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Dietary intake and plasma phospholipid concentrations of saturated, monounsaturated and *trans* fatty acids and colorectal cancer risk in the EPIC cohort

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Abbreviations used: BHT, butylated hydroxytoluene, BMI, Body mass index; CLA, conjugated linoleic acid; CRC, colorectal cancer; EPIC, European Prospective Investigation into Cancer and Nutrition; FAME, Fatty acid methyl ester; HR, Hazard ratio; IARC, International Agency for Research on Cancer; iTFA, industrial TFA; MUFA, monounsaturated fatty acids; OR, Odds ratio; PPAR, peroxisome proliferator-activated receptor; PUFA, polyunsaturated fatty acid; rTFA, ruminant TFA;

SCD-1, stearoyl-CoA desaturase-1; SFA, saturated fatty acid; TFA, *trans* fatty acid; USDA, United States Department of Agriculture; WHR, waist-to-hip ratio

Novelty and impact: The role of specific fatty acids in cancer development is not fully understood. In a large-scale European cohort, we examined dietary and blood levels of saturated, monounsaturated, industrial-processed trans and ruminant-sourced trans fatty acids in relation to colorectal cancer risk. Dietary myristic and palmitic acids were inversely associated with colorectal cancer risk. Similarly, plasma myristic acid levels were inversely associated with colon cancer risk. Dietary industrial trans fatty acids were positively associated with rectal cancer.

Abstract

Epidemiologic studies examining the association between specific fatty acids and colorectal cancer (CRC) risk are inconclusive. We investigated the association between dietary estimates and plasma levels of individual and total saturated (SFA), monounsaturated (MUFA), industrial-processed *trans* (iTFA), and ruminant-sourced *trans* (rTFA) fatty acids, and CRC risk in the European Prospective Investigation into Cancer and Nutrition (EPIC). Baseline fatty acid intakes were estimated in 450,112 participants (6,162 developed CRC, median follow-up=15 years). In a nested case-control study, plasma phospholipid fatty acids were determined by gas chromatography in 433 colon cancer cases and 433 matched controls. Multivariable-adjusted hazard ratios (HRs) and odds ratios (ORs) with 95% confidence intervals (CIs) were computed using Cox and conditional logistic regression, respectively. Dietary total SFA (highest *vs.* lowest quintile, $HR_{Q5vs.Q1}$=0.80; 95%CI:0.69-0.92), myristic acid ($HR_{Q5vs.Q1}$=0.83, 95%CI:0.74-0.93) and palmitic acid ($HR_{Q5vs.Q1}$=0.81, 95%CI:0.70-0.93) were inversely associated with CRC risk. Plasma myristic acid was also inversely associated with colon cancer risk (highest *vs.* lowest quartile, $OR_{Q4vs.Q1}$=0.51; 95%CI:0.32-0.83), whereas a borderline positive association was found for plasma stearic acid ($OR_{Q4vs.Q1}$=1.63; 95%CI:1.00-2.64). Dietary total MUFA was inversely associated with colon cancer (per one-standard deviation increment, $HR_{1\text{-}SD}$=0.92, 95%CI: 0.85-0.98), but not rectal cancer ($HR_{1\text{-}SD}$=1.04, 95%CI:0.95-1.15, $P_{heterogeneity}$=*0.027*). Dietary iTFA, and particularly elaidic acid, was positively associated with rectal cancer ($HR_{1\text{-}SD}$ =1.07, 95%CI:1.02-1.13). Our results suggest that total and individual saturated fatty acids and fatty acids of industrial origin may be relevant to the aetiology of CRC. Both dietary and plasma myristic acid levels were inversely associated with colon cancer risk, which warrants further investigation.

Introduction

Colorectal cancer (CRC) is the third most commonly diagnosed cancer worldwide with an estimated 1.8 million new cases in 2018 [1]. There is evidence that the adoption of "Western" dietary patterns characterised by usual consumption of large amounts of animal products including processed meats are associated with CRC [2-4]. A possible link between Western diets and CRC may lie in their high content of saturated (SFAs) and *trans* fatty acids (TFAs). In animal models, SFAs and TFAs have been shown to promote CRC through systemic inflammation by disrupting the integrity of the cell membranes, increasing the formation of pro-inflammatory prostaglandins E2, and activating toll-like receptors [5-7]. However, previous experimental studies have shown that TFAs from different dietary sources have divergent activity; industrial TFA (iTFAs) produced through partial hydrogenation promote low-grade inflammation and oxidative stress whereas natural ruminant-derived TFAs (rTFA) may decrease low-grade inflammation [8].

Epidemiologic studies that have investigated dietary intakes of SFA, iTFA and rTFA and CRC risk have been inconclusive. A meta-analysis of eighteen prospective cohort studies reported no association between SFA intakes and the risk for CRC [9]. Further, prospective studies showed no association between intakes of individual or total iTFA and CRC [10-13], though one study reported a positive association for distal colon cancer [14]. Few prospective studies have evaluated the associations between rTFA and CRC and those published reported null associations [11]. However, previous studies have not systematically investigated the associations by dietary food sources of fatty acids, or by colorectal cancer tumour location, or for individual fatty acids as well as subgroups such as iTFA or rTFA.

Studies of fatty acids and CRC have generally relied on dietary questionnaires and are therefore limited by the imprecision of dietary questionnaires and the incompleteness of food composition tables [8, 15]. The use of biomarkers such as plasma phospholipid fatty acids is an alternative approach and is complementary to dietary estimation to investigate fatty acids and disease associations. The measurement of plasma phospholipid concentrations, which reliably reflect weeks-to-monthly intakes of specific fatty acids including TFA and odd-chain SFA, has been successfully applied to epidemiological studies [16-18]. The use of biomarkers also enables the estimation of endogenous conversion of individual SFA into corresponding monounsaturated fatty acids (MUFA) through the activity of the stearoyl-CoA desaturase-1 (SCD-1) enzyme [19]. The activity of SCD-1 calculated as the ratio of specific MUFA over SFA has been found to be associated with colon cancer risk [20]. However, prospective cohort studies that have examined the associations between plasma phospholipid concentrations of SFA, MUFA, iTFA and rTFA and CRC risk were inconclusive [10, 20].

In this study, we examined the associations between dietary estimates and plasma phospholipid concentrations of SFA, MUFA, iTFA, rTFA, and SCD-1 proxies and the risk of CRC in the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort. Additional analyses were conducted

by fatty acid dietary sources and tumour anatomical subsites. This study did not include polyunsaturated fatty acids (PUFAs), as the data on PUFAs and CRC have been reported in a prior publication [21].

Materials and methods

Study participants

A total of 521,324 participants (35-75 years old) were recruited between 1992 and 2000 from 23 centres located in 10 European countries (Denmark, France, Germany, Greece, Italy, the Netherlands, Norway, Spain, Sweden, and the United Kingdom) [22]. Anthropometric measures and questionnaires on demographics, diet and other lifestyle factors were collected at recruitment for all the participants. Blood was collected at baseline and stored at -196°C at the main EPIC biobank located in the International Agency for Research on Cancer (IARC) facilities (Lyon, France), or in local biobanks in Denmark (Copenhagen, at -150°C), and Sweden (Malmö and Umeå, at -80°C). The EPIC study was approved by the IARC Ethical Committee and the local ethics committees pertaining to each participating centre. Informed consent was obtained from all the participants. Participants diagnosed with cancer prior to recruitment (n=25,184), or missing follow-up (n=4,148) or dietary (n=6,259) information, or those in the extreme highest or lowest 1% energy intake versus requirements (n=9,573) were excluded. The current analysis included data from all EPIC countries with the exception of Greece (n=26,048). The final analytical dataset included 450,112 participants (131,426 men and 318,686 women).

Anthropometry, diet and lifestyle collection

At baseline, participants completed extensive questionnaires on lifestyle and dietary intakes including questions on education, smoking, alcohol intake, physical activity, and previous illness. Physical activity was determined according to the Cambridge physical activity index: inactive (sedentary job and no recreational activity), moderately inactive (sedentary job with less than 0.5 hour recreational activity daily/or standing job with no recreational activity), moderately active (sedentary job with 0.5 to 1 hour recreational activity daily/ or standing job with 0.5 hour recreational activity daily/ or physical job with no recreational activity) or active (sedentary job with >1 hour recreational activity daily/or standing job with >0.5 hour recreational activity daily/or physical job with at least some recreational activity/or heavy manual job) [23]. Body weight and standing height were measured in all centres, except for the centres in Oxford, France, and Norway, where they were self-reported. Body mass index (BMI) was calculated. Dietary intake was assessed at baseline with validated centre-specific dietary assessment methods, mainly food frequency questionnaires [24]. EPIC food items were matched to the food composition database Release 26, developed by the United States Department of Agriculture (USDA) as part of the Nutrient Database for Standard Reference publications [25] (Release 26, October 2013, available at https://ndb.nal.usda.gov/ndb/). The method has been described previously [26]. The USDA database includes data for over 50 individual fatty acids and was used to calculate total SFA (Σ all cis

14:0, 15:0, 16:0, 17:0, 18:0, 20:0, 22:0, 24:0), total MUFA (Σ all cis: 16:1; 17:1; 18:1; 20:1; 22:1; 24:1), total iTFA (Σ *trans*-18:1; *trans*-18:2, *trans*, *trans*-18:2) and total rTFA (Σ*trans*-16:1, *trans*-18:1n-7; and conjugated linoleic acid, CLA).

