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Vegetable, fruit and nitrate intake in relation to the risk of Barrett's oesophagus in a large Dutch cohort

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Abstract

There are few epidemiological data on the dietary risk factors of Barrett's oesophagus, a precursor of oesophageal adenocarcinoma. The present study investigated the association between vegetable, fruit and nitrate intake and Barrett's oesophagus risk in a large prospective cohort. The Netherlands Cohort Study recruited 120 852 individuals aged 55–69 years in 1986. Vegetable and fruit intake was assessed using a 150-item FFQ, and nitrate intake from dietary sources and drinking water was determined. After 16·3 years of follow-up, 433 cases (241 men and 192 women) of Barrett's oesophagus with specialised intestinal metaplasia and 3717 subcohort members were analysed in a case–cohort design using Cox proportional hazards models while adjusting for potential confounders. Men exhibited a lower risk of Barrett's oesophagus in the highest *v.* the lowest quintile of total (multivariable-adjusted hazard ratio (HR): 0·66, 95 % CI 0·43, 1·01), raw (HR 0·63, 95 % CI 0·40, 0·99), raw leafy (HR 0·55, 95 % CI 0·36, 0·86) and Brassica (HR 0·64, 95 % CI 0·41, 1·00) vegetable intake. No association was found for other vegetable groups and fruits. No significant associations were found between vegetable and fruit intake and Barrett's oesophagus risk among women. Total nitrate intake was inversely associated with Barrett's disease risk in men (HR 0·50, 95 % CI 0·25, 0·99) and positively associated with it in women (HR 3·77, 95 % CI 1·68, 8·45) (*P* for interaction=0·04). These results suggest that vegetable intake may contribute to the prevention of Barrett's oesophagus. The possible differential effect in men and women should be evaluated further.

Key words: Barrett's oesophagus: Vegetables: Fruits: Nitrate: Cohort studies

The incidence of oesophageal adenocarcinoma has risen in recent decades in several developed countries of the world[1–5]. Barrett's oesophagus, a premalignant metaplastic condition of the distal oesophagus, is a precursor of oesophageal adenocarcinoma[6]. Chronic gastro-oesophageal reflux, male sex, Caucasian ethnicity, age, obesity and cigarette smoking are risk factors for the development of Barrett's oesophagus[7]. The majority of these risk factors are difficult or impossible to modify. The identification of modifiable environmental risk factors of Barrett's disease, including dietary factors, may help in the formulation of prevention strategies for oesophageal adenocarcinoma.

Few studies have focused on dietary factors in relation to Barrett's oesophagus risk[8], and they have suggested an inverse association with vegetable and fruit consumption. However, no prospective cohort study has investigated this relationship. Case–control studies are prone to bias due to the possibility that individuals with preclinical symptoms may voluntarily, or on a physician's advice, change their diet.

Green vegetables are a major source of dietary nitrate intake. Nitrate may have several beneficial health effects mediated through reactive N intermediates, including antibacterial effects and effects on gastric mucosal integrity[9]. Nitrate content may explain part of the potentially protective effect of vegetables in various health conditions. On the other hand, nitrate is also involved in the endogenous formation of *N*-nitroso compounds through reduction by anaerobic bacteria to nitrite and subsequent formation of nitrosating agents[10].

Abbreviation: HR, hazard ratio.

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The *N*-nitrosation process can be inhibited by antioxidants such as vitamin C. Epidemiological studies have not shown an association of nitrate intake[11,12], or nitrate from plant sources[13], with oesophageal adenocarcinoma. No study has evaluated the association between nitrate intake and Barrett's oesophagus risk.

The aim of the present study was to investigate the association between vegetable and fruit consumption, as well as nitrate intake, and Barrett's oesophagus risk in a large prospective cohort of Dutch men and women.

Subjects and methods

Population and follow-up

The Netherlands Cohort Study was started in September 1986. At baseline, 58 279 men and 62 573 women aged 55–69 years were recruited, and a self-administered questionnaire was completed by the study participants[14]. Ethical approval to conduct the study was obtained from the institutional review boards of the University Hospital Maastricht and TNO Nutrition and Food Research. The cohort was followed for 16·3 years until 31 December 2002 for incident Barrett's disease cases by computerised record linkage to the nationwide network and registry of histo- and cytopathology in The Netherlands (PALGA)[15]. After manual check for false-positive linkages, excerpts of all pathology records of cases were reviewed independently by a pathologist (A. L. C. D.) and a pathologist in training (C. J. R. H.) who were blinded to exposure. Of 974 cases, 106 were excluded due to uncertain diagnosis, seventy-six due to prevalent cancer or Barrett's disease at baseline, fifty-eight due to oesophageal or gastric cancer diagnosed before or within half a year of Barrett's disease diagnosis, and 148 cases because of incomplete or inconsistent dietary data or missing data on confounders. Histology was not completely specified in the excerpts available from the central PALGA database for 250 cases. Full pathology reports of these cases were retrieved from the local pathology laboratories and were reviewed by the pathologists to identify the type of metaplasia. A total of 603 cases were available for analysis, of which 433 cases had specialised intestinal metaplasia. A case–cohort design was employed and hence a random subcohort (n 5000) was selected at baseline. Due to prevalent cancer or Barrett's disease at baseline and due to inconsistent or missing data, 230 and 1053 subcohort members were excluded from further analysis, respectively.

