RESEARCH ARTICLE

Causal association between dietary factors and esophageal diseases: A Mendelian randomization study

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Abstract

Background

Using Mendelian randomization (MR) approach, our objective was to determine whether there was a causal association between dietary factors and gastroesophageal reflux disease (GERD), Barrett's esophagus (BE), or esophageal cancer (EC).

Methods

Genome-wide association study (GWAS) data for eighteen types of dietary intake were obtained from the UK Biobank. GWAS data for GERD, BE, and EC were sourced from the FinnGen consortium. We performed univariable and multivariable MR analysis to assess the cause effect between dietary factors and esophageal diseases. MR results were expressed as odds ratios (OR) with 95% confidence intervals (CI).

Results

Raw vegetable intake was associated with a lower risk of GERD (OR = 0.478; P = 0.011). On the contrary, cooked vegetable intake increased the risk of GERD (OR = 1.911; P = 0.024). Bread intake was associated with increased odds of BE (OR = 6.754; P = 0.007), while processed meat intake was associated with reduced risk of BE (OR = 0.210; P = 0.035). We also observed evidence that increased consumption of dried fruit (OR = 0.087; P = 0.022) and salt added to food (OR = 0.346; P = 0.045) could prevent EC. The results of multivariable MR showed that the protective effect of consumption of salt added to food on EC was no longer significant after adjusting for the consumption of dried fruit.

Conclusion

Vegetable consumption was associated with GERD, whereas consumption of bread and processed meat was associated with BE. Dried fruit intake was associated with a lower risk of EC, and the protective effect of consumption of salt added food on EC may also be mediated by consumption of dried fruit. Future research should be performed to investigate the

mechanisms behind these cause-and-effect relationships to reduce the burden of disease caused by dietary habits.

Introduction

Esophageal cancer (EC) is one of the most commonly diagnosed malignancies worldwide and ranks sixth in cancer-related mortality [1]. Estimates predicted a total of 604,100 new cases and 544,076 deaths from EC in 2020 alone [2]. Esophageal adenocarcinoma (EAC) is increasing rapidly and has become the predominant subtype of EC in European populations [3]. Barrett's esophagus (BE) refers to the metaplastic alteration of the esophageal mucosa from a normal squamous epithelium to a columnar epithelium, which is recognized as a precursor lesion for the development of EAC [4, 5]. The development of EAC is typically characterized by a sequential progression from BE metaplasia to dysplasia, culminating in the emergence of invasive carcinoma. Approximately 10–15% of patients suffering from gastroesophageal reflux disease (GERD) will experience the development of BE, which is caused by a metaplastic transformation resulting from chronic mucosal injury due to repeated episodes of acid reflux [6]. With the worldwide increase in the incidence of EAC in recent decades [7], it is important to identify modifiable risk factors that may contribute to the development of GERD.

Research has been carried out since the twentieth century to determine the risk factors for esophageal diseases. The association between dietary factors and esophageal diseases has been investigated in different parts of the world, but with different and sometimes contradictory findings. Alcohol, for example, may have a direct toxic effect on the esophageal mucosa and reduces lower esophageal sphincter (LES) pressure, especially when consumed in large quantities [8]. However, epidemiological research on alcohol intake and esophageal diseases has shown contradictory results [9–12]. Most physiological research has not been able to determine the role of diet (especially fat) in GERD [13]. Some researchers even advocate a low-fat diet for people with GERD, but this is inappropriate [14]. Conversely, a number of previous researchers have demonstrated an association between fat and GERD [15]. In conclusion, the association between dietary factors and esophageal diseases has attracted considerable attention. However, establishing a link between dietary and esophageal diseases has been a challenge since clinical case-control studies are susceptible to bias from confounders, such as reverse causality.

Randomized controlled trials (RCTs) are considered the gold standard for the establishment of causality. However, RCTs are not always practical as they can be excessively costly and even unethical. RCTs cannot provide answers to many causal questions, such as the long-term consequences of using addictive substances or those of potentially harmful substances. The instrumental variables approach is another statistical method used to investigate the causality of associations between exposure and outcome. In fact, the concept of the instrumental variables approach was first introduced by econometricians about a century ago and later applied to medical statistics [16, 17]. Mendelian randomization (MR) is a novel method of epidemiological research that uses genetic variants as instrumental variables to assess the presence or absence of causal effects between exposure and outcome [18–20]. Evidence of causal effects analyzed in this method greatly reduces the bias caused by confounders in observational studies, because the genetic variants are randomly assigned at the time of conception [21]. Genetic analysis of traits with modest heritability (e.g., diet) is now possible in large-scale biobanks. A genome-wide association analysis of 85 dietary patterns based on the UK Biobank food frequency questionnaire by Cole et al. provides a deeper exploration of the causal relationship

between diet and disease [22]. In this study, we used MR approach to evaluate the causal effect of eighteen dietary patterns of genetic susceptibility on the risk of GERD, BE, and EC, providing an indication for primary disease prevention.

Materials and methods

Study design

A two-sample MR analysis was used to investigate the causal relationship between 18 dietary habits and esophageal disease (Fig 1). Representative phenotypic single-nucleotide polymorphisms (SNPs) were selected as instrumental variables [23]. The instrumental variables for phenotypes should meet the three main assumptions: (1) SNPs should be strongly associated with corresponding phenotype; (2) SNPs should be unaffected by potential confounders of the exposure-outcome association; (3) direct links between SNPs and outcome are not available [24]. The data used in this study were obtained from public databases that had already been ethically approved for the original study. Therefore, no additional ethical approval was required for this study.

Data source

Eighteen exposure factors related to dietary habits were included in this study, including beverage intake (alcohol, coffee, and tea), staple food intake (cereal and bread), fruit intake (fresh fruit and dried fruit), vegetable intake (cooked vegetable and raw vegetable), meat intake (beef, pork, lamb, processed meat, poultry, oily fish, and non-oily fish), salt added to food, and another food intake (cheese). These GWAS summary data were obtained directly or indirectly from the UK Biobank (http://www.nealelab.is/uk-biobank) by the integrative epidemiology unit (IEU) open GWAS project (https://gwas.mrcieu.ac.uk/) [25, 26]. Information on the 18 dietary patterns was collected retrospectively using a dietary frequency questionnaire, which is publicly available in the UK Biobank (https://biobank.ctsu.ox.ac.uk/crystal/label.cgi?id= 100052). Questionnaire submission was rejected if participants' answers were unrealistic. Details of the 18 dietary questionnaires are given in S1 Table (S1 Appendix). The GWAS summary data for esophageal diseases were extracted from the FinnGen consortium R8 release (https://www.finngen.fi/en) [27], which includes 22,867 cases and 292,256 controls for GERD, 946 cases and 292,256 controls for BE, and 503 cases and 259,583 non-cancer controls for EC. There was little overlap between the exposure and outcome samples.
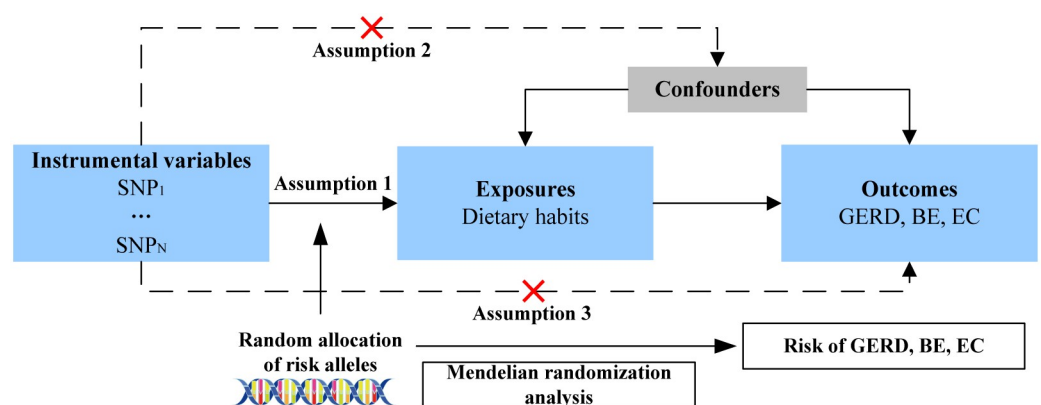

Fig 1. Flowchart describing research process.

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Genetic instrumental variables selection

It is known that $P < 5 \times 10^{-8}$ is the threshold for genome-wide significance. Therefore, we extracted SNPs strongly associated with each dietary pattern based on a threshold of $P < 5 \times 10^{-8}$. Linkage disequilibrium (LD) clumping was performed using parameters (r2 < 0.001 and window size = 10,000 kb) to ensure the independence of these SNPs. In addition, the F statistic was calculated to assess the bias of the weak instrumental variables [28]. When the F statistic > 10, the bias of weak instrumental variables can be ignored [28]. Data on exposure and outcome were merged and harmonized by effect alleles [29]. We discarded SNPs associated to outcomes from the merged data [30].

