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Dietary Antioxidant and Mineral Intake in Humans Is Associated with Reduced Risk of Esophageal Adenocarcinoma but Not Reflux Esophagitis or Barrett's Esophagus[1,2]

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Abstract

The role of antioxidants in the pathogenesis of reflux esophagitis (RE), Barrett's esophagus (BE), and esophageal adenocarcinoma (EAC) remains unknown. We evaluated the associations among dietary antioxidant intake and these diseases. We performed an assessment of dietary antioxidant intake in a case control study of RE (n = 219), BE (n = 220), EAC (n = 224), and matched population controls (n = 256) (the Factors Influencing the Barrett's Adenocarcinoma Relationship study) using a modification of a validated FFQ. We found that overall antioxidant index, a measure of the combined intake of vitamin C, vitamin E, total carotenoids, and selenium, was associated with a reduced risk of EAC [odds ratio (OR) = 0.57; 95% CI = 0.33–0.98], but not BE (OR = 0.95; 95% CI = 0.53–1.71) or RE (OR = 1.60; 95% CI = 0.86–2.98), for those in the highest compared with lowest category of intake. Those in the highest category of vitamin C intake had a lower risk of EAC (OR = 0.37; 95% CI = 0.21–0.66; P-trend = 0.001) and RE (OR = 0.46; 95% CI = 0.24–0.90; P-trend = 0.03) compared with those in the lowest category. Vitamin C intake was not associated with BE, and intake of vitamin E, total carotenoids, zinc, copper, or selenium was not associated with EAC, BE, or RE. In conclusion, the overall antioxidant index was associated with a reduced risk of EAC. Higher dietary intake of vitamin C was associated with a reduced risk of EAC and RE. These results suggest that antioxidants may play a role in the pathogenesis of RE and EAC and may be more important in terms of progression rather than initiation of the disease process. J. Nutr. 140: 1757–1763, 2010.

Introduction

Barrett's esophagus (BE),[8] a condition in which the native squamous mucosa of the lower esophagus is replaced by columnar mucosa, is an important risk factor for the development of esophageal adenocarcinoma (EAC) (1). However, most patients with BE never develop EAC, and both environmental and genetic susceptibility factors are thought to be important in determining individual risk. Although the relationship of these conditions to reflux esophagitis (RE) is controversial (2,3), it is thought that RE precedes the BE-EAC spectrum. It remains unclear why some patients with frequent symptoms of gastroesophageal reflux develop RE and/or BE whereas others do not; host circumstances are likely to be important in this regard. The rapid rise in incidence of EAC in recent decades (4) means that a greater understanding of the relationships between, and pathogenesis of, these diseases is required.

Dietary factors are an important modifier of risk of EAC. Several studies of EAC have reported inverse associations with consumption of fruit and vegetables (5–11). When specific micronutrients were examined, vitamin C was associated with a reduced risk of EAC in most (8,12–16) but not all (9) studies. Total vitamin A (total carotenoids, i.e. retinol and β-carotene) has been associated with significant (14,15) and nonsignificant (8,9,13) reductions in risk of EAC. Studies that reported separate estimations of vitamin A from plant and animal sources suggested that an increased risk was associated with vitamin A derived from animal sources (16), although this has not been consistently demonstrated (8). Vitamin E has been associated with a reduced risk of EAC (9,15), although 1 study found no association (14).

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[8] Abbreviations used: BE, Barrett's esophagus; EAC, esophageal adenocarcinoma; NSAID, nonsteroidal antiinflammatory drug; OR, odds ratio; RDA, recommended daily allowance; RE, reflux esophagitis.
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Studies assessing plasma concentrations of antioxidants have reported lower plasma vitamin C concentrations in patients with BE (47.3 μmol/L) compared with normal controls (59.5 μmol/L; $P = 0.013$) (17) and among patients with BE (18.3 μmol/L) compared with RE patients (26.6 μmol/L; $P = 0.05$) (18).

A recent study from California (19) reported an inverse association between intakes of vitamin C, vitamin E, and β-carotene and risk of BE. A meta-analysis of 10 studies showed inverse associations between intakes of vitamin C, vitamin E, and β-carotene and risk of EAC (20). Only 1 previous study has examined dietary intake in gastroesophageal reflux disease, BE, and EAC, reporting significant inverse associations between vitamin C and all 3 diseases (21). The association between intake of other potentially important nutrients, such as copper and zinc, has not been examined. Zinc, copper, and selenium are essential dietary trace metals and play an important role in maintaining DNA integrity by preventing oxidative DNA damage. As such, they could be related to the risk of RE, BE, and EAC or may play a role in the disease progression of patients with RE or BE to EAC.