Assessment of determinants

Vegetable and fruit intake. Food and beverage intake during the year preceding the Netherlands Cohort Study baseline was assessed using a 150-item semi-quantitative FFQ. The participants were queried about vegetable consumption frequency in summer and that in winter separately[16]. The participants could choose one of six categories ranging from 'never or less than once per month' to 'three to seven times per week'. The subjects were asked about usual serving sizes only for string beans and cooked endive; the mean of these values served as an indicator to derive the serving sizes of all the cooked vegetables using a vegetable-specific factor calculated based on the results of a pilot study, which showed an intra-individual correlation between serving sizes of different vegetables. Vegetables that were eaten regularly, but were not specifically queried about in the questionnaire, could be entered in an open-ended question along with consumption frequency and amount consumed on each occasion. For most of the vegetables, the questionnaire explicitly specified whether the vegetable was eaten raw or cooked.

The participants were queried about fruit consumption frequency and amount of fruits consumed using categories ranging from 'never or less than once per month' to 'six or seven times per week'.

Data were key-entered and processed in a standardised manner, blinded to case/subcohort status. Mean daily intakes were calculated from frequencies and serving sizes[16].

The FFQ was validated against a 9 d diet record[17]. The Spearman correlation coefficients were 0·38 for total vegetable consumption and 0·60 for total fruit consumption. The FFQ appeared to slightly overestimate vegetable consumption, on average, while underestimating fruit consumption when compared with the diet records.

Nitrate intake from diet. Food composition values for nitrate were derived from the databank on contaminants in food from the State Institute for Quality Control of Agricultural Products (RIKILT). Estimations were based on the mean nitrate contents between 1985 and 1989. Distinction between summer and winter was made while calculating nitrate intake from some vegetables (i.e. endive (raw/cooked) and lettuce), and information on nitrate losses during preparation (washing, cutting or cooking) was considered. For several vegetables, experimental data were available regarding nitrate losses during preparation[18,19]. Nitrate loss percentages used to construct the nitrate table were 16, 31, 42, 20 and 49 % for endive, spinach, chicory, cabbage and potatoes, respectively. For other vegetables consumed after cooking, nitrate losses were estimated to be 40 %. For lettuce, a 20 % loss was estimated.

Nitrate intake from drinking water. Information on nitrate content in drinking water from all the pumping stations in The Netherlands in 1986 (Vereniging van Exploitanten van Waterleiding bedrijven in Nederland (VEWIN) 1989) was used to determine the nitrate concentration in drinking water for each home address by postal code. To calculate nitrate intake from water, we used information from the questionnaire about the amount of water, coffee, tea and soup consumed. Total nitrate intake was calculated by summing dietary nitrate intake and nitrate intake from water. In the subcohort, the median proportion of dietary nitrate to total nitrate was 99 %, and the median proportion of intake from vegetables was 90 %. The major source of nitrate intake among vegetables was leafy vegetables in line with studies of nitrate intake in the Dutch population[20].

Vitamin C intake. Daily vitamin C intake was calculated from the FFQ data using the Dutch food composition table.

Assessment of potential confounders. Information on education (primary school, lower vocational, high school or higher vocational/university), cigarette smoking status (never-smoker, ex-smoker or current smoker), smoking history (number of cigarettes smoked and duration of smoking), total energy intake (kJ/d), BMI (kg/m^2), non-occupational physical activity (<30, 30–60, 60–90 or >90 min/d), alcohol intake (g/d), and long-term (more than half year) use of non-steroidal anti-inflammatory drugs and lower oesophageal sphincter-relaxing medications (nitroglycerins, aminophyllines, β-blockers, anticholinergics, nifedipine and benzodiazepines) was obtained from the baseline questionnaire.

Statistical analyses

The characteristics of cases and subcohort members are described using percentages, means, standard deviations, medians and interquartile ranges. Incidence rate ratios and 95 % CI for Barrett's oesophagus were estimated using Cox proportional hazards models comparing quintiles of intakes and using continuous variables. Analyses were carried out using Prentice's method of weighting for case–cohort designs[21]. Men and women were analysed separately. Standard errors were calculated using a robust variance estimator[22]. Results were similar while using time on study and age as the time scale; hence, only results with the former are presented. Tests

Table 1. Characteristics of Barrett's disease cases and subcohort members in the Netherlands Cohort Study on diet and cancer, 1986–2002 (Median values and interquartile ranges (IQR))

