Dietary Flavonoid Intake and Colorectal Cancer Risk in the European Prospective Investigation into Cancer and Nutrition (EPIC) Cohort

Raul Zamora-Ros[1,2], Dinesh Barupal[1,3], Joseph A. Rothwell[1], Mazda Jenab[1], Veronika Fedirko[4], Isabelle Romieu[1], Krasimira Aleksandrova[5], Kim Overvad[6], Cecilie Kyrø[7], Anne Tjønneland[7], Aurélie Affret[8,9], Mathilde His[8,9], Marie-Christine Boutron-Ruault[8,9], Verena Katzke[10], Tilman Kühn[10], Heiner Boeing[5], Antonia Trichopoulou[11,12], Androniki Naska[11,12], Maria Kritikou[11], Calogero Saieva[13], Claudia Agnoli[14], Maria Santucci de Magistris[15], Rosario Tumino[16], Francesca Fasanelli[17,18], Elisabete Weiderpass[19,20,21,22], Guri Skeie[19], Susana Merino[23], Paula Jakszyn[2], Maria-José Sánchez[24,25], Miren Dorronsoro[25,26], Carmen Navarro[25,27,28], Eva Ardanaz[25,29,30], Emily Sonestedt[31], Ulrika Ericson[31], Lena Maria Nilsson[32], Stina Bodén[33], H Bas Bueno-de-Mesquita[34,35,36], Petra H. Peeters[35,37], Aurora Perez-Cornago[38], Nicholas J. Wareham[39], Kay-Thee Khaw[40], Heinz Freisling[1], Amanda J Cross[35], Elio Riboli[35], Augustin Scalbert[1,*]

Author affiliations

[1]Section of Nutrition and Metabolism, International Agency for Research on Cancer (IARC-WHO), Lyon, France.

[2]Unit of Nutrition and Cancer, Cancer Epidemiology Research Program, Catalan Institute of Oncology, Bellvitge Biomedical Research Institute (IDIBELL), Barcelona, Spain.

[3]Genome Center, University of California, Davis, US.

[4]Rollins School of Public Health, Winship Cancer Institute, Emory University, Atlanta, GA.

[5]Department of Epidemiology, German Institute of Human Nutrition Potsdam-Rehbruecke, Nuthetal, Germany.

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[6]Department of Public Health, Section for Epidemiology, Aarhus University, Aarhus, Denmark.

[7]Danish Cancer Society Research Center, Copenhagen, Denmark.

[8]Université Paris-Saclay, Université Paris-Sud, UVSQ, CESP, INSERM, Villejuif, France.

[9]Institut Gustave Roussy, F-94805, Villejuif, France.

[10]Division of Cancer Epidemiology, German Cancer Research Center, Heidelberg, Germany.

[11]Hellenic Health Foundation, Athens, Greece.

[12]WHO Collaborating Center for Nutrition and Health, Unit of Nutritional Epidemiology and Nutrition in Public Health, Dept. of Hygiene, Epidemiology and Medical Statistics, University of Athens Medical School, Greece.

[13]Cancer Risk Factors and Life-Style Epidemiology Unit, Cancer Research and Prevention Institute – ISPO, Florence, Italy.

[14]Nutritional Epidemiology Unit, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy.

[15]Department of Clinical and Experimental Medicine, Federico II University, Naples, Italy.

[16]Cancer Registry and Histopathology Unit, "Civic M.P. Arezzo" Hospital, ASP Ragusa, Italy

[17]Unit of Cancer Epidemiology, Department of Medical Sciences, University of Turin, Turin, Italy.

[18]Città della Salute e della Scienza Hospital, Turin, Italy.

[19]Department of Community Medicine, Faculty of Health Sciences, UiT The Artic University of Tromsø, Tromsø, Norway.

[20]Department of Research, Cancer Registry of Norway, Institute of Population-Based Cancer Research, Oslo, Norway.

[21]Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden.

[22]Genetic Epidemiology Group, Folkhälsan Research Center, Helsinki, Finland.

[23]Public Health Directorate, Asturias, Spain.

[24]Escuela Andaluza de Salud Pública. Instituto de Investigación Biosanitaria ibs.Granada. Hospitales Universitarios de Granada/Universidad de Granada, Granada, Spain.

[25]CIBER de Epidemiología y Salud Pública (CIBERESP), Spain.

[26]Public Health Division of Gipuzkoa, Regional Government of the Basque Country, Spain

[27]Department of Epidemiology, Murcia Regional Health Council, IMIB-Arrixaca, Murcia, Spain.

[28]Department of Health and Social Sciences, Universidad de Murcia, Murcia, Spain.