Ascertainment of CRC cases

Cancer cases ascertainment (until December 2014) was done using the data from cancer registries (Denmark, Italy, the Netherlands, Norway, Spain, Sweden, and the United Kingdom) or a combination of sources including health insurance records, cancer and pathology registries, active follow-up of the participants and their next of kin family members (France, Germany). CRC cases were defined as tumours located in the colon (C18) or the rectum (C19-C20) according to the International Classification of Diseases for Oncology (ICD-O, codes C18-C20). Colon cancer included tumours in the proximal (from cecum to splenic flexure, ICD-O codes: C18.0-C18.5) or the distal segment (from descending colon down to sigmoid colon, ICD-O codes: C18.6-C18.7), while rectal cancer included tumours that occurred from the recto-sigmoid junction (C19) down to the rectum (C20). Tumours that arose in the anal canal were excluded in this analysis.

Nested case-control study of biomarkers of fatty acids and colon cancer

Pre-diagnostic plasma samples from 433 colon cancer cases (178 proximal colon cases, 213 distal colon cancer cases, and 42 overlapping cases) and 433 matched controls were included in the nested case-control analysis of circulating SFA, MUFA, iTFA and rTFA and colon cancer. Controls were selected by incidence density sampling from all the participants free of cancer at the time of diagnosis of the index case. Cases and controls were matched by sex, study centre, age at blood collection, time of the day at blood collection, fasting status at blood collection; and among women, by menopausal status, and among premenopausal women, by phase of menstrual cycle and hormonal therapy use at time of blood collection.

Laboratory analysis of plasma phospholipid fatty acids

Plasma concentrations of fatty acids were determined by gas chromatography in the Biomarkers Laboratory at IARC (Lyon, France) following a methodology previously described in detail elsewhere [27, 28]. Briefly, total lipids were extracted from plasma samples by chloroform-methanol 2:1 (v/v) containing butylated hydroxytoluene (BHT) as an antioxidant, and L-α-phosphatidylcholine-dimyristoyl-d54 as internal standard. Phospholipids were purified by adsorption chromatography on silica tubes. Fatty acid methyl esters (FAMEs) were formed by transmethylation with Methyl-Prep II (Alltech, Deerfield, IL, USA). Analyses were carried out on the gas chromatograph 7890A (Agilent Technologies, CA, USA). The individual fatty acids were separated and identified by comparison of their respective retention characteristics with those of purchased standard methyl ester fatty acids (Sigma, St Louis, MO, USA). Intra- and inter-batch coefficients of variation (CV) were assessed by

using two quality control samples within each batch. Coefficients of variation for intra- and inter-assay ranged between 0.013% for larger peaks (palmitic acid), to 9.34% for the smallest peak (18, trans). Total SFA, MUFA, iTFA and rTFA were calculated as the sum of the specific individual fatty acids of their family and expressed as percentages of total fatty acids or in μmol/ml based on the quantity of the methyl deuterated internal standard.

Fatty acids considered in the analysis

For both dietary estimates and plasma phospholipid fatty acids the following individual fatty acids were considered: (i) SFAs, myristic acid (14:0), pentadecanoic acid (15:0), palmitic acid (16:0), heptadecanoic acid (17:0), stearic acid (18:0); (ii) MUFA, palmitoleic acid (16:1n-7), *cis*-vaccenic acid (18:1n-7), oleic acid (18:1n-9); (iii) iTFA, elaidic acid (18:1n-9/12), *trans*-18:2, *trans,trans*-18:2; and (iv) rTFA, palmitelaidic acid (16:1n-7/9), *trans*-vaccenic acid (18:1n-7), and conjugated linoleic acid (CLA). We calculated the desaturation indices as the ratio of product to substrate, for oleic acid to stearic acid (SCD-18) or for palmitoleic acid to palmitic acid (SCD-16), as biomarkers of stearoyl-CoA desaturase activity [29].

Statistical analyses

Descriptive analyses

Means and standard deviations (SD) for continuous and normally distributed variables, and frequencies for categorical variables were calculated for cases and non-cases. For plasma phospholipid fatty acids, geometric means and 95% confidence interval (CI) were computed.

Full cohort analysis

Cox proportional hazards regression models stratified by age at recruitment, sex, and study centre were run to estimate hazard ratios (HRs) and 95% CI for the association between individual or total dietary SFAs, MUFAs, iTFA, and rTFA, and CRC risk. Age at recruitment was considered as time at entry whereas exit time was set as the age when any of the following first occurred: ascertained CRC diagnosis, death, emigration, or last date at which follow-up was considered complete. Dietary estimates of individual and groupings of fatty acids were divided into quintiles. Trends were evaluated using median value of categories fitted continuously. Analyses were also conducted using continuous variables for fatty acids intakes (per SD increment). We found no deviation from the proportional hazards assumption when we assessed the proportionality using Schoenfeld residuals over time [30].

All the models were adjusted for established or putative risk factors for CRC including as continuous variables BMI, height, and intakes of energy (kcal/day), alcohol (g/day), calcium (g/day), red and processed meat (g/day) and fibre (g/day), and as categorical variables physical activity (inactive, moderately inactive, moderately active, active), smoking (never, 1-15cigarettes/day, 16-25 cigarettes/day, over 26 cigarettes/day, former smokers who quit<10 years, former smokers who quit 11-

20 years, former smokers who quit>20 years, current pipe-cigar and occasional smokers), education (none, primary, technical/professional, secondary, higher). Analysis by dietary sources of the fatty acids was conducted considering the contribution of specific food groups. Participants with missing data for physical activity (1.9%), education (2.3%), and smoking (3.3%) were distinctly coded as an additional category. We evaluated possible nonlinear associations by flexibly modelling restricted cubic splines [31]. We tested the linearity of the associations between fatty acids and CRC by using the likelihood ratio test, comparing the model with only the linear term to the model with the linear and the cubic spline terms. All associations, with the exception of circulating CLA, were linear after evaluation with splines.

We also assessed the associations between the intakes of SFA, MUFA, iTFA, and rTFA and the risk by tumour anatomical subsite (proximal and distal colon cancers and rectum). Differences in associations by tumour site were tested using competing risk analyses [32]. We also evaluated heterogeneity by sex and country by adding multiplicative interaction terms in the models. To evaluate the possible impact of reverse causation, all the analyses were re-run excluding participants with less than 4 years of follow-up.

Nested case-control study of biomarkers of fatty acids

For the analysis of the association between plasma phospholipid SFA, MUFA iTFA, and rTFA and colon cancer, we conducted multivariate conditional logistic regression models to compute odds ratios (ORs) and 95% CIs. We divided the concentrations of fatty acids into quartiles based on the distributions in the control group. Multivariable models were adjusted for the same covariates included in the aforementioned dietary fatty acid intake analyses. We conducted trend test by using the median values of each quartile included in the model as continuous variables. We ran analyses for proximal and distal colon cancer anatomical subsites.

Sensitivity analysis

In sensitivity analysis, we excluded the cases that occurred within two years of follow-up and re-run the analysis. We also replicated the analysis with dietary intake in the nested case-control study to verify whether this population is as representative as possible to the full cohort.

The P-values obtained were corrected for multiple testing using the false discovery rate method by Benjamini-Hochberg [33]. All statistical analyses were carried out using Stata 14.0 (StataCorp, College Station, TX, USA). We considered two-sided *P*-values < 0.05 as statistically significant. The figures were prepared using R software (Foundation for Statistical Computing, Vienna, Austria).

Results

Dietary sources of fatty acids and correlations with circulating levels

Over a median follow-up of 15 years, 6,162 CRC cases (3,997 colon cancer cases and 2,165 rectal cancer cases) were ascertained in the cohort. Dairy products and meats represented the major dietary sources for the majority of fatty acids except for elaidic acid and *trans*-18:2 which came predominantly from margarine (**Supplementary Table 1**). The correlations between dietary intakes and blood concentrations of these fatty acids are shown in **Supplementary Tables 2**. A positive correlation was observed between dietary and plasma levels of myristic acid, pentadecanoic acid, oleic acid, CLA and elaidic acid, while no correlation was observed for palmitic acid, stearic acid or palmitoleic acid.

