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Citation	Jenab, Mazda, H. Bas Bueno-de-Mesquita, Pietro Ferrari, Franzel J. B van Duijnhoven, Teresa Norat, Tobias Pischon, Eugène H. J. M. Jansen, et al. 2010. Association between pre-diagnostic circulating vitamin D concentration and risk of colorectal cancer in European populations: a nested case-control study. <i>BMJ : British Medical Journal</i> 340.
Published Version	doi:10.1136/bmj.b5500
Accessed	February 19, 2015 12:04:02 AM EST
Citable Link	http://nrs.harvard.edu/urn-3:HUL.InstRepos:4553309
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Association between pre-diagnostic circulating vitamin D concentration and risk of colorectal cancer in European populations: a nested case-control study

Mazda Jenab, scientist,¹ H Bas Bueno-de-Mesquita, senior scientist,^{2,33} Pietro Ferrari, scientist,^{1,3} Franzel J B van Duijnhoven, scientist,^{2,4} Teresa Norat, principal research fellow,⁵ Tobias Pischon, scientist,⁶ Eugène H J M Jansen, scientist,² Nadia Slimani, scientist, group head,¹ Graham Byrnes, statistician,¹ Sabina Rinaldi, scientist,¹ Anne Tjønneland, department head,⁷ Anja Olsen, scientist,⁷ Kim Overvad, professor of epidemiology,⁸ Marie-Christine Boutron-Ruault, senior scientist,⁹ Françoise Clavel-Chapelon, department head,⁹ Sophie Morois, research fellow,⁹ Rudolf Kaaks, professor, division head,¹⁰ Jakob Linseisen, unit head,^{10,34} Heiner Boeing, professor, department chair,⁶ Manuela M Bergmann, scientist,⁶ Antonia Trichopoulou, professor of nutrition,^{11,12} Gesthimani Misirli, research associate,¹¹ Dimitrios Trichopoulos, professor of cancer prevention, professor of epidemiology,¹³ Franco Berrino, department chief,¹⁴ Paolo Vineis, chair of environmental epidemiology, unit chief,^{5,15} Salvatore Panico, professor of internal medicine, unit chief,¹⁶ Domenico Palli, unit chief,¹⁷ Rosario Tumino, director,¹⁸ Martine M Ros, junior scientist,^{2,19} Carla H van Gils, associate professor of clinical epidemiology,⁴ Petra H Peeters, professor of epidemiology,⁴ Magritt Brustad, researcher,²⁰ Eiliv Lund, professor,²⁰ María-José Tormo, unit chief, scientist,^{21,22} Eva Ardanaz, scientist,²³ Laudina Rodríguez, section chief,²⁴ María-José Sánchez, lecturer, director,^{25,26} Miren Dorronsoro, unit chief,²⁷ Carlos A Gonzalez, scientist, unit chief,²⁸ Göran Hallmans, professor,²⁹ Richard Palmqvist, senior lecturer, consultant,³⁰ Andrew Roddam, visiting research fellow,³¹ Timothy J Key, deputy director,³¹ Kay-Tee Khaw, professor of clinical gerontology,³² Philippe Autier, scientist, section head,¹ Pierre Hainaut, scientist, section head,¹ Elio Riboli, director⁵

¹International Agency for Research on Cancer (IARC-WHO), Lyon, France

²National Institute for Public Health and the Environment (RIVM), Bilthoven, Netherlands

³Data Collection and Exposure Unit, European Food Safety Authority (EFSA), Parma, Italy

⁴Julius Centre for Health Sciences and Primary Care, University Medical Centre, Utrecht, Netherlands

⁵School of Public Health, Imperial College, London, UK

⁶Department of Epidemiology, German Institute of Human Nutrition Potsdam-Rehbruecke, Nuthetal, Germany

⁷Institute of Cancer Epidemiology, Danish Cancer Society, Copenhagen, Denmark

⁸Department of Epidemiology, School of Public Health, Aarhus University, Aarhus, Denmark

⁹Inserm (Institut National de la Santé et de la Recherche Médicale) ERI 20, EA 4045, and Institut Gustave Roussy, Villejuif, France

ABSTRACT

Objective To examine the association between pre-diagnostic circulating vitamin D concentration, dietary intake of vitamin D and calcium, and the risk of colorectal cancer in European populations.

Design Nested case-control study.

Setting The study was conducted within the EPIC study, a cohort of more than 520 000 participants from 10 western European countries.

Participants 1248 cases of incident colorectal cancer, which developed after enrolment into the cohort, were matched to 1248 controls

Main outcome measures Circulating vitamin D concentration (25-hydroxy-vitamin-D, 25-(OH)D) was measured by enzyme immunoassay. Dietary and lifestyle data were obtained from questionnaires. Incidence rate ratios and 95% confidence intervals for the risk of colorectal cancer by 25-(OH)D concentration and levels of dietary calcium and vitamin D intake were estimated from multivariate conditional logistic regression models, with adjustment for potential dietary and other confounders.

Results 25-(OH)D concentration showed a strong inverse linear dose-response association with risk of colorectal cancer (P for trend <0.001). Compared with a pre-defined mid-level concentration of 25-(OH)D (50.0-75.0 nmol/l), lower levels were associated with higher colorectal cancer

risk (<25.0 nmol/l: incidence rate ratio 1.32 (95% confidence interval 0.87 to 2.01); 25.0-49.9 nmol/l: 1.28 (1.05 to 1.56), and higher concentrations associated with lower risk (75.0-99.9 nmol/l: 0.88 (0.68 to 1.13); ≥100.0 nmol/l: 0.77 (0.56 to 1.06)). In analyses by quintile of 25-(OH)D concentration, patients in the highest quintile had a 40% lower risk of colorectal cancer than did those in the lowest quintile (P<0.001). Subgroup analyses showed a strong association for colon but not rectal cancer (P for heterogeneity=0.048). Greater dietary intake of calcium was associated with a lower colorectal cancer risk. Dietary vitamin D was not associated with disease risk. Findings did not vary by sex and were not altered by corrections for season or month of blood donation.

Conclusions The results of this large observational study indicate a strong inverse association between levels of pre-diagnostic 25-(OH)D concentration and risk of colorectal cancer in western European populations. Further randomised trials are needed to assess whether increases in circulating 25-(OH)D concentration can effectively decrease the risk of colorectal cancer.