[29]Navarre Public Health Institute, Pamplona, Spain.

[30]IdiSNA, Navarra Institute for Health Research, Pamplona, Spain

[31]Department of Clinical Sciences Malmö, Lund University, Malmö, Sweden

[32]Nutritional Research and Arcum, Arctic Research Centre at Umeå University, Umeå, Sweden.

[33]Department of Radiation Sciences, Oncology, Umeå University, Umeå, Sweden.

[34]Department for Determinants of Chronic Diseases (DCD), National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands

[35]School of Public Health, Imperial College London, London, United Kingdom.

[36]Department of Social & Preventive Medicine, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia

[37]Department of Epidemiology, Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht, The Netherlands

[38]Cancer Epidemiology Unit, University of Oxford, UK

[39]MRC Epidemiology Unit, University of Cambridge School of Clinical Medicine, Cambridge, UK

[40]Department of Public Health and Primary Care, University of Cambridge, UK

[*]To whom correspondence should be addressed:

Augustin Scalbert, PhD

International Agency for Research on Cancer, Lyon, France, E-mail: ScalbertA@iarc.fr

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LIST OF ABBREVIATIONS: BMI, body mass index; CRC, colorectal cancer; CI, confidence interval; EPIC, European Prospective Investigation into Cancer and Nutrition; HR, hazard ratio; ICD, International Classification of Diseases; NOS, not otherwise specified; SD, standard deviation.

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NOVELTY AND IMPACT OF THE WORK:

The present study, conducted in Europe, is the largest prospective cohort to date evaluating the associations between the intake of total flavonoids and flavonoid subclasses, expressed as flavonoid glycosides and aglycones, and the risk of colorectal cancer (CRC). Our results do not support protection by flavonoid intake against CRC. Our findings were practically identical whether flavonoid intake was expressed as glycosides or aglycone equivalents.

ABSTRACT

Flavonoids have been shown to inhibit colon cancer cell proliferation *in vitro* and protect against colorectal carcinogenesis in animal models. However, epidemiological evidence on the potential role of flavonoid intake in colorectal cancer (CRC) development is still sparse and inconsistent. We evaluated the association between dietary intakes of total flavonoids and their subclasses and risk of development of CRC, within the European Prospective Investigation into Cancer and Nutrition (EPIC) study. A cohort of 477,312 adult men and women were recruited in 10 European countries. At baseline, dietary intakes of total flavonoids and individual subclasses were estimated using centre-specific validated dietary questionnaires and composition data from the Phenol-Explorer database. During an average of 11 years of follow-up, 4,517 new cases of primary CRC were identified, of which 2,869 were colon (proximal=1,298 and distal=1,266) and 1,648 rectal tumours. No association was found between total flavonoid intake and the risk of overall CRC (HR for comparison of extreme quintiles 1.05, 95% CI 0.93–1.18; p-trend=0.58) or any CRC subtype. No association was also observed with any intake of individual flavonoid subclasses. Similar results were observed for flavonoid intake expressed as glycosides or aglycone equivalents. Intake of total flavonoids and flavonoid subclasses, as estimated from dietary questionnaires, did not show any association with risk of CRC development.

INTRODUCTION

In the last two decades, much attention has been given to flavonoids and their proposed anti-carcinogenic properties, especially regarding gastrointestinal cancers.[1] Flavonoids have been shown to inhibit colon cell proliferation, minimize the effect of mutation, inhibit DNA oxidation, induce phase I and phase II enzymes, modulate cell growth signalling pathways and mediate inflammatory response *in vitro*.[2,3]

Flavonoids are a major class of polyphenols naturally occurring in plant-based foods and beverages, such as fruits, vegetables, tea, wine and juices.[4] In nature, flavonoids are generally bound to sugars as glycosides, except for flavan-3-ols. During digestion, flavonoid glycosides are usually hydrolysed and absorbed as aglycones (free forms). Bioavailability varies widely between flavonoids, with recovery in urine varying between 0.3% and 43% depending on the chemical structure of the aglycone and the sugar moiety.[5] Non-absorbed flavonoids reach the colon in relatively high concentrations, particularly for polymeric flavonoids, where they are extensively metabolized by the gut microbiota and absorbed as small phenolic acids. Thus, flavonoids may exert their protective effects in the intestinal lumen and at systemic level after absorption.[5]

All epidemiological data available are based on the calculation of flavonoids as aglycone equivalents and show inconsistent results. Several case-control studies have found an inverse association between flavonoid intake and colorectal cancer (CRC) risk.[4,6,7] In two cohort studies: the Iowa Women's Health Study and the Netherlands Cohort Study, an inverse association between flavanol intake and rectal cancer risk was also suggested.[8,9] However, no association has been observed in the vast majority of prospective studies, mostly US-based cohorts.[4,6,7,10] To date there are two prospective

studies measuring nutritional biomarkers of flavonoids and they only evaluate some flavonoid subclasses. The first one showed null associations with either urinary or plasma isoflavonoid concentrations[11] and the second one, an inverse association between two urinary flavanol concentrations and colon cancer risk.[12] Therefore, more longitudinal studies are required, especially in European countries.