Baseline characteristics of study participants

The baseline characteristics of CRC cases and non-cases are shown in **Table 1**. In both the full cohort and the nested case-control study, participants who became cases tended to have higher BMI and consumed more alcohol and red and processed meats at baseline. The characteristics of the participants in the full cohort are similar to those in the nested case-control study, except for differences in the sex ratio and attendant slight effects on variables such as smoking and physical activity. Oleic acid was the most consumed fatty acid (mean intake in non-cases: 27.1 g/day), followed respectively by palmitic acid (mean intake in non-cases: 14.8 g/day), cis-vaccenic acid (mean intake in non-cases: 8.59 g/day), and stearic acid (mean intake in non-cases: 6.55 g/day) (**Supplementary Table 3**). Palmitic and stearic acid (both SFAs) were the most abundant fatty acids in the circulation with geometric means of 25.3% and 14.1% of total fatty acids in the controls, respectively.

Dietary fatty acids and CRC risk

Table 2 presents the HRs and 95%CI for the associations between dietary estimates of fatty acids and the risk for CRC. Total SFA intake was inversely associated with CRC risk (HR comparing highest to lowest quintile $HR_{Q5vs.Q1}$=0.80; 95%CI: 0.69-0.92), with similar associations found for the individual even-chain SFAs: myristic acid ($HR_{Q5vs.Q1}$=0.83; 95%CI: 0.74-0.93) and palmitic acid ($HR_{Q5vs.Q1}$=0.81; 95%CI: 0.70-0.93). Dietary intakes of odd-chain SFAs were not associated with CRC risk (pentadecanoic acid, $HR_{Q5vs.Q1}$=1.05, 95%CI: 0.95 - 1.17; heptadecanoic acid, $HR_{Q5vs.Q1}$=1.04, 95%CI: 0.93 - 1.16). Intake of palmitoleic acid was inversely associated with CRC risk ($HR_{Q5vs.Q1}$=0.88; 95%CI: 0.77-1.00, *P* for trend=*0.042*) while no significant associations were observed for all other MUFAs. Also, no significant associations were observed for the intakes of individual or total iTFAs and rTFAs. After correcting for false discovering rate, only the association obtained with dietary myristic acid remained statistically significant (P-value corrected using Benjamini-Hochberg P_{BH}=*0.036*). Sex-specific analysis showed no differences between men and women (All *P* values for heterogeneity >=0.10), expect for pentadecanoic acid (*P* for heterogeneity by sex=*0.021*). Further analyses showed no statistical differences in the association for groups of SFA, MUFA, iTFA and rTFA across countries (data not shown).

Analyses by dietary sources revealed that the inverse associations observed for myristic acid, palmitic acid, and palmitoleic acid were mainly driven by their dairy origin, while positive associations were found when they originate from meat and meat products, margarine and deep-frying fats (**Figure 1**). Stratified analyses by anatomical subsites (**Figure 2**) showed that total dietary MUFA intake was inversely associated with colon cancer (HR per 1 standard deviation increment, HR per 1 SD=0.92, 95%CI: 0.85-0.98), but not with rectal cancer (HR per 1 SD=1.04, 95%CI: 0.95-1.15, *P* for heterogeneity=*0.027*). *Cis*-vaccenic acid was associated with higher rectal cancer risk (HR per SD intake: HR per 1 SD=1.09, 95%CI: 1.02-1.17), but not colon cancer (HR per 1 SD=0.97, 95%CI: 0.92-1.02, *P* for heterogeneity=*0.006*). The inverse association observed with total MUFA and colon cancer was driven by oleic acid (HR per 1 SD=0.92, 95%CI: 0.85-0.99) and palmitoleic acid (HR per 1 SD=0.94, 95%CI: 0.88-1.00). We also observed a higher risk of rectal cancer with dietary iTFA (HR per 1 SD=1.07, 95%CI: 1.02-1.13), an association driven by elaidic acid, although heterogeneity for rectal *vs.* colon cancer was not significant (*P* for heterogeneity=*0.063*). When dietary sources of the main iTFA, elaidic acid were considered, the increased risk observed with rectal cancer was found to be driven by their margarine origin (HR per 1SD=1.06, 95%CI: 1.01-1.10) (**Figure 3**). Further analyses by colon cancer subsites showed oleic acid to be inversely associated with the risk of distal colon cancer (HR=0.82, 95%CI: 0.74-0.92), while a null association was observed for proximal colon cancer (HR=0.98, 95%CI: 0.87-1.09, *P* for heterogeneity=*0.046*) (**Supplementary Figure 1**). Odd-chain SFA (pentadecanoic and heptadecanoic acids) as well as other SFAs were all inversely associated with distal colon cancer risk. Dietary palmitelaidic acid and CLA, as well as industrial *trans*-18:2 was also inversely associated with distal colon cancer risk. The exclusion of the participants with less than 4 years of follow-up did not materially change the results (**Table 2**).

Plasma phospholipid fatty acids and CRC risk

Table 3 presents the ORs and 95%CI for the associations between plasma phospholipid fatty acids and colon cancer risk. Myristic acid levels were inversely associated with colon cancer risk (OR comparing highest to lowest quartile $OR_{Q4vs.Q1}$=0.51; 95%CI: 0.32-0.83; P for trend=*0.005*). A statistically non-significant positive association was found between plasma stearic acid levels and colon cancer risk ($OR_{Q4vs.Q1}$=1.63; 95%CI: 1.00-2.64, P for trend=*0.087*). Plasma CLA showed a non-linear association with colon cancer (*P* non-linearity=*0.038*, $OR_{Q4vs.Q1}$=0.89, 95%CI: 0.51-1.54, *P* for trend=*0.823*; OR per SD increase=0.79, 95%CI: 0.64-0.98). Overall, none of the associations remained significant after correcting for false discovery rate.

Additional analyses by colonic anatomical subsites showed no differences in the associations across anatomical subsites, except for rTFA which showed an inverse association with distal colon cancer (OR=0.63; 95%CI: 0.41-0.95), driven mostly by CLA (**Supplementary Figure 2**).

Desaturation indices

We observed no statistically significant associations between SCD-16 ($OR_{Q4vs.Q1}$=0.91; 95%CI: 0.55-1.50) or SCD-18 ($OR_{Q4vs.Q1}$=0.77; 95%CI: 0.49-1.23) and colon cancer risk (**Supplementary Table 4**).

Sensitivity analysis

The exclusion of cases that occurred within two years of follow-up did not materially change our results (data not shown). We also investigated the association between dietary fatty acids and colon cancer risk in the nested case-control study population. In general, these analyses yielded similar trends as observed with the full cohort, although none of the associations were statistically significant. For example, the ORs for myristic acid and elaidic acid were 1.02 (95%CI=0.81-1.29) and 1.15 (95%CI=0.92-1.42), respectively.

Discussion

In this large prospective cohort study, we found that higher intakes of some individual and total SFAs and MUFAs were associated with lower colon or rectal cancer risk. The inverse associations observed with SFA and MUFA were restricted to their dairy sources, showing that the dietary origin of the fatty acids may play an important role in their relationship to CRC. We also found that iTFA, particularly elaidic acid from margarine, was associated with increased rectal cancer risk. The associations observed with dietary estimates were not consistently found with plasma phospholipid concentrations, except for plasma phospholipid myristic acid which was also inversely associated with colon cancer risk.

Our findings suggest that fatty acids from the same major family (i.e SFAs such as myristic and stearic acid in the plasma) may have divergent associations with CRC depending on their dietary sources, and these associations may also differ by the location of the tumour. Experimental models and clinical analysis of tumours studies suggest that tumours that arise in the rectum may be genetically, and aetiologically different from those that originate in the colon; and even in the colon, risk factors may differ for tumours in distal or proximal sites [34, 35]. We observed that dietary intake of myristic acid and its levels in plasma were consistently inversely associated with the risk of colon cancer. The inverse association observed between dietary myristic acid and CRC were restricted to dairy products sources and these results are in concordance with previous studies which also showed that higher intakes of myristic acid from dairy products were associated with lower CRC risk [36]. Dairy products have been consistently linked to lower risk of CRC [4]. The mechanisms underlying this relationship are not understood but have been posited to entail greater exposure to calcium as well as specific fatty acids [37]. In our analysis, the inverse association between dietary myristic acid and CRC remained statistically significant after further adjustment for dietary calcium suggesting a mechanism independent of calcium. Myristic acid is involved in several anti-tumorigenic mechanisms such as the production of myristoleic acid (14: 1n-5) which has been shown to induce apoptosis in tumours [38]. Myristic acid can acylate proteins through myristoylation, a process which further synthetises dihydroceramide D4-desaturases

involved in *de novo* ceramide synthesis with possible tumour preventive properties [39]. This is in line with rodent studies, which showed that diets high in myristic acid prevent hyperplasia [40] and administering myristic acid delayed the initiation of tumours [41]. It is therefore possible that myristic acid may prevent CRC through several mechanisms and findings from the current study should inform future research into this area.

