed links between EC and diet, we sought to examine whether SMD may influence risk of EC incidence in a US population.

Materials and methods

Study population

The sample population used in this study was derived from the Prostate, Lung, Colorectal, and Ovarian (PLCO) Cancer Screening Trial, which was a large population-based randomized controlled study. This trial aimed to ascertain whether a few screening procedures, such as colonoscopy, chest X-ray, digital rectal examination, prostate-specific antigen (PSA), cancer antigen 125 (CA-125), etc., could reduce the risk of death from PLCO malignancies [16]. In this trial, subjects were selected to participate in between November 1993 and September 2001, finally, 154,887 participants aged between 55 and 74 years were registered [16]. The registered participants were then randomly divided into the control and screening groups. Participants in the control group received standard cares, whereas those in the screening group were subjected to above-mentioned specific screening tests. All participants were asked to answer questionnaires related to demographics, lifestyle, dietary habits, health behaviors, medication use, diagnoses of diseases, and other exposures of interest. The Supplemental Questionnaire (SQX), Baseline Questionnaire (BQ), and Diet History Questionnaire (DHQ) were some of the specific questionnaires that were used in this study.

Based on the objectives of our study, the following participants were excluded: (1) Participants who failed to submit the BQ (n=4918); (2) Participants who submitted an invalid DHQ (n=38,462) (Invalid DHQ refers to DHQs that missed the completion date, subjects were deceased before DHQ completion, containing 8 or more missing/multiple frequency responses on DHQ, and having extreme energy consumption on DHQ [participants within the first and last percentile by gender]); (3) Participants having a history of cancer (excluding the nonmelanoma skin cancer) before DHQ analysis entry (n=9684); and (4) participants with outcome events (diagnoses of EC, death, loss of follow-up, and the end of follow-up) occurred before DHQ completion (n=71). The whole baseline sample included 101,752 individuals (Fig. 1). Written informed permission was required from every participant. The National Cancer Institute approved the experimental procedures and protocols used in this study (Project ID: PLCO-1134).

Assessment of sulfur microbial diet scores

The DHQ, a self-administered, 124-item food frequency questionnaire, was used to collect dietary data in this study. The DHQ was designed to assess the serving sizes and frequency of food consumed for the 12 months prior to registration, its validity and dependability have already been described [17]. Participants' consumption of each food was calculated by multiplying the serving sizes and food frequency.

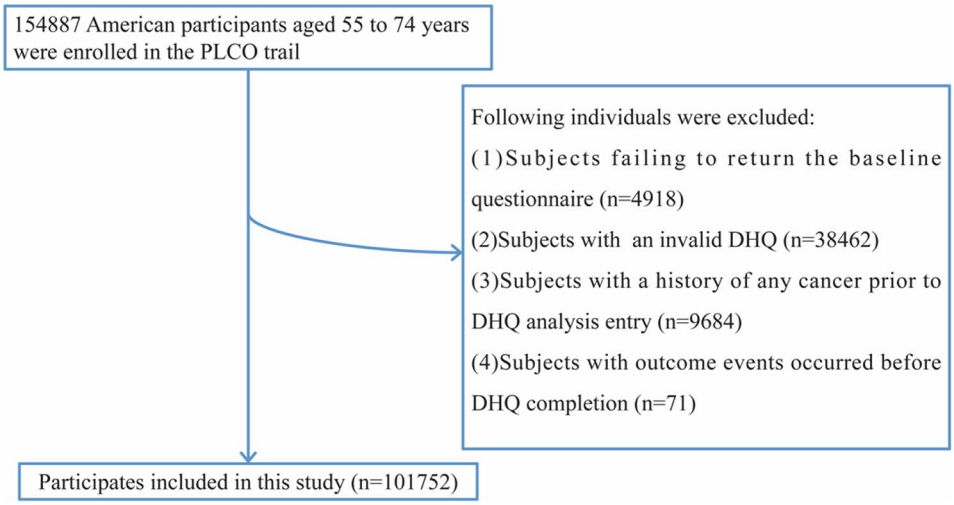

Fig. 1 The flow chart of identifying eligible subjects. PLCO: Prostate, Lung, Colorectal, and Ovarian; BQ: Baseline Questionnaire; DHQ: Diet History Questionnaire

SMD scores were calculated to quantify the participants' adherence to SMD following an established method [11]. The food component groups in SMD included liquor, processed meat, low-calorie drinks, beer, fruit juice, legumes, other vegetables (including corn, eggplant, mixed vegetables, mushrooms, celery, green pepper, and summer squash), and sweets/desserts. Each food group was ranked into quartiles and was given positive or negative scores. For example, in the case of liquor, processed meat, and low-calorie drinks, participants below the lowest quartile, received a score of 1, whereas those above the highest quartile of a food group received a score of 4. As for beer, fruit juice, legumes, other vegetables, and whole grains, the scoring pattern was reversed (Supplementary Table 1). The SMD score was computed by summing the scores of the above-mentioned 8 food groups, and the total score of every participant ranged from 8 to 32. Higher SMD scores reflected greater adherence to the SMD pattern.

Assessment of the covariates

In this study, we evaluated the individual and population level risks associated with 15 potentially modifiable risk factors. The self-reported BQ was used to gather information regarding the demographic and lifestyle characteristics, including sex, race, marital status, body mass index (BMI), educational level, smoking status, regular consumption of aspirin, history of diabetes, history of hypertension, pack-years of smoking, and family history of EC. The terms "white" and "non-white" were used to represent the race, the marital status was described as "married or living as married" and "other", and the educational level of the participants were described as "college below", "college graduate", and "postgraduate". BMI was computed by dividing the weight (kg) by height (m^2). Other risk factors that were evaluated using the aforementioned DHQ included age at DHQ completion, energy consumption from the diet, intake of pickled vegetables and fruits. The SQX was used to collect data on physical activity levels, which were calculated as the weekly sum of self-reported minutes of moderate to vigorous activity.

Outcome ascertainment

To gather data regarding newly diagnosed EC cases, the date of diagnosis, and more detailed relative information, participants were emailed a self-reporting annual study update form. Researchers checked the participants' medical records after obtaining the consent of them to validate the diagnosis. Family reports and death certificates were used as supplemental information to identify deaths. It should be noted that the diagnosis of EC (ICD-O-2 codes: C150-C15, C153-155, and C158-C159) was the primary outcome of this study. The diagnoses of esophageal squamous cell carcinoma (ESCC) and esophageal adenocarcinoma (EA) were the secondary endpoints (ICD-O-2 codes: ESCC: 8070, and 8071; EA: 8140, 8480, and 8481).

