

# **Dietary intake of total polyphenol and polyphenol classes and the risk of colorectal cancer in the European Prospective Investigation into Cancer and Nutrition (EPIC) Cohort**

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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.

## 1 **ABSTRACT**

2 Polyphenols may play a chemopreventive role in colorectal cancer (CRC);  
3 however, epidemiological evidence supporting a role for intake of individual  
4 polyphenol classes, other than flavonoids is insufficient. We evaluated the  
5 association between dietary intakes of total and individual classes and  
6 subclasses of polyphenols and CRC risk and its main subsites, colon and  
7 rectum, within the European Prospective Investigation into Cancer and Nutrition  
8 (EPIC) study. The cohort included 476,160 men and women from 10 European  
9 countries. During a mean follow-up of 14 years, there were 5,991 incident CRC  
10 cases, of which 3,897 were in the colon and 2,094 were in the rectum.  
11 Polyphenol intake was estimated using validated centre/country specific dietary  
12 questionnaires and the Phenol-Explorer database. In multivariable-adjusted Cox  
13 regression models, a doubling in total dietary polyphenol intake was not  
14 associated with CRC risk in women ( $HR_{\log 2} = 1.06$ , 95 % CI 0.99-1.14) or in  
15 men ( $HR_{\log 2} = 0.97$ , 95 % CI 0.90-1.05), respectively. Phenolic acid intake,  
16 highly correlated with coffee consumption, was inversely associated with colon  
17 cancer in men ( $HR_{\log 2} = 0.91$ , 95 % CI 0.85-0.97) and positively associated with  
18 rectal cancer in women ( $HR_{\log 2} = 1.10$ , 95 % CI 1.02-1.19); although  
19 associations did not exceed the Bonferroni threshold for significance. Intake of  
20 other polyphenol classes was not related to colorectal, colon or rectal cancer  
21 risks. Our study suggests a possible inverse association between phenolic acid  
22 intake and colon cancer risk in men and positive with rectal cancer risk in  
23 women.

24

## 25 INTRODUCTION

26 Colorectal cancer (CRC) is the third most common cancer and the fourth most  
27 common cause of death from cancer worldwide, with 1.4 million new cases and  
28 694,000 deaths in 2012 (1). Lifestyle (physical inactivity, body fatness, tobacco  
29 smoking and alcohol consumption) and dietary factors, such as a high intake of  
30 red and processed meat and low intake of fruit and vegetables, are known to  
31 increase CRC risk (2).

32 Polyphenols are bioactive compounds naturally contained in plant-based foods,  
33 such as tea, coffee, wine, fruit, vegetables, whole-grain cereals, and cocoa (3).  
34 Experimental studies have shown anti-carcinogenic properties of polyphenols  
35 against CRC through several plausible biological mechanisms including  
36 modulation of nuclear factor (NF)- $\kappa$ B genes involved in inflammation and  
37 carcinogenesis, reduction of oxidative damage to lipids and DNA, induction of  
38 phase I and II enzymes, inhibition of angiogenesis, stimulation of DNA repair  
39 and apoptosis (4-7). Based on their chemical backbone, polyphenols are  
40 divided into 4 main classes: flavonoids, phenolic acids, lignans, and stilbenes  
41 (3). Polyphenols can be absorbed in the small intestine, although the vast  
42 majority, from 50 to 99% depending on the polyphenol, transit down to the colon  
43 where they can be metabolized by the gut microbiota and partially absorbed in  
44 the con as small phenolic acids (8). Furthermore, polyphenols can modulate gut  
45 microbiota, both in quantity and type of species (9). Imbalanced gut microbiota,  
46 called dysbiosis, can alter both metabolism and absorption of polyphenols, and  
47 may also induce aberrant molecular signalling, triggering the CRC pathogenesis  
48 (10).

49 To date, several case-control studies suggest an inverse association between  
50 flavonoid and lignan intake and CRC risk (3). However, no association in cohort  
51 studies has been observed so far (3;11;12) including our previous results in the  
52 European Prospective Investigation into Cancer and Nutrition (EPIC) study with  
53 a shorter follow-up (13); except for the Iowa Women's Health study, in which an  
54 inverse association between flavanol intake and rectal cancer risk was shown  
55 (14). To our knowledge, there is only one case-control study investigating the  
56 relationships with other polyphenol classes, such as phenolic acids, stilbenes  
57 and other minor subclasses in Japan (15). In this previous study, intakes of  
58 coffee polyphenols and consequently coffee consumption were inversely  
59 associated with CRC risk in men and women, especially with colon cancer (15).

60 The Phenol-Explorer ([www.phenol-explorer.eu](http://www.phenol-explorer.eu)) (16), a food composition  
61 database on all known dietary polyphenols, greatly facilitates the assessment of  
62 relationships between polyphenol intake and chronic disease risk. The aim of  
63 the present study was to investigate the associations between the intake of total  
64 polyphenols and individual polyphenol subclasses and CRC risk and by subsite  
65 (colon and rectum) in the EPIC study, a large cohort with a high variability in  
66 polyphenol intake and a long follow-up (17).

## 67 **MATERIALS AND METHODS**

### 68 **Subjects and study design**

69 EPIC is an on-going cohort consisting of 521,324 adult participants, mostly  
70 recruited from the general population, enrolled between 1992 and 2000 from 23  
71 centres in 10 European countries: Denmark, France, Germany, Greece, Italy,

72 the Netherlands, Norway, Spain, Sweden and the United Kingdom (18). All  
73 participants gave written informed consent, and the study was approved by the  
74 local ethics committees in the participating countries and the ethical review  
75 board of the International Agency for Research on Cancer (IARC). We excluded  
76 participants with prevalent cancer other than non-melanoma skin cancer at  
77 baseline or with missing information on date of diagnosis or incomplete follow-  
78 up data (n=29,332), missing data on dietary or lifestyle factors (n=6,259),  
79 extreme energy intake and/or expenditure (participant in the top or the bottom  
80 1% of the distribution of the ratio of total energy intake to energy requirement;  
81 n=9,573). In the current analysis, 476,160 men and women were included.

## 82 **Identification and follow-up of colorectal cancer cases**

83 Cancer cases were identified through population cancer registries in Denmark,  
84 Italy, the Netherlands, Norway, Spain, Sweden and the United Kingdom. In  
85 France, Germany, Greece and Naples-Italy, a combination of methods was  
86 used including health insurance records, cancer and pathology registries, and  
87 by active follow-up of study participants and their next of kin. Vital status was  
88 collected from regional or national mortality registries.

