Risk of Colorectal Cancer Is Linked to Erythrocyte Compositions of Fatty Acids as Biomarkers for Dietary Intakes of Fish, Fat, and Fatty Acids

Kiyonori Kuriki,[1,2] Kenji Wakai,[1] Kaoru Hirose,[1] Keitaro Matsuo,[1] Hidemi Ito,[1] Takeshi Suzuki,[1] Toshiko Saito,[1] Yukihide Kanemitsu,[3] Takashi Hirai,[3] Tomoyuki Kato,[3] Masae Tatematsu,[2] and Kazuo Tajima[1,4]

Divisions of [1]Epidemiology and Prevention and [2]Oncological Pathology, Aichi Cancer Center Research Institute; [3]Department of Gastroenterological Surgery, Aichi Cancer Center Hospital, and [4]Department of Epidemiology, Nagoya University Graduate School of Medicine, Nagoya, Japan

Abstract

Consumption of fish rich in n-3 polyunsaturated fatty acids (PUFAs), such as docosahexaenoic acid, is suggested to reduce colorectal cancer risk through inhibition of the arachidonic acid (AA) cascade related to tumorigenesis and cell proliferation. High intake of saturated fatty acids (SFAs) may increase the risk. To examine associations between colorectal cancer risk and fatty acid compositions in erythrocyte membranes, as biomarkers for dietary intakes of fish, fat, and fatty acids, we conducted a case-control study with 74 incident cases and 221 noncancer controls (matched by age, sex, and season of sample collection). Erythrocyte fatty acids were measured using an accelerated solvent extraction and a gas-liquid chromatography. Colorectal cancer had no association with dietary intakes of meat, fish, fat, and fatty acids. However, the risk was inversely associated with erythrocyte compositions of docosahexaenoic acid, AA, and PUFAs [the highest to the lowest tertile, odds ratios, 0.36, 0.42, and 0.15; 95% confidence intervals, 0.14-0.93, 0.18-0.95, and 0.05-0.46; $P_{trend} < 0.05$, respectively] and positively with those of palmitic acid, SFAs, and the ratio of SFAs/PUFAs (odds ratios, 6.46, 8.20, and 9.45; 95% confidence intervals, 2.41-17.26, 2.86-23.52, and 2.84-31.43; $P_{trend} < 0.005$, respectively). In conclusion, we could clearly show decreased and increased risks for colorectal cancer related to PUFAs and SFAs compositions in erythrocyte membranes, respectively, but further research is needed to investigate the discrepancy between our findings and the generally accepted role of the AA cascade. (Cancer Epidemiol Biomarkers Prev 2006;15(10):1791–8)

Introduction

Dietary linoleic acid (18:2n-6) is desaturated and elongated to arachidonic acid (AA; 20:4n-6) and then converted into n-6 polyunsaturated fatty acid (PUFA)–derived eicosanoids. Such eicosanoids are prostaglandin E_2, thromboxane A_2, leukotriene B_4, and prostacyclins, which are commonly linked to inflammation, tumorigenesis, angiogenesis, cell proliferation, and inhibition of apoptosis (1, 2). The AA cascade is thought to play critical roles in colorectal tumor development. On the other hand, fish rich in eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3) has been hypothesized to reduce colorectal cancer risk (3). EPA and DHA, either from fish in the diet or by metabolism from α-linolenic acid (18:3n-3) in vegetable oils, are converted into n-3 PUFA-derived eicosanoids (4, 5). Therefore, n-3 PUFAs, especially n-3 highly unsaturated fatty acids (HUFAs = EPA + docosapentaenoic acid + DHA), are thought to compete with n-6 PUFAs for incorporation of phospholipids into cell membranes and alter PUFAs components in the membranes (6). A high composition of n-3 HUFAs in tissue has been shown to decrease the risk of colorectal adenoma (7).

Although fish consumption in Japanese continues to be among the highest in the world, dietary intakes of meat, animal fat, and/or saturated fatty acids (SFAs), such as 16:0 and 18:0, have remarkably increased because of adoption of a westernized diet (8, 9). High intake of SFAs may elevate colorectal cancer risk through increased bile acid production and an elevated concentration of diacylglycerol (10, 11), but the latest reports in Japan and America-European countries are conflicting (12-16). In erythrocyte membranes, the composition of 18:0 has been reported to be higher in colorectal cancer patients than in noncancer controls (17). Although the erythrocyte ratio of 18:0/18:1n-9 (i.e., saturation $index_{n-9}$), suggested to reflect activity of the rate-limiting enzyme Δ9 desaturase (catalyzing conversion from 18:0 to 18:1n-9), is associated with cancer risk in the colorectum and breast, findings are inconsistent (18-21).