Statistical analysis

This study includes a few variates with missing data. For variables containing < 5% missing values, categorical covariates such as marital status, educational level, aspirin consumption, history of diabetes and hypertension, family history of EC, and smoking status were imputed by the modal value; while the continuous covariates namely BMI and pack-years of smoking were imputed by the median value. In our analysis, the "physical activity levels" variable with > 25% missing values was assumed to be missing at random and implement using the Multivariate

Imputation by Chained Equations (MICE) approach [18], which is a robust method of data imputation. In this method, missing data were replaced by iteratively drawing from the fitted conditional distributions of partially observed variables, given the observed and imputed values of the remaining variables in the imputation model. In this study, 25 imputed datasets were created for physical activity. Considering the heterogeneity of the imputed results, we took the mean value of the 25 imputed results for physical activity and applied it as a covariate in the final analysis. Supplementary Tables 2 and Supplementary Table 3 presents additional details regarding the relevant data imputation.

To calculate the hazard ratios (HRs) and 95% confidence intervals (CIs) of the EC, ESCC and EA incidence related to SMD scores, the Cox proportional hazards regression models were utilized. The follow-up period spanned from the DHQ completion date to the date of EC diagnosis, death, loss of follow-up, or the completion of follow-up (December 31, 2009), whichever occurred first (Fig. 2). Person-years were calculated by summing up the follow-up time of each participant and were used as the time variable. For all analyses, the SMD scores were divided into quartiles, and the first quartile (Q1) was set as the reference group. To estimate the linear trends of the association, the median scores of SMD in every quartile was assigned to every participant in this quartile, and the scores were regarded as continuous variables to conduct Cox regression analyses and acquire the P-value for trend. To adjust for potential relevant confounders, two multivariate Cox proportional hazards regression models were used. Particularly, Model 1 adjusted for a variety of demographic variables, including sex, age, race, educational level, and marital status. Potential effect moderating covariates, such as BMI, regular consumption of aspirin, levels of physical activity, history of hypertension, history of diabetes, energy intake from diet, smoking status, pack-years of smoking, and consumption of pickled vegetables/fruit, were further adjusted in Model 2. The multivariable-adjusted HRs and 95% CIs associated with a 1-point increment in SMD score were also estimated. In this study, we employed a restricted cubic spline (RCS) model with three strategically placed knots at the 10th, 50th, and 90th percentiles to investigate the nonlinear association between the SMD score and the risk of EC and its subtypes after adjusting for confounders. The structure of the RCS model, with its first knot dictating linear behavior at the lower exposure range, internal knots allowing the model to introduce nonlinearity within these intervals, and the final knot ensuring linear behavior at the upper range, was designed to effectively captures the complexity of the dose-response relationship. The "second spline" coefficient, representing the spline function's coefficient after the first knot, is key to identifying the nonlinearity between the variable and the response.

Prespecified subgroup analyses were conducted to assess the probable effect of the interaction factors, such as sex (male or female), age (>65 or ≤65 years), BMI (>30 kg/m^2 or ≤30 kg/m^2), regular consumption of aspirin (yes or no), smoking status (never or current/former), history of diabetes (yes or no), history of hypertension (yes or no), dietary energy intake (>medium or ≤medium), and physical activity levels (>medium or ≤medium). We used the likelihood ratio test as a mean to obtain P value in assessing the effect of adding the interaction term into a multivariate linear mixed-effects model. To test the robustness of the findings, some

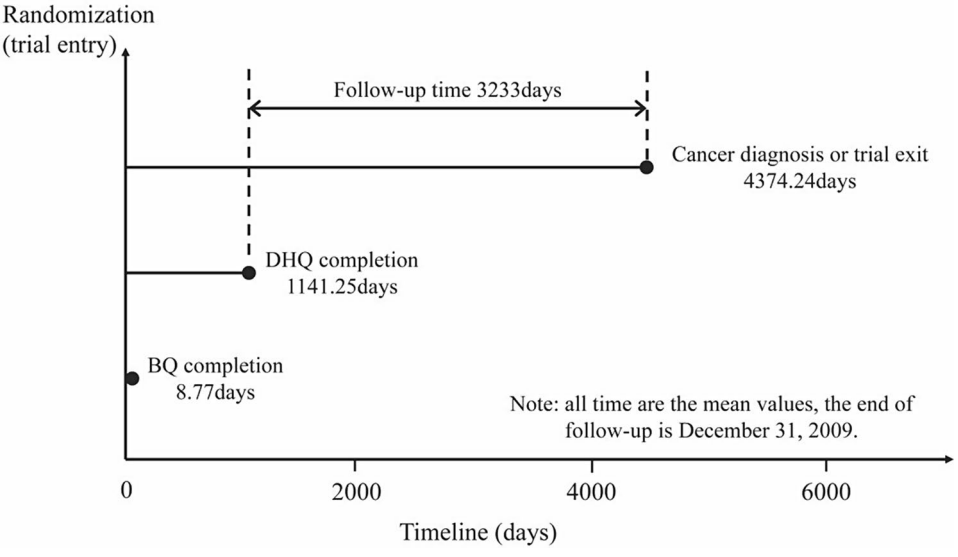

Fig. 2 The timeline and follow-up scheme of our study

sensitivity analyses were carried out as follows: (1) Firstly, the participants with family history of EC were excluded; (2) Secondly, the participants in the control group were excluded; (3) Thirdly, participants with extreme BMI (defined as top 1% and bottom 1%) were excluded; (4) Additionally, participants with unbelievable energy intake (>4000 kcal/day or <500 kcal/day) [19] was excluded; (5) Physical activity were excluded from covariates to help better rule out the impact of such high missingness; (6) Finally, cases observed within the first 1 years of follow-up were exclude to test the potential inverse causation.

Cox regression analyses for each of these SMD components in relation to the risk of EC were further conducted to ascertain the principal contributing components to this relationship.

All data were statistically analyzed using the R (ver. 4.2.1) software. A two-tailed P-value<0.05 indicated the significance level.

Results

Participant characteristics

A total of 101,752 participants were included in our analysis, which consisted of 49,494 (48.64%) males and 52,258 (51.36%) females, with the average (standard deviation) age of 65.53 (5.73). The mean (standard deviation) SMD score of subjects was 20.14 (3.09). According to the SMD scores, subjects were divided into quartiles [Quartile 1 (SMD score≤18), *n*=30685; Quartile 2 (SMD score 19–20), *n*=25085; Quartile 3 (SMD score 21–22), *n*=23103; Quartile 4 (SMD score≥23), *n*=22879] (Table 1). The higher the SMD scores, the greater the adherence to SMD. In comparison to the participants included in Quartile 1, those included in Quartile 4 were more likely to be male, white, and current/former smokers. In addition, the participants in the highest quartile of SMD scores showed higher pack-years of smoking, higher BMI, no aspirin regularly consumption, lower physical activity levels, energy intake from diet, and pickled vegetables/fruit intake versus the lowest quartiles.