The objective of the current study was to examine the association between dietary vitamin C, vitamin E, total carotenoids, selenium, copper, and zinc within a population-based case control study of RE, BE, and EAC, the Factors Influencing the Barrett's Adenocarcinoma Relationship (FINBAR) study.

Methods

Design. The study methods have been described in detail elsewhere (22). Briefly, the FINBAR study collected data and samples between March 2002 and December 2004. The study recruited 3 groups of Caucasian participants from throughout the island of Ireland: patients with EAC, patients with long segment BE, and normal population controls. RE participants were recruited in Northern Ireland only from September 2004 to July 2005.

EAC cases (aged ≤85 y) were patients with a histological confirmation of adenocarcinoma within the esophagus. Cases from Northern Ireland were identified from electronic pathology records from all pathology laboratories within the province. Republic of Ireland cases were identified from the main hospitals involved in the diagnosis and treatment of esophageal cancer. BE patients were eligible for inclusion if ≥3 cm of typical Barrett's mucosa was seen at endoscopy and the presence of specialized intestinal metaplasia was confirmed by histological examination of biopsy specimens. Patients with dysplasia on histological examination were excluded. RE patients were included if there was macroscopically visible erosive esophagitis at endoscopy. Erosive esophagitis was defined as mucosal breaks or erosions within the esophagus (grades 2–4 in the Savary Miller/Hetzel-Dent classification or grades B, C, or D in the Los Angeles classification were included). Eligible control participants were adults without a history of esophageal or other gastrointestinal cancer or a known diagnosis of BE. Controls were frequency-matched by sex and 5-y age band to the distribution of EAC patients and selected at random from the General Practice Master Index (a province-wide database of all persons registered with a general practitioner). Republic of Ireland controls were selected at random from 4 selected general practices representing urban and rural areas (Dublin and Cork). The response rates of eligible participants were 74, 82, and 69% for EAC, BE, and RE cases, respectively, and 42% for controls.

Interview. All participants underwent a structured computerized interview with trained interviewers. The questions included demographic characteristics, occurrence of gastroesophageal reflux symptoms, smoking, alcohol intake, dietary history, medication use, occupational history, and anthropometric measures. Dietary intake was assessed using a semiquantitative version of the European Prospective Investigation into Cancer FFQ (23), which was adapted for the Irish population. Participants were asked to report their habitual intake of food items over the 12-mo period 5 y prior to interview. Nutrient intakes were calculated using Q-Builder (Tinuviel Software), which utilizes McCance and Widdowson's Food composition table (6th edition) and 5th edition supplements.

Blood sampling. A 30-mL sample of peripheral venous blood (nonfasting) was taken, transported on ice, and centrifuged within 2 h and stored at -80^0C. Serum samples from cases and controls were analyzed using Western-blot assay (Helico Blot 2.1, Genelabs Diagnostics), which is a qualitative assay for the detection of IgG antibodies to different *H. pylori* antigens in human serum or plasma. Batches of assays contained samples from both cases and controls and the operator was unaware of the participant group. Sera were defined as *H. pylori* and CagA seropositive based on the presence of the specific combinations of indicator bands in accordance with the manufacturer's protocol. Equivocal tests were repeated to obtain a clear result or were excluded from the analyses. Weak bands, i.e., clearly present but less intense than the positive control, were interpreted as positive.