We recently estimated the intake of 234 flavonoid glycosides and aglycones in the European Prospective Investigation into Cancer and Nutrition (EPIC) using the Phenol-Explorer database,[13] which contains food composition data for both flavonoid glycosides and aglycones.[14] It differs in this respect from the USDA database in which flavonoid food composition data are expressed as aglycone equivalents with 26 compounds documented.[15,16] In the present study, we used the new data on intake on flavonoid glycosides and aglycones in the EPIC cohort to prospectively investigate the associations between flavonoid intake and CRC risk, overall and by anatomical subtype, colon (proximal and distal) and rectum.

SUBJECTS AND METHODS

Subjects and study design

EPIC is an on-going multicentre study that has been described in detail elsewhere.[17] Briefly, a total of 521,448 participants (~70% women), mostly aged 35-70 years, were recruited between 1992 and 2000 from 23 centres in 10 European countries, primarily from the general population, with some exceptions, including the recruitment of volunteer blood donors (parts of the Spanish and Italian cohorts), a health conscious group (Oxford), women attending breast cancer screenings (Utrecht and Florence) and school employees (France). All participants gave written informed consent, and the

study was approved by the local ethics committees in the participating countries and the ethical review board of the International Agency for Research on Cancer (IARC).

Dietary data collection used validated country/centre-specific dietary assessment methods, mostly food frequency questionnaires for 150 to 250 food items depending on the country/centre, enquiring about usual intakes in the previous 12 months.[17] The nature of polyphenol-rich foods included in the food frequency questionnaires differed between countries depending on their significance in the diet. For example, consumption of black berries was reported in the Scandinavian countries but not in Spain and red and white wines were differentiated in countries where wine is widely consumed like France, Spain and Italy. Dietary flavonoid intakes were estimated using the Phenol-Explorer database[14] making appropriate adjustments for cooked and processed foods[18] as previously described.[13] Briefly, all animal foods that contain none or only traces of flavonoids were excluded. Generic or unspecified dietary questionnaire food items were matched to multiple more specific related foods from Phenol-Explorer using weighing factors based on centre-specific frequencies of consumption obtained from the 24-hour diet recall collected in the EPIC calibration subsample[19] and recipes provided by local dietitians. Total flavonoids, expressed as glycosides, were calculated as the sum of the following subclasses: anthocyanins, chalcones, dihydrochalcones, dihydroflavonols, flavanols (including flavan-3-ol monomers, proanthocyanidins, and theaflavins), flavanones, flavones, flavonols, and isoflavones. Data on flavonoids was also expressed as aglycone equivalents, after converting flavonoid glycoside contents into aglycone contents using their respective molecular weights. Data on anthropometric data, and questionnaires on sociodemographic factors, lifestyles, physical activity and health history were also collected.[17]

Cancer cases were identified through population cancer registries in Denmark, Italy, the Netherlands, Norway, Spain, Sweden and the United Kingdom. In France, Germany and Greece, a combination of methods was used including health insurance records, cancer and pathology registries, and by active follow-up of study participants and their next of kin. CRC included tumours coded as C18-C20 in the 10th revision of the international classification of diseases (ICD-10), and the second revision of the International Classification of Disease for Oncology (ICDO-2). CRC cases were classified by anatomic location: proximal colon (C18.0–18.5), distal colon (C18.6–18.7), not otherwise specified (NOS) colon (C18.8–18.9), and finally rectum (C19–C20). Vital status was collected from regional or national mortality registries. Complete follow-up censoring dates varied among centres, ranging between 2005 and 2010.[20]

The analytic cohort excluded: prevalent cancer cases other than non-melanoma skin cancer at baseline or subjects with missing information on date of diagnosis or incomplete follow-up data (n=28,283), missing data on dietary or lifestyle factors (n=6,253), extreme energy intake and/or expenditure (participant in the top or the bottom 1% of the distribution of the ratio of total energy intake to energy requirement) (n=9,600). The final dataset included 477,312 subjects.