89 Cancer incidence data were coded according to the 10<sup>th</sup> revision of the  
90 International Statistical Classification of Diseases, Injuries and Causes of Death  
91 (ICD-10) and the second revision of the International Classification of Diseases  
92 for Oncology (ICDO-2). Proximal colon cancers included those within the  
93 cecum, appendix, ascending colon, hepatic flexure, transverse colon, and  
94 splenic flexure (C18.0–18.5). Distal colon cancers included those within the  
95 descending (C18.6) and sigmoid (C18.7) colon. Overlapping (C18.8) and

96 unspecified (C18.9) lesions of the colon were grouped among all colon cancers  
97 only (C18.0-C18.9). Cancer of the rectum included tumours occurring at the  
98 recto sigmoid junction (C19) and rectum (C20). Five hundred and fourteen  
99 cases were censored because they were carcinoma in situ (n=193), non-  
100 adenocarcinoma, mixed types or not well defined (n=312), unknown histology of  
101 the cancer (n=5), or a CRC originating from other organs (n=4).

## 102 **Dietary assessment and data collection**

103 At recruitment, validated country/centre-specific dietary questionnaires were  
104 used for recording habitual diet over the previous 12 months (18;19). Most  
105 centres utilized a self-administered food frequency questionnaire. In the  
106 remaining centres (Greece, Spain, and Ragusa and Naples-Italy), a face-to-face  
107 diet history questionnaire was employed to collect dietary information. In  
108 Malmö-Sweden, a method combining a food frequency questionnaire with a 7-  
109 day dietary diary and 1h interview was used. Total energy, alcohol, and nutrient  
110 intakes were estimated by using the standardized EPIC Nutrient Database (20).

111 Lifestyle questionnaires were collected to obtain information on lifetime and  
112 smoking status, physical activity classified according to the Cambridge Physical  
113 Activity Index (21), education, menstrual and reproductive history. Height and  
114 weight were measured at baseline in all centres except for Norway, France, and  
115 the majority of participants in EPIC-Oxford where anthropometric measures  
116 were self-reported (18).

## 117 **Polyphenol intake**

118 Dietary polyphenol intake was estimated using the Phenol-Explorer database  
119 (16) accounting for cooking and processing of foods via retention factors (22),  
120 as previously described (17;23). Total polyphenols was calculated as the sum of  
121 all classes of polyphenols: flavonoids [anthocyanidins, chalcones,  
122 dihydrochalcones, dihydroflavonols, flavanols (including flavan-3-ol monomers,  
123 proanthocyanidins, theaflavins), flavanones, flavones, flavonols, and  
124 isoflavones], phenolic acids (hydroxybenzoic acids, hydroxycinnamic acids, and  
125 hydroxyphenylacetic acids), lignans, stilbenes, and other minor polyphenols  
126 (alkylphenols, tyrosols, alkylmethoxyphenols, furanocoumarins,  
127 hydroxybenzaldehydes, and hydroxycoumarins). The content of polyphenols  
128 was expressed in mg/100 g of food fresh weight.

### 129 **Statistical analysis**

130 Polyphenol intakes were analysed as categorical variables based on quintiles of  
131 the distribution among the entire EPIC cohort and by sex. Tests for linear trend  
132 were performed by assigning the medians of each quintile as scores.  
133 Polyphenol intakes were also analysed as continuous variables, after log<sub>2</sub>  
134 transformation to improve normality of intake distributions. Each increase of one  
135 unit corresponded to a doubling in intake.

136 Multivariable Cox proportional hazard models were used to calculate hazard  
137 ratios (HR) and 95% confidence intervals (CIs) of the associations between  
138 total, classes and subclasses of polyphenol intakes and CRC risk. A chi-  
139 squared test based upon the scaled Schoenfeld residuals was used to ensure  
140 that the assumptions of proportional hazards were met. Age was the primary  
141 time variable in all models. Entry time was age at recruitment and exit time was

142 age at diagnosis, death or censoring date (lost or end of follow-up), whichever  
143 came first. Model 1 was stratified by centre (to control for differences in  
144 questionnaires, follow-up procedures) and age at baseline (1-y interval). Model  
145 2 was additionally adjusted for non-dietary variables: smoking status and  
146 intensity (never, former quit <11 years, former quit 11–20 years, former quit >20  
147 years, current <16 cigarettes/d, current 16–25 cigarettes/d, current >25  
148 cigarettes/d, current occasional, and not specified), physical activity (inactive,  
149 moderately inactive, moderately active, active, and not specified), education  
150 level (none, primary school, technical/professional school, secondary school,  
151 university or higher, and not specified), and body mass index (BMI, continuous  
152 kg/m<sup>2</sup>); and in women also for menopausal status (pre-, peri-, post-menopausal,  
153 surgical menopause), hormone replacement therapy use (yes, no, and  
154 unknown), and oral contraceptive use (yes, no, and unknown). Model 3 was  
155 further adjusted for dietary variables: total energy intake (kJ/d), alcohol (g/d),  
156 red and processed meat (g/d), fibre (g/d) and calcium (mg/d) intakes. The  
157 multivariable model for phenolic acids was additionally adjusted for coffee  
158 intake, because coffee is its main food source by far (17). Moreover, model 1  
159 and 2 were also adjusted for total energy intake to assess the effect of absolute  
160 versus relative intakes of polyphenols in the diet. Results of Cox models with  
161 and without adjusting for total energy intake were almost identical. Furthermore,  
162 polyphenol intakes were also included in the statistical models as nutrient  
163 density (mg/8240kJ day) (24). This energy-adjustment method did not modify  
164 the results appreciably.

