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 fatty acid measurements to objectively assess individual FA and FA categories are scarce.

Objective: 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.

Design: 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 results obtained for eicosapentaenoic acid support a protective effect of fish consumption on CRC risk. The findings for stearic acid reflect differences in FA intake and metabolism between cancer cases and matched controls, assessed in RBCs obtained prior to diagnosis. As for stearic acid, the results obtained for long-chain n-6 PUFA deserve further investigation.

Short title: Erythrocyte fatty acids and colorectal cancer in EPIC

Keywords: colorectal cancer; fatty acids; erythrocytes; cohort study; biomarker; EPIC

Introduction

Colorectal cancer (CRC) is associated with Western lifestyle [1]. 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 [2]. Experimental and epidemiological evidence indicates that nutritional and nutrition-related factors modulate CRC risk [3]. 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 [3]. Fatty acids are among the nutrients which are hypothesized to affect the risk of CRC [4, 5]. Among these, the role of n-3 and n-6 polyunsaturated fatty acids (PUFA) is of particular interest [6-8].

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 [9]. However, in humans these associations are less clear [7, 10].

Higher fish intake, the main source of EPA and DHA, has been consistently reported as potentially protective for CRC [7]. 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 to overcome important limitations of dietary intake data [11-13]. Adipose tissue composition reflects best long-term 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 more easily to collect. Compared to fatty acids in plasma phospholipids (as a possible alternative specimen), 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. However, it has to be acknowledged that RBCs are not fully comparable to eicosanoid producing target cells in the colon in terms of their enzymatic properties, e.g. expression of delta-5 and delta-6 desaturases. Due to their long half-life time of about 120 days, RBC fatty acids may reflect medium-term fatty acid supply from the diet, at least for some fatty acids [13, 14]. Significant correlations between dietary fatty acid intake (FFQ-derived) and their proportion in RBC membranes have been reported for very-long chain PUFA, especially fish oil fatty acids (EPA, DHA), odd-numbered fatty acids as markers of dairy fat intake (pentadecanoic acid, heptadecanoic acid), and trans fatty acids [15-17]. This has been confirmed in a randomized cross-over intervention study with chemical analysis of the diet [18]. 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 [18]. To date, only a few prospective studies have assessed the role of fatty acid levels in CRC development by measuring circulating biomarkers of FA [5, 19-22], 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 [23-25].

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 [24].

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.

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 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 etc.), follow-up time since blood collection, time of the day at blood collection (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 [26]. 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).

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), educational level (none/primary, secondary, or higher degree), physical activity (inactive, moderately inactive, active), self-reported diabetes status at baseline (yes, no), and season of blood collection. Analyses of dietary sources of fatty acids were not conducted.

Additionally, sub-analyses were performed for 769 cases and their matched controls adjusting for 25-hydroxyvitamin D plasma concentrations. 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 of the colon cancers were attributed to the distal colon and 40% to the proximal colon (table 1). In comparison to the controls, rectum cancer cases were more

likely to have high alcohol consumption (≥ 40g/day). There was little difference in mean BMI between cases and controls for both colon and rectum cancer.

For the control group, the fatty acid composition of RBC membranes and the sums of 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 saturated fatty acids (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.

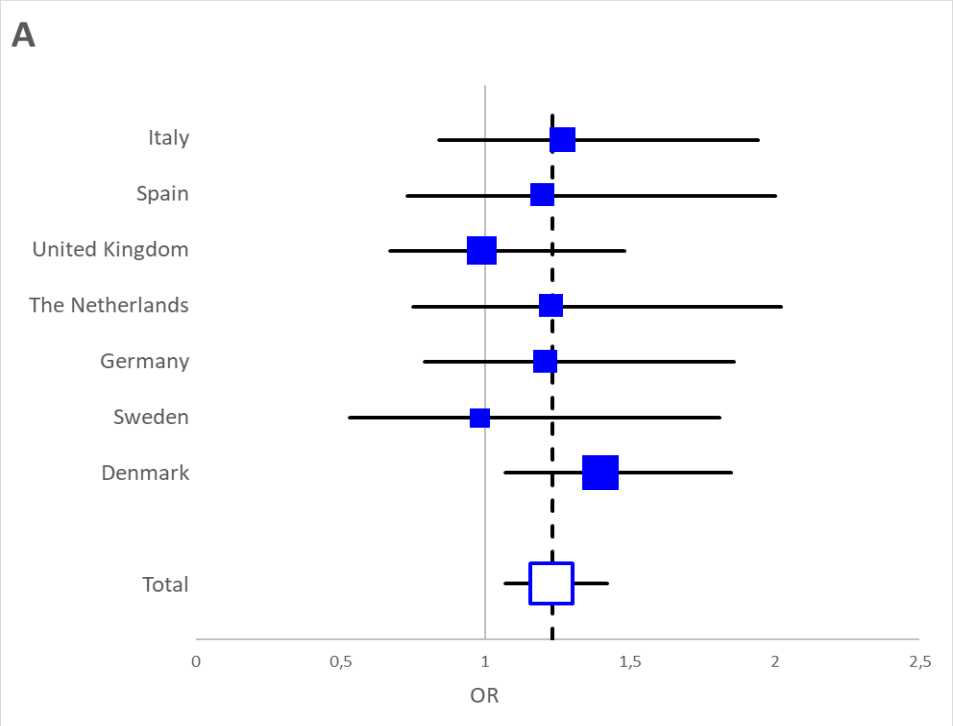
The odd-numbered fatty acid heptadecanoic acid as a putative biomarker of dairy consumption was inversely associated with CRC risk (OR=0.49; 95%CI=0.33-0.80). Likewise, C18:1 trans fatty acids (sum of vaccenic and elaidic acid; their peaks could not be separated with the chosen laboratory methods) showed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