INTRODUCTION

Vitamin D can be derived from the diet but in most populations it is mainly produced endogenously from

sun exposure.¹ The primary role of vitamin D is the maintenance of calcium homeostasis and bone metabolism. Vitamin D might also play an important part in cancer control by modulating cellular growth and apoptosis and by reducing angiogenesis.²

An effect of vitamin D on cancer may be important in the colorectum because both normal and neoplastic colon cells can produce the active hormone from the main circulating form 25-hydroxy-vitamin D (25-(OH)D), suggesting that it may play a direct role in controlling the growth of normal and neoplastic colonic cells.^{3,4} However, the epidemiological evidence is not conclusive and almost no pre-diagnostic data are available from European populations.

Most of the epidemiological data available are based on dietary vitamin D intake and show mixed findings from both case-control and prospective cohort studies.^{5,6} These studies often do not account for endogenous vitamin D production from sun exposure and are limited by measurement errors from the various dietary assessment methods and food composition tables used to assess its dietary intake. Such limitations can be overcome by measuring circulating 25-(OH)D concentration. This biomarker provides an overall estimate of vitamin D status and integrates vitamin D derived from endogenous production and from dietary intake.⁷ However, in addition to some evidence on colorectal adenomas,⁸⁻¹² only a few prospective studies have measured blood 25-(OH)D concentrations, with all but one¹³ reporting an inverse association with the risk of either colorectal cancer or its anatomic subsites.¹⁴⁻¹⁹ Nevertheless, many of these studies were small, and all but two^{16,18} were based on North American populations.

Although vitamin D metabolism might be modulated by some dietary factors, particularly intake levels of calcium,²⁰ retinol²¹, and alcohol,^{22,23} the potential interactions have not been well studied in previous considerations of the vitamin D-colorectal cancer hypothesis. We therefore did a nested case-control study within the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort to examine the association between pre-diagnostic 25-(OH)D concentration and dietary intakes of vitamin D and calcium with colorectal cancer risk in European populations.

METHODS

Study population and data collection

The rationale and methods of the EPIC study, including information on dietary assessment methods, blood collection protocols, and follow-up procedures, have been reviewed previously.²⁴ EPIC is a large prospective cohort study with more than 520 000 participants enrolled from 23 centres in Denmark, France, Greece, Germany, Italy, the Netherlands, Norway, Spain, Sweden, and the United Kingdom. Individuals who were eligible for the study were selected from the general population of a specific geographical area, town, or province. Exceptions included the French sub-cohort, which is based on members of the health insurance system or state-school employees, and the Utrecht

(Netherlands) sub-cohort, which is based on women who underwent screening for breast cancer. Between 1992 and 1998, standardised lifestyle and personal history questionnaires, anthropometric data, and blood samples were collected from most participants at recruitment, before disease onset or diagnosis. Diet over the previous 12 months was assessed at recruitment by validated country-specific questionnaires designed to ensure high compliance and improved measures of local dietary habits.²⁴ Blood samples were stored at the International Agency for Research on Cancer (Lyon, France; -196°C, liquid nitrogen) for all countries except Denmark (-150°C, nitrogen vapour) and Sweden (-80°C freezers). Values for dietary intake of total energy, vitamin D, calcium, and retinol were computed using country-specific food composition tables. Data on the intake of vitamin D from dietary supplements were only available from a subset of participants and are not included as a component of the dietary vitamin D variable presented here.

Follow-up for cancer incidence and vital status

Vital status follow-up (98.4% complete) is collected by record linkage with regional and/or national mortality registries in all countries except Germany and Greece, where data are collected actively. Cancer incidence is determined through record linkage with regional cancer registries (Denmark, Italy, the Netherlands, Norway, Spain, Sweden, and the United Kingdom; complete up to June 2003) or via a combination of methods, including the use of health insurance records, contacts with cancer and pathology registries, and active follow-up through participants and their next-of-kin (France, Germany, and Greece; complete up to June 2002).

Nested case-control design and participant selection

Case ascertainment and selection

Colon cancers were defined as tumours in the cecum, appendix, ascending colon, hepatic flexure, transverse colon, splenic flexure, descending and sigmoid colon (C18.0-C18.7 as per the 10th Revision of the International Statistical Classification of Diseases, Injury and Causes of Death), and overlapping or unspecified origin tumours (C18.8 and C18.9). Rectal cancers were defined as tumours occurring at the rectosigmoid junction (C19) or rectum (C20). Anal canal cancers were excluded. Colorectal cancer is the combination of the colon and rectal cancer cases.

After exclusions (56 cases for missing matching information, 31 cases for missing laboratory 25-(OH)D data for the case-control set), a total of 1248 first incident colorectal cases (colon cancer=785; rectal cancer=463) were identified. Cases were not selected from Norway (blood samples only recently collected; few colorectal cancers diagnosed after blood donation) and the Malmö centre of Sweden. The numbers of cases for analyses of dietary vitamin D, calcium, and retinol were 772 colon and 448 rectal because of missing nutrient intake values from Greece.

Control selection

Controls were selected (1:1) by incidence density sampling from all cohort members alive and free of cancer at the time of diagnosis of the cases and were matched by age (plus or minus six months at recruitment), sex, study centre (to account for centre-specific differences such as questionnaire design and blood collection procedures), time of the day at blood collection, and fasting status at the time of blood collection (less than three hours, three to six hours, and more than six hours). Women were further matched by menopausal status (pre-menopausal, post-menopausal, peri-menopausal/unknown), phase of menstrual cycle at time of blood collection, and usage of hormone replacement therapy at time of blood collection (yes/no). The additional matching criteria for women were needed for other studies that were being done using the same matched case-control sets. The numbers of case-control matched sets from each country are shown in table 1.

Laboratory assays

The feasibility and reliability of measuring 25-(OH)D in EPIC samples has been previously established.²⁵ Vitamin D status was quantitatively determined by measuring 25-(OH)D in 25 µl of serum (heparin plasma for Swedish samples) using a commercially available enzyme immunoassay kit (OCTEIA 25-(OH)D Kit, Immuno Diagnostic Systems, Boldon, UK) at the Laboratory for Health Protection Research, National Institute for Public Health and the Environment, the Netherlands. The kit is specific for 100% of vitamin D3 origin and 75% of vitamin D2 origin. For technical reasons, some case-control sets were not measured in the same analytical batch. However, batch-to-batch differences are considered to be minor: the coefficient of variation (inter-assay) as determined with two kit control samples was minimal (5.9% at the level of 20.3 nmol/L and 5.4% at the level of 77.4 nmol/l), no significant between-day drift, time shifts, or other trends were observed and the percentage of variance attributable to batch-to-batch differences was 4.5%. For all analyses, laboratory technicians were blinded to the case-control status of the samples.

