

A prospective evaluation of plasma polyphenol levels and colon cancer risk

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Key words: colon cancer, polyphenols, biomarkers, EPIC, nested case-control study

Additional Supporting Information may be found in the online version of this article.

Disclosure of Potential Conflicts of Interest: The authors declare no potential conflicts of interest.

For information on how to submit an application for gaining access to EPIC data and/or biospecimens, please follow the instructions at <http://epic.iarc.fr/access/index.php>

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Grant sponsors: European Commission (DG-SANCO), International Agency for Research on Cancer, Danish Cancer Society (Denmark), Ligue Contre le Cancer, Institut Gustave Roussy, Mutuelle Générale de l'Éducation Nationale, Institut National de la Santé et de la Recherche Médicale (INSERM) (France), German Cancer Aid, German Cancer Research Center (DKFZ), Federal Ministry of Education and Research (BMBF), Deutsche Krebshilfe, Deutsches Krebsforschungszentrum, Federal Ministry of Education and Research (Germany), Hellenic Health Foundation (Greece), Associazione Italiana per la Ricerca sul Cancro-AIRC-Italy, National Research Council (Italy), Dutch Ministry of Public Health, Welfare and Sports (VWS), Netherlands Cancer Registry (NKR), LK Research Funds, Dutch Prevention Funds, Dutch ZON (Zorg Onderzoek Nederland), World Cancer Research Fund (WCRF), Statistics Netherlands (The Netherlands), ERC-2009-AdG 232997 and Nordforsk, Nordic Centre of Excellence programme on Food, Nutrition and Health; **Grant sponsor:** Health Research Fund (FIS); **Grant numbers:** PI13/00061, PI13/01162; **Grant sponsor:** Regional Governments of Andalucía, Asturias, Basque Country, Murcia and Navarra, ISCIII RETIC; **Grant number:** RD06/0020; **Grant sponsors:** Swedish Cancer Society, Swedish Research Council and County Councils of Skåne and Västerbotten; **Grant sponsor:** Cancer Research UK; **Grant numbers:** 14136, C570/A16491, C8221/A19170; **Grant sponsor:** Medical Research Council; **Grant numbers:** 1000143, MR/M012190/1; **Grant sponsor:** Institute of Health Carlos III and the European Social Fund (ESF); **Grant number:** CP15/00100

DOI: 10.1002/ijc.31563

History: Received 7 Dec 2017; Accepted 26 Mar 2018; Online 26 Apr 2018

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Polyphenols have been shown to exert biological activity in experimental models of colon cancer; however, human data linking specific polyphenols to colon cancer is limited. We assessed the relationship between pre-diagnostic plasma polyphenols and colon cancer risk in a case-control study nested within the European Prospective Investigation into Cancer and Nutrition study. Using high pressure liquid chromatography coupled to tandem mass spectrometry, we measured concentrations of 35 polyphenols in plasma from 809 incident colon cancer cases and 809 matched controls. We used multivariable adjusted conditional logistic regression models that included established colon cancer risk factors. The false discovery rate (q_{values}) was computed to control for multiple comparisons. All statistical tests were two-sided. After false discovery rate correction and in continuous \log_2 -transformed multivariable models, equol (odds ratio [OR] per \log_2 -value, 0.86, 95% confidence interval [95% CI] = 0.79–0.93; $q_{\text{value}} = 0.01$) and homovanillic acid (OR per \log_2 -value, 1.46, 95% CI = 1.16–1.84; $q_{\text{value}} = 0.02$) were associated with colon cancer risk. Comparing extreme fifths, equol concentrations were inversely associated with colon cancer risk (OR = 0.61, 95% CI = 0.41–0.91, $p_{\text{trend}} = 0.003$), while homovanillic acid concentrations were positively associated with colon cancer development (OR = 1.72, 95% CI = 1.17–2.53, $p_{\text{trend}} < 0.0001$). No heterogeneity for these associations was observed by sex and across other colon cancer risk factors. The remaining polyphenols were not associated with colon cancer risk. Higher equol concentrations were associated with lower risk, and higher homovanillic acid concentrations were associated with greater risk of colon cancer. These findings support a potential role for specific polyphenols in colon tumorigenesis.

What's new?

Polyphenols are antioxidants abundant in food, and some polyphenols have demonstrated an anti-cancer effect. Here, the authors looked for an association between polyphenols and colon cancer risk by conducting a nested case-control analysis within the EPIC cohort. Rather than employing questionnaires that rely on participant memories of their diet, this study directly measured 35 polyphenols in plasma samples and compared them with colon cancer risk. Only 2 chemicals showed any association. Concentrations of equol, which is metabolized from soy foods, were inversely associated with colon cancer risk. Higher levels of homovanillic acid, on the other hand, were associated with increased risk.

Polyphenols are plant-based bioactive compounds characterized by the presence of phenolic group(s) in their structure. Polyphenols are abundant in the human diet and dietary sources such as fruits, vegetables, nuts, legumes, whole-grain cereals, cocoa, soya beans, tea, coffee and wine.^{1,2} Over 500 different polyphenols have been identified from dietary

sources and can be broadly divided into four common classes: phenolic acids, flavonoids, lignans and stilbenes.¹ Polyphenols are the most abundant antioxidants in the human diet,³ and are postulated to confer anti-inflammatory and estrogenic effects (*e.g.*, isoflavonoids).^{4,5} Once ingested, they can be metabolized by the intestinal microbiota into low-

molecular weight phenolic acids and into various conjugated forms (e.g., glucuronides) in human tissues.^{1,6}

