Dietary Polyphenols in the Aetiology of Crohn's Disease and Ulcerative Colitis–A Multicenter European Prospective Cohort Study (EPIC)

Yunxia Lu, MD, PhD,[1,2,3] Raul Zamora-Ros, PhD,[4,5] Simon Chan, MB BChir, PhD,[6,7] Amanda J. Cross, PhD,[1] Heather Ward, PhD,[1] Paula Jakszyn, MPH, PhD,[5] Robert Luben, BSc,[8] Jorrit L. Opstelten, MD, MSc,[9] Bas Oldenburg, MD, PhD,[9] Göran Hallmans, MD, PhD,[10] Pontus Karling, PhD,[11] Olof Grip, MD, PhD,[12] Timothy Key, DPhil, PhD,[13] Manuela M. Bergmann, PhD,[14] Heiner Boeing, PhD,[14] Kim Overvad, PhD,[15] Domenico Palli, MD, MPH,[16] Giovanna Masala, MD, MPH,[16] Kay-Tee Khaw, MB BChir, FRCP,[8] Antoine Racine, MD, MSc,[17,18,19] Franck Carbonnel, MD, PhD,[17,18,19] Marie-Christine Boutron-Ruault, MD, PhD,[17,18] Vibeke Andersen, MD,[20,21,22] Anja Olsen, PhD, MSc,[23] Anne Tjonneland, PhD, DMSc,[23] Rudolf Kaaks, PhD,[24] Rosario Tumino, MD, MSc,[25] Antonia Trichopoulou, MD, PhD,[26] Augustin Scalbert, PhD,[4] Elio Riboli, MD, MPH,[1] and Andrew R. Hart, MB ChB, MD[6,7]

Background: Oxidative stress may be involved in the aetiology of inflammatory bowel disease and whether dietary polyphenols, which possess antioxidants properties, prevent its development is unknown.

Methods: A total of 401,326 men and women aged 20 to 80 years from 8 countries were recruited between 1991 and 1998 and at baseline completed validated food frequency questionnaires. Dietary polyphenol intake was measured using Phenol-Explorer, a database with information on the content of 502 polyphenols. Incident cases of Crohn's diseases (CD) and ulcerative colitis (UC) were identified during the follow-up period of up to December 2010. A nested case–control study using conditional logistic regression estimated the odds ratios (ORs), and 95% confidence intervals, for polyphenol intake (categories based on quartiles) and developing CD or UC.

Results: In total, 110 CD (73% women) and 244 UC (57% women) cases were identified and matched to 440 and 976 controls, respectively. Total polyphenol intake was not associated with CD (P trend = 0.17) or UC (P trend = 0.16). For flavones and CD, there were reduced odds for all quartiles, which were statistically significant for the third ($OR_{3rd \text{ versus } 1st \text{ quartile}}$ = 0.33; 95% confidence interval, 0.15–0.69) and there was an inverse trend across quartiles (P = 0.03). Similarly, for resveratrol, there was an inverse association with CD ($OR_{4th \text{ versus } 1st \text{ quartile}}$ = 0.40; 95% confidence interval,

0.20–0.82) with an inverse trend across quartiles ($P = 0.02$). No significant associations between subtypes of polyphenols and UC were found. Effect modification by smoking in CD was documented with borderline statistical significance.

Conclusions: The data supports a potential role of flavones and resveratrol in the risk of developing CD; future aetiological studies should investigate these dietary components and further examine the potential for residual confounding.

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Key Words: polyphenols, Crohn's diseases, ulcerative colitis, antioxidants

The incidence of inflammatory bowel disease (IBD) namely ulcerative colitis (UC) and Crohn's disease (CD) is rising, particularly in westernized nations.[1] IBD is an important clinical problem, as patients have to take medications lifelong, which may require surgery and can develop serious complications, such as colorectal cancer.[2,3] The aetiology of IBD has yet to be elucidated, although it probably involves an abnormal immunological response to gastrointestinal luminal antigens in a genetically susceptible host.[2] Documented risk factors include a positive family history,[3] smoking,[4,5] and dietary components including intake of n-3 and n-6 fatty acids.[6–8]

There are plausible biological mechanisms for how dietary polyphenols may prevent several gastrointestinal diseases, including IBD.[9] These bioactive compounds form a large group of related organic molecules in plants and are most abundant in fruits, some vegetables, tea, coffee, cocoa, wine, herbs, and spices.[10–12] Plant polyphenols are subclassified according to the number of phenol rings in their chemical structure. The largest class is flavonoids, which is further subdivided into flavonols, flavones, isoflavones, flavanones, anthocyanidins, and flavanols. Other major polyphenol classes are the stilbenes (resveratrol), phenolic acids (including hydroxybenzoic acids and hydroxycinnamic acids), and lignans. The subclasses of polyphenols vary in different foods e.g., flavonols are widely present in berries, apples, and red onions; flavanols are mainly found in tea, cocoa, and chocolate; isoflavones are commonly available in soybeans; lignans are abundant in flaxseed and sesame seed; resveratrol is affluent in grapes and wine; and flavones are contained mainly in thyme, rosemary, and oregano.

A potential mechanism for how polyphenols may prevent IBD is through inhibiting the biological process of oxidative stress and the associated damage to DNA, lipids, and proteins. The gastrointestinal mucosa is constantly exposed to luminal pro-oxidants in foods such as alcohol, cholesterol oxides, and fatty acids. Polyphenol compounds ameliorate oxidative stress by acting as free radical scavengers and inducing cellular antioxidant defence-modulating proteins and genes expression.[13,14] Furthermore, experimental evidence suggests that polyphenols may attenuate intestinal inflammation by reducing the expression of proinflammatory proteins through influencing mitogen-activated protein kinases and the transcription factor nuclear factor kB signaling pathways.[15]

The molecular mechanisms for how polyphenols may prevent IBD need to be supported by population-based epidemiological studies, demonstrating that initially well people who eat lower amounts of polyphenols are more susceptible to developing UC and CD than those eating more of these micronutrients. To date, no such epidemiological investigations have investigated the polyphenol hypothesis, including prospective studies, the most robust observational study design in nutritional epidemiology. The aim of this aetiological investigation was to conduct the first such prospective cohort study investigating if dietary polyphenols are inversely associated with the development of IBD in participants enrolled into the European Prospective Investigation into Cancer and Nutrition Study (EPIC). Demonstrating associations would support encouraging higher dietary intakes of polyphenols to reduce the incidence of IBD and possibly their assessment in randomized controlled trials as treatments in patients to improve clinical outcomes.