Some of PUFAs, especially in membrane phospholipids of erythrocytes (120 days half-life), are useful as biomarkers in assessing dietary intakes of fat, fatty acids, and fish because they are not biosynthesized *in vivo* (22-24). Erythrocyte compositions of such PUFAs have been shown to correlate with dietary intakes of the corresponding fatty acids and also reflect dietary supplementation with fish, fish oil, EPA, and DHA in validity and feeding studies (25-28). EPA and DHA are preferentially incorporated into phospholipids rich in erythrocyte membranes and the proportion of 18:2n-6 may increase with DHA supplementation (28). Recently, we have developed an automatic extraction method for measuring fatty acids in biomaterials by gas-liquid chromatography with high precision and accuracy, making it feasible to use small volume multisamples routinely, rapidly, and cheaply (29).[5] To examine

Received 3/9/06; revised 6/28/06; accepted 8/7/06.

Grant support: Grant-in-Aid for Scientific Research on Priority Areas for Cancer (grant no. 12218242 and 17015052) from the Ministry of Education, Science, Sports, Culture and Technology of Japan; a Grant-in-Aid for a Research Fellow of the Japan Society for the Promotion of Science; and a Grant-in-Aid for the 3rd Term Comprehensive 10-Year Strategy for Cancer Control. K. Kuriki was supported by a Research Resident Fellowship from the Foundation for Promotion of Cancer Research (Japan) for the 3rd Term Comprehensive 10-Year Strategy for Cancer Control during the performance of much of this work.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Requests for reprints: Kiyonori Kuriki, Division of Epidemiology and Prevention, Aichi Cancer Center Research Institute, 1-1 Kanokoden, Chikusa-ku, Nagoya 464-8681, Japan. Phone: 81-52-762-6111; Fax: 81-52-763-5233. E-mail: kkuriki@aichi-cc.jp

doi:10.1158/1055-9965.EPI-06-0180

[5] A patent for this method to quantitatively measure fatty acids in biomaterials was applied (Japanese Patent applied No. 2005-080461).

whether associations exist between colorectal cancer risk and biomarkers of fatty acid compositions in erythrocyte membranes, we therefore conducted a case-control study in a Japanese population using the new analytic method.

Materials and Methods

Study Design. From December 2002 to June 2004, subjects, ages 20 to 80 years, were recruited in the framework of the Hospital-based Epidemiologic Research Program at Aichi Cancer Center. The study design has been elsewhere described (30). As a part of Hospital-based Epidemiologic Research Program at Aichi Cancer Center, a large number of blood variables for Aichi Fatty Acid (AiFat) Research are routinely measured to clarify associations with cancers in several sites. Briefly, all first-visit outpatients (n = 10,703), including all cancer cases (n = 2,230) on each site, are asked to fill out a self-administrated questionnaire about their lifestyle as well as to provide 7 mL blood. Trained interviewers systematically collect and check information from the questionnaire, which is completed by 97.6% of 9,371 eligible subjects. Three thousand six hundred and twenty-two subjects (39.6%) have provided blood samples. The participation rates for all cancer cases, colorectal cancer cases, and controls were 39.6%, 40.3%, and 39.3%, in that order. All subjects for the present study were Japanese, living in and around Aichi Prefecture, central Japan.

Case Ascertainment and Control Subjects. Seventy-four incident cases, who donated the completed questionnaire and blood samples during the study period, were histologically diagnosed as having colorectal cancers (58.1%, 40.5%, and 1.4% in the colon, rectum, and both, in that order) at Aichi Cancer Center Hospital (ACCH). The control subjects were randomly selected from first-visit outpatients who visited ACCH at the same period and confirmed to have no cancer or any prior history of cancer according to a questionnaire. Moreover, they were checked for current and/or previous history of cancer based on the cancer registry system in Aichi Prefecture. Subjects with the following current diseases or the history were excluded; hepatitis (3.4% of all subjects), liver cirrhosis (1.6%), chronic nephritis (2.5%), diabetes (6.0%), angina (3.3%), stroke (1.7%), ovary resection (3.7%), and uterus resection (5.1%). Finally, 221 controls were individually matched for age ($\pm$5 years), sex, and season of sample collection to cases with a 1:3 case-control ratio. We here took into consideration seasonal variation in biomarkers for dietary intakes of fish, fat, and fatty acids because plasma fatty acid concentrations exhibit significant differences between seasons (31). Our previous study showed that it is feasible to use noncancer outpatients at ACCH as controls in epidemiologic studies because their general lifestyles are accordant with those of general population randomly selected from the electoral roll in Nagoya City, Aichi Prefecture (32). All subjects were provided with an explanatory document and gave their written informed consent for participation in this study, which was approved by the Ethics Committee of the Aichi Cancer Center.