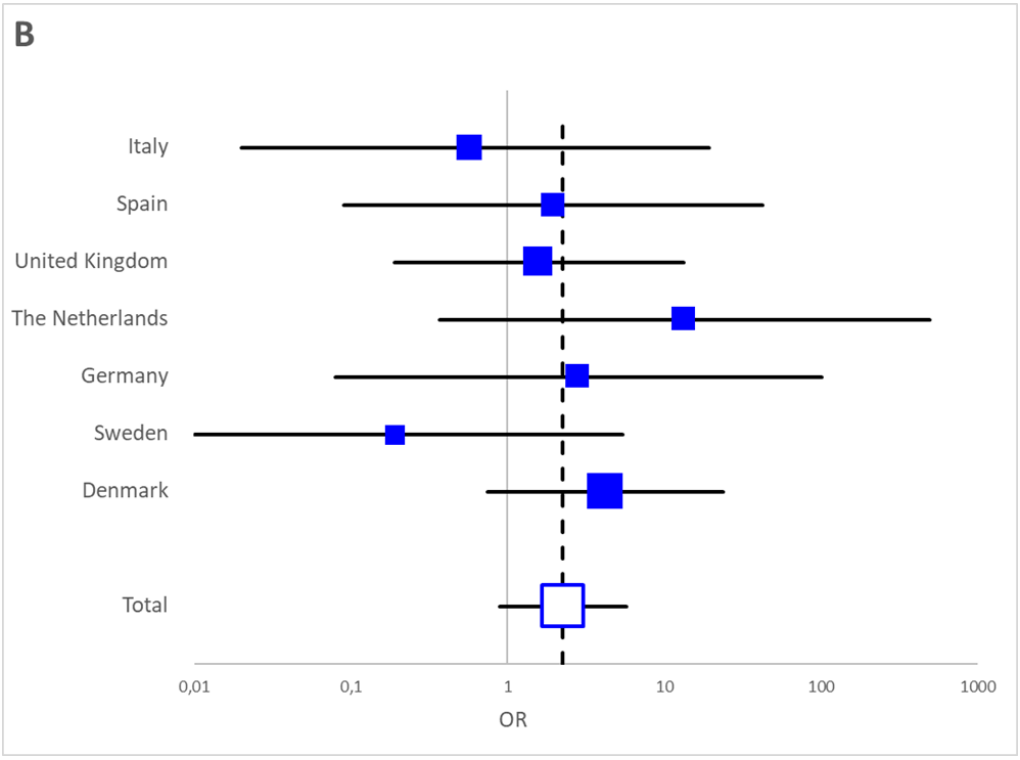
Concerning n-6 PUFA, a statistically significant positive relationship between the AA content in RBC membranes and CRC risk was seen for the 3rd (OR=1.53; 95%CI= 1.12-2.07) and 4th quintile (OR=1.46; 95%CI=1.05-2.02) compared to the 1st quintile; using the continuous variable, OR estimates increased 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), AA (Figure 1B), and EPA (Figure 1C).





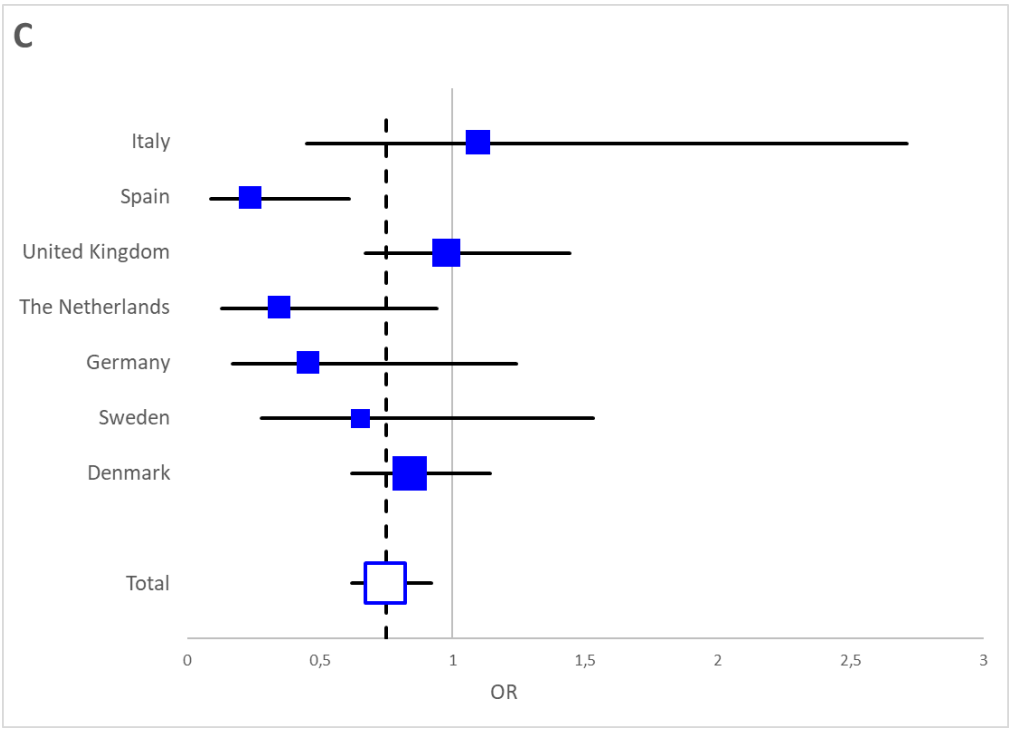


Figure 1: Forest plots: Odds Ratios for CRC and their corresponding 95% CIs for the RBC content of stearic acid (Figure 1A)*, of arachidonic acid (Figure 1B)* and of eicosapentaenoic acid (Figure 1C)*, for each country and total