METHODS

Study Population

The methodology of the main EPIC study has been previously described[16]; in brief, 520,000 men and women were recruited in 23 collaborating centers in 10 European countries during the years 1991 to 1998. EPIC was originally designed to investigate habitual diet and the aetiology of common cancers in the general population. The EPIC-IBD study is a subcohort of 401,326 initially healthy men and women without CD or UC at recruitment (see Table 1, Supplemental Digital Content 1, http://links.lww.com/IBD/B492). The age of participants at recruitment ranged between 20 and 80 years in the 12 centers from 8 European countries, namely Denmark, Germany, Greece, France, Italy, the Netherlands, Sweden, and the United Kingdom. At recruitment, lifestyle factors were measured including habitual diet using country-specific food frequency questionnaires (FFQs) that included information on smoking, alcohol intake, and physical activity. The FFQs listed approximately 200 different foods and up to 9 frequency of intake categories. From these FFQs, dietary polyphenol intake was assessed using the Phenol-Explorer database, which contains data on the content of 502 polyphenol compounds in 452 plant-based foods.[17] The effects of cooking and food processing on the polyphenol content of foods were accounted for by applying polyphenol-specific retention factors from Phenol-Explorer to the relevant foods.[17] A retention factor describes the loss or gain of a micronutrient during food processing e.g., a value of 0.40 means that 40% of the compound has been retained during processing.

In addition to total polyphenol intake, the 4 main subclasses, namely flavonoids, lignans, stilbenes, and phenolic acids, were computed from Phenol-Explorer. Total polyphenol content was calculated as the sum of the contents of the individual compounds expressed in mg/100 g food fresh weight. All animal foods that contain no or only traces of polyphenols were excluded from the database. Total daily energy intake was also derived from the FFQs.

Follow-up and Ascertainment of IBD

After recruitment, the subcohort was subsequently monitored until at least May 2004, and in some centers until December 2010, to identify incident cases of both CD and UC. New cases of IBD were detected by several methods depending on the center, namely local and national IBD registries, self-reported follow-up questionnaires, pathology databases, and hospital in-patient records. All the clinical notes of potential cases were reviewed by local physicians to confirm the diagnoses. Those participants who developed indeterminate or microscopic colitis or who had prevalent IBD at recruitment (including IBD diagnosed less than 18 months after recruitment) were excluded. The latter helped to ensure that the nutritional data were more likely to represent habitual diet before the development of symptoms and the subsequent diagnosis of IBD.

Statistical Analysis

The analysis was a nested case–control design within a prospective cohort study. Four controls per case were randomly selected and matched by age at recruitment (± 6 months), sex, date of recruitment into the study (± 3 months), and study center. The controls were alive on the date of diagnosis of the matched case to ensure a similar follow-up time. Absolute differences in polyphenol intake between cases and controls were compared with either a student's t test or Wilcoxon–Mann–Whitney test, according to the nature of the distributions. The intakes of polyphenols were divided into quartiles across the distribution of the cohort. Conditional logistic regression calculated the odds ratios (ORs) and 95% confidence intervals (CIs) for the dietary intake of total polyphenols and the subtypes: flavonoids, lignans, stilbenes, phenolic acid, and the development of CD and UC separately. All the models were analyzed by adjusting for total energy intake (continuous variable), dietary fat, and protein. The latter adjustment did not alter the observed associations and therefore we only kept total energy in the final model. The models were adjusted by educational level (none/primary school, technical/professional school, secondary school, and longer education [including university]) and smoking (never, current, or former). As cigarettes contain pro-oxidants, effect modification by smoking status (never smokers versus ever smokers) was evaluated. All analyses were performed using SAS (SAS Statistical Package. version 9.3; SAS institute, Gary, NC).

RESULTS

Overall, 110 incident cases of CD (73% women; mean age at diagnosis = 55.4 years, SD 11.1) and 244 incident cases of UC (57% women; mean age at diagnosis 57.5 years, SD = 10.3) were

identified (Table 1). Being an ever smoker was more frequent among CD and UC cases than among controls (Table 1). There were no significant statistical differences in the median intakes of either total or any of the subtypes of polyphenols between cases and controls (total polyphenols and CD = 1091.6 mg/d versus 1164.8 mg/d; for UC = 1226.1 mg/d versus 1203.0 mg/d, respectively; Table 1).

In the multivariable analysis for total polyphenol intake and CD, and using the first quartile as the reference category, lower odds were found for all higher quartiles, which was statistically significant in the third ($OR_{3 \text{ versus } 1 \text{ quartile}} = 0.38$; 95% CI, 0.18–0.79) but with no trend across quartiles (P value for trend = 0.17) (Table 2). In subgroups of polyphenols, for flavones, lower odds were documented in all quartiles and was statistically significant in the third (OR = 0.33; 95% CI, 0.15–0.69), with an inverse trend across quartiles (P for trend = 0.03). Similarly, for resveratrol, all higher quartiles were associated with lower odds, which was statistically significant for the second quartile ($OR_{2 \text{ versus } 1 \text{ quartile}} = 0.47$; 95% CI, 0.25–0.90) and highest quartile (OR = 0.40; 95% CI, 0.20–0.82), with an inverse trend across quartiles (P = 0.02). For most other polyphenol subtypes, there were inverse, but not statistically significant, associations with higher quartiles, and no trends across categories. For isoflavones, the third quartile was positively associated with the odds of CD ($OR_{3 \text{ versus } 1 \text{ quartile}} = 2.26$; 95% CI, 1.13–4.53), but no trend across categories (P = 0.11). For total polyphenol intake and UC, there were no statistically significant associations (Table 3). For subtypes of polyphenols, there were no associations within quartiles, apart from a higher odds ratio in the third quartile of flavanols ($OR_{3 \text{ versus } 1 \text{ quartile}} = 1.71$; 95% CI, 1.09–2.69) and no trends across quartiles.

