Red blood cell fatty acids and risk of colorectal cancer in the European Prospective Investigation into Cancer and Nutrition (EPIC).

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Abstract

Background: A growing body of evidence suggests that alterations of dietary fatty acid (FA) profiles are associated with colorectal cancer (CRC) risk. However, data from large-scale epidemiological studies using circulating FA measurements to objectively assess individual FA and FA categories are scarce.

Methods: To investigate the association between red blood cell (RBC) membrane FAs and risk of CRC in a case-control study nested within a large prospective cohort. After a median follow-up of 6.4 years, 1069 incident CRC cases were identified and matched to 1069 controls among participants of the European Prospective Investigation into Cancer and Nutrition (EPIC). The FA composition of RBC phospholipids (in mol%) was analyzed by gas chromatography, and their association with risk of CRC was estimated by multivariable adjusted conditional logistic regression models.

Results: After correction for multiple testing, subjects with higher concentrations of RBC stearic acid were at higher risk for CRC (OR=1.23; 95% CI=1.07-1.42, per 1 mol%). Conversely, CRC incidence decreased with increasing proportions of RBC n-3 PUFA, particularly eicosapentaenoic acid (0.75; 0.62-0.92, per 1 mol%). The findings for the n-6 PUFA arachidonic acid were inconsistent.

Conclusion: The positive association between pre-diagnostic RBC stearic acid and CRC reflects putative differences in FA intake and metabolism between cancer cases and matched controls which deserve further investigation. The inverse relationship between EPA and CRC is in line with the repeatedly reported protective effect of fish consumption on CRC risk.

Impact: These findings add to the evidence on CRC prevention.

Colorectal cancer (CRC) is one the most frequent malignancies worldwide. In 2018, CRC was the second most common cancer (12.8% of all cancers) diagnosed in Europe accounting for approximately 500,000 incident, and 242,500 fatal cases [1]. Experimental and epidemiological evidence indicates that nutritional and nutrition-related factors modulate CRC risk [2]. Obesity and physical inactivity, a diet high in red and processed meat or low in wholegrains and dairy products, and high alcohol consumption were shown to be associated with an increased CRC risk, while a reduced risk was reported for diets high in fibre and calcium [2]. Fatty acids are among the nutrients which are hypothesized to affect the risk of CRC [3, 4]. Among these, the role of n-3 and n-6 polyunsaturated fatty acids (PUFA) is of particular interest [5-7].

In most studies in rodents, diets high in n-6 PUFA such as linoleic acid (LA) and arachidonic acid (AA) have shown a tumor-promoting effect, whereas diets high in n-3 PUFA, especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), were protective against colorectal neoplasms [8]. However, in humans these associations are less clear [6, 9].

Higher fish intake, the main source of EPA and DHA, has been consistently reported as potentially protective for CRC [6]. However, the interpretation of dietary intake data derived from food frequency questionnaires are hampered by substantial imprecision due to potential measurement errors, arguing for the use of objective biomarkers [10-12]. Adipose tissue composition reflects best long-term (several years) dietary intake of fatty acids, but due to its invasive methodology, it is not feasible for prospective studies, however, specimens of red blood cells (RBCs) are relatively easy to collect. Compared to fatty acids in plasma phospholipids or cholesterol esters (as possible alternative specimens reflecting short-term intake in days up to few weeks), membrane-bound fatty acids are released by phospholipase A₂, and - in case of AA and EPA - may serve as substrates for enzymes of the AA and

eicosanoid pathways. Thus, the fatty acid composition of RBCs is close to the site with an expected direct impact on metabolic processes involved in the development of CRC. Due to their long half-life time of about 120 days, RBC fatty acids may reflect medium-term fatty acid supply from the diet [12, 13]. Significant correlations between dietary fatty acid intake (FFQ-derived) and their proportion in RBC membranes have been reported for very-long chain PUFA (EPA, DHA), odd-numbered fatty acids (pentadecanoic acid, heptadecanoic acid) as markers of dairy fat intake, and trans fatty acids, with correlation coefficients between 0.2 to 0.5 [14-16]. In addition palmitic acid, oleic acid, and AA were added to the list of fatty acids for which evidence was found that dietary intake could directly modulate their content in RBC [17]. To date, only a few prospective studies have assessed the role of fatty acids in CRC development by measuring circulating biomarkers of fatty acids [4, 18-21], and to our knowledge, this is the first study using RBC fatty acid composition to investigate this association prospectively. Here, we conducted a nested case-control study embedded in the European Prospective Investigation into Cancer and Nutrition (EPIC), a large multinational cohort of more than 520,000 participants across Europe with considerable variation in fat consumption and dietary fat quality [22-24].

Material and methods

Study Population and Collection of Blood Samples

The detailed recruitment procedures and collection of questionnaire data, anthropometric measurements and blood samples for the EPIC cohort have been published elsewhere [23]. Briefly, dietary and non-dietary variables were assessed using standardized questionnaires that were administered between 1992 and 2000 to 519,978 individuals in ten European countries. Blood samples were collected at baseline from 385,747 participants. Fasting prior to blood samples collection was not systematic, but time since last consumption of food or

drink was recorded. The present study included incident CRC cases that occurred after baseline assessment and matched control subjects from eight of the ten participating countries. At setup of this nested case-control study, few Norwegian CRC cases with available blood samples had been identified, and the EPIC center in Malmö, Sweden, did not provide RBCs for fatty acid analysis. Participants from Greece were not included in this analysis for formal reasons.

At recruitment, plasma was obtained from blood samples that were drawn into monovettes containing sodium citrate as an anti-coagulant except in Umeå, Sweden where EDTA or heparin-containing vials were used. After centrifugation of the monovettes and pipetting of the plasma and buffy coat (PBMC) layer, the remaining RBC suspension was aliquoted and frozen. RBC samples were stored in heat-sealed straws (0.5 ml) in liquid nitrogen (-196°C) at the biobank facility of the International Agency for Research on Cancer (IARC; Lyon, France) for all participating countries except Denmark and Sweden where samples were stored locally and under different protocols (Denmark: aliquots of 1.0ml stored locally in Nunc tubes at -150°C under nitrogen vapour, Sweden: stored in -80°C freezers).

This study was approved by the Ethical Review Committee of the IARC (Lyon/France), and ethical committees pertaining to all EPIC centers. All EPIC participants have provided written consent for the use of their blood samples and data.

Follow-up for cancer incidence and vital status

In Denmark, Italy, the Netherlands, Spain, Sweden and the UK, incident cancer cases were identified through record linkage with regional or national cancer registries. In Germany and France, follow-up was based on a combination of methods, including health insurance records, cancer and pathology registries, and active follow-up through study subjects and their next-of-kin. Data on vital status in most EPIC study centers was collected from mortality registries at the regional or national level, in combination with data collected by active

follow-up. For each EPIC study center, closure dates of the study period were defined as the latest dates of complete follow-up for both cancer incidence and vital status. By March 2007, complete follow-up data had been reported up to December 2003 or December 2004 for most centers. For Germany, the censoring date was considered to be the date of the last known contact, or date of cancer diagnosis or death, whichever came first.