* Cases from France were excluded from these figures;

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 association for former smokers. There was also evidence for significant heterogeneity for the association between stearic acid and CRC by country (P = 0.018) (results not shown).

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 [20] or found no statistically significant association for the total SFA content [19]. 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 (Elov1-5, Elov1-6) 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]. SFA, including stearic acid, resulted in reduced accumulation of p53, compromised induction of p21 and Bax expression and increased cell proliferation in response to double stranded breaks and single stranded DNA in primary mouse fibroblasts [35]. High intake of SFA may also modulate CRC risk through an increased bile acid production [36] and elevated diacylglycerol levels [37]. Bile acids have been shown

to cause DNA damage [38] and possibly play a role in the modulation of COX-2 expression [39], which is thought to play a role in CRC development.

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 [40]; however, it can also be of endogenous origin. 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 [3, 41]. The protective effect of dairy products is likely due to their high calcium content. Results from a previous analysis in EPIC confirmed that a higher intake of dietary calcium was associated with a reduced risk of CRC [42] as well as colorectal adenoma [43]. 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 does-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]. However, a Japanese cohort study reported no association between serum n-6 PUFA and CRC risk [19]. Another nested case-control study found a positive association between colon cancer and the ratio of plasma AA/LA as indicator of fatty acid desaturase activity [22].

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 [5, 7] and fits with the reported inverse association between dietary fish intake or the intake of n-3 PUFA in EPIC [5] or in a recent meta-analyses of prospective studies [7]. This recently published meta-analysis found a small inverse dose response relationship between blood levels of n-3 PUFA and CRC risk [7]. 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 [44].

N-3 and n-6 PUFA use the same enzymes for conversion to different eicosanoids with different biological properties [45]. N-3 PUFA were shown to have an effect on cell proliferation and apoptosis, and exert anti-inflammatory functions [46]. Inhibition of the synthesis of pro-inflammatory cytokines, e.g., interleukin-1 beta (IL-1ß) and tumor necrosis factor alpha (TNF-α), has been observed with supplementation of n-3 PUFA in humans [47]. This effect is likely to be mediated via decreased activity of the NF-κB 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 E_2, are well described [48]. PGE_2 itself can promote tumor growth by activating signaling pathways which control cell proliferation and apoptosis [49]. 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 [50], 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 [51] as well as alteration in membrane fluidity and subsequent signalling processes [52]. 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 [53]. Direct evidence for suppression of inflammation-

driven tumor progression by n-3 PUFA has been reported using fat-1 transgenic mice [54]. These mice, which convert endogenous n-6 PUFA to n-3 PUFA in multiple tissues, were at reduced risk of colitis-associated colon cancer. Further, the crucial balance between colonic epithelial cell proliferation and apoptosis was shown to be favourably affected by dietary n-3 PUFA [55-57]. Apoptotic processes are progressively inhibited during colon cancer development [58] which may be restored by n-3 PUFA. Cox-2 downregulation appears to be the mechanism underlying the apoptotic effect of n-3 PUFA in colon cancer cells [59]. However, proteasome-dependent degradation of b-catenin and downregulation of survivin, an important anti-apoptotic factor, may be implicated as well [60].

Increasingly, an interaction between PUFA and the epigenome has been reported, with effects at the global as well as the gene-specific level [61]. 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 [62]. Stearic and palmitic acid, in mice, were shown to modulate methylation levels of the PPARγ promoter in murine macrophages triggering expression of proinflammatory factors suggesting one mechanism via which these SFAs may promote colon tumorigenesis [63]. This favouring of the proinflammatory milieu and dysregulation of lipid metabolism through epigenetic mechanisms triggered by n-6 PUFA and SFA is also supported by other pieces of research as summarized in a recent review [64].

The major strength of our study is its large sample size and its prospective design. Blood samples were collected, at baseline of the study, prior to diagnosis, and thus we could exclude the possibility that the cancer itself, subsequent medication, or a change in the patients` dietary habits following the cancer diagnosis and treatment, could subsequently have entailed changes in the fatty acid composition of the erythrocyte phospholipids. Sensitivity analysis

with exclusion of cases diagnosed within the first two years of follow-up complemented this approach. Additional robustness of results was obtained by correction for multiple comparisons. Although having adjusted for several common CRC risk factors, residual and unmeasured confounding cannot be excluded, and thus still remains an important limitation. 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. The lack 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 (±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 (±7.1)	58.9 (±7.1)		58.0 (±6.5)	58.0 (±6.5)	
Height (cm)		168.4 (±9.3)	167.3 (±9.1)	*<0.001*	168.7 (±9.1)	169.0 (±9.1)	*0.553*
Weight (kg)		76.7 (±14.5)	74.4 (±12.7)	*<0.001*	76.3 (±13.6)	75.7 (±14.3)	*0.414*
BMI (kg/m²)		27.0 (±4.4)	26.6 (±3.9)	*0.034*	26.8 (±4.0)	26.4 (±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	56 (8.4%)			
	Rectosigmoid junction			98 (24.6%)	
	Rectum, NOS			301 (75.4%)	

