

Vitamin D, calcium and dairy intake, and risk of oesophageal adenocarcinoma and its precursor conditions

Helen G. Mulholland*, Liam J. Murray, Lesley A. Anderson and Marie M. Cantwell on behalf of the FINBAR study group

Cancer Epidemiology and Health Services Research Group, Centre for Public Health, Queens University Belfast, Mulhouse Building, RVH Site, Grosvenor Road, Belfast BT12 6BJ, UK

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Abstract

Evidence is accumulating that vitamin D may be protective against carcinogenesis, although exceptions have been observed for some digestive tract neoplasms. The aim of the present study was to explore the association between dietary vitamin D and related nutrients and the risk of oesophageal adenocarcinoma and its precursor conditions, Barrett's oesophagus and reflux oesophagitis. In an all-Ireland case–control study conducted between March 2002 and July 2005, 218 oesophageal adenocarcinoma patients, 212 Barrett's oesophagus patients, 208 reflux oesophagitis patients and 252 population-based controls completed a 101-item FFQ, and provided lifestyle and demographic information. Multiple logistic regression analysis was applied to examine the association between dietary intake and disease risk. Oesophageal adenocarcinoma risk was significantly greater for individuals with the highest compared with the lowest tertile of vitamin D intake (OR 1.99, 95% CI 1.03, 3.86; *P* for trend=0.02). The direct association could not be attributed to a particular vitamin D food source. Vitamin D intake was unrelated to Barrett's oesophagus and reflux oesophagitis risk. No significant associations were observed for Ca or dairy intake and oesophageal adenocarcinoma, Barrett's oesophagus or reflux oesophagitis development. High vitamin D intake may increase oesophageal adenocarcinoma risk but is not related to reflux oesophagitis and Barrett's oesophagus. Ca and dairy product intake did not influence the development of these oesophageal lesions. These findings suggest that there may be population subgroups at an increased risk of oesophageal adenocarcinoma if advice to improve vitamin D intake from foods is implemented. Limited work has been conducted in this area, and further research is required.

Key words: Diet: Vitamin D: Reflux oesophagitis: Barrett's oesophagus: Oesophageal adenocarcinoma

Reflux oesophagitis and Barrett's oesophagus represent early transitional stages in the progression of normal oesophageal tissue to oesophageal adenocarcinoma, arising from the repetitive reflux of gastric acid and bile. This spectrum of oesophageal disorders is of growing concern in developed nations. Although relatively rare, the widely reported rise in oesophageal adenocarcinoma incidence in the UK has continued into the new millennium⁽¹⁾. A systematic analysis of time trends has also confirmed that the prevalence of both gastro-oesophageal reflux disease and oesophagitis has increased since the 1980s, particularly in North America and Europe⁽²⁾. Although Barrett's oesophagus is a designated premalignant condition for oesophageal adenocarcinoma⁽³⁾, progression along the carcinogenic pathway is selective, with approximately 0.5% of patients going on to develop this malignancy^(4–6). Such selective development has generated interest in identifying modifiable risk factors for cancer progression in this small subsample of patients.

Despite certain dietary triggers such as fat, chocolate, mints, onions, citrus fruit and tomatoes being observed for reflux episodes, there is little evidence to suggest that these play a role in the subsequent development of oesophageal adenocarcinoma⁽⁷⁾. Only a limited number of studies have explored diet as an aetiological agent for these oesophageal lesions, with studies to date largely focusing on fruit and vegetable, macronutrient and antioxidant vitamin intake^(8,9–13).

There is considerable evidence in the scientific literature citing a protective role for vitamin D against the development of many cancers⁽¹⁴⁾. Vitamin D has been shown to have several generic anti-carcinogenic effects such as suppressing cell proliferation, promoting cell differentiation and regulating apoptosis⁽¹⁵⁾. Consistent inverse associations are evident for optimum vitamin D status and colorectal cancer and adenoma risk in particular^(16,17), and such findings are fuelling momentum for randomised controlled trials of vitamin D supplementation in cancer prevention. However, the hypothesised

Abbreviation: FINBAR, Factors Influencing the Barrett's Adenocarcinoma Relationship.

* **Corresponding author:** Dr H. G. Mulholland, fax +44 2890235900, email h.mulholland@qub.ac.uk

protective effects of vitamin D in cancer prevention may not be universal. There have been notable exceptions to this protective relationship though from studies of vitamin D and other digestive tract neoplasms⁽¹⁸⁾. Increased risks of oesophageal squamous cell carcinoma and its precursor, squamous dysplasia of the oesophagus, have been demonstrated for individuals with the highest serum concentrations of 25-hydroxyvitamin D in a Chinese population^(19,20).