	Men				Women			
	Subcohort (*n* 1833)		Cases (*n* 241)		Subcohort (*n* 1884)		Cases (*n* 192)	
Characteristics	Median	IQR	Median	IQR	Median	IQR	Median	IQR
Age (years)								
Mean	61·2		61·1		61·4		61·4	
SD	4·2		4·1		4·2		4·1	
BMI (kg/m^2)								
Mean	24·9		25·0		25·0		26·1	
SD	2·6		2·4		3·5		3·0	
Cigarette smoking (%)								
Never-smoker	14		12		59		61	
Former smoker	53		61		20		26	
Current smoker	33		27		21		14	
Level of education (%)								
Primary school	23		24		32		35	
Lower vocational	20		17		23		24	
High school	37		34		36		35	
Higher vocational/university	20		24		10		6	
Non-occupational physical activity (%)								
< 30 min/d	17		18		23		31	
30–< 60 min/d	31		27		32		30	
60–< 90 min/d	20		23		23		21	
≥ 90 min/d	32		32		22		18	
Family history of oesophageal or gastric cancer (%)	8		7		7		6	
Use of non-steroidal anti-inflammatory drugs (%)	5		8		8		11	
Use of lower oesophageal sphincter-relaxing medication (%)	15		18		14		17	
Daily intake in subcohort*†								
Alcohol (g/d)	9·7	2·2–23·1	9·6	2·2–22·7	1·7	0–8·0	1·1	0–3·7
Energy (kJ/d)								
Mean	9065		8931		7045		6759	
SD	2087		2092		1628		1643	
Fruits (g/d)	136	76–210	134	77–218	177	113–257	177	116–268
Vegetables (g/d)	177	136–227	178	122–220	182	141–231	181	140–236
Raw vegetables (g/d)	30·7	16·6–49·5	27·3	14·2–45·4	36·7	21·6–56·8	32·8	17·8–53·5
Cooked vegetables (g/d)	143·1	106·5–184·5	138·0	101·0–177·8	142·9	109·0–183·3	147·8	111·3–174·2
Raw leafy vegetables (g/d)	7·1	3·6–13·9	6·4	3·6–11·5	7·2	3·6–14·2	7·1	3·6–14·2
Cooked leafy vegetables (g/d)	18·7	10·7–29·2	17·2	9·3–27·3	19·0	10·0–28·7	20·4	13·7–29·8
Allium vegetables (g/d)	22·5	11·0–40·2	21·9	11·0–40·2	25·3	11·0–42·3	22·3	12·4–41·7
Legumes (g/d)	29·9	19·4–44·3	28·5	16·6–43·5	26·7	16·8–39·7	25·5	14·6–36·5
Brassica vegetables (g/d)	29·0	18·8–42·8	27·5	16·9–40·6	27·9	17·9–40·5	28·8	14·8–40·5
Citrus fruits (g/d)	42·0	11·4–94·6	46·8	11·2–90·1	76·6	29·7–124·4	69·1	30·5–118·5
Nitrate from food (mg/d)	98·8	75·7–125·2	95·1	70·8–114·8	96·6	73·1–123·9	96·2	77·9–121·1
Nitrate from water (mg/d)	1·2	0·4–2·2	1·4	0·6–2·6	1·2	0·4–2·3	1·3	0·5–2·3
Total nitrate (mg/d)	100·4	76·9–126·9	96·7	72·9–117·6	97·8	74·8–125·7	97·1	78·9–123·6

*Vegetables included string/French beans, cauliflower, lettuce, carrots (raw/cooked), endive (raw/cooked), Brussels sprouts, sauerkraut, tomatoes, onions, spinach, beetroot, kale, cabbage, leek, mushrooms, broad beans, sweet peppers, rhubarb and gherkins.

†Fruits included apples, pears, strawberries, oranges and fresh orange juice, grapes, mandarins, bananas, grapefruits and grapefruit juice, raisins, and other dried fruits.

Table 2. Hazard ratios (HR) for Barrett's oesophagus with specialised intestinal metaplasia in men by vegetable and fruit intake in the Netherlands Cohort Study on diet and cancer, 1986–2002

(Hazard ratios and 95 % confidence intervals)

		Subcohort					
	Cases (*n*)	Person-years	Median intake (g/d)	HR*	95 % CI	HR†	95 % CI
Total vegetable intake							
Q1	66	4875	103·5	1		1	
Q2	39	5279	144·1	0·53	0·35, 0·82	0·54	0·35, 0·83
Q3	59	5167	178·2	0·83	0·57, 1·22	0·84	0·57, 1·24
Q4	34	5163	216·9	0·48	0·31, 0·74	0·47	0·30, 0·74
Q5	43	4715	287·4	0·66	0·43, 1·00	0·66	0·43, 1·01
P for trend					0·07		0·08
Continuous (25 g/d increment)				0·96	0·91, 1·01	0·96	0·91, 1·01
Raw vegetable intake							
Q1	57	4851	6·9	1		1	
Q2	55	5003	19·2	0·92	0·62, 1·38	0·94	0·62, 1·42
Q3	51	5003	30·6	0·86	0·57, 1·29	0·85	0·55, 1·29
Q4	40	5346	45·5	0·62	0·40, 0·96	0·62	0·40, 0·98
Q5	38	4996	73·1	0·63	0·41, 0·98	0·63	0·40, 0·99
P for trend					0·01		0·02
Continuous (25 g/d increment)				0·87	0·76, 1·00	0·87	0·75, 1·01
Cooked vegetable intake							
Q1	57	4965	79·3	1		1	
Q2	46	5204	114·9	0·75	0·50, 1·15	0·76	0·50, 1·17
Q3	50	5255	143·8	0·82	0·54, 1·23	0·81	0·53, 1·22
Q4	46	5130	176·9	0·77	0·50, 1·17	0·75	0·49, 1·16
Q5	42	4645	236·3	0·77	0·50, 1·19	0·78	0·50, 1·21
P for trend					0·33		0·34
Continuous (25 g/d increment)				0·97	0·91, 1·03	0·97	0·91, 1·03
Raw leafy vegetable intake							
Q1	76	6232	2·0	1		1	
Q2	42	3578	4·4	0·95	0·63, 1·44	0·96	0·63, 1·45
Q3	46	5168	7·1	0·72	0·48, 1·06	0·71	0·47, 1·05
Q4	41	5078	11·8	0·66	0·44, 0·99	0·66	0·43, 1·00
Q5	36	5142	21·6	0·56	0·37, 0·85	0·55	0·36, 0·86
P for trend					0·004		0·005
Continuous (25 g/d increment)				0·55	0·35, 0·88	0·55	0·34, 0·89
Cooked leafy vegetable intake							
Q1	56	4982	4·3	1		1	
Q2	50	5245	12·3	0·84	0·55, 1·26	0·82	0·53, 1·26
Q3	47	5008	18·8	0·84	0·55, 1·28	0·79	0·51, 1·22
Q4	42	5185	27·1	0·71	0·47, 1·10	0·69	0·45, 1·07
Q5	46	4779	41·8	0·85	0·56, 1·30	0·84	0·55, 1·29
P for trend					0·42		0·39
Continuous (25 g/d increment)				0·85	0·68, 1·07	0·85	0·67, 1·07
Allium vegetable intake							
Q1	73	7030	5·3	1		1	
Q2	28	3126	15·5	0·86	0·54, 1·37	0·85	0·53, 1·36
Q3	51	5301	23·7	0·92	0·62, 1·34	0·90	0·61, 1·33
Q4	46	5070	36·8	0·86	0·58, 1·28	0·85	0·56, 1·28
Q5	43	4672	61·2	0·87	0·58, 1·31	0·87	0·58, 1·33
P for trend					0·53		0·55
Continuous (25 g/d increment)				0·95	0·82, 1·11	0·95	0·81, 1·12
Legume intake							
Q1	66	5080	11·6	1		1	
Q2	40	5101	21·5	0·61	0·40, 0·94	0·63	0·41, 0·96
Q3	50	5141	29·9	0·75	0·51, 1·12	0·77	0·51, 1·16
Q4	42	5167	40·9	0·64	0·42, 0·97	0·63	0·41, 0·96
Q5	43	4710	63·2	0·72	0·47, 1·08	0·73	0·48, 1·12
P for trend					0·22		0·24
Continuous (25 g/d increment)				0·89	0·76, 1·04	0·89	0·76, 1·05
Brassica vegetable intake							
Q1	57	4954	10·7	1		1	
Q2	52	5266	21·0	0·84	0·56, 1·27	0·87	0·57, 1·32
Q3	44	5062	29·3	0·74	0·49, 1·13	0·74	0·48, 1·13
Q4	52	5161	39·8	0·86	0·57, 1·29	0·86	0·57, 1·30
Q5	36	4756	59·0	0·65	0·41, 1·01	0·64	0·41, 1·00
P for trend					0·09		0·08
Continuous (25 g/d increment)				0·88	0·72, 1·06	0·88	0·72, 1·07