Further stratified analyses were conducted by smoking status because this may hypothetically modify the effects of polyphenols. For nonsmokers, there were no associations with either total or subtypes of polyphenols with CD or UC (see Tables 2 and 3, Supplemental Digital Content 1, http://links.lww.com/IBD/B492); the exceptions were an inverse association with the second quartile of lignans and CD ($OR_{2 \text{ versus } 1 \text{ quartile}} = 0.06$; 95% CI, 0.01–0.39) and borderline statistical significant trends for UC with flavonols (P = 0.08) and isoflavones (P = 0.07). In ever smokers (former plus current smokers), there were borderline significant trends across quartiles for flavanones (P = 0.07), flavones (P = 0.07), and lignans (P = 0.06) for CD (Table 4). In ever smokers, total polyphenol intake was positively associated with UC (P trend = 0.05), although only the third quartile was statistically significant ($OR_{3 \text{ versus } 1 \text{ quartile}} = 2.00$; 95% CI, 1.04–3.85) (Table 5). There were positive associations between phenolic acids and UC ($OR_{3 \text{ versus } 1 \text{ quartile}} = 2.11$; 95% CI, 1.06–4.22; $OR_{4 \text{ versus } 1 \text{ quartile}} = 2.14$; 95% CI, 1.03–4.46; P trend = 0.05) because of the contribution of hydroxycinnamic acid ($OR_{3 \text{ versus } 1 \text{ quartile}} = 2.01$; 95% CI, 0.99–4.07; $OR_{4 \text{ versus } 1 \text{ quartile}} = 2.35$; 95% CI, 1.13–4.91; P tend = 0.04).

DISCUSSION

We found no associations between total dietary polyphenol intake and the odds of developing either CD or UC. However,

TABLE 1. Characteristics of Participants in a Nested Case–control Study Within the EPIC-IBD Study

Category	CD		UC	
	Cases (n = 110)	Controls (n = 440)	Cases (n = 244)	Controls (n = 976)
Sex (female %)	80 (72.7)	320 (72.3)	140 (57.4)	565 (57.9)
Age at recruitment, yrs, mean ± SD	50.1 ± 10.8	50.1 ± 10.7	51.7 ± 10.5	51.7 ± 10.5
Age at diagnosis, yrs, mean ± SD	55.4 ± 11.1	—	57.5 ± 10.3	—
Interval between recruitment and diagnosis, mean ± SD	5.5 ± 2.9	—	5.8 ± 3.3	—
Education, n (%)				
Primary school	22 (20.0)	107 (24.3)	68 (27.8)	253 (26.0)
Technical/professional school	33 (30.0)	96 (21.8)	62 (25.4)	246 (25.2)
Secondary school	30 (27.3)	105 (23.9)	52 (21.3)	201 (21.0)
Longer education (including university)	25 (22.7)	125 (28.4)	54 (22.1)	245 (25.1)
Missing	0 (0.0)	7 (1.6)	8 (3.3)	31 (3.2)
Smoking status, n (%)		[a]		[a]
Never smoked	41 (37.3)	209 (47.5)	65 (26.6)	418 (42.8)
Former smoker	28 (25.5)	121 (27.5)	91 (37.3)	281 (28.8)
Current smoker	38 (34.6)	107 (24.3)	82 (33.6)	255 (26.1)
Missing	3 (2.7)	3 (0.7)	6 (2.5)	22 (2.3)
Daily median intakes, mg/d, (interquartile range)				
Total polyphenols	1091.6 (848.8–1517.4)	1164.8 (888.8–1517.4)	1226.1 (911.3–1598.5)	1203.0 (872.1–1595.3)
Flavonoids	474.6 (276.6–770.6)	462.8 (293.4–707.8)	512.2 (306.6–739.8)	475.5 (311.4–762.6)
Anthocyanidins	25.7 (9.9–53.7)	30.4 (13.8–55.5)	23.7 (11.5–49.7)	24.0 (11.5–49.0)
Flavanols	318.4 (192.6–627.6)	327.8 (192.7–527.1)	379.3 (218.1–581.1)	338.2 (203.7–585.1)
Flavonols	28.2 (15.2–56.9)	29.9 (18.0–52.7)	30.6 (17.0–59.5)	31.5 (17.8–59.1)
Flavanones	21.8 (9.0–48.9)	24.8 (11.3–51.7)	23.9 (10.0–50.4)	26.1 (10.5–59.3)
Flavones	7.7 (5.2–14.4)	9.5 (6.0–14.1)	9.5 (5.3–14.5)	8.9 (5.1–14.5)
Isoflavones	0.04 (0.01–0.11)	0.03 (0.01–0.08)	0.03 (0.01–0.08)	0.03 (0.01–0.08)
Lignans	1.4 (1.0–2.0)	1.4 (1.1–1.9)	1.4 (1.1–2.0)	1.4 (1.0–2.0)
Stilbenes	0.3 (0.1–1.3)	0.5 (0.1–2.1)	0.5 (0.1–2.1)	0.4 (0.1–2.1)
Resveratrol	0.1 (0.0–0.2)	0.1 (0.0–0.2)	0.1 (0.0–0.3)	0.1 (0.0–0.2)
Phenolic acids	560.9 (428.4–806.9)	573.4 (396.1–815.0)	592.4 (388.1–868.8)	571.3 (365.0–821.3)
Hydroxybenzoic acid	23.7 (8.7–79.0)	26.1 (11.1–65.9)	27.9 (8.4–82.7)	28.6 (10.4–79.8)
Hydroxycinnamic acid	522.0 (328.3–777.4)	529.3 (341.1–770.5)	546.9 (340.4–818.4)	518.4 (301.0–777.3)
Hydroxyphenylacetic	0.1 (0.0–0.3)	0.1 (0.0–0.3)	0.1 (0.0–0.34)	0.1 (0.0–0.3)