Vitamin D and Ca are highly related in physiological terms, with low serum Ca status known to induce the synthesis of the active form of vitamin D from 25-hydroxyvitamin D in the kidney⁽²¹⁾. Adequate Ca consumption has been demonstrated to reduce the risk of colorectal cancer, although reports of its relationship with the risk of other cancers have been inconsistent⁽¹²⁾. These equivocal findings are suggested to be due to confounding from high fat contents of common food sources, particularly dairy products.

The most recent World Cancer Research Fund global report on food, nutrition, physical activity and the prevention of cancer could not draw any conclusions about the association of vitamin D, Ca or dairy product intake with oesophageal cancer risk due to a lack of available evidence⁽¹²⁾. Furthermore, no studies to date have investigated the association between vitamin D, Ca or dairy intake and the risk of the precursor conditions, reflux oesophagitis and Barrett's oesophagus.

The aim of the present investigation was to explore the association between vitamin D, Ca and dairy intake and the risk of reflux oesophagitis, Barrett's oesophagus and oesophageal adenocarcinoma in an all-Ireland case-control study.

Methods

Study design

The Factors Influencing the Barrett's Adenocarcinoma Relationship (FINBAR) study is an all-Ireland population-based case-control study that was established to investigate the aetiology of reflux oesophagitis, Barrett's oesophagus and oesophageal adenocarcinoma, as described previously^(9,13,22). Incident cases of oesophageal adenocarcinoma (n 227) and long-segment Barrett's oesophagus (n 224), and normal population controls (n 260) were recruited throughout the island of Ireland between March 2002 and July 2005. Reflux oesophagitis cases (n 230) were recruited in Northern Ireland only. Barrett's oesophagus, reflux oesophagitis and control subjects were frequency matched within 5-year age and sex strata to the distribution of oesophageal adenocarcinoma patients, up to a maximum age of 85 years. The present study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects/patients were approved by the Research Ethics Committee of the Queen's University Belfast (Northern Ireland); Clinical Research Ethics Committee of Cork Teaching Hospitals; and Research Ethics Committee Board of St James's Hospital (Dublin, Ireland). Written informed consent was obtained from all subjects.

Study participants

Incident cases of histologically confirmed oesophageal adenocarcinoma were identified from hospital clinical records in the Republic of Ireland or via electronic pathology records in Northern Ireland. Patients with endoscopically diagnosed long-segment Barrett's oesophagus (≥ 3 cm in length) were recruited if the presence of specialised intestinal metaplasia had been histologically confirmed. Patients with dysplasia were excluded. Reflux oesophagitis patients were included if they had been diagnosed with mucosal erosions in the oesophagus, classified as grades 2-4 or grades B, C or D using the Savary Miller/Hetzel-Dent or Los Angeles methods⁽²³⁾, respectively, at the time of endoscopy. Population-based controls were adults with no previous history of Barrett's oesophagus, oesophageal or other gastrointestinal cancers. Controls were recruited at random in Northern Ireland via the General Practice Master Index and in the Republic of Ireland from four General Practices representing urban and rural areas (Dublin and Cork). The response rates of eligible participants ranged from 42% for controls to 69, 82 and 74% for reflux oesophagitis, Barrett's oesophagus and oesophageal adenocarcinoma cases, respectively.

Data collection

Trained interviewers collected data from study participants using an electronic questionnaire. Dietary intake was assessed using a 101-item FFQ, adapted for the Irish population from a version of the European Prospective Investigation into Cancer and Nutrition FFQ⁽²⁴⁾, by incorporating foods reported as commonly eaten in the North/South Ireland Food Consumption Survey⁽²⁵⁾. The FFQ did not specifically ask for information on vitamin D or Ca supplement usage. Mean daily dietary intakes were calculated from the FFQ using Q-Builder (Tinuviel Software, Llanfechell, Anglesey, UK). Participants were asked to recall their dietary habits over the 12-month period 5 years before the interview. BMI 5 years before the interview was calculated using self-reported weight (kg) divided by current height (m^2), as measured by the interviewer. *Helicobacter pylori* infection status was assessed from serum samples using a Western blot assay, as described previously⁽²⁶⁾.

Statistical analysis

A total of twenty-two study participants failed to provide any dietary information, and information on dairy product intake was missing for a further twenty-nine participants, and therefore these individuals were excluded from the present analysis. This equated to twenty-two reflux oesophagitis cases, twelve Barrett's oesophagus cases, nine oesophageal adenocarcinoma cases and eight controls being excluded, leaving 208 reflux oesophagitis patients, 212 Barrett's oesophagus patients, 218 oesophageal adenocarcinoma patients and 252 population controls for consideration in this analysis. The comparison group for all analysis exploring reflux oesophagitis risk was restricted to controls recruited in Northern Ireland,

since reflux oesophagitis patients were recruited in Northern Ireland only.