Palmitic acid and stearic acid are the most abundant SFA in the diet and they have been hypothesised to increase CRC risk due to their potential to promote endoplasmic reticulum stress and reactive oxygen species in experimental studies [19, 42]. Also, palmitic acid and stearic acid have been shown to negatively impact upon colon membrane integrity, leading to the promotion of CRC development [43]. Dysbiosis of the gut microbiome is often indicated as a possible pathway through which palmitic acid and stearic acid may increase CRC risk [44, 45]. However, a study using a murine model showed that supplementation with these SFAs sustains the microbial equilibria of the distal colon [46]. This is in line with our findings of inverse associations between individual and total SFA intakes with distal colon cancer while no associations were observed with proximal colon cancer. Another study in mice reported that diets high in SFA such as palmitic acid and stearic acid were associated with reduced inflammation and were protective against CRC [47].

We did not observe significant correlations between dietary and plasma palmitic acid and stearic acid, which confirms that plasma levels of these fatty acids, do not reflect dietary intake but rather endogenous production through the actions of desaturases. Therefore, the positive association observed between plasma stearic acid and colon cancer risk in our study may be explained by endogenous production, possibly in addition to the activity of SCD-1. Interestingly, our study showed that palmitic acid from dairy products was inversely associated with CRC risk, while when from meats and meat products, it was associated with higher CRC risk, suggesting that the dietary source is important in the role of SFA in CRC risk. Additional research is required to explore how SFA from different dietary sources may influence inflammation and other mechanisms within the distal and proximal colon mucosa, and possibly explain differential associations with CRC risk.

Previous epidemiological evidence did not support an association between MUFA intake and CRC risk [9]. Consistent with this, we found no association between total MUFA intake and CRC risk. However, we found differential associations across dietary intake of individual MUFAs, with an inverse association for palmitoleic acid, a positive association for *cis*-vaccenic acid, and a null association for oleic acid. The null association observed with MUFA was mainly driven by oleic acid which constitutes over 70% of the total MUFA from the diet. The null association observed specifically with oleic acid is similar to the findings from previous prospective studies [10, 13]. However, when considering tumour location, intake of oleic acid was not associated with rectal cancer risk but was inversely associated with colon cancer risk, specifically distal but not proximal colon cancer. It is possible that the effects of oleic acid on different colon locations may depend on its dietary sources. This may explain the inverse association between oleic acid and colon cancer in our population where olive oil is the major

dietary source, particularly in Southern European countries [48] whereas in Australia where a major contributor to oleic acid intake is meat and meat products, Hodge et al. [10] found a positive association for rectal cancer. It is therefore possible that the dietary sources might be the most important factor when it comes to MUFA intakes and CRC risk.

rTFA are mostly consumed from ruminant dairy products while iTFA are industrially formed during the partial hydrogenation of vegetable oils. Previous prospective studies have found null associations between total TFA, iTFA or rTFA intakes and CRC risk [10-13]. However, similar to our findings, Vinikoor et al. [14] observed a higher risk of rectal cancer associated with dietary TFA intakes in the population-based case-control North Carolina Colon Cancer Study II. The authors suggested that TFAs in the fecal matter may directly irritate the colon and rectal mucosa, leading to inflammation and oxidative stress. Although this hypothesis has not been clearly demonstrated in previous studies, Ohmori et al. [49] have shown that colon cells exposed to elaidic acid resulted in the rapid growth of pre-existent tumour, and subsequent metastasis. As fecal matter moves through the bowel, gradual absorption of water may concentrate unabsorbed fractions of deleterious substances including iTFA, resulting in higher levels in contact with the rectal mucosa [50]. Dhibi et al. [51] have shown that the consumption of margarine, the major source of elaidic acid, induced excessive oxidative stress in rats. In a case-control study, Le Marchand et al. [52] found an increased risk of rectal cancer with margarine intake, which is consistent with our findings. iTFA intake has been decreasing in Europe due to the legislation to ban or discourage iTFAs in foods. Similarly, the WHO is supporting global programmes to decrease iTFA in foods through its REPLACE initiative [53]; thus it is difficult to evaluate how previous iTFA intake has impacted CRC risk, prior to the current bans. These findings were supported by plasma phospholipids levels. The mechanism of action of CLAs is likely through the reduction of deleterious COX-2 metabolites, and by binding peroxisome proliferator-activated receptors (PPARs) [54]. Additional studies are required to investigate the actions of elaidic acid across the colon and rectum mucosa, and how margarine intake or sources of iTFA participates or enhances these actions.

Strengths of our study include the large heterogeneous population, a prospective study design, the use of both dietary and biomarker data, and the consideration of endogenous conversion activity proxies and dietary sources of the fatty acids. However, our study is limited by only one collection of dietary information and blood at baseline, and the absence of rectal cancer cases in our biomarker analyses. In addition, although comprehensive adjustment for several covariates and relevant sensitivity analysis were conducted, it is still possible that some of our findings may be the results of residual confounding.

In conclusion, we found an inverse association between dietary SFA and risk of CRC, but this association was restricted to SFAs of dairy origin. Dietary intake and plasma phospholipid concentrations of myristic acid were inversely associated with colon cancer risk. Dietary MUFA intake was inversely associated with colon cancer risk. iTFA and particularly elaidic acid from margarine intake were found to be associated with higher risk of rectal cancer whereas rTFA showed inverse

associations with distal colon cancer. This study contributes new knowledge on the role of dietary fat and fatty acids in CRC development and suggests that studies on fatty acids and CRC should consider their dietary origin.

Conflict of interest: The authors declare no potential conflicts of interest.

Ethics statement: The EPIC study was approved by the IARC Ethical Committee and the local ethics committees pertaining to each participating centre.

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Data Availability Statement: For information on how to submit an application for gaining access to EPIC data and/or biospecimens, please follow the instructions at http://epic.iarc.fr/access/index.php. Further details and other data that support the findings of this study are available from the corresponding author upon request.

Disclaimer: Where authors are identified as personnel of the International Agency for Research on Cancer / World Health Organization, the authors alone are responsible for the views expressed in this article and they do not necessarily represent the decisions, policy or views of the International Agency for Research on Cancer / World Health Organization.