Table 2. *Continued*

		Subcohort					
	Cases (*n*)	Person-years	Median intake (g/d)	HR*	95 % CI	HR†	95 % CI
Fruit intake							
Q1	49	4653	31·2	1		1	
Q2	53	5051	88·4	0·97	0·64, 1·47	0·93	0·61, 1·42
Q3	45	5062	134·6	0·81	0·53, 1·25	0·78	0·50, 1·22
Q4	36	5169	187·2	0·64	0·41, 1·02	0·64	0·40, 1·03
Q5	58	5264	292·8	1·00	0·66, 1·51	1·00	0·65, 1·53
P for trend					0·76		0·87
Continuous (25 g/d increment)				0·99	0·96, 1·02	0·99	0·96, 1·02
Citrus fruit intake							
Q1	47	4877	0	1		1	
Q2	47	5115	15·7	0·94	0·61, 1·46	0·96	0·61, 1·50
Q3	45	4951	41·0	0·92	0·59, 1·43	0·91	0·58, 1·43
Q4	61	5098	83·3	1·22	0·81, 1·84	1·22	0·79, 1·88
Q5	41	5157	166·7	0·80	0·51, 1·26	0·81	0·51, 1·28
P for trend					0·53		0·54
Continuous (25 g/d increment)				0·99	0·94, 1·04	0·99	0·94, 1·04

Q, quintile.
* Adjusted for age (years); calculated using Cox proportional hazards model.
† Adjusted for age (years), smoking status (current *v.* non-current smoker), duration of cigarette smoking (years), number of cigarettes smoked per d, total energy intake (kJ/d), BMI (quintiles), alcohol intake (g/d), levels of education (four categories), non-occupational physical activity (four categories) and use of lower oesophageal sphincter-relaxing medications (yes/no); calculated using Cox proportional hazards model.

of linear trend in the incidence rate ratios were carried out by fitting models with the median values of each exposure quintile as a continuous variable. The proportional hazards assumption was assessed using the scaled Schoenfeld residuals[23] and by introducing time–covariate interactions into the models and examining estimates and testing their significance using the Wald test. Substantial deviation from the assumption was not detected for any of the exposure variables. Effect modification by sex and vitamin C intake was tested using cross-product terms. All the models were adjusted for age, and multivariable models were additionally adjusted for smoking status (current: yes/no), number of cigarettes smoked per d, duration of smoking (years), total energy intake (kJ/d), alcohol intake (g/d), BMI categories (quintiles), levels of education (four categories), non-occupational physical activity (four categories) and long-term use of lower oesophageal sphincter-relaxing medications (yes/no). Models for nitrate intake were also adjusted for vegetable intake (g/d). Primary analysis was carried out using only cases with intestinal metaplasia (*n* 433). Long-term use of reflux medications and vitamin C supplement use were also considered in multivariable models, but were not included in the final reported models as they had only minor effects on the estimates. Additional analyses were carried out restricted to individuals who reported having similar vegetable and fruit intakes 5 years before baseline. Furthermore, we carried out an analysis by excluding the first 2 years of follow-up.

Based on a method proposed by Cai & Zeng[24] and using the available number of cases and subcohort members, we estimated that an 80 % power could be achieved to show hazard ratio (HR) of 0·55 and 0·5 between quintiles of exposure with a two-sided type 1 error of 0·05 among men and women, respectively.