Key characteristics and mean nutrient intakes of cases in relation to controls were examined using independent *t* tests for continuous variables and χ^2 tests for categorical variables. Unconditional multiple logistic regression analysis was utilised to explore the association between vitamin D, Ca and dairy intake and disease risk by tertiles of intake, resulting in OR and corresponding 95% CI. In order to test for trend, each individual within a particular tertile was assigned the median intake value for that tertile before inclusion in the regression model. In multivariate-adjusted models, confounding variables were age (years), sex, energy intake (kJ/d), smoking status (current/previous/never), education (years), BMI 5 years before the interview (kg/m²), occupation (manual/non-manual), alcohol intake (g/d), regular non-steroidal inflammatory drug use (weekly use for at least 6 months' duration), *H. pylori* infection (seronegative/seropositive), energy-adjusted glycaemic index intake (units/4184 kJ per d), energy-adjusted saturated fat intake (g/4184 kJ per d) and location (Northern Ireland/Republic of Ireland). Reflux oesophagitis analyses were restricted to Northern Ireland controls only. Covariates were chosen based on previously known risk factors for oesophageal lesions. In a separate multivariate-adjusted regression model, we further tested for regular gastro-oesophageal reflux symptoms (ever/never), since it is debatable whether reflux symptoms may confound or be on the causal pathway between disease risk and the dietary variables of interest. Stratified analyses were conducted according to BMI categories, smoking and *H. pylori* infection status, which were further adjusted for each other, and the likelihood ratio test was applied to evaluate potential interactions. Statistical analysis was performed using Intercooled STATA version 9.2 (StataCorp, College Station, TX, USA).

Results

Table 1 summarises the key characteristics and dietary variables of interest for all three case groups and their respective controls. Reflux oesophagitis and Barrett's oesophagus cases did not differ from their respective controls by daily energy, vitamin D, Ca or dairy product intakes. In contrast, oesophageal adenocarcinoma cases had significantly higher energy and vitamin D intakes compared with controls, although Ca and dairy intakes were similar between the groups. Vitamin D and Ca intakes represent intakes from foods only and do not include intakes from supplements, which we were unable to assess.

As illustrated in Table 2, Ca intake was inversely associated with odds of reflux oesophagitis when comparing the highest with the lowest tertile of intake (OR 0.37, 95% CI 0.17, 0.80; *P* for trend=0.01) in age- and energy-adjusted regression analysis. However, no significant associations were identified for Ca, vitamin D or total dairy product intake and reflux oesophagitis risk once the adjusted for potential confounders.

No significant associations were found between vitamin D or Ca intakes and Barrett's oesophagus risk either before or after multivariate adjustments, as shown in Table 2. There

was also little evidence of an association between dairy product consumption and odds of Barrett's oesophagus in multivariate-adjusted regression analysis. Once the analysis was further adjusted for gastro-oesophageal reflux symptoms, however, a significant positive association emerged, indicating a nearly twofold increased risk of Barrett's oesophagus for individuals with the highest tertile of dairy product intake.

A significant direct association was observed between the highest tertile *v.* the lowest tertile of vitamin D intake and odds of oesophageal adenocarcinoma, even after adjustment for confounders (OR 1.99, 95% CI 1.03, 3.86; *P* for trend=0.02). This was attenuated slightly after adjustment for gastro-oesophageal reflux symptoms, but a significant trend remained (*P* for trend=0.03). Consumption of dairy products also tended to be positively associated with oesophageal adenocarcinoma risk, although this did not achieve statistical significance until multivariate-adjusted analyses further accounted for gastro-oesophageal reflux symptoms (OR 1.89, 95% CI 1.02, 3.50; *P* for trend=0.04). No significant relationship was found between dietary Ca and oesophageal adenocarcinoma risk (Table 2).

To investigate the unexpected direct association observed between vitamin D and oesophageal adenocarcinoma risk further, we explored intake of foods commonly consumed in the Irish diet that are rich sources of vitamin D. Oily fish, egg, margarine and total red meat intakes were not significantly associated with oesophageal adenocarcinoma risk and could not explain the association observed (Table 3). Individuals who consumed liver were at heightened odds of oesophageal adenocarcinoma compared with non-consumers after adjusting for potential confounders (OR 1.73, 95% CI 1.01, 2.97). However, further adjustment for liver intake actually strengthened, rather than attenuated, the positive association between vitamin D and oesophageal adenocarcinoma risk (OR 1.99, 95% CI 1.03, 3.86) when comparing extreme categories of intake. Furthermore, individuals who consumed liver also had elevated odds of Barrett's oesophagus compared with non-consumers (OR 1.77, 95% CI 1.06, 2.98), thus failing to demonstrate the specificity to oesophageal adenocarcinoma that was shown by the results of vitamin D. Additional adjustment for total vitamin A intake could not explain the deleterious effect of liver, although it was not possible to investigate the effects of retinyls and pro-vitamin A carotenoids separately. It should be noted that unlike in the USA, milk is not fortified with vitamin D in either Northern Ireland or the Republic of Ireland and was therefore not considered in the vitamin D-related food analysis.