165 Interactions between polyphenol intakes (continuous as mg/day) and sex, age  
166 (<55 years, 55 to 65 years, or >65 years), BMI (BMI<25, 25 to <30, ≥30 kg/m<sup>2</sup>),

167 tobacco smoking status (never, former, current smokers) and alcohol  
168 consumption (for women <15g/d and ≥15g/d; and for men <30g/d and ≥30g/d)  
169 were evaluated in separate analyses. The statistical significance of interactions  
170 on the multiplicative scale was assessed using the likelihood ratio test.  
171 Separate sex-specific models were fitted because a statistically significant  
172 interaction between sex and intake of total polyphenols was detected. In  
173 addition, we assessed separate models by smoking status category because a  
174 statistically significant interaction with smoking status (never, former, and  
175 current smokers) was observed. The Wald test statistic was used to evaluate  
176 heterogeneity by anatomical subsites of CRC (colon, proximal colon, distal  
177 colon, and rectum). Additional analyses by length of follow-up [censoring data at  
178 3-, 6-, 9-, 12-, 15-, 18-years, and maximum of follow-up (22.8 years)] were  
179 performed. Sensitivity analyses were performed by repeating main analyses  
180 after the exclusion of 462 CRC cases diagnosed during the first 2 years of  
181 follow-up (279 colon and 183 rectum cancer cases). All P values presented are  
182 2-tailed and were considered to be statistically significant when  $P < 0.05$ . To  
183 account for multiple testing for the subclasses of polyphenols, Bonferroni  
184 correction was used and then results were considered statistically significant if  
185  $P < 0.05/26$  (number of tests for the intakes of all polyphenol subclasses)  $< 0.002$ .  
186 All statistical analyses were conducted using R 3.2.1 software (R Foundation for  
187 Statistical Computing, Vienna, Austria).

## 188 **RESULTS**

189 During 13.9 (4.0) years of mean (SD) follow-up, 5,991 (56.8% in women)  
190 incident primary CRC cases were diagnosed, of which 3,897 were identified as  
191 colon cancers (including 1,877 proximal, 1,743 distal, and 277 overlapping or

192 unspecified colon cancers) and 2,094 as rectum cancers. The number of  
193 participants and distribution of CRC cases by country and sex are presented in  
194 **Table 1**. The highest estimated median of total polyphenol intakes among both  
195 sexes were in Denmark; whereas the lowest intakes amongst women and men  
196 were observed in Norway and Spain, respectively (Table 1). Phenolic acids  
197 were the main contributors to total polyphenols (51.0%), followed by flavonoids  
198 (44.2%), other minor polyphenol classes (4.4%), lignans (0.2%) and stilbenes  
199 (0.2%). Baseline characteristics of study participants by quintile of total  
200 polyphenol intake are shown in **Supplementary Table 1**. Men and women in  
201 the higher polyphenol intake groups were older, more physically active, had a  
202 lower BMI, higher educational level, and had a lower proportion of never  
203 smokers. Higher total polyphenol intake was also associated with higher  
204 average intakes of total energy, alcohol, calcium, fibre and red meat compared  
205 to participants with lower total polyphenol intakes. Furthermore, women with  
206 higher total polyphenol intakes were more likely to be post-menopausal and  
207 users of hormone replacement therapy and oral contraceptives than those with  
208 lower total polyphenol intakes.

209 In multivariable models, total polyphenol intake was not associated with CRC  
210 risk in either women ( $HR_{\log 2} = 1.06$ , 95 % CI 0.99 - 1.14) or men ( $HR_{\log 2} = 0.97$ ,  
211 95 % CI 0.90 - 1.05) ( $P_{\text{sex-interaction}} < 0.001$ ) (**Table 2**). Null associations were  
212 also observed with the risk of colon cancer and its anatomical subsites  
213 (proximal and distal) in women; although a borderline statistically significant  
214 inverse association was observed in men for colon cancer, especially for  
215 proximal cancer ( $HR_{\log 2} = 0.85$ , 95 % CI 0.73 – 0.99). Higher intakes of total  
216 polyphenols were significantly associated with a higher rectal cancer in women

217 (HR<sub>log2</sub> = 1.25, 95 % CI 1.10 - 1.41) but not in men (HR<sub>log2</sub> = 1.08, 95 % CI 0.95 -  
218 1.23) (P<sub>sex-interaction</sub> = 0.026).

219 For CRC, no statistically significant relationships were observed between any of  
220 the classes and subclasses of polyphenols neither in women nor in men (**Table**  
221 **3**). For colon cancers, inverse associations with the intake of total phenolic  
222 acids (HR<sub>log2</sub> = 0.91, 95 % CI 0.85 - 0.97; P=0.005) (P<sub>sex-interaction</sub> < 0.001) and its  
223 main subclass hydroxycinnamic acids (HR<sub>log2</sub> = 0.92, 95 % CI 0.87 - 0.97;  
224 P=0.004), as well as for methoxyphenols (HR<sub>log2</sub> = 0.99, 95 % CI 0.98 - 1.00;  
225 P=0.007) were found only in men. For rectal cancers, positive associations  
226 were observed in women with the intake of phenolic acids (HR<sub>log2</sub> = 1.10, 95 %  
227 CI 1.02 - 1.19; P=0.013) (P<sub>sex-interaction</sub> = 0.22), and its subclasses  
228 hydroxybenzoic acids (HR<sub>log2</sub> = 1.05, 95 % CI 1.00 - 1.10; P=0.039), and  
229 hydroxycinnamic acids (HR<sub>log2</sub> = 1.07, 95 % CI 1.00 - 1.15; P=0.038), as well as  
230 for flavanones (HR<sub>log2</sub> = 1.03, 95 % CI 1.00 - 1.07; P=0.048),  
231 alkylmethoxyphenols (HR<sub>log2</sub> = 1.04, 95 % CI 1.00 - 1.08; P=0.031), and  
232 methoxyphenols (HR<sub>log2</sub> = 1.02, 95 % CI 1.00 - 1.03; P=0.036). In women, a  
233 significant positive association was also detected between the risk of rectal  
234 cancer and flavonoid intake using the continuous variable (HR<sub>log2</sub> = 1.09, 95 %  
235 CI 1.00 - 1.18; P=0.039), but not using the quintiles (HR<sub>Q5 vs Q1</sub> = 1.23, 95 % CI  
236 0.94 - 1.60; P-trend=0.41). In men, an inverse association was found between  
237 hydroxybenzaldehyde intake and rectal cancer (HR<sub>log2</sub> = 0.97, 95 % CI 0.95 -  
238 1.00; P=0.035). However, none of these associations exceeded the Bonferroni  
239 significance threshold.

240 There were no evidence that age, BMI, and baseline alcohol intake modified the  
241 association between total polyphenol intake and CRC risk in the multivariable

242 models. Since a statistically significant interaction between smoking status  
243 (never, former, and current smoker) and total polyphenol ( $P_{\text{interaction}} = 0.033$ ) and  
244 flavonoid ( $P_{\text{interaction}} = 0.037$ ) intake in relation to CRC risk was observed in  
245 women, we stratified the statistical models by smoking status (**Supplementary**  
246 **table 2**). In most of cases, stronger associations were detected in either never  
247 or current smokers, although the results obtained were similar to those of the  
248 entire cohort.