SMD scores and the risk of esophageal cancer and its subtypes

During follow-up period (900,654 person years, median follow-up time 9.4 years), 154 incident EC events, consisted of 41 ESCC cases and 97 EA cases, occurred with an incidence rate of 1.7 per 10,000 person-years. In the unadjusted model, participants in the highest quartile had a significantly higher risk of EC than those in the lowest quartile ($HR_{Q4\ vs.\ Q1}$: 2.05; 95% CI: 1.33, 3.14; $P<0.001$ for trend; HR per 1-point increment: 1.11; 95% CI: 1.05, 1.17; $P<0.001$) (Table 2). After full adjustment for potential confounders, positive association of the SMD score with the risk of EC was also observed ($HR_{Q4\ vs.\ Q1}$: 1.64; 95% CI: 1.05, 2.56; $P=0.016$ for trend; HR per 1-point increment: 1.08; 95% CI: 1.02, 1.14; $P=0.005$) (Table 2). A similar positive relationship between SMD score and the risk of ESCC was documented ($HR_{Q4\ vs.\ Q1}$: 2.37; 95% CI: 1.02, 5.47; $P=0.031$ for trend; HR per 1-point increment: 1.14; 95% CI: 1.03, 1.26; $P=0.014$). However, in the Cox regression analysis for EA, we did not observe significant positive association ($HR_{Q4\ vs.\ Q1}$: 1.41; 95% CI: 0.81, 2.45; $P=0.144$ for trend; HR per 1-point increment: 1.07; 95% CI: 1.00, 1.14; $P=0.062$) (Table 2). The restricted cubic spline model indicated that SMD scores were positively related to EC and ESCC incidence in a linear dose-dependent manner ($P>0.05$ for nonlinearity) (Fig. 3).

Additional analyses

In subgroup analyses of age, sex, BMI, regular consumption of aspirin, smoking status, food energy intake from diet, history of hypertension, history of diabetes, and physical activity levels, the association between SMD score and EC risk was stronger in participants with no regular consumption of aspirin ($HR_{Q4\ vs.\ Q1}$: 1.90; 95% CI: 1.04, 3.47) than in those using aspirin regularly ($HR_{Q4\ vs.\ Q1}$: 1.37; 95% CI: 0.71, 2.66) ($P=0.008$ for interaction) (Table 3). A high robustness of our primary result was obtained with a series of sensitive analyses (Table 4). The positive association between the SMD score and the EC incidence was still recorded when participants with a family history of EC, and in the control group were separately excluded. Additionally, after excluding participants with extreme BMI levels (defined as top 1% and bottom 1%) or extreme levels of energy intake (>4000 kcal/day or <500 kcal/day) [19], no significant alterations in the final results were observed (Table 4). Furthermore, when excluded physical activity from covariates, the positive association between SMD and EC still existed. Excluding cases observed within the first 1 years of follow-up, the main result remained (with a marginal P value=0.052) (Table 4).

Individual components of SMD and the risk of esophageal cancer

According to Supplementary Table 4, for processed meat, liquor, low-calorie drinks, beer, fruit juice, legumes, other vegetables, and sweets/desserts, no significant associations were observed with the risk of EC.

Discussion

In the multicenter cohort of American adults from PLCO Cancer Screening Trail, we found that greater adherence to SMD pattern was associated with an increased risk of EC and ESCC. Interestingly, a significant stronger positive associate between SMD pattern and EC was found in participants without regular consumption of aspirin. The restricted cubic spline models showed that the

Table 1 Baseline characteristics of study population according to sulfur microbial diet score*

		Quartiles of sulfur microbial diet score			
Characteristics	**Overall**	**Quartile 1 (≤ 18)**	**Quartile 2 (19–20)**	**Quartile 3 (21–22)**	**Quartile 4 (≥ 23)**
Number of participants	101,752	30,685	25,085	23,103	22,879
sulfur microbial diet score	20.14 ± 3.09	16.56 ± 1.49	19.51 ± 0.50	21.47 ± 0.50	24.31 ± 1.44
Age	65.53 ± 5.73	65.92 ± 5.80	65.63 ± 5.74	65.44 ± 5.69	64.99 ± 5.63
Sex					
Male	49,494 (48.64%)	13,081 (42.63%)	12,069 (48.11%)	11,867 (51.37%)	12,477 (54.53%)
Female	52,258 (51.36%)	17,604 (57.37%)	13,016 (51.89%)	11,236 (48.63%)	10,402 (45.47%)
Race					
White	94,063 (92.44%)	27,887 (90.88%)	23,133 (92.22%)	21,501 (93.07%)	21,542 (94.16%)
Non-white	7689 (7.56%)	2798 (9.12%)	1952 (7.78%)	1602 (6.93%)	1337 (5.84%)
Education level					
College below	64,953 (63.83%)	19,144 (62.39%)	16,015 (63.84%)	14,930 (64.62%)	14,864 (64.97%)
College graduate	17,847 (17.54%)	5307 (17.30%)	4415 (17.60%)	4062 (17.58%)	4063 (17.76%)
Postgraduate	18,952 (18.63%)	6234 (20.32%)	4655 (18.56%)	4111 (17.79%)	3952 (17.27%)
Marital status					
Married or living as married	79,823 (78.45%)	23,928 (77.98%)	19,882 (79.26%)	18,205 (78.80%)	17,808 (77.84%)
Other	21,929 (21.55%)	6757 (22.02%)	5203 (20.74%)	4898 (21.20%)	5071 (22.16%)
Body mass index (kg/m^2)	27.22 ± 4.79	26.64 ± 4.65	27.26 ± 4.80	27.45 ± 4.79	27.74 ± 4.88
Smoking status					
Never	48,579 (47.74%)	16,472 (53.68%)	12,372 (49.32%)	10,657 (46.13%)	9078 (39.68%)
Current	9401 (9.24%)	2185 (7.12%)	2069 (8.25%)	2257 (9.77%)	2890 (12.63%)
Former	43,772 (43.02%)	12,028 (39.20%)	10,644 (42.43%)	10,189 (44.10%)	10,911 (47.69%)
Smoking pack-years	17.65 ± 26.59	14.22 ± 24.04	16.53 ± 25.82	18.76 ± 27.36	22.37 ± 29.02
Regular consumption of aspirin					
No	53,953 (53.02%)	16,635 (54.21%)	13,299 (53.02%)	12,141 (52.55%)	11,878 (51.92%)
Yes	47,799 (46.98%)	14,050 (45.79%)	11,786 (46.98%)	10,962 (47.45%)	11,001 (48.08%)
Arm					
Intervention	51,816 (50.92%)	15,561 (50.71%)	12,866 (51.29%)	11,889 (51.46%)	11,500 (50.26%)
Control	49,936 (49.08%)	15,124 (49.29%)	12,219 (48.71%)	11,214 (48.54%)	11,379 (49.74%)
History of diabetes					
No	94,947 (93.31%)	29,355 (95.67%)	23,496 (93.67%)	21,419 (92.71%)	20,677 (90.38%)
Yes	6805 (6.69%)	1330 (4.33%)	1589 (6.33%)	1684 (7.29%)	2202 (9.62%)
History of hypertension					
No	68,707 (67.52%)	21,395 (69.72%)	16,905 (67.39%)	15,439 (66.83%)	14,968 (65.42%)
Yes	33,045 (32.48%)	9290 (30.28%)	8180 (32.61%)	7664 (33.17%)	7911 (34.58%)
Family history of esophageal cancer					
No	98,281 (96.59%)	29,640 (96.59%)	24,264 (96.73%)	22,292 (96.49%)	22,085 (96.53%)
Yes	813 (0.80%)	264 (0.86%)	184 (0.73%)	177 (0.77%)	188 (0.82%)
Possible	2658 (2.61%)	781 (2.55%)	637 (2.54%)	634 (2.74%)	606 (2.65%)
Physical activity level (min/week)	122.75 ± 108.73	134.60 ± 111.96	124.37 ± 108.43	117.52 ± 107.21	110.38 ± 104.39
Energy intake from diet (kcal/day)	1738.64 ± 736.43	1884.10 ± 751.86	1754.13 ± 742.20	1664.84 ± 719.77	1601.07 ± 688.22
Pickled Vegetables/Fruit intake (g/day)	2.10 ± 2.43	2.19 ± 2.43	2.18 ± 2.45	2.07 ± 2.44	1.93 ± 2.41
Components of sulfur microbial diet intake					
Processed meat (g/day)	17.02 ± 18.84	13.13 ± 16.39	16.51 ± 18.32	18.22 ± 19.25	21.60 ± 20.83
Liquor (g/day)	10.68 ± 46.12	4.27 ± 24.24	8.26 ± 36.56	12.14 ± 45.67	20.48 ± 70.30
Low-calorie drinks (g/day)	218.58 ± 393.85	107.95 ± 245.79	182.88 ± 333.89	245.94 ± 405.08	378.46 ± 526.13
Beer (g/day)	99.19 ± 485.05	100.69 ± 450.04	98.16 ± 468.04	102.79 ± 521.02	94.65 ± 510.30
Fruit juice (g/day)	45.90 ± 157.27	71.64 ± 188.44	49.22 ± 163.67	34.48 ± 132.09	19.26 ± 116.81
Legumes (g/day)	35.38 ± 43.76	53.63 ± 57.48	36.34 ± 40.65	26.62 ± 29.75	18.69 ± 23.98
Other Vegetables (g/day)	20.79 ± 44.67	37.86 ± 58.01	20.96 ± 44.00	12.31 ± 32.28	6.28 ± 22.59
Sweets & desserts (g/day)	39.62 ± 42.66	47.58 ± 43.66	41.99 ± 44.23	37.07 ± 42.50	28.93 ± 36.86