Statistical analysis

Multivariable Cox proportional hazard models were used to estimate hazard ratios (HR) and 95% confidence intervals (CIs) for the associations between intake of total flavonoids and flavonoid subclasses and risk of CRC and CRC subtype (colon [proximal and distal] and rectum). Tests and graphs based on Schoenfeld residuals were used to assess the proportional hazards assumption, which was satisfied. Age was the primary time variable in all models. Model 1 was stratified by sex, age at recruitment

(1-y interval) and centre. Model 2 was model 1 additionally adjusted for non-dietary variables: smoking status and intensity (never, former quit <11 years, former quit 11–20 years, former quit >20 years, current <15 cigarettes/d, current 15–25 cigarettes/d, current >25 cigarettes/d, current occasional, and not specified), Cambridge physical activity index (inactive, moderately inactive, moderately active, active, and not specified), education level (none, primary school, technical/professional school, secondary school, university or higher, and not specified), body mass index (BMI, kg/m^2), and in women for menopausal status (pre-menopausal, post-menopausal, peri-menopausal/unknown menopausal status, or surgical postmenopausal), hormone replacement therapy use (yes, no, and unknown), and oral contraceptive use (yes, no, and unknown). Model 3 was model 1 additionally adjusted for total energy intake (kcal/d), because flavonoid intake increases with total energy consumption (Supplementary table 1). Model 4 was model 3 additionally adjusted for non-dietary variables as in the model 2. Finally, model 5 was model 4 additionally adjusted for dietary variables: alcohol (g/d), red and processed meat (g/d), fibre (g/d) and calcium (mg/d) intakes. Fibre intake was included in the model as potential mediator, since many of the main flavonoid food sources are important food sources of fibre as well, except tea, wine and juices.

Flavonoid intakes were analysed as categorical variables based on quintiles of the distribution among the entire EPIC cohort. Quintile 1 and 2 of theaflavin intakes were grouped together because 38.8% of subjects were non-consumers. Chalcones were not included in the analysis due to the low daily intake (median <0.01mg/d; percentile 5^{th} = 0 and percentile 95^{th} = 0.02mg/d). Tests for linear trend were performed by assigning the medians of each quintile as scores. Flavonoid intakes were additionally analysed as continuous variables following a $\log_2$ transformation. To account for multiple

comparisons Bonferroni correction was applied and the significance level was set at $P<0.004$, since 13 individual flavonoid subclasses were evaluated (0.05/13).

Interactions between flavonoid intakes and sex, age (<55 years, 55 to 65 years, or >65 years), BMI (BMI<25, 25 to <30, ≥30 kg/m^2), tobacco smoking (never, former, current smokers), fibre and alcohol (quartiles) consumption were evaluated in separate analyses. Additional analyses by length of follow-up (censoring data at 3 years, 6 years, and 9 years) were performed. The statistical significance of effect modification (on the multiplicative scale) was assessed using likelihood ratio tests based on the models with and without the interaction terms. Sensitivity analyses were performed by repeating the models after the exclusion of 502 CRC cases diagnosed during the first 2 years of follow-up (306 colon and 196 rectum cancer cases).

R 3.1.2 software (R Foundation for Statistical Computing, Vienna, Austria) was used for all analyses.

RESULTS

After a mean (standard deviation, SD) follow-up of 11.0 (2.8) years, 4,517 (70.2% women) incident primary CRC cases were diagnosed, of which 2,869 were identified as colon tumours (including 1,298 proximal, 1,266 distal and 305 NOS) and 1,648 as rectum tumours. **Table 1** shows the mean (SD), median, percentiles (5th and 95th) of both total and individual subclasses of flavonoids. Subjects in the highest quintile of total flavonoid intake had the lowest BMI, tobacco consumption and processed meat intake (**Supplementary Table 1**). Moreover, these subjects had the highest educational level and intake of alcohol, energy, fibre and calcium, and tended to be more physically active than subjects in the lowest quintile of total flavonoid intake.

Similar results were obtained for the five Cox models (**Supplementary Table 2**), and therefore in the main results only model 1 and 5 are presented (**Table 2**). In Model 5, neither total flavonoid intake (HR for highest vs. lowest quintile 1.05; 95 % CI: 0.93–1.18; *P*-trend = 0.58) nor intake of any individual flavonoid subclass was associated with CRC risk (Table 2). By anatomical sub-site, only borderline significant inverse relationships were found between dihydroflavonol intake and proximal colon cancer risk (HR_{log2} 0.99; 95% CI: 0.98-0.99; *P*-value = 0.026) (**Supplementary Table 3**); although it disappeared after applying Bonferroni correction.