249 In additional analysis, the relationships between the intake of total polyphenols  
250 and their main classes (flavonoids and phenolic acids) and the risk of overall  
251 CRC and by anatomical subsite (colon and rectal cancers) (**Figure 1**) were  
252 performed by length of follow-up [at 3 years, 6 years, 9 years, 12 years, 15  
253 years, 18 years, and maximum of follow-up (22.8 years)]. When censoring data  
254 at 3 years of follow-up, no associations were observed. At 6 years, all  
255 associations were similar to those found after the longest follow-up, although  
256 not all of them were statistically significant. The strongest results were found  
257 censoring data at 9 years of follow-up, while in longer follow-ups (>9 years) the  
258 associations were progressively attenuated.

259 In a separate sensitivity analysis in which the 462 CRC cases diagnosed within  
260 the first 2 years of follow-up were excluded, the associations between the intake  
261 of total polyphenols and polyphenol classes and overall CRC risk and by  
262 anatomical subsite were practically identical to results based on the whole  
263 cohort (data not shown).

## 264 **DISCUSSION**

265 In the present European prospective multi-country study, no statistically  
266 significant association between total polyphenol intake and overall CRC risk  
267 was observed. This is in line with findings of the Fukuoka colorectal case-  
268 control study (15). However, we observed a suggestive inverse association  
269 between total polyphenols intake and colon cancer risk in men and a positive  
270 one with rectal cancer risk in women. These findings for total polyphenol intake  
271 were almost identical to those found for phenolic acid intake.

272 Phenolic acids are the main contributors to total polyphenol intake (49.0% and  
273 54.7% in Mediterranean and non-Mediterranean EPIC countries, respectively)  
274 and coffee is, by far, their principal food source (70.6-74.6%) (17). In the current  
275 study, we did not see an association between phenolic acid intake and CRC risk  
276 in either men or women. Similar results were also observed after adjustment for  
277 coffee intake, implying that other food sources of phenolic acids were not  
278 related to CRC risk. In a nested case-control study within EPIC, no associations  
279 were found between concentrations of phenolic acids in plasma (including  
280 caffeic and ferulic acids which are major phenolic acids associated with coffee  
281 intake) (25) and colon cancer risk, except that homovanillic acid was associated  
282 with an increased risk (26). Plasma homovanillic acid is most probably  
283 associated with the metabolism of catecholamines and cannot be directly linked  
284 to phenolic acid intake. In the Fukuoka colorectal case-control study a  
285 borderline statistically significant inverse association between coffee polyphenol  
286 intake (which accounts for most phenolic acids) and colon cancer risk was  
287 reported in both sexes, but not for rectal cancer risk (15). In the EPIC study, null  
288 results were previously shown between coffee intake and overall CRC risk (27)  
289 and CRC mortality (28), although inverse associations with colon cancer risk in

290 men and positive associations with rectal cancer risk in women (27) and CRC  
291 mortality in women (28) were noted. In two recent meta-analyses, coffee intake  
292 was not associated with the risk of both overall CRC and rectum cancers in  
293 cohort studies (29;30); although higher doses of coffee (>5cups/day) has been  
294 reported to decrease the risk of colon cancer (30). However, the evidence is  
295 inconsistent; in an Australian-based case-control study, iced coffee  
296 consumption was associated with a higher risk of rectal cancer (31).  
297 Interestingly, in a recent meta-analysis of coffee intake, including 8 Japanese  
298 cohorts, a significant decreased risk of colon cancer was observed in women,  
299 but not in men (32). Moreover, no association was observed with rectal cancer  
300 risk in both sexes; although a significant increase was detected after excluding  
301 cases diagnosed within 3 years of the baseline only in women. Despite the  
302 suggestive epidemiological evidence regarding sex and anatomical location,  
303 there is heterogeneity in the association between phenolic acid and coffee in  
304 relation to CRC, thus further research is needed to confirm these results and to  
305 elucidate the underlying mechanisms of action. Part of these discrepancies  
306 might be because different types of coffee have different polyphenol  
307 compositions and contents, which are difficult to take into account in large  
308 epidemiological studies, such as in EPIC (33). In an Israeli-based case-control  
309 study, a significant inverse association was found between CRC risk and the  
310 intake of boiled and espresso coffees but not instant and filter coffees, with  
311 stronger associations for colon cancer (34). Phenolic acid intake is highly  
312 correlated with coffee intake (35) and therefore, other coffee constituents such  
313 as caffeine, cafestol and kahweol may also contribute to any association with  
314 CRC risk (36). No associations between total, caffeinated or decaffeinated

315 coffee and CRC risk were found in the Prostate, Lung, Colorectal, and Ovarian  
316 Cancer Screening Trial (37). Indeed, CYP1A2 and NAT2 genotypes, enzymes  
317 involved in caffeine metabolism, did not affect associations between coffee  
318 consumption and CRC risk (27). Therefore, caffeine does not seem to play a  
319 role in CRC pathogenesis. Another potential explanation for these differences in  
320 the relationships between cancer sites and sexes is due to endogenous factors,  
321 such as metabolic heterogeneity and gut microbiota, which may influences  
322 coffee bioavailability and therefore the bioactivity and bioefficacy of its  
323 constituents. Gut microbiota composition slightly varies between sexes (38),  
324 and especially, depend on the interaction between sex and diet (39).

325 We did not observe clear associations between flavonoid intake, the second  
326 major contributor to total polyphenols (44.3%), and CRC risk, and anatomical  
327 subsites in both men and women. These results were in concordance with our  
328 previous study with shorter follow-up (13), and three meta-analyses of  
329 prospective studies (40-42), although some protective associations have been  
330 systematically reported in case-control studies (41;42). In these prospective  
331 studies and in agreement with the present findings, no association was  
332 observed either with any of the flavonoid subclasses. However, some inverse  
333 associations have been reported between CRC risk and specific flavonoid  
334 compounds such as tea polyphenols and isoflavones. Urinary biomarkers of  
335 green tea polyphenols were also associated with a reduced risk of developing  
336 colon cancer in Chinese men (43); however, in Europe black tea is the type  
337 usually consumed. Plasma equol concentration, but not other isoflavones, was  
338 inversely related to colon cancer risk in a previous nested case-control study  
339 within EPIC (26). In contrast, no association was found with plasma and urinary

340 isoflavone levels in the EPIC-Norfolk study (44) or with dietary isoflavone  
341 intakes in a meta-analysis of cohort studies (11).