Statistical analysis

Differences between cases and controls in mean dietary variables, circulating 25-(OH)D levels and baseline covariates were tested by paired *t*-tests of the values in each case-control set for colon and rectum anatomical sub-sites separately. For categorical variables (smoking status, physical activity, education level), case-control differences were assessed by conditional logistic regression.

Conditional logistic regression, stratified by the case-control set, was used to estimate the risk and 95% confidence intervals of colorectal cancers and cancers of the colon and rectum in relation to levels of intake of dietary variables and circulating 25-(OH)D concentrations (SAS statistical software, version 9, SAS Institute,

Cary, NC). In a nested case-control study where controls are selected using incidence density sampling, this procedure estimates the incidence rate ratio which, given the rarity of the disease, is roughly equal to the odds ratio.²⁶ For dietary vitamin D and calcium, quintile cut-points were based on the variable distributions in all the controls combined. Circulating 25-(OH)D concentration was divided into five categories with predefined cut-points on the basis of proposed levels of vitamin D deficiency/insufficiency:²⁷⁻³⁰ category 1: <25.0 nmol/l, category 2: ≥25.0 to <50.0 nmol/l, category 3 (referent): ≥50.0 to <75.0 nmol/l, category 4: ≥75.0 to 100.0 nmol/l, category 5: ≥100.0 nmol/l. A level of between ≥50.0 and <75.0 nmol/l was assumed as a central, mid-range reference category in order to provide stability in the statistical analyses and for a clearer ascertainment of the cancer risk consequences of both lower and higher 25-(OH)D concentrations. As an additional analysis, circulating 25-(OH)D concentration was also divided by quintiles based on the distribution in the control members, with the lowest category chosen as the referent. Quintile cut-points are described in web table 1.

For all variables of interest, risk estimates were computed as both univariate analyses based on the matching factors, and multivariate analyses, with additional adjustments for potential confounders including body mass index (kg/m²), physical activity (combined recreational and household activity; expressed as sex-specific categories of metabolic equivalents), duration/status/intensity of smoking (table 1), education level (an indicator variable for socioeconomic status), total energy intake (in quartiles), total intake of fruits (quartiles), total intake of vegetables (quartiles), total intake of red and processed meats (quartiles), and total alcohol intake (categorical cut-points for men: non-consumers, 1 to 10, 11 to 20, 21 to 40, >40 g/day; cut-points for women: non-consumers, 1 to 5, 6 to 15, 16 to 25, >25 g/day). Models similar to the above were also run with variables included in the model as log transformed continuous variables with the incidence rate ratio estimated for the risk related to a 10% increase in the value of the variable. Potential effects of dietary fibre intake, as well as consumption of dairy products and fish (rich dietary sources of vitamin D) were examined, but they did not provide appreciable changes in risk estimates and were not included in the final models. For all models, tests for linear trend were performed using a score variable with values from 1 to 5, consistent with the category/quintile grouping.

To assess any effects of the season or month of blood collection, two different approaches were used. As a first approach, incident rate ratios and 95% confidence intervals were calculated as described above but with an additional adjustment for season of blood collection (categorical variable: winter, spring, summer, autumn). In a second approach, circulating 25-(OH)D concentrations were standardised and the standardised values were then used in conditional regression models as described above. The results were then compared with those of the non-standardised 25-(OH)D. Circulating

Table 1 | Description of cases and matched controls, by anatomical site

	Colon			Rectum		
	Cases*	Matched controls	P value difference†	Cases*	Matched controls	P value difference†
Men, n	369	369	—	251	251	—
Women, n	416	416	—	212	212	—
Total	785	785		463	463	
Mean age, years (SD), minimum-maximum value:						
At recruitment	58.5 (7.2), 30.1 to 76.9	58.6 (7.2), 30.3 to 76.6	0.65	58.0 (6.8), 38.1 to 75.0	58.0 (6.9), 38.4 to 75.3	0.33
At blood collection	58.7 (7.3) 30.1 to 76.9	58.7 (7.3) 30.3 to 76.6	0.57	58.1 (6.9) 38.1 to 75.0	58.0 (6.9) 38.4 to 75.3	0.21
Mean years of follow up (SD), maximum value	3.8 (2.2), 11.5	—	—	3.9 (2.2), 10.3	—	—
Mean BMI (SD), minimum-maximum value	26.8 (4.4), 17.6 to 52.5	26.3 (3.9), 17.2 to 49.3	0.01	26.5 (4.1), 15.7 to 41.1	26.4 (3.9), 17.6 to 44.5	0.52
Smoking status/duration/intensity, n (%):			0.52			0.63
Never smokers	330 (42.0)	356 (45.4)		179 (38.7)	179 (38.7)	
Ex-smokers, duration of smoking <10 years	44 (5.6)	37 (4.7)		18 (3.9)	21 (4.5)	
Ex-smokers, duration of smoking ≥10 years	200 (25.5)	203 (25.9)		127 (27.4)	115 (24.8)	
Ex-smokers, missing duration of smoking	19 (2.4)	15 (1.9)		5 (1.1)	9 (1.9)	
Smokers, <15 cigarettes a day	66 (8.4)	71 (9.0)		55 (11.9)	49 (10.6)	
Smokers, ≥15 to <25 cigarettes a day	66 (8.4)	57 (7.3)		36 (7.8)	51 (11.0)	
Smokers, ≥25 cigarettes a day	15 (1.9)	16 (2.0)		14 (3.0)	13 (2.8)	
Missing smoking status	45 (5.7)	30 (3.8)		29 (6.3)	26 (5.6)	
Physical activity, n (%):			0.27			0.24
Inactive	170 (21.7)	139 (17.7)		80 (17.3)	86 (18.6)	
Moderately inactive	167 (21.3)	186 (23.7)		112 (24.2)	93 (20.1)	
Moderately active	198 (25.4)	198 (25.2)		100 (21.6)	107 (23.1)	
Active	204 (26.0)	214 (27.3)		145 (31.3)	144 (31.1)	
Missing/unspecified	46 (5.9)	48 (6.1)		26 (5.6)	33 (7.1)	
Education level, n (%):			0.55			0.40
None/primary	292 (27.2)	310 (39.5)		160 (34.6)	176 (38.0)	
Technical/professional	186 (23.7)	185 (23.6)		127 (27.6)	128 (27.6)	
Secondary	150 (19.1)	131 (16.7)		68 (14.7)	64 (13.8)	
University or higher	130 (16.6)	137 (17.5)		91 (20.0)	86 (18.6)	
Missing/unspecified	27 (3.4)	22 (2.8)		16 (3.5)	9 (1.9)	
Dietary variables (g/day):						
Total energy (MJ/day)	9.0 (3.1)	8.9 (2.7)	0.31	9.2 (2.9)	9.1 (2.6)	0.20
Total vegetables	183.7 (122.2)	190.3 (123.3)	0.20	184.2 (162.9)	182.9 (123.9)	0.87
Total fruits	233.2 (192.1)	241.2 (188.5)	0.35	218.9 (168.7)	222.4 (168.6)	0.71
Meats and meat products	112.6 (77.9)	110.2 (58.2)	0.48	123.2 (65.6)	116.8 (63.9)	0.08
Dairy and dairy products	335.0 (251.7)	350.7 (248.2)	0.20	339.3 (276.1)	374.3 (271.5)	0.05
Alcohol	16.1 (22.4)	14.7 (19.2)	0.11	20.4 (25.1)	16.8 (21.6)	0.01
Vitamin D (µg/day)	4.1 (2.6)	4.0 (2.5)	0.83	4.2 (2.7)	4.3 (2.7)	0.43
Calcium (mg/day)	985.8 (429.2)	1000.9 (406.4)	0.46	981.6 (418.0)	1038.6 (439.6)	0.05
Retinol (µg/day)	940.1 (938.0)	918.5 (890.1)	0.61	1011.9 (840.2)	999.2 (966.5)	0.83
Circulating 25-(OH)D geometric mean (5th-95th percentile):						
Vitamin D (nmol/l)	51.7 (24.1-104.4)	57.2 (28.0-114.8)	<0.01	54.9 (26.3-111.0)	55.4 (24.7-116.5)	0.75