* chi² test or t-test for matched pairs;

Abbreviations: 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)

Abbreviations:

[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)
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)

Fatty acids	Colon cancer cases	Matched Controls	Rectal cancer cases	Matched Controls
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		
	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§
OCFA, total [e]	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[#]

[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;

Abbreviations: § log-transformed; [$] Wald test statistics; [+] Sum of vaccenic acid and elaidic acid; [#] significant after correction for multiple comparisons (Benjamini-Hochberg); OR, adjusted ‡ conditional logistic regression adjusted for BMI, smoking status, education, physical activity, alcohol intake, history of diabetes, and season of blood collection

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		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
	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

Fatty acids		Quintiles					Continuous variable	
		Q1	Q2	Q3	Q4	Q5	OR (95% CI) ‡	p trend$
	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
C22:6n3	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§

[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; § log-transformed; $ Wald test statistics

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

Supplementary Table S1: Odds Ratio (OR) and 95% confidence interval (CI) of colorectal cancer by red blood cell fatty acid composition, first two years excluded

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)	131 / 150	183 / 167	148 / 169	174 / 160	176 / 166		
	OR, adjusted	1.00 (ref.)	1.26 (0.89; 1.79)	1.01 (0.69; 1.47)	1.25 (0.85; 1.83)	1.23 (0.85; 1.78)	1.28 (0.52; 3.17)	0.589
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)	138 / 150	169 / 164	180 / 166	157 / 156	168 / 176		
	OR, adjusted	1.00 (ref.)	1.03 (0.74; 1.44)	1.13 (0.80; 1.59)	0.98 (0.69; 1.40)	0.91 (0.62; 1.32)	0.93 (0.81; 1.07)	0.343
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)	154 / 172	144 / 157	186 / 165	151 / 172	177 / 146		
	OR, adjusted	1.00 (ref.)	1.07 (0.77; 1.49)	1.33 (0.97; 1.84)	1.06 (0.76; 1.48)	1.51 (1.07; 2.14)	1.25 (1.06; 1.46)	0.007
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)	157 / 162	157 / 171	150 / 157	173 / 161	175 / 161		
	OR, adjusted	1.00 (ref.)	0.98 (0.69; 1.38)	1.03 (0.70; 1.52)	1.19 (0.78; 1.79)	1.29 (0.81; 2.05)	1.07 (0.96; 1.19)	0.221
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)	168 / 148	160 / 171	139 / 162	181 / 166	164 / 165		
	OR, adjusted	1.00 (ref.)	0.78 (0.55; 1.12)	0.71 (0.48; 1.05)	0.96 (0.64; 1.43)	0.92 (0.58; 1.44)	0.89 (0.30; 2.63)	0.834
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)	175 / 163	172 / 165	182 / 168	155 / 156	128 / 160		
	OR, adjusted	1.00 (ref.)	1.00 (0.72; 1.37)	1.06 (0.76; 1.47)	0.92 (0.65; 1.30)	0.77 (0.54; 1.10)	0.52 (0.30; 0.91)§	0.023§

OCFA, total [e]	Range	0.18 - 0.58	0.59 - 0.69	0.69 - 0.78	0.78 - 0.89	0.89 - 1.47		
	Cases/Controls (n)	167 / 156	175 /162	157 / 165	156 / 168	157 / 161		
	OR, adjusted	1.00 (ref.)	1.05 (0.75; 1.47)	0.90 (0.62; 1.30)	0.85 (0.58; 1.25)	0.85 (0.56; 1.32)	0.60 (0.27; 1.36)	0.221
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)	174 / 147	170 / 161	173 / 163	153 / 161	182 / 180		
	OR, adjusted	1.00 (ref.)	1.09 (0.78; 1.52)	1.09 (0.77; 1.56)	0.98 (0.68; 1.43)	0.99 (0.67; 1.46)	0.85 (0.49; 1.49)	0.575
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)	142 / 148	179 / 157	179 / 169	148 / 171	164 / 167		
	OR, adjusted	1.00 (ref.)	1.18 (0.85; 1.65)	1.15 (0.82; 1.62)	0.91 (0.64; 1.30)	1.01 (0.70; 1.44)	0.99 (0.90; 1.08)	0.805
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)	181 / 156	162 / 157	145 / 161	169 / 171	153 / 167		
	OR, adjusted	1.00 (ref.)	0.93 (0.67; 1.28)	0.77 (0.55; 1.09)	0.80 (0.55; 1.15)	0.74 (0.49; 1.13)	0.66 (0.36; 1.20)	0.174
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)	173 / 151	152 / 163	143 / 165	169 / 171	175 / 162		
	OR, adjusted	1.00 (ref.)	0.84 (0.60; 1.16)	0.73 (0.51; 1.03)	0.85 (0.59; 1.22)	0.93 (0.64; 1.36)	0.97 (0.90; 1.05)	0.471
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)	165 / 158	149 / 160	166 / 171	172 / 169	160 / 154		
	OR, adjusted	1.00 (ref.)	1.00 (0.72; 1.39)	1.00 (0.72; 1.38)	1.08 (0.77; 1.52)	1.14 (0.78; 1.65)	1.02 (0.95; 1.10)	0.628
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)	160 / 168	162 / 159	167 / 160	160 / 162	163 / 163		
	OR, adjusted	1.00 (ref.)	1.07 (0.78; 1.48)	1.04 (0.76; 1.44)	1.00 (0.72; 1.38)	1.00 (0.72; 1.39)	0.98 (0.72; 1.33)	0.888
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)	134 / 156	177 / 172	196 / 167	154 / 157	151 / 160		