342 No association between lignan intake and CRC risk was observed in our study,  
343 as previously reported in a meta-analysis of cohort studies. No association was  
344 found with urinary and plasma lignan concentrations in EPIC (26;44) and in a  
345 Dutch cohort (45). However an inverse association between intakes of dietary  
346 enterolignan and enterodiol and CRC risk were found in women but not in men  
347 from EPIC-Norfolk (44).

348 No significant association between any minor subclasses of polyphenols and  
349 CRC risk was observed in our study. Methoxyphenols (guaiacol is the only  
350 polyphenol in this class) showed a similar pattern of associations to phenolic  
351 acids, because the main food source is coffee (17). In agreement with present  
352 observations, plasma concentrations of stilbenes and tyrosols were not related  
353 to colon cancer (26), although an inverse association between plasma  
354 alkylresorcinols, biomarkers of whole-grain wheat and rye intake, and distal  
355 colon cancer risk (46) was observed in a previous nested case-control study  
356 within EPIC.

357 We also investigated the relationships between polyphenol intake and CRC risk  
358 over the years of follow-up. The strongest associations were found from 6 to 9  
359 years of follow-up, which may be the presumable period of progression from  
360 asymptomatic precancerous polyps to CRC (47;48). Results from longer follow-  
361 ups tended to be attenuated, which could be due to misclassification bias. The  
362 longer the follow-up the higher the chance of change of dietary and lifestyle  
363 habits by the participants. This can be evaluated with periodic reassessments of

364 the main exposure and the cofounders. Despite this attenuation, our findings  
365 after a mean of 14 years of follow-up maintained their significance because  
366 accrual of more cases meant there was greater statistical power to detect  
367 associations.

368 The major strengths of the present study are its prospective design, its long  
369 follow-up, its large size and number of cases, and the coverage of several  
370 European countries with large dietary heterogeneity. This study also has  
371 several potential limitations. First, diet and other lifestyle variables were only  
372 available at baseline, and therefore, changes in these variables could not be  
373 taken into account in these analyses. The second limitation may be the  
374 measurement error in collecting dietary intake, but centre/country-specific  
375 validated questionnaires for polyphenol-rich foods were used (19). Moreover,  
376 the Phenol-Explorer is the most comprehensive food composition database on  
377 polyphenols available nowadays (16). The third limitation is the potential  
378 modification of diet during the early prediagnostic period of the disease;  
379 however, sensitivity analyses excluding incident cases diagnosed in the first 2  
380 years of follow-up did not alter the associations. The fourth limitation is the  
381 potential impact of residual confounding, since several lifestyle and other dietary  
382 factors related to CRC were different according to polyphenol intake. Although  
383 we have included them in the statistical models, measurement error and  
384 changes during follow-up may affect our results. Finally, we realize that our  
385 study is prone to the well-known drawback of multiple comparisons. We have  
386 therefore applied the Bonferroni correction and none of the tested associations  
387 remained statistically significant. Despite this rather conservative method, we  
388 were still able to observe borderline statistically significant associations.

389 In summary, we found that higher intakes of phenolic acids, reflecting high  
390 coffee consumption, were associated with a lower risk of colon cancer in men  
391 and a higher risk of rectal cancer in women, although the findings were no  
392 longer significant after Bonferroni correction. Further studies are warranted to  
393 evaluate the potential role of the intakes of phenolic acids and coffee in CRC  
394 development.

395

396 **CONFLICT OF INTEREST**

397 The authors declare that they have no conflict of interest.

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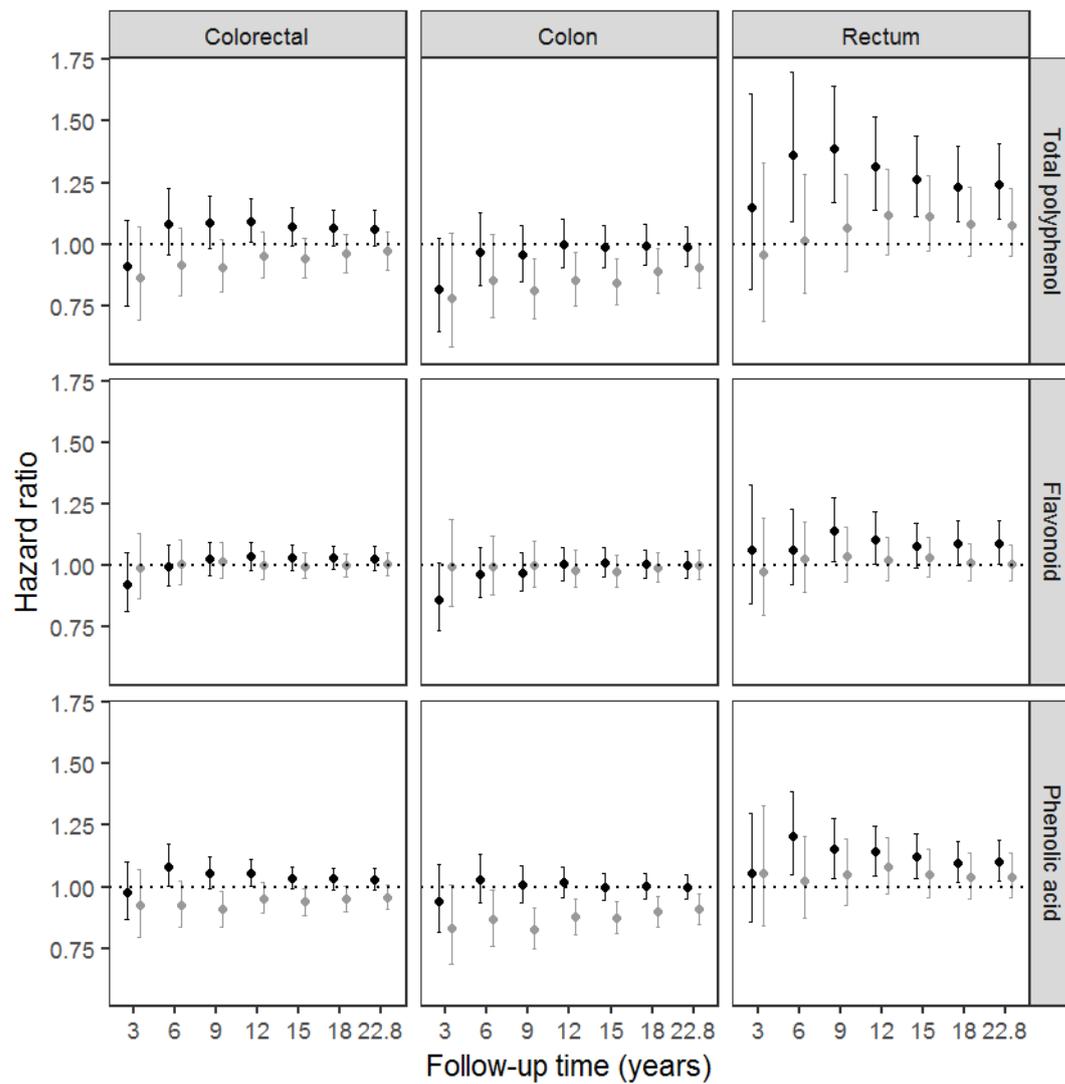


Figure 1. Hazard ratios and (95% CI) for colorectal cancer and subsites by sex and length of follow-up, according to double the intake ( $\log_2$ ) of total polyphenol, flavonoid, and phenolic acid in women (black circles) and men (grey circles) from the EPIC study.