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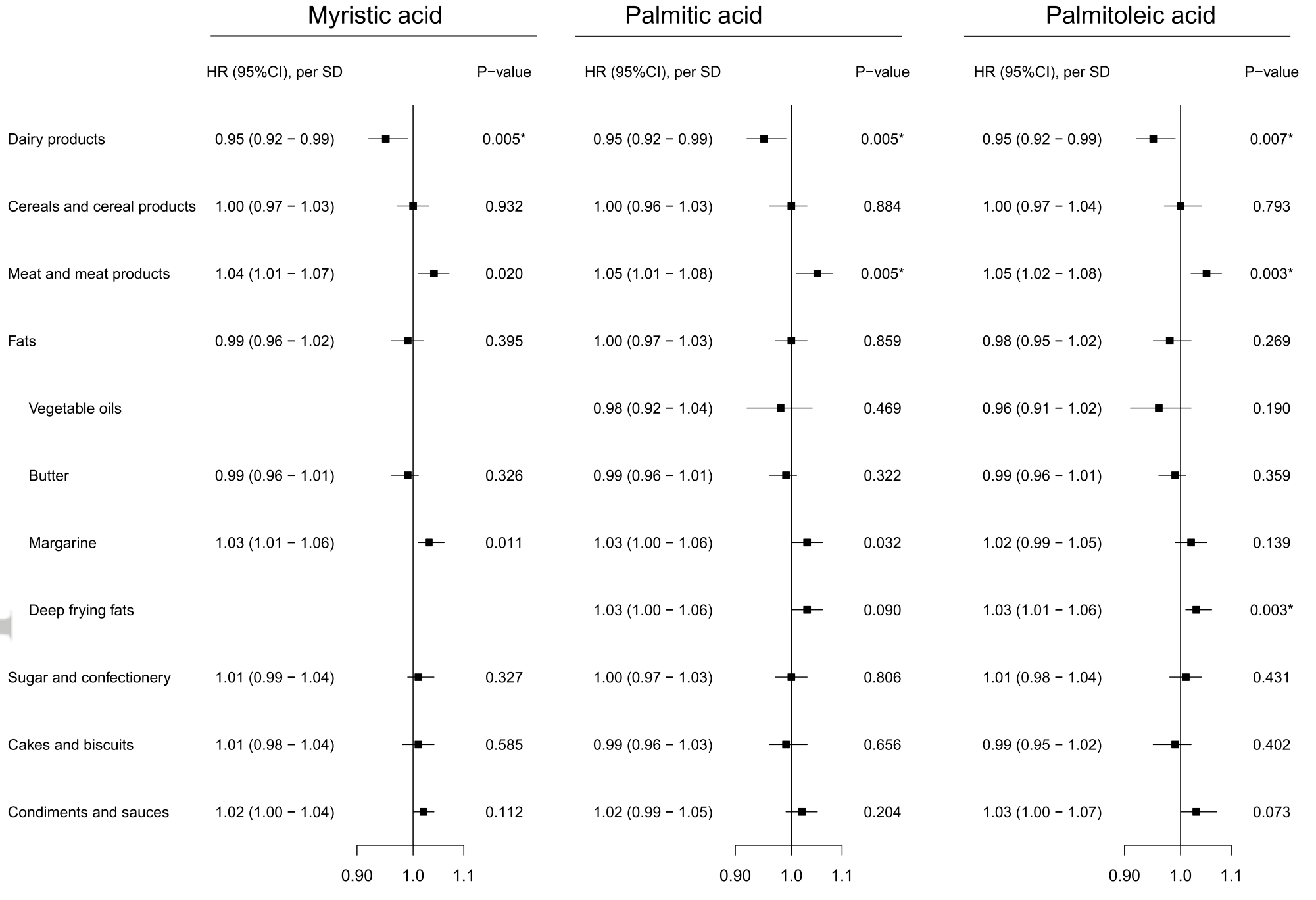
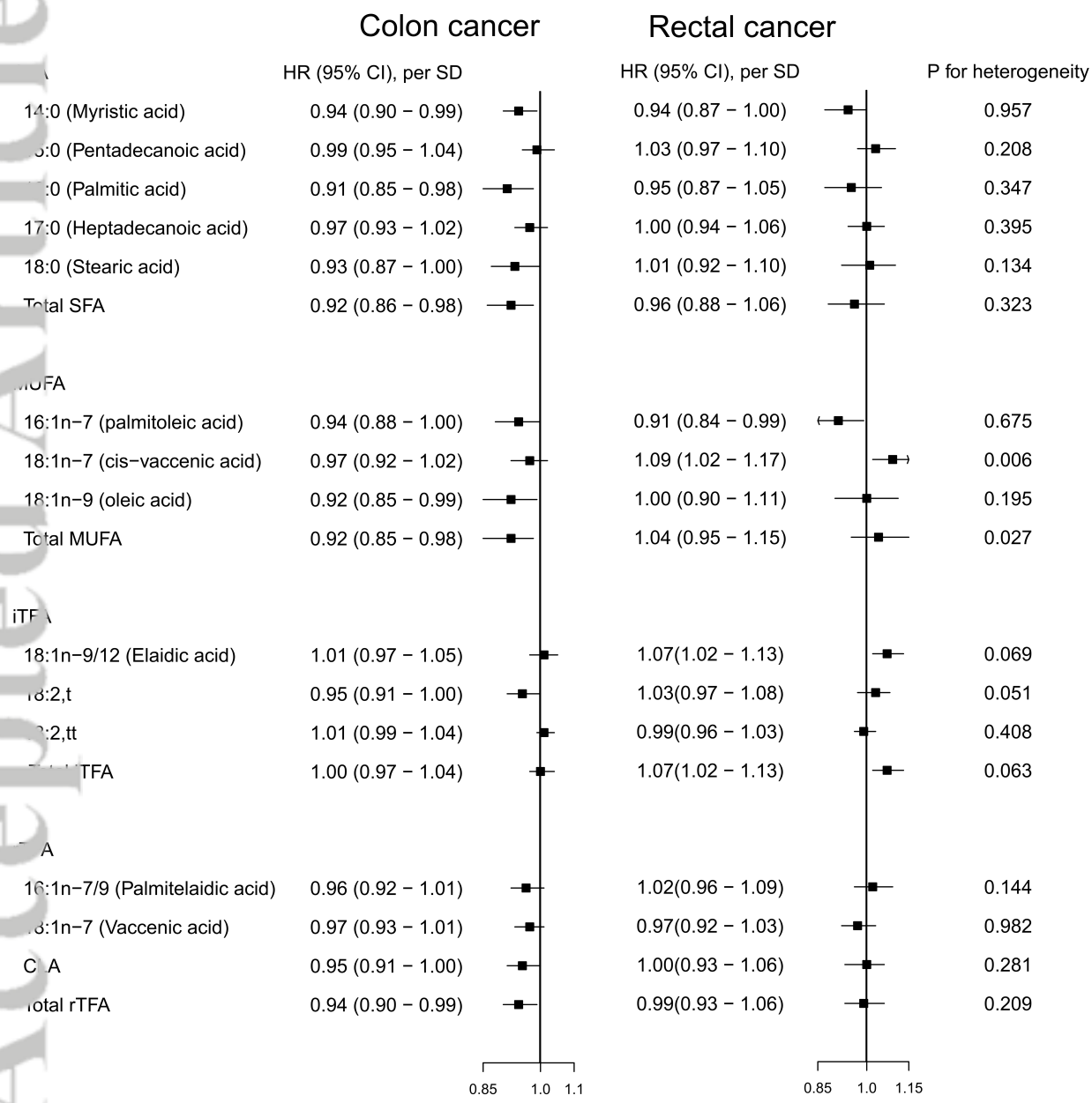
Figure legends

Figure 1: Dietary intake of selected SFA and MUFA and colorectal cancer risk
Dietary contributions represent on average, the amount of the fatty acids that came from the major food items. Negligible contribution (<0.1%) were not considered in the analysis. Fats included vegetable oils, butter, margarine and deep-frying fats. HR and 95%CI for dietary sources of two SFA (myristic acid and palmitic acid) and a MUFA (palmitoleic acid) were determined using multivariable-adjusted Cox models. All the models were multivariable-adjusted Cox models adjusted for BMI (continuous), height (continuous), physical activity (inactive, moderately inactive, moderately active, active), smoking (never, 1-15cigarettes/day, 16-25 cigarettes/day, over 26 cigarettes/day, former smokers who quit<10 years, former smokers who quit 11-20 years, former smokers who quit>20 years, current pipe-cigar and occasional smokers), education (none, primary, technical and professional, secondary, higher education), and dietary intakes of energy (continuous), alcohol (continuous), and calcium (continuous) and stratified by age, sex, and centre.
*P-value significant after correcting for multiple testing.

Figure 2: Dietary intake of SFA, MUFA, iTFA and rTFA and colon and rectal cancer risk
All the models were multivariable-adjusted Cox models adjusted for BMI (continuous), height (continuous), physical activity (inactive, moderately inactive, moderately active, active), smoking (never, 1-15cigarettes/day, 16-25 cigarettes/day, over 26 cigarettes/day, former smokers who quit<10 years, former smokers who quit 11-20 years, former smokers who quit>20 years, current pipe-cigar and occasional smokers), education (none, primary, technical and professional, secondary, higher education), and dietary intakes of energy (continuous), red and processed meats (continuous), fibre (continuous), alcohol (continuous), and calcium (continuous) and stratified by age, sex, and centre.

Figure 3: Dietary contribution and sources of elaidic acid and risk of colon and rectal cancers
Dietary contributions represent on average, the amount of the elaidic acid that came from the major food items. Negligible contribution (<0.1%) were not considered in the analysis. Fats included vegetable oils, butter, margarine and deep-frying fats. HRs and 95%CI were determined using multivariable-adjusted Cox models adjusted for BMI (continuous), height (continuous), physical activity (inactive, moderately inactive, moderately active, active), smoking (never, 1-15cigarettes/day, 16-25 cigarettes/day, over 26 cigarettes/day, former smokers who quit<10 years, former smokers who quit 11-20 years, former smokers who quit>20 years, current pipe-cigar and occasional smokers), education (none, primary, technical and professional, secondary, higher education), and dietary intakes of energy (continuous), alcohol (continuous), and calcium (continuous) and stratified by age, sex, and centre