Heterogeneity and pleiotropy analysis

Cochran's Q test was performed to evaluate heterogeneity and a significant P value indicates the presence of heterogeneity [31, 32]. MR Egger intercept analysis was used to assess horizontal pleiotropy [33]. In the presence of pleiotropy ($P < 0.05$), we used MR-PRESSO to identify potential outliers [23]. The MR analysis was re-conducted after removing the outliers.

Univariable and multivariable MR analysis

The inverse variance weighting (IVW) method was used as the primary analysis to assess the causal effect of dietary habits on the risk of esophageal diseases. Two additional MR methods (MR Egger and weighted median) were used as a complement. We used multivariable MR analysis to adjust for potential confounders between different dietary patterns. MR results were expressed as odds ratios (OR) with 95% confidence intervals (CI). A bilateral P value < 0.05 was considered statistically significant. In this study, all analysis were performed using the "TwoSampleMR (version 0.5.6)" package in the R software (version 4.2.2).

Results

Instrumental variables for dietary factors

Overall, eighteen diet-related GWAS data were included in our study for analysis (Table 1). The amounts of SNPs for each dietary pattern ranged from 8 to 106. Detailed information about these SNPs is presented in S2 Table (S1 Appendix). The F statistics are all greater than 10, indicating that the bias of weak instrumental variables on the results of this study can be ignored.

Univariable MR analysis of esophageal diseases

In the primary univariable MR analysis, six causal associations from eighteen dietary patterns to esophageal diseases were identified (Figs 2–4; S3-S5 Tables in S1 Appendix). Raw vegetable intake was associated with a lower risk of GERD (OR = 0.478; 95% CI = 0.271 to 0.841; $P = 0.011$) but not with BE (OR = 0.286; 95% CI = 0.019 to 4.419; $P = 0.370$) and EC (OR = 2.315; 95% CI = 0.065 to 82.076; $P = 0.645$). On the contrary, cooked vegetable intake levels increased the risk of GERD (OR = 1.911; 95% CI = 1.090 to 3.352; $P = 0.024$). Although the results of the MR Egger intercept analysis suggested possible pleiotropy ($P = 0.027$), the MR-PRESSO analysis found no outliers ($P_{\text{global test}} = 0.182$) and the Cochran's Q test found no heterogeneity ($P = 0.172$). Bread intake was strongly associated with an increased risk of BE (OR = 6.754; 95% CI = 1.676 to 27.229; $P = 0.007$). Processed meat intake reduced the risk of BE (OR = 0.210; 95% CI = 0.049 to 0.899; $P = 0.035$). We also observed evidence that consumption of dried fruit (OR = 0.087; 95% CI = 0.011 to 0.701; $P = 0.022$) and salt added to food (OR = 0.346; 95% CI = 0.122 to 0.979; $P = 0.045$) could prevent EC. Based on the results of the

Table 1. Summary of eighteen dietary habits.

GWAS id	Exposure	Number of SNPs	Sample	R^2 (%)	F
ukb-a-25	Alcohol intake frequency	44	336965	0.78	59.85
ukb-b-11348	Bread intake	32	452236	0.30	41.93
ukb-b-1489	Cheese intake	65	460006	0.56	39.23
ukb-b-15926	Cereal intake	43	451486	0.44	45.22
ukb-b-16576	Dried fruit intake	43	441640	0.43	42.07
ukb-b-3881	Fresh fruit intake	55	421764	0.57	46.20
ukb-b-17627	Non-oily fish intake	11	460880	0.11	44.84
ukb-b-2209	Oily fish intake	63	435435	0.61	45.18
ukb-b-1996	Raw vegetable intake	22	460443	0.19	38.40
ukb-b-8089	Cooked vegetable intake	17	461053	0.14	37.63
ukb-b-2862	Beef intake	17	446462	0.15	41.53
ukb-b-5640	Pork intake	14	428860	0.11	37.72
ukb-b-14179	Lamb intake	32	460162	0.28	39.70
ukb-b-6324	Processed meat intake	23	447485	0.19	38.60
ukb-b-8006	Poultry intake	8	461981	0.06	32.55
ukb-b-5237	Coffee intake	40	461900	0.68	73.15
ukb-b-6066	Tea intake	41	448651	0.56	61.12
ukb-b-8121	Salt added to food	106	462630	1.15	50.77

GWAS, Genome-Wide Association Studies; SNPs, Single-nucleotide polymorphisms; F, F statistics; R^2, phenotype variance explained by genetics.

IVW-MR analysis, there are some critical values that we should be of concern. For the GERD, consumption of dried fruit (OR = 0.685; P = 0.052) and fresh fruit (OR = 0.738; P = 0.072) tended to decrease disease risk, whereas consumption of poultry tended to increase disease risk (OR = 3.778; P = 0.059). For the BE, lamb intake had a tendency to reduce disease risk (OR = 5.824; P = 0.061). For the EC, there was a trend for fresh fruit intake to decrease disease risk (OR = 0.127; P = 0.052). In addition to the causal associations identified by the IVW method described above, weighted median method also identified several indicative results that showed trends consistent with IVW method. Based on weighted median method, dried fruit intake (OR = 0.549; P = 0.008) was suggested to be associated with a decreased risk of GERD, cooked vegetable intake (OR = 0.023; P = 0.030) and beef intake (OR = 0.041; P = 0.040) were suggested to be associated with a decreased risk of BE, lamb intake (OR = 18.188; P = 0.028) was suggested to be associated with an increased risk of BE, and non-oily fish intake (OR = 123.033; P = 0.039) was suggested to be associated with an increased risk of EC (S3-S5 Tables in S1 Appendix).

Multivariable MR analysis of esophageal diseases

Although univariable MR analysis identified causal associations between dietary habits and esophageal diseases, dietary habit may not be a single element. Therefore, we performed a multivariable MR analysis based on the causal associations identified by the IVW method described above (Fig 5; S6 Table in S1 Appendix). The association between raw vegetable intake (adjusted for cooked vegetable intake: OR = 0.314; P = 0.005) or cooked vegetable intake (adjusted for raw vegetable intake: OR = 3.407; P = 0.002) and GERD remained significant in multivariate MR analysis. Bread intake (adjusted for processed meat intake: OR = 12.453; P = 0.001) or processed meat intake (adjusted for bread intake: OR = 0.070; P = 0.002) also had similar significant causal effects on BE. Higher dried fruit intake levels also were a protective
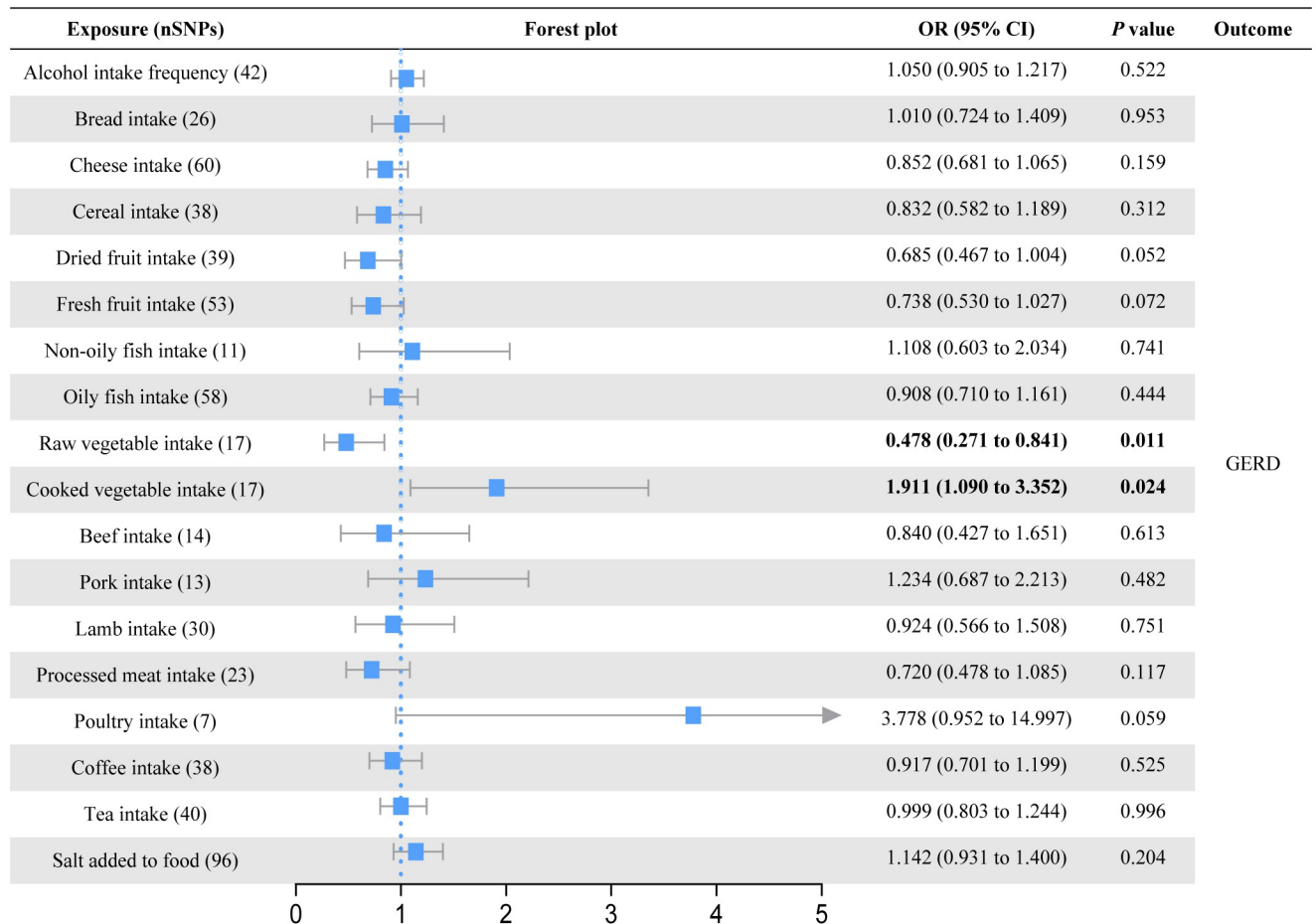
Exposure (nSNPs)	Forest plot	OR (95% CI)	P value	Outcome
Alcohol intake frequency (42)		1.050 (0.905 to 1.217)	0.522	
Bread intake (26)		1.010 (0.724 to 1.409)	0.953	
Cheese intake (60)		0.852 (0.681 to 1.065)	0.159	
Cereal intake (38)		0.832 (0.582 to 1.189)	0.312	
Dried fruit intake (39)		0.685 (0.467 to 1.004)	0.052	
Fresh fruit intake (53)		0.738 (0.530 to 1.027)	0.072	
Non-oily fish intake (11)		1.108 (0.603 to 2.034)	0.741	
Oily fish intake (58)		0.908 (0.710 to 1.161)	0.444	
Raw vegetable intake (17)		**0.478 (0.271 to 0.841)**	**0.011**	GERD
Cooked vegetable intake (17)		**1.911 (1.090 to 3.352)**	**0.024**	
Beef intake (14)		0.840 (0.427 to 1.651)	0.613	
Pork intake (13)		1.234 (0.687 to 2.213)	0.482	
Lamb intake (30)		0.924 (0.566 to 1.508)	0.751	
Processed meat intake (23)		0.720 (0.478 to 1.085)	0.117	
Poultry intake (7)		3.778 (0.952 to 14.997)	0.059	
Coffee intake (38)		0.917 (0.701 to 1.199)	0.525	
Tea intake (40)		0.999 (0.803 to 1.244)	0.996	
Salt added to food (96)		1.142 (0.931 to 1.400)	0.204	