Statistical analysis. Micronutrient intakes were log-transformed and a chi-square test was used to test for differences in categorical variables between cases and controls, and the Kruskal-Wallis test was used to test differences in continuous variables. All analyses related to the RE group were limited to controls recruited in Northern Ireland only, because RE cases were recruited from only this region. Unconditional multiple logistic regression analysis was used to examine the association between energy-adjusted (intake per 4179 kJ/d) dietary vitamin C, vitamin E, total carotenoids, selenium, copper, or zinc intake and case status compared with controls, using tertiles of intake, to attain odds ratios (OR) and 95% CI. Tertile cutpoints were based on the distribution of intake among controls. To test for trend, each person within a particular tertile was assigned the median intake value for that tertile prior to inclusion in the regression model. Confounding variables that were adjusted for in multivariate analysis were: age (y), sex, BMI (kg/m^2), energy intake (kJ/d), smoking status (current/previous/never), education (y), occupation (manual/nonmanual), daily alcohol intake (g/d), regular nonsteroidal antiinflammatory drugs (NSAID) use (weekly use for at least a 6-mo duration), gastroesophageal reflux symptoms (ever/never), and *H. pylori* infection (seropositive/seronegative). We also evaluated each participant's total antioxidant intake using an antioxidant index as described by Kubo et al. (19). In brief, the antioxidant index summed the decile category of vitamin C, vitamin E, total carotenoids, and selenium intakes for each participant. For example, a participant who was in the second decile of intake for each of the 4 nutrients had an antioxidant index of 8, whereas a person in the 10th decile of intake for each of the 4 nutrients had an antioxidant index of 40. Statistical analysis was performed using Stata 9.0 (StataCorp, Stata statistical software). All statistical tests were 2-sided and a P-value < 0.05 was considered significant.

The FINBAR study was approved by the Research Ethics Committee of Queen's University Belfast, the Clinical Research Ethics Committee of Cork Teaching Hospitals, and the Research Ethics Committee Board of St. James's Hospital, Dublin.

Power calculation. Based on a sample size of 224 participants with EAC, the study provided over 85% power to detect a 20 mg/d difference in vitamin C intake between healthy controls and those with EAC.

Results

Population characteristics. Data from 219 patients with RE, 220 patients with BE, 224 patients with EAC, and 256 healthy controls were available for this analysis (**Table 1**). RE cases were younger and more likely to be male compared with the Northern Ireland controls. BE cases had a higher energy intake, were more likely to work in manual jobs, and had received less education than controls. Compared with controls, EAC cases had a higher BMI, higher energy intake, were more likely to smoke, were less educated, were less likely to hold manual occupations, and consumed less alcohol.

TABLE 1 Characteristics of normal controls and cases of RE, BE, and EAC[1]

Characteristics	Northern Ireland controls	RE	*P*-value[3]	All controls	BE	*P*-value[4]	EAC	*P*-value[4]
n	119	219		256	220		224	
Age, *y*	68 ± 10	62 ± 12	<0.01	63 ± 13	62 ± 12	0.50	64 ± 11	0.25
Sex, *n* (%)								
Men	83 (69.8)	183 (83.6)		216 (84.4)	181 (82.3)		189 (84.4)	
Women	36 (30.2)	36 (16.4)	0.03	40 (15.6)	39 (17.7)	0.54	35 (15.6)	1.00
BMI 5 y prior, *kg/m*^{2}	27.2 ± 4.1	27.7 ± 4.5	0.25	27.1 ± 3.9	26.9 ± 4.0	0.74	28.6 ± 4.9	<0.01
Waist:hip ratio	0.96 ± 0.07	0.94 ± 0.08	0.03	0.96 ± 0.08	0.96 ± 0.08	0.65	0.96 ± 0.07	0.74
Education, *y*	11 ± 3	11 ± 2	0.15	12 ± 3	11 ± 3	0.02	11 ± 3	<0.01
Occupation type, *n* (%)								
Manual	54 (47.0)	106 (49.5)		123 (49.4)	127 (58.5)		87 (39.9)	
Nonmanual	61 (53.0)	108 (50.5)	0.66	126 (50.6)	90 (41.5)	0.05	131 (60.1)	0.02
GER symptoms,[2] *n* (%)								
Ever	28 (23.5)	87 (39.7)		49 (19.2)	159 (72.3)		108 (48.2)	
Never	91 (76.5)	132 (60.2)	<0.01	206 (80.8)	61 (27.7)	<0.01	116 (51.8)	<0.01
Smoking status, *n* (%)								
Current	22 (18.8)	46 (21.5)		44 (17.7)	50 (22.8)		76 (34.7)	
Previous	44 (37.6)	62 (29.0)		105 (42.2)	82 (37.5)		98 (44.8)	
Never	51 (43.6)	106 (49.5)	0.27	100 (40.1)	87 (39.7)	0.33	45 (20.5)	<0.01
Alcohol intake, *g/d*	20.2 ± 22.7	22.0 ± 21.5	0.55	26.3 ± 23.3	22.3 ± 25.4	0.13	19.8 ± 22.0	<0.01
H pylori positive, %	56.8	50.0	0.24	55.3	50.5	0.13	49	0.06
Location, *n* (%)								
Northern Ireland	119 (100)	219 (100)		119 (46.5)	150 (68.2)		114 (50.9)	
Republic of Ireland	0 (0)	0 (0)		137 (53.5)	70 (31.8)	<0.01	110 (49.1)	0.34
Energy intake, *kJ/d*	10835 ± 3739	11270 ± 3128	−0.26	10785 ± 3395	11392 ± 3224	0.05	11539 ± 3404	0.02
Vitamin C, *mg/d*	160.4 (2–448)	134.9 (25–435)	0.04	141.8 (2–448)	129.8 (19–461)	0.37	121.0 (4–425)	0.02
Vitamin E, *μg/d*	13.7 (3.4–35.8)	14.00 (2.4–39.4)	0.96	11.0 (1.9–35.8)	12.2 (2.7–41.6)	0.41	11.1 (0.6–34)	1.00
Total carotenoids, *mg/d*	3.9 (0.26–10.78)	3.4 (0.33–11.39)	0.10	3.4 (0.06–10.78)	3.6 (0.75–13.34)	0.78	3.6 (0.02–9.96)	0.70
Zinc, *mg/d*	12.3 (5.1–26.4)	12.3 (4.3–23.5)	1.00	12.2 (4.4–26.4)	12.6 (4.8–21.4)	0.53	13.4 (3.2–28)	<0.01
Copper, *mg/d*	1.3 (0.5–3.3)	1.3 (0.5–3.5)	0.03	1.3 (0.5–3.3)	1.4 (0.6–3.0)	0.94	1.5 (0.5–3.9)	<0.01
Selenium, *μg/d*	65.2 (19–118)	73.6 (22–155)	0.02	64.3 (19–153)	67.3 (14–128)	0.55	68.9 (28–147)	0.17