Results

The baseline characteristics of 241 male and 192 female cases and 3717 subcohort members are given in Table 1. Cases were more often men, were less likely to be current smokers at baseline, and were somewhat more likely to have used lower oesophageal sphincter-relaxing medications and non-steroidal anti-inflammatory drugs. The median intake of total vegetables was 179 g/d (interquartile range 138–229 g/d) in the subcohort and was higher in women than in men (Kruskal–Wallis χ^2(df = 1): 7·3, P=0·007). The intakes of different groups of vegetables were comparable between cases and subcohort members (Table 1). Vegetables consumed in the largest amount in the subcohort were tomatoes, onions, string beans and cauliflower (median 19, 17, 17 and 13 g/d, respectively). Median fruit consumption in the subcohort was 157 g/d (interquartile range 94–234 g/d) with a higher intake in women (Kruskal–Wallis χ^2(df = 1): 153, P=0·0001).

Total nitrate intake strongly correlated with vegetable intake in the subcohort (r 0·83) and median intake was comparable between cases and subcohort members (Table 1).

Among men, total vegetable intake was inversely associated with the risk of Barrett's oesophagus with intestinal metaplasia (Table 2), but a clear linear trend was not observed. A strong inverse association with a clear trend was observed with raw leafy vegetable consumption. The multivariable-adjusted model with continuous exposure variables estimated a 45 % decrease in risk per 25 g/d increase in raw leafy vegetable intake. An inverse association was also found for total raw vegetable and Brassica vegetable intake. No association was found for cooked vegetable and fruit intake.

Associations between vegetable and fruit intake and Barrett's oesophagus risk among women are summarised in Table 3. The strongest inverse association was observed for Brassica

Table 3. Hazard ratios (HR) for Barrett's oesophagus with specialised intestinal metaplasia in women by vegetable and fruit intake in the Netherlands Cohort Study on diet and cancer, 1986–2002

(Hazard ratios and 95 % confidence intervals)

		Subcohort					
	Cases (*n*)	Person-years	Median intake (g/d)	HR*	95 % CI	HR†	95 % CI
Total vegetable intake							
Q1	39	5440	106·3	1		1	
Q2	32	5799	148·7	0·77	0·47, 1·25	0·82	0·49, 1·35
Q3	47	5997	182·8	1·09	0·70, 1·71	1·21	0·76, 1·93
Q4	46	5874	223·6	1·10	0·70, 1·72	1·22	0·75, 1·97
Q5	28	4924	298·8	0·80	0·48, 1·33	0·98	0·57, 1·67
P for trend					0·78		0·61
Continuous (25 g/d increment)				0·98	0·93, 1·03	1·00	0·95, 1·05
Raw vegetable intake							
Q1	48	5604	9·4	1		1	
Q2	41	5413	24·7	0·89	0·57, 1·38	0·89	0·56, 1·42
Q3	37	5842	36·5	0·74	0·47, 1·16	0·84	0·52, 1·34
Q4	36	5772	51·7	0·73	0·46, 1·14	0·83	0·52, 1·34
Q5	30	5403	78·0	0·65	0·40, 1·05	0·77	0·47, 1·27
P for trend					0·06		0·31
Continuous (25 g/d increment)				0·90	0·77, 1·03	0·95	0·82, 1·09
Cooked vegetable intake							
Q1	35	5426	80·5	1		1	
Q2	36	5648	115·9	0·99	0·61, 1·61	1·04	0·64, 1·71
Q3	41	6207	144·0	1·01	0·63, 1·63	1·10	0·67, 1·79
Q4	47	5766	177·7	1·27	0·80, 2·03	1·36	0·85, 2·19
Q5	33	4987	234·1	1·04	0·63, 1·70	1·18	0·71, 1·99
P for trend					0·60		0·32
Continuous (25 g/d increment)				0·99	0·93, 1·05	1·01	0·95, 1·07
Raw leafy vegetable intake							
Q1	49	7176	2·1	1		1	
Q2	34	4225	4·8	1·20	0·75, 1·90	1·26	0·78, 2·05
Q3	39	5788	7·6	0·99	0·64, 1·54	0·99	0·62, 1·57
Q4	35	5634	13·0	0·92	0·59, 1·45	0·93	0·58, 1·51
Q5	35	5211	22·8	0·99	0·63, 1·57	1·14	0·70, 1·84
P for trend					0·71		0·90
Continuous (25 g/d increment)				1·10	0·69, 1·75	1·27	0·78, 2·05
Cooked leafy vegetable intake							
Q1	28	5689	4·3	1		1	
Q2	29	5668	11·9	1·04	0·61, 1·78	1·04	0·60, 1·80
Q3	51	5720	19·2	1·81	1·12, 2·94	1·95	1·18, 3·23
Q4	45	5585	27·0	1·64	1·00, 2·69	1·71	1·02, 2·86
Q5	39	5372	41·8	1·47	0·89, 2·45	1·53	0·91, 2·59
P for trend					0·05		0·04
Continuous (25 g/d increment)				1·10	0·92, 1·32	1·12	0·92, 1·36
Allium vegetable intake							
Q1	47	7379	3·8	1		1	
Q2	48	5205	18·3	1·46	0·95, 2·23	1·57	1·01, 2·42
Q3	32	5013	27·6	1·00	0·62, 1·59	1·12	0·68, 1·82
Q4	27	5061	40·1	0·84	0·52, 1·38	0·99	0·59, 1·65
Q5	38	5377	60·3	1·11	0·71, 1·75	1·27	0·79, 2·03
P for trend					0·80		0·69
Continuous (25 g/d increment)				1·01	0·86, 1·19	1·06	0·90, 1·25
Legume intake							
Q1	45	5241	10·2	1		1	
Q2	28	5960	18·0	0·55	0·33, 0·90	0·56	0·34, 0·93
Q3	48	5638	26·6	1·00	0·65, 1·54	1·02	0·64, 1·62
Q4	41	5828	37·0	0·83	0·53, 1·29	0·83	0·52, 1·32
Q5	30	5367	58·9	0·66	0·41, 1·07	0·71	0·42, 1·20
P for trend					0·32		0·49
Continuous (25 g/d increment)				0·81	0·67, 0·98	0·83	0·67, 1·01
Brassica vegetable intake							
Q1	50	5454	10·5	1		1	
Q2	32	5784	19·8	0·60	0·38, 0·96	0·61	0·38, 0·99
Q3	32	5851	28·2	0·60	0·38, 0·95	0·60	0·37, 0·98
Q4	47	5699	38·2	0·91	0·59, 1·39	0·93	0·60, 1·45
Q5	31	5246	57·8	0·65	0·41, 1·05	0·65	0·40, 1·07
P for trend					0·37		0·40
Continuous (25 g/d increment)				0·88	0·72, 1·08	0·89	0·71, 1·11