Fig 2. Forest plot of the causal effect of dietary habits on GERD using the inverse variance-weighted method.

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factor against EC (adjusted for the consumption of salt added to food: OR = 0.109; P = 0.029). However, the protective effects of consumption of salt added to food was no longer statistically significant after adjusting for dried fruit intake (adjusted OR = 0.331; P = 0.069).

Discussion

In light of the rapid surge in incidence rates of esophageal diseases over recent decades, pinpointing modifiable risk factors, such as dietary habits, represents an appealing avenue to thwart the initiation and progression of these conditions. In this study, MR analysis was conducted to investigate the potential causal relationship between dietary intake and three different types of esophageal diseases. Using large GWAS data on dietary intake, GERD, BE, and EC, we identified six causal associations, including intake of raw and cooked vegetables with GERD, intake of bread and processed meat with BE, and intake of dried fruit and salt added to food with EC.

Alcohol is one of the most concerning dietary intakes for esophageal diseases. Alcohol consumption has the potential to increase reflux symptoms, damage the esophageal mucosa and even promote carcinogenesis [34]. A small study in 25 healthy volunteers performed by Hamoui et al. showed that the consumption of alcohol reduced LES pressure, which led to an
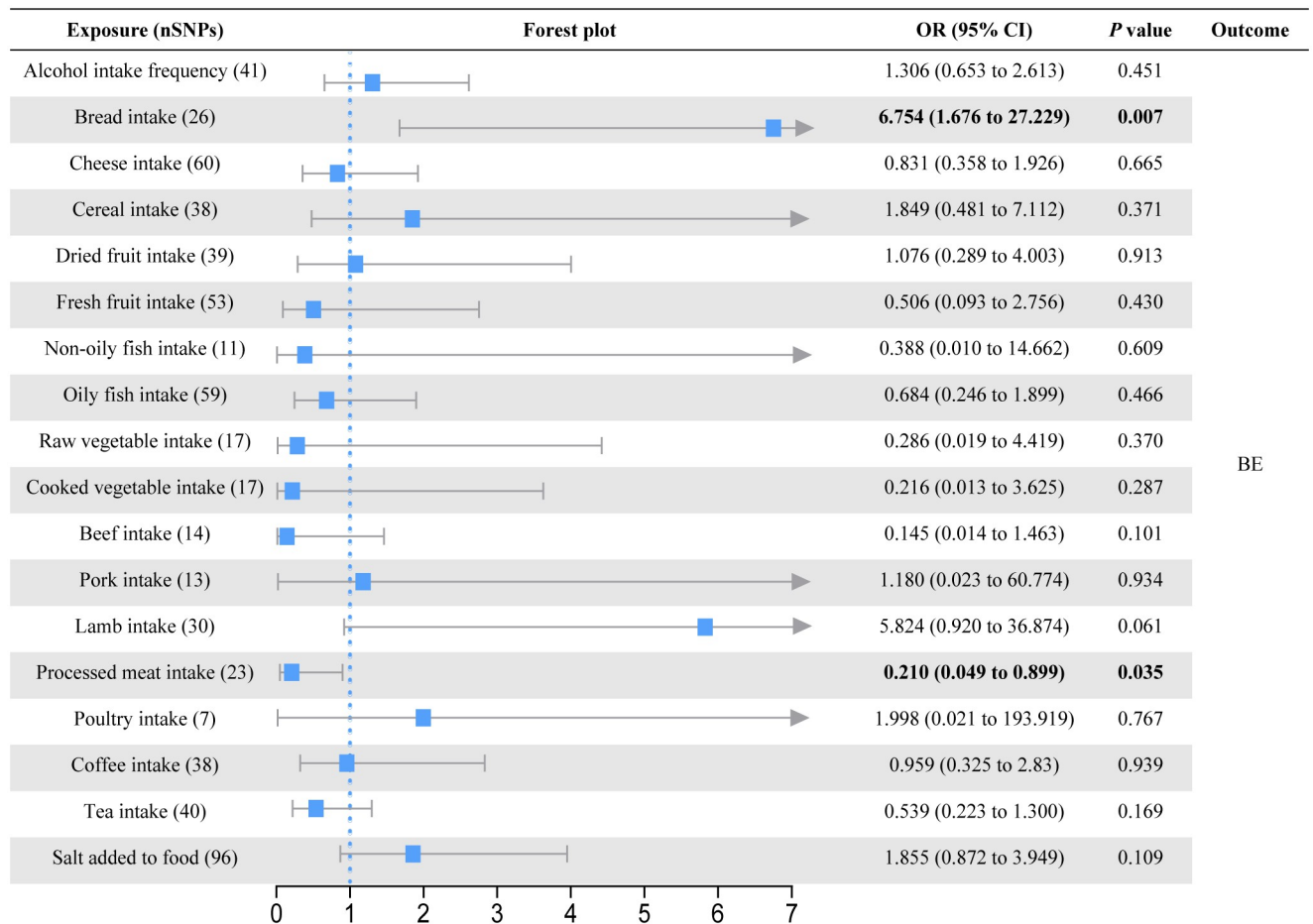
Exposure (nSNPs)	Forest plot	OR (95% CI)	P value	Outcome
Alcohol intake frequency (41)		1.306 (0.653 to 2.613)	0.451	
Bread intake (26)		**6.754 (1.676 to 27.229)**	**0.007**	
Cheese intake (60)		0.831 (0.358 to 1.926)	0.665	
Cereal intake (38)		1.849 (0.481 to 7.112)	0.371	
Dried fruit intake (39)		1.076 (0.289 to 4.003)	0.913	
Fresh fruit intake (53)		0.506 (0.093 to 2.756)	0.430	
Non-oily fish intake (11)		0.388 (0.010 to 14.662)	0.609	
Oily fish intake (59)		0.684 (0.246 to 1.899)	0.466	
Raw vegetable intake (17)		0.286 (0.019 to 4.419)	0.370	
Cooked vegetable intake (17)		0.216 (0.013 to 3.625)	0.287	BE
Beef intake (14)		0.145 (0.014 to 1.463)	0.101	
Pork intake (13)		1.180 (0.023 to 60.774)	0.934	
Lamb intake (30)		5.824 (0.920 to 36.874)	0.061	
Processed meat intake (23)		**0.210 (0.049 to 0.899)**	**0.035**	
Poultry intake (7)		1.998 (0.021 to 193.919)	0.767	
Coffee intake (38)		0.959 (0.325 to 2.83)	0.939	
Tea intake (40)		0.539 (0.223 to 1.300)	0.169	
Salt added to food (96)		1.855 (0.872 to 3.949)	0.109	