	OR, adjusted	1.00 (ref.)	1.18 (0.85; 1.64)	1.50 (1.05; 2.13)	1.23 (0.84; 1.79)	1.13 (0.76; 1.69)	2.17 (0.72; 6.50)§	0.168§
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)	142 / 163	161 / 168	170 / 158	178 / 157	161 / 166		
	OR, adjusted	1.00 (ref.)	1.10 (0.79; 1.52)	1.32 (0.93; 1.86)	1.37 (0.97; 1.95)	1.21 (0.83; 1.76)	1.18 (0.98; 1.42)	0.083
	Range	14.94 - 26.24	26.25 - 27.58	27.58 - 29.02	29.02 - 30.45	30.46 - 37.73		
n-6 PUFA, total [c]	Cases/Controls (n)	149 / 158	154 / 174	189 / 164	149 / 156	171 / 160		
	OR, adjusted	1.00 (ref.)	1.04 (0.75; 1.44)	1.39 (0.98; 1.96)	1.15 (0.80; 1.67)	1.34 (0.90; 2.01)	2.84 (0.67; 12.01)§	0.157§
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)	142 / 151	164 / 152	169 / 169	172 / 170	164 / 170		
	OR, adjusted	1.00 (ref.)	1.07 (0.77; 1.50)	1.02 (0.71; 1.47)	1.03 (0.71; 1.51)	1.00 (0.67; 1.50)	1.17 (0.31; 4.45)	0.821
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)	168 / 153	183 / 158	153 / 171	152 / 164	156 / 166		
	OR, adjusted	1.00 (ref.)	1.03 (0.75; 1.41)	0.72 (0.51; 1.03)	0.71 (0.49; 1.05)	0.74 (0.50; 1.09)	0.79 (0.63; 0.99)	0.037
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)	163 / 157	159 / 160	148 / 154	168 / 160	174 / 181		
	OR, adjusted	1.00 (ref.)	0.87 (0.59; 1.27)	0.89 (0.60; 1.33)	0.94 (0.62; 1.42)	0.87 (0.57; 1.33)	0.90 (0.65; 1.23)	0.500
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)	179 / 167	183 / 161	155 / 164	146 / 159	149 / 161		
	OR, adjusted	1.00 (ref.)	0.97 (0.71; 1.32)	0.77 (0.55; 1.07)	0.76 (0.55; 1.07)	0.75 (0.52; 1.09)	0.70 (0.40; 1.21)§	0.199§
	Range	2.33 - 8.14	8.14 - 9.04	9.04 - 9.94	9.94 - 10.99	10.99 - 18.69		
n-3 PUFA, total [d]	Cases/Controls (n)	181 / 160	173 / 168	154 / 152	140 / 170	164 / 162		
	OR, adjusted	1.00 (ref.)	0.87 (0.64; 1.19)	0.77 (0.55; 1.09)	0.61 (0.42; 0.89)	0.82 (0.56; 1.20)	0.94 (0.88; 1.01)	0.076

[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; § log-transformed; [+] Sum of vaccenic acid and elaidic acid; [$] Wald test statistics; OR, adjusted ‡ conditional logistic regression adjusted for BMI, smoking status, education, physical activity, alcohol intake, history of diabetes, and season of blood collection

Supplementary Table S2: Odds Ratio (OR) and 95% confidence interval (CI) of colorectal cancer by red blood cell fatty acid composition, total FU, adjusted for plasma 25-hydroxy- vitamin D concentration