Selection of case and control subjects

The 10th Revision of the International Statistical Classification of Diseases, Injuries and Causes of Death (ICD) was used to code the cancer sites. Colon cancers were defined as tumors in the cecum, appendix, ascending colon, hepatic flexure, transverse colon, splenic flexure, descending and sigmoid colon (C18.0-C18.7), as well as tumors that were overlapping or of unspecified origin (C18.8 and C18.9). Stratified analyses were performed for cancers located in the proximal colon (C18.0 – C18.5) and distal colon (C18.6 – C18.7). Cancers of the rectum were defined as tumors occurring at the recto-sigmoid junction (C19) or in the rectum (C20). Anal canal tumors were excluded.

Controls were randomly selected from all cohort members alive and free of cancer (except non-melanoma skin cancer) at the time of diagnosis of the cases and were matched by age at recruitment (± 6 months), sex, study center (to account for center specific differences in questionnaire design, blood collection procedures, differences in ascertaining the outcome, etc.), follow-up time since blood collection (matched by monthly intervals), time of the day at blood collection (± 1 hour) (to account for any potential changes that may have occurred in the blood samples over time during storage), and fasting status at the time of blood donation (<3 , $3-6$, >6 hours). Women were further matched by menopausal status (pre-menopausal, post-menopausal, peri-menopausal/unknown) and phase of menstrual cycle at blood collection. The latter matching criteria was of necessity to other EPIC nested case-control studies that were being conducted using the same matched case-control sets.

Laboratory analysis

Laboratory analyses were conducted at National Institute for Public Health and Environment (RIVM), Bilthoven, Netherlands. A detailed description of the methods was published elsewhere [25]. Briefly, the phospholipids from RBC membranes were extracted and subsequently methylated with a mixture of toluene and BF₃/MeOH. The fatty acid methyl esters (FAME) were separated by means of gas chromatography and results are reported as percent of the total of 32 fatty acids, on a molar basis (i.e., mol%). The trivial names or the systematic names of the fatty acids are given in Table 2, along with the short version; in the other tables, only the short version is given. Throughout the text, we use common trivial names (and abbreviations of) where possible.

Statistical analysis

Study participants' baseline characteristics and fatty acid concentrations were compared between cases and controls using the paired t-test (for normally distributed continuous data), the Wilcoxon signed-rank test (for not normally distributed continuous data) or the χ^2 -test for matched pairs (for categorical data).

We focused on fatty acids which are biomarkers for fatty acid intake or for which a relation to cancer development was postulated. The association between fatty acids and CRC risk was estimated using conditional logistic regression models conditioned on the matching variables. The results are given as odds ratios (OR), considered as relative risk, and 95% confidence intervals (CI). Fatty acids were categorized into quintiles based on their distribution among the controls. Also, fatty acids data were analysed as continuous variables (per 1 mol%), and the corresponding P-values (Wald statistics) can be interpreted as p for trend. Three fatty acids, heptadecanoic acid, AA, and DHA were natural log-transformed for normality. All models were adjusted for BMI (continuous), smoking status (never, former or current), alcohol intake (continuous [g/day]), educational level (none/primary, secondary, or

higher degree), physical activity (inactive, moderately inactive, active (moderately active and active combined) –combining recreational and household activity, expressed as sex-specific categories of metabolic equivalents [26]), self-reported diabetes status at baseline (yes, no), and season of blood collection. These covariates were either known confounders for the association of RBC fatty acids and CRC [18-21], or related to specific variables. Analyses of dietary sources of fatty acids were not conducted because the analysis of dietary intake of fatty acids was not in the scope of the current biomarker project.

Additionally, sub-analyses were performed for 769 cases and their matched controls adjusting for 25-hydroxyvitamin D plasma concentrations to evaluate a putative effect of RBC fatty acids on CRC risk by possibly interacting with vitamin D metabolism for which a protective effect on colon carcinogenesis has been suggested. Information on family history of CRC was not available; in addition, data on waist and hip circumference was missing in one center (Umeå); however, sub-analyses with inclusion of waist-to-hip ratio as confounder showed no meaningful difference in risk estimates as compared to the main analysis. In sensitivity analyses, cases of CRC diagnosed within the first two years of follow-up were excluded, as the tumor might have already started growing and affecting biomarkers when the blood samples were taken.

We also conducted stratified analyses by anatomical sub-sites of the tumors (distal and proximal colon, rectum), sex, smoking status, and country to assess potential effect modification. Likelihood ratio chi-square tests were used to examine heterogeneity of the association by strata. All statistical analyses were performed using SAS software package, version 9.1 (SAS Institute, Cary, NC). All P values reported were two-tailed and a P value < 0.05 was considered statistically significant. The Benjamini-Hochberg correction was used to control for multiple comparisons in the main analysis [27].

Results

The present study included a total of 1069 incident CRC cases, 670 cancers of the colon and 399 rectal cancer cases, and 1069 matched controls; their baseline characteristics were shown in Table 1. About half (51%) of the colon cancers were attributed to the distal colon and 40% to the proximal colon (table 1).

For the control group, the fatty acid composition of RBC membranes and the sums of saturated fatty acids (SFA), MUFA, n-6 PUFA and n-3 PUFA are given in table 2. Even though the tests for differences between countries for most of the listed fatty acids were statistically significant (data not shown), the absolute differences between countries were within a fairly limited range. The mean fatty acid content (in mol%) in RBC membranes in colon and rectal cancer cases and controls are shown in Table 3.

The odds ratios of CRC for fatty acids and fatty acid groups are presented in table 4. After adjustment for established CRC risk factors, there was a significant positive association between the stearic acid content in RBC membranes and CRC incidence using categorical as well as the continuous variables. The OR (95%CI) increased by 23% (7-42%, p trend =0.005) per 1 mol% increase in stearic acid. No significant associations were seen for other SFAs, thus the result for the sum of SFA (OR [per 1 mol%] =1.13; 95%CI=1.03-1.24) was driven by the association observed for stearic acid.

The odd-numbered fatty acid heptadecanoic acid as a putative biomarker of dairy consumption was inversely associated with CRC risk (OR=0.49; 95%CI=0.33-0.80). Likewise, C18:1 trans fatty acids (sum of vaccenic and elaidic acid; their peaks could not be separated with the chosen laboratory methods) showed a significant inverse association with CRC (OR=0.56; 95%CI=0.33-0.96). The RBC membrane content of the cis monounsaturated fatty acids was unrelated to CRC risk.

Concerning n-6 PUFA, a statistically significant positive relationship between the AA content in RBC membranes and CRC risk was seen for the 3rd (OR=1.53; 95%CI= 1.12-2.07) and 4th quintile (OR=1.46; 95%CI=1.05-2.02) compared to the 1st quintile; using the continuous variable, OR estimates increased but failed to reach statistical significance. However, docosatetraenoic acid (C22:4n6) was significantly associated with CRC incidence (OR=1.22; 95%CI=1.04-1.45; p trend=0.018). We observed no association between the RBC membranes content of LA or dihomo- γ -linolenic acid (C20:3n6) and CRC risk.

The content of EPA and DHA and the sum of n-3 PUFA in RBC membrane lipids was inversely associated with the risk of CRC. The odds ratios of CRC were 0.68 (0.49-0.96) and 0.70 (0.51-0.97) for the highest versus lowest quintile of EPA and DHA, respectively. Per 1 mol% increase in EPA, the cancer risk decreased by 25% (8-38%, p for trend=0.005).

After adjustment for multiple comparisons, significance of associations was confirmed for stearic acid and the total sum of SFA, EPA and the sum of n-3PUFA, and borderline significance for docosatetraenoic acid. Risk estimates for CRC and corresponding 95% CI by EPIC country are presented in figure 1 for the RBC content of stearic acid (Figure 1A) and EPA (Figure 1B) and for the RBC content of AA in supplementary figure SF1.