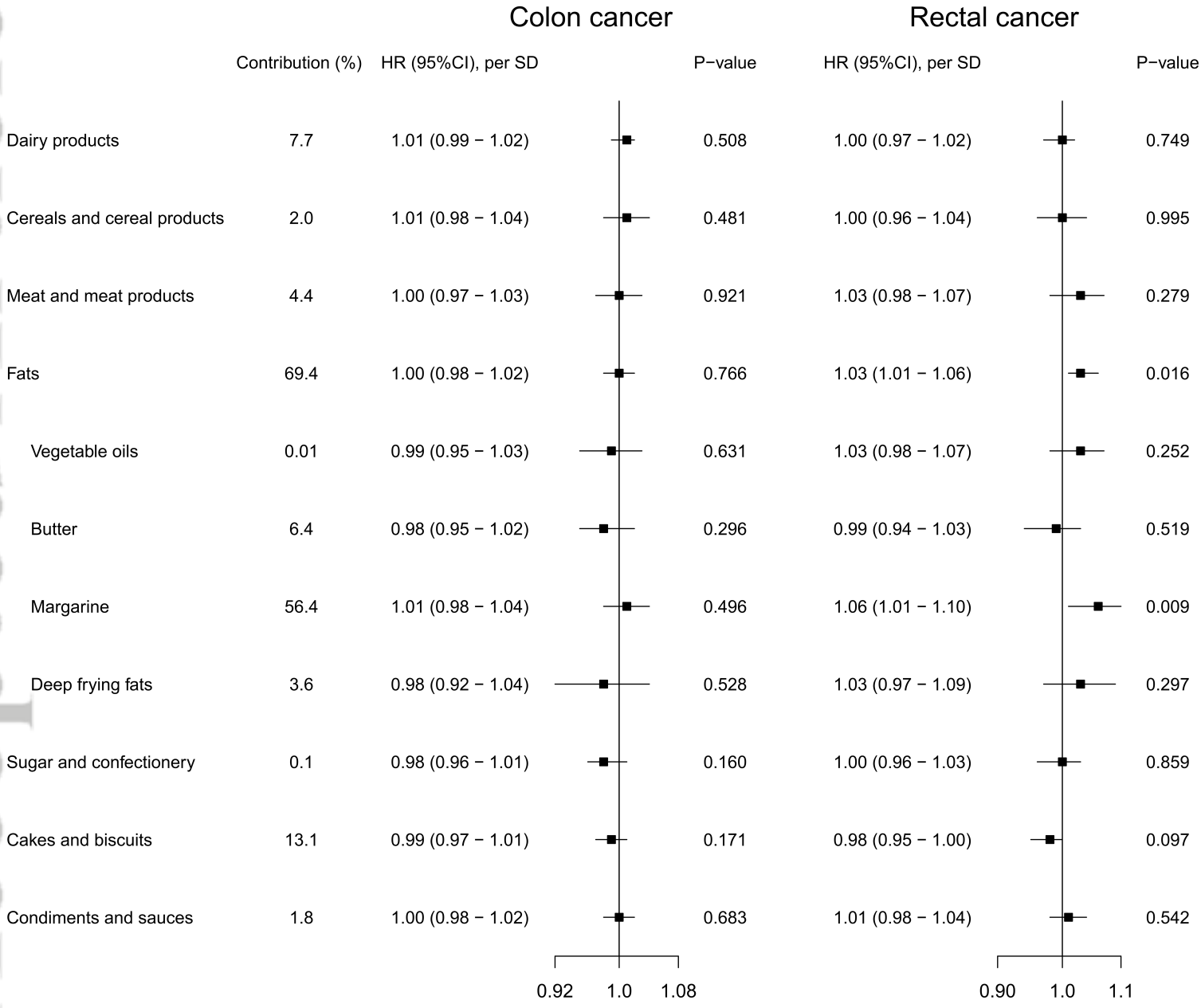


Table 1: Selected baseline demographic and lifestyle characteristics of study participants by colorectal cancer status, EPIC cohort study, 1992-2014

	Full cohort		Nested case-control	
	CRC Cases (n=6,162)	Non-cases (n=443,950)	Colon cancer cases (n=433)	Controls (n=433)
Men, %	43.0	29.0	39.3	39.3
Age at recruitment, years	57.1 ± 7.79	51.0 ± 9.75	56±7.8	56±7.7
Follow-up duration, years	8.78 ± 4.73	13.7 ± 3.93	6.5±3.5	15.2±2.6
Anthropometry				
Body mass index, kg/m²	26.3 ± 4.21	25.2 ± 4.19	26.9±4.5	26.5±3.6
Waist circumference, cm	89.4 ± 13.2	84.6 ± 12.9	89.5±13.3	87.7±11.4
Waist-to-hip ratio	0.88 ± 0.10	0.84 ± 0.10	0.87±0.1	0.86±0.09
Socio-demographic and lifestyle				
Education status (%)				
None	4.72	4.45	12.0	11.7
Primary school	32.1	25.9	40.3	41.6
Technical and professional school	25.2	22.5	14.1	17.9
Secondary school	15.6	20.8	17.4	13.1
Higher education	19.0	24.2	14.8	14
Smoking status (%)				
Never	37.2	43.2	41.2	44.8
Current, 1 to <16cigarettes/day	11.0	11.6	9.26	9.66
Current, 16-<26 cigarettes/day	6.29	6.23	6.48	5.52
Current, >26 cigarettes/day	1.72	1.82	2.78	0.69
Former, quit <10 years	10.6	9.53	13	9.43
Former, quit 11-<20 years	10.1	8.14	9.03	10.6
Former, quit >20 years	11.8	7.83	9.26	8.51
Current, pipe-cigar-occasional	8.28	8.42	7.87	10.1
Physical activity (%)				
Inactive	24.9	20.9	30.1	30.6
Moderately inactive	32.5	32.9	44.0	39.5
Moderately active	22.5	26.4	15.3	16.3
Active	18.4	17.9	10.7	13.6
Dietary intake				
Energy, kcal/day	2107 ± 614	2076 ± 619	2205±818	2168±629
Alcohol, g/day	15.4 ± 20.4	11.9 ± 17.0	15.3±19.6	13±17.3
Calcium, g/day	0.99±0.40	1.00±0.41	1±0.43	1.01±0.4
Red and processed meat, g/day	83.7 ± 52.9	75.8 ± 51.7	75.8±81	76.9±44
Fibre, g/day	22.7 ± 8.04	22.9 ± 8.14	22.7±7.6	23.2±8
Dairy products, g/day	333.7 ± 245.1	326.5 ± 235.4	281.3±235	295.3±210.0
Cereals, g/day	213 ± 112	219 ± 111	258.8±137.3	256.7±161.7
Fats, g/day	80.9 ± 29.4	81.4 ± 29.9	83.7±42.9	82.8±28.1
Sugar and confectionery, g/day	47.2 ± 59.8	41.6 ± 47.7	39.8±50.8	36.9±30.1
Cakes and biscuits, g/day	39.2 ± 42.4	40.9 ± 42.4	46.6±57.3	43.7±41.2

Condiments and sauces, g/day	20.9 ± 21.2	21.1 ± 20.0	15.9±15.3	16.4±16.1

Abbreviations: CRC, colorectal cancer
Descriptive statistics are presented as mean±standard deviation, unless otherwise specified
Frequencies may not add up to 100 due to missing value

Table 2: Hazard ratios (HRs) and 95% confidence intervals (CIs) for colorectal cancer risk associated with dietary SFA, MUFA, iTFA and rTFA (quintiles and continuous), EPIC cohort study, 1992-2014

	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	P_{trend}*	P_{BH}†	Continuous, per SD increase	Continuous, per SD increase ‡
SFA									
14:0 (myristic acid)									
Range, g/day	<1.58	1.58-<2.23	2.23-<2.93	2.93-<3.95	≥ 3.95				
Cases	1349	1321	1332	1188	972				
HR (95%CI)	1.00 (Ref.)	0.94 (0.87 - 1.01)	0.95 (0.88 - 1.04)	0.89 (0.81 - 0.98)	0.83 (0.74 - 0.93)	*0.002*	*0.036*	0.94 (0.90-0.98)	0.94(0.90 - 0.98)
15:0 (pentadecanoic acid)									
Range, g/day	<0.02	0.02-<0.03	0.03-<0.06	0.06-<0.10	≥ 0.10				
Cases	1087	1443	1283	1230	1129				
HR (95%CI)	1.00 (Ref.)	1.04 (0.96 - 1.13)	1.02 (0.94 - 1.12)	1.03 (0.93 - 1.13)	1.05 (0.95 - 1.17)	*0.479*	*0.752*	1.01 (0.98-1.05)	1.01(0.97 - 1.05)
16:0 (palmitic acid)									
Range, g/day	<9.93	9.93-<12.8	12.8-<15.4	15.4-<19.2	≥ 19.2				
Cases	1241	1314	1289	1248	1070				
HR (95%CI)	1.00 (Ref.)	0.99 (0.91 - 1.07)	0.95 (0.87 - 1.04)	0.94 (0.84 - 1.04)	0.81 (0.70 - 0.93)	*0.017*	*0.102*	0.92 (0.87-0.98)	0.94(0.88 - 1.00)
17:0 (heptadecanoic acid)									
Range, g/day	<0.05	0.05-<0.08	0.08-<0.11	0.11-<0.15	≥ 0.15				
Cases	1317	1317	1315	1174	1039				
HR (95%CI)	1.00 (Ref.)	1.06 (0.98 - 1.15)	1.13 (1.04 - 1.23)	1.05 (0.96 - 1.16)	1.04 (0.93 - 1.16)	*0.515*	*0.752*	0.98 (0.95-1.02)	0.98(0.94 - 1.02)
18:0 (stearic acid)									
Range, g/day	<4.20	4.20-<5.48	5.48-<6.79	6.79-<8.60	≥ 8.60				
Cases	1122	1256	1252	1321	1211				
HR (95%CI)	1.00 (Ref.)	1.00 (0.92 - 1.09)	0.95 (0.86 - 1.04)	0.96 (0.86 - 1.07)	0.88 (0.76 - 1.01)	*0.095*	*0.252*	0.95 (0.90-1.01)	0.96(0.90 - 1.02)
Total SFA									
Range, g/day	<16.1	16.1 - <20.7	20.7 - <25.4	25.4 - <31.9	≥31.9				