Fig 3. Forest plot of the causal effect of dietary habits on BE using the inverse variance-weighted method.

increase in reflux episodes [35]. This is inconsistent with the results of large cohort studies, for example, a case-controlled assessment of 3,153 people with GERD compared with 40,210 people without GERD by Nilsson et al. showed no association [36]. The two main types of EC are squamous cell carcinoma (ESCC) and EAC. Patients with EAC had a higher total alcohol intake compared to controls [37–39]. However, some researchers have also reported moderate alcohol consumption as a protective factor for EAC [40, 41], whereas some studies found no effect [42–45]. Alcohol consumption has been shown to be a risk factor for ESCC in most studies [39, 43, 46]. Again, a number of studies found an association between lifetime alcohol consumption and a lower risk of BE [45, 47], but some reports found no association [48–52]. According to Kubo et al, an increased risk mediated by alcohol consumption was only found when BE cases were compared with GERD controls [53]. Similar to alcohol, some studies reported that tea or coffee significantly reduced LES pressure and lower esophageal pH [54, 55]. In this MR analysis, we found no evidence for a causal association between the consumption of alcohol, coffee, or tea and GERD, BE, or EC. Differences in results between studies may be due to the heterogeneity of the cases or controls studied in many trials, as controls from the general population sampled may have undiagnosed GERD or BE. In addition, observational or retrospective studies may be subject to unavoidable confounders that interfere with the
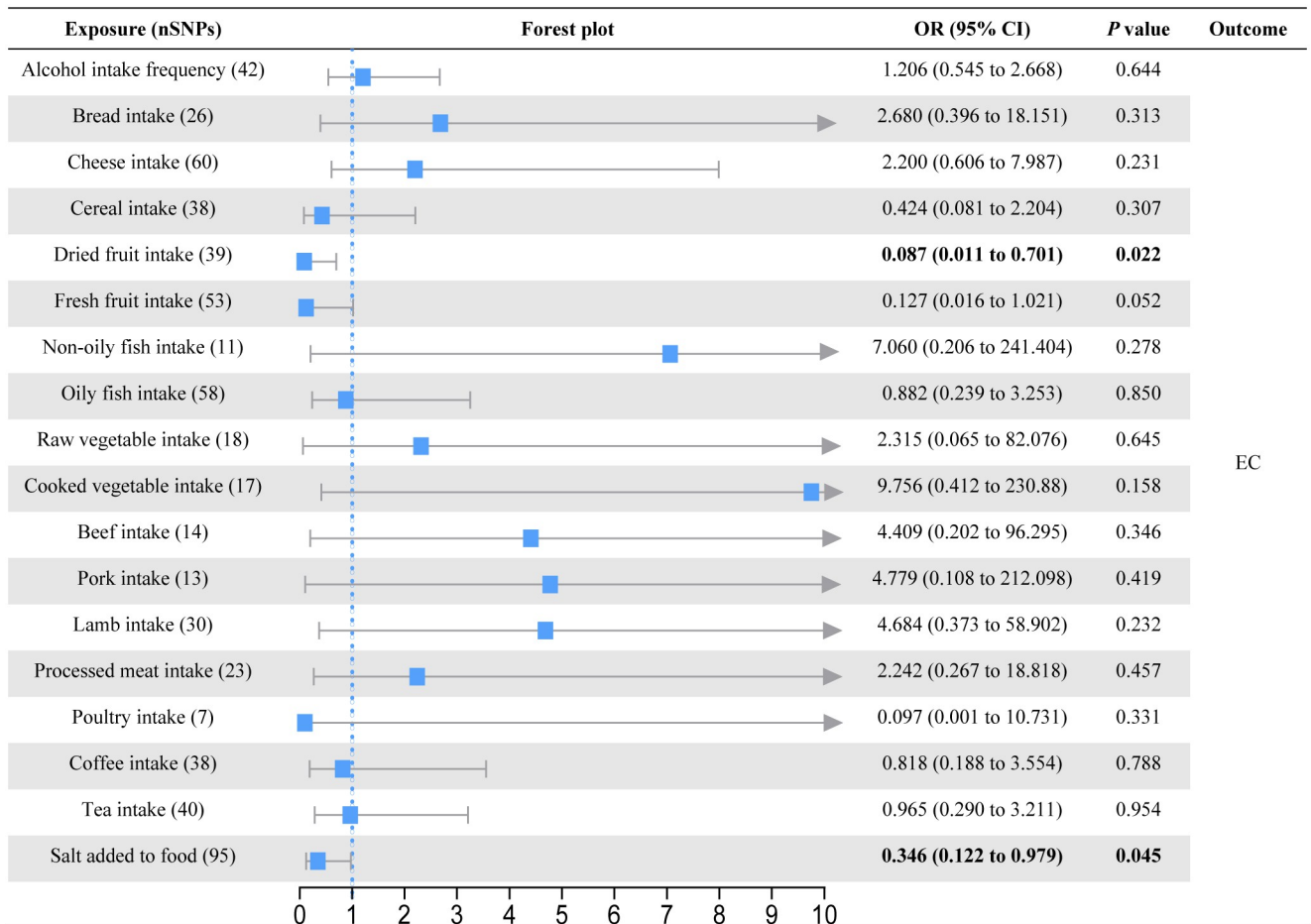
Exposure (nSNPs)	Forest plot	OR (95% CI)	P value	Outcome
Alcohol intake frequency (42)		1.206 (0.545 to 2.668)	0.644	
Bread intake (26)		2.680 (0.396 to 18.151)	0.313	
Cheese intake (60)		2.200 (0.606 to 7.987)	0.231	
Cereal intake (38)		0.424 (0.081 to 2.204)	0.307	
Dried fruit intake (39)		**0.087 (0.011 to 0.701)**	**0.022**	
Fresh fruit intake (53)		0.127 (0.016 to 1.021)	0.052	
Non-oily fish intake (11)		7.060 (0.206 to 241.404)	0.278	
Oily fish intake (58)		0.882 (0.239 to 3.253)	0.850	
Raw vegetable intake (18)		2.315 (0.065 to 82.076)	0.645	EC
Cooked vegetable intake (17)		9.756 (0.412 to 230.88)	0.158	
Beef intake (14)		4.409 (0.202 to 96.295)	0.346	
Pork intake (13)		4.779 (0.108 to 212.098)	0.419	
Lamb intake (30)		4.684 (0.373 to 58.902)	0.232	
Processed meat intake (23)		2.242 (0.267 to 18.818)	0.457	
Poultry intake (7)		0.097 (0.001 to 10.731)	0.331	
Coffee intake (38)		0.818 (0.188 to 3.554)	0.788	
Tea intake (40)		0.965 (0.290 to 3.211)	0.954	
Salt added to food (95)		**0.346 (0.122 to 0.979)**	**0.045**	

Fig 4. Forest plot of the causal effect of dietary habits on EC using the inverse variance-weighted method.

estimation of exposure-outcome correlations and weaken the power of the findings to make precise causal decisions. This means that a direct causal relationship could not be proven, although one observational or retrospective study reported a potentially strong association. The MR analysis approach largely avoids the interference of potential confounders by introducing genetic variants, thereby providing a relatively precise estimate of causal associations.
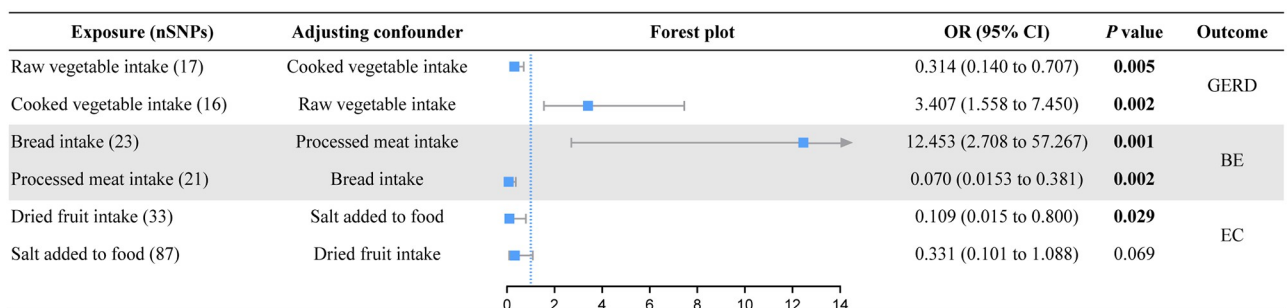
Exposure (nSNPs)	Adjusting confounder	Forest plot	OR (95% CI)	P value	Outcome
Raw vegetable intake (17)	Cooked vegetable intake		0.314 (0.140 to 0.707)	**0.005**	GERD
Cooked vegetable intake (16)	Raw vegetable intake		3.407 (1.558 to 7.450)	**0.002**	
Bread intake (23)	Processed meat intake		12.453 (2.708 to 57.267)	**0.001**	BE
Processed meat intake (21)	Bread intake		0.070 (0.0153 to 0.381)	**0.002**	
Dried fruit intake (33)	Salt added to food		0.109 (0.015 to 0.800)	**0.029**	EC
Salt added to food (87)	Dried fruit intake		0.331 (0.101 to 1.088)	0.069	

Fig 5. Forest plot of the results of multivariable MR.