After exclusion of CRC cases diagnosed within the first two years of follow-up, significant associations persisted for stearic acid, heptadecanoic acid and EPA (supplementary table S1). In a sub-analysis with additional adjustment of the main model for plasma 25-hydroxy-vitamin D concentrations associations remained statistically significant for the sum of SFA, heptadecanoic acid and odd-chain fatty acids and borderline significant for EPA (supplementary table S2).

In table 5, the associations between fifths of fatty acids and the risk of cancer stratified by tumor site (colon, proximal colon, distal colon, and rectal cancer) are shown. Only the results for fatty acids identified from the main analysis as being associated with CRC risk are

presented. For most fatty acids, including stearic acid, docosatetraenoic acid, EPA, and DHA we found no clear indication for differential effects by cancer sub-site.

We found no evidence for heterogeneity of the results between men and women (supplementary table S3). There was suggestion of heterogeneity by smoking status for EPA, with current smokers having a lower risk of CRC with increasing EPA concentrations (P for heterogeneity = 0.012), whereas there was no respective association for former or never smokers.

Discussion

In this multi-center case-control study nested in the prospective EPIC cohort, we observed a positive association between stearic acid content in RBC membrane lipids and the risk of CRC, and an inverse association with EPA, the major very long-chain n-3 PUFA. We got indication for a risk-increasing association for the n-6 PUFA AA and docosatetraenoic acid, but for AA, a dose-response relationship could not be established. Inverse associations with CRC risk were also noted for the odd-numbered fatty acid heptadecanoic acid and the sum of C18:1 trans fatty acids (vaccenic acid and elaidic acid). Correction for multiple testing as well as the results of sensitivity analyses confirmed especially the associations reported for stearic acid and the sum of SFA as well as for EPA and the sum of n-3 PUFA.

We observed that a higher proportion of stearic acid was associated with a higher risk of both colon and rectal cancer. This has also been found in a few very small human studies measuring fatty acid composition in plasma, RBC or tumor tissue (versus normal tissue) [28-30] and strong indication for a causal relationship was provided by using the Mendelian randomisation approach [31]. Two nested case-control studies with fatty acid measurements in serum and blood did either not report the results for SFA [19] or found no statistically

significant association for the total SFA content [18]. Stearic acid in blood is poorly correlated with dietary intake, and is endogenously synthesized and also metabolized to the corresponding monounsaturated fatty acid. Studies in rodents have shown that long-chain fatty acid elongase (Elovl-5, Elovl-6) activities are tightly regulated by diet and fasting, hormones, drugs, and also in chronic disease [32]. Fatty acid synthesis is increased in many tumors and fatty acid synthase (FASN), the primary enzyme involved in de novo lipogenesis from carbohydrates, has been suggested as a drug target for cancer therapy [33]. Interestingly, increased expression of FASN has been detected in more than 80% of aberrant crypt foci, the earliest identified monoclonal lesion in the colon [34], suggesting an involvement of fatty acid metabolism in very early colorectal tumorigenesis. Emerging evidence indicates also a role of SFAs in DNA damage response [35]. High intake of SFA may also modulate CRC risk through an increased bile acid production [36] and elevated diacylglycerol levels [37].

In our study, odd-numbered fatty acids in RBCs, especially heptadecanoic acid, were inversely associated with CRC risk. Being highly correlated with habitual consumption, heptadecanoic acid can be interpreted as a marker for milk and dairy products consumption [38]. A protective association has been found for the consumption of total dairy products and CRC risk in EPIC, which is consistent with data from other prospective studies [2, 39]. The protective effect of dairy products is likely due to their high calcium content[40].. Intake of vaccenic acid, the C18:1trans fatty acid originating from ruminant microbiota activity, is also associated with dairy intake. However, vaccenic and elaidic acid could not be separated, thus, a summary estimate for both combined was presented. Since elaidic acid, produced by industrial fat hydrogenation, may have distinctly different biological activities as compared to vaccenic acid, our results are difficult to interpret.

Though we found no significant trend and thus no clear dose-response relationship for AA, we obtained significant positive associations for 3rd and 4th quintile of AA and for the n-6

PUFA docosatetraenoic acid. Using plasma fatty acid data, a Mendelian randomisation study supported a causal link between AA and CRC incidence [31]. Data from other prospective studies reported conflicting results.

For long-chain n-3 PUFA, EPA and DHA, our data demonstrated a lower risk of CRC and significant association was confirmed by correction for multiple comparisons; this finding is in accordance with previously published results [4, 6] and fits with the reported inverse association between dietary fish intake or the intake of n-3 PUFA in EPIC [4] or in a recent meta-analyses of prospective studies [6]. This recently published meta-analysis found a small inverse dose response relationship between blood levels of n-3 PUFA and CRC risk [6]. An inverse association between advanced colorectal adenomas and the levels of EPA and DHA in erythrocyte membrane phospholipids was also observed in the E3N-EPIC cohort [41].

N-3 and n-6 PUFA use the same enzymes for conversion to different eicosanoids with different biological properties [42]. N-3 PUFA were shown to have an effect on cell proliferation and apoptosis, and exert anti-inflammatory functions [43]. Inhibition of the synthesis of pro-inflammatory cytokines, e.g., interleukin-1 beta (IL-1 β) and tumor necrosis factor alpha (TNF- α), has been observed with supplementation of n-3 PUFA in humans [44]. This effect is likely to be mediated via decreased activity of the NF- κ B system, a crucial regulator of apoptotic processes. In addition, effects through inhibition of cyclooxygenase-2 (COX-2) and thus decreased production of pro-inflammatory eicosanoids derived from AA, especially prostaglandin E₂, are well described [43, 45]. PGE₂ itself can promote tumor growth by activating signaling pathways which control cell proliferation and apoptosis [45]. Regular use of aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs) have protective associations with colorectal adenoma and CRC development, most likely via inhibition of COX-1 and COX-2 enzymes [46], thus underlining the importance of this pathway. Enrichment of bio-membranes with EPA in subjects with high fish consumption or

supplementation of fish oil may have pleiotropic effects on various molecular pathways, including cellular oxidative stress responses [47] as well as alteration in membrane fluidity and subsequent signalling processes [48]. Interference in these pathways via dietary interventions appears to be an interesting alternative to the use of chemopreventive drugs, which may exert harmful side-effects [49]. EPA potentially can be considered as a chemopreventive agent, and a diet rich in fish (and thus n-3 PUFA) can be recommended for the majority of the population. The apparently stronger CRC protective effect for EPA seen particularly among current smokers with their expected higher inflammatory potential point into the direction of EPA as anti-inflammatory compound. Direct evidence for suppression of inflammation-driven tumor progression by n-3 PUFA has been reported using fat-1 transgenic mice [50]. Further, the crucial balance between colonic epithelial cell proliferation and apoptosis was shown to be favourably affected by dietary n-3 PUFA [51-53].

Increasingly, an interaction between PUFA and the epigenome has been reported, with effects at the global as well as the gene-specific level [54]. PUFA, particularly EPA, were shown to change the expression and activity of crucial epigenomic regulators such as DNMTs and TET proteins. Among the differentially methylated sites are important factors for colon carcinogenesis such as FAS death receptor and the HLTF tumor suppressor protein [55]. Epigenetic mechanisms triggered by n-6 PUFA and SFA were also described [56].