Experimental evidence supports a chemo-preventive role for certain polyphenols against colon cancer development. Phenolic acids, such as caffeic acid and ferulic acid, have been shown to have anti-proliferative effects on colon cancer in *in vitro* and *in vivo* investigations.^{7–9} Flavonoids have been demonstrated to induce apoptosis and inhibit colon cancer cell line growth.^{10–12} Nevertheless, prospective epidemiological studies of colon cancer which measured polyphenol intakes *via* dietary questionnaires have reported largely null results. In the Nurses' Health Study, Health Professionals Follow-up Study, and the European Prospective Investigation into Cancer and Nutrition (EPIC) study, dietary intakes of flavonoids and its sub-classes were unrelated to colon cancer risk.^{13–15} Such analyses are reliant on participant's self-reporting previous dietary intakes accurately and the availability of reliable data from databases on the polyphenol content of foods. A more objective, and potentially more accurate approach to assess how polyphenols are associated with colon cancer risk, is to directly measure levels of polyphenols directly in bio-specimens. Previous studies, which have undertaken such analyses have been of relatively small size, focused on small selections of polyphenols, and have produced inconclusive results.^{16–18}

To provide more conclusive evidence on the association of polyphenol levels and colon cancer we conducted a nested case-control analysis within the EPIC cohort, in which we measured 35 polyphenols in prospectively collected plasma samples using a recently developed method based on mass spectrometry and studied their associations with colon cancer risk.

Methods

Study population and collection of blood samples and data

EPIC is a multicenter prospective cohort of 521,330 participants, mostly aged ≥ 35 , who were recruited in 1992–2000, predominantly from the general population of 10 European countries.^{19,20} Additional detail on the study population is provided in the Supporting Information Methods. Blood samples were collected at recruitment according to standardized procedures^{19,20} and stored at the International Agency for Research on Cancer (-196°C , liquid-nitrogen) for all countries except Denmark (-150°C , nitrogen-vapor) and Sweden (-80°C , freezers). Participants completed standardized lifestyle and personal history questionnaires at recruitment, and most participants also had anthropometric measurements and blood samples taken at recruitment before disease onset or diagnosis. Diet over the previous 12-months was assessed at recruitment using validated country/center-specific dietary questionnaires.^{19,20} All participants provided written informed consent. Ethical approval for the EPIC study was obtained from the review boards of the International Agency for Research on Cancer (IARC) and local participating centers.

Follow-up for cancer incidence

Cancer incidence was determined through record linkage with regional cancer registries (Denmark, Italy, the Netherlands, Spain, Sweden, United Kingdom) 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, Greece). Colon cancer cases were defined using the tenth revision of the International Classification of Diseases (ICD-10) and the second revision of the International Classification of Diseases for Oncology (ICDO-2). Colon cancers include those within the proximal (C18.0–18.5), distal (C18.6–C18.7), overlapping (C18.8) and unspecified (C18.9) regions.

Selection of cases and controls

Controls were selected from the all participants who were alive and free of cancer (except non-melanoma skin cancer) at the time of diagnosis of the cases, using incidence density sampling, and with controls matched to cases by age, sex, study center, follow-up time since blood collection, time of day at blood collection, fasting status, menopausal status and phase of menstrual cycle at blood collection. The current study included 809 incident colon cancer cases and 809 matched controls.

Laboratory methods

Plasma polyphenol measurements were undertaken at IARC (France) using a differential isotope labeling method and mass spectrometry (Achainre *et al.* under review). The 35 polyphenols measured are listed in Table 2. Citrated plasma samples were first treated with a β -glucuronidase/sulfatase enzyme mixture containing ascorbic acid and the resulting polyphenol aglycones were extracted twice with ethyl acetate. Quenching was applied by evaporation under vacuum followed by reconstitution with water/acetonitrile. Quantitative dansylation of phenolic hydroxyl groups was carried out with either ^{13}C -labeled dansyl chloride (samples) or non-labeled dansyl chloride (well-characterized reference pooled sample). Each ^{13}C -dansylated sample was mixed with the ^{12}C -dansylated reference sample, and the relative concentrations in samples over the reference sample were determined in batches of 50 samples by ultra-high pressure liquid chromatography coupled to tandem mass spectrometry *via* an electrospray interface. Limits of quantification for the polyphenols varied between 0.11 nmol/L for apigenin and 44.4 nmol/L for quercetin. Intra-batch coefficients of variation varied between 2.3% and 9%. Inter-batch coefficients of variations were $< 20\%$ for all except four polyphenols (quercetin, gallic acid, hydroxytyrosol and enterodiol). Spiking experiments showed good accuracy for 29 polyphenols (72%–122% recovery) and satisfactory for the others (43%–62%). Dilution experiments also showed good recovery (90%–120%) for 30 of the 35 compounds tested.

Statistical analysis

Spearman's rank correlation coefficients were calculated to assess the correlations between plasma concentrations of polyphenols and dietary variables among control participants. Multivariable conditional logistic regression, stratified by case-control set, was used to compute odds ratios (ORs) and 95% confidence intervals (95% CI) for the associations between log₂-transformed plasma polyphenol concentrations and colon cancer risk. The basic model was conditioned on the matching factors only, while the multivariable model included additional adjustment for body mass index (BMI), height, physical activity, smoking status, prevalent diabetes, education level, alcohol consumption, ever use of postmenopausal hormone therapy (HT) and dietary intakes of total energy, red and processed meats and fiber. False discovery rate (FDR; q_{value}) correction was performed using the Benjamini-Hochberg method.²¹ Additional analyses were then conducted for polyphenols which were associated with colon cancer after correction for multiple comparisons ($q_{\text{value}} < 0.05$). For these analyses, participants were divided into fifths based on polyphenol concentrations in the control group, and multivariable conditional logistic regression models were undertaken. Statistical tests for trend (p_{trend}) for a given analyte were calculated using the ordinal quartile entered into the models as a continuous variable. Possible nonlinear effects were modeled using restricted cubic spline models. Sub-group analyses were conducted by anatomical site (proximal colon and distal colon), sex, BMI, ever use of HT, age at recruitment and dairy product consumption (above and below median based on distribution of the control group), and formally tested for heterogeneity using χ^2 tests. To assess whether preclinical disease may have influenced the results, cases diagnosed within the first 4-years were excluded. Statistical tests used in the analysis were all two-sided.