Table 1. Distribution of subjects and colorectal cancer cases according to anatomical subsite and medians (5th–95th percentiles) of total polyphenol intake in 10 participating countries in the EPIC Study.

Country	N	Colorectal cancer cases, N						Polyphenol intake (mg/d)	Flavonoid intake (mg/d)	Phenolic acid intake (mg/d)
		Overall	Colon	Proximal	Distal	NOS	Rectum			
Women										
Denmark	28,720	533	363	170	161	32	170	1,552 (802-2,481)	514 (133-1,459)	890 (320-1,547)
France	67,403	410	264	129	125	10	146	1,320 (552-2,603)	514 (188-1,226)	679 (165-1,848)
Germany	27,379	177	121	66	53	2	56	1,033 (549-1,927)	414 (153-1,051)	504 (194-1,074)
Greece	15,233	41	25	11	7	7	16	759 (345-1,556)	247 (101-528)	416 (105-1,105)
Italy	30,513	342	264	119	116	29	78	853 (443-1,438)	413 (175-791)	377 (118-757)
Norway	33,975	297	195	104	86	5	102	653 (263-1,090)	184 (61-400)	371 (66-844)
Spain	24,850	218	154	57	79	18	64	671 (254-1,407)	282 (80-684)	311 (61-907)
Sweden	26,368	442	305	182	108	15	137	838 (418-1,465)	272 (89-678)	488 (166-971)
The Netherlands	26,912	387	268	154	109	5	119	1,158 (631-1,760)	514 (185-1,008)	574 (186-985)
United Kingdom	52,566	555	381	216	132	33	174	1,443 (662-2,240)	873 (317-1,495)	469 (129-1,054)
TOTAL	333,919	3,402	2,340	1,208	976	156	1,062	1,054 (415-2,148)	420 (116-1,239)	508 (123-1,318)
Men										
Denmark	26,294	709	395	161	202	32	314	1,594 (809-2,460)	397 (107-1,271)	993 (359-1,629)
France	-	-	-	-	-	-	-	-	-	-
Germany	21,178	258	141	59	67	15	117	1,093 (554-2,079)	402 (140-1,056)	549 (199-1,226)
Greece	10,815	51	31	10	10	11	20	967 (469-1,921)	302 (126-614)	538 (153-1,377)
Italy	14,032	228	160	55	86	19	68	1,009 (522-1,695)	493 (202-964)	428 (156-805)
Norway	-	-	-	-	-	-	-	-	-	-
Spain	15,139	339	220	81	126	13	119	834 (333-1,725)	425 (118-1,085)	315 (92-769)
Sweden	22,306	473	284	142	136	6	189	888 (442-1,568)	252 (75-664)	544 (193-1,064)

The Netherlands	9,627	119	58	29	26	3	61	1,155 (601-1,854)	398 (137-910)	674 (178-1,198)
United Kingdom	22,850	412	268	132	114	22	144	1,509 (735-2,309)	916 (334-1,519)	517 (157-1,076)
<u>TOTAL</u>	<u>142,241</u>	<u>2,589</u>	<u>1,557</u>	<u>669</u>	<u>767</u>	<u>121</u>	<u>1,032</u>	<u>1,150 (505-2,159)</u>	<u>419 (117-1,246)</u>	<u>562 (162-1,396)</u>

Table 2. HRs (95% CIs) for colorectal cancer (CRC) and subsites, according to quintile of intake of total polyphenols in women and men from the EPIC study.

		Overall CRC HR (95% CI)	Colon HR (95% CI)	Proximal HR (95% CI)	Distal HR (95% CI)	P- value <sup>1</sup>	Rectum HR (95% CI)	P- value <sup>2</sup>
Women								
Model 1	Quintile 1	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)		1.00 (ref)	
	Quintile 2	1.00 (0.89-1.13)	0.96 (0.83-1.10)	1.02 (0.84-1.24)	0.86 (0.70-1.06)		1.13 (0.91-1.40)	
	Quintile 3	1.11 (0.99-1.26)	1.02 (0.89-1.18)	1.06 (0.87-1.30)	0.96 (0.77-1.19)		1.37 (1.10-1.71)	
	Quintile 4	1.10 (0.97-1.25)	0.99 (0.85-1.16)	1.14 (0.92-1.41)	0.81 (0.64-1.02)		1.39 (1.10-1.76)	
	Quintile 5	1.12 (0.98-1.28)	0.99 (0.85-1.17)	1.12 (0.89-1.41)	0.85 (0.66-1.09)		1.45 (1.34-1.86)	
	P-trend	0.09	0.93	0.26	0.22		0.004	
	Continuous (log <sub>2</sub> )	1.06 (0.99-1.13)	0.99 (0.92-1.07)	1.05 (0.94-1.16)	0.92 (0.83-1.03)	0.11	1.22 (1.09-1.37)	0.002
Model 3	Quintile 1	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)		1.00 (ref)	
	Quintile 2	1.00 (0.89-1.13)	0.96 (0.83-1.10)	1.02 (0.84-1.24)	0.86 (0.70-1.07)		1.13 (0.91-1.41)	
	Quintile 3	1.12 (0.99-1.26)	1.02 (0.88-1.18)	1.05 (0.86-1.30)	0.97 (0.78-1.21)		1.39 (1.11-1.74)	
	Quintile 4	1.10 (0.97-1.26)	0.99 (0.85-1.17)	1.13 (0.90-1.41)	0.82 (0.64-1.06)		1.41 (1.10-1.80)	
	Quintile 5	1.13 (0.97-1.30)	1.00 (0.83-1.19)	1.11 (0.86-1.42)	0.87 (0.66-1.14)		1.49 (1.14-1.94)	
	P-trend	0.10	0.92	0.35	0.36		0.006	
	Continuous (log <sub>2</sub> )	1.06 (0.99-1.14)	0.99 (0.91-1.07)	1.04 (0.93-1.17)	0.93 (0.82-1.06)	0.22	1.25 (1.10-1.41)	0.002
Men								
Model 1	Quintile 1	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)		1.00 (ref)	
	Quintile 2	1.02 (0.90-1.16)	1.08 (0.92-1.27)	0.95 (0.74-1.22)	1.23 (0.98-1.54)		0.92 (0.75-1.13)	
	Quintile 3	0.96 (0.84-1.10)	1.02 (0.86-1.21)	0.94 (0.73-1.20)	1.14 (0.90-1.44)		0.88 (0.71-1.08)	
	Quintile 4	0.94 (0.82-1.08)	0.93 (0.78-1.11)	0.81 (0.62-1.06)	1.07 (0.83-1.37)		0.95 (0.77-1.18)	
	Quintile 5	0.89 (0.77-1.02)	0.82 (0.68-0.99)	0.78 (0.59-1.03)	0.84 (0.64-1.11)		0.97 (0.78-1.22)	
	P-trend	0.05	0.010	0.05	0.07		0.94	
	Continuous (log <sub>2</sub> )	0.94 (0.88-1.01)	0.88 (0.81-0.97)	0.85 (0.74-0.97)	0.91 (0.80-1.04)	0.43	1.05 (0.93-1.17)	0.022