*Values are means (standard deviation) for continuous variables and percentages for categorical variables

Table 2 Hazard ratios of the association of sulfur microbial diet scores with the risk of esophageal cancer and its subtypes

	No. of participants	No. of cases	Hazard ratio (95% confidence interval)			
Quartiles of SMD score			**Person-years**	**Unadjusted**	**Model 1[a]**	**Model 2[b]**
Esophageal Cancer						
Quartile 1 (≤ 18)	30,685	35	274480.10	1.00 (reference)	1.00 (reference)	1.00 (reference)
Quartile 2 (19–20)	25,085	28	222019.20	0.99 (0.60, 1.63)	0.93 (0.56, 1.53)	0.90 (0.55, 1.49)
Quartile 3 (21–22)	23,103	39	204082.00	1.50 (0.95, 2.37)	1.35 (0.85, 2.13)	1.29 (0.81, 2.05)
Quartile 4 (≥ 23)	22,879	52	200072.70	2.05 (1.33, 3.14)	1.80 (1.17, 2.77)	1.64 (1.05, 2.56)
P for trend				< 0.001	0.004	0.016
SMD as a continuous variable (per 1 score)				1.11 (1.05, 1.17)	1.09 (1.04, 1.15)	1.08 (1.02, 1.14)
P for trend				< 0.001	0.001	0.005
Esophageal Squamous Cell Carcinoma						
Quartile 1 (≤ 18)	30,685	10	274480.10	1.00 (reference)	1.00 (reference)	1.00 (reference)
Quartile 2 (19–20)	25,085	6	222019.20	0.75 (0.27, 2.05)	0.76 (0.28, 2.09)	0.80 (0.29, 2.22)
Quartile 3 (21–22)	23,103	10	204082.00	1.35 (0.56, 3.25)	1.39 (0.58, 3.35)	1.51 (0.62, 3.70)
Quartile 4 (≥ 23)	22,879	15	200072.70	2.08 (0.93, 4.62)	2.21 (0.99, 4.97)	2.37 (1.02, 5.47)
P for trend				0.051	0.038	0.031
SMD as a continuous variable (per 1 score)				1.12 (1.01, 1.23)	1.13 (1.02, 1.25)	1.14 (1.03, 1.26)
P for trend				0.027	0.018	0.014
Esophageal Adenocarcinoma						
Quartile 1 (≤ 18)	30,685	23	274480.10	1.00 (reference)	1.00 (reference)	1.00 (reference)
Quartile 2 (19–20)	25,085	16	222019.20	0.86 (0.45, 1.63)	0.78 (0.41, 1.48)	0.74 (0.39, 1.41)
Quartile 3 (21–22)	23,103	25	204082.00	1.46 (0.83, 2.58)	1.25 (0.71, 2.21)	1.16 (0.65, 2.06)
Quartile 4 (≥ 23)	22,879	33	200072.70	1.97 (1.16, 3.36)	1.62 (0.95, 2.76)	1.41 (0.81, 2.45)
P for trend				0.007	0.046	0.144
SMD as a continuous variable (per 1 score)				1.11 (1.04, 1.18)	1.08 (1.02, 1.16)	1.07 (1.00, 1.14)
P for trend				0.001	0.015	0.062

a: model 1 was adjusted for age(years), sex (male, female), race (white, non-white), marital status (married or living as married, other), education level (college below, college graduate, postgraduate)

b: model 2 added other potential risk factors: smoking status (never, current, former), pack-years of smoking (continuous), body mass index (kg/m^2), aspirin use (no, yes), history of diabetes (no, yes), history of hypertension (no, yes), energy intake from diet (kcal/day), physical activity (min/week), intake of pickled vegetables/fruit(g/day)

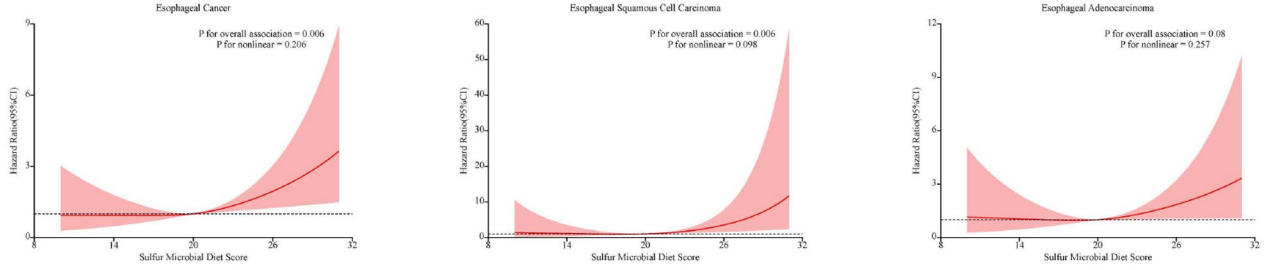

Fig. 3 Dose-response analysis on the association of sulfur microbial diet score with the risk of esophageal cancer. Hazard ratio was adjusted for age (years), sex (male, female), race (white and non-white), marital status (married or living as married, other), smoking status (never, current, former), pack-years of smoking, educational level (college below, college graduate, post graduate), physical activity levels (min/week), body mass index (kg/m^2), aspirin use (yes, no), pickled vegetables/fruit intake (g/day), history of diabetes (yes, no), history of hypertension (yes, no), and energy intake from diet (kcal/day)

relationships were linear and dose-dependent, and the finding was validated by multiple sensitivity analyses.