Data are mean (SD) unless otherwise indicated.

*Indicates number of cases and matched controls for circulating 25-(OH)D analyses. The distribution of cases (colon/rectum) by country was: Denmark=186/167, France=28/8, Greece=12/14, Germany=93/55, Italy=104/42, Netherlands=93/48, Spain=78/41, UK=150/64, and Sweden=41/24.

†P values for differences in means between cases and controls determined by paired *t*-tests, except for the categorical variables smoking status, physical activity, and education level, where P values were determined by conditional logistic regression.

25-(OH)D concentrations were standardised using two different methods: (a) by the month of blood collection calculated by adding the overall mean of the circulating 25-(OH)D for all subjects to the residuals derived from a simple regression model fitted to circulating 25-(OH)D concentration by month of blood collection and (b) by the method of Munger et al.³¹

All analysis models were run separately for colorectal cancer and by anatomical sub-site: colon, left colon,

right colon, and rectum using the same categorical cut-points as for colorectal cancer.

Since a primary function of vitamin D is maintenance of calcium homeostasis, a potential interaction of the effect of circulating 25-(OH)D concentration with the level of dietary calcium intake on colorectal cancer risk was explored by including a single degree of freedom interaction term formed by the product of the 25-(OH)D category value (cut-points: <50.0 nmol/l,

≥ 50.0 to < 75.0 nmol/l, ≥ 75.0 nmol/l) and the dietary calcium tertile. The statistical significance of a linear interaction was assessed using the likelihood ratio test. Similar models were used to test for interaction of 25-(OH)D with amount of alcohol consumption and dietary retinol intake.

For all the main variables, heterogeneity of effects by sex and anatomical subsite were assessed by χ^2 statistics. To assess the potential for reverse causality (lower 25-(OH)D level due to the presence of disease at enrolment) and to assess the effect of follow-up time in greater detail, analyses were also performed excluding cases diagnosed with less than two years of follow-up. All statistical tests were two tailed.

RESULTS

Description of study population

Mean age of participants at recruitment was similar in both the colon (cases: 59.6 (SD 7.2); controls: 58.6 (SD 7.2)) and rectum (cases: 58.0 (SD 6.8); controls: 58.0 (SD 6.9) anatomical sub-sites (table 1). The average time between blood collection and diagnosis was 3.8 years for colon cancer cases and 3.9 years for rectal cancer cases.

Circulating 25-(OH)D level

Compared with a level of ≥ 50.0 to < 75.0 nmol/l, higher circulating 25-(OH)D level (categories 4 and 5) was associated with a reduced risk, and lower circulating 25-(OH)D concentration (categories 2 and 1) was associated with an increased risk of colon cancer (table 2). A linear trend was evident for colorectal cancer (P for trend < 0.001) and colon cancer (P for trend < 0.001) but not rectal cancer (P for trend = 0.320). In the multivariate adjusted continuous model, the cancer risks (incidence rate ratio (95% confidence interval))

associated with 10% higher level of circulating 25-(OH)D were: colorectal 0.97 (0.95 to 0.99); colon 0.95 (0.93 to 0.98); rectum 1.00 (0.97 to 1.03).

Additional analyses of risk estimates using a 25-(OH)D concentration based on quintiles with the lowest category as the referent are shown in web table 1. Higher circulating 25-(OH)D concentration was associated with lower colorectal risk in a dose-response manner (multivariate incidence rate ratio for the lowest versus highest quintile 0.60 (95% confidence interval 0.46 to 0.80); P for trend < 0.001). The association was stronger for the colon (0.47 (0.33 to 0.68)) than the rectum (0.89 (0.56 to 1.43); web table 1).

Country-specific incidence rate ratios and 95% confidence intervals indicating the colorectal cancer risk for a 10% increase in circulating 25-(OH)D concentration were: Denmark 0.98 (0.96 to 1.01); France 0.88 (0.79 to 0.98); Germany 0.91 (0.86 to 0.97); Greece 1.00 (0.88 to 1.15); Italy 0.98 (0.94 to 1.04); the Netherlands 1.02 (0.98 to 1.09); Spain 0.96 (0.89 to 1.03); Sweden 0.93 (0.83 to 1.04); and the United Kingdom 0.93 (0.88 to 0.97).