Cases	1,226	1,336	1,272	1,274	1,054				
HR (95%CI)	1.00 (Ref.)	1.00(0.92 - 1.09)	0.93(0.85 - 1.02)	0.93(0.84 - 1.04)	0.80(0.69 - 0.92)	*0.006*	*0.054*	0.93 (0.88-0.98)	0.94(0.88 - 1.00)
MUFA									
16:1n-7 (palmitoleic acid)									
Range, g/day	<0.77	0.77-<1.05	1.05-<1.33	1.33-<1.69	≥ 1.69				
Cases	1205	1265	1280	1198	1214				
HR (95%CI)	1.00 (Ref.)	0.97 (0.89 - 1.06)	0.98 (0.89 - 1.08)	0.92 (0.82 - 1.03)	0.88 (0.77 - 1.00)	*0.042*	*0.189*	0.93 (0.89-0.98)	0.95(0.90 - 1.00)
18:1n-7 (cis-vaccenic acid)									
Range, g/day	<4.46	4.46-<6.53	6.53-<8.82	8.82-<12.1	≥ 12.1				
Cases	1072	1242	1,201	1285	1,362				
HR (95%CI)	1.00 (Ref.)	1.11 (1.02 - 1.21)	1.05 (0.96 - 1.16)	1.12 (1.01 - 1.24)	1.12 (0.99 - 1.27)	*0.098*	*0.252*	1.01 (0.97-1.05)	1.02(0.97 - 1.06)
18:1n-9 (oleic acid)									
Range, g/day	<17.1	17.1-<22.1	22.1-<27.5	27.5-<35.5	≥ 35.5				
Cases	1313	1333	1348	1195	973				
HR (95%CI)	1.00 (Ref.)	1.00 (0.92 - 1.08)	1.03 (0.94 - 1.13)	0.96 (0.86 - 1.08)	0.93 (0.80 - 1.08)	*0.419*	*0.752*	0.95 (0.90-1.01)	0.96(0.90 - 1.03)
Total MUFA									
Range, g/day	<24.9	24.9-<31.8	31.8-<38.7	38.7-<48.4	≥ 48.4				
Cases	1313	1273	1278	1230	1068				
HR (95%CI)	1.00 (Ref.)	0.96 (0.88 - 1.04)	1.00 (0.91 - 1.09)	0.98 (0.88 - 1.09)	0.92 (0.80 - 1.06)	*0.593*	*0.752*	0.97 (0.91-1.02)	0.97(0.92 - 1.03)
iTFA									
18:1n-9/12 (elaidic acid)									
Range, g/day	<0.51	0.51 - <0.99	0.99 - <1.67	1.67 - <2.97	≥2.97				
Cases	1,189	955	1,125	1,337	1,556				
HR (95%CI)	1.00 (Ref.)	0.97(0.88 - 1.07)	1.06(0.95 - 1.18)	1.04(0.93 - 1.16)	1.03(0.92 - 1.16)	*0.399*	*0.752*	1.03 (1.00-1.06)	1.04(1.00 - 1.07)
18:2, t									

Range, g/day	<0.01	0.01 - <0.02	0.02 - <0.04	0.04 - <0.07	≥0.07				
Cases	1,437	1,255	1,206	1,181	1,083				
HR (95%CI)	1.00 (Ref.)	1.05(0.97 - 1.14)	1.06(0.97 - 1.16)	1.05(0.95 - 1.15)	1.03(0.93 - 1.14)	*0.627*	*0.752*	0.98(0.95 - 1.02)	0.98(0.94 - 1.02)
18:2, tt									
Range, g/day	<0	0 - <0.001	0.001 - <0.002	0.002 - <0.005	≥0.005				
Cases	1,272	1,159	1,025	1,204	1,502				
HR (95%CI)	1.00 (Ref.)	1.02(0.94 - 1.12)	0.94(0.84 - 1.04)	1.06(0.94 - 1.18)	1.07(0.95 - 1.21)	*0.093*	*0.252*	1.01(0.98 - 1.03)	1.01(0.98 - 1.03)
Total iTFA									
Range, g/day	<0.54	0.54 - <1.04	1.04 - <1.73	1.73 - <3.03	≥3.03				
Cases	1,190	965	1,116	1,343	1,548				
HR (95%CI)	1.00 (Ref.)	0.97(0.88 - 1.08)	1.05(0.94 - 1.17)	1.05(0.94 - 1.17)	1.03(0.92 - 1.16)	*0.380*	*0.752*	1.03(1.00 - 1.06)	1.04(1.00 - 1.07)
rTFA									
16:1n-7/9 (palmitelaidic acid)									
Range, g/day	<0.001	0.001 - <0.003	0.003 - <0.006	0.006 - <0.017	≥0.017				
Cases	1,494	1,415	1,170	1,105	978				
HR (95%CI)	1.00 (Ref.)	1.04(0.96 - 1.13)	1.06(0.96 - 1.16)	1.01(0.91 - 1.12)	1.03(0.92 - 1.16)	*0.711*	*0.753*	0.99 (0.96-1.03)	0.99(0.95 - 1.03)
18:1n-7 (trans vaccenic acid)									
Range, g/day	0		<0.001	0.001-<0.003	≥ 0.003				
Cases	3358	-	753	866	1,185				
HR (95%CI)	1.00 (Ref.)	-	0.93 (0.82 - 1.06)	0.95 (0.84 - 1.08)	0.97 (0.86 - 1.10)	*0.671*	*0.753*	0.97 (0.94-1.00)	0.96(0.93 - 1.00)
CLA									
Range, g/day	<0.01	0.01-<0.02	0.02-<0.03	0.03-<0.06	≥ 0.06				
Cases	1,649	1,302	1,190	1,108	913				
HR (95%CI)	1.00 (Ref.)	1.08 (0.99 - 1.17)	1.08 (0.99 - 1.19)	1.04 (0.95 - 1.15)	0.99 (0.89 - 1.10)	*0.810*	*0.810*	0.97 (0.94-1.01)	0.47(0.18 - 1.19)
Total rTFA									

Range, g/day	<0.01	0.01 - <0.03	0.03 - <0.05	0.05 - <0.08	≥0.08				
Cases	1,685	1,236	1,194	1,118	929				
HR (95%CI)	1.00 (Ref.)	1.02(0.94 - 1.10)	1.03(0.94 - 1.13)	1.01(0.91 - 1.11)	0.97(0.87 - 1.08)	*0.579*	*0.752*	0.96 (0.93-1.00)	0.96(0.92 - 1.00)

Abbreviations: CLA, conjugated linoleic acid; FA, fatty acid; MUFA, monounsaturated fatty acid; iTFA, industrial trans fatty acid; rTFA, ruminant trans fatty acid; SFA, saturated fatty acid. All the models were multivariable-adjusted Cox models adjusted for BMI (continuous), height (continuous), physical activity (inactive, moderately inactive, moderately active, active), smoking (never, 1-15cigarettes/day, 16-25 cigarettes/day, over 26 cigarettes/day, former smokers who quit<10 years, former smokers who quit 11-20 years, former smokers who quit>20 years, current pipe-cigar and occasional smokers), education (none, primary, technical and professional, secondary, higher education), and dietary intakes of energy (continuous), red and processed meats (continuous), fibre (continuous), alcohol (continuous), and calcium (continuous) and stratified by age, sex, and centre

*Determined by using median value of the quintiles as continuous in the model

†P-values corrected for multiple testing using the Benjamini-Hochberg approach

‡Analyses excluding cases that occurred within 4 years of follow-up (n=1042 cases excluded)