[a]$P < 0.05$.

certain subtypes of polyphenols, namely flavones and resveratrol, were associated with lower odds of CD. There was some evidence of effect modification in ever smokers with suggestive inverse associations for CD with flavanones, flavones, and lignans, but no associations in nonsmokers for both UC and CD. The plausible biological mechanisms, magnitudes of these effect sizes, biological gradients, temporal data collection, and adjustment for potential confounders suggest that these inverse associations could be protective ones. Mechanistically, certain polyphenols may prevent CD through inhibiting oxidative stress,[13] which in the intestinal tract may contribute to the pathogenesis and progression of IBD.[18,19] Recent data reported a positive correlation between reactive oxygen species, reactive nitrogen species, and gastrointestinal mucosal inflammation, which was associated with increased proinflammatory cytokines such as interleukin 6, interleukin-17A, and interleukin−23.[20]

The suggestion of effect modification for certain polyphenols and CD in smokers, a process which induces oxidative stress, but not in nonsmokers, provides evidence for the role of this metabolic process in the aetiology of CD. Oxidative stress develops through mechanisms including bacteriologic and immunologic pathways, which may support the varying associations of polyphenols with CD and UC; for example, antibiotic treatment of CD but not UC, and the distinct immune responses of

TABLE 2. Odds Ratios (OR) of Developing CD According to Quartiles of Polyphenol Intakes

Polyphenols	Quartile of Intake				P for Trend
	Quartile 1	Quartile 2	Quartile 3	Quartile 4	
Total polyphenols					
Cases (n)	32	30	17	31	
OR (95% CI)	1.00	0.78 (0.41–1.47)	0.38 (0.18–0.79)[a]	0.70 (0.33–1.49)	0.17
Flavonoids					
Cases (n)	32	23	23	32	
OR (95% CI)	1.00	0.76 (0.41–1.43)	0.72 (0.37–1.38)	0.94 (0.45–1.97)	0.71
Anthocyanidins					
Cases (n)	36	26	20	28	
OR (95% CI)	1.00	0.80 (0.43–1.48)	0.56 (0.29–1.11)	0.67 (0.33–1.36)	0.18
Flavanols					
Cases (n)	28	32	18	32	
OR (95% CI)	1.00	1.14 (0.63–2.05)	0.67 (0.34–1.34)	1.11 (0.51–2.42)	0.75
Flavonols					
Cases (n)	31	26	22	31	
OR (95% CI)	1.00	0.80 (0.42–1.51)	0.68 (0.32–1.44)	1.00 (0.45–2.22)	0.89
Flavanones					
Cases (n)	37	24	23	26	
OR (95% CI)	1.00	0.64 (0.35–1.17)	0.57 (0.30–1.10)	0.60 (0.32–1.13)	0.11
Flavones					
Cases (n)	36	32	12	30	
OR (95% CI)	1.00	0.88 (0.49–1.60)	0.33 (0.15–0.69)[a]	0.61 (0.28–1.30)	0.03[a]
Isoflavones					
Cases (n)	22	20	40	28	
OR (95% CI)	1.00	0.98 (0.50–1.92)	2.26 (1.13–4.53)[a]	1.59 (0.72–3.49)	0.11
Lignans					
Cases (n)	36	18	27	29	
OR (95% CI)	1.00	0.47 (0.24–0.93)[a]	0.71 (0.37–1.38)	0.67 (0.30–1.48)	0.43
Stilbenes					
Cases (n)	37	32	25	21	
OR (95% CI)	1.00	0.91 (0.50–1.66)	0.67 (0.35–1.28)	0.53 (0.26–1.08)	0.05
Resveratrol					
Cases (n)	40	20	29	21	
OR (95% CI)	1.00	0.47 (0.25–0.90)[a]	0.60 (0.32–1.11)	0.40 (0.20–0.82)[a]	0.02[a]
Phenolic acids					
Cases (n)	24	32	28	26	
OR (95% CI)	1.00	1.21 (0.65–2.27)	1.11 (0.58–2.11)	0.83 (0.41–1.69)	0.55
Hydroxybenzoic acid					
Cases (n)	30	27	24	29	
OR (95% CI)	1.00	0.82 (0.43–1.59)	0.77 (0.37–1.59)	0.85 (0.37–1.95)	0.66
Hydroxycinnamic acid					
Cases (n)	25	32	26	27	
OR (95% CI)	1.00	1.23 (0.67–2.28)	0.95 (0.50–1.82)	0.91 (0.46–1.79)	0.60
Hydroxyphenylacetic					
Cases (n)	24	26	36	24	
OR (95% CI)	1.00	0.94 (0.50–1.78)	1.39 (0.76–2.56)	0.77 (0.37–1.60)	0.87

Adjusted for the following: educational level, smoking, and total energy.
[a]P < 0.05.