Adjustments for the season of blood collection did not alter any of the findings (results not shown). Differences were apparent in circulating 25-(OH)D concentration by month of blood collection with cases exhibiting lower mean circulating 25-(OH)D concentration than controls in each month (web figure 1). Assessment of colorectal cancer risk using circulating 25-(OH)D concentration standardised by month of blood collection showed a similar pattern of results (web table 2; results in quintiles shown in web table 3) compared with models using non-standardised 25-(OH)D (table 2, web table 1). Results for standardisation by the method of Munger et al³¹ (data not shown) were not different from those presented in web tables 2 and 3.

Table 2 | Circulating 25-(OH)D concentration and the risk of cancers of the colorectum, colon, and rectum

Type of cancer	Pre-defined category cut-points (nmol/l)					P for trend
	1 (< 25.0)	2 (≥ 25.0 to < 50.0)	3 (≥ 50.0 to < 75.0) (reference)	4 (≥ 75.0 to < 100.0)	5 (≥ 100.0)	
Colorectum						
Mean (SD), median (nmol/l)	19.6 (5.0), 21.4	38.7 (6.7), 39.3	61.2 (7.3), 60.0	85.1 (6.8), 84.5	125.7 (37.6), 116.1	
No of cases/controls	64/52	473/400	448/461	173/209	90/126	
Matching factors*	1.32 (0.89 to 1.97)	1.25 (1.03 to 1.52)	1.00	0.86 (0.68 to 1.10)	0.72 (0.53 to 0.97)	< 0.001
Multivariate adjusted†	1.32 (0.87 to 2.01)	1.28 (1.05 to 1.56)	1.00	0.88 (0.68 to 1.13)	0.77 (0.56 to 1.06)	< 0.001
Colon						
Mean (SD), median (nmol/l)	19.8 (3.5), 20.4	38.9 (6.7), 39.3	60.9 (7.3), 59.9	85.5 (7.0), 85.0	123.5 (29.6), 116.3	
No of cases/controls	45/27	300/249	286/295	104/138	50/76	
Matching factors*	1.84 (1.10 to 3.08)	1.31 (1.03 to 1.67)	1.00	0.81 (0.60 to 1.09)	0.66 (0.44 to 0.99)	< 0.001
Multivariate adjusted†	1.90 (1.10 to 3.29)	1.36 (1.05 to 1.76)	1.00	0.86 (0.62 to 1.17)	0.71 (0.46 to 1.08)	< 0.001
Rectum						
Mean (SD), median (nmol/l)	19.4 (6.3), 22.4	38.4 (6.7), 39.2	61.5 (7.3), 60.9	84.2 (6.2), 83.1	128.9 (47.5), 115.4	
Matching factors*	0.76 (0.39 to 1.47)	1.19 (0.87 to 1.62)	1.00	0.99 (0.66 to 1.49)	0.80 (0.50 to 1.29)	0.288
Multivariate adjusted†	0.77 (0.37 to 1.59)	1.17 (0.84 to 1.65)	1.00	0.93 (0.60 to 1.45)	0.82 (0.48 to 1.40)	0.320

Data are incidence rate ratio (95% CI) unless indicated.

*Model based on matching factors only.

†Model based on matching factors plus further adjustments for smoking status/duration/intensity, body mass index, total physical activity, education level, total dietary energy consumption, and intake of total fruits, vegetables, meat or meat products, and alcohol. Values for mean and median are based on control participants only.

Table 3 | Dietary intakes of vitamin D and calcium and the risk of cancers of the colorectum, colon, and rectum

	Quintiles of dietary intake					P for trend
	1 (reference)	2	3	4	5	
Vitamin D intake (µg/day)	<2.1	≥2.1 to < 3.1	≥3.1 to < 4.2	≥4.2 to < 5.8	≥5.8	
Colorectum:						
Mean (SD), median (µg/day)	1.5 (0.5), 1.6	2.5 (0.3), 2.7	3.6 (0.3), 3.6	4.9 (0.9), 4.9	8.2 (2.7), 7.3	
No of cases/controls	251/243	255/245	249/244	235/246	230/244	
Matching factors*	1.00	1.00 (0.78 to 1.28)	0.97 (0.74 to 1.26)	0.90 (0.68 to 1.19)	0.88 (0.66 to 1.17)	0.277
Multivariate adjusted†	1.00	1.03 (0.79 to 1.34)	0.96 (0.72 to 1.28)	0.88 (0.65 to 1.20)	0.84 (0.60 to 1.17)	0.187
Colon:						
Mean (SD), median (µg/day)	1.5 (0.5), 1.6	2.7 (0.3), 2.7	3.6 (0.3), 3.6	4.9 (0.5), 4.9	8.0 (2.7), 7.3	
No of cases/controls	165/163	169/154	155/149	135/160	148/147	
Matching factors*	1.00	1.07 (0.78 to 1.46)	1.00 (0.72 to 1.40)	0.82 (0.58 to 1.15)	0.96 (0.67 to 1.38)	0.365
Multivariate adjusted†	1.00	1.11 (0.80 to 1.55)	1.05 (0.73 to 1.50)	0.83 (0.56 to 1.22)	1.00 (0.66 to 1.53)	0.514
Rectum:						
Mean (SD), median (µg/day)	1.5 (0.4), 1.6	2.7 (0.3), 2.7	3.6 (0.3), 3.6	4.9 (0.5), 4.9	8.3 (2.8), 7.4	
No of cases/controls	86/80	86/91	94/95	100/86	82/97	
Matching factors*	1.00	0.87 (0.57 to 1.34)	0.93 (0.60 to 1.45)	1.09 (0.68 to 1.76)	0.78 (0.49 to 1.25)	0.546
Multivariate adjusted†	1.00	0.85 (0.52 to 1.36)	0.79 (0.48 to 1.30)	0.85 (0.49 to 1.48)	0.61 (0.34 to 1.09)	0.142
Quintiles of dietary calcium intake (mg/day)	<667.2	≥667.2 to <854.7	≥854.7 to <1047.6	≥1047.6 to <1299.4	≥1299.4	
Colorectum:						
Mean (SD), median (mg/day)	523.9 (104.9), 544.6	766.4 (53.5), 769.7	949.2 (57.7), 947.7	1163.6 (70.3), 1163.1	1667.5 (358.0), 1574.4	
No of cases/controls	269/243	258/247	258/243	213/245	222/244	
Matching factors*	1.00	0.93 (0.72 to 1.20)	0.94 (0.73 to 1.22)	0.77 (0.59 to 1.00)	0.81 (0.62 to 1.05)	0.042
Multivariate Adjusted†	1.00	0.88 (0.67 to 1.15)	0.90 (0.67 to 1.20)	0.72 (0.52 to 0.96)	0.69 (0.50 to 0.96)	0.016
Colon:						
Mean (SD), median (mg/day)	517.0 (107.1), 538.1	766.0 (52.8), 769.0	948.7 (57.0), 949.6	1161.1 (69.9), 1161.4	1677.1 (345.8), 1590.9	
No of cases/controls	164/150	167/168	167/163	139/145	135/147	
Matching factors*	1.00	0.90 (0.66 to 1.23)	0.93 (0.68 to 1.27)	0.87 (0.62 to 1.22)	0.83 (0.60 to 1.16)	0.287
Multivariate adjusted†	1.00	0.86 (0.61 to 1.21)	0.88 (0.62 to 1.27)	0.80 (0.54 to 1.18)	0.72 (0.47 to 1.10)	0.176
Rectum:						
Mean (SD), median (mg/day)	534.7 (100.5), 565.5	767.2 (55.0), 770.9	950.1 (59.1), 947.5	1167.8 (71.1), 1166.8	1652.9 (376.5), 1536.4	
No of cases/controls	105/93	91/79	91/80	74/100	87/97	
Matching factors*	1.00	1.01 (0.66 to 1.55)	0.99 (0.63 to 1.55)	0.62 (0.40 to 0.97)	0.78 (0.51 to 1.20)	0.054
Multivariate adjusted†	1.00	0.88 (0.55 to 1.42)	0.91 (0.54 to 1.42)	0.52 (0.30 to 0.90)	0.63 (0.36 to 1.11)	0.025