Model 3	Quintile 1	1.00 (ref)					
	Quintile 2	1.03 (0.91-1.17)	1.10 (0.93-1.29)	0.95 (0.74-1.22)	1.27 (1.01-1.59)	0.93 (0.75-1.14)	
	Quintile 3	0.97 (0.85-1.11)	1.04 (0.87-1.23)	0.93 (0.72-1.21)	1.18 (0.92-1.51)	0.88 (0.71-1.10)	
	Quintile 4	0.96 (0.83-1.11)	0.95 (0.79-1.15)	0.80 (0.61-1.06)	1.12 (0.86-1.46)	0.96 (0.77-1.21)	
	Quintile 5	0.94 (0.80-1.10)	0.89 (0.72-1.09)	0.79 (0.58-1.08)	0.95 (0.71-1.28)	1.01 (0.79-1.29)	
	P-trend	0.30	0.09	0.10	0.36	0.65	
	Continuous (log <sub>2</sub> )	0.97 (0.90-1.05)	0.91 (0.82-1.01)	0.85 (0.73-0.99)	0.97 (0.84-1.12)	0.21	1.08 (0.95-1.23) 0.036

<sup>1</sup>P-value for heterogeneity for proximal vs distal colon cancer

<sup>2</sup>P-value for heterogeneity for colon vs rectum cancer

Model 1: Cox model was stratified by age and centre.

Model 3: Cox model was additionally adjusted for smoking status and intensity, physical activity, education level, body mass index, total energy intake, alcohol, red and processed meat, fibre (g/d) and calcium (mg/d) intakes and in women also for menopausal status, hormone replacement therapy use, and oral contraceptive use.

Table 3. Hazard ratios (95% CIs) for colorectal cancer and subsites, according to double the intake of polyphenol classes and subclasses by sex in the EPIC study.