Fruit and vegetables are the main sources of antioxidants in the diet. The most protective was dark-green (mustard, spinach) and red-orange (tomatoes, carrots) vegetables, apples, berry juices, and citrus fruits [56]. Antioxidants provide protection by interacting with reactive oxygen species to attenuate oxidation/nitrosation reactions caused by the overproduction or overaccumulation of these highly reactive molecules. Oxidative/nitrosative stress has been implicated in the development of many human diseases, including EC [57–59]. In patients with BE, GERD-induced esophageal inflammation can lead to the production of free radicals, which promote carcinogenesis by directly damaging DNA and inhibiting apoptosis [60]. A case-control study by Terry et al found that higher dietary intake of antioxidants (β-carotene and vitamin C) was associated with a lower risk of EAC, particularly in patients with GERD, who are thought to have higher levels of oxidative stress [61]. It was found that a higher intake of vegetables was more effective than fruit, which could be explained by the low sugar and energy content [62]. In contrast, an investigation carried out by Zheng et al. [63] did not establish a significant association between the consumption of fruit or vegetables and the occurrence of GERD. Another study conducted by Jarosz and Taraszewska [64] similarly failed to demonstrate any statistically significant associations between fruit intake and GERD (OR = 1.04, P = 0.930). No evidence of protection against EC was found in the systematic review performed by Bjelakovic et al. [65–67]. In addition, they found that vitamin A and β-carotene supplementation appeared to be associated with an increased risk of EC. In our study, the weighted median-based approach suggested that dried fruit intake may be associated with a reduced risk of GERD; whereas the IVW-based approach suggested that dried fruit intake was associated with a reduced risk of EC, but the latter may be influenced by salt-added food intake. We found significant effects of vegetable intake (both raw and cooked) on GERD, but part of the causal association was no longer significant after adjusting for confounders. Interestingly, raw vegetable intake was a protective factor for GERD, while cooked vegetable intake was a risk factor. The reason for these differences may be that cooking reduces the antioxidant capacity of vegetables [68]. A study performed by Gunathilake et al. also showed that the total antioxidant capacity of the steamed leaves of *O.zeylanica* was significantly lower compared with its raw leaves [69]. Based on the weighted median approach, the consumption of cooked vegetables was suggested to reduce the risk of BE, while raw vegetables were not. There was no evidence in our study that the consumption of vegetables was associated with EC.

In the European prospective investigation, a positive but not statistically significant association between total intake of processed meat or red meat and EAC was found [70]. Kubo and colleagues reported that meat consumption was associated with an increased risk of BE compared with population controls, but among patients with GERD, this was not a risk factor for BE [71]. A case-control study in the USA found a higher risk of EAC with a high intake of meat in the diet (especially red meat) [72]. Gallus and La Vecchia [73], Castellsagué et al. [74] also reported a positive association between high risk of EAC and consumption of processed or red meat, and a negative association with consumption of fish and white meat. In contrast, a high intake of fat or red meat reduced the risk of EAC, according to Wu et al. [75]. High-fat foods release large amounts of cholecystokinin, which delays gastric emptying by stimulating vagal afferent fibers and predisposes to gastro-esophageal reflux [76]. However, in a double-blind randomized controlled trial, low-fat meals with isocaloric delivery had no effect on the number of reflux episodes and mean LES pressure compared to high-fat meals [14]. This contrasts with previous studies that reported changes in the LES pressure and duration of esophageal acid exposure after fat ingestion [77, 78]. Our study found no association between meat consumption (including beef, pork, lamb, processed meat and poultry) and GERD or EC, but did find that eating processed meat reduced the risk of BE. Ganesh et al. reported a 20% reduction in the incidence of EC with increased consumption of fresh fish in a case-control study

conducted in Mumbai [79]. However, we did not find a causal relationship between genetically predicted fish intake (oily and non-oily) and GERD, BE or EC based on IVW-MR analysis.

Cereals and legumes are the groups of plants with the highest nutritional requirements in the world. An earlier retrospective cohort study by Yu et al. in Linxian, an area of China with a high prevalence of EC, reported a positive association between increased cereal intake and EC risk [80]. A HELGA cohort study by Skeie et al, consisting of three sub-cohorts from Norway, Sweden and Denmark, reported a negative association [81]. A possible reason for the discrepancy in results is that earlier studies did not take into account the type of cereal. Our MR results did not support a causal association between cereal intake and GERD, BE or EC. An observational study in the USA found no effect of dairy consumption on GERD symptoms [82], while another randomized controlled trial also reported no association between the risk of GERD and the consumption of dairy products (both reduced-fat and full-fat cheeses) [83]. These findings are consistent with our MR results, where we did not find a causal relationship between genetic susceptibility to cheese consumption and GERD, BE or EC. However, we found that bread intake significantly increased the risk of BE, independent of GERD or BE. In addition, we also observed an intriguing finding that increasing the consumption of salt added to food could reduce the risk of EC. After performing multivariable MR analysis, we found that the protective effect of salt-added food intake on EC may be mediated by dried fruit intake (adjusted for the consumption of salt added to food: OR = 0.109; P = 0.029; adjusted for the consumption of dried fruit intake: OR = 0.331; P = 0.069). Therefore, some caution is needed in interpreting the result that salt added to food may act as a protective factor for EC.

The present study has three strengths. Firstly, to our knowledge, this is the first report to use large-scale GWAS data for a two-sample MR approach to assess the causal association between food intake factors and esophageal diseases. Compared to previous observational and retrospective studies, this approach is less susceptible to the influence of confounders. Secondly, our study reports a causal link between a variety of dietary habits and three types of esophageal disease, some of which have not been reported in previous studies. The rigorous selection of instrumental variables ($P < 5×10^{-8}$ and $F > 10$) and the large sample size with little overlap ensure the reliability of our results. Thirdly, multivariable MR analysis assessed and adjusted for potential confounders.

Our study also has limitations. First, MR results are subject to bias due to pleiotropy, which is currently a challenge for all MR analyses. In this study, most of our results were stable. Second, the strength of the evidence provided by the MR results depends largely on the plausibility of the instrumental variable assumptions, and acquired development may buffer the representativeness of the instrumental variables. Third, although we investigated the causal relationship between dietary intake and three esophageal diseases, we did not perform analysis of the subtypes of esophageal diseases. For example, EAC and ESCC are the two main subtypes of EA, and there are differences in the risk factors for these two subtypes. Therefore, further MR analysis using dietary intake as the exposure and disease subtype as the outcome would provide informative results. Unfortunately, there is no large GWAS summary data available for EAC and ESCC. The present study presents findings on the causal association between dietary intake and esophageal disease, which will facilitate future subtype analysis.

Conclusion

In conclusion, we thoroughly examined the potential causal association between dietary habits and esophageal diseases. Consumption of raw vegetable reduced the risk of GERD, while the opposite was true for cooked vegetable. Bread intake was positively associated with BE risk, while processed meat intake was negatively associated. Dried fruit intake was associated with a

lower risk of EC, and the protective effect of consumption of salt added food on EC may also be mediated by consumption of dried fruit. Future research should be performed to investigate the mechanisms behind these cause-and-effect relationships to reduce the burden of disease caused by dietary habits.

Supporting information

S1 Appendix. S1-S6 Tables are included in file.
(XLSX)

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References

1. Burki TK. Definitions of oesophageal cancer. The Lancet Oncology. 2017; 18(2):e71. Epub 2017/01/17. https://doi.org/10.1016/S1470-2045(17)30018-9 PMID: 28089633.

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: a cancer journal for clinicians. 2021; 71(3):209–49. Epub 2021/02/05. https://doi.org/10.3322/caac.21660 PMID: 33538338.