TABLE 3. Odds Ratios (OR) of Developing UC According to Quartiles of Polyphenols Intake

	Quartile of Intake				
	Quartile 1	Quartile 2	Quartile 3	Quartile 4	*P* for Trend
Total polyphenols					
Cases (n)	54	61	66	63	
OR (95% CI)	1.00	1.46 (0.93–2.31)	1.59 (0.97–2.61)	1.52 (0.88–2.63)	0.16
Flavonoids					
Cases (n)	66	43	78	57	
OR (95% CI)	1.00	0.77 (0.49–1.22)	1.43 (0.92–2.24)	1.10 (0.66–1.86)	0.26
Anthocyanidins					
Cases (n)	61	62	53	68	
OR (95% CI)	1.00	1.15 (0.76–1.74)	1.03 (0.65–1.62)	1.31 (0.82–2.10)	0.36
Flavanols					
Cases (n)	60	46	80	58	
OR (95% CI)	1.00	0.85 (0.54–1.35)	1.71 (1.09–2.69)[a]	1.30 (0.76–2.20)	0.07
Flavonols					
Cases (n)	63	64	53	64	
OR (95% CI)	1.00	1.10 (0.71–1.71)	0.91 (0.55–1.50)	1.22 (0.73–2.03)	0.63
Flavanones					
Cases (n)	66	61	66	51	
OR (95% CI)	1.00	1.00 (0.66–1.52)	1.06 (0.70–1.62)	0.79 (0.50–1.24)	0.38
Flavones					
Cases (n)	62	50	67	65	
OR (95% CI)	1.00	0.97 (0.62–1.51)	1.33 (0.85–2.08)	1.37 (0.81–2.34)	0.14
Isoflavones					
Cases (n)	68	61	61	54	
OR (95% CI)	1.00	0.91 (0.59–1.40)	0.84 (0.52–1.37)	0.66 (0.37–1.20)	0.19
Lignans					
Cases (n)	70	56	48	70	
OR (95% CI)	1.00	0.82 (0.54–1.26)	0.72 (0.45–1.15)	0.99 (0.58–1.70)	0.75
Stilbenes					
Cases (n)	64	53	64	63	
OR (95% CI)	1.00	0.96 (0.62–1.49)	1.16 (0.74–1.82)	1.09 (0.68–1.75)	0.57
Resveratrol	64	48	67	65	
	1.00	0.79 (0.50–1.24)	1.30 (0.82–2.04)	1.26 (0.75–2.11)	0.32
Phenolic acids					
Cases (n)	55	59	60	70	
OR (95% CI)	1.00	1.17 (0.74–1.85)	1.21 (0.76–1.94)	1.33 (0.81–2.19)	0.28
Hydroxybenzoic acid					
Cases (n)	73	49	59	63	
OR (95% CI)	1.00	0.67 (0.43–1.04)	0.83 (0.53–1.32)	1.00 (0.59–1.58)	0.92
Hydroxycinnamic acid					
Cases (n)	52	61	56	75	
OR (95% CI)	1.00	1.31 (0.82–2.07)	1.22 (0.75–1.97)	1.60 (0.98–2.63)	0.10
Hydroxyphenylacetic					
Cases (n)	63	57	55	69	
OR (95% CI)	1.00	1.01 (0.66–1.55)	0.93 (0.59–1.46)	1.14 (0.72–1.82)	0.67

Adjusted for the following: educational level, smoking, and total energy.
[a]$P < 0.05$.

TABLE 4. Odds Ratios (OR) of Developing CD According to Quartiles of Polyphenol Intake in Ever Smokers

Polyphenols	Quartile of Intake				P for Trend
	Quartile 1	Quartile 2	Quartile 3	Quartile 4	
Total polyphenols					
Cases (n)	20	19	9	18	
OR (95% CI)	1.00	0.73 (0.29–1.83)	0.28 (0.10–0.84)[a]	0.56 (0.20–1.63)	0.13
Flavonoids total					
Cases (n)	22	14	10	20	
OR (95% CI)	1.00	0.69 (0.28–1.70)	0.50 (0.18–1.38)	1.14 (0.36–3.55)	0.67
Anthocyanidins					
Cases (n)	24	13	13	16	
OR (95% CI)	1.00	0.65 (0.26–1.66)	0.51 (0.20–1.28)	0.69 (0.24–1.96)	0.30
Flavanols					
Cases (n)	20	21	5	20	
OR (95% CI)	1.00	1.35 (0.57–3.20)	0.21 (0.05–0.82)[a]	1.82 (0.51–6.51)	0.66
Flavonols					
Cases (n)	20	18	11	17	
OR (95% CI)	1.00	1.14 (0.46–2.83)	0.89 (0.28–2.82)	1.07 (0.31–3.65)	0.99
Flavanones					
Cases (n)	23	13	12	18	
OR (95% CI)	1.00	0.33 (0.13–0.82)[a]	0.37 (0.13–1.06)	0.40 (0.17–0.97)[a]	0.07
Flavones					
Cases (n)	25	19	5	17	
OR (95% CI)	1.00	0.67 (0.28–1.59)	0.15 (0.04–0.54)[a]	0.56 (0.20–1.58)	0.07
Isoflavones					
Cases (n)	11	10	25	20	
OR (95% CI)	1.00	0.96 (0.32–2.87)	3.69 (1.15–11.85)[a]	2.32 (0.66–8.11)	0.12
Lignans					
Cases (n)	23	14	14	15	
OR (95% CI)	1.00	0.52 (0.21–1.29)	0.38 (0.13–1.12)	0.38 (0.12–1.19)	0.06
Stilbenes					
Cases (n)	21	18	14	13	
OR (95% CI)	1.00	1.03 (0.43–2.51)	0.66 (0.25–1.73)	0.57 (0.20–1.64)	0.19
Resveratrol					
Cases (n)	25	9	19	13	
OR (95% CI)	1.00	0.42 (0.16–1.13)	0.72 (0.30–1.73)	0.39 (0.14–1.07)	0.14
Phenolic acids					
Cases (n)	14	22	16	14	
OR (95% CI)	1.00	1.14 (0.45–2.89)	0.90 (0.33–2.49)	0.71 (0.26–1.96)	0.37
Hydroxybenzoic acid					
Cases (n)	17	19	14	16	
OR (95% CI)	1.00	1.28 (0.49–3.35)	1.32 (0.47–3.74)	1.42 (0.39–5.15)	0.58
Hydroxycinnamic acid					
Cases (n)	14	22	14	16	
OR (95% CI)	1.00	1.71 (0.67–4.41)	1.00 (0.36–2.79)	1.10 (0.40–3.01)	0.71
Hydroxyphenylacetic					
Cases (n)	14	16	20	16	
OR (95% CI)	1.00	0.82 (0.33–2.04)	1.56 (0.64–3.80)	1.01 (0.37–2.74)	0.56

Adjusted for educational level and total energy.
[a]$P < 0.05$.