Researches on the relationship between dietary patterns and EC risk began as early as the 20th century. These included studies on the Dietary Inflammatory Index [20], Mediterranean Diet [21], and glycemic index and glycemic load diet [22]. It is worth noting that our study was the first to identify the positive association between SMD pattern and the risk of EC and ESCC. Previous studies have shown that adherence to SMD may lead to the enrichment of sulfur-metabolizing microbes, including *Bacteroides*, *Acidaminococcus*, *Escherichia coli (E. coli)*, *Parabacteroides*, *Streptococcus*, and *Veillonella sp.*, et al., in the human gut [14]. Dysbiosis of gut microbiota is possible response for the occurrence and development of EC. Several existing studies have provided insights into this relationship. Zaidi et al. figured out that *E. coli* primarily existed in Barrett's esophagus and

Table 3 Subgroup analyses on the association of SMD scores with the risk of esophageal cancer

Subgroup variable	No. of cases	Hazard Ratio (95% Confidence Interval) Sulfur Microbial Diet Scores [a]					*P* for trend	*P* for interaction
		Person-years	Quartile 1 (≤ 18)	Quartile 2 (19–20)	Quartile 3 (21–22)	Quartile 4 (≥ 23)		
Age (years)								0.600
≤ 65	61	470143.53	1.00 (reference)	0.86 (0.37, 1.99)	1.09 (0.49, 2.42)	1.87 (0.92, 3.80)	0.042	
> 65	93	430510.50	1.00 (reference)	0.90 (0.48, 1.68)	1.34 (0.76, 2.38)	1.36 (0.76, 2.43)	0.236	
Sex								0.523
Male	131	433166.85	1.00 (reference)	1.03 (0.60, 1.80)	1.45 (0.87, 2.43)	1.74 (1.05, 2.86)	0.018	
Female	23	467487.18	1.00 (reference)	0.48 (0.13, 1.79)	0.75 (0.22, 2.48)	1.42 (0.50, 4.02)	0.597	
Body mass index (kg/m^2)								0.236
≤ 30	114	699902.04	1.00 (reference)	1.23 (0.70, 2.17)	1.50 (0.86, 2.62)	2.07 (1.22, 3.52)	0.005	
> 30	40	200751.99	1.00 (reference)	0.31 (0.10, 0.97)	0.82 (0.35, 1.93)	0.84 (0.37, 1.91)	0.951	
Physical activity (min/week)								0.184
≤Medium [b]	62	457406.83	1.00 (reference)	0.77 (0.36, 1.64)	1.00 (0.49, 2.03)	0.92 (0.45, 1.86)	0.891	
> Medium [b]	92	443247.20	1.00 (reference)	1.02 (0.53, 1.98)	1.58 (0.86, 2.93)	2.58 (1.46, 4.57)	0.001	
Smoking status								0.981
Never	34	436496.59	1.00 (reference)	0.73 (0.26, 2.02)	1.26 (0.50, 3.17)	1.49 (0.58, 3.79)	0.373	
Current/Former	120	464157.45	1.00 (reference)	0.96 (0.54, 1.71)	1.31 (0.76, 2.25)	1.71 (1.03, 2.85)	0.022	
Aspirin consumption								**0.008**
No	82	481469.63	1.00 (reference)	1.30 (0.70, 2.43)	0.85 (0.42, 1.74)	1.90 (1.04, 3.47)	0.045	
Yes	72	419184.40	1.00 (reference)	0.47 (0.19, 1.14)	1.71 (0.91, 3.23)	1.37 (0.71, 2.66)	0.177	
History of diabetes								0.626
No	143	844507.89	1.00 (reference)	0.98 (0.59, 1.64)	1.36 (0.84, 2.21)	1.79 (1.12, 2.84)	0.008	
Yes	11	56146.14	1.00 (reference)	0.24 (0.02, 2.33)	0.62 (0.12, 3.16)	0.57 (0.12, 2.75)	0.655	
History of hypertension								0.502
No	97	613252.76	1.00 (reference)	1.00 (0.52, 1.92)	1.68 (0.94, 3.02)	1.94 (1.09, 3.46)	0.014	
Yes	57	287401.27	1.00 (reference)	0.77 (0.35, 1.67)	0.78 (0.35, 1.71)	1.23 (0.61, 2.46)	0.505	
Energy intake from diet (kcal/day)								0.437
≤Medium [c]	67	450579.80	1.00 (reference)	0.88 (0.39, 1.96)	1.36 (0.67, 2.79)	1.18 (0.58, 2.42)	0.554	
> Medium [c]	87	450074.23	1.00 (reference)	0.90 (0.47, 1.70)	1.15 (0.61, 2.14)	2.00 (1.15, 3.49)	0.007	

[a]Hazard ratios were adjusted for age(years), sex (male, female), race (white, non-white), marital status (married or living as married, other), education level (college below, college graduate, postgraduate), smoking status (never, current, former), pack-years of smoking (continuous), body mass index (kg/m^2), aspirin use (no, yes), history of diabetes (no, yes), history of hypertension (no, yes), energy intake from diet (kcal/day), physical activity (min/week), intake of pickled vegetables/fruit(g/day)

[b]The median value of physical activity was 105 min/week in the study

[c]The median value of energy intake from diet was 1607.53 kcal/day in this study

esophageal adenocarcinoma in their analysis of 54 samples [23]. Another study of Narikiyo M et al. suggested the adverse effects of *Streptococcus* on EC and the eradication of *Streptococcus* can reduce the risk of EC recurrence [24]. In addition, *Bacteroidetes* were also proved to significantly increase in EC samples [25, 26]. H_2S, as one of the major metabolic products of sulfur-metabolizing microbes, has been found to be associated with tumor formation. Some studies have indicated that excessive production of H_2S is linked to the development of tumors [27, 28], and the production of H_2S increased in many cancers including colorectal cancer, gastric cancer, etc [29]. However, there is no consensus on the association between H_2S and EC.