[1] Values are mean ± SD, mean (range), or *n* (%).
[2] GER, Gastroesophageal reflux.
[3] Cases compared with Northern Ireland controls only.
[4] Cases compared with all controls.

Overall antioxidant index. The overall antioxidant index was not associated with RE (OR 1.60; 95% CI = 0.86–2.98) (**Table 2**) or BE (OR = 0.95; 95% CI = 0.53–1.71) (**Table 3**) but was associated with a reduced risk of EAC (OR = 0.57; 95% CI = 0.33–0.98) (Table 3) for those in the highest compared with the lowest category of intake.

Vitamin C. Patients in the highest category of vitamin C intake had a lower risk of EAC (OR = 0.37; 95% CI = 0.21–0.66; *P*-trend = 0.001) (**Table 3**) and RE (OR = 0.46; 95% CI = 0.24–0.90; *P*-trend = 0.03) (Table 2) compared with those in the lowest category. Analyses were also stratified by smoking status, which showed a further reduction in risk among current smokers with EAC with the highest intake of vitamin C compared with those with the lowest intake (OR = 0.23; 95% CI = 0.07–0.76). In addition, analyses stratified by BMI demonstrated that the inverse association between vitamin C intake and EAC risk was only apparent for those who were not obese (>30 kg/m^2). No association was observed between vitamin C and risk of BE (Table 3). However, risk of BE was lower among current smokers with the highest vitamin C intake compared with those with the lowest intake (OR = 0.21; 95% CI = 0.05–0.82).

Total carotenoids. Total carotenoid intake was inversely associated with RE in unadjusted and age- and energy-adjusted analyses, although the results were not significant in the multivariate analyses (*P* = 0.07) (Table 2). Total carotenoid intake was not associated with BE or EAC risk (Table 3).

Vitamin E, selenium, copper, and zinc. Vitamin E intake was not associated with risk of RE (*P* = 0.07) (Table 2), BE (*P* = 0.33) (Table 3), or EAC (*P* = 0.21) (Table 3). Selenium intake was higher in RE cases compared with controls; however, we found no main effect of intake on RE (*P* = 0.09) (Table 2), BE (*P* = 1.08) (Table 3), or EAC (*P* = 0.55) (Table 3). Copper intake was not associated with risk of RE (*P* = 0.57) (Table 2), BE (*P* = 0.58) (Table 3), or EAC (*P* = 0.79) (Table 3). Similarly, there was no significant association between zinc intake and risk of RE (*P* = 0.14) (Table 2), BE (*P* = 0.74) (Table 3), or EAC (*P* = 0.33) (Table 3).