Results

Descriptive data analysis

Compared with the control group participants, colon cancer cases had a higher BMI and homovanillic acid concentrations, and lower equol concentrations (Tables 1 and 2). Weak correlations were observed between concentrations of the 35 plasma polyphenols and dietary intakes, except for tea ((+)-epigallocatechin, $r = 0.64$; gallic acid, $r = 0.51$) and coffee (ferulic acid, $r = 0.48$; caffeic acid, $r = 0.46$) (Fig. 1). The strongest correlations (all $p < 0.0001$) between polyphenol concentrations were found for 3,5-dihydroxybenzoic acid and 3,5-dihydroxyphenylpropionic acid ($r = 0.90$); genistein and daidzein ($r = 0.81$); hesperetin and naringenin ($r = 0.76$) and quercetin and kaempferol ($r = 0.71$). Median (5th–95th percentile) concentrations of polyphenols among controls by country are shown in Supporting Information Table S1.

Table 1. Baseline characteristics of colon cancer cases and controls

Baseline characteristic	Cases	Controls
Colon cancer cases (<i>n</i>)	809	809
Men (<i>n</i>)	323 (39.9)	323 (39.9)
Women (<i>n</i>)	486 (60.1)	486 (60.1)
Age at blood collection (years) ¹	56.8 (7.5)	56.8 (7.4)
Years of follow-up ¹	6.6 (3.7)	-
Anthropometrics ¹		
Body mass index (kg/m ²)	26.9 (4.4)	26.3 (3.8)
Height (cm)	166.4 (9.4)	165.5 (9.5)
Smoking status ²		
Never	378 (46.7)	404 (49.9)
Former	242 (29.9)	226 (27.9)
Current	180 (22.3)	175 (21.6)
Physical activity ²		
Inactive	228 (28.2)	212 (26.2)
Moderately inactive	295 (36.5)	275 (34.0)
Moderately active	159 (19.7)	160 (19.8)
Active	125 (15.5)	159 (19.7)
Education level ²		
None/primary school completed	371 (46.2)	384 (47.7)
Technical/professional/secondary school	304 (37.9)	292 (36.3)
Longer education (including university degree)	122 (15.2)	122 (15.2)
Ever menopausal hormone therapy use ²		
No	330 (42.4)	346 (44.1)
Yes	126 (16.2)	115 (14.7)
Prevalent diabetes ²		
No	739 (95.1)	740 (95.2)
Yes	32 (4.1)	32 (4.1)
Dietary intakes ¹		
Alcohol consumption (g/day)	13.8 (19.2)	12.9 (17.4)
Total energy (kcal/day)	2111.9 (751.0)	2101.5 (639.9)
Red and processed meat (g/day)	78.9 (69.1)	78.8 (48.4)
Fiber (g/day)	22.6 (7.9)	23.1 (8.0)

¹Values are mean (standard deviation). ²Values are *n* (%) with participants with any missing/unknown values for baseline characteristics excluded.

Polyphenol concentrations and risk of colon cancer

Of the 35 polyphenols included in this analysis, in the multivariable models after FDR correction ($q_{\text{value}} < 0.05$), equol and homovanillic acid were significantly associated with colon cancer risk. In continuous log₂-transformed multivariable models, equol was inversely (OR per log₂-value, 0.86, 95% CI = 0.79–0.93; $q_{\text{value}} = 0.01$; OR[comparing extreme fifths] = 0.61, 95% CI = 0.41–0.91, $p_{\text{trend}} = 0.003$; Table 4) and

Table 2. Median (5th–95th percentile) concentrations of plasma polyphenols (nmol/L) among colon cancer cases and controls

Polyphenol class/subclass	Cases			Controls			<i>p</i> for differences
	Median	5th	95th	Median	5th	95th	
Phenolic acids/Hydroxybenzoic acids (nmol/L)							
4-Hydroxybenzoic acid	202.0	152.0	367.0	201.0	150.0	353.0	0.56
3-Hydroxybenzoic acid	19.1	7.1	59.4	19.5	7.1	61.0	0.49
Protocatechuic acid	155.0	121.0	194.0	155.0	120.0	195.0	0.44
Gallic acid	38.0	22.0	91.0	37.0	21.0	96.0	0.62
Vanillic acid	178.0	95.0	353.0	176.0	95.0	359.0	0.60
3,5-Dihydroxybenzoic acid	28.6	7.0	150.6	29.0	6.6	156.0	0.97
Gallic acid ethyl ester	1.1	1.1	11.1	1.1	1.1	8.6	0.41
Phenolic acids/Hydroxyphenylacetic acids (nmol/L)							
4-Hydroxyphenylacetic acid	324.0	185.0	888.0	315.0	194.0	888.0	0.18
3-Hydroxyphenylacetic acid	58.4	2.2	236.4	55.3	2.2	238.9	0.95
3,4-Dihydroxyphenylacetic acid	40.8	23.8	76.5	40.0	24.2	78.0	0.40
Homovanillic acid	88.0	51.0	182.0	84.0	51.0	172.0	0.007
Phenolic acids/Hydroxyphenylpropanoic acids (nmol/L)							
3,4-Dihydroxyphenylpropionic acid	193.5	133.0	375.0	195.0	133.0	426.0	0.51
3,5-Dihydroxyphenylpropionic acid	41.2	10.8	161.0	39.7	10.8	162.8	0.78
Phenolic acids/Hydroxycinnamic acids (nmol/L)							
<i>p</i> -Coumaric acid	17.9	11.9	37.1	17.7	11.7	33.6	0.26
<i>m</i> -Coumaric acid	9.7	2.2	79.2	10.6	2.2	88.8	0.07
Caffeic acid	430.0	336.0	615.0	427.0	341.0	606.0	0.93
Ferulic acid	92.0	49.0	407.0	93.0	49.0	409.0	0.90
Flavonoids/Flavonols (nmol/L)							
Kaempferol	93.0	63.0	143.5	92.0	64.0	138.0	0.58
Quercetin	349.0	222.0	633.0	342.0	213.0	585.0	0.29
Flavonoids/Flavanols (nmol/L)							
(+)-Catechin	19.4	5.6	66.8	18.1	5.6	64.4	0.10
(-)-Epicatechin	17.7	5.6	116.5	17.0	5.6	102.0	0.30
(+)-Gallocatechin	11.1	11.1	26.5	11.1	11.1	27.3	0.89
(+)-Epigallocatechin	11.1	11.1	68.7	11.1	11.1	70.6	0.97
Flavonoids/Flavones (nmol/L)							
Apigenin	14.7	11.5	19.5	14.7	11.5	18.8	0.51
Flavonoids/Flavanones (nmol/L)							
Naringenin	3.8	1.7	69.6	4.0	1.7	78.3	0.23
Hesperetin	2.0	0.6	145.2	2.3	0.6	122.9	0.32
Flavonoids/Isoflavonoids (nmol/L)							
Daidzein	12.8	2.6	128.6	12.1	2.6	185.9	0.76
Genistein	4.9	1.4	47.2	4.9	1.3	66.1	0.36
Equol	0.37	0.1	2.3	0.42	0.1	2.9	0.002
Flavonoids/Dihydrochalcones (nmol/L)							
Phloretin	1.1	1.1	9.5	1.1	1.1	8.3	0.73
Stilbenes (nmol/L)							
Resveratrol	2.6	1.1	16.1	2.6	1.1	14.1	0.98
Tyrosols (nmol/L)							
Tyrosol	3.0	1.4	9.2	2.9	1.4	9.7	0.57
Hydroxytyrosol	28.1	17.2	63.7	27.3	18.1	61.0	0.47