In multi-centre studies, heterogeneity due to sampling procedures are not uncommon and the magnitude varies between the different participating centres. Thus, analyses were matched for the variable “centre”, following also the EPIC-internal suggestions. For the single participating countries, varying sample size affected robustness of the results. Comprising different regions of whole Europe, this large multi-center study provides data of differing dietary habits. This heterogeneity should be reflected also in the respective biomarkers which enhances the chance to detect exposure-disease associations. The major strength of our study

is its large sample size and its prospective design. Additional robustness of results was obtained by correction for multiple comparisons. Although having adjusted for several common CRC risk factors, residual and unmeasured confounding, due to e.g. medication use, social or environmental factors, cannot be excluded, and thus still remains an important limitation. Likewise, possible misclassification of outcomes cannot be entirely excluded. For a substantial subset of this study, we could adjust for vitamin D status and thus confirm that the major findings reported here are independent of possible vitamin D effects on colorectal carcinogenesis. Data on family history of CRC was not available. As family disposition would capture genetic predisposition, this is a potential limitation, though we focused especially on sporadic CRC. Furthermore, the impossibility of separation of the C18:1 trans fatty acids with the applied analytic method is a clear limitation of our study.

In conclusion, the results from this large case-control study nested within EPIC provide evidence for a positive association between stearic acid and probably also long-chain n-6 PUFA (AA, docosatetraenoic acid) in RBC membranes and the risk of CRC. Inverse associations were observed for the RBC long-chain n-3 PUFA, especially EPA, and CRC. These associations can partly be explained by well described biologic mechanisms. However, more research integrating genetic as well as epigenetic data is recommended to further decipher the differential effects of individual fatty acids in colorectal carcinogenesis.

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Table 1: Baseline characteristics of colon and rectal cancer cases and matched controls [Mean ($\pm$ SD) or N (%)]

Characteristic		Colon cancer cases	Matched controls	P-value*	Rectal cancer cases	Matched controls	P-value*
N		n = 670	n = 670		n = 399	n = 399	
Sex	Men	342 (51.0%)	342 (51.0%)		233 (58.4%)	233 (58.4%)	
	Women	328 (49.0%)	328 (49.0%)		166 (41.6%)	166 (41.6%)	
Age at baseline (years)		58.8 ($\pm$ 7.1)	58.9 ($\pm$ 7.1)		58.0 ($\pm$ 6.5)	58.0 ($\pm$ 6.5)	
Height (cm)		168.4 ($\pm$ 9.3)	167.3 ($\pm$ 9.1)	<0.001	168.7 ($\pm$ 9.1)	169.0 ($\pm$ 9.1)	0.553
Weight (kg)		76.7 ($\pm$ 14.5)	74.4 ($\pm$ 12.7)	<0.001	76.3 ($\pm$ 13.6)	75.7 ($\pm$ 14.3)	0.414
BMI (kg/m ²)		27.0 ($\pm$ 4.4)	26.6 ($\pm$ 3.9)	0.034	26.8 ($\pm$ 4.0)	26.4 ($\pm$ 3.7)	0.144
Smoking status	Never	278 (41.5%)	296 (44.2%)	0.420	155 (38.8%)	147 (36.8%)	0.916
	Former	235 (35.1%)	227 (33.9%)		130 (32.6%)	134 (33.6%)	
	Current	151 (22.5%)	145 (21.6%)		112 (28.1%)	115 (28.8%)	
	Unknown, missing	6 (0.9%)	2 (0.3%)		2 (0.5%)	3 (0.8%)	
Alcohol intake (g/day)	≤ 7.9	330 (49.3%)	346 (51.6%)	0.334	165 (41.4%)	181 (45.4%)	0.046
	8.0 - 15.9	125 (18.7%)	113 (16.9%)		74 (18.5%)	66 (16.5%)	
	16.0 - 39.9	125 (18.7%)	138 (20.6%)		79 (19.8%)	97 (24.3%)	
	≥ 40.0	90 (13.4%)	73 (10.9%)		81 (20.3%)	55 (13.8%)	
Physical activity	Inactive	100 (14.9%)	84 (12.5%)	0.262	58 (14.5%)	51 (12.8%)	0.683
	Moderately inactive	183 (27.3%)	166 (24.8%)		101 (25.3%)	93 (23.3%)	
	Active	335 (50.0%)	370 (55.2%)		209 (52.4%)	218 (54.6%)	
	Missing	52 (7.8%)	50 (7.5%)		31 (7.8%)	37 (9.3%)	
Educational attainment	None or primary school completed	258 (38.5%)	268 (40.0%)	0.819	134 (33.6%)	150 (37.6%)	0.492
	Technical, professional, secondary school	283 (42.2%)	284 (42.4%)		167 (41.9%)	157 (39.3%)	

	Longer education (incl. University degree)	104 (15.5%)	98 (14.6%)	85 (21.3%)	84 (21.1%)
	Missing/ not specified	25 (3.7%)	20 (3.0%)	13 (3.3%)	8 (2.0%)
Age at diagnosis		62.6 (± 7.4)		61.9 (± 6.7)	
Site of cancer	Cecum	109 (16.3%)			
	Appendix	1 (0.1%)			
	Ascending colon	70 (10.4%)			
	Hepatic flexure of colon	17 (2.5%)			
	Transverse colon	48 (7.2%)			
	Splenic flexure of colon	26 (3.9%)			
	Descending colon	52 (7.8%)			
	Sigmoid colon	284 (42.4%)			
	Overlapping lesion of colon	7 (1.0%)			
	Colon, NOS ^a	56 (8.4%)			
	Rectosigmoid junction			98 (24.6%)	
	Rectum, NOS ^a			301 (75.4%)	

* chi² test or t-test for matched pairs; a) NOS = not otherwise specified;

Table 2: Red blood cell fatty acid (FA) composition among controls by EPIC country [mol%; Median (25th-75th percentile)]