No statistically significant interactions between total flavonoid intake and CRC risk in the multivariable models (Model 5) stratified by sex ($P_{interaction}$ = 0.35), age ($P_{interaction}$ = 0.83), BMI ($P_{interaction}$ = 0.26), fibre ($P_{interaction}$ = 0.67) and baseline alcohol intake ($P_{interaction}$ = 0.73) were observed. Since a statistically significant interaction between smoking status (never, former, and current smoker) and total flavonoid intake in relation to CRC risk was observed ($P_{interaction}$ = 0.001), we stratified the statistical models by smoking status. Some of the flavonoid subclasses (anthocyanins, flavan-3-ol monomers, theaflavins and flavonols) were inversely associated with CRC risk in former smokers; but no association was found between CRC cancer risk and any specific flavonoid subclass in either never or current smokers (**Table 3**). Similar results were observed after stratifying by smoking status in men ($P_{interaction}$ = 0.072) and women ($P_{interaction}$ = 0.037) (data not shown). The magnitude of the risk was influenced by the length of follow-up and similar results were observed at 3 years, 6 years, 9 years (data not shown), and with complete follow-up.

In sensitivity analyses after excluding cases diagnosed during the first 2 years of follow-up, the multivariable hazard ratios were nearly identical to the results based on the whole cohort (data not shown). The results obtained for total flavonoid or flavonoid

subclasses, expressed as aglycone equivalents, did not show any CRC risk associations either (**Supplementary Table 4**).

DISCUSSION

In the present large multi-country European cohort, we observed no association between habitual intake of either total flavonoids or any flavonoid subclass and CRC risk, and tumour subsites, after multivariable adjustment. Similar null results were obtained whether flavonoid intake was expressed as glycosides or aglycone equivalents.

Our results are in agreement with a meta-analysis on previous prospective studies,[6] and especially with a recent study based on two large US cohorts, the Health Professionals Follow-Up Study and the Nurses' Health Study,[10] showing no association between the intake of total flavonoids and flavonoid subclasses and risk of CRC or its sub-sites. The only two studies showing significant inverse associations were the Iowa Women's Health and the Netherlands Cohort Study both suggesting an inverse association between intake of flavan-3-ol monomers and rectal cancer risk in postmenopausal women and in overweight men.[8,9] Moreover, in the Netherlands Cohort Study, the intake of flavonols and flavan-3-ol monomers may be associated with a decreased risk in colorectal cancer.[9] In the EPIC-Norfolk study, isoflavonoid and lignan biomarkers were also found to show no association with CRC risk,[11] but some urinary flavanols were inversely related to colon cancer risk in the Shanghai cohort study.[12] To our knowledge, no prospective studies evaluating the relationship between biomarkers of other flavonoid subclasses and CRC risk has been conducted.

In contrast, most of the retrospective studies (i.e., case–control studies), which are susceptible to recall bias, showed inverse associations with total and some flavonoid subclasses (flavan-3-ol monomers, proanthocyanidins, anthocyanins, flavonols,

flavones and isoflavones).[4,6,21,22] The flavonoid intake in these studies was comparable to those in EPIC countries with a low flavonoid intake. Furthermore, in the Polyp Prevention Trial, flavonol intake was inversely associated with advanced recurrent adenoma risk, a precursor for CRC.[23] Similarly, in a small clinical trial, long-term daily intervention with a flavonoid mixture (20 mg apigenin + 20 mg epigallocatechin gallate) was shown to reduce colon cancer recurrence risk in patients with resected colon cancer.[24] However, no significant association was observed between flavonoid intake and both the overall survival and CRC recurrence in CRC patients in an observational study.[25]

We also need to be very cautious interpreting the protective results of some flavonoid classes against CRC risk observed in former smokers only. Tobacco smoking may lead to higher oxidative stress, and both oxidative stress[26] and smoking tobacco[27] are related to increased CRC risk. In previous EPIC studies, a stronger inverse association with dietary flavonoids in current smokers than in non-smokers was found for other cancer sites, such as gastric[28] and oesophageal cancer,[29] but the opposite was observed in CRC risk in Scotland.[30] An interaction of tobacco consumption in the association between fruit and vegetable consumption and CRC risk was observed in a previous EPIC study, also showing a significant protective association in former smokers.[31] More studies are warranted to clarify whether tobacco consumption interacts with flavonoid intake and influences CRC risk.