Table 2. Median (5th–95th percentile) concentrations of plasma polyphenols (nmol/L) among colon cancer cases and controls (Continued)

Polyphenol class/subclass	Cases			Controls			<i>p</i> for differences
	Median	5th	95th	Median	5th	95th	
Lignans (nmol/L)							
Enterodiol	1.1	0.2	10.8	1.0	0.2	11.5	0.88
Enterolactone	9.6	0.8	53.8	10.1	1.0	55.8	0.47

¹Calculated using *t*-tests.

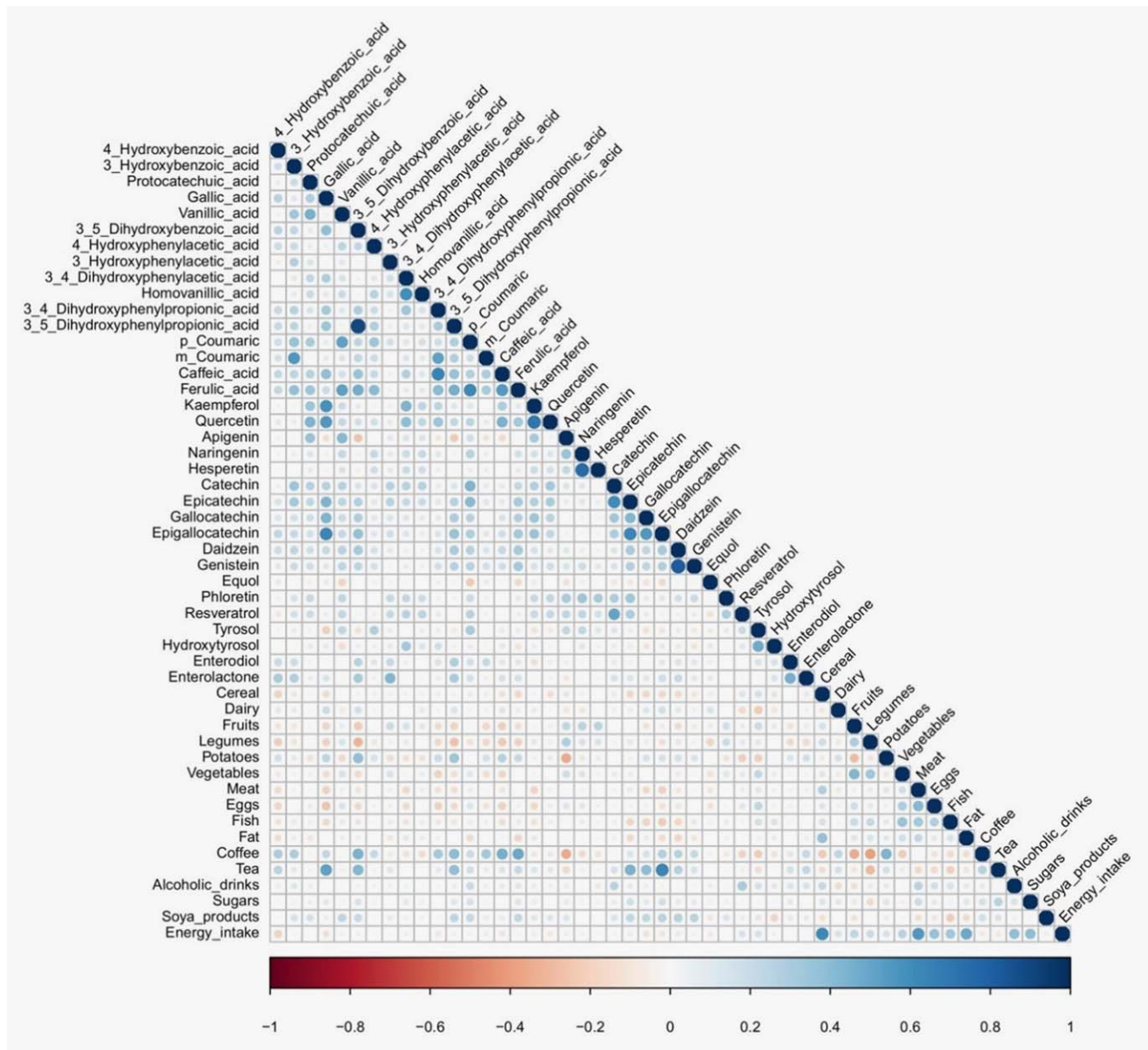


Figure 1. Spearman correlation heatmap among polyphenols and dietary intakes at recruitment among all controls (*n* = 809). Circle color reflects the strength and direction of the correlation. The size of the circle reflects the *p*_{value} (the bigger the circle, the stronger the *p*-value). Where blank, this reflects statistically non-significant correlations (*p*_{value} > 0.05). [Color figure can be viewed at wileyonlinelibrary.com]

homovanillic acid was positively (OR per log₂-value, 1.46, 95% CI = 1.16–1.84; *q*_{value} = 0.02; (OR[comparing extreme fifths] = 1.72, 95% CI = 1.17–2.53, *p*_{trend} < 0.0001)

associated with colon cancer risk (Table 3). The remaining 33 polyphenols were not associated with colon cancer risk (Table 3).