Several mechanisms could be used to explain the detrimental effect of sulfur-metabolizing microbes on the incidence of EC. (1) DNA damage: Gut microbes can regulate the bile acid metabolism through different hydrolase enzyme activities, whereas the dysregulation of intestinal microbiota could lead to an increase in deoxycholate secretion, which induces DNA damage [30]. High concentrations of H_2S, as the main metabolite of sulfur-metabolizing microbes, can also cause DNA damage [31, 32]. (2) Altered host cell proliferation and death: Excessive H_2S can diffuse into blood [33] and enter the esophageal tissue, where it promotes cell proliferation, anti-apoptosis, and angiogenesis by the up-regulation of HSP90, which causes cancerization and migration of

Table 4 Sensitivity analyses on the association of sulfur microbial diet scores with the risk of esophageal cancer [a]

Categories	No. of Participants	No. of Cases	Hazard Ratio (95% Confidence Interval) by Sulfur Microbial Diet Scores [a]				*P* for trend
			Quartile 1 (≤ 18)	Quartile 2 (19–20)	Quartile 3 (21–22)	Quartile 4 (≥ 23)	
Excluded participants with a family history of esophageal cancer [b]	98,281	147	1.00 (reference)	0.87 (0.52, 1.45)	1.31 (0.82, 2.10)	1.62 (1.03, 2.55)	0.020
Excluded participants who were in control group [c]	51,816	69	1.00 (reference)	1.53 (0.68, 3.42)	1.62 (0.73, 3.61)	2.59 (1.23, 5.45)	0.008
Excluded participants with extreme body mass index [d]	99,761	152	1.00 (reference)	0.92 (0.56, 1.52)	1.31 (0.82, 2.09)	1.62 (1.04, 2.55)	0.020
Excluded participants with extreme energy intake [e]	100,276	150	1.00 (reference)	0.88 (0.53, 1.47)	1.26 (0.79, 2.01)	1.60 (1.02, 2.51)	0.023
Excluded physical activity from covariates [f]	101,752	154	1.00 (reference)	0.90 (0.54, 1.48)	1.27 (0.80, 2.02)	1.61 (1.03, 2.50)	0.020
Excluded cases observed within the first 1 years of follow-up	101,740	142	1.00 (reference)	0.88 (0.52, 1.47)	1.23 (0.76, 1.98)	1.51 (0.95, 2.39)	0.052

a HR were adjusted for age(years), sex (male, female), race (white, non-white), marital status (married or living as married, other), education level (college below, college graduate, postgraduate), smoking status (never, current, former), pack-years of smoking (continuous), body mass index (kg/m^2), aspirin use (no, yes), history of diabetes (no, yes), history of hypertension (no, yes), energy intake from diet (kcal/day), physical activity (min/week), and intake of pickled vegetables/fruit(g/day)

b HR was not adjusted for family history of esophageal cancer

c HR was not adjusted for control group

d HR was not adjusted for extreme BMI

e HR was not adjusted for extreme energy intake

f physical activity has a high missingness of more than 25%, which may have a significant impact on the results

cells [34]. (3) Inflammation: The gut microbes and their metabolites promote inflammation. Some Gram-negative rods, such as *E. coli*, can induce chronic inflammation and promote a cascade of reactions leading to EC [35]. A few of the compounds like Lipopolysaccharide (LPS), activated the NF-κB signaling pathway, which led to an increase in the inflammatory cytokine levels [36]. The accumulation of inflammatory cytokine causes angiogenesis, cell proliferation and invasion, mutation, and finally tumor formation [37]. (4) Immune regulation: Massive accumulation of sulfur-metabolizing microbes and H_2S lead to an increase in intestinal permeability, which allows the gut microbes to translocate into the body, and disrupt immunological homeostasis by inducing innate and adaptive immune responses [38–40] and affect the development of immune cells like neutrophils, T lymphocytes, B lymphocytes, etc [41]. The immune system can also be stimulated by the structural components of bacteria, such as flagellin, LPS, etc [42]. Interestingly, gut microorganisms can cause immunosuppression, which lowers anticancer immunity [43]. However, there is currently no research focusing on the mechanisms underlying the relationship between sulfur-metabolizing microbes, H_2S and EC.

The SMD pattern showed a positive association with EC and ESCC, but only a statistically significant association between each 1 score increment of SMD and EA in our results. ESCC and EA are two common histological subtypes of EC, and they display considerable differences in pathogenesis, epidemiological characteristics, and other aspects [44]. ESCC is closely related to factors such as smoking, alcohol consumption, intake of hot foods, and chronic esophagitis, whereas EA is more closely related to gastroesophageal reflux disease, Barrett's esophagus, and obesity. The incidence of ESCC is higher in Asian and African regions, while EA is more prevalent in Western countries. Our study population is based in the United States, and we only recorded 39 cases of ESCC, which may be related to the lower incidence of ESCC in Western countries. Moreover, this study aimed to explore the association between diet and EC, and previous research suggested that the link between ESCC and diet was more pronounced, which may explain why we found a positive correlation in ESCC but not in EA. Of course, given that we only recorded 39 cases of ESCC, we cannot dismiss the possibility that this is a chance finding, and larger population studies are required to confirm our conclusions.

In our subgroup analyses, we found a stronger inverse correlation between SMD score and EC incidence in participants without regular consumption of aspirin. In fact, as early as 2003, Douglas A Corley et al. discovered the protective effect of aspirin on EC [45]. Their meta-analysis results showed that aspirin/NSAIDs could reduce the risk of EC by 43%. In addition, they found that regular use of aspirin was associated with a lower risk of EC compared to intermittent use. The possible mechanisms include: (1) Aspirin can inhibit the activity of cyclooxygenase-2 (COX-2), which act as Prostaglandin Synthetase and is highly expressed in EC tissue and involved in

some key cellular activities such as cell proliferation and apoptosis [46]; (2) Aspirin can promote tumor apoptosis through activation of the NF-κB signaling pathway or upregulation of anti-cancer gene expression [46, 47]; (3) Aspirin can regulate human gut microbes: increase beneficial bacteria and reduce pathogenic bacteria, such as *Bacteroides* [48]. Therefore, in populations using aspirin regularly, the adverse effects of SMD on EC may be counteracted by the action of aspirin, which needs more research to testify.

A major strength of this study is that we firstly estimate the correlation between SMD (which is a microbe-associated dietary pattern) and the risk of EC. This study offered additional insights into the pathogenesis of EC. Secondly, the data collection instrument used for collecting the dietary data was well-validated. As a prospective designed cohort analysis, the recall and selection bias were minimized. Thirdly, by focusing on dietary patterns rather than specific food items, the interactions between foods could be taken into consideration, and the probability of accidental statistical significance was reduced. Lastly, we could adjust for multiple confounding factors in the prospective cohort study.

Despite the above superiorities, this study exhibited a few limitations. Firstly, a limited number of EC patients were studied in this study, there were only 41 cases of ESCC and 97 cases of EA. There were limited data regarding the molecular subtypes, which affected our ability to determine the associations between different subtypes and the robustness of the statistical outcomes. Secondly, all participants in this study were American and aged more than 55, which affected the generalization of the findings. Thirdly, no study has determined the probable mechanism between the SMD, sulfur-metabolizing microbes and EC to date. Finally, many studies have already confirmed the impact of cooking methods and temperatures on EC [49, 50], relevant data was lack in our study, so we cannot take it into consideration. Further researches are needed to fill these gaps.