TABLE 5. Odds Ratios (OR) of Developing UC and Polyphenol Intake in Ever Smokers

| | Quartile of Intake | | | | |
Polyphenols	Quartile 1	Quartile 2	Quartile 3	Quartile 4	*P* for Trend
Total polyphenols					
Cases (n)	39	38	49	47	
OR (95% CI)	1.00	1.41 (0.76–2.64)	2.00 (1.04–3.85)[a]	2.04 (0.95–4.36)	0.05
Flavonoids total					
Cases (n)	49	33	53	38	
OR (95% CI)	1.00	0.86 (0.48–1.55)	1.41 (0.79–2.52)	1.23 (0.61–2.47)	0.30
Anthocyanidins					
Cases (n)	43	44	40	46	
OR (95% CI)	1.00	1.45 (0.83–2.52)	1.37 (0.75–2.49)	1.50 (0.81–2.78)	0.27
Flavanols					
Cases (n)	47	35	52	39	
OR (95% CI)	1.00	0.89 (0.51–1.56)	1.44 (0.81–2.57)	1.26 (0.64–2.51)	0.26
Flavonols					
Cases (n)	49	48	36	40	
OR (95% CI)	1.00	0.97 (0.55–1.74)	0.70 (0.36–1.36)	1.06 (0.54–2.09)	0.88
Flavanones					
Cases (n)	42	46	47	38	
OR (95% CI)	1.00	1.71 (0.97–3.03)	1.33 (0.76–2.32)	1.05 (0.58–1.91)	0.95
Flavones					
Cases (n)	47	31	51	44	
OR (95% CI)	1.00	0.93 (0.52–1.65)	1.55 (0.88–2.74)	1.34 (0.66–2.72)	0.19
Isoflavones					
Cases (n)	47	45	42	39	
OR (95% CI)	1.00	1.04 (0.59–1.80)	0.80 (0.43–1.50)	0.55 (0.26–1.16)	0.12
Lignans					
Cases (n)	50	41	36	46	
OR (95% CI)	1.00	0.96 (0.56–1.65)	0.78 (0.43–1.42)	1.00 (0.48–2.08)	0.74
Stilbenes					
Cases (n)	44	41	45	43	
OR (95% CI)	1.00	1.08 (0.60–1.94)	1.21 (0.65–2.25)	0.98 (0.52–1.87)	0.99
Resveratrol					
Cases (n)	44	38	46	45	
OR (95% CI)	1.00	0.92 (0.51–1.65)	1.37 (0.76–2.47)	1.02 (0.54–1.90)	0.69
Phenolic acids					
Cases (n)	31	43	46	53	
OR (95% CI)	1.00	1.70 (0.88–3.29)	2.11 (1.06–4.22)[a]	2.14 (1.03–4.46)[a]	**0.05**
Hydroxybenzoic acid					
Cases (n)	56	36	41	40	
OR (95% CI)	1.00	0.71 (0.41–1.23)	0.78 (0.43–1.40)	0.85 (0.45–1.61)	0.60
Hydroxycinnamic acid					
Cases (n)	28	46	44	55	
OR (95% CI)	1.00	1.85 (0.96–3.56)	2.01 (0.99–4.07)	2.35 (1.13–4.91)[a]	0.04[a]
Hydroxyphenylacetic					
Cases (n)	48	41	36	48	
OR (95% CI)	1.00	0.93 (0.53–1.63)	0.61 (0.33–1.15)	1.01 (0.54–1.88)	0.78

Adjusted for educational level and total energy.
[a]*P* < 0.05.

CD (TH1 cytokine profile) and UC (TH2 profile) (the TH1 cytokine profile is involved in immune response to infection but not TH2 profile) support a possible role for the gut microbiota in CD.[21] Furthermore, specific polyphenols, such as stilbenes (particularly resveratrol) may alter the composition of the gut microbial ecosystem, as supported by evidence in rats.[22] Moreover, patients with active CD, compared with active UC, may have higher levels of oxidative stress characterized by higher superoxide dismutase activity leading to H_2O_2 production increasing lipid peroxidation and inhibiting mitochondrial function.[23] Patients with CD have higher $ONOO^-$ (peroxynitrite) content, a by-product of the inducible isoform of nitric oxide synthases that is highly expressed in activated macrophages and neutrophils of colonic mucosa.[24] Finally, polyphenols possess not only antioxidant properties but also other bioactive functions, including vasodilatory, antiviral, antibacterial, anti-inflammatory, and anticarcinogenic effects, as well modulating signaling pathways influencing inflammation such as phospholipase A2, cyclooxygenase, lipoxygenase, and the induction of nuclear factor kB and mitogen-activated protein kinase signaling pathways.[25] Most polyphenols were associated with decreased odds of CD, although the trends were only statistically significant for flavones and resveratrol. This may suggest that either different polyphenols have varying biological activities in preventing CD or there were insufficient cases to detect significant associations in other groups. Experimental work to elucidate any differential properties of specific polyphenols and how these may influence aetiology, plus further cohort follow-up to accrue more cases is required to clarify if there are associations. In UC, there was a positive association between hydroxycinnamic acid and UC among ever smokers, but not nonsmokers, although we are not aware of any biological mechanisms to explain this, and we cannot discount the possibility that this is a false-positive result. Hydroxycinnamic acid is mainly obtained from coffee, and the possibility of residual confounding exists, if there is another component of coffee which increases the risk of UC.