Table 3. Risk of colon cancer for log₂-transformed polyphenol concentrations (sorted by multivariable model false discovery rate [FDR] q_{values})

	Basic model				Multivariable model			
	OR per log ₂ -value	95% CI	p_{value}	FDR (q_{value})	OR per log ₂ -value	95% CI	p_{value}	FDR (q_{value})
Equol	0.88	0.82–0.95	0.0010	0.03	0.86	0.79–0.93	0.0003	0.01
Homovanillic acid	1.37	1.12–1.68	0.0020	0.03	1.46	1.16–1.84	0.0012	0.02
<i>m</i> -Coumaric acid	0.94	0.88–1.00	0.0684	0.53	0.94	0.88–1.01	0.0944	0.63
Quercetin	1.49	0.96–2.30	0.0751	0.53	1.49	0.91–2.43	0.1148	0.63
3,5-Dihydroxyphenylpropionic acid	1.02	0.92–1.13	0.7488	0.97	1.09	0.96–1.24	0.1604	0.63
<i>p</i> -Coumaric acid	1.17	0.93–1.47	0.1787	0.73	1.18	0.92–1.52	0.1830	0.63
Naringenin	0.96	0.90–1.02	0.1946	0.73	0.95	0.89–1.02	0.1876	0.63
Gallic acid	1.09	0.86–1.37	0.4750	0.73	1.20	0.91–1.57	0.1897	0.63
(+)-Catechin	1.10	0.99–1.22	0.0655	0.53	1.08	0.96–1.21	0.1944	0.63
4-Hydroxyphenylacetic acid	1.15	0.96–1.37	0.1190	0.69	1.14	0.94–1.38	0.1966	0.63
(–)-Epicatechin	1.06	0.97–1.17	0.1987	0.73	1.07	0.96–1.19	0.2011	0.63
3,5-Dihydroxybenzoic acid	1.00	0.92–1.10	0.9396	0.99	1.07	0.96–1.19	0.2467	0.63
Apigenin	1.22	0.77–1.94	0.4012	0.73	1.34	0.81–2.21	0.2545	0.63
Protocatechuic acid	1.46	0.76–2.81	0.2512	0.73	1.53	0.73–3.21	0.2604	0.63
Phloretin	1.02	0.92–1.13	0.6793	0.95	1.07	0.95–1.20	0.2714	0.63
Hesperetin	0.98	0.94–1.02	0.2639	0.73	0.97	0.93–1.02	0.2889	0.63
3,4-Dihydroxyphenylacetic acid	1.15	0.89–1.48	0.2752	0.73	1.16	0.87–1.55	0.3068	0.63
3-Hydroxyphenylacetic acid	1.00	0.95–1.06	0.9461	0.99	1.03	0.97–1.10	0.3432	0.67
Kaempferol	1.18	0.79–1.78	0.4215	0.73	1.24	0.77–2.01	0.3718	0.68
4-Hydroxybenzoic acid	1.12	0.83–1.51	0.4768	0.73	1.16	0.82–1.63	0.3984	0.70
Resveratrol	1.00	0.91–1.10	0.9795	0.99	0.96	0.86–1.07	0.4173	0.70
Vanillic acid	1.11	0.87–1.42	0.3897	0.73	1.11	0.85–1.45	0.4555	0.72
Genistein	0.97	0.91–1.03	0.3203	0.73	0.98	0.91–1.05	0.5092	0.76
Caffeic acid	0.97	0.61–1.52	0.8854	0.99	1.19	0.70–2.00	0.5218	0.76
Daidzein	0.99	0.93–1.06	0.7577	0.97	0.98	0.91–1.05	0.5729	0.76
Hydroxytyrosol	1.09	0.88–1.34	0.4393	0.73	1.07	0.85–1.35	0.5759	0.76
Gallic acid ethyl ester	1.06	0.95–1.18	0.3226	0.73	1.04	0.91–1.18	0.5831	0.76
Enterolactone	0.98	0.92–1.04	0.4450	0.73	0.99	0.93–1.06	0.7286	0.90
3,4-Dihydroxyphenylpropionic acid	0.89	0.68–1.16	0.3749	0.73	0.95	0.70–1.29	0.7481	0.90
(+)-Epigallocatechin	1.00	0.84–1.19	0.9845	0.99	1.03	0.84–1.26	0.8023	0.92
Tyrosol	1.05	0.92–1.19	0.4714	0.73	1.02	0.88–1.17	0.8133	0.92
3-Hydroxybenzoic acid	0.97	0.87–1.08	0.5863	0.85	0.99	0.88–1.12	0.9111	0.97
Ferulic acid	0.98	0.84–1.14	0.7794	0.97	1.01	0.85–1.19	0.9211	0.97
(+)-Gallocatechin	0.97	0.69–1.37	0.8685	0.99	1.01	0.66–1.54	0.9557	0.97
Enterodiol	1.00	0.94–1.07	0.9856	0.99	1.00	0.93–1.07	0.9711	0.97

Sorted by false discovery rate (FDR) q_{values} . OR per log₂ value (95% CI)—therefore the ORs correspond to a doubling in polyphenol level. Basic model was conditioned on matching factors only. Multivariable model was conditioned on matching factors, with additional adjustment for body mass index, height, physical activity, smoking status, education level, alcohol consumption, prevalent diabetes, ever use of menopausal hormone therapy, and dietary intakes of total energy, red and processed meats and fiber.