Conclusion

In conclusion, our results indicated positive associations between SMD pattern and EC, as well as ESCC, and the association between SMD and EC was more pronounced in participants with no regular consumption of aspirin. Therefore, avoiding SMD pattern may be beneficial for EC prevention.

Abbreviations

SMD	Sulfur microbial diet
EC	Esophageal cancer
ESCC	Esophageal squamous cell carcinoma
EA	Esophageal adenocarcinoma
BMI	Body mass index
DHQ	Dietary history questionnaire
BQ	Baseline questionnaire
SQX	Supplemental questionnaire
HRs	Hazard ratios
CIs	Confidence intervals
NCI	National cancer institute
PLCO	Prostate lung colorectal and ovarian
SD	Standard deviation
H_2S	Hydrogen sulfide gas
E. coli	Escherichia coli
LPS	Lipopolysaccharide

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12937-024-01035-y.

Supplementary Material 1: Supplementary Table 1. Criteria for determining the sulfur microbial diet scores. Supplementary Table 2. Distribution of variables with missing data before and after imputation. Supplementary Table 3. The distribution of missing categories by sulfur microbial diet score. Supplementary Table 4. Association between sulfur microbial diet components (8 food groups) and the risk of esophageal cancer stratified according to their scores

Acknowledgements

All authors sincerely thank the PLCO study team and the National Cancer Institute for access to data collected by the PLCO trial.

Author contributions

XR, LX (Li Xin), YT, and HG contributed to the study design and data analysis. XR, and LX (Li Xin) contributed to the data interpretation and writing of the manuscript. XR, LP, YX, ZZ (Zhihang Zhou), HL, ZZ (Zhiyong Zhu), YJ, QW, HH, LX (Ling Xiang), YW, YT, and HG contributed to the data collection, and data curation of the present analysis. XR, and HG assisted with statistical analysis and the funding acquisition. All of the authors reviewed or revised the manuscript. All authors contributed to the article and approved the submitted manuscript. The work reported in the paper has been performed by the authors, unless clearly specified in the text.

Funding

This work was supported by 2018 Medical Research Project of Chongqing Health Commission and Chongqing Science and Technology Commission (No. 2018MSXM079), The General Project of Chongqing Natural Science Foundation, Chongqing Science and Technology Commission, China [cstc2021jcyj-msxmX0153], [cstc2021jcyj-msxmX0112], and [CSTB2022NSCQ-MSX1005], and Kuanren Talents Project of the Second Affiliated Hospital of Chongqing Medical University in China [kryc-yq-2110].

Data availability

The raw data is unavailable due to the National Cancer Institute's data policy. Please contact the National Cancer Institute by mail if you want access to the dataset.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Author details

[1]Department of Gastrointestinal Surgery, The Second Affiliated Hospital of Chongqing Medical University, No.288 Tianwen Avenue, Nan'an District, Chongqing 400010, China
[2]Department of Gastroenterology, The Second Affiliated Hospital of Chongqing Medical University, Chongqing, China

[3]Department of Clinical Nutrition, The Second Affiliated Hospital of Chongqing Medical University, Chongqing, China

Received: 30 June 2023 / Accepted: 15 October 2024
Published online: 07 November 2024