Table 3. *Continued*

		Subcohort					
	Cases (*n*)	Person-years	Median intake (g/d)	HR*	95 % CI	HR†	95 % CI
Fruit intake							
Q1	43	5538	64·5	1		1	
Q2	36	5564	124·9	0·84	0·53, 1·34	0·93	0·58, 1·52
Q3	29	5680	176·9	0·65	0·40, 1·07	0·72	0·43, 1·20
Q4	41	5800	236·8	0·91	0·58, 1·42	0·91	0·57, 1·46
Q5	43	5451	342·6	1·01	0·64, 1·58	1·12	0·69, 1·81
P for trend					0·74		0·58
Continuous (25 g/d increment)				1·00	0·97, 1·03	1·00	0·97, 1·04
Citrus fruit intake							
Q1	42	5550	8·2	1		1	
Q2	44	5858	38·2	0·99	0·64, 1·55	1·05	0·66, 1·66
Q3	36	5618	77·7	0·85	0·53, 1·35	0·87	0·54, 1·42
Q4	31	5626	110·9	0·72	0·44, 1·18	0·79	0·47, 1·31
Q5	39	5383	187·5	0·96	0·60, 1·52	1·05	0·65, 1·69
P for trend					0·62		0·89
Continuous (25 g/d increment)				0·98	0·93, 1·03	0·98	0·93, 1·04

Q, quintile.
* Adjusted for age (years); calculated using Cox proportional hazards model.
† Adjusted for age (years), smoking status (current *v.* non-current smoker), duration of cigarette smoking (years), number of cigarettes smoked per d, total energy intake (kJ/d), BMI (quintiles), alcohol intake (g/d), levels of education (four categories), non-occupational physical activity (four categories) and use of lower oesophageal sphincter-relaxing medications (yes/no); calculated using Cox proportional hazards model.

vegetables, but significant inverse associations were not detected. HR estimates for cooked leafy vegetables suggested positive associations, but the estimate for the highest category was not significant. HR estimates were generally higher than those found among men, and interaction analyses with continuous variables suggested effect modification by sex for leafy vegetables (Wald: $P=0{\cdot}04$ and $P=0{\cdot}07$ for raw and cooked leafy vegetables, respectively), but not for other vegetable groups.

Table 4. Hazard ratios (HR) for Barrett's oesophagus with specialised intestinal metaplasia in men by nitrate intake from diet and water in the Netherlands Cohort Study on diet and cancer, 1986–2002

(Hazard ratios and 95 % confidence intervals)

		Subcohort					
	Cases (*n*)	Person-years	Median intake (mg/d)	HR*	95 % CI	HR†	95 % CI
Nitrate intake from food							
Q1	58	4724	58·3	1		1	
Q2	47	5280	80·0	0·71	0·47, 1·08	0·72	0·47, 1·11
Q3	57	5258	99·3	0·88	0·59, 1·31	0·89	0·57, 1·41
Q4	45	5094	119·8	0·71	0·46, 1·07	0·72	0·42, 1·23
Q5	34	4842	157·6	0·56	0·36, 0·88	0·59	0·30, 1·18
P for trend					0·02		0·19
Continuous (10 mg/d increment)				0·96	0·92, 1·00	0·96	0·90, 1·03
Nitrate intake from water							
Q1	34	4855	0·15	1		1	
Q2	44	4967	0·50	1·28	0·80, 2·05	1·30	0·80, 2·09
Q3	48	5012	1·16	1·36	0·85, 2·17	1·38	0·86, 2·21
Q4	52	5180	1·93	1·44	0·91, 2·28	1·48	0·93, 2·35
Q5	61	4707	3·60	1·83	1·17, 2·87	1·92	1·22, 3·04
P for trend					0·008		0·005
Continuous (1 mg/d increment)				1·09	1·03, 1·15	1·10	1·04, 1·17
Total nitrate intake‡							
Q1	59	4757	59·5	1		1	
Q2	48	5250	81·7	0·73	0·48, 1·10	0·71	0·46, 1·10
Q3	57	5298	100·8	0·86	0·58, 1·28	0·85	0·54, 1·34
Q4	45	5042	121·5	0·71	0·47, 1·08	0·68	0·40, 1·15
Q5	32	4852	159·4	0·52	0·33, 0·82	0·50	0·25, 0·99
P for trend					0·008		0·07
Continuous (10 mg/d increment)				0·96	0·92, 1·00	0·97	0·91, 1·04