Since equol is produced by intestinal bacteria by metabolic transformation of daidzein, an isoflavone present within soya food products, the models were adjusted for daidzein and soya; the risk estimates remained unchanged after these additional adjustments (OR per log₂-value with adjustment for

daidzein, 0.84, 95% CI = 0.76–0.92; OR per log₂-value with adjustment for soya, 0.86, 95% CI = 0.79–0.93) (Table 4). The inverse relationship was also insensitive to additional adjustment for dietary intakes of dairy products and milk, which are known dietary sources of pre-formed equol²²

Table 4. Risk of colon cancer for fifths of equol and homovanillic acid after further adjustment for other dietary exposures and polyphenol concentrations

	1	2	3	4	5	<i>P</i> _{trend}	OR per log ₂ -value
Equol (nmol/L)	<0.22	0.22 to <0.35	0.35 to <0.54	0.54 to <1.01	≥1.01		
Basic	1.00	0.77 (0.55–1.07)	0.75 (0.54–1.05)	0.62 (0.44–0.87)	0.70 (0.49–1.00)	0.017	0.88 (0.82–0.95)
Multivariable	1.00	0.69 (0.47–1.00)	0.66 (0.45–0.95)	0.50 (0.34–0.74)	0.61 (0.41–0.91)	0.003	0.86 (0.79–0.93)
Plus dairy consumption	1.00	0.71 (0.48–1.03)	0.67 (0.46–0.98)	0.51 (0.35–0.76)	0.62 (0.42–0.93)	0.005	0.86 (0.79–0.93)
Plus milk consumption	1.00	0.69 (0.47–1.02)	0.68 (0.47–0.99)	0.53 (0.36–0.78)	0.63 (0.42–0.95)	0.007	0.86 (0.79–0.94)
Plus dietary calcium intake	1.00	0.71 (0.49–1.04)	0.69 (0.47–1.00)	0.52 (0.35–0.77)	0.64 (0.42–0.96)	0.007	0.86 (0.79–0.94)
Plus soya product consumption	1.00	0.68 (0.46–0.99)	0.64 (0.44–0.93)	0.50 (0.34–0.74)	0.61 (0.41–0.91)	0.003	0.86 (0.79–0.93)
Plus daidzein concentration	1.00	0.61 (0.42–0.91)	0.62 (0.42–0.92)	0.48 (0.32–0.72)	0.54 (0.35–0.84)	0.002	0.84 (0.76–0.92)
Plus genistein concentration	1.00	0.68 (0.46–0.99)	0.66 (0.45–0.95)	0.51 (0.35–0.76)	0.60 (0.40–0.91)	0.004	0.86 (0.79–0.94)
Plus daidzein and genistein concentration	1.00	0.61 (0.41–0.91)	0.62 (0.42–0.92)	0.49 (0.32–0.74)	0.54 (0.35–0.84)	0.002	0.84 (0.76–0.92)
Homovanillic acid (nmol/L)	<65	65 to <78	78 to <91	91 to <118	≥118		
Basic	1.00	0.97 (0.70–1.36)	1.05 (0.76–1.45)	1.57 (1.14–2.16)	1.54 (1.09–2.16)	<0.0001	1.37 (1.12–1.68)
Multivariable	1.00	0.97 (0.67–1.42)	1.06 (0.73–1.52)	1.77 (1.23–2.53)	1.72 (1.17–2.53)	<0.0001	1.46 (1.16–1.84)
Plus 3,4-Dihydroxyphenylacetic acid concentration	1.00	0.93 (0.63–1.38)	1.06 (0.72–1.56)	1.71 (1.16–2.51)	1.59 (1.02–2.47)	0.002	1.50 (1.12–1.99)

Values are ORs for fifths of equol and homovanillic acid, and OR per log₂ value (95% CI). Multivariable model was conditioned on matching factors, with additional adjustment for body mass index, height, physical activity, smoking status, education level, alcohol consumption, prevalent diabetes, ever use of menopausal hormone therapy and dietary intakes of total energy, red and processed meats and fiber.