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 - 0.32	0.32 - 0.38	0.38 - 0.43	0.43 - 0.50	0.50 - 1.65		
	Cases/Controls (n)	154 / 153	156 / 153	139 / 155	153 / 154.	167 / 154		
	OR, adjusted	1.00 (ref.)	0.98 (0.69; 1.39)	0.84 (0.58; 1.22)	0.90 (0.62; 1.31)	0.96 (0.67; 1.38)	1.06 (0.43; 2.58)	0.902
C16:0	Range	17.81 - 20.52	20.52 - 20.99	20.99 - 21.39	21.39 - 21.86	21.86 - 30.40		
	Cases/Controls (n)	133 / 152	163 / 154	145 / 155	154 / 154	174 / 154		
	OR, adjusted	1.00 (ref.)	1.16 (0.83; 1.62)	1.05 (0.75; 1.48)	1.09 (0.77; 1.56)	1.17 (0.80; 1.72)	1.08 (0.94; 1.25)	0.267
C18:0	Range	11.29 - 13.54	13.54 - 13.88	13.88 - 14.18	14.18 - 14.58	14.58 - 17.90		
	Cases/Controls (n)	153 / 152	133 / 155	161 / 154	151 / 153	171 / 155		
	OR, adjusted	1.00 (ref.)	0.90 (0.64; 1.26)	1.08 (0.77; 1.51)	1.07 (0.76; 1.50)	1.25 (0.87; 1.80)	1.17 (0.99; 1.39)	0.065
Saturated FA,	Range	35.86 - 40.28	40.30 - 41.04	41.05 - 41.78	41.78 - 42.64	42.64 - 55.18		
total [a]	Cases/Controls (n)	124 / 153	156 / 153	144 / 154	175 / 154	170 / 155		
	OR, adjusted	1.00 (ref.)	1.55 (1.05; 2.30)	1.54 (1.01; 2.34)	1.88 (1.21; 2.93)	1.84 (1.14; 2.97)	1.12 (1.01; 1.24)	0.033
Odd-chain fatty acids (OCFA)								
C15:0	Range	0.03 - 0.26	0.26 - 0.33	0.33 - 0.39	0.39 - 0.49	0.49 - 1.20		
	Cases/Controls (n)	164 / 153	175 / 153	133 / 155	144 / 153	153 / 155		
	OR, adjusted	1.00 (ref.)	1.07 (0.75; 1.52)	0.74 (0.49; 1.11)	0.74 (0.47; 1.15)	0.81 (0.49; 1.34)	0.60 (0.18; 1.98)	0.404
C17:0	Range	0.11 - 0.30	0.30 - 0.34	0.34 - 0.37	0.37 - 0.41	0.41 - 0.60		
	Cases/Controls (n)	182 / 152	152 / 154	177 / 154	143 / 155	115 / 154		

	OR, adjusted	1.00 (ref.)	0.85 (0.61; 1.18)	1.02 (0.73; 1.42)	0.78 (0.55; 1.12)	0.63 (0.43; 0.90)	0.39 (0.21; 0.70)§	0.002§
OCFA, total [e]	Range	0.24 - 0.58	0.58 - 0.68	0.68 - 0.76	0.76 - 0.88	0.88 - 1.47		
	Cases/Controls (n)	162 / 152	187 /155	138 / 153	148 / 154	134 / 155		
	OR, adjusted	1.00 (ref.)	1.21 (0.86; 1.69)	0.88 (0.60; 1.28)	0.84 (0.56; 1.26)	0.69 (0.44; 1.09)	0.37 (0.16; 0.90)	0.028
Monounsaturated fatty acids (MUFA)								
C16:1n7c	Range	0.05 - 0.35	0.35 - 0.45	0.45 - 0.52	0.52 - 0.61	0.61 - 2.15		
	Cases/Controls (n)	132 / 152	169 / 155	150 / 154	132 / 153	186 / 155		
	OR, adjusted	1.00 (ref.)	1.24 (0.88; 1.76)	1.03 (0.72; 1.48)	0.96 (0.66; 1.39)	1.17 (0.79; 1.73)	1.24 (0.68; 2.28)	0.483
C18:1n9c	Range	9.24 - 11.60	11.61 - 12.26	12.26 - 12.84	12.85 - 13.57	13.58 - 18.35		
	Cases/Controls (n)	128 / 153	170 / 154	158 / 153	151 / 154	162 / 155		
	OR, adjusted	1.00 (ref.)	1.29 (0.92; 1.80)	1.24 (0.89; 1.74)	1.12 (0.79; 1.59)	1.15 (0.79; 1.65)	1.04 (0.94; 1.14)	0.473
C18:1n7t+n9t [+]	Range	0.00 - 0.41	0.41 - 0.49	0.49 - 0.57	0.57 - 0.72	0.72 - 2.46		
	Cases/Controls (n)	183 / 152	152 / 154	138 / 155	163 / 153	133 / 155		
	OR, adjusted	1.00 (ref.)	0.86 (0.62; 1.20)	0.77 (0.55; 1.07)	0.85 (0.59; 1.24)	0.67 (0.44; 1.04)	0.59 (0.31; 1.14)	0.116
MUFA, total [b]	Range	13.59 - 16.88	16.88 - 17.70	17.71 - 18.41	18.41 - 19.25	19.25 - 25.41		
	Cases/Controls (n)	133 / 152	156 / 155	151 / 153	157 / 154	172 / 155		
	OR, adjusted	1.00 (ref.)	1.21 (0.86; 1.70)	1.11 (0.78; 1.58)	1.15 (0.79; 1.66)	1.20 (0.82; 1.78)	1.04 (0.96; 1.12)	0.375
n-6 Polyunsaturated fatty acids (n-6 PUFA)								
C18:2n6c	Range	6.56 - 9.49	9.49 - 10.28	10.28 - 10.92	10.93 - 11.91	11.91 - 18.25		
	Cases/Controls (n)	134 / 153.	141 / 153	156 / 155	174 / 153	164 / 155		
	OR, adjusted	1.00 (ref.)	0.80 (0.58; 1.11)	0.82 (0.59; 1.14)	0.95 (0.68; 1.33)	0.78 (0.54; 1.12)	0.97 (0.90; 1.05)	0.468
C20:3n6	Range	0.80 - 1.33	1.33 - 1.48	1.49 - 1.64	1.64 - 1.85	1.85 - 3.41		
	Cases/Controls (n)	158 / 152	155 / 154	153 / 155	147 / 154	156 / 154		
	OR, adjusted	1.00 (ref.)	1.00 (0.72; 1.39)	0.91 (0.65; 1.28)	0.89 (0.64; 1.25)	0.90 (0.64; 1.28)	0.87 (0.63; 1.20)	0.387
C20:4n6	Range	3.87 - 11.78	11.78 - 12.61	12.61 - 13.42	13.42 - 14.34	14.34 - 17.45		