3. Thrift AP. Global burden and epidemiology of Barrett oesophagus and oesophageal cancer. Nature reviews Gastroenterology & hepatology. 2021; 18(6):432–43. Epub 2021/02/20. https://doi.org/10.1038/s41575-021-00419-3 PMID: 33603224.

4. Shaheen NJ, Richter JE. Barrett's oesophagus. Lancet (London, England). 2009; 373(9666):850–61. Epub 2009/03/10. https://doi.org/10.1016/S0140-6736(09)60487-6 PMID: 19269522.

5. Schneider JL, Corley DA. A review of the epidemiology of Barrett's oesophagus and oesophageal adenocarcinoma. Best practice & research Clinical gastroenterology. 2015; 29(1):29–39. Epub 2015/03/07. https://doi.org/10.1016/j.bpg.2014.11.008 PMID: 25743454.

6. Schlottmann F, Molena D, Patti MG. Gastroesophageal reflux and Barrett's esophagus: a pathway to esophageal adenocarcinoma. Updates in surgery. 2018; 70(3):339–42. Epub 2018/07/25. https://doi.org/10.1007/s13304-018-0564-y PMID: 30039277.

7. Holmes RS, Vaughan TL. Epidemiology and pathogenesis of esophageal cancer. Seminars in radiation oncology. 2007; 17(1):2–9. Epub 2006/12/23. https://doi.org/10.1016/j.semradonc.2006.09.003 PMID: 17185192.

8. Pehl C, Wendl B, Pfeiffer A, Schmidt T, Kaess H. Low-proof alcoholic beverages and gastroesophageal reflux. Digestive diseases and sciences. 1993; 38(1):93–6. Epub 1993/01/01. https://doi.org/10.1007/BF01296779 PMID: 8420765.

9. Nilsson M, Johnsen R, Ye W, Hveem K, Lagergren J. Lifestyle related risk factors in the aetiology of gastro-oesophageal reflux. Gut. 2004; 53(12):1730–5. Epub 2004/11/16. https://doi.org/10.1136/gut.2004.043265 PMID: 15542505.

10. Nocon M, Labenz J, Willich SN. Lifestyle factors and symptoms of gastro-oesophageal reflux—a population-based study. Alimentary pharmacology & therapeutics. 2006; 23(1):169–74. Epub 2006/01/06. https://doi.org/10.1111/j.1365-2036.2006.02727.x PMID: 16393294.

11. Watanabe Y, Fujiwara Y, Shiba M, Watanabe T, Tominaga K, Oshitani N, et al. Cigarette smoking and alcohol consumption associated with gastro-oesophageal reflux disease in Japanese men. Scandinavian journal of gastroenterology. 2003; 38(8):807–11. Epub 2003/08/28. https://doi.org/10.1080/00365520310004506 PMID: 12940431.

12. Bagnardi V, Rota M, Botteri E, Tramacere I, Islami F, Fedirko V, et al. Alcohol consumption and site-specific cancer risk: a comprehensive dose-response meta-analysis. British journal of cancer. 2015; 112(3):580–93. Epub 2014/11/26. https://doi.org/10.1038/bjc.2014.579 PMID: 25422909.

13. Colombo P, Mangano M, Bianchi PA, Penagini R. Effect of calories and fat on postprandial gastro-oesophageal reflux. Scandinavian journal of gastroenterology. 2002; 37(1):3–5. Epub 2002/02/15. https://doi.org/10.1080/003655202753387266 PMID: 11843031.

14. Pehl C, Waizenhoefer A, Wendl B, Schmidt T, Schepp W, Pfeiffer A. Effect of low and high fat meals on lower esophageal sphincter motility and gastroesophageal reflux in healthy subjects. The American journal of gastroenterology. 1999; 94(5):1192–6. Epub 1999/05/11. https://doi.org/10.1111/j.1572-0241.1999.01064.x PMID: 10235191.

15. Penagini R, Mangano M, Bianchi PA. Effect of increasing the fat content but not the energy load of a meal on gastro-oesophageal reflux and lower oesophageal sphincter motor function. Gut. 1998; 42 (3):330–3. Epub 1998/05/13. https://doi.org/10.1136/gut.42.3.330 PMID: 9577336.

16. Greenland S. An introduction to instrumental variables for epidemiologists. International journal of epidemiology. 2000; 29(4):722–9. Epub 2000/08/03. https://doi.org/10.1093/ije/29.4.722 PMID: 10922351.

17. Thomas DC, Conti DV. Commentary: the concept of 'Mendelian Randomization'. International journal of epidemiology. 2004; 33(1):21–5. Epub 2004/04/13. https://doi.org/10.1093/ije/dyh048 PMID: 15075141.

18. Burgess S, Davey Smith G, Davies NM, Dudbridge F, Gill D, Glymour MM, et al. Guidelines for performing Mendelian randomization investigations. Wellcome open research. 2019; 4:186. Epub 2020/08/11. https://doi.org/10.12688/wellcomeopenres.15555.2 PMID: 32760811.

19. Lawlor DA, Harbord RM, Sterne JA, Timpson N, Davey Smith G. Mendelian randomization: using genes as instruments for making causal inferences in epidemiology. Statistics in medicine. 2008; 27 (8):1133–63. Epub 2007/09/22. https://doi.org/10.1002/sim.3034 PMID: 17886233.

20. Evans DM, Davey Smith G. Mendelian Randomization: New Applications in the Coming Age of Hypothesis-Free Causality. Annual review of genomics and human genetics. 2015; 16:327–50. Epub 2015/05/06. https://doi.org/10.1146/annurev-genom-090314-050016 PMID: 25939054.

21. Smith GD, Ebrahim S. Mendelian randomization: prospects, potentials, and limitations. International journal of epidemiology. 2004; 33(1):30–42. Epub 2004/04/13. https://doi.org/10.1093/ije/dyh132 PMID: 15075143.

22. Cole JB, Florez JC, Hirschhorn JN. Comprehensive genomic analysis of dietary habits in UK Biobank identifies hundreds of genetic associations. Nature communications. 2020; 11(1):1467. Epub 2020/03/21. https://doi.org/10.1038/s41467-020-15193-0 PMID: 32193382.

23. Verbanck M, Chen CY, Neale B, Do R. Detection of widespread horizontal pleiotropy in causal relationships inferred from Mendelian randomization between complex traits and diseases. Nature genetics. 2018; 50(5):693–8. Epub 2018/04/25. https://doi.org/10.1038/s41588-018-0099-7 PMID: 29686387.

24. Davies NM, Holmes MV, Davey Smith G. Reading Mendelian randomisation studies: a guide, glossary, and checklist for clinicians. BMJ (Clinical research ed). 2018; 362:k601. Epub 2018/07/14. https://doi.org/10.1136/bmj.k601 PMID: 30002074 interests and declare that we have no competing interests.

25. Sudlow C, Gallacher J, Allen N, Beral V, Burton P, Danesh J, et al. UK biobank: an open access resource for identifying the causes of a wide range of complex diseases of middle and old age. PLoS medicine. 2015; 12(3):e1001779. Epub 2015/04/01. https://doi.org/10.1371/journal.pmed.1001779 PMID: 25826379

26. Hemani G, Zheng J, Elsworth B, Wade KH, Haberland V, Baird D, et al. The MR-Base platform supports systematic causal inference across the human phenome. eLife. 2018; 7. Epub 2018/05/31. https://doi.org/10.7554/eLife.34408 PMID: 29846171.

27. Mitja IK, Juha K, Priit P, Timo PS, Kati K, Kati D, et al. FinnGen: Unique genetic insights from combining isolated population and national health register data. medRxiv. 2022:2022.03.03.22271360. https://doi.org/10.1101/2022.03.03.22271360

28. Palmer TM, Lawlor DA, Harbord RM, Sheehan NA, Tobias JH, Timpson NJ, et al. Using multiple genetic variants as instrumental variables for modifiable risk factors. Statistical methods in medical research. 2012; 21(3):223–42. Epub 2011/01/11. https://doi.org/10.1177/0962280210394459 PMID: 21216802.

29. Emdin CA, Khera AV, Kathiresan S. Mendelian Randomization. Jama. 2017; 318(19):1925–6. Epub 2017/11/23. https://doi.org/10.1001/jama.2017.17219 PMID: 29164242.

30. Chen B, Han Z, Geng L. Mendelian randomization analysis reveals causal effects of food intakes on inflammatory bowel disease risk. Frontiers in immunology. 2022; 13:911631. Epub 2022/10/11. https://doi.org/10.3389/fimmu.2022.911631 PMID: 36211374.

31. Bowden J, Del Greco MF, Minelli C, Davey Smith G, Sheehan N, Thompson J. A framework for the investigation of pleiotropy in two-sample summary data Mendelian randomization. Statistics in medicine. 2017; 36(11):1783–802. Epub 2017/01/24. https://doi.org/10.1002/sim.7221 PMID: 28114746.

32. Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. BMJ (Clinical research ed). 2003; 327(7414):557–60. Epub 2003/09/06. https://doi.org/10.1136/bmj.327.7414.557 PMID: 12958120.

33. Bowden J, Davey Smith G, Burgess S. Mendelian randomization with invalid instruments: effect estimation and bias detection through Egger regression. International journal of epidemiology. 2015; 44 (2):512–25. Epub 2015/06/08. https://doi.org/10.1093/ije/dyv080 PMID: 26050253.

34. Keshavarzian A, Rizk G, Urban G, Willson C. Ethanol-induced esophageal motor disorder: development of an animal model. Alcoholism, clinical and experimental research. 1990; 14(1):76–81. Epub 1990/02/01. https://doi.org/10.1111/j.1530-0277.1990.tb00450.x PMID: 2178477.

35. Hamoui N, Lord RV, Hagen JA, Theisen J, Demeester TR, Crookes PF. Response of the lower esophageal sphincter to gastric distention by carbonated beverages. Journal of gastrointestinal surgery: official journal of the Society for Surgery of the Alimentary Tract. 2006; 10(6):870–7. Epub 2006/06/14. https://doi.org/10.1016/j.gassur.2005.11.010 PMID: 16769544.

36. Nilsson M, Johnsen R, Ye W, Hveem K, Lagergren J. Prevalence of gastro-oesophageal reflux symptoms and the influence of age and sex. Scandinavian journal of gastroenterology. 2004; 39(11):1040–5. Epub 2004/11/17. https://doi.org/10.1080/00365520410003498 PMID: 15545159.

37. Vaughan TL, Davis S, Kristal A, Thomas DB. Obesity, alcohol, and tobacco as risk factors for cancers of the esophagus and gastric cardia: adenocarcinoma versus squamous cell carcinoma. Cancer epidemiology, biomarkers & prevention: a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 1995; 4(2):85–92. Epub 1995/03/01. PMID: 7742727.

38. Kabat GC, Ng SK, Wynder EL. Tobacco, alcohol intake, and diet in relation to adenocarcinoma of the esophagus and gastric cardia. Cancer causes & control: CCC. 1993; 4(2):123–32. Epub 1993/03/01. https://doi.org/10.1007/BF00053153 PMID: 8481491.

39. Garidou A, Tzonou A, Lipworth L, Signorello LB, Kalapothaki V, Trichopoulos D. Life-style factors and medical conditions in relation to esophageal cancer by histologic type in a low-risk population. International journal of cancer. 1996; 68(3):295–9. Epub 1996/11/04. https://doi.org/10.1002/(SICI)1097-0215(19961104)68:3<295::AID-IJC5>3.0.CO;2-X PMID: 8903469

40. Freedman ND, Murray LJ, Kamangar F, Abnet CC, Cook MB, Nyrén O, et al. Alcohol intake and risk of oesophageal adenocarcinoma: a pooled analysis from the BEACON Consortium. Gut. 2011; 60 (8):1029–37. Epub 2011/03/17. https://doi.org/10.1136/gut.2010.233866 PMID: 21406386.

41. Gammon MD, Schoenberg JB, Ahsan H, Risch HA, Vaughan TL, Chow WH, et al. Tobacco, alcohol, and socioeconomic status and adenocarcinomas of the esophagus and gastric cardia. Journal of the National Cancer Institute. 1997; 89(17):1277–84. Epub 1997/09/18. https://doi.org/10.1093/jnci/89.17.1277 PMID: 9293918.

42. Veugelers PJ, Porter GA, Guernsey DL, Casson AG. Obesity and lifestyle risk factors for gastroesophageal reflux disease, Barrett esophagus and esophageal adenocarcinoma. Diseases of the esophagus: official journal of the International Society for Diseases of the Esophagus. 2006; 19(5):321–8. Epub 2006/09/21. https://doi.org/10.1111/j.1442-2050.2006.00602.x PMID: 16984526.

43. Hashibe M, Boffetta P, Janout V, Zaridze D, Shangina O, Mates D, et al. Esophageal cancer in Central and Eastern Europe: tobacco and alcohol. International journal of cancer. 2007; 120(7):1518–22. Epub 2007/01/06. https://doi.org/10.1002/ijc.22507 PMID: 17205526.

44. Anderson LA, Cantwell MM, Watson RG, Johnston BT, Murphy SJ, Ferguson HR, et al. The association between alcohol and reflux esophagitis, Barrett's esophagus, and esophageal adenocarcinoma. Gastroenterology. 2009; 136(3):799–805. Epub 2009/01/24. https://doi.org/10.1053/j.gastro.2008.12.005 PMID: 19162028.

45. Yates M, Cheong E, Luben R, Igali L, Fitzgerald R, Khaw KT, et al. Body mass index, smoking, and alcohol and risks of Barrett's esophagus and esophageal adenocarcinoma: a UK prospective cohort

study. Digestive diseases and sciences. 2014; 59(7):1552–9. Epub 2014/02/07. https://doi.org/10.1007/s10620-013-3024-z PMID: 24500448.

46. Lagergren J, Bergström R, Lindgren A, Nyrén O. The role of tobacco, snuff and alcohol use in the aetiology of cancer of the oesophagus and gastric cardia. International journal of cancer. 2000; 85(3):340–6. Epub 2000/02/01. PMID: 10652424.

47. Thrift AP, Kramer JR, Richardson PA, El-Serag HB. No significant effects of smoking or alcohol consumption on risk of Barrett's esophagus. Digestive diseases and sciences. 2014; 59(1):108–16. Epub 2013/10/12. https://doi.org/10.1007/s10620-013-2892-6 PMID: 24114046.

48. Koek GH, Sifrim D, Lerut T, Janssens J, Tack J. Multivariate analysis of the association of acid and duodeno-gastro-oesophageal reflux exposure with the presence of oesophagitis, the severity of oesophagitis and Barrett's oesophagus. Gut. 2008; 57(8):1056–64. Epub 2008/04/12. https://doi.org/10.1136/gut.2006.119206 PMID: 18403496.

49. Steevens J, Schouten LJ, Driessen AL, Huysentruyt CJ, Keulemans YC, Goldbohm RA, et al. A prospective cohort study on overweight, smoking, alcohol consumption, and risk of Barrett's esophagus. Cancer epidemiology, biomarkers & prevention: a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2011; 20(2):345–58. Epub 2010/12/22. https://doi.org/10.1158/1055-9965.EPI-10-0636 PMID: 21173169.

50. Edelstein ZR, Bronner MP, Rosen SN, Vaughan TL. Risk factors for Barrett's esophagus among patients with gastroesophageal reflux disease: a community clinic-based case-control study. The American journal of gastroenterology. 2009; 104(4):834–42. Epub 2009/03/26. https://doi.org/10.1038/ajg.2009.137 PMID: 19319131.

51. Conio M, Filiberti R, Blanchi S, Ferraris R, Marchi S, Ravelli P, et al. Risk factors for Barrett's esophagus: a case-control study. International journal of cancer. 2002; 97(2):225–9. Epub 2002/01/05. https://doi.org/10.1002/ijc.1583 PMID: 11774268.

52. Johansson J, Håkansson HO, Mellblom L, Kempas A, Johansson KE, Granath F, et al. Risk factors for Barrett's oesophagus: a population-based approach. Scandinavian journal of gastroenterology. 2007; 42(2):148–56. Epub 2007/03/01. https://doi.org/10.1080/00365520600881037 PMID: 17327933.

53. Kubo A, Levin TR, Block G, Rumore GJ, Quesenberry CP Jr., Buffler P, et al. Alcohol types and sociodemographic characteristics as risk factors for Barrett's esophagus. Gastroenterology. 2009; 136 (3):806–15. Epub 2008/12/30. https://doi.org/10.1053/j.gastro.2008.11.042 PMID: 19111726.

54. Gudjonsson H, McAuliffe TL, Kaye MD. [The effect of coffee and tea upon lower esophageal sphincteric function.]. Laeknabladid. 1995; 81(6):484–8. Epub 1995/06/01. PMID: 20065484.

55. Newberry C, Lynch K. The role of diet in the development and management of gastroesophageal reflux disease: why we feel the burn. Journal of thoracic disease. 2019; 11(Suppl 12):S1594–s601. Epub 2019/09/07. https://doi.org/10.21037/jtd.2019.06.42 PMID: 31489226.

56. Ekström AM, Serafini M, Nyrén O, Hansson LE, Ye W, Wolk A. Dietary antioxidant intake and the risk of cardia cancer and noncardia cancer of the intestinal and diffuse types: a population-based case-control study in Sweden. International journal of cancer. 2000; 87(1):133–40. Epub 2000/06/22. PMID: 10861464.

57. Halliwell B. Oxidative stress and cancer: have we moved forward? The Biochemical journal. 2007; 401 (1):1–11. Epub 2006/12/08. https://doi.org/10.1042/BJ20061131 PMID: 17150040.