When analysis was stratified by BMI categories, as shown in Table 4, a 2.5-fold elevated OR for oesophageal adenocarcinoma was limited to overweight and obese individuals with the highest category of vitamin D intake, and was not observed in normal-weight individuals. The direct association between vitamin D and oesophageal adenocarcinoma risk also appeared to be modified by *H. pylori* status, and was only evident in individuals with the highest intakes who were *H. pylori*-positive (OR 3.78, 95% CI 1.44, 9.91; *P* for trend=0.008) and not *H. pylori*-negative cases (OR 0.98, 95% CI 0.35, 2.77; *P* for trend =0.72). Ever smokers were

Table 1. Characteristics and nutrient intakes of reflux oesophagitis, Barrett's oesophagus and oesophageal adenocarcinoma patients and controls
(Mean values, standard deviations, number of patients and percentages)

Characteristics	Northern Ireland controls (<i>n</i> 115)		Reflux oesophagitis (<i>n</i> 208)		<i>P</i> *	All controls (<i>n</i> 252)		Barrett's oesophagus (<i>n</i> 212)		<i>P</i> †	Oesophageal adenocarcinoma (<i>n</i> 218)		<i>P</i> †
	Mean	SD	Mean	SD		Mean	SD	Mean	SD		Mean	SD	
Age (years)	67.8	10.3	62.2	11.5	< 0.001	62.8	12.8	62.3	12.1	0.67	64.4	11.2	0.15
BMI 5 years before the interview (kg/m ²)	27.2	4.1	27.8	4.1	0.24	27.1	3.9	26.9	4.0	0.01	28.5	4.7	< 0.001
Energy intake (MJ/d)	10.87	3.79	11.29	3.16	0.29	10.79	3.41	11.38	3.18	0.06	11.46	3.34	0.03
Vitamin D intake (µg/d)	2.9	1.7	2.8	1.6	0.56	2.8	1.6	2.9	1.5	0.44	3.2	1.5	< 0.01
Ca intake (mg/d)	1161.3	401.1	1120.2	374.4	0.36	1126.3	370.9	1169.1	389.6	0.23	1185.6	393.7	0.09
Total dairy product intake (g/d)‡	404.8	204.1	366.1	220.1	0.12	408.2	226.9	413.7	231.6	0.80	436.0	250.8	0.21
Alcohol intake (g/d)	19.9	22.2	22.1	21.7	0.48	26.3	23.1	21.8	23.7	0.08	19.4	21.8	< 0.01
Education (years)	11.1	2.6	10.7	2.1	0.12	12.0	3.2	11.2	2.9	< 0.01	10.6	2.5	< 0.001
Sex													
Men					0.001					0.57			0.86
<i>n</i>	80		178			213		175			183		
%	69.6		85.6			84.5		82.6			83.9		
Women													
<i>n</i>	35		30			39		37			35		
%	30.4		14.4			15.5		17.4			16.1		
Occupation type													
Manual					0.71					0.06			0.02
<i>n</i>	52		100			121		122			127		
%	46.8		49.0			49.4		58.4			59.9		
Non-manual													
<i>n</i>	59		104			124		87			85		
%	53.2		51.0			50.6		41.6			40.1		
GOR symptoms§													
Ever					< 0.01					< 0.001			< 0.001
<i>n</i>	28		84			49		153			106		
%	24.4		59.6			19.5		72.2			48.6		
Never													
<i>n</i>	87		124			202		59			112		
%	75.6		40.4			80.5		27.8			51.4		
Smoking status													
Current					0.60					0.40			< 0.001
<i>n</i>	22		45			44		48			75		
%	19.5		22.2			18.0		22.7			35.2		
Previous													
<i>n</i>	40		61			101		78			94		
%	35.4		30.0			41.2		37.0			44.1		
Never													
<i>n</i>	51		97			100		85			44		
%	45.1		47.8			40.8		40.3			20.7		
NSAID use													
Ever					0.43					0.42			0.67
<i>n</i>	15		33			31		28			22		
%	13.1		15.9			12.3		13.2			10.1		
Never													
<i>n</i>	98		174			219		184			195		
%	85.2		30.0			86.9		86.8			89.4		
Unknown													

Table 1. Continued

Characteristics	Northern Ireland controls (n 115)			Reflux oesophagitis (n 208)			P*	All controls (n 252)			P†	Barrett's oesophagus (n 212)			P‡	Oesophageal adenocarcinoma (n 218)		
	Mean	SD		Mean	SD			Mean	SD			Mean	SD			Mean	SD	
n	2			1				2				0				1		
%	1.7			47.8				0.8				0.0				0.5		
<i>Helicobacter pylori</i> positive (%)	63.5			44.7			0.001	57.4				50.7				47.5		
Location																		
Northern Ireland																		
n	115			208				115				146				108		
%	100			100				45.6				68.9				49.5		
Republic of Ireland																		
n	0			0				137				66				110		
%	0			0				54.4				31.1				50.5		

GOR, gastro-oesophageal reflux; NSAID, non-steroidal anti-inflammatory drug.