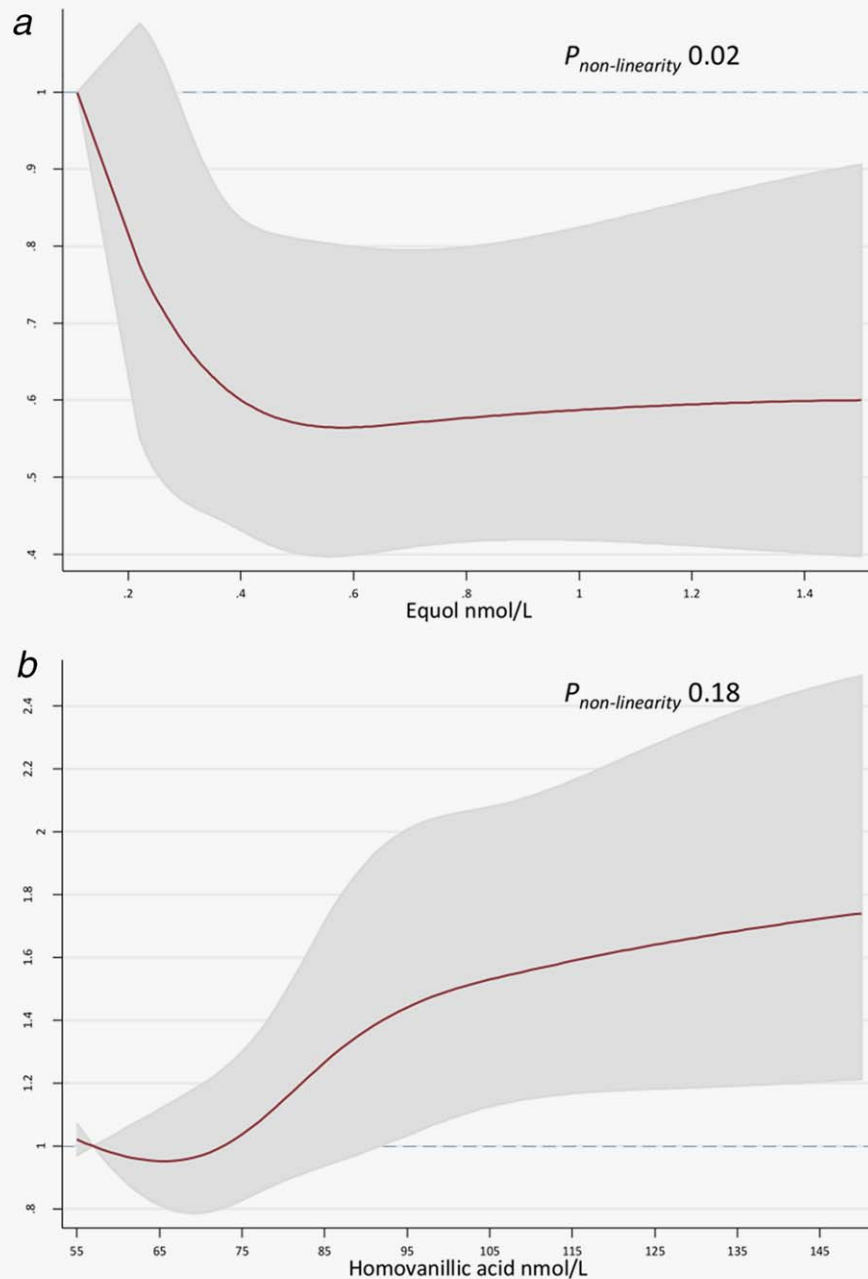


Figure 2. Association between circulating (a) equol, (b) homovanillic acid with colon cancer allowing for nonlinear effects (restricted cubic spline). Solid lines indicate the odds ratio, and shaded gray areas indicate the 95% confidence intervals. Multivariable model was conditioned on matching factors, with additional adjustment for body mass index, height, physical activity, smoking status, education level, alcohol consumption, prevalent diabetes, ever use of menopausal hormone therapy and dietary intakes of total energy, red and processed meats and fiber. The references for these restricted cubic spline plots (with five knots placed at the 10th, 25th, 50th, 75th and 95th percentiles) were 57 nmol/L for homovanillic acid and 0.11 nmol/L for equol. [Color figure can be viewed at wileyonlinelibrary.com]

(Table 4). A non-linear relationship was observed between equol concentrations and colon cancer risk ($p_{\text{non-linear}} = 0.02$), with the risk plateauing at an OR of 0.60 at plasma concentrations of 0.6 nmol/L and above (Fig. 2a). Similar strength inverse associations between equol and colon cancer were observed for women and men ($p_{\text{heterogeneity}} = 0.54$) (Supporting Information Table S2), and for tumors located in the proximal

and distal colon ($p_{\text{heterogeneity}} = 0.17$) (Supporting Information Table S3). A similar association was observed for equol when colon cancer cases which occurred within the first 4-years of follow-up were excluded (OR per \log_2 -value, 0.89, 95% CI = 0.81–0.97). Heterogeneity for the equol and colon cancer risk relationship was found by BMI ($p_{\text{heterogeneity}} = 0.003$), with an inverse association only apparent for the BMI < 25 kg/m²

group (OR per \log_2 -value, 0.81, 95% CI = 0.63–1.04), and not for the BMI ≥ 25 kg/m² group (OR per \log_2 -value, 0.97, 95% CI = 0.85–1.10) (data not shown). No heterogeneity was observed for the relationships between equol and colon cancer risk across strata of ever HT use ($p_{\text{heterogeneity}} = 0.28$), age at recruitment ($p_{\text{heterogeneity}} = 0.84$) and dairy consumption ($p_{\text{heterogeneity}} = 0.97$) (data not shown).

The magnitude of this positive homovanillic acid relationship was unchanged after additional statistical adjustment for concentrations of 3,4-dihydroxyphenylacetic acid, from which homovanillic acid is predominantly formed (*via* O-methylation) (Table 4). In the restricted cubic spline model, no evidence of non-linearity for the relationship between homovanillic acid and colon cancer was observed ($p_{\text{non-linear}} = 0.18$) (Fig. 2b). The positive relationship for homovanillic acid was similar for women and men ($p_{\text{heterogeneity}} = 0.87$) (Supporting Information Table S2), and for tumors located in the proximal and distal colon ($p_{\text{heterogeneity}} = 0.66$) (Supporting Information Table S3). A similar positive relationship was observed for homovanillic acid when colon cancer cases which occurred within the first 4-years of follow-up were excluded (OR per \log_2 -value, 1.39, 95% CI = 1.06–1.82). No heterogeneity was observed for the relationships between homovanillic acid and colon cancer risk strata of BMI ($p_{\text{heterogeneity}} = 0.34$) and age at recruitment ($p_{\text{heterogeneity}} = 0.25$) (data not shown).

Discussion

In this large-scale study that assessed polyphenols in pre-diagnostic plasma samples, circulating concentrations of equol were inversely, and homovanillic acid were positively, associated with colon cancer risk, even after control for established colon cancer risk factors and statistical correction for multiple comparisons. We did not observe any relationships between the other measured polyphenols and colon cancer risk.