The strengths of the our study are its prospective and population-based design, detailed information on diet and lifestyle factors, and a large sample of CRC cases, which allows sufficient statistical power for subgroup analyses. Our study also has several limitations. Firstly, diet and other lifestyle variables were only assessed at baseline, and any potential changes during follow-up are unaccounted for. In addition, underestimation of

flavonoid intake is possible because i) dietary questionnaires were not specifically designed to estimate flavonoids,[17] although questionnaires were country specific and previously validated and reproducible for some flavonoid-rich foods using 24-h dietary recalls.[32] Moreover, these foods in the 24-h dietary recall were significantly correlated with urinary flavonoid biomarkers;[33] and ii) they did not include herb/plant supplement intakes, which are consumed by up to 5% of the population in Denmark.[34] Furthermore, Phenol-Explorer database has, in some cases, missing or insufficient food composition data.[13] For example, polymeric flavonoids such as thearubigins can be particularly abundant in foods such as fruits and tea and accurate methods for their estimation in foods are still missing.[35] These polymeric flavonoids are also not easily absorbed in the small intestine because of their high molecular weight and transit down to the colon where they reach high local concentrations.[36,37] This misclassification is likely to be random and therefore any association between intake of such polymeric flavonoids and disease risk is likely underestimated. Another limitation is linked to inter-individual variations in flavonoid absorption and metabolism.[38] Studies using flavonoid biomarkers, which may better reflect individual exposures to flavonoids and improve subject classification, should be carried out.[4,33] Finally, another limitation is the potential modification of diet during the early pre-diagnostic period of the disease; however, sensitivity analyses excluding incident cases diagnosed in the first 2 years of follow-up did not alter the associations. Overall, our findings and recent results from the literature do not support a protection by flavonoid intake against CRC.

CONFLICT OF INTEREST

The authors are not aware of any conflicts of interest.

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Table 1. Dietary intake of flavonoids (mg/d)* in the EPIC study.

	Mean	SD	Median	Percentile 5	Percentile 95
Total flavonoids	516	358	418	116	1,240
Anthocyanins	40	54	25	3.4	117
Dihydrochalcones	2.4	2.6	1.7	0.11	6.4
Dihydroflavonols	2.6	5.1	0.54	<0.01	12.8
Flavanols	379	305	284	63	1,019
Flavan-3-ol monomers	118	159	41	6.6	462
Proanthocyanidins	238	165	203	52	539
Theaflavins	23	39	1.5	<0.01	106
Flavanones	40	45	25	1.9	119
Flavones	11.5	8.6	9.3	2.6	28
Flavonols	40	34	28	7.2	112
Isoflavones	1.7	9.1	0.03	<0.01	7.3

*Intake values are expressed as either flavonoid glycosides or aglycones as found in foods.

Table 2. Hazard ratios and 95% confidence intervals for the associations between intake of total flavonoids and flavonoid subclasses (mg/d) and risk of colorectal cancer in the EPIC study.

	Intake	No of cases	Person-years	Model 1[1] HR (95% CI)	Model 5[2] HR (95% CI)
Total flavonoids					
Quintile 1	<223	839	1,076,813	1.00 (ref)	1.00 (ref)
Quintile 2	223-346	888	1,082,475	1.05 (0.96-1.16)	1.09 (0.99-1.20)
Quintile 3	347-507	904	1,084,052	1.06 (0.96-1.17)	1.10 (0.99-1.22)
Quintile 4	508-771	932	1,079,804	1.01 (0.91-1.12)	1.07 (0.96-1.20)
Quintile 5	>771	954	1,077,491	0.98 (0.87-1.09)	1.05 (0.93-1.18)
P trend				0.30	0.58
Continuous (log_2)				0.98 (0.95-1.02)	1.01 (0.97-1.05)
Anthocyanins					
Quintile 1	<10.3	982	1,115,820	1.00 (ref)	1.00 (ref)
Quintile 2	10.3-19.1	886	1,097,974	0.94 (0.85-1.03)	0.95 (0.87-1.04)
Quintile 3	19.2-32.5	855	1,079,949	0.94 (0.86-1.04)	0.96 (0.87-1.06)
Quintile 4	32.6-58.9	878	1,068,432	1.00 (0.91-1.10)	1.00 (0.91-1.10)
Quintile 5	>58.9	916	1,038,460	1.04 (0.94-1.15)	1.01 (0.91-1.13)
P trend				0.14	0.43
Continuous (log_2)				1.00 (0.98-1.01)	1.00 (0.99-1.00)
Dihydrochalcones					
Quintile 1	>0.58	894	1,084,773	1.00 (ref)	1.00 (ref)
Quintile 2	0.58-1.26	818	1,069,797	0.98 (0.89-1.08)	1.02 (0.93-1.13)
Quintile 3	1.27-2.07	801	1,058,361	0.99 (0.89-1.09)	1.05 (0.95-1.16)
Quintile 4	2.08-3.52	979	1,097,278	0.96 (0.88-1.06)	1.05 (0.95-1.16)
Quintile 5	>3.52	1,025	1,090,426	0.92 (0.84-1.01)	1.05 (0.94-1.16)
P trend				0.07	0.43
Continuous (log_2)				0.99 (0.98-1.00)	1.01 (0.99-1.03)
Dihydroflavonols					
Quintile 1	<0.01	988	1,110,156	1.00 (ref)	1.00 (ref)
Quintile 2	0.01-0.26	801	1,042,150	1.03 (0.93-1.14)	1.02 (0.92-1.12)
Quintile 3	0.27-1.24	860	1,113,183	0.99 (0.90-1.09)	0.97 (0.88-1.07)
Quintile 4	1.25-3.76	826	1,054,337	0.94 (0.85-1.03)	0.90 (0.81-0.99)
Quintile 5	>3.76	1,042	1,080,809	1.07 (0.97-1.18)	0.94 (0.84-1.05)
P trend				0.11	0.29
Continuous (log_2)				1.00 (0.99-1.00)	0.99 (0.98-1.01)
Flavanols					
Quintile 1	<135	806	1,070,803	1.00 (ref)	1.00 (ref)
Quintile 2	135-228	873	1,078,479	1.05 (0.95-1.15)	1.08 (0.98-1.19)
Quintile 3	229-356	935	1,085,840	1.10 (1.00-1.22)	1.15 (1.04-1.28)
Quintile 4	357-584	949	1,082,593	1.04 (0.94-1.16)	1.10 (0.99-1.23)