Q, quintile.
* Adjusted for age (years); calculated using Cox proportional hazards model.
† Adjusted for age (years), smoking status (current *v.* non-current smoker), duration of cigarette smoking (years), number of cigarettes smoked per d, total energy intake (kJ/d), vegetable intake (g/d), fruit intake (g/d), BMI (quintiles), alcohol intake (g/d), levels of education (four categories), non-occupational physical activity (four categories) and use of lower oesophageal sphincter-relaxing medications (yes/no); calculated using Cox proportional hazards model.
‡ Total nitrate is the sum of nitrate intake from food and nitrate intake from water.

Table 5. Hazard ratios (HR) for Barrett's oesophagus with specialised intestinal metaplasia in women by nitrate intake from diet and water in the Netherlands Cohort Study on diet and cancer, 1986–2002

(Hazard ratios and 95 % confidence intervals)

		Subcohort					
	Cases (*n*)	Person-years	Median intake (mg/d)	HR*	95 % CI	HR†	95 % CI
Nitrate intake from food							
Q1	29	5468	57·4	1		1	
Q2	39	5818	77·3	1·27	0·77, 2·09	1·51	0·88, 2·60
Q3	49	5928	97·0	1·56	0·96, 2·52	2·26	1·31, 3·92
Q4	39	5793	119·4	1·28	0·78, 2·12	2·12	1·12, 4·01
Q5	36	5027	158·3	1·35	0·81, 2·25	3·25	1·46, 7·23
P for trend					0·35		0·005
Continuous (10 mg/d increment)				1·00	0·97, 1·04	1·07	1·00, 1·15
Nitrate intake from water							
Q1	25	5473	0·16	1		1	
Q2	42	5536	0·48	1·68	1·00, 2·81	1·84	1·08, 3·13
Q3	42	5305	1·16	1·76	1·05, 2·94	2·01	1·18, 3·41
Q4	40	5718	1·97	1·54	0·92, 2·60	1·71	1·00, 2·92
Q5	38	5524	3·86	1·52	0·90, 2·58	1·67	0·97, 2·89
P for trend					0·52		0·39
Continuous (1 mg/d increment)				1·01	0·95, 1·07	1·01	0·96, 1·07
Total nitrate intake‡							
Q1	28	5534	58·6	1		1	
Q2	44	5721	79·3	1·53	0·93, 2·51	1·84	1·07, 3·15
Q3	45	5980	98·5	1·49	0·91, 2·44	2·15	1·22, 3·79
Q4	37	5764	121·7	1·28	0·77, 2·15	2·19	1·15, 4·18
Q5	38	5035	159·5	1·50	0·90, 2·49	3·77	1·68, 8·45
P for trend					0·33		0·004
Continuous (10 mg/d increment)				1·00	0·97, 1·04	1·07	1·00, 1·15

Q, quintile.

* Adjusted for age (years); calculated using Cox proportional hazards model.

† Adjusted for age (years), smoking status (current *v.* non-current smoker), duration of cigarette smoking (years), number of cigarettes smoked per d, total energy intake (kJ/d), vegetable intake (g/d), fruit intake (g/d), BMI (quintiles), alcohol intake (g/d), levels of education (four categories), non-occupational physical activity (four categories) and use of lower oesophageal sphincter-relaxing medications (yes/no); calculated using Cox proportional hazards model.

‡ Total nitrate is the sum of nitrate intake from food and nitrate intake from water.

Associations between nitrate intake and Barrett's oesophagus risk in men and women are summarised in Tables 4 and 5, respectively. The highest *v.* the lowest category of total nitrate and nitrate intake from food was inversely associated with Barrett's oesophagus risk among men after adjustment for confounders including vegetables. However, the estimate for nitrate intake from food in the multivariable model was not significant. Nitrate intake from water was positively associated with Barrett's oesophagus risk. Among women, estimates for the highest category suggested moderately strong positive associations for all the three nitrate variables. Test for interaction with sex was significant for age-adjusted and multivariable models with categorical exposure variables for total nitrate intake ($P=0{\cdot}04$). Interaction between nitrate intake and vitamin C intake was not significant ($P=0{\cdot}10$ and $P=0{\cdot}99$ in men and women, respectively).

Analysis restricted to individuals who reported having similar vegetable and fruit intakes 5 years earlier was based on 104 male and 89 female cases and 1632 subcohort members. An association with total vegetable intake was no longer apparent among men (HR for the highest *v.* the lowest category: 0·81, 95 % CI 0·40, 1·65). The HR for the highest category of raw vegetable intake compared with the lowest category was 0·38 (95 % CI 0·18, 0·80) in men and 0·94 (95 % CI 0·45, 1·95) in women (*P* for interaction=0·79). Analyses for nitrate intake were similar to the primary analysis with somewhat attenuated HR estimates (data not shown). Analyses excluding the first 2 years of follow-up did not substantially change the estimates for vegetable/fruit intake and nitrate intake (data not shown).