* Cases compared with Northern Ireland controls only.

† Cases compared with all controls.

‡ Dairy product intake defined as milk, yogurt, Greek yogurt, cheese, processed cheese and cottage cheese.

\$ Heartburn/acid reflux symptoms experienced at least once weekly or >50 times/year >5 years before the interview date.

|| Ever use defined as use at least once weekly for ≥6 months' duration.

also at increased odds of oesophageal adenocarcinoma with increasing vitamin D intakes, which appeared to be largely due to previous smoking habits. Notably though, all formal tests for the interaction between vitamin D and these stratified factors failed to reach statistical significance.

Discussion

Findings from this population-based case-control study indicate that high vitamin D intakes from foods are associated with elevated oesophageal adenocarcinoma risk but are unlikely to influence earlier stages of the carcinogenic pathway. Ca intake was not related to the development of any of the oesophageal lesions investigated. The association between dairy product intake and Barrett's oesophagus and oesophageal adenocarcinoma risk only became significant once analyses were further adjusted for gastro-oesophageal reflux symptoms. This would suggest that individuals are consuming dairy products to soothe their reflux symptoms, rather than being associated with the aetiology of these conditions.

The present findings that high vitamin D intake increases oesophageal adenocarcinoma risk are stronger than those of a US population-based case-control study, which identified a non-significant 10% increased risk of oesophageal adenocarcinoma comparing the 75th centile with the 25th centile of intake⁽²⁷⁾. Findings from the same study corroborate ours, in that Ca intake was also unrelated to oesophageal adenocarcinoma risk; however, an increased risk was identified for those individuals with high-fat dairy intake^(27,28). No such connection was observed for low-fat dairy intake, and so the authors have concluded that fat content was responsible for the elevated oesophageal adenocarcinoma risk⁽²⁸⁾. We were unable to disentangle low- and high-fat dairy product intake in the present study; however, all analyses were adjusted for saturated fat intake.

A biologically plausible mechanism for the overall direct association between dietary vitamin D and oesophageal adenocarcinoma risk can only be speculated. Undoubtedly, the evidence for anti-carcinogenic properties of vitamin D in the body⁽²⁹⁾ far outweighs that describing an opposing role. Specific evidence to explain an increased risk of oesophageal malignancy is also lacking. A feeding study of a tumorigenic diet administered to mice in addition to vitamin D was found to have no significant effects in oesophagus tissue⁽³⁰⁾. To fully probe the association, we first investigated common sources of vitamin D in the Irish diet to explore whether there was a nefarious effect of a particular food. Only liver was directly related to oesophageal adenocarcinoma risk; however, further adjustment for liver intake only served to strengthen the association observed for vitamin D. It is therefore unlikely that an alternative carcinogenic agent in vitamin D food sources is responsible for the association observed.

We then performed stratified analysis to determine whether the direct association was limited to a certain subgroup of individuals. The increased risk was heightened among overweight individuals, *H. pylori*-positive individuals and ever, particularly past, smokers, although interactions between these factors and vitamin D intake were not significant.

Table 2. Vitamin D, calcium and dairy product intake and risk of reflux oesophagitis, Barrett's oesophagus and oesophageal adenocarcinoma (Number of patients, odds ratios and 95 % confidence intervals)