Fatty acids	France	Italy	Spain	UK	Netherlands	Germany	Sweden	Denmark
N	8	138	116	172	122	122	76	315
Saturated fatty acids (SFA)								
C14:0, Myristic acid	0.5 (0.4-0.6)	0.3 (0.3-0.4)	0.3 (0.2-0.3)	0.4 (0.3-0.5)	0.5 (0.4-0.5)	0.4 (0.4-0.5)	0.4 (0.4-0.5)	0.4 (0.4-0.5)
C16:0, Palmitic acid	21.9 (21.4-22.5)	21.0 (20.6-21.5)	20.3 (19.7-21.0)	21.3 (20.8-21.8)	21.2 (20.7-21.6)	21.3 (20.8-21.9)	21.2 (20.6-21.5)	21.4 (20.9-21.9)
C18:0, Stearic acid	13.5 (12.9-14.0)	13.9 (13.6-14.3)	14.2 (13.7-14.7)	13.7 (13.3-14.1)	14.1 (13.7-14.6)	14.3 (14.0-14.7)	14.1 (13.8-14.4)	14.0 (13.6-14.5)
SFA, total ^a	42.7 (41.7-43.2)	40.9 (40.0-41.6)	40.1 (39.4-41.0)	40.9 (40.2-41.7)	41.8 (40.9-42.8)	42.0 (41.3-42.8)	40.5 (40.1-41.1)	41.9 (41.1-42.8)
Odd-chain fatty acids								
C15:0, Pentadecanoic acid	0.4 (0.4-0.5)	0.4 (0.3-0.5)	0.4 (0.3-0.4)	0.5 (0.4-0.5)	0.5 (0.3-0.6)	0.5 (0.2-0.7)	0.4 (0.3-0.4)	0.3 (0.3-0.3)
C17:0, Heptadecanoic acid	0.4 (0.4-0.5)	0.4 (0.3-0.4)	0.4 (0.4-0.4)	0.4 (0.3-0.4)	0.3 (0.3-0.4)	0.3 (0.3-0.4)	0.3 (0.3-0.4)	0.3 (0.3-0.4)
Monounsaturated fatty acids (MUFA)								
C16:1n7c, palmitoleic acid	0.7 (0.5-0.8)	0.4 (0.3-0.5)	0.3 (0.2-0.4)	0.5 (0.4-0.6)	0.5 (0.4-0.6)	0.5 (0.4-0.6)	0.5 (0.4-0.6)	0.5 (0.4-0.6)
C18:1n9c, Oleic acid	12.5 (12.3-13.6)	13.4 (12.8-14.2)	13.1 (12.1-14.3)	12.5 (11.5-13.4)	11.9 (11.3-12.6)	12.1 (11.6-12.8)	13.4 (12.7-14.2)	12.5 (11.9-13.2)
C18:1n7t+n9t, Sum of vaccenic acid and elaidic acid	0.5 (0.4-0.5)	0.4 (0.3-0.5)	0.5 (0.4-0.5)	0.8 (0.6-0.9)	0.8 (0.6-0.9)	0.5 (0.4-0.7)	0.6 (0.5-0.7)	0.5 (0.4-0.6)
MUFA, total ^b	18.3 (17.5-19.2)	19.2 (18.0-20.5)	18.9 (17.3-20.4)	17.5 (16.6-18.3)	16.4 (15.6-17.7)	17.5 (17.1-18.3)	18.5 (17.8-19.1)	18.3 (17.7-19.1)
n-6 polyunsaturated fatty acids								

(n-6 PUFA)								
C18:2n6c, Linoleic acid, LA	10.6 (9.5-10.7)	9.8 (9.0-10.4)	10.4 (9.3-11.7)	11.4 (10.5-12.5)	11.3 (10.4-12.5)	10.3 (9.6-11.1)	10.3 (9.8-11.2)	10.6 (9.8-11.4)
C20:3n6, Dihomo-γ-linolenic acid, DGLA	1.6 (1.4-1.6)	1.8 (1.5-2.1)	1.6 (1.4-1.8)	1.7 (1.4-1.8)	1.6 (1.4-1.9)	1.5 (1.3-1.7)	1.6 (1.4-1.8)	1.5 (1.3-1.7)
C20:4n6, Arachidonic acid, AA	12.5 (11.8-13.0)	14.3 (13.5-15.1)	13.6 (12.6-14.4)	12.8 (12.0-13.6)	13.8 (13.0-14.6)	13.7 (12.9-14.5)	13.0 (12.4-13.8)	12.1 (11.3-13.0)
C22:4n6, Docosatetraenoic acid	2.3 (2.1-2.5)	3.2 (2.8-3.6)	2.6 (2.3-3.0)	2.5 (2.2-3.0)	3.0 (2.7-3.4)	2.8 (2.4-3.2)	2.3 (2.1-2.6)	2.1 (1.8-2.5)
n-6 PUFA, total ^c	27.3 (25.9-27.5)	29.6 (28.1-30.8)	28.7 (26.9-30.6)	28.8 (27.4-30.8)	30.2 (28.9-32.1)	28.7 (27.4-30.1)	28.0 (26.4-28.9)	26.9 (25.4-28.2)
n-3 polyunsaturated fatty acids (n-3 PUFA)								
C18:3n3, α-Linolenic acid, ALA	0.1 (0.1-0.1)	0.1 (0.0-0.1)	0.1 (0.0-0.1)	0.1 (0.1-0.2)	0.1 (0.1-0.2)	0.1 (0.1-0.2)	0.2 (0.2-0.2)	0.2 (0.1-0.2)
C20:5n3, Eicosapentaenoic acid, EPA	1.0 (1.0-1.3)	0.6 (0.5-0.8)	0.9 (0.7-1.2)	1.0 (0.7-1.3)	0.8 (0.6-1.0)	1.0 (0.7-1.2)	1.4 (1.1-1.7)	1.3 (1.1-1.7)
C22:5n3, Docosapentaenoic acid, DPA	2.3 (2.0-2.3)	2.1 (1.9-2.3)	1.9 (1.7-2.0)	2.6 (2.4-2.8)	2.6 (2.4-2.8)	2.5 (2.3-2.7)	2.8 (2.5-3.0)	2.6 (2.4-2.8)
C22:6n3, Docosahexaenoic acid, DHA	5.5 (5.3-6.2)	5.1 (4.6-5.8)	6.8 (6.3-7.4)	5.8 (5.1-6.6)	5.0 (4.4-5.7)	5.3 (4.8-6.1)	6.2 (5.4-7.0)	6.3 (5.6-7.0)
n-3 PUFA, total ^d	8.9 (8.6-9.6)	7.9 (7.2-8.9)	9.6 (8.8-10.6)	9.6 (8.5-10.8)	8.5 (7.7-9.5)	9.0 (8.1-9.9)	10.5 (9.6-11.5)	10.4 (9.5-11.6)

a) sum of C14:0, C16:0, C18:0, C20:0; b) sum of C16:1n7c, C18:1n9c, C18:1n7c, C20:1n9c; c) sum of C18:2n6c, C18:3n6, C20:2n6, C20:3n6, C20:4n6, C22:4n6;

d) sum of C18:3n3, C20:5n3, C22:5n3, C22:6n3

Table 3: Red blood cell fatty acid composition for cases and matched controls [mol%; Median (25th-75th percentile)]