To our knowledge, this study is the first to report an inverse relationship between equol concentrations and colon cancer risk. Equol is produced by intestinal bacteria through the metabolic conversion of daidzein, an isoflavone present within soya food products.²³ In Western populations, circulating equol levels are relatively low due to minimal consumption of soya food products, and only ~25%–30% of the adult population are classified as “equol producers,”²³ *i.e.*, harboring the colonic bacteria required for the conversion of daidzein into equol. Dairy products can also be a direct source of pre-formed equol as cows fed soybean products in their feed are capable of producing equol, which is then secreted into milk,²² in agreement with weak correlations ($r = 0.33$) observed between urinary equol concentrations and the consumption of dairy products reported in dietary 24-hr recalls in a subset of EPIC participants.²⁴ The lower risk of colon cancer we observed with high equol concentrations is consistent with an analysis of dietary equol intake and colorectal cancer in the EPIC-Norfolk cohort, but inconsistent with an analysis of circulating equol levels and colorectal

cancer (221 cases) in the same study (OR per \log_2 equal levels, 1.04, 95% CI = 0.99–1.09).^{16,25} This discrepancy may have been a consequence of the lower equal concentrations in the EPIC-Norfolk population (median = 0.04 nmol/L)¹⁶ compared to concentrations across all EPIC countries (median = 0.42 nmol/L). Nonetheless, compared to Asian populations, where soya consumption is much greater and ~50%–60% of the adult population are classified as “equal producers,”²³ equal levels in our study were low. In a Japanese study, which reported an inverse relationship between equal concentrations and prostate cancer risk, a median equal level of 19.4 nmol/L was recorded.²⁶ However, despite lower concentrations of equal in our European study, we nevertheless observed an inverse association with colon cancer risk.

Equol is a phytoestrogen, and in some studies higher concentrations in blood or urine have been associated with lower risks of certain reproductive system cancers such as prostate cancer²⁶ and breast cancer.²⁷ Equol shares structural similarity with mammalian estrogens and can similarly bind to estrogen receptors, in particular estrogen receptor- β (ER β).²⁸ Several lines of evidence support the role of estrogens potentially being beneficial against the development of colon cancer. First, experimental data have shown that expression of ER β results in the inhibition of proliferation and G1 phase cell-cycle arrest in colon cancer cells,²⁹ and in xenograft mouse studies ER β inhibits *cMyc* expression and tumor growth.²⁹ Second, expression of ER β is low in human colon cancer cells³⁰ and is inversely associated with stage of colon cancer,³¹ suggesting a possible role in disease progression. Third, lower incidence of colorectal cancer has been observed among postmenopausal HT users when compared with non-users.^{32–34} Finally, endogenous circulating estrogens have recently been associated with reduced risk of colon cancer in a case-control study nested within the Women’s Health Initiative study.³⁵

It is also possible that our observed inverse relationship between equal and colon cancer, rather than being a consequence of any phytoestrogenic effects of equal, is instead a marker of a particular community of intestinal microbiota which may be protective against the development of colon cancer.³⁶ Evidence suggests that a consortium of intestinal bacteria produce equal from the metabolism of daidzein. Strains of intestinal bacteria which have been shown to convert daidzein into equal in experimental models include, amongst others, *Enterococcus faecium* EPI1, *Finegoldia magna* EPI3, *Lactobacillus mucosae* EPI2 and an unknown strain of *Veillonella* sp.^{37–39} Evidence also suggests that being an “equal producer” may also be associated with a lowering of circulating insulin-like growth factor-I levels,⁴⁰ which have been positively associated with colorectal cancer risk.⁴¹ Overall, the inverse relationship between equal and colon cancer observed in the current study while supported by plausible biological mechanisms requires validation by additional prospective studies.

To our knowledge, this was the first study to observe that higher homovanillic acid levels were associated with greater colon cancer risk. This relationship was consistent by sex, follow-up time and across strata of other colon cancer risk factors. Homovanillic acid is formed from the O-methylation of 3,4-dihydroxyphenylacetic acid, which is sourced from colonic microbiota metabolism of other polyphenols, such as quercetin, or from dopamine metabolism.⁴² In our study, homovanillic acid and 3,4-dihydroxyphenylacetic acid were moderately correlated ($r = 0.59$), and adjustment of the multivariable model for 3,4-dihydroxyphenylacetic acid levels did not alter the homovanillic acid–colon cancer association. Experimental data on the effects of homovanillic acid on colon cancer development are lacking but it is possible that homovanillic acid is a marker of dopamine-related metabolism. Dopamine receptors have been identified throughout the gastrointestinal tract, and human colon cancer tissue has been shown to have 3- to 10-fold lower concentrations of dopamine than normal tissue.⁴³ In addition, experimental evidence suggests that dopamine regulates tumor angiogenesis and inhibits the growth of colon cancer cell lines in pre-clinical models.⁴⁴ Epidemiological investigations of pre-diagnostic dopamine levels and colon cancer risk have not been undertaken. Further, it is not known how plasma concentrations of homovanillic acid and dopamine are correlated in healthy individuals and colon cancer patients. Additional prospective studies are required to corroborate the positive relationship we observed between homovanillic acid and colon cancer. Such investigations should also measure circulating dopamine levels to better understand how the degradation of dopamine and its by-products relate to colon cancer development.

Strengths of this study include the application of a newly developed analytical method able to simultaneously quantify pre-diagnostic concentrations of 35 polyphenols spanning all major classes found in the diet. The direct measurement of plasma concentrations of polyphenols captures all their possible sources or precursors and circumvents potential biases inherent to questionnaire-based data acquisition. Additional strengths of our study include the large sample size, the diverse lifestyles of participants from nine European countries which have been well characterized and allowed us to control for other risk factors which may confound the polyphenols and colon cancer relationships.⁴⁵ Nevertheless, as is the case in all observational epidemiological studies, we cannot rule out the possibility that residual confounding influenced the relationships we observed. A limitation is that most polyphenols show a relatively short half-life in plasma, resulting in moderate-to-high intra-individual variability depending on the compounds, unless their sources are regularly consumed.^{1,46} Single plasma samples from baseline were used to assess the observed relationships, meaning that intra-individual variation in circulating polyphenol levels were unaccounted for, which could have caused attenuation of the observed risk estimates.⁴⁶

In conclusion, in this prospective analysis which measured circulating concentrations of 35 polyphenols, levels of equol were inversely, and those of homovanillic acid positively, associated with colon cancer development. Further studies of the relationships between equol and homovanillic acid and colon cancer are now warranted, but these novel findings suggest that circulating levels of these polyphenols may be indicative of direct effects of polyphenols on colon cancer development or could represent specific pathways or phenotypes that are relevant for colon tumorigenesis.

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