	Controls (n)	Cases (n)	Age- and energy-adjusted		Multivariate-adjusted*		Multivariate + GOR-adjusted†	
			OR	95 % CI	OR	95 % CI	OR	95 % CI
Reflux oesophagitis‡	115	208						
Vitamin D (µg/d)								
< 2.08	36	70	1.00		1.00		1.00	
2.08– < 3.13	41	76	0.94	0.52, 1.71	0.59	0.25, 1.38	0.59	0.25, 1.39
≥ 3.13–9.2	38	62	0.80	0.41, 1.57	0.64	0.25, 1.63	0.63	0.25, 1.62
P for trend				0.50		0.49		0.48
Ca (mg/d)								
< 957	38	78	1.00		1.00		1.00	
957– < 1316.3	39	76	0.87	0.47, 1.61	0.97	0.38, 2.51	1.00	0.39, 2.60
≥ 1316.3–2796.0	38	54	0.37	0.17, 0.80	0.40	0.13, 1.29	0.41	0.13, 1.30
P for trend				0.01		0.08		0.08
Dairy products (g/d)§								
< 285	39	84	1.00		1.00		1.00	
285– < 472.4	38	64	0.77	0.44, 1.36	0.78	0.45, 1.36	0.70	0.32, 1.53
≥ 472.2–1032.0	38	60	0.77	0.43, 1.36	0.73	0.42, 1.28	0.69	0.31, 1.51
P for trend				0.39		0.29		0.37
Barrett's oesophagus	252	212						
Vitamin D (µg/d)								
< 2.05	87	71	1.00		1.00		1.00	
2.05– < 3.0	79	53	0.74	0.46, 1.21	0.75	0.40, 1.43	0.64	0.33, 1.28
≥ 3.0–9.7	86	88	1.05	0.65, 1.70	1.24	0.68, 2.29	1.18	0.61, 2.29
P for trend				0.59		0.29		0.33
Ca (mg/d)								
< 929.3	85	66	1.00		1.00		1.00	
929.3– < 1262.0	83	65	0.85	0.52, 1.38	0.68	0.35, 1.33	0.65	0.32, 1.32
≥ 1262.0–2796.0	84	81	0.91	0.52, 1.68	0.80	0.36, 1.77	0.66	0.28, 1.56
P for trend				0.78		0.71		0.43
Dairy products (g/d)§								
< 281.5	84	59	1.00		1.00		1.00	
281.5– < 493.2	84	84	1.43	0.91, 2.25	1.63	0.91, 2.92	1.83	0.97, 3.42
≥ 493.2–999.9	84	69	1.21	0.76, 1.92	1.45	0.80, 2.64	1.94	1.01, 3.71
P for trend				0.63		0.36		0.09
Oesophageal adenocarcinoma	252	218						
Vitamin D (µg/d)								
< 2.05	87	48	1.00		1.00		1.00	
2.05– < 3.0	79	68	1.43	0.87, 2.34	1.11	0.56, 2.21	1.00	0.50, 2.01
≥ 3.0–9.7	86	102	1.85	1.12, 3.05	1.99	1.03, 3.86	1.92	0.98, 3.76
P for trend				0.02		0.02		0.03
Ca (mg/d)								
< 929.3	85	62	1.00		1.00		1.00	
929.3– < 1262.0	83	73	1.06	0.66, 1.73	0.97	0.49, 1.92	0.94	0.47, 1.88
≥ 1262.0–2796.0	84	83	1.00	0.57, 1.75	0.88	0.38, 2.03	0.80	0.34, 1.87
P for trend				0.95		0.75		0.59
Dairy products (g/d)§								
< 281.5	84	60	1.00		1.00		1.00	
281.5– < 493.2	84	71	1.17	0.74, 1.86	1.30	0.70, 2.42	1.34	0.71, 2.52
≥ 493.2–999.9	84	87	1.50	0.96, 2.35	1.67	0.91, 3.06	1.89	1.02, 3.50
P for trend				0.07		0.10		0.04

GOR, gastro-oesophageal reflux.

* Multivariate adjustments: age (years); sex; energy intake (kJ/d); smoking status (current/previous/never); BMI 5 years before the interview; education (years); occupation (manual/non-manual); alcohol (g/d); regular non-steroidal anti-inflammatory drug use (ever/never: ever, weekly use for at least 6 months' duration); *Helicobacter pylori* infection (seropositive/seronegative); energy-adjusted glycaemic index intake (units/4184 kJ per d); energy-adjusted saturated fat intake (g/4184 kJ per d); location (Northern Ireland/Republic of Ireland).

† GOR symptoms experienced at least once weekly or >50 times/year >5 years before the interview date.

‡ Analysis limited to Northern Ireland controls only.

§ Dairy product intake defined as milk, yogurt, Greek yogurt, cheese, processed cheese and cottage cheese.

Nevertheless, BMI is a well-established correlate of vitamin D status, with obesity linked with lower circulating 25-hydroxy-vitamin D concentrations^(31–33), which makes the present findings even more surprising. This association is possibly confounded by lower outdoor physical activity levels among overweight individuals. Similar converse results were

observed for *H. pylori* positivity, which is a highly significant protective factor in oesophageal adenocarcinoma development⁽²⁶⁾, and yet we observed a heightened risk among *H. pylori*-positive individuals with the highest vitamin D intakes. A significant positive correlation between serum vitamin D concentrations and *H. pylori* status has been

Table 3. Vitamin D-related foods and risk of oesophageal adenocarcinoma (OAC)
(Number of patients, odds ratios and 95 % confidence intervals)