Table 3: Odds ratios and 95% confidence intervals (CI) for colorectal cancer risk associated with plasma phospholipid SFA, MUFA, iTFA, and rTFA (Quartiles and continuous), EPIC cohort study, 1992-2014

	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P_{trend}*	P_{BH}†	Continuous, per SD increase
SFA							
14:0 (myristic acid)							
Range, in %	<0.21	0.22-<0.26	0.27-<0.33	>0.34			
Cases/Controls	146/121	94/101	101/105	92/106			
OR (95%CI)	1.00 (Ref.)	0.69 (0.45 - 1.05)	0.58 (0.37 - 0.90)	0.51 (0.32 - 0.83)	*0.005*	*0.090*	0.76 (0.63-0.92)
15:0 (pentadecanoic acid)							
Range, in %	<0.13	0.14-<0.17	0.18-<0.2	>0.21			
Cases/Controls	130/105	139/146	80/84	84/98			
OR (95%CI)	1.00 (Ref.)	0.79 (0.53 - 1.18)	0.77 (0.47 - 1.27)	0.63 (0.37 - 1.06)	*0.105*	*0.630*	0.77 (0.63-0.94)
16:0 (palmitic acid)							
Range, in %	<24.2	24.2-<25.3	25.3-<26.2	>26.3			
Cases/Controls	101/106	114/110	97/108	121/109			
OR (95%CI)	1.00 (Ref.)	1.02 (0.68 - 1.53)	0.73 (0.46 - 1.17)	0.88 (0.54 - 1.43)	*0.396*	*0.809*	0.80 (0.66-0.98)
17:0 (heptadecanoic acid)							
Range, in %	<0.34	0.35-<0.39	0.4-<0.43	>0.44			
Cases/Controls	135/109	119/131	92/93	87/100			
OR (95%CI)	1.00 (Ref.)	0.81 (0.55 - 1.20)	0.91 (0.58 - 1.44)	0.81 (0.50 - 1.32)	*0.539*	*0.809*	0.88 (0.73-1.05)
18:0 (stearic acid)							
Range, in %	<13.3	13.3-<14.1	14.1-<14.8	>14.8			
Cases/Controls	83/111	124/107	121/112	105/103			
OR (95%CI)	1.00 (Ref.)	1.69 (1.07 - 2.65)	1.61 (1.03 - 2.52)	1.63 (1.00 - 2.64)	*0.087*	*0.630*	1.17 (0.98-1.39)
Total SFA							
Range, in %	<39.4	39.4-<40.2	40.2-<41.0	>41.0			

Cases/Controls	97/106	107/111	110/113	119/103			
OR (95%CI)	1.00 (Ref.)	1.05 (0.69 - 1.58)	0.92 (0.59 - 1.42)	1.04 (0.64 - 1.68)	*0.952*	*0.952*	0.90 (0.74-1.09)
MUFA							
16:1n-7 (palmitoleic acid)							
Range, in %	<0.49	0.49-<0.63	0.63-<0.82	>0.82			
Cases/Controls	102/105	110/111	114/111	107/106			
OR (95%CI)	1.00 (Ref.)	0.93 (0.60-1.44)	0.84 (0.52-1.37)	0.75 (0.44-1.27)	*0.252*	*0.809*	0.88 (0.73-1.06)
18:1n-7 (cis-vaccenic acid)							
Range, in %	<1.32	1.32-<1.46	1.46-<1.64	>1.64			
Cases/Controls	112/101	112/112	117/115	92/105			
OR (95%CI)	1.00 (Ref.)	0.94 (0.63-1.40)	0.97 (0.65-1.46)	0.82 (0.53-1.28)	*0.462*	*0.809*	0.94 (0.80-1.10)
18:1n-9 (oleic acid)							
Range, in %	<9.34	9.34-<10.6	10.6-<12.1	>12.1			
Cases/Controls	111/105	110/113	102/115	110/100			
OR (95%CI)	1.00 (Ref.)	0.90 (0.60-1.37)	0.83 (0.55-1.26)	0.91 (0.57-1.45)	*0.579*	*0.809*	1.00 (0.85-1.17)
Total MUFA							
Range, in %	<11.9	11.9-<13.3	13.3-<14.6	>14.6			
Cases/Controls	117/106	102/112	95/113	119/102			
OR (95%CI)	1.00 (Ref.)	0.83 (0.54-1.25)	0.75 (0.49-1.12)	0.97 (0.61-1.54)	*0.690*	*0.828*	0.98 (0.83-1.14)
iTFA							
18:1n-9/12 (elaidic acid)							
Range, in %	<0.13	0.14-<0.19	0.2-<0.29	>0.3			
Cases/Controls	106/102	129/119	87/102	111/110			
OR (95%CI)	1.00 (Ref.)	1.16 (0.77 - 1.74)	0.88 (0.56 - 1.38)	1.06 (0.63 - 1.79)	*0.833*	*0.882*	0.88 (0.71-1.10)

18:2, t							
Range, in %	<0.01	0.01-<0.02	0.02-0.03	>=0.03			
Cases/Controls	112/101	112/112	117/115	92/105			
OR (95%CI)	1.00 (Ref.)	1.36(0.91 - 2.03)	1.29(0.75 - 2.20)	0.47(0.22 - 1.01)	*0.361*	*0.809*	0.86(0.71 - 1.05)
18:2, tt							
Range, in %	<0.04	0.04-<0.05	0.05-<0.07	>=0.07			
Cases/Controls	83/110	124/108	121/112	105/103			
OR (95%CI)	1.00 (Ref.)	0.96(0.61 - 1.50)	1.04(0.68 - 1.61)	0.63(0.35 - 1.17)	*0.397*	*0.809*	0.87(0.69 - 1.10)
Total iTFA							
Range, in %	<0.42	0.43-<0.59	0.6-<0.99	>1.01			
Cases/Controls	106/106	108/108	118/107	101/112			
OR (95%CI)	1.00 (Ref.)	0.96(0.63 - 1.47)	0.86(0.54 - 1.36)	0.92(0.52 - 1.62)	*0.633*	*0.814*	0.85(0.68 - 1.07)
rTFA							
16:1n-7/9 (palmitelaidic acid)							
Range, in %	<0.20	0.20-<0.26	0.26-<0.39	>=0.39			
Cases/Controls	122/109	84/109	120/105	107/110			
OR (95%CI)	1.00 (Ref.)	0.77 (0.48 - 1.24)	1.65 (0.79 - 3.47)	1.57 (0.61 - 4.00)	*0.584*	*0.809*	1.14 (0.87-1.52)
18:1n-7 (trans vaccenic acid)							
Range, in %	<0.09	0.09-<0.13	0.13-<0.20	>0.20			
Cases/Controls	124/113	107/102	99/110	103/108			
OR (95%CI)	1.00 (Ref.)	0.90 (0.60-1.36)	0.85 (0.54-1.34)	0.84 (0.48-1.44)	*0.488*	*0.809*	0.92 (0.74-1.13)
CLA							
Range, in %	<0.11	0.11-<0.15	0.15-<0.23	>0.23			
Cases/Controls	121/123	88/89	127/118	97/103			
OR (95%CI)	1.00 (Ref.)	1.11 (0.71-1.73)	1.20 (0.74-1.94)	0.89 (0.51-1.54)	*0.823*	*0.882*	0.79 (0.64-0.98)

Total rTFA							
Range, in %	<1.77	1.77-<1.99	1.99-<2.35	>=2.35			
Cases/Controls	128/121	82/93	132/111	91/108			
OR (95%CI)	1.00 (Ref.)	0.97(0.61 - 1.54)	0.99(0.61 - 1.62)	0.72(0.36 - 1.44)	0.528	*0.809*	0.93(0.73 - 1.18)

Abbreviations: CLA, conjugated linoleic acid; FA, fatty acid; MUFA, monounsaturated fatty acid; iTFA, industrial trans fatty acid; rTFA, ruminant trans fatty acid; SFA, saturated fatty acid

All the models were conditional logistic regression models conditioned on the matching factors and adjusted for BMI (continuous), height (continuous), physical activity (inactive, moderately inactive, moderately active, active), smoking (never, 1-15cigarettes/day, 16-25 cigarettes/day, over 26 cigarettes/day, former smokers who quit<10 years, former smokers who quit 11-20 years, former smokers who quit>20 years, current pipe-cigar and occasional smokers), education (none, primary, technical and professional, secondary, higher education), and dietary intakes of energy (continuous), red and processed meats (continuous), fibre (continuous), alcohol (continuous), and calcium (continuous).

*Calculated by using median value of the quartiles as continuous in the model.

[†]P-values corrected for multiple testing using the Benjamini-Hochberg approach