Discussion

This is the largest case control study to examine the association between dietary intake of antioxidant vitamins and minerals in humans and the risk of RE, BE, and EAC utilizing the same control group. Overall antioxidant intake was inversely associ-

TABLE 2 Risk of RE in relation to vitamin C, vitamin E, total carotenoid, zinc, copper, and selenium intakes and to the overall antioxidant index

	NI controls, *n*	RE, *n*	Unadjusted OR (95%CI)	Age- and energy-adjusted OR (95%CI)	Multivariate-adjusted OR (95%CI)[1]
Vitamin C, *mg/d*					
<100	39	88	1.00	1.00	1.00
100 to <166	40	80	0.62 (0.35–1.10)	0.71 (0.40–1.26)	0.80 (0.43–1.47)
≥166	40	51	0.32 (0.18–0.58)	0.43 (0.23–0.78)	0.46 (0.24–0.90)
P-trend			<0.001	0.007	0.03
Vitamin E, *μg/d*					
<7.16	39	45	1.00	1.00	1.00
7.16 - <11.34	40	78	0.89 (0.45–1.72)	0.98 (0.50–1.92)	1.10 (0.53–2.30)
≥11.34	40	96	0.64 (0.34–1.20)	0.61 (0.33–1.16)	0.67 (0.34–1.33)
P-trend			0.11	0.05	0.07
Total carotenoids, *mg/d*					
<2.6	39	83	1.00	1.00	1.00
2.6 to <3.8	40	70	0.53 (0.30–0.96)	0.60 (0.33–1.08)	0.61 (0.33–1.15)
≥3.8	40	66	0.45 (0.25–0.78)	0.51 (0.28–0.93)	0.56 (0.30–1.06)
P-trend			0.008	0.04	0.07
Zinc, mg/d					
<10.9	39	91	1.00	1.00	1.00
10.9 to <13.2	40	75	0.75 (0.44–1.27)	0.78 (0.45–1.35)	0.77 (0.43–1.37)
≥13.2	40	53	0.57 (0.32–0.99)	0.61 (0.34–1.12)	0.60 (0.33–1.11)
P-trend			0.05	0.13	0.14
Copper, *mg/d*					
<1.13	39	89	1.00	1.00	1.00
1.13 to <1.44	40	85	0.87 (0.52–1.46)	0.90 (0.53–1.53)	0.97 (0.56–1.69)
≥1.44	40	45	0.63 (0.35–1.12)	0.74 (0.40–1.34)	0.79 (0.43–1.47)
P-trend			0.13	0.35	0.57
Selenium, μg/d					
<53	39	49	1.00	1.00	1.00
53 to <72	40	70	1.05 (0.59–1.86)	1.04 (0.58–1.88)	1.04 (0.56–1.95)
≥72	40	100	1.61 (0.91–2.85)	1.58 (0.87–1.88)	1.56 (0.84–2.91)
P-trend			0.07	0.10	0.09
Antioxidant index					
Tertile 1	39	52	1.00	1.00	1.00
Tertile 2	40	85	1.67 (1.06–2.66)	1.82 (1.14–2.92)	1.58 (0.88–2.85)
Tertile 3	40	82	1.42 (0.90–2.25)	1.68 (1.04–2.71)	1.60 (0.86–2.98)
P-trend			0.04	0.05	0.17

[1] Adjusted for age (y), sex, BMI (kg/m^2), energy intake (kJ/d), smoking status (current/previous/never), education (y), occupation (manual/nonmanual), alcohol (g/d), regular NSAID use (weekly use for at least a 6-mo duration), and *H. pylori* infection (seropositive/seronegative).

ated with EAC risk. Specifically, vitamin C intake was inversely associated with both RE and EAC.

Vitamin C, total carotenoids, and vitamin E. The inverse association between vitamin C intake and EAC risk shown here is in agreement with previous studies. Most studies, but not all (9), have reported either a significant reduced risk (8,13,15), or nonsignificant trends of reduced risk (12,14,16). It remains unclear whether this relationship with vitamin C is due to the antioxidant properties of vitamin C per se or whether it is merely a proxy measure for fruit and vegetable intake. We have previously reported a 50% reduction in risk of EAC among participants in the highest tertile of fruit intake (11). Our current findings that an overall antioxidant index, as well as total dietary vitamin C intake, are also inversely related to EAC risk strengthens the plausibility of this finding. We think that the majority of the effect size from antioxidant intake is due to the contribution of vitamin C intake, because it is strongly inversely associated with risk of RE and EAC. Vitamin C may therefore be more important in the progression of BE and/or RE to EAC rather than initiation of the disease process. Indeed, the inverse association between vitamin C intake and risk of EAC was even stronger among current smokers as was the inverse association between intake and BE risk, suggesting that vitamin C may offset any increased risk associated with smoking. This finding needs to be confirmed in future studies.