Food group	Controls (n)	OAC (n)	Age- and energy-adjusted		Multivariate-adjusted*		Multivariate-adjusted + GOR†	
			OR	95 % CI	OR	95 % CI	OR	95 % CI
Oily fish (portions/week)								
0	85	82	1.00		1.00		1.00	
0.5	101	76	0.74	0.48, 1.13	0.80	0.45, 1.44	0.82	0.45, 1.48
≥ 1	66	60	0.90	0.56, 1.43	1.21	0.65, 2.27	1.20	0.63, 2.28
P for trend				0.58		0.60		0.63
Eggs (portions/week)								
< 1.5	90	72	1.00		1.00		1.00	
1.5– < 3.5	82	60	0.88	0.56, 1.40	1.05	0.57, 1.93	1.00	0.54, 1.86
≥ 3.5	80	86	1.32	0.85, 2.04	1.19	0.65, 2.17	1.18	0.64, 2.18
P for trend				0.11		0.57		0.55
Margarine (portions/week)								
0	108	101	1.00		1.00		1.00	
Any (median 17.5 tsp/week)	144	117	0.92	0.64, 1.33	1.09	0.66, 1.80	1.10	0.66, 1.83
P for trend				0.66		0.72		0.71
Total red meat (portions/week)‡								
< 4.5	64	48	1.00		1.00		1.00	
4.5– < 7	101	85	1.12	0.70, 1.80	1.12	0.58, 2.16	1.14	0.58, 2.22
≥ 7	87	85	1.29	0.80, 2.09	1.03	0.53, 2.01	1.03	0.52, 2.03
P for trend				0.29		0.99		0.99
Liver (portions/week)								
0	192	135	1.00		1.00		1.00	
Any (median 0.5)	60	83	1.94	1.30, 2.89	1.73	1.01, 2.97	1.67	0.97, 2.89
P for trend				0.001		0.05		0.07

GOR, gastro-oesophageal reflux; tsp, teaspoon.

* Multivariate adjustments: age (years); sex; energy intake (kJ/d); smoking status (current/previous/never); BMI 5 years before the interview (kg/m²); education (years); occupation (manual/non-manual); alcohol (g/d); regular non-steroidal anti-inflammatory drug use (weekly use for at least 6 months' duration); location (Northern Ireland/Republic of Ireland); *Helicobacter pylori* infection (seropositive/seronegative); energy-adjusted saturated fat intake (g/4184 kJ per d); energy-adjusted glycaemic index intake (units/4184 kJ per d).

† GOR symptoms experienced at least once weekly or > 50 times/year > 5 years before the interview date.

‡ Total red meat intake: beef + pork + ham + lamb + liver.

reported in a small cross-sectional study of end-stage renal disease patients⁽³⁴⁾, suggesting that vitamin D may modulate *H. pylori* activity, but this hypothesis requires considerable investigation to elucidate any potential pathways. A history of smoking also appeared to increase oesophageal adenocarcinoma risk for high vitamin D consumers. Correspondingly, results from a pooled sample of upper gastrointestinal cancers illustrated a significant reduced risk for never smokers with low vitamin D status⁽³⁵⁾. Given the lack of statistical significance in interaction tests in the present study though, it is likely that the associations observed in stratified analysis are subject to differential misclassification bias and should be interpreted with caution.

Finally, it may be that nutrient–gene interactions are explaining the associations observed. The vitamin D receptor has been demonstrated to regulate bile acid metabolism *in vivo*⁽³⁶⁾, and bile acids are known to induce DNA damage that will ultimately encourage malignant growth in human gastrointestinal tissue⁽³⁷⁾. Therefore, the association between vitamin D receptor polymorphisms and oesophageal adenocarcinoma risk will provide a useful insight into potential mechanisms involved.

The direct association between dietary vitamin D and oesophageal adenocarcinoma risk is perhaps not entirely surprising though. An international consortium has recently published their findings from a Vitamin D Pooling Project of

Rarer Cancers⁽³⁸⁾. Combining data of circulating 25-hydroxyvitamin D concentrations, the research team found that high vitamin D status significantly doubled the risk of pancreatic cancer⁽³⁹⁾, while there was no evidence of an association between 25-hydroxyvitamin D concentrations and the combined risk of oesophageal and gastric cancers, which included 104 oesophageal adenocarcinomas⁽³⁵⁾. The research team deemed that 'careful consideration' should be taken when recommending healthy individuals to increase their vitamin D intake⁽³⁹⁾. Importantly, these findings do not negate the proven inverse association between vitamin D and colorectal cancer and adenoma risk^(16,17), but suggest that more appropriate targeting of individuals is required for advising an increased intake of vitamin D from foods or conduction of supplementation trials in future.

The present study has several strengths, including its population-based design, which maximises the generalisability of the findings, and a large number of cases included. A wide variety of confounding variables were able to be accounted for in regression analyses, including *H. pylori* infection, which was not available in the Cohort Consortium Vitamin D Pooling Project⁽³⁵⁾. However, there is the possibility that some residual confounding may be occurring in these analyses. We do not underestimate the key limitation that this study examines vitamin D intake only and does not account for additional consumption via supplements, nor serum

Table 4. Stratified analysis of vitamin D and oesophageal adenocarcinoma risk by BMI, *Helicobacter pylori* and smoking categories*
(Number of patients, odds ratios and 95 % confidence intervals)