Data are incidence rate ratio (95% confidence interval) unless stated otherwise.

*Model based on matching factors only.

†Model based on matching factors plus further adjustments for smoking status/duration/intensity, body mass index, total physical activity, education level, total dietary energy consumption, and intake of total fruits, vegetables, meats/meat products and alcohol. For dietary vitamin D and calcium, the number of total colorectal cancer case-control sets is 1220 due to missing dietary data from Greece. Values for mean and median are based on control participants only.

Dietary vitamin D

Dietary vitamin D intake did not show an association with colorectal cancer risk (table 3). P values for linear trend tests were: colorectal 0.187; colon 0.496; rectum 0.145. The risk associated with a 10% increase in dietary vitamin D intake was (multivariate adjusted incidence rate ratio, 95% confidence interval): colorectal 1.00 (0.98 to 1.01); colon 1.01 (0.99 to 1.03); rectum 0.98 (0.95 to 1.01).

Dietary calcium

Higher intake of dietary calcium showed some evidence of association with a reduced cancer risk association, particularly in the rectal anatomical sub-site (table 3). P values for linear trend tests were: colorectal=0.013, colon=0.152, rectum=0.026. The risk associated with a 10% increase in dietary calcium intake was (multivariate adjusted incidence rate ratio, 95%

confidence interval): colorectal 0.97 (0.94 to 0.99); colon 0.98 (0.94 to 1.00); rectum 0.94 (0.90 to 0.99).

Interactions with dietary factors

The dose-response analysis of the interaction between circulating 25-(OH)D concentration and dietary calcium intake (P=0.154) showed that the inverse association between colorectal cancer risk and circulating 25-(OH)D concentration was apparent across levels of dietary calcium. The lowest level of both variables was associated with an increased colorectal cancer risk (incidence rate ratio 1.33 (95% confidence interval 1.16 to 1.55); table 4). No interaction on colorectal cancer risk was observed between circulating 25-(OH)D concentration and the level of alcohol consumption (P for interaction=0.283; table 5). However, the highest colorectal cancer risk was seen in those with the lowest circulating levels of 25-(OH)D and the

Table 4 | Incidence rate ratios for risk of colorectal cancer by increasing levels of circulating 25-(OH)D and dietary calcium

Teriles of dietary calcium intake level (mg/day)	Categories of serum 25-(OH)D (nmol/l)		
	1 (<50.0)	2(≥50.0 to <75.0)	3(≥75.0)
1 (<797.5)	1.33 (1.14 to 1.54)	1.11 (1.01 to 1.22)	0.85 (0.69 to 1.02)
2 (≥797.5 to <1113.5)	1.17 (1.07 to 1.27)	1.00	0.80 (0.69 to 0.90)
3 (≥1113.5)	0.95 (0.80 to 1.13)	0.85 (0.73 to 0.98)	0.72 (0.57 to 0.91)

Data are incidence rate ratio (95% confidence interval) derived from the multivariate adjusted models described in the text based on a dose-response analysis with predefined categories of circulating 25-(OH)D and tertiles of dietary calcium intake. P value for statistical interaction of circulating 25-(OH)D with dietary calcium=0.154. For this analysis, the total number of colorectal cancer case-control sets is 1220 due to missing nutrient data from Greece.

highest level of alcohol consumption (incidence rate ratio 1.46, 95% confidence interval 1.16 to 1.83). The dose-response analysis of the interaction between circulating 25-(OH)D concentration and level of dietary retinol intake (P for interaction=0.030) indicates that the inverse colorectal cancer risk association of higher 25-(OH)D was stronger at lower intakes of retinol (table 5).

The cancer risk associations did not differ by sex (P for heterogeneity: 25-(OH)D=0.782, dietary vitamin D=0.600, dietary calcium=0.500). For circulating 25-(OH)D, the cancer risk association showed evidence of heterogeneity between the colon and rectal anatomical sub-sites (P for heterogeneity=0.048), but this was not the case for dietary vitamin D (P for heterogeneity=0.400) or dietary calcium (P for heterogeneity=0.300). Comparison of findings for the proximal and distal anatomical sub-sites within the colon suggested some heterogeneity for dietary calcium (P for heterogeneity=0.010), but not for the other variables (P for heterogeneity: 25-(OH)D=0.500, dietary vitamin D=0.600). The exclusion of cases with less than two years of follow-up did not change any of the results (data not shown).

DISCUSSION

The results of this study, which is the largest to date and one of the first based on European populations, show that, compared with a mid-range concentration of 50 to 75.0 nmol/l, circulating 25-(OH)D levels lower than 50.0 nmol/l are associated with an increased risk of colorectal cancer. Although levels higher than 75.0 nmol/L were associated with a reduced colorectal cancer risk, the association was not significant compared with the mid-range concentration. Analyses by quintile of 25-(OH)D concentration, showed a dose-response decrease in colorectal cancer risk with increasing 25-(OH)D concentration. Participants in the highest quintile had a significant 40% lower risk of colorectal cancer than did those in the lowest quintile. Additionally, higher consumption of dietary calcium, but not dietary vitamin D, was found to be associated with a reduced risk of colorectal cancer.