Fatty acids	Colon cancer cases	Matched Controls	Rectal cancer cases	Matched Controls
N	670	670	399	399
Saturated fatty acids				
C14:0	0.4 (0.3-0.5)	0.4 (0.3-0.5)	0.4 (0.3-0.5)	0.4 (0.3-0.5)
C16:0	21.1 (20.7-21.7)	21.2 (20.6-21.7)	21.3 (20.8-21.9)	21.2 (20.7-21.8)
C18:0	14.1 (13.6-14.5)	14.0 (13.6-14.5)	14.0 (13.7-14.5)	14.0 (13.6-14.4)
SFA, total ^a	41.4 (40.5-42.4)	41.3 (40.4-42.3)	41.6 (40.7-42.6)	41.4 (40.5-42.3)
Odd-chain fatty acids				
C15:0	0.4 (0.3-0.5)	0.4 (0.3-0.5)	0.3 (0.3-0.5)	0.3 (0.3-0.5)
C17:0	0.4 (0.3-0.4)	0.4 (0.3-0.4)	0.3 (0.3-0.4)	0.4 (0.3-0.4)
Monounsaturated fatty acids				
C16:1n7c	0.5 (0.4-0.6)	0.5 (0.4-0.6)	0.5 (0.4-0.6)	0.5 (0.4-0.6)
C18:1n9c	12.7 (11.9-13.5)	12.6 (11.8-13.4)	12.5 (11.8-13.3)	12.8 (11.9-13.5)
C18:1n7t+n9t	0.5 (0.4-0.7)	0.5 (0.4-0.7)	0.5 (0.4-0.6)	0.5 (0.4-0.7)
MUFA, total ^b	18.1 (17.1-19.1)	17.9 (17.1-19.0)	18.1 (17.3-19.0)	18.2 (17.4-19.2)
n-6 Polyunsaturated fatty acids				
C18:2n6c	10.6 (9.6-11.6)	10.6 (9.7-11.7)	10.4 (9.6-11.4)	10.5 (9.7-11.6)
C20:3n6	1.6 (1.4-1.8)	1.6 (1.4-1.8)	1.5 (1.3-1.8)	1.5 (1.4-1.8)
C20:4n6	13.2 (12.4-14.2)	13.2 (12.2-14.3)	13.0 (12.2-14.0)	13.0 (12.0-13.9)
C22:4n6	2.6 (2.2-3.1)	2.6 (2.2-3.1)	2.6 (2.2-3.0)	2.5 (2.1-3.0)
n-6 PUFA, total ^c	28.5 (27.0-30.2)	28.4 (26.8-30.3)	28.1 (26.6-29.6)	28.3 (26.6-29.7)
n-3 Polyunsaturated fatty acids				
C18:3n3	0.1 (0.1-0.2)	0.1 (0.1-0.2)	0.1 (0.1-0.2)	0.1 (0.1-0.2)
C20:5n3	0.9 (0.7-1.3)	1.0 (0.7-1.3)	1.0 (0.8-1.4)	1.1 (0.8-1.5)
C22:5n3	2.5 (2.2-2.7)	2.5 (2.2-2.7)	2.5 (2.2-2.8)	2.5 (2.3-2.8)

Fatty acids	Colon cancer cases	Matched Controls	Rectal cancer cases	Matched Controls
C22:6n3	5.7 (4.9-6.6)	5.9 (5.0-6.7)	5.8 (5.1-6.6)	5.9 (5.2-6.7)
n-3 PUFA, total ^d	9.2 (8.2-10.5)	9.4 (8.3-10.7)	9.4 (8.4-10.6)	9.7 (8.6-10.8)

a) sum of C14:0, C16:0, C18:0, C20:0; b) sum of C16:1n7c, C18:1n9c, C18:1n7c, C20:1n9c; c) sum of C18:2n6c, C18:3n6, C20:2n6, C20:3n6, C20:4n6, C22:4n6; d) sum of C18:3n3, C20:5n3, C22:5n3, C22:6n3;

Table 4: Odds Ratio (OR) and 95% confidence interval (CI) of colorectal cancer by red blood cell fatty acid composition

Fatty acids		Quintiles					Continuous variable, per 1 mol% increment	
		Q1	Q2	Q3	Q4	Q5	OR (95% CI)	p trend [§]
Saturated fatty acids (SFA)								
C14:0	Range	0.07 - 0.31	0.31 - 0.38	0.38 - 0.43	0.43 - 0.50	0.50 - 1.65		
	Cases/Controls (n)	200 / 213	234 / 213	184 / 214	223 / 214	228 / 215		
	OR, adjusted	1.00 (ref.)	1.16 (0.86; 1.55)	0.90 (0.66; 1.24)	1.08 (0.78; 1.49)	1.08 (0.79; 1.48)	1.10 (0.52; 2.34)	0.803
C16:0	Range	17.81 - 20.49	20.49 - 20.96	20.96 - 21.38	21.38 - 21.87	21.87 - 30.40		
	Cases/Controls (n)	179 / 212	219 / 215	224 / 214	221 / 214	226 / 214		
	OR, adjusted	1.00 (ref.)	1.17 (0.87; 1.56)	1.24 (0.92; 1.67)	1.17 (0.86; 1.59)	1.14 (0.82; 1.59)	1.03 (0.92; 1.16)	0.588
C18:0	Range	10.03 - 13.52	13.52 - 13.86	13.86 - 14.18	14.18 - 14.57	14.57 - 18.17		
	Cases/Controls (n)	201 / 212	175 / 215	240 / 213	210 / 215	243 / 214		
	OR, adjusted	1.00 (ref.)	0.89 (0.67; 1.20)	1.27 (0.96; 1.69)	1.11 (0.83; 1.49)	1.37 (1.01; 1.86)	1.23 (1.07; 1.42)	0.005 [#]
SFA, total ^a	Range	35.71 - 40.24	40.24 - 40.99	40.99 - 41.70	41.70 - 42.54	42.55 - 55.18		
	Cases/Controls (n)	194 / 213	198 / 213	202 / 215	225 / 214	250 / 214		
	OR, adjusted	1.00 (ref.)	1.12 (0.82; 1.53)	1.19 (0.84; 1.67)	1.36 (0.94; 1.95)	1.72 (1.15; 2.56)	1.13 (1.03; 1.24)	0.009 [#]
Odd-chain fatty acids (OCFA)								
C15:0	Range	0.03 - 0.26	0.26 - 0.33	0.33 - 0.39	0.39 - 0.50	0.50 - 1.20		
	Cases/Controls (n)	239 / 213	215 / 213	175 / 214	224 / 215	216 / 214		
	OR, adjusted	1.00 (ref.)	0.86 (0.63; 1.17)	0.67 (0.48; 0.95)	0.91 (0.64; 1.31)	0.93 (0.62; 1.38)	0.86 (0.33; 2.24)	0.758
C17:0	Range	0.07 - 0.30	0.30 - 0.34	0.34 - 0.37	0.37 - 0.41	0.41 - 0.60		
	Cases/Controls (n)	245 / 212	216 / 214	237 / 214	196 / 214	175 / 215		