Total carotenoid intake was inversely associated with RE in unadjusted and age- and energy-adjusted analyses, although the results were not significant in the multivariate analyses. No associations were observed with BE and EAC. Comparison of our results with those of previous studies is difficult, because carotenoid intake had been reported in different ways, i.e. total carotenoid (total vitamin A) intake or separate intakes of retinol and β-carotene. Some studies have reported significant reductions in risk of EAC for total vitamin A, but not for retinol (14,15), suggesting a beneficial effect of β-carotene. Other studies reported no association when β-carotene and total vitamin A were analyzed separately (9). One further difficulty in

TABLE 3 Risk of BE and EAC in relation to vitamin C, vitamin E, total carotenoid, zinc, copper, and selenium intakes and to the overall antioxidant index

	Controls, *n*	BE, *n*	Unadjusted OR (95%CI)	Age- and energy-adjusted OR (95%CI)	Multivariate-adjusted OR (95%CI)[1]	EAC, *n*	Unadjusted OR (95%CI)	Age- and energy-adjusted OR (95%CI)	Multivariate-adjusted OR (95%CI)[1]
Vitamin C, *mg/d*									
<100	85	89	1.00	1.00	1.00	117	1.00	1.00	1.00
100 to <166	86	78	0.87 (0.57–1.33)	0.88 (0.57–1.35)	0.91 (0.54–1.52)	66	0.56 (0.36–0.85)	0.54 (0.35–0.84)	0.63 (0.39–1.03)
≥166	85	53	0.60 (0.38–0.94)	0.64 (0.40–1.03)	0.64 (0.36–1.13)	41	0.35 (0.22–0.56)	0.34 (0.21–0.55)	0.37 (0.21–0.66)
P-trend			0.24	0.07	0.12		<0.001	<0.001	0.001
Vitamin E, *μg/d*									
<7.16	85	68	1.00	1.00	1.00	72	1.00	1.00	1.00
7.16 to <11.34	86	74	1.08 (0.69–1.68)	1.09 (0.70–1.71)	1.21 (0.69–2.10)	93	1.27 (0.83–1.96)	1.29 (0.84–1.99)	1.46 (0.84–2.38)
≥11.34	85	78	1.13 (0.73–1.76)	1.16 (0.75–1.82)	0.85 (0.48–1.50)	59	0.82 (0.52–1.29)	0.85 (0.54–1.35)	0.84 (0.48–1.47)
P-trend			0.61	0.54	0.33		0.18	0.24	0.21
Total carotenoids, *mg/d*									
<2.6	85	85	1.00	1.00	1.00	91	1.00	1.00	1.00
2.6 to <3.8	86	71	0.83 (0.53–1.28)	0.86 (0.55–1.33)	0.68 (0.40–1.16)	58	0.63 (0.40–0.98)	0.66 (0.42–1.03)	0.53 (0.32–0.89)
≥3.8	85	64	0.76 (0.49–1.18)	0.86 (0.54–1.37)	0.66 (0.39–1.13)	75	0.82 (0.54–1.25)	0.93 (0.59–1.47)	0.81 (0.49–1.34)
P-trend			0.25	0.55	0.16		0.45	0.86	0.54
Zinc, *mg/d*									
<10.9	85	92	1.00	1.00	1.00	65	1.00	1.00	1.00
10.9 to <13.2	86	53	0.57 (0.36–0.89)	0.61 (0.39–0.97)	0.67 (0.39–1.15)	66	1.00 (0.64–1.58)	1.18 (0.74–1.89)	0.96 (0.57–1.64)
≥13.2	85	75	0.82 (0.53–1.25)	0.97 (0.61–1.55)	0.91 (0.53–1.55)	93	1.43 (0.92–2.21)	2.04 (1.25–3.31)	1.28 (0.75–2.18)
P-trend			0.36	0.91	0.74		0.09	0.003	0.33
Copper, *mg/d*									
<1.13	85	95	1.00	1.00	1.00	73	1.00	1.00	1.00
1.13 to <1.44	86	62	0.94 (0.61–1.44)	0.67 (0.43–1.04)	0.74 (0.44–1.26)	73	0.99 (0.64–1.54)	1.04 (0.67–1.63)	1.19 (0.72–1.97)
≥1.44	85	63	0.51 (0.32–0.82)	0.72 (0.46–1.13)	0.87 (0.51–1.49)	78	1.07 (0.69–1.66)	1.17 (0.74–1.83)	0.95 (0.56–1.60)
P-trend			0.07	0.15	0.58		0.76	0.50	0.79
Selenium, *μg/d*									
<53	85	68	1.00	1.00	1.00	64	1.00	1.00	1.00
53 to <72	86	70	1.02 (0.65–1.59)	1.06 (0.67–1.66)	1.07 (0.62–1.83)	78	1.20 (0.77–1.88)	1.28 (0.81–2.01)	1.26 (0.75–2.11)
≥72	85	82	1.19 (0.77–1.85)	1.32 (0.84–2.08)	1.08 (0.64–1.83)	82	1.27 (0.81–1.97)	1.49 (0.94–2.36)	1.20 (0.72–2.00)
P-trend			0.41	0.21	0.79		0.32	0.09	0.55
Antioxidant index									
Tertile 1	83	69	1.00	1.00	1.00	99	1.00	1.00	1.00
Tertile 2	81	82	1.22 (0.78–1.90)	1.31 (0.84–2.06)	1.12 (0.65–1.84)	60	0.62 (0.40–0.97)	0.65 (0.42–1.03)	0.64 (0.38–1.08)
Tertile 3	92	69	0.90 (0.58–1.41)	1.04 (0.65–1.65)	0.95 (0.53–1.71)	65	0.59 (0.38–0.91)	0.64 (0.41–1.01)	0.57 (0.33–0.98)
P-trend			0.70	0.90	0.86		0.02	0.04	0.04