Since the first suggestion that vitamin D may have a role in colorectal cancer risk,³² the association has been evaluated pre-diagnostically by only a few small studies.¹³⁻¹⁹ Most of these studies have shown inverse associations with colorectal cancer risk but the results

have been based mainly on North American populations with different dietary and lifestyle habits than their western European counterparts. The strong inverse associations of the present study suggest that further research efforts should concentrate less on observational findings and more on clinically relevant studies to determine whether vitamin D has a causal role in colorectal cancer prevention or whether it is a marker of other events.

An important consideration for circulating 25-(OH)D levels is what concentration should be deemed sufficient for colorectal cancer protection. Results of a recent review and meta-analysis, which included around half as many cases as in our study suggest that a blood 25-(OH)D level of about 80.0 nmol/l results in a colorectal cancer risk reduction of roughly 50%.³³ This level of risk reduction is in line with that observed in our study when comparing the highest to the lowest quintiles of 25-(OH)D. However, there is debate over the definition of a sufficient level of circulating 25-(OH)D and suggestions range from about 50 nmol/l³⁰ to higher.²⁷⁻²⁹ In the present study, although cancer risks for 25-(OH)D levels above 75 nmol/l were lower than those in the 50-75 nmol/l mid-range reference, the differences were not statistically significant. This finding suggests that raising very low levels of 25-(OH)D to the mid-range may protect against colorectal cancer, and that levels above 75 nmol/l might not significantly reduce the cancer risks any further, but this needs to be proven in a clinical trial. In light of accumulating evidence for a possible beneficial role of increased circulating vitamin D levels in reducing the risk of a range of different diseases³⁴⁻³⁷ as well as cancer specific³⁸ and total mortality,³⁹ there is growing advocacy for vitamin D supplementation and the maintenance of higher circulating levels. However, there has been little study into the long term health effects of very high circulating 25-(OH)D concentrations potentially obtainable from supplementation regimens or widespread fortification of food products. Our findings suggest that the potential cancer risk benefits of higher vitamin D levels should be balanced with caution for the toxic potential. In fact, any public health advocacy of higher circulating 25-(OH)D concentrations should be based on clear and conclusive evidence from double blind randomised trials, as for any drug.

We know of only one previously published clinical trial with a primary objective of assessing the effect of vitamin D and calcium supplementation on incidence of colorectal cancer.⁴⁰ It was conducted within the Women's Health Initiative and showed no effect of supplementation (1000 mg/day of calcium and 400 IU/day of vitamin D) on colorectal cancer incidence. Critiques of this large trial include a low level of vitamin D supplementation, a short duration of follow-up, low compliance, potentially sufficient levels of vitamin D and calcium intake at baseline, and lack of information on actual changes in circulating 25-(OH)D due to supplementation.⁴¹ Two other trials have also published data on supplementation of these nutrients and the incidence of all cancers, but in both studies the

Table 5 | Incidence rate ratios for risk of colorectal cancer by increasing levels of circulating 25-(OH)D and alcohol and dietary retinol

	Categories of circulating 25-(OH)D (nmol/l)		
	1 (<50.0)	2 (50.0 to <75.0)	3 (≥75.0)
Sex specific categories of dietary alcohol intake level (g/day)			
1 (men <1.0, women <1.0)	1.13 (1.03 to 1.26)	1.00	0.82 (0.70 to 0.96)
2 (men 1.0 to <25.0, women 1.0 to <15.0)	1.19 (1.07 to 1.32)	1.04 (1.00 to 1.08)	0.85 (0.75 to 0.98)
3 (men ≥25.0, women ≥15.0)	1.46 (1.16 to 1.83)	1.25 (1.02 to 1.52)	1.01 (0.74 to 1.29)
Tertiles of dietary retinol intake level (µg/day)			
1 (<503.5)	1.23 (1.10 to 1.37)	1.00	0.73 (0.62 to 0.86)
2 (503.5 to <998.8)	1.20 (1.08 to 1.33)	1.01 (0.96 to 1.06)	0.78 (0.67 to 0.90)
3 (≥998.8)	1.12 (0.90 to 1.34)	1.04 (0.87 to 1.22)	0.94 (0.71 to 1.22)

Data are incidence rate ratio (95% confidence interval) derived from the multivariate adjusted models described in the text based on a dose-response analysis with pre-defined categories of circulating 25-(OH)D and sex specific categories of alcohol, and tertiles of retinol intake. Alcohol P value for interaction=0.283. Dietary retinol P value for interaction=0.030. For the analyses involving retinol, the total number of colorectal cancer case-control sets is 1220 due to missing nutrient data from Greece.

assessment of cancer outcomes was a secondary objective.^{42,43} Their findings were conflicting, showing either no reduction in incidence of colorectal or all cancers⁴² or a significant reduction in incidence of all cancers, albeit with a very small number of events (n=50).⁴³ Whether alteration of circulating vitamin D concentration can change the risk for colorectal cancer remains to be determined. In order to establish appropriate public health and safety guidelines, future efforts should concentrate on the conduct of new clinical trials of vitamin D supplementation to assess whether increases in circulating concentration can effectively change colorectal cancer risk.

One of the key functions of vitamin D is the maintenance of calcium homeostasis. Given that there is some epidemiological evidence for a possible inverse association between higher calcium intake and the risk of colorectal cancer,⁴⁴⁻⁴⁷ a biological interaction of circulating 25-(OH)D levels and dietary calcium intake may exist. However, to date the relation between dietary calcium and blood 25-(OH)D concentration has been primarily considered in studies of colorectal adenomas, showing either that both nutrients act together to reduce the risk of adenoma^{8,48} or that the inverse association with higher 25-(OH)D concentration is apparent only in those with lower calcium intakes.¹¹ In the present study, a significant statistical interaction was not observed between circulating 25-(OH)D concentration and dietary calcium intake. Although this finding may not discount a potential biological interaction, it does suggest that some of the modes of action of these two factors in the gastrointestinal tract might be unconnected. For example, the main proposed colorectal cancer protective mechanisms of calcium action (binding bile acids and fatty acids)⁴⁶ could pertain largely to its concentration in the colorectal milieu rather than to a direct vitamin D-mediated effect.