No previous epidemiological studies, as far as we are aware, have investigated polyphenols in the aetiology of either CD or UC. However, other foods that contain these micronutrients, such as fiber-rich vegetables and fruits, have been studied.[12,26] A meta-analysis (of 2 cohort studies and 5 case–control studies) reported that total dietary fiber was associated with a decreased risk of CD (pooled OR = 0.44; 95% CI, 0.29–0.69), but not UC (pooled OR = 0.80; 95% CI, 0.64–1.00).[27] This inverse association for CD with fiber may be due to micronutrients in plant-based foods, such as polyphenols. Another meta-analysis of 14 case–control studies documented that consumption of vegetables was inversely associated with the risk of UC, but not CD. However, in a subgroup analysis for vegetables and CD, there was a significantly inverse association for studies performed in Europe, but not in Asia.[27] The inverse association with vegetables in the European population is consistent with our findings because some polyphenols e.g., lignans, are abundant in western foods. The increased

odds associated with some categories of isoflavones in CD might be linked to other conditions, such as lactose intolerance often undiagnosed in patients with IBD who avoid dairy foods but have increased intakes of soybean foods that are rich in isoflavones.[28–30] To help clarify if inverse associations do exist with polyphenols, further observational studies, ideally prospective cohort investigations, are required.

This observational study has several methodological strengths and limitations. The strengths include the prospective cohort design, which minimized both selection bias and recall bias for dietary polyphenol intake, and a full and detailed assessment of dietary polyphenol intake was derived from interpreting the FFQ using the polyphenol database specifically developed for EPIC.[17] FFQs do have several limitations compared with 24-hour dietary recall or food diaries, in that a set list of certain foods' portion sizes and frequencies of intake are listed, therefore not allowing for any variations in individuals' specific intakes. Dietary assessments using weighed records of intake over a set time are not pragmatic in large-scale epidemiological studies. The online resource, Phenol-Explore (http://phenol-explorer.eu/), is the first comprehensive database on polyphenol content of foods to be used in epidemiological studies. The latest version also includes detailed data on the effects of food processing and cooking. A further methodological strength was that all the case notes of potential patients with IBD were reviewed by physicians to confirm the diagnoses to reduce measurement bias. Follow-up bias should be minimal as the number of cases identified was similar to that in a large incidence study conducted in many European countries.[31] However, more cases in the analyses, particularly according to smoking status, would have allowed a better estimate of the precision of effect sizes. Further follow-up of the cohort is now being planned, including in centers not in the original EPIC-IBD study. Our findings may only be applicable to a middle-aged to elderly patient group with IBD, although the peak prevalence of both UC and CD is in younger people. However, the results are generalizable in that we studied both male and female participants from 8 countries with variation in their dietary habits and the site of disease in the gastrointestinal tract was similar to that expected. Residual confounding may exist, namely there are other variables, possibly dietary or other health-conscious behavior, associated with polyphenols which are actually the true aetiological exposures. Polyphenols are present in several foods including fruits and vegetables, and it may be other micronutrients in these food groups that actually influence risk. The EPIC-IBD study is continuing to investigate other dietary hypotheses and polyphenols will be adjusted for any new dietary variables for which associations are detected. Furthermore, participants' diet may change over time, and as we only had 1 dietary measure, namely that at recruitment, this could introduce measurement error, making any smaller associations difficult to detect. However, such measurement error would introduce an underestimate of effect sizes, rather than a spurious overestimate. The 2 forms of potential error for measuring diet, namely accuracy of the FFQ compared with weighed records, and just 1 measure of diet, plus relatively small numbers may

explain why significant associations were found for quartile 1 versus quartile 3, but not for quartile 4. However, the suggestion of a biological gradient for flavones and plausible biological mechanisms suggests further investigation of certain polyphenols is justified in a larger cohort size. Measurement error may be reduced by controlling for total energy intake. Relatively small numbers of cases, particularly according to smoking status, would mean that small effect sizes may not be identified and further cohort follow-up is required to detect such differences. The higher proportions of smoking in UC was unexpected and maybe because there was just 1 measure of smoking, namely at recruitment which was several years before diagnosis. This may be relevant if recent cessation of smoking before the development of symptoms is important in aetiology. Finally, in this investigation multiple comparisons between polyphenols and the odds of IBD were undertaken, which could result in false-positive results. Although this is possible, it is less likely as there are plausible biological mechanisms for the inverse associations, including the suggestion of effect modification in the smoking group, and for several of these micronutrients, there were biological gradients for the ORs across quartiles.

In conclusion, we have reported that higher intakes of some polyphenols, namely flavones and resveratrol, are associated with a lower risk of CD, but no associations with polyphenol intake for UC. In CD, inverse associations were documented for some subgroups of polyphenols in smokers, but not nonsmokers, supporting a role for oxidative stress in the aetiology. Residual confounding for other dietary variables associated with polyphenols is a possibility. Further large prospective cohort studies are warranted to measure polyphenol intake and determine whether the associations are consistent. If so, increasing the dietary intake of these micronutrients may help reduce the risk of developing CD and support their assessment as therapies in patients with established disease.

REFERENCES

1. Molodecky NA, Soon IS, Rabi DM, et al. Increasing incidence and prevalence of the inflammatory bowel diseases with time, based on systematic review. *Gastroenterology.* 2012;142:46–54.e42; quiz e30.
2. Sartor RB. Microbial influences in inflammatory bowel diseases. *Gastroenterology.* 2008;134:577–594.
3. Childers RE, Eluri S, Vazquez C, et al. Family history of inflammatory bowel disease among patients with ulcerative colitis: a systematic review and meta-analysis. *J Crohn's Colitis.* 2014;8:1480–1497.

4. Mahid SS, Minor KS, Soto RE, et al. Smoking and inflammatory bowel disease: a meta-analysis. *Mayo Clinic Proc.* 2006;81:1462–1471.