Quintile 5	>584	954	1,082,921	0.97 (0.86-1.09)	1.05 (0.93-1.19)
P trend				0.19	0.85
Continuous (log_2)				0.98 (0.95-1.01)	1.00 (0.99-1.00)
Flavan-3-ol monomers					
Quintile 1	<14.9	795	1,060,569	1.00 (ref)	1.00 (ref)
Quintile 2	14.9-28.2	869	1,085,585	0.97 (0.88-1.08)	1.00 (0.91-1.11)
Quintile 3	28.3-63.0	891	1,081,610	0.98 (0.88-1.09)	1.00 (0.90-1.12)
Quintile 4	63.1-243.0	945	1,084,007	1.03 (0.93-1.15)	1.06 (0.95-1.19)
Quintile 5	>243.0	1,017	1,088,863	0.93 (0.83-1.05)	1.00 (0.88-1.13)
P trend				0.27	0.90
Continuous (log_2)				0.99 (0.97-1.00)	1.00 (0.99-1.00)
Proanthocyanidins					
Quintile 1	<108	834	1,081,370	1.00 (ref)	1.00 (ref)
Quintile 2	108-170	917	1,078,924	1.07 (0.97-1.17)	1.10 (1.00-1.21)
Quintile 3	171-239	936	1,077,493	1.03 (0.93-1.13)	1.07 (0.97-1.19)
Quintile 4	240-344	929	1,080,981	0.98 (0.89-1.09)	1.04 (0.93-1.16)
Quintile 5	>344	901	1,081,867	0.98 (0.88-1.09)	1.04 (0.92-1.18)
P trend				0.28	0.96
Continuous (log_2)				0.98 (0.95-1.01)	1.00 (0.99-1.00)
Theaflavins					
Quintile 1&2	<0.1	1,753	2,092,861	1.00 (ref)	1.00 (ref)
Quintile 3	0.1-5.1	854	1,128,732	0.96 (0.87-1.07)	0.99 (0.89-1.09)
Quintile 4	5.2-42.9	894	1,088,472	0.98 (0.89-1.09)	1.02 (0.92-1.13)
Quintile 5	>42.9	1016	1,090,570	0.90 (0.80-1.01)	0.97 (0.86-1.09)
P trend				0.10	0.61
Continuous (log_2)				1.00 (0.99-1.00)	1.00 (0.99-1.00)
Flavanones					
Quintile 1	<8.2	1,051	1,074,793	1.00 (ref)	1.00 (ref)
Quintile 2	8.2-18.1	944	1,091,388	0.95 (0.87-1.04)	0.96 (0.88-1.05)
Quintile 3	18.2-33.3	886	1,081,232	0.97 (0.89-1.06)	0.99 (0.91-1.09)
Quintile 4	33.4-65.9	791	1,077,364	0.92 (0.84-1.02)	0.95 (0.86-1.05)
Quintile 5	>65.9	845	1,075,858	0.96 (0.88-1.06)	1.00 (0.91-1.10)
P trend				0.53	0.91
Continuous (log_2)				0.99 (0.98-1.01)	1.00 (0.99-1.00)
Flavones					
Quintile 1	<5.1	1,079	1,074,611	1.00 (ref)	1.00 (ref)
Quintile 2	5.1-7.8	964	1,078,369	0.98 (0.90-1.08)	1.01 (0.93-1.11)
Quintile 3	7.9-11.0	823	1,080,829	0.90 (0.82-0.98)	0.94 (0.85-1.03)
Quintile 4	11.1-16.5	861	1,082,844	0.98 (0.89-1.08)	1.04 (0.94-1.15)
Quintile 5	>16.5	790	1,083,982	0.96 (0.86-1.07)	1.04 (0.92-1.17)
P trend				0.53	0.43
Continuous (log_2)				0.97 (0.94-1.00)	1.00 (0.99-1.01)
Flavonols					
Quintile 1	<13.9	870	1,071,927	1.00 (ref)	1.00 (ref)
Quintile 2	13.9-23.0	897	1,083,395	1.00 (0.90-1.10)	1.02 (0.92-1.13)