OCFA, total ^e	OR, adjusted	1.00 (ref.)	0.91 (0.68; 1.20)	1.02 (0.77; 1.36)	0.81 (0.60; 1.10)	0.73 (0.54; 1.00)	0.49 (0.30; 0.80) §	0.004§
	Range	0.18 - 0.58	0.59 - 0.69	0.69 - 0.78	0.78 - 0.89	0.89 - 1.47		
	Cases/Controls (n)	231 / 213	237 / 214	193 / 213	205 / 215	203 / 214		
	OR, adjusted	1.00 (ref.)	1.08 (0.81; 1.45)	0.87 (0.63; 1.20)	0.88 (0.63; 1.24)	0.85 (0.58; 1.23)	0.56 (0.27; -1.14)	0.110
Monounsaturated fatty acids (MUFA)								
C16:1n7c	Range	0.05 - 0.35	0.35 - 0.44	0.44 - 0.52	0.52 - 0.62	0.62 - 2.74		
	Cases/Controls (n)	183 / 213	232 / 213	220 / 214	197 / 214	237 / 215		
	OR, adjusted	1.00 (ref.)	1.22 (0.91; 1.63)	1.13 (0.83; 1.54)	1.02 (0.74; 1.40)	1.10 (0.78; 1.55)	1.00 (0.61; 1.64)	0.987
C18:1n9c	Range	9.08 - 11.63	11.63 - 12.31	12.31 - 12.94	12.94 - 13.72	13.73 - 22.46		
	Cases/Controls (n)	185 / 212	241 / 215	237 / 213	199 / 214	207 / 215		
	OR, adjusted	1.00 (ref.)	1.26 (0.95; 1.67)	1.30 (0.97; 1.73)	1.05 (0.78; 1.41)	1.05 (0.76; 1.43)	1.00 (0.93; 1.09)	0.914
C18:1n7t+n9t ⁺	Range	0.00 - 0.41	0.41 - 0.50	0.50 - 0.59	0.59 - 0.74	0.74 - 2.46		
	Cases/Controls (n)	259 / 213	215 / 214	187 / 213	216 / 214	192 / 215		
	OR, adjusted	1.00 (ref.)	0.86 (0.65; 1.13)	0.70 (0.52; 0.95)	0.78 (0.57; 1.08)	0.66 (0.46; 0.95)	0.56 (0.33; 0.96)	0.035
MUFA, total ^b	Range	13.11 - 16.92	16.92 - 17.71	17.72 - 18.41	18.41 - 19.30	19.30 - 27.87		
	Cases/Controls (n)	217 / 212	202 / 214	202 / 214	214 / 214	234 / 215		
	OR, adjusted	1.00 (ref.)	0.95 (0.72; 1.26)	0.90 (0.67; 1.21)	0.96 (0.70; 1.31)	1.03 (0.74; 1.42)	0.99 (0.93; 1.06)	0.775
n-6 Polyunsaturated fatty acids (n-6 PUFA)								
C18:2n6c	Range	6.56 - 9.48	9.48 - 10.24	10.25 - 10.93	10.93 - 12.00	12.00 - 18.25		
	Cases/Controls (n)	242 / 212	197 / 215	211 / 213	227 / 215	192 / 214		
	OR, adjusted	1.00 (ref.)	0.87 (0.66; 1.15)	0.92 (0.70; 1.22)	1.01 (0.76; 1.34)	0.85 (0.62; 1.16)	0.98 (0.91; 1.04)	0.452
C20:3n6	Range	0.07 - 1.33	1.33 - 1.49	1.49 - 1.65	1.65 - 1.86	1.86 - 3.41		
	Cases/Controls (n)	212 / 212	217 / 214	203 / 215	214 / 213	223 / 215		
	OR, adjusted	1.00 (ref.)	1.02 (0.77; 1.35)	0.90 (0.68; 1.20)	0.95 (0.72; 1.26)	0.97 (0.72; 1.29)	0.93 (0.71; 1.21)	0.572

C20:4n6	Range	3.87 - 11.86	11.86 - 12.72	12.72 - 13.47	13.47 - 14.39	14.39 - 17.45		
	Cases/Controls (n)	174 / 213	220 / 213	244 / 214	226 / 215	205 / 214		
	OR, adjusted	1.00 (ref.)	1.28 (0.95; 1.71)	1.53 (1.12; 2.07)	1.46 (1.05; 2.02)	1.27 (0.90; 1.79)	2.26 (0.89; 5.74) §	0.085§
C22:4n6	Range	0.68 - 2.04	2.04 - 2.37	2.37 - 2.70	2.71 - 3.15	3.15 - 8.54		
	Cases/Controls (n)	186 / 212	194 / 214	225 / 215	237 / 213	227 / 215		
	OR, adjusted	1.00 (ref.)	1.05 (0.78; 1.40)	1.29 (0.95; 1.74)	1.43 (1.04; 1.95)	1.39 (0.99; 1.94)	1.22 (1.04; 1.45)	0.018
n-6 PUFA, total ^c	Range	14.94 - 26.24	26.25 - 27.58	27.58 - 29.02	29.02 - 30.45	30.46 - 37.73		
	Cases/Controls (n)	191 / 212	200 / 214	263 / 215	197 / 214	218 / 214		
	OR, adjusted	1.00 (ref.)	1.14 (0.85; 1.52)	1.53 (1.14; 2.06)	1.17 (0.85; 1.62)	1.33 (0.94; 1.89)	1.89 (0.55; 6.45) §	0.310§
n-3 Polyunsaturated fatty acids (n-3 PUFA)								
C18:3n3	Range	0.00 - 0.07	0.07 - 0.11	0.11 - 0.15	0.15 - 0.20	0.20 - 1.38		
	Cases/Controls (n)	195 / 212	218 / 214	233 / 214	225 / 214	198 / 215		
	OR, adjusted	1.00 (ref.)	1.04 (0.78; 1.40)	1.13 (0.83; 1.55)	1.10 (0.79; 1.53)	0.98 (0.69; 1.40)	0.95 (0.29; 3.12)	0.927
C20:5n3	Range	0.10 - 0.66	0.66 - 0.90	0.90 - 1.13	1.13 - 1.48	1.48 - 5.82		
	Cases/Controls (n)	232 / 212	245 / 214	207 / 215	191 / 214	194 / 214		
	OR, adjusted	1.00 (ref.)	1.01 (0.76; 1.33)	0.77 (0.57; 1.05)	0.68 (0.49; 0.95)	0.68 (0.49; 0.96)	0.75 (0.62; 0.92)	0.005 [#]
C22:5n3	Range	0.64 - 2.11	2.11 - 2.38	2.39 - 2.58	2.58 - 2.79	2.79 - 5.71		
	Cases/Controls (n)	229 / 212	208 / 215	199 / 214	202 / 213	231 / 215		
	OR, adjusted	1.00 (ref.)	0.78 (0.56; 1.09)	0.80 (0.57; 1.13)	0.79 (0.55; 1.13)	0.91 (0.63; 1.31)	0.90 (0.69; 1.18)	0.458
C22:6n3	Range	1.34 - 4.85	4.86 - 5.56	5.56 - 6.18	6.18 - 6.92	6.92 - 10.55		
	Cases/Controls (n)	236 / 213	239 / 214	207 / 213	192 / 214	195 / 215		
	OR, adjusted	1.00 (ref.)	0.92 (0.70; 1.21)	0.77 (0.58; 1.03)	0.72 (0.54; 0.97)	0.70 (0.51; 0.97)	0.61 (0.38; 0.97) §	0.038§
n-3 PUFA, total ^d	Range	2.33 - 8.14	8.14 - 9.04	9.04 - 9.94	9.94 - 10.99	10.99 - 18.69		
	Cases/Controls (n)	238 / 213	232 / 214	219 / 214	178 / 214	202 / 214		

OR, adjusted	1.00 (ref.)	0.91 (0.69; 1.20)	0.78 (0.58; 1.05)	0.63 (0.46; 0.87)	0.73 (0.52; 1.01)	0.93 (0.88; 0.98)	0.013 [#]
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a) sum of C14:0, C16:0, C18:0, C20:0; b) sum of C16:1n7c, C18:1n9c, C18:1n7c, C20:1n9c; c) sum of C18:2n6c, C18:3n6, C20:2n6, C20:3n6, C20:4n6, C22:4n6;

d) sum of C18:3n3, C20:5n3, C22:5n3, C22:6n3; e) sum of C15:0, C17:0;

OR, adjusted: conditional logistic regression adjusted for BMI, smoking status, education, physical activity, alcohol intake, history of diabetes, and season of blood collection

§ log-transformed; \$ Wald test statistics; + Sum of vaccenic acid and elaidic acid; # significant after correction for multiple comparisons (Benjamini-Hochberg);

Table 5: Multivariable adjusted Odds Ratio (OR) and 95% confidence interval (CI) of colorectal cancer by sub-site in association with red blood cell fatty acid composition