[1] Adjusted for: age (y), sex, BMI (kg/m^2), energy intake (kJ/d), smoking status (current/previous/never), education (y), occupation (manual/nonmanual), alcohol (g/d), regular NSAID use (weekly use for at least a 6-mo duration), Gastroesophageal reflux disease (ever/never), location (Northern Ireland/Republic of Ireland), and *H. pylori* infection (seropositive/seronegative).

interpreting vitamin A results is that it is derived from both plant (poorly bioavailable carotenoids) and animal (easily absorbed retinyl palmitate) sources (24). Indeed, 1 study that provided separate estimates from each source of vitamin A reported an increased risk of EAC and gastric cancer with vitamin A from animal sources but no association with vitamin A from plant sources (16), although this has not been consistently demonstrated (8). A study conducted in Greece found a significant reduction in risk of EAC with total vitamin A intake (13); a high intake of vitamin A from plant sources in this population may explain this finding.

The relationship between vitamin E intake and EAC risk remains equivocal. We found no association, in agreement with a previous study (14). Other investigators reported an inverse association (9) and a borderline inverse association (15). The discrepancies in these findings may reflect that vitamin E has a small effect size that previous studies have not had sufficient power to detect (type 2 error); alternatively, positive findings may have been due to a false positive finding (type 1 error) arising from multiple hypothesis testing. Larger studies will be required to resolve these issues.

Selenium, copper, and zinc. We found no main effect from dietary selenium intake and RE, BE, or EAC in agreement with the only other published study on this topic (25). It should be noted that the mean dietary selenium intake in participants in this study was greater than the recommended daily allowance (RDA) of 50 μg/d. However, it is possible that the range of intake may still have been insufficient to detect an association if one truly exists. It should also be noted that measurement of dietary selenium intake is very difficult, because the selenium content of food depends on the selenium content of the soil on which the food is grown. A biomarker of selenium intake such as toenail selenium, which reflects long-term status, is particularly

useful in case control studies, because it eliminates the possibility of recall bias. Steevens et al. (25) have recently examined the association between selenium status and upper gastrointestinal cancer as assessed by toenail analysis. Although there was no association between selenium status and overall EAC risk, there was an inverse association between selenium and EAC risk among certain subgroups such as women, those who never smoked, and those with low antioxidant intake. One U.S. cross sectional study found an inverse association between serum selenium and markers of neoplastic progression (e.g. 17p loss of heterozygosity) in BE patients (26). Previous research has also shown modest inverse associations between serum and dietary selenium and cell proliferation (27). A recent Cochrane review concluded that the potential cancer preventive effect of selenium should be tested in adequately conducted randomized trials (28).