Advantages and limitations

In addition to its large size and scope, another key advantage of our study is that it is based on geographically diverse European populations, thus encompassing

many different lifestyle patterns (including sun exposure) and wide dietary heterogeneity. Further advantages are its prospective design (participants recruited before disease onset) and pre-diagnostic measurement of circulating 25-(OH)D concentration to collectively account for dietary consumption (limited dietary sources include fatty fish, egg yolk, fortified dairy products), intake of supplements, and endogenous production. The use of this biomarker compensates for our lack of data on total sun exposure, sun tanning habits, and vitamin D supplement intake.

Although the study was larger than other prospective studies on the same topic, it may still be limited for consideration of 25-(OH)D-diet interactions. However, a more important limitation may be the fairly short follow-up time. Cases identified within a short period after the start of the study might have had some symptoms, leading to dietary or lifestyle changes and hence possible alterations in the circulating 25-(OH)D concentration or dietary calcium level (reverse causality bias). However, exclusion of cases with less than two years of follow-up did not alter any of the findings, suggesting that cases diagnosed close to study entry might not be different from those diagnosed later. But, in view of the long term nature of colorectal cancer development and the short follow-up time, some caution is still necessary in the interpretation of these results. An additional potential limitation applicable to all observational studies is the possibility for residual or uncontrolled confounding. The dietary and lifestyle data of the EPIC study have been well measured and validated. Nevertheless, the possibility of residual confounding cannot ever be wholly discounted. Uncontrolled confounding is unlikely because the multivariate adjusted model presented here addressed a large number of potentially important confounding variables including anthropometry, smoking, physical activity, socioeconomic status, total energy intake, and consumption of fruits, vegetables, red and processed meats, and alcohol. A potential confounding variable not controlled for here is colorectal cancer screening, which is still uncommon in Europe⁴⁹ and thus not likely to have had an effect on our findings.

Conclusions

This comprehensive study, based on western European populations in the prospective EPIC cohort, has shown that pre-diagnostic concentrations of circulating 25-(OH)D below 50 nmol/l are associated with an increased risk of colon cancer, whereas concentrations above 75.0 nmol/l are associated with a non-significant reduced risk. Comparison of lowest to highest quintiles of 25-(OH)D concentration showed that participants in the highest quintile had a significant 40% lower colorectal cancer risk. However, before any public health recommendations can be made for vitamin D supplementation, new randomised trials are needed to test the hypothesis that increases in circulating 25-(OH)D concentration are effective in reducing colorectal cancer risk without inducing serious adverse events.

WHAT IS ALREADY KNOWN ON THIS TOPIC

Higher pre-diagnostic concentrations of circulating vitamin D have been suggested to be associated with lower risk of colorectal cancer, but information from western European populations is scarce

Findings of randomised trials of vitamin D supplementation on colorectal cancer risk have been inconsistent

WHAT THIS STUDY ADDS

In a western European population, higher pre-diagnostic circulating vitamin D concentration was inversely associated with risk of colorectal cancer in a dose-response manner

In subgroup analyses this association was noted for colon cancer but not rectal cancer

New randomised trials are needed to assess whether increases in circulating 25-(OH)D concentration can effectively decrease colorectal cancer risk without inducing serious adverse events

We are very grateful to Sheila Bingham who contributed greatly to the present manuscript, but sadly died between the time from submission to final acceptance and did not have an opportunity to approve the final version. We also thank Paolo Boffetta for his many comments and input into the manuscript, C Biessy and B Hemon for their assistance in database preparation and statistical analyses, and J Cremers and P Beekhof for their laboratory assistance in the vitamin D analyses.

Contributors: ER is the overall coordinator of the EPIC study, which he conceptualised, designed, and implemented in collaboration with the main investigators in the collaborating centres: **Denmark:** ATJønneland, KO; **France:** MCBR, FCC; **Germany:** RK, JL, HB; **Greece:** ATrichopoulou, DT; **Italy:** FB, PV, SP, DP, RT; **Netherlands:** HBBdM, PHP; **Norway:** EL; **Spain:** MJT, EA, LRS; **MJS, MD, CAG;** **Sweden:** GH; **UK:** TJK, KTK, Sheila Bingham; **IARC:** Paolo Boffetta. All authors contributed to recruitment, data collection/acquisition and/or biological sample collection, and are responsible for the ongoing follow-up and management of the EPIC cohort. All coauthors commented on and approved the study proposal. This article was written by MJ with assistance from HBBdM, FJBvd, TN, NS, SR, TP, EHJM, PA, and ER, and taking into account the comments and suggestions of the coauthors. All coauthors had the opportunity to comment on the analysis and interpretation of the findings and approved the final version for publication. The grant application for this study was written by MJ and ER. Statistical expertise and input was provided by PF, GB, and AR. The laboratory analyses were done by EHJM.

Funding: We thank the World Cancer Research Fund (WCRF, London, UK; grant number 2005/12) for grant funding for the present study. The EPIC study was supported by the Europe Against Cancer Programme of the European Commission (SANCO); Ligue contre le Cancer (France); Mutuelle Générale de l'Éducation Nationale; Institut National de la Santé et de la Recherche Médicale (INSERM); German Cancer Aid; German Cancer Research Center; German Federal Ministry of Education and Research; Danish Cancer Society; Health Research Fund (FIS) of the Spanish Ministry of Health (RETIC-RD06/0020); the participating regional governments and institutions of Spain; The ISCIII Red de Centro RCESP (CO3/09); Cancer Research UK; Medical Research Council, UK; the Stroke Association, UK; British Heart Foundation; Department of Health, UK; Food Standards Agency, UK; the Wellcome Trust, UK; Greek Ministry of Health and Social Solidarity; Hellenic Health Foundation and Stavros Niarchos Foundation; Greek Ministry of Education; Italian Association for Research on Cancer; Italian National Research Council; Compagnia di San Paolo; Dutch Ministry of Public Health, Welfare and Sports; Dutch Ministry of Health; Dutch Prevention Funds; LK Research Funds; Dutch ZON (Zorg Onderzoek Nederland); Swedish Cancer Society; Swedish Scientific Council; Regional Governments of Skane and Vasterbotten, Sweden; and Norwegian Cancer Society.

Competing interests: None declared.

Ethical approval: This study was approved by the ethics review boards of the International Agency for Research on Cancer and individual EPIC centres. EPIC participants provided written consent for the use of their blood samples and all data.

Data sharing: No additional data available.

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