Oesophageal adenocarcinoma	Cases/ controls (<i>n</i>)	BMI ($<25\text{ kg/m}^2$)†		Cases/ controls (<i>n</i>)	BMI ($\geq 25\text{ kg/m}^2$)†		Cases/ controls (<i>n</i>)	<i>H. pylori</i> ‡ negative		Cases/ controls (<i>n</i>)	<i>H. pylori</i> positive‡	
		OR	95 % CI		OR	95 % CI		OR	95 % CI		OR	95 % CI
Vitamin D ($\mu\text{g/d}$)												
< 2.05	12/25	1.00		36/62	1.00		25/33	1.00		19/51	1.00	
$2.05 - < 3.0$	21/21	1.38	0.34, 5.59	47/58	1.01	0.45, 2.25	34/41	0.57	0.21, 1.49	26/35	2.32	0.78, 6.88
≥ 3.0	17/31	0.91	0.22, 3.87	85/55	2.49	1.16, 5.37	47/30	0.98	0.35, 2.77	51/54	3.78	1.44, 9.91
<i>P</i> for trend		0.76			0.008			0.72			0.008	
per 1 $\mu\text{g/d}$ increment	50/77	0.95	0.67, 1.35	168/175	1.32	1.06, 1.65	106/104	1.04	0.78, 1.38	96/140	1.33	1.04, 1.70
<i>P</i> for interaction 0.50				<i>P</i> for interaction 0.57								
		Never smokers§		Cases/ controls (<i>n</i>)	Previous smokers§		Cases/ controls (<i>n</i>)	Current smokers§		Cases/ controls (<i>n</i>)	Ever smokers§ (previous+ current)	
		OR	95 % CI		OR	95 % CI		OR	95 % CI		OR	95 % CI
Vitamin D												
per 1 $\mu\text{g/d}$ increment	44/100	1.16	0.80, 1.70	94/101	1.46	1.04, 2.05	75/44	1.04	0.77, 1.40	169/145	1.23	0.99, 1.52
<i>P</i> for interaction 0.14												

* Multivariate adjustments: age (years); sex; energy intake (kJ/d); education (years); occupation (manual/non-manual); alcohol (g/d); regular non-steroidal anti-inflammatory drug use (weekly use for at least 6 months' duration); location (Northern Ireland/Republic of Ireland); energy-adjusted saturated fat intake (g/4184 kJ per d); energy-adjusted glycaemic index intake (units/4184 kJ per d).

† BMI analysis further adjusted for *H. pylori* infection (seropositive/seronegative) and smoking status (current/previous/never).

‡ *H. pylori* analysis further adjusted for BMI 5 years before the interview and smoking status (current/previous/never).

§ Smoking status analysis further adjusted for BMI 5 years before the interview and *H. pylori* infection (seropositive/seronegative).

25-hydroxyvitamin D concentrations, and therefore does not account for cutaneous vitamin D production from sunlight. Indeed, recent data from the USA confirm that dietary vitamin D is only partially correlated with serum 25-hydroxyvitamin D concentrations and therefore does not fully reflect vitamin D status⁽⁴⁰⁾. Unfortunately, it would be inappropriate to assess vitamin D status in the present study due to the possibility of reverse causation, and it would be useful for serum 25-hydroxyvitamin D and oesophageal adenocarcinoma risk to be assessed in future prospective studies. It is possible that dietary recall bias among the oesophageal adenocarcinoma cases or dietary measurement error from the FFQ utilised may be explaining the association observed. The potential for selection bias may also have been introduced by the lower response rate among our population-based controls compared with cases. However, mean vitamin D intakes observed in the present study are in line with those measured in the representative North/South Ireland Food Consumption Survey of 3.7 µg/d⁽⁴¹⁾, as were Ca intakes⁽⁴²⁾. Moreover, supplements were found to contribute to only 6.2 and 11.4 % of vitamin D intakes by men and women in the same nationally representative survey, respectively⁽⁴¹⁾. Vitamin D insufficiency is highly prevalent in healthy Irish adults⁽⁴³⁾, prompting calls for a nationwide fortification or supplementation programme. Yet, it cannot be ignored that certain population subgroups may be at risk of harm if advice to increase vitamin D intake from foods was implemented on a widespread basis. These findings provide specific evidence for a research direction call on vitamin D to identify population groups that may be at risk with vitamin D intake modification. Many Barrett's oesophagus patients remain asymptomatic and therefore undiagnosed⁽⁴⁴⁾, and so any necessary restrictions should perhaps be extended to regular sufferers of gastro-oesophageal reflux symptoms. However, further research is required to confirm the direct association between vitamin D intake and oesophageal adenocarcinoma risk and to elucidate the potential mechanism involved.

In conclusion, intake of vitamin D-containing foods was directly associated with oesophageal adenocarcinoma risk in this Irish population but was not related to the risk of its precursor conditions. Ca and dairy product intakes did appear to contribute to the development of these oesophageal lesions.

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