	Cases/Controls (n)	131 / 153	159 / 153	183 / 155	158 / 154	138 / 154		
	OR, adjusted	1.00 (ref.)	1.22 (0.86; 1.72)	1.40 (0.97; 2.02)	1.27 (0.85; 1.88)	1.04 (0.68; 1.59)	1.24 (0.39; 3.92)§	0.717§
C22:4n6	Range	0.68 - 1.98	1.98 - 2.34	2.34 - 2.68	2.68 - 3.15	3.15 - 8.54		
	Cases/Controls (n)	139 / 152	154 / 154	166 / 154	169 / 154	141 / 155		
	OR, adjusted	1.00 (ref.)	1.10 (0.78; 1.55)	1.22 (0.85; 1.75)	1.21 (0.84; 1.76)	1.01 (0.67; 1.51)	1.05 (0.86; 1.28)	0.663
	Range	14.94 - 25.98	25.98 - 27.49	27.50 - 28.91	28.91 - 30.40	30.41 - 37.73		
n-6 PUFA, total [c]	Cases/Controls (n)	139 / 153	167 / 153	188 /155	137 / 154	138 / 154		
	OR, adjusted	1.00 (ref.)	1.19 (0.85; 1.67)	1.35 (0.96; 1.91)	1.01 (0.68; 1.49)	0.99 (0.64; 1.52)	0.75 (0.17; 3.43)§	0.715§
n-3 Polyunsaturated fatty acids (n-3 PUFA)								
C18:3n3	Range	0.00 - 0.08	0.08 - 0.12	0.12 - 0.15	0.15 - 0.20	0.20 - 1.38		
	Cases/Controls (n)	146/153	148 / 154	154 / 153	169 / 155	152 / 154		
	OR, adjusted	1.00 (ref.)	0.95 (0.66; 1.35)	1.07 (0.74; 1.57)	1.14 (0.77; 1.69)	1.07 (0.70; 1.64)	1.21 (0.30; 4.93)	0.788
C20:5n3	Range	0.10 - 0.68	0.68 - 0.93	0.93 - 1.18	1.18 - 1.51	1.52 - 4.95		
	Cases/Controls (n)	158 / 153	181 / 154	148 / 153	132 / 154	150 / 155		
	OR, adjusted	1.00 (ref.)	1.06 (0.75; 1.51)	0.77 (0.52; 1.14)	0.72 (0.48; 1.09)	0.80 (0.52; 1.22)	0.79 (0.63; 1.00)	0.054
C22:5n3	Range	0.64 - 2.13	2.14 - 2.40	2.40 - 2.59	2.59 - 2.78	2.78 - 5.71		
	Cases/Controls (n)	167 / 153	149 / 154	137 / 154	143 / 153	173 / 155		
	OR, adjusted	1.00 (ref.)	0.78 (0.53; 1.16)	0.78 (0.52; 1.17)	0.78 (0.51; 1.19)	0.93 (0.60; 1.43)	0.86 (0.62; 1.18)	0.347
C22:6n3	Range	1.34 - 4.84	4.85 - 5.61	5.61 - 6.23	6.23 - 6.96	6.97 - 10.11		
	Cases/Controls (n)	157 / 152	175 / 154	157 / 154	139 / 155	141 / 154		
	OR, adjusted	1.00 (ref.)	1.03 (0.74; 1.43)	0.89 (0.63; 1.25)	0.81 (0.56; 1.15)	0.81 (0.55; 1.19)	0.72 (0.41; 1.26)§	0.249§
	Range	2.33 - 8.19	8.19 - 9.16	9.18 - 9.97	9.98 - 11.04	11.05 - 18.65		
n-3 PUFA, total [d]	Cases/Controls (n)	158 / 153	184 / 154	138 / 153	134 / 155	155 / 154		
	OR, adjusted	1.00 (ref.)	1.14 (0.83; 1.57)	0.82 (0.58; 1.15)	0.76 (0.53; 1.11)	0.90 (0.62; 1.31)	0.95 (0.88; 1.01)	0.120

[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; § log-transformed; [+] Sum of vaccenic acid and elaidic acid; [$] Wald test statistics; OR, adjusted ‡ conditional logistic regression adjusted for BMI, smoking status, education, physical activity, alcohol intake, vitamin D, history of diabetes, and season of blood collection

Supplementary Table S3: Multivariable adjusted Odds Ratio (OR) ‡ and 95% confidence interval (CI) of colorectal cancer by red blood cell fatty acid composition (continuous variable), stratified by gender and smoking status