58. Federico A, Morgillo F, Tuccillo C, Ciardiello F, Loguercio C. Chronic inflammation and oxidative stress in human carcinogenesis. International journal of cancer. 2007; 121(11):2381–6. Epub 2007/09/26. https://doi.org/10.1002/ijc.23192 PMID: 17893868.

59. Reuter S, Gupta SC, Chaturvedi MM, Aggarwal BB. Oxidative stress, inflammation, and cancer: how are they linked? Free radical biology & medicine. 2010; 49(11):1603–16. Epub 2010/09/16. https://doi.org/10.1016/j.freeradbiomed.2010.09.006 PMID: 20840865.

60. Wetscher GJ, Hinder RA, Klingler P, Gadenstätter M, Perdikis G, Hinder PR. Reflux esophagitis in humans is a free radical event. Diseases of the esophagus: official journal of the International Society for Diseases of the Esophagus. 1997; 10(1):29–32; discussion 3. Epub 1997/01/01. https://doi.org/10.1093/dote/10.1.29 PMID: 9079270.

61. Terry P, Lagergren J, Ye W, Nyrén O, Wolk A. Antioxidants and cancers of the esophagus and gastric cardia. International journal of cancer. 2000; 87(5):750–4. Epub 2000/08/05. PMID: 10925371.

62. Sardana RK, Chhikara N, Tanwar B, Panghal A. Dietary impact on esophageal cancer in humans: a review. Food & function. 2018; 9(4):1967–77. Epub 2018/04/05. https://doi.org/10.1039/c7fo01908d PMID: 29616242.

63. Zheng Z, Nordenstedt H, Pedersen NL, Lagergren J, Ye W. Lifestyle factors and risk for symptomatic gastroesophageal reflux in monozygotic twins. Gastroenterology. 2007; 132(1):87–95. Epub 2007/01/24. https://doi.org/10.1053/j.gastro.2006.11.019 PMID: 17241862.

64. Jarosz M, Taraszewska A. Risk factors for gastroesophageal reflux disease: the role of diet. Przeglad gastroenterologiczny. 2014; 9(5):297–301. Epub 2014/11/15. https://doi.org/10.5114/pg.2014.46166 PMID: 25396005.

65. Bjelakovic G, Nikolova D, Simonetti RG, Gluud C. Systematic review: primary and secondary prevention of gastrointestinal cancers with antioxidant supplements. Alimentary pharmacology & therapeutics. 2008; 28(6):689–703. Epub 2009/01/16. https://doi.org/10.1111/j.1365-2036.2008.03785.x PMID: 19145725.

66. Bjelakovic G, Nikolova D, Simonetti RG, Gluud C. Antioxidant supplements for preventing gastrointestinal cancers. The Cochrane database of systematic reviews. 2008;(3):Cd004183. Epub 2008/08/05. https://doi.org/10.1002/14651858.CD004183.pub3 PMID: 18677777.

67. Bjelakovic G, Nikolova D, Simonetti RG, Gluud C. Antioxidant supplements for prevention of gastrointestinal cancers: a systematic review and meta-analysis. Lancet (London, England). 2004; 364 (9441):1219–28. Epub 2004/10/07. https://doi.org/10.1016/S0140-6736(04)17138-9 PMID: 15464182.

68. Jiménez-Monreal AM, García-Diz L, Martínez-Tomé M, Mariscal M, Murcia MA. Influence of cooking methods on antioxidant activity of vegetables. Journal of food science. 2009; 74(3):H97–h103. Epub 2009/04/29. https://doi.org/10.1111/j.1750-3841.2009.01091.x PMID: 19397724.

69. Gunathilake K, Ranaweera K, Rupasinghe HPV. Effect of Different Cooking Methods on Polyphenols, Carotenoids and Antioxidant Activities of Selected Edible Leaves. Antioxidants (Basel, Switzerland). 2018; 7(9). Epub 2018/09/12. https://doi.org/10.3390/antiox7090117 PMID: 30200223.

70. González CA, Jakszyn P, Pera G, Agudo A, Bingham S, Palli D, et al. Meat intake and risk of stomach and esophageal adenocarcinoma within the European Prospective Investigation Into Cancer and Nutrition (EPIC). Journal of the National Cancer Institute. 2006; 98(5):345–54. Epub 2006/03/02. https://doi.org/10.1093/jnci/djj071 PMID: 16507831.

71. Kubo A, Levin TR, Block G, Rumore GJ, Quesenberry CP, Jr., Buffler P, et al. Dietary patterns and the risk of Barrett's esophagus. American journal of epidemiology. 2008; 167(7):839–46. Epub 2008/01/26. https://doi.org/10.1093/aje/kwm381 PMID: 18218607.

72. Navarro Silvera SA, Mayne ST, Risch H, Gammon MD, Vaughan TL, Chow WH, et al. Food group intake and risk of subtypes of esophageal and gastric cancer. International journal of cancer. 2008; 123 (4):852–60. Epub 2008/06/10. https://doi.org/10.1002/ijc.23544 PMID: 18537156.

73. Gallus S, La Vecchia C. Is there a link between diet and esophageal cancer? Nature clinical practice Gastroenterology & hepatology. 2007; 4(1):2–3. Epub 2007/01/05. https://doi.org/10.1038/ncpgasthep0697 PMID: 17203078.

74. Castellsagué X, Muñoz N, De Stefani E, Victora CG, Castelletto R, Rolón PA. Influence of mate drinking, hot beverages and diet on esophageal cancer risk in South America. International journal of cancer. 2000; 88(4):658–64. Epub 2000/11/04. https://doi.org/10.1002/1097-0215(20001115)88:4<658::aid-ijc22>3.0.co;2-t PMID: 11058886.

75. Wu AH, Tseng CC, Hankin J, Bernstein L. Fiber intake and risk of adenocarcinomas of the esophagus and stomach. Cancer causes & control: CCC. 2007; 18(7):713–22. Epub 2007/06/15. https://doi.org/10.1007/s10552-007-9014-8 PMID: 17562192.

76. Lacy BE, Carter J, Weiss JE, Crowell MD. The effects of intraduodenal nutrient infusion on serum CCK, LES pressure, and gastroesophageal reflux. Neurogastroenterology and motility: the official journal of the European Gastrointestinal Motility Society. 2011; 23(7):631–e256. Epub 2011/03/26. https://doi.org/10.1111/j.1365-2982.2011.01701.x PMID: 21435103.

77. Becker DJ, Sinclair J, Castell DO, Wu WC. A comparison of high and low fat meals on postprandial esophageal acid exposure. The American journal of gastroenterology. 1989; 84(7):782–6. Epub 1989/07/01. PMID: 2741888.

78. Iwakiri K, Kobayashi M, Kotoyori M, Yamada H, Sugiura T, Nakagawa Y. Relationship between postprandial esophageal acid exposure and meal volume and fat content. Digestive diseases and sciences. 1996; 41(5):926–30. Epub 1996/05/01. https://doi.org/10.1007/BF02091532 PMID: 8625764.

79. Ganesh B, Talole SD, Dikshit R. Tobacco, alcohol and tea drinking as risk factors for esophageal cancer: A case-control study from Mumbai, India. Cancer epidemiology. 2009; 33(6):431–4. Epub 2009/10/23. https://doi.org/10.1016/j.canep.2009.09.002 PMID: 19846360.

80. Yu Y, Taylor PR, Li JY, Dawsey SM, Wang GQ, Guo WD, et al. Retrospective cohort study of risk-factors for esophageal cancer in Linxian, People's Republic of China. Cancer causes & control: CCC. 1993; 4(3):195–202. Epub 1993/05/01. https://doi.org/10.1007/BF00051313 PMID: 8318635.

81. Skeie G, Braaten T, Olsen A, Kyrø C, Tjønneland A, Landberg R, et al. Intake of whole grains and incidence of oesophageal cancer in the HELGA Cohort. European journal of epidemiology. 2016; 31 (4):405–14. Epub 2015/06/21. https://doi.org/10.1007/s10654-015-0057-y PMID: 26092139.

82. El-Serag HB, Satia JA, Rabeneck L. Dietary intake and the risk of gastro-oesophageal reflux disease: a cross sectional study in volunteers. Gut. 2005; 54(1):11–7. Epub 2004/12/14. https://doi.org/10.1136/gut.2004.040337 PMID: 15591498.

83. Fernando I, Schmidt KA, Cromer G, Burhans MS, Kuzma JN, Hagman DK, et al. The impact of low-fat and full-fat dairy foods on symptoms of gastroesophageal reflux disease: an exploratory analysis based on a randomized controlled trial. European journal of nutrition. 2022; 61(5):2815–23. Epub 2022/03/17. https://doi.org/10.1007/s00394-022-02855-6 PMID: 35294608.