To our knowledge, this is the first study to examine the association between copper intake and RE, BE, and EAC risk. We did not show any significant associations between copper intake and these diseases and all subgroups met the RDA of 900 μg/d. It is likely that a serum measure of copper would provide a more reliable measure of bioavailability, because its absorption is negatively affected by fiber intake. The confounding affect of fiber on the association between copper intake and EAC risk was demonstrated by the attenuation of the OR in the regression models that included fiber as a confounder (data not shown).

Dietary intake of zinc was not associated with RE, BE, or EAC in our study. Previous studies have produced discordant findings: some have reported a reduced risk of EAC with increasing zinc intake (9,14), while one has shown an increase in risk (15). Although all groups in this study had a mean zinc intake similar to the RDA of 11 mg, only 20% of dietary zinc is absorbed and its bioavailability is inhibited by other dietary components such as fiber and phytic acid. It is likely, therefore, that exposure based on a measure of dietary intake alone is higher than the bioavailable amount. We included fiber intake as a confounding variable in the regression models, but the results were not altered substantially (data not shown).

We found no association between antioxidant intake and risk of BE in contrast to previous studies that reported inverse associations (19,21). Possible explanations for this discrepancy include differences in the confounders adjusted for in the analyses. Unlike previous studies, we included adjustment based on *H. pylori* status. *H. pylori* is associated with a reduced risk of RE, BE, and EAC (29), partly through the induction of gastric atrophy and reduced gastric acid secretion. This may in turn affect absorption of micronutrients. The discrepancy could also be due to differences in total antioxidant intake. For example, total intake of vitamin C in our study (130 mg/d) was lower than in the study from California (210 mg/d) (19) as was total intake of vitamin E (12 μg/d and 35 μg/d, respectively).

We presented age- and energy-adjusted results in addition to multivariate results to demonstrate that there is little difference between the age- and energy-adjusted values and the multivariate adjusted models, indicating that age and energy are the 2 most important confounders. This is shown, e.g., in the association between vitamin C and RE risk (Table 2). By including all of the confounders in the multivariate analyses, some study participants were excluded due to missing data for the entire list of confounders, so a less precise estimate of risk is produced with wider CI. We have also presented our unadjusted or minimally adjusted (age- and energy-adjusted) results so that our results can be included in any future meta-analysis. Fully adjusted models can differ substantially between studies and so are difficult to combine.

The limitations of this study include the potential for dietary recall bias and the possibility of residual confounding. Also, the response rate was considerably lower among controls compared with cases, which may have introduced some selection bias. In addition, recruitment of participants was from throughout the island of Ireland, with the exception of RE participants, who were recruited in Northern Ireland only. Because of this, all analyses related to the RE group were limited to controls recruited in Northern Ireland only. Strengths of the FINBAR study include its population-based design, stringent inclusion criteria, and large size, which provided over 85% power to detect a 20-mg/d difference in vitamin C intake between healthy controls and those with EAC. Also, unlike many previous studies, we corrected our analyses for symptoms of gastroesophageal reflux, a potential confounder or effect modifier in the diet-cancer association. In addition, because there is evidence that *H. pylori* infection may protect against RE, BE, and EAC (29) and affect nutrient absorption, we adjusted all analyses for *H. pylori* status. Finally, we have provided data regarding the absolute amount of antioxidant intake, allowing future comparative studies utilizing analyses according to fixed levels of nutrient intake to be performed.

In summary, we have shown that the overall antioxidant index, a measure of the combined intake of vitamin C, vitamin E, total carotenoids, and selenium, was associated with lower risk of EAC. In addition, a higher dietary intake of vitamin C was associated with a reduced risk of RE and EAC. This finding needs to be replicated by future studies that evaluate vitamin C intake in relation to preclinical conditions associated with EAC such as RE and BE as well as EAC per se. Future areas of research could include assessment of these vitamins and minerals in a prospective study design to investigate their impact on the progression of patients with RE or BE to EAC. Dietary intervention studies are ultimately required to define the role of these nutrients as chemopreventive agents for EAC.

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