Fatty acids	Men		Women		.	Never smoker		Former smoker		Current smoker		
	OR (95%CI)	P-value[$]	OR (95%CI)	P-value[$]	P het*	OR (95%CI)	P-value[$]	OR (95%CI)	P-value[$]	OR (95%CI)	P-value[$]	P het*
Saturated fatty acids												
C14:0	2.28 (0.76; 6.87)	0.143	0.61 (0.21; 1.81)	0.374	0.251	1.41 (0.60; 3.29)	0.427	0.82 (0.36; 1.90)	0.651	1.16 (0.43; 3.14)	0.766	0.370
C16:0	1.17 (0.99; 1.38)	0.067	0.91 (0.76; 1.08)	0.265	0.131	0.99 (0.87; 1.13)	0.897	0.94 (0.82; 1.08)	0.397	1.13 (0.99; 1.29)	0.074	0.351
C18:0	1.27 (1.04; 1.55)	0.022	1.21 (0.98; 1.48)	0.074	0.975	1.08 (0.93; 1.25)	0.323	1.08 (0.92; 1.26)	0.356	1.14 (0.95; 1.36)	0.167	0.818
SFA, total	1.17 (1.03; 1.33)	0.018	1.10 (0.96; 1.26)	0.175	0.624	1.03 (0.95; 1.12)	0.426	0.99 (0.90; 1.09)	0.800	1.10 (1.01; 1.19)	0.033	0.307
Odd-chain fatty acids												
C15:0	0.57 (0.14; 2.32)	0.431	1.17 (0.31; 4.43)	0.816	0.714	0.80 (0.37; 1.72)	0.569	1.11 (0.55; 2.26)	0.768	1.09 (0.38; 3.13)	0.876	0.650
C17:0§	0.16 (0.02; 1.22)	0.077	0.08 (0.01; 0.76)	0.028	0.956	0.52 (0.09; 2.94)	0.459	0.53 (0.09; 3.22)	0.490	0.13 (0.01; 1.16)	0.068	0.370
Monounsaturated fatty acids												
C16:1n7c	1.31 (0.67; 2.59)	0.431	0.71 (0.33; 1.55)	0.391	0.294	1.15 (0.64; 2.06)	0.643	0.84 (0.47; 1.48)	0.541	1.21 (0.64; 2.27)	0.561	0.756
C18:1n9c	1.04 (0.93; 1.16)	0.484	0.95 (0.84; 1.07)	0.371	0.578	1.02 (0.93; 1.11)	0.722	0.99 (0.90; 1.09)	0.835	0.98 (0.89; 1.09)	0.758	0.970
MUFA, total	1.01 (0.92; 1.10)	0.864	0.96 (0.87; 1.05)	0.367	0.686	1.00 (0.93; 1.07)	0.990	0.98 (0.91; 1.06)	0.634	1.00 (0.92; 1.08)	0.967	0.394
n-6 Polyunsaturated fatty acids												
C18:2n6c	0.94 (0.86; 1.04)	0.230	1.01 (0.92; 1.11)	0.769	0.440	1.00 (0.93; 1.07)	0.968	0.98 (0.91; 1.06)	0.645	0.99 (0.90; 1.08)	0.739	0.818
C20:3n6	1.18 (0.81; 1.72)	0.399	0.75 (0.51; 1.11)	0.153	0.353	1.01 (0.75; 1.35)	0.954	0.90 (0.66; 1.22)	0.490	1.02 (0.67; 1.56)	0.928	0.710
C20:4n6§	1.05 (0.95; 1.16)	0.347	1.11 (0.98; 1.24)	0.089	0.796	1.03 (0.95; 1.12)	0.432	1.03 (0.95; 1.12)	0.465	1.03 (0.92; 1.14)	0.610	0.891
C22:4n6	1.19 (0.94; 1.51)	0.141	1.26 (0.99; 1.61)	0.062	0.790	1.09 (0.91; 1.31)	0.344	1.01 (0.85; 1.21)	0.895	1.21 (0.94; 1.56)	0.141	0.142
n-6 PUFA, total§	1.01 (0.95; 1.07)	0.756	1.05 (0.98; 1.12)	0.160	0.622	1.01 (0.97; 1.06)	0.531	1.00 (0.95; 1.05)	0.941	1.01 (0.96; 1.07)	0.661	0.903
n-3 Polyunsaturated fatty acids												
C18:3n3	1.15 (0.26; 5.09)	0.855	0.63 (0.08; 5.24)	0.669	0.508	1.63 (0.41; 6.56)	0.491	0.99 (0.26; 3.74)	0.993	0.60 (0.12; 3.01)	0.532	0.095
C20:5n3	0.74 (0.57; 0.97)	0.031	0.74 (0.55; 1.00)	0.049	0.847	0.87 (0.69; 1.11)	0.256	0.99 (0.81; 1.22)	0.922	0.66 (0.48; 0.90)	0.009	0.012
C22:5n3	0.92 (0.63; 1.32)	0.638	0.86 (0.57; 1.31)	0.494	0.757	1.01 (0.75; 1.36)	0.966	0.91 (0.68; 1.22)	0.545	0.79 (0.53; 1.16)	0.227	0.170
C22:6n3§	0.92 (0.82; 1.04)	0.195	0.89 (0.79; 1.02)	0.084	0.799	0.93 (0.84; 1.02)	0.114	1.04 (0.94; 1.14)	0.452	0.91 (0.80; 1.03)	0.121	0.313
n-3 PUFA, total	0.94 (0.86; 1.01)	0.103	0.92 (0.84; 1.00)	0.052	0.718	0.95 (0.89; 1.02)	0.158	1.01 (0.95; 1.08)	0.736	0.91 (0.84; 0.99)	0.034	0.084

[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; § log-transformed; [$] Wald test statistics; * P heterogeneity; [‡] conditional logistic regression adjusted for BMI, education, physical activity, alcohol intake, history of diabetes, and season of blood collection