References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. CA Cancer J Clin. 2020;70:7–30.
2. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer statistics 2020: GLOBOCAN estimates of incidence and Mortality Worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2021;71:209–49.
3. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2021. CA Cancer J Clin. 2021;71:7–33.
4. Merkow RP, Bilimoria KY, Keswani RN, Chung J, Sherman KL, Knab LM, et al. Treatment trends, risk of lymph node metastasis, and outcomes for localized esophageal cancer. J Natl Cancer Inst. 2014;106:dju133.
5. Steinmetz KA, Potter JD. Vegetables, fruit, and cancer. I. Epidemiology. Cancer Causes Control. 1991;2:325–57.
6. Bravi F, Edefonti V, Randi G, Garavello W, La Vecchia C, Ferraroni M, et al. Dietary patterns and the risk of esophageal cancer. Ann Oncol. 2012;23:765–70.
7. Vingeliene S, Chan DSM, Vieira AR, Polemiti E, Stevens C, Abar L, et al. An update of the WCRF/AICR systematic literature review and meta-analysis on dietary and anthropometric factors and esophageal cancer risk. Ann Oncol. 2017;28:2409–19.
8. Dawsey SM, Fagundes RB, Jacobson BC, Kresty LA, Mallery SR, Paski S, et al. Diet and esophageal disease. Ann N Y Acad Sci. 2014;1325:127–37.
9. Qin X, Jia G, Zhou X, Yang Z. Diet and Esophageal Cancer Risk: an Umbrella Review of systematic reviews and Meta-analyses of Observational studies. Adv Nutr. 2022;13:2207–16.
10. Prabhu A, Obi KO, Rubenstein JH. The synergistic effects of alcohol and tobacco consumption on the risk of esophageal squamous cell carcinoma: a meta-analysis. Am J Gastroenterol. 2014;109:822–7.
11. Nguyen LH, Ma W, Wang DD, Cao Y, Mallick H, Gerbaba TK, et al. Association between Sulfur-Metabolizing Bacterial communities in Stool and Risk of Distal Colorectal Cancer in men. Gastroenterology. 2020;158:1313–25.
12. Magee EA, Richardson CJ, Hughes R, Cummings JH. Contribution of dietary protein to sulfide production in the large intestine: an in vitro and a controlled feeding study in humans. Am J Clin Nutr. 2000;72:1488–94.
13. Beerens H, Romond C. Sulfate-reducing anaerobic bacteria in human feces. Am J Clin Nutr. 1977;30:1770–6.
14. Wang Y, Nguyen LH, Mehta RS, Song M, Huttenhower C, Chan AT. Association between the Sulfur Microbial Diet and Risk of Colorectal Cancer. JAMA Netw Open. 2021;4:e2134308.
15. Nguyen LH, Cao Y, Hur J, Mehta RS, Sikavi DR, Wang Y, et al. The Sulfur Microbial Diet is Associated with increased risk of early-onset Colorectal Cancer precursors. Gastroenterology. 2021;161:1423–e14324.
16. Prorok PC, Andriole GL, Bresalier RS, Buys SS, Chia D, Crawford ED, et al. Design of the prostate, lung, colorectal and ovarian (PLCO) Cancer Screening Trial. Control Clin Trials. 2000;21:S273–309.
17. Csizmadi I, Boucher BA, Lo Siou G, Massarelli I, Rondeau I, Garriguet D, et al. Using national dietary intake data to evaluate and adapt the US Diet History Questionnaire: the stepwise tailoring of an FFQ for Canadian use. Public Health Nutr. 2016;19:3247–55.
18. White IR, Royston P, Wood AM. Multiple imputation using chained equations: issues and guidance for practice. Stat Med. 2011;30:377–99.
19. Zhang Y, Zhong G, Zhu M, Chen L, Wan H, Luo F. Association between Diabetes Risk Reduction Diet and Lung Cancer Risk in 98,159 participants: results from a prospective study. Front Oncol. 2022;12:855101.
20. Shivappa N, Zucchetto A, Serraino D, Rossi M, La Vecchia C, Hébert JR. Dietary inflammatory index and risk of esophageal squamous cell cancer in a case-control study from Italy. Cancer Causes Control. 2015;26:1439–47.
21. Wang Q, Hao J, Guan Q, Yuan W. The Mediterranean diet and gastrointestinal cancers risk. Recent Pat Food Nutr Agric. 2014;6:23–6.
22. Eslamian G, Jessri M, Hajizadeh B, Ibiebele TI, Rashidkhani B. Higher glycemic index and glycemic load diet is associated with increased risk of esophageal squamous cell carcinoma: a case-control study. Nutr Res. 2013;33:719–25.
23. Zaidi AH, Kelly LA, Kreft RE, Barlek M, Omstead AN, Matsui D, et al. Associations of Microbiota and toll-like receptor signaling pathway in esophageal adenocarcinoma. BMC Cancer. 2016;16:52.
24. Narikiyo M, Tanabe C, Yamada Y, Igaki H, Tachimori Y, Kato H, et al. Frequent and preferential infection of Treponema denticola, Streptococcus mitis, and Streptococcus anginosus in esophageal cancers. Cancer Sci. 2004;95:569–74.
25. Yang L, Lu X, Nossa CW, Francois F, Peek RM, Pei Z. Inflammation and intestinal metaplasia of the distal esophagus are associated with alterations in the microbiome. Gastroenterology. 2009;137:588–97.
26. Nasrollahzadeh D, Malekzadeh R, Ploner A, Shakeri R, Sotoudeh M, Fahimi S, et al. Variations of gastric corpus microbiota are associated with early esophageal squamous cell carcinoma and squamous dysplasia. Sci Rep. 2015;5:8820.
27. Role of hydrogen sulfide. donors in cancer development and progression - PubMed [Internet]. [cited 2023 Jun 19]. https://pubmed.ncbi.nlm.nih.gov/33390834/
28. Hydrogen Sulfide Biology and Its Role in. Cancer - PubMed [Internet]. [cited 2023 Jun 19]. https://pubmed.ncbi.nlm.nih.gov/35684331/
29. Wang R-H, Chu Y-H, Lin K-T. The hidden role of Hydrogen Sulfide Metabolism in Cancer. Int J Mol Sci. 2021;22:6562.
30. Quante M, Bhagat G, Abrams JA, Marache F, Good P, Lee MD, et al. Bile acid and inflammation activate gastric cardia stem cells in a mouse model of Barrett-like metaplasia. Cancer Cell. 2012;21:36–51.
31. Attene-Ramos MS, Wagner ED, Gaskins HR, Plewa MJ. Hydrogen sulfide induces direct radical-associated DNA damage. Mol Cancer Res. 2007;5:455–9.
32. Schwabe RF, Jobin C. The microbiome and cancer. Nat Rev Cancer. 2013;13:800–12.
33. Shen X, Carlström M, Borniquel S, Jädert C, Kevil CG, Lundberg JO. Microbial regulation of host hydrogen sulfide bioavailability and metabolism. Free Radic Biol Med. 2013;60:195–200.
34. Lei Y, Zhen Y, Zhang W, Sun X, Lin X, Feng J, et al. Exogenous hydrogen sulfide exerts proliferation, anti-apoptosis, angiopoiesis and migration effects via activating HSP90 pathway in EC109 cells. Oncol Rep. 2016;35:3714–20.
35. Al-Haddad S, El-Zimaity H, Hafezi-Bakhtiari S, Rajendra S, Streutker CJ, Vajpeyi R, et al. Infection and esophageal cancer. Ann N Y Acad Sci. 2014;1325:187–96.
36. Pikarsky E, Porat RM, Stein I, Abramovitch R, Amit S, Kasem S, et al. NF-kappaB functions as a tumour promoter in inflammation-associated cancer. Nature. 2004;431:461–6.
37. Sharma T, Gupta A, Chauhan R, Bhat AA, Nisar S, Hashem S, et al. Cross-talk between the microbiome and chronic inflammation in esophageal cancer: potential driver of oncogenesis. Cancer Metastasis Rev. 2022;41:281–99.
38. Gensollen T, Iyer SS, Kasper DL, Blumberg RS. How colonization by microbiota in early life shapes the immune system. Science. 2016;352:539–44.
39. Palm NW, de Zoete MR, Flavell RA. Immune-Microbiota interactions in health and disease. Clin Immunol. 2015;159:122–7.
40. Kinashi Y, Hase K. Partners in Leaky Gut Syndrome: intestinal dysbiosis and autoimmunity. Front Immunol. 2021;12:673708.
41. Pickard JM, Zeng MY, Caruso R, Núñez G. Gut microbiota: role in pathogen colonization, immune responses, and inflammatory disease. Immunol Rev. 2017;279:70–89.
42. Vijay-Kumar M, Gewirtz AT. Flagellin: key target of mucosal innate immunity. Mucosal Immunol. 2009;2:197–205.
43. Garrett WS. Cancer and the microbiota. Science. 2015;348:80–6.
44. Gupta B, Kumar N. Worldwide incidence, mortality and time trends for cancer of the oesophagus. Eur J Cancer Prev. 2017;26:107–18.
45. Corley DA, Kerlikowske K, Verma R, Buffler P. Protective association of aspirin/NSAIDs and esophageal cancer: a systematic review and meta-analysis. Gastroenterology. 2003;124:47–56.
46. Qiao Y, Yang T, Gan Y, Li W, Wang C, Gong Y, et al. Associations between aspirin use and the risk of cancers: a meta-analysis of observational studies. BMC Cancer. 2018;18:288.
47. Bosetti C, Rosato V, Gallus S, Cuzick J, La Vecchia C. Aspirin and cancer risk: a quantitative review to 2011. Ann Oncol. 2012;23:1403–15.
48. Zhao R, Coker OO, Wu J, Zhou Y, Zhao L, Nakatsu G, et al. Aspirin reduces colorectal Tumor Development in mice and gut microbes reduce its Bioavailability and Chemopreventive effects. Gastroenterology. 2020;159:969–e9834.

49. Lagiou P, Talamini R, Samoli E, Lagiou A, Ahrens W, Pohlabeln H, et al. Diet and upper-aerodigestive tract cancer in Europe: the ARCAGE study. Int J Cancer. 2009;124:2671–6.
50. S FIAPDNFK. F, R S, Tea drinking habits and oesophageal cancer in a high risk area in northern Iran: population based case-control study. BMJ (Clinical research ed) [Internet]. 2009 [cited 2023 Jun 17];338. https://pubmed.ncbi.nlm.nih.gov/19325180/

Publisher's note