Quintile 3	23.1-34.8	862	1,082,236	1.01 (0.91-1.12)	1.03 (0.93-1.15)
Quintile 4	34.9-61.7	893	1,077,446	0.97 (0.87-1.08)	0.99 (0.88-1.11)
Quintile 5	>61.7	995	1,085,632	0.95 (0.85-1.07)	1.00 (0.89-1.14)
P trend				0.31	0.82
Continuous (log_2)				0.98 (0.95-1.01)	1.00 (0.99-1.00)
Isoflavones					
Quintile 1	<0.009	936	1,107,247	1.00 (ref)	1.00 (ref)
Quintile 2	0.009-0.021	1,062	1,090,665	1.02 (0.93-1.11)	1.01 (0.93-1.11)
Quintile 3	0.022-0.047	974	1,061,796	1.05 (0.95-1.15)	1.02 (0.93-1.13)
Quintile 4	0.048-0.171	875	1,059,762	1.08 (0.97-1.20)	1.05 (0.95-1.17)
Quintile 5	>0.171	670	1,081,165	0.97 (0.85-1.11)	0.99 (0.86-1.14)
P trend				0.27	0.60
Continuous (log_2)				1.00 (0.99-1.01)	0.97 (0.86-1.09)

1Cox model was stratified by sex, age and centre.

[2]Model 1 was additionally adjusted for smoking status and intensity, physical activity, education level, body mass index, total energy, alcohol, red and processed meat, fibre and calcium intakes. The model was additionally adjusted for menopausal status, hormone replacement therapy use, and oral contraceptive use in women.

Table 3. Hazard ratios and 95% confidence intervals for the associations between total and subclasses of flavonoid intakes (log_2) and risk of total colorectal cancer, stratified by smoking status, in the EPIC study.

	Never smokers (1,223 cases)	Former smokers (1,514 cases)	Current smokers (1,060 cases)	P for interaction
	HR (95% CI)	HR (95% CI)	HR (95% CI)	
Total flavonoids	1.03 (0.97-1.10)	0.94 (0.88-1.01)	1.04 (0.97-1.11)	0.001
Anthocyanins	0.99 (0.96-1.02)	0.96 (0.93-0.99)	1.01 (0.98-1.05)	0.28
Dihydrochalcones	1.00 (0.98-1.02)	0.99 (0.98-1.01)	1.00 (0.98-1.02)	0.62
Dihydroflavonols	0.99 (0.99-1.00)	0.99 (0.99-1.00)	1.00 (0.99-1.01)	0.45
Flavanols	1.01 (0.96-1.06)	0.95 (0.90-1.01)	1.03 (0.98-1.10)	<0.001
Flavan-3-ol monomers	1.01 (0.98-1.04)	0.96 (0.92-0.99)	1.01 (0.97-1.05)	<0.001
Proanthocyanidins	1.00 (0.94-1.06)	0.96 (0.91-1.02)	1.05 (0.98-1.12)	0.005
Theaflavins	1.01 (1.00-1.01)	0.99 (0.98-0.99)	1.00 (0.99-1.00)	0.001
Flavanones	1.00 (0.97-1.02)	0.99 (0.97-1.02)	0.98 (0.95-1.01)	0.68
Flavones	1.02 (0.96-1.08)	0.96 (0.90-1.01)	0.99 (0.93-1.06)	0.28
Flavonols	1.02 (0.97-1.08)	0.92 (0.87-0.98)	1.02 (0.95-1.09)	<0.001
Isoflavones	1.01 (0.99-1.02)	1.00 (0.99-1.02)	1.00 (0.98-1.01)	0.50

Model 5: Cox model was stratified by sex, age and centre and adjusted for physical activity, education level, body mass index, total energy, alcohol, red and processed meat, fibre and calcium intakes. The model was additionally adjusted for menopausal status, hormone replacement therapy use, and oral contraceptive use in women.