Fatty acids	Site	Quintiles					Continuous variable	
		Q1	Q2	Q3	Q4	Q5	OR (95% CI) [‡]	p trend [§]
C17:0	Colon	1.00 (ref.)	0.87 (0.61; 1.24)	1.06 (0.74; 1.53)	0.81 (0.55; 1.20)	0.61 (0.41; 0.91)	0.36 (0.19; 0.69)§	0.002§
	Colon prox.	1.00 (ref.)	1.37 (0.74; 2.54)	1.91 (1.05; 3.46)	1.08 (0.58; 2.03)	1.10 (0.56; 2.15)	0.64 (0.23; 1.79) §	0.395§
	Colon dist.	1.00 (ref.)	0.65 (0.39; 1.08)	0.65 (0.38; 1.12)	0.46 (0.26; 0.82)	0.40 (0.23; 0.71)	0.16 (0.06; 0.43)§	0.000§
	Rectum	1.00 (ref.)	1.04 (0.65; 1.68)	1.02 (0.63; 1.65)	0.84 (0.50; 1.39)	1.03 (0.61; 1.73)	0.76 (0.35; 1.66)§	0.492§
C18:0	Colon	1.00 (ref.)	0.95 (0.65; 1.38)	1.18 (0.82; 1.70)	1.05 (0.72; 1.52)	1.42 (0.96; 2.12)	1.22 (1.02; 1.46)	0.034
	Colon prox.	1.00 (ref.)	1.12 (0.61; 2.03)	1.28 (0.70; 2.33)	1.16 (0.64; 2.13)	2.44 (1.25; 4.77)	1.38 (1.01; 1.89)	0.042
	Colon dist.	1.00 (ref.)	0.89 (0.51; 1.53)	1.23 (0.73; 2.08)	1.11 (0.65; 1.89)	1.16 (0.66; 2.05)	1.18 (0.92; 1.52)	0.184
	Rectum	1.00 (ref.)	0.87 (0.54; 1.43)	1.48 (0.92; 2.38)	1.39 (0.84; 2.30)	1.38 (0.84; 2.27)	1.29 (1.01; 1.64)	0.040
C18:1n7t+n9t	Colon	1.00 (ref.)	0.79 (0.55; 1.15)	0.69 (0.47; 1.02)	0.83 (0.55; 1.25)	0.77 (0.48; 1.22)	0.80 (0.42; 1.51)	0.492
	Colon prox.	1.00 (ref.)	1.05 (0.57; 1.94)	0.60 (0.31; 1.18)	0.69 (0.35; 1.37)	0.92 (0.44; 1.92)	0.94 (0.37; 2.38)	0.892
	Colon dist.	1.00 (ref.)	0.61 (0.36; 1.04)	0.71 (0.41; 1.25)	0.86 (0.48; 1.54)	0.57 (0.29; 1.12)	0.53 (0.20; 1.39)	0.200
	Rectum	1.00 (ref.)	0.94 (0.60; 1.47)	0.77 (0.48; 1.23)	0.77 (0.45; 1.31)	0.53 (0.28; 0.99)	0.27 (0.10; 0.73)	0.010
C20:4n6	Colon	1.00 (ref.)	1.39 (0.95; 2.05)	1.70 (1.15; 2.52)	1.57 (1.03; 2.37)	1.38 (0.89; 2.12)	3.28 (1.02; 10.62)§	0.047§
	Colon prox.	1.00 (ref.)	1.85 (0.99; 3.45)	1.79 (0.98; 3.30)	1.59 (0.82; 3.08)	1.09 (0.54; 2.22)	2.05 (0.29; 14.34)§	0.468§
	Colon dist.	1.00 (ref.)	1.29 (0.73; 2.28)	1.86 (1.03; 3.36)	1.63 (0.89; 2.98)	1.68 (0.88; 3.19)	6.10 (1.08; 34.36)§	0.041§
	Rectum	1.00 (ref.)	1.20 (0.74; 1.92)	1.40 (0.84; 2.35)	1.35 (0.79; 2.32)	1.30 (0.72; 2.36)	1.76 (0.36; 8.56)§	0.486§
C22:4n6	Colon	1.00 (ref.)	1.06 (0.73; 1.54)	1.25 (0.84; 1.86)	1.50 (1.00; 2.25)	1.30 (0.85; 1.99)	1.22 (0.99; 1.50)	0.062
	Colon prox.	1.00 (ref.)	0.75 (0.39; 1.44)	1.02 (0.52; 2.03)	1.06 (0.54; 2.07)	1.18 (0.57; 2.44)	1.24 (0.89; 1.73)	0.194
	Colon dist.	1.00 (ref.)	1.04 (0.62; 1.76)	1.50 (0.85; 2.64)	1.89 (1.03; 3.48)	1.50 (0.81; 2.80)	1.39 (1.00; 1.94)	0.048
	Rectum	1.00 (ref.)	1.05 (0.64; 1.72)	1.41 (0.86; 2.30)	1.33 (0.80; 2.22)	1.58 (0.89; 2.79)	1.23 (0.92; 1.65)	0.157
C20:5n3	Colon	1.00 (ref.)	0.99 (0.71; 1.40)	0.76 (0.53; 1.10)	0.80 (0.53; 1.22)	0.81 (0.53; 1.23)	0.79 (0.62; 1.01)	0.062
	Colon prox.	1.00 (ref.)	0.92 (0.54; 1.57)	0.58 (0.32; 1.04)	0.55 (0.28; 1.10)	0.72 (0.36; 1.44)	0.86 (0.58; 1.27)	0.451

Fatty acids	Site	Quintiles					Continuous variable	
		Q1	Q2	Q3	Q4	Q5	OR (95% CI) [‡]	p trend [§]
C22:6n3	Colon dist.	1.00 (ref.)	0.98 (0.59; 1.64)	0.83 (0.49; 1.41)	0.87 (0.48; 1.57)	0.77 (0.41; 1.45)	0.63 (0.43; 0.91)	0.015
	Rectum	1.00 (ref.)	0.98 (0.58; 1.64)	0.68 (0.38; 1.20)	0.47 (0.26; 0.85)	0.49 (0.27; 0.89)	0.67 (0.48; 0.94)	0.021
	Colon	1.00 (ref.)	0.91 (0.65; 1.27)	0.76 (0.53; 1.10)	0.66 (0.46; 0.95)	0.72 (0.49; 1.07)	0.57 (0.31; 1.04)§	0.065§
	Colon prox.	1.00 (ref.)	1.02 (0.59; 1.76)	0.59 (0.31; 1.11)	0.85 (0.48; 1.50)	0.81 (0.42; 1.56)	0.55 (0.21; 1.48)§	0.236§
	Colon dist.	1.00 (ref.)	0.84 (0.51; 1.38)	0.88 (0.53; 1.46)	0.53 (0.31; 0.89)	0.63 (0.36; 1.11)	0.46 (0.19; 1.11)§	0.084§
	Rectum	1.00 (ref.)	0.95 (0.59; 1.55)	0.82 (0.49; 1.36)	0.88 (0.52; 1.49)	0.65 (0.37; 1.13)	0.66 (0.29; 1.46)§	0.302§

[‡] conditional logistic regression adjusted for BMI, smoking status, education, physical activity, alcohol intake, history of diabetes, and season of blood collection;

[§] Wald test statistics; § log-transformed;

Figure 1: The forest plots of figure 1 show the odds ratios for CRC and their corresponding 95% CIs, total and for each country participating in the EPIC cohort, for the RBC content of stearic acid (Figure 1A)* and of eicosapentaenoic acid (Figure 1B)*

* Cases from France were excluded from these figures;

1A

1B

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