Discussion

This is the first prospective cohort study to examine the association between vegetable and fruit consumption, as well as nitrate intake, and Barrett's oesophagus risk. The results indicate an inverse association between total vegetable intake and Barrett's oesophagus risk among men. Raw vegetable intake, especially raw leafy vegetable intake, was inversely associated with Barrett's oesophagus risk in men and the highest category of Brassica vegetable intake also suggested a protective effect. Estimates among women, in general, do not suggest an inverse association. Fruit consumption was not associated with Barrett's disease risk in men or in women. In men, an inverse association with nitrate intake was observed, but not with nitrate intake from water sources. In women, nitrate intake was positively associated with Barrett's disease risk. Effects were not different across the levels of vitamin C intake.

Epidemiological studies have suggested an inverse association between vegetable and fruit intake and oesophageal adenocarcinoma, and stronger inverse associations have been reported with the intake of green leafy, raw and

cruciferous vegetables in several studies[25]. Few studies have examined the association between vegetable and fruit intake and Barrett's disease, a precursor lesion for adenocarcinoma. Case–control studies have observed an inverse association with vegetable and/or fruit intake[26–29], a 'health-conscious' diet including fruits and vegetables[30], and fibre intake[31,32]. A case–control study carried out in the USA has evaluated specific subtypes of vegetables and found an inverse association with dark green vegetables[33]. The present results suggest that a possible protective effect could be due to raw leafy vegetable and cruciferous vegetable consumption, in line with previous findings for oesophageal adenocarcinoma[25] and Barrett's oesophagus[33].

Vegetables are a major source of nitrate in human diet. Nitrate intake might be responsible for some of the effects of raw leafy vegetables found in the present study. Absorbed nitrate is excreted by the salivary glands in high concentrations and is reduced to nitrite by anaerobic bacteria[9]. High concentrations of NO are generated from nitrite at the gastro-oesophageal junction under acidic conditions[34], and high concentrations of NO can also be found within the columnar lined oesophagus of patients with Barrett's oesophagus during acid reflux[35]. It has thus been suggested that high doses of NO in the oesophagus may be important in the development of Barrett's oesophagus[36], possibly through its effect on oesophageal tissue damage[37]. It has also been shown that dietary nitrate may induce *N*-nitrosation in juxtaluminal compartments of the upper gastrointestinal tract of healthy subjects and patients with Barrett's oesophagus via the generation of nitric oxide[38]. In the absence of vitamin C and under acidic conditions, nitrite is converted to nitrous acid and nitrosating agents that can react with secondary amines to form *N*-nitrosamines[39]. On the other hand, ingested nitrate also has several beneficial effects through the formation of NO[40], including protection of gastric mucosa from damage[41,42] and from gastrointestinal infections[43,44]. We are not aware of any previous epidemiological studies that have evaluated the association between nitrate intake and Barrett's oesophagus risk. Studies assessing the risk of oesophageal adenocarcinoma did not show associations with nitrate intake from diet and/or drinking water[11–13]. Although the present results cannot be unambiguously explained by the possible positive and negative effects of nitrate in the gastro-oesophageal junction, our findings provide additional support to the hypothesis that increased nitrate intake may increase or decrease the risk of health outcomes in different populations[40].

Previous epidemiological studies did not evaluate the possible differences between men and women regarding the association of Barrett's disease risk with vegetable intake[26–28]. The present results suggest a differential effect of raw vegetables in men and women, which might be partly explained by the sex differences in the occurrence of Barrett's oesophagus and differences in oesophageal pathophysiology. Barrett's disease occurs more often in men with a male to female ratio of 2:1 estimated in a meta-analysis[45], and male sex is an independent risk factor for oesophagitis[46,47]. Studies comparing men and women with reflux symptoms suggest sex-specific differences in oesophageal acid exposure, presence of defective oesophageal sphincter and hiatus hernia[48]. Animal studies have suggested that female sex hormones might have an effect on parietal cell mass and decrease basal acid secretion[49], and in rat models of reflux oesophagitis, oesophageal damage has been shown to be more prominent in male rats than in female rats in the presence of NO administration[50]. These sex-specific differences support the hypothesis that dietary factors might have differential effects on the progression from reflux disease to Barrett's oesophagus. However, if these experimental results translate to effects on human risks, they would suggest more protective effects of sex hormones in women rather than in men.

Differential bias could also partly explain our dissimilar findings in men and women. Since the absence of Barrett's disease in the cohort of the present study cannot be verified, false-negative cases might have occurred. A previous study investigating the presence of Barrett's oesophagus among asymptomatic individuals has found a prevalence of 3% in men and 1% among women[51]. We cannot rule out the possibility that different false-negative proportions among men and women could have led to differential bias in the present study.

Other limitations include the lack of information on gastro-oesophageal reflux and *Helicobacter pylori* infection, which has been shown to be inversely associated with Barrett's oesophagus[52]. Additionally, it is difficult to measure vegetable and fruit intake in large epidemiological studies. Low correlations were observed for vegetable intake in the validation of the FFQ[17]. We would expect to find attenuations of HR estimates, and it is unlikely that differential bias across cases and non-cases would have been introduced. Similarly, attenuation of estimates might have resulted from the lack of repeated exposure measurements over time.

The prospective design, the large number of cases and the availability of full pathology reports to identify intestinal metaplasia are the most important strengths of the present study.

In conclusion, results obtained for this large prospective cohort are consistent with an inverse association between vegetable intake, especially green leafy vegetable intake, and Barrett's oesophagus risk. These findings add to the limited number of published epidemiological research on the relationship between vegetable intake and Barrett's oesophagus risk. However, the possibility that the beneficial effects of vegetables might be stronger among men should be evaluated further.

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