	Women					Men					
	Intake (mg/d) median (P5%-P95%)	Colorectal HR (95% CI)	Colon HR (95% CI)	Rectum HR (95% CI)	P-value <sup>1</sup>	Intake (mg/d) median (P5%-P95%)	Colorectal HR (95% CI)	Colon HR (95% CI)	Rectum HR (95% CI)	P-value <sup>1</sup>	P-value <sup>2</sup>
Flavonoid subclasses	419.7 (116.3-1238.9)	1.03 (0.98-1.08)	1.00 (0.95-1.06)	1.09 (1.00-1.18)*	0.10	418.8 (117.4-1245.8)	1.00 (0.96-1.05)	1.00 (0.94-1.06)	1.01 (0.94-1.09)	0.87	0.030
Anthocyanins	25.5 (3.7-116.1)	1.01 (0.99-1.04)	1.00 (0.97-1.03)	1.02 (0.97-1.06)	0.46	22.9 (2.8-120.5)	0.99 (0.97-1.01)	1.00 (0.97-1.02)	0.98 (0.95-1.01)	0.49	0.09
Dihydrochalcones	1.8 (0.1-6.3)	1.00 (0.99-1.02)	1.00 (0.99-1.02)	1.01 (0.98-1.03)	0.76	1.5 (0.1-6.9)	1.00 (0.99-1.01)	0.99 (0.98-1.01)	1.00 (0.98-1.01)	0.61	0.038
Dihydroflavonols	0.4 (0.0-9.6)	1.00 (1.00-1.01)	1.00 (0.99-1.00)	1.00 (0.99-1.01)	0.20	1.0 (0.0-18.4)	0.99 (0.99-1.00)	0.99 (0.99-1.00)	0.99 (0.98-1.00)	0.64	0.027
Flavanols	285.6 (62.4-1015.5)	1.02 (0.98-1.06)	1.00 (0.95-1.04)	1.05 (0.98-1.13)	0.19	283.5 (65.1-1028.8)	1.01 (0.97-1.05)	0.99 (0.94-1.04)	1.01 (0.95-1.08)	0.51	0.043
Flavan-3-ol monomers	39.8 (6.4-460.4)	1.01 (0.99-1.03)	0.99 (0.97-1.02)	1.04 (1.00-1.09)	0.08	42.8 (7.4-466.1)	1.00 (0.97-1.02)	0.99 (0.96-1.02)	1.00 (0.96-1.04)	0.63	0.08
Proanthocyanidins	202.9 (52.4-532.0)	1.04 (0.99-1.08)	1.01 (0.96-1.07)	1.06 (0.98-1.15)	0.29	203.7 (51.5-552.1)	1.00 (0.96-1.05)	0.99 (0.93-1.04)	1.00 (0.93-1.07)	0.56	0.020
Theaflavins	1.6 (0.0-106.4)	1.00 (1.00-1.01)	1.00 (1.00-1.00)	1.01 (1.00-1.01)	0.26	1.5 (0.0-112.0)	1.00 (1.00-1.01)	1.00 (1.00-1.01)	1.00 (1.00-1.01)	0.74	0.06
Flavanones	25.6 (1.8-118.3)	1.00 (0.99-1.02)	0.99 (0.97-1.02)	1.03 (1.00-1.07)*	0.05	24.2 (2.2-120.0)	0.99 (0.97-1.01)	0.99 (0.96-1.01)	1.00 (0.96-1.03)	0.69	0.10
Flavones	9.3 (2.7-26.6)	1.02 (0.98-1.06)	1.02 (0.97-1.07)	1.02 (0.95-1.10)	0.09	9.4 (2.3-30.4)	0.98 (0.94-1.03)	0.96 (0.91-1.02)	0.97 (0.90-1.04)	0.42	0.027
Flavonols	27.9 (6.9-112.0)	1.01 (0.97-1.05)	0.99 (0.94-1.04)	1.07 (0.99-1.15)	0.08	29.5 (7.9-113.3)	1.01 (0.96-1.05)	0.99 (0.94-1.05)	1.00 (0.93-1.07)	0.57	0.23
Isoflavonoids	0.0 (0.0-7.3)	1.01 (1.00-1.02)	1.01 (1.00-1.02)	1.00 (0.99-1.02)	0.48	0.0 (0.0-5.0)	0.99 (0.98-1.01)	0.99 (0.98-1.00)	1.00 (0.98-1.02)	0.28	0.001
Phenolic acid subclasses	508.2 (122.8-1317.8)	1.03 (0.99-1.08)	1.00 (0.95-1.05)	1.10 (1.02-1.19)*	0.038	561.9 (162.1-1395.7)	0.96 (0.91-1.01)	0.91 (0.85-0.97)**	1.04 (0.95-1.14)	0.015	0.001
Hydroxybenzoics	19.5 (1.3-155.0)	1.00 (0.98-1.03)	0.99 (0.96-1.02)	1.05 (1.00-1.10)*	0.03	23.0 (3.1-159.5)	1.00 (0.97-1.04)	1.00 (0.96-1.04)	1.01 (0.96-1.06)	0.93	0.10
Hydroxycinnamic	474.6 (95.5-1279.3)	1.02 (0.98-1.06)	1.01 (0.96-1.05)	1.07 (1.00-1.15)*	0.10	513.6 (118.2-1356.5)	0.96 (0.92-1.01)	0.92 (0.87-0.97)**	1.03 (0.96-1.11)	0.017	0.002
Hydroxyphenylacetic	0.1 (0.0-0.6)	0.99 (0.98-1.01)	0.99 (0.98-1.01)	1.00 (0.98-1.03)	0.54	0.2 (0.0-1.3)	1.00 (0.98-1.01)	0.99 (0.97-1.01)	0.98 (0.96-1.01)	0.12	0.40
Stilbenes	0.4 (0.0-6.6)	1.00 (0.98-1.01)	1.00 (0.98-1.02)	1.00 (0.97-1.03)	0.74	0.8 (0.0-11.8)	0.98 (0.96-0.99)	0.98 (0.96-1.00)	0.97 (0.95-1.00)	0.70	0.042
Lignans	1.4 (0.7-4.9)	1.01 (0.94-1.08)	0.98 (0.90-1.06)	1.08 (0.95-1.21)	0.20	1.6 (0.8-5.3)	1.06 (0.98-1.15)	1.11 (1.01-1.22)	0.99 (0.86-1.13)	0.17	0.83
Other polyphenol classes											
Alkylphenols	24.4 (2.0-80.1)	1.00 (0.97-1.03)	1.00 (0.97-1.04)	1.00 (0.95-1.06)	0.95	39.7 (2.3-113.5)	0.99 (0.96-1.02)	0.99 (0.95-1.02)	1.01 (0.95-1.06)	0.57	<0.001
Tyrosol	3.5 (0.3-30.2)	0.99 (0.97-1.00)	0.99 (0.97-1.01)	0.97 (0.94-1.00)	0.26	4.5 (0.4-49.8)	0.99 (0.97-1.01)	1.00 (0.97-1.02)	0.97 (0.94-1.00)	0.22	0.20
Alkylmethoxyphenols	2.2 (0.1-6.2)	1.01 (0.99-1.02)	1.00 (0.98-1.02)	1.04 (1.00-1.08)*	0.036	2.7 (0.3-7.3)	0.99 (0.98-1.01)	0.99 (0.97-1.01)	1.00 (0.97-1.03)	0.49	0.005
Furanocoumarins	0.0 (0.0-0.4)	1.00 (0.99-1.00)	1.00 (0.99-1.01)	0.99 (0.98-1.00)	0.39	0.0 (0.0-0.3)	1.00 (0.99-1.01)	1.00 (0.99-1.01)	1.01 (0.99-1.02)	0.31	0.87
Hydroxybenzaldehydes	0.1 (0.0-1.5)	0.99 (0.98-1.01)	0.99 (0.98-1.01)	1.01 (0.98-1.03)	0.39	0.3 (0.0-2.5)	0.99 (0.98-1.01)	0.98 (0.96-1.00)	0.97 (0.95-1.00)*	0.10	0.008
Hydroxycoumarins	0.0 (0.0-0.4)	0.99 (0.99-1.00)	1.00 (0.99-1.01)	0.99 (0.98-1.01)	0.42	0.2 (0.0-1.3)	1.00 (0.99-1.01)	0.99 (0.98-1.01)	0.99 (0.98-1.00)	0.13	0.003
Hydroxyphenylpropenes	0.0 (0.0-4.0)	1.00 (1.00-1.01)	1.00 (1.00-1.01)	1.00 (0.99-1.01)	0.59	0.2 (0.0-5.8)	1.00 (1.00-1.01)	1.01 (1.00-1.01)	1.00 (0.99-1.01)	0.22	0.18
Methoxyphenols	0.3 (0.0-0.8)	1.01 (1.00-1.02)	1.00 (0.99-1.01)	1.02 (1.00-1.03)*	0.17	0.3 (0.0-0.8)	0.99 (0.98-1.00)	0.99 (0.98-1.00)**	0.99 (0.98-1.00)	0.73	<0.001

\*P-value<0.05; \*\*P-value<0.01; any association exceeds the Bonferroni threshold ( $P < 0.05/26$ ) < 0.002

<sup>1</sup>P-value for heterogeneity for colon vs rectum cancer

<sup>2</sup>P-value for interaction by sex in colorectal cancer

Cox model was stratified by age and centre, and additionally adjusted for smoking status and intensity, physical activity, education level, body mass index, total energy intake, alcohol, red and processed meat, fibre (g/d) and calcium (mg/d) intakes and in women also for menopausal status , hormone replacement therapy use, and oral contraceptive use