5. Jones DT, Osterman MT, Bewtra M, et al. Passive smoking and inflammatory bowel disease: a meta-analysis. *Am J Gastroenterol.* 2008;103:2382–2393.

6. Tjonneland A, Overvad K, Bergmann MM, et al. Linoleic acid, a dietary n-6 polyunsaturated fatty acid, and the aetiology of ulcerative colitis: a nested case-control study within a European prospective cohort study. *Gut.* 2009;58:1606–1611.

7. Bassaganya-Riera J, Hontecillas R. Dietary conjugated linoleic acid and n-3 polyunsaturated fatty acids in inflammatory bowel disease. *Curr Opin Clin Nutr Metab Care.* 2010;13:569–573.

8. Chan SS, Luben R, Olsen A, et al. Association between high dietary intake of the n-3 polyunsaturated fatty acid docosahexaenoic acid and reduced risk of Crohn's disease. *Aliment Pharmacol Ther.* 2014;39:834–842.

9. Dryden GW, Song M, McClain C. Polyphenols and gastrointestinal diseases. *Curr Opin Gastroenterol.* 2006;22:165–170.

10. Manach C, Scalbert A, Morand C, et al. Polyphenols: food sources and bioavailability. *Am J Clin Nutr.* 2004;79:727–747.

11. Scalbert A, Williamson G. Dietary intake and bioavailability of polyphenols. *J Nutr.* 2000;130(8S suppl):2073S–2085S.

12. Hou JK, Abraham B, El-Serag H. Dietary intake and risk of developing inflammatory bowel disease: a systematic review of the literature. *Am J Gastroenterol.* 2011;106:563–573.

13. Kruidenier L, Verspaget HW. Review article: oxidative stress as a pathogenic factor in inflammatory bowel disease–radicals or ridiculous? *Aliment Pharmacol Ther.* 2002;16:1997–2015.

14. Hollman PC, Cassidy A, Comte B, et al. The biological relevance of direct antioxidant effects of polyphenols for cardiovascular health in humans is not established. *J Nutr.* 2011;141:989S–1009S.

15. Rosillo MA, Sanchez-Hidalgo M, Cardeno A, et al. Protective effect of ellagic acid, a natural polyphenolic compound, in a murine model of Crohn's disease. *Biochem Pharmacol.* 2011;82:737–745.

16. Riboli E, Hunt KJ, Slimani N, et al. European prospective investigation into cancer and nutrition (EPIC): study populations and data collection. *Public Health Nutr.* 2002;5:1113–1124.

17. Zamora-Ros R, Knaze V, Rothwell JA, et al. Dietary polyphenol intake in Europe: the European prospective investigation into cancer and nutrition (EPIC) study. *Eur J Nutr.* 2016;55:1359–1375.

18. Lam G, Apostolopoulos V, Zulli A, et al. NADPH oxidases and inflammatory bowel disease. *Curr Med Chem.* 2015;22:2100–2109.

19. Pereira C, Gracio D, Teixeira JP, et al. Oxidative stress and DNA damage: implications in inflammatory bowel disease. *Inflamm Bowel Dis.* 2015;21:2403–2417.

20. Piechota-Polanczyk A, Fichna J. Review article: the role of oxidative stress in pathogenesis and treatment of inflammatory bowel diseases. *Naunyn-Schmiedeberg's Arch Pharmacol.* 2014;387:605–620.

21. Sartor RB. Mechanisms of disease: pathogenesis of Crohn's disease and ulcerative colitis. *Nat Clin Pract Gastroenterol Hepatol.* 2006;3:390–407.

22. Etxeberria U, Arias N, Boque N, et al. Reshaping faecal gut microbiota composition by the intake of trans-resveratrol and quercetin in high-fat sucrose diet-fed rats. *J Nutr Biochem.* 2015;26:651–660.

23. Beltran B, Nos P, Dasi F, et al. Mitochondrial dysfunction, persistent oxidative damage, and catalase inhibition in immune cells of naive and treated Crohn's disease. *Inflamm Bowel Dis.* 2010;16:76–86.

24. Rachmilewitz D, Karmeli F, Okon E, et al. Experimental colitis is ameliorated by inhibition of nitric oxide synthase activity. *Gut.* 1995;37:247–255.

25. Kolacek M, Muchova J, Dvorakova M, et al. Effect of natural polyphenols (Pycnogenol) on oxidative stress markers in children suffering from Crohn's disease–a pilot study. *Free Radic Res.* 2013;47:624–634.

26. Ananthakrishnan AN, Khalili H, Konijeti GG, et al. A prospective study of long-term intake of dietary fiber and risk of Crohn's disease and ulcerative colitis. *Gastroenterology.* 2013;145:970–977.

27. Liu X, Wu Y, Li F, et al. Dietary fiber intake reduces risk of inflammatory bowel disease: result from a meta-analysis. *Nutr Res.* 2015;35:753–758.

28. Nolan-Clark D, Tapsell LC, Hu R, et al. Effects of dairy products on crohn's disease symptoms are influenced by fat content and disease location but not lactose content or disease activity status in a New Zealand population. *J Am Diet Assoc.* 2011;111:1165–1172.

29. Zallot C, Quilliot D, Chevaux JB, et al. Dietary beliefs and behavior among inflammatory bowel disease patients. *Inflamm Bowel Dis.* 2013;19:66–72.

30. Brasil Lopes M, Rocha R, Castro Lyra A, et al. Restriction of dairy products; a reality in inflammatory bowel disease patients. *Nutr Hosp.* 2014;29:575–581.

31. Shivananda S, Lennard-Jones J, Logan R, et al. Incidence of inflammatory bowel disease across Europe: is there a difference between north and south? Results of the European Collaborative Study on Inflammatory Bowel Disease (EC-IBD). *Gut.* 1996;39:690–697.