Questionnaire. The participants completed a questionnaire that included height, weight, dietary habit, habitual exercise, drinking habit, and smoking status. The assessment of various food and nutrient consumption was according to a semiquantitative food frequency questionnaire (SQFFQ) with 47 food items. The methods for developing the SQFFQ and computing the average daily consumption of energy and selected nutrients have been described elsewhere (33, 34). Compared with 3-day weighed diet records, an acceptable relative validity of the SQFFQ for assessing dietary intakes of most nutrients has been shown, and the Spearman's correlation coefficients for fatty acids were 0.13 (for n-6 PUFAs) to 0.52 (SFAs) for men and 0.16 (PUFAs) to 0.34 (SFAs) for women, respectively (35). Therefore, it seemed appropriate to use the SQFFQ for the study subjects, as well as the general population. Moreover, intake of some dietary fatty acids based on the SQFFQ has been shown to have significant correlations with the corresponding fatty acid concentrations (mmol/L) in plasma, assessed as biomarkers (36). The obtained Spearman's correlation coefficients were 0.38 and 0.26 for n-3 HUFAs and 0.43 and 0.37 for the ratio of n-6 PUFAs/n-3 PUFAs among men and women, respectively. The SQFFQ inquired about habitual dietary intake during the latest 1 year for 47 foods/food groups with division of frequency into eight categories: never or seldom, one to three times/monthly, one to two times weekly, three to four times weekly, five to six times weekly, once daily, twice daily, and three or more times daily. We asked the case subjects to provide information about their lifestyle before the onset of disease and the control subjects at the study enrollment.

Fat and fatty acids were computed by multiplying the standard portion size (in grams), frequency of consumption, and the content (per gram) of fat or each fatty acid in foods as listed in the Standard Tables of Food Consumption and the Follow-up of the Standard Tables of Food Consumption (37, 38). Dietary intakes (g/day) of meat, fish, other seafood, fat, and fatty acids in 47 foods/food groups were calculated. Dietary intakes (g/day) of each fatty acid were summed up as SFAs, monounsaturated fatty acids (MUFAs), n-6 PUFAs, n-3 PUFAs, and n-3 HUFAs. These foods and fatty acids were adjusted for total energy intake of each person and tertile cut points (g/1,000 kcal) in control subjects were used to designate low, moderate, and high. Lifestyle factors were also classified into three groups (i.e., for habitual exercise, which was other than work), less than once weekly ''low,'' one to twice weekly ''moderate,'' and three or more times weekly ''high''; for drinking habit, less than once weekly ''low,'' one to four times weekly ''moderate,'' and five or more times weekly ''high''; and for smoking status, current, former, and never smokers. We defined former smokers as those who quit smoking >2 years before the questionnaire study.

Analysis of Fatty Acids in Erythrocytes. Our approach using an accelerated solvent extractor and a gas-liquid chromatography for measuring fatty acids in biomaterials can be summarized as follows (23, 29).[5] In short, erythrocytes were collected using an EDTA-2Na tube and centrifuged at 2,000 $\times$ g for 15 minutes at 4°C. At least 500 μL of unwashed erythrocytes was kept at −80°C until the time of extraction. The membranes (white ghosts) from 50 μL erythrocytes were prepared with sodium phosphate buffer to exclude iron of hemoglobin for preventing oxidative degradation of fatty acids. Butylate hydroxytoluene was used as an antioxidant. The accelerated solvent extractor (ASE®) 200 (Nippon Dionex, Osaka, Japan) was applied for extracting lipids in erythrocyte membranes (first extraction) and fatty acid methyl esters (second extraction) with chloroform-methanol (1:2) by volume and petroleum ether, respectively, as solvents. The two extraction processes were automatically achieved with computerized programs (i.e., 75°C, 1,500 p.s.i., and 8.5 minutes/sample and 50°C, 1,500 p.s.i., and 3.5 minutes/sample for first and second extraction, respectively). Although Folch's solvent (chloroform-methanol, 2:1, by volume) and chloroform-isopropanol (7:11, by volume) are usually used to extract fatty acids in biomaterials (39), our mixture rates for the two solvents were optimal for our method. All samples from the first extraction were treated with hydrochloride-methanol reagent for fatty acid conversion from lipids and subsequent methyl transformation of fatty acids. The fatty acid methyl esters from the second extraction were analyzed by Shimadzu GC-2010 gas chromatography (Shimadzu, Kyoto, Japan) on a capillary column DB-225 (J&W Scientific, Folsom, CA), equipped with an autoinjector, autosampler, and flame ionization detector, under the conditions described elsewhere

Table 1. Mean and SD of some variables possibly related to colorectal cancer in case and control subjects

	Case subjects (n = 74)		Control subjects (n = 221)		P, χ^2 or t test
	Mean	SD	Mean	SD	
Age (years)	58.4	9.6	58.0	9.6	NS
Men/women (% for women)	45/29	(39.2%)	134/87	(39.4%)	NS
BMI (kg/m^2)	23.0	2.9	23.0	2.7	NS
Family history of colorectal cancer in parents and/or siblings, n (%)	10 (13.5)		11 (5.0)		<0.05
Habitual exercise, n (%)					
High	12 (16.2)		61 (27.6)		NS
Moderate	42 (56.8)		111 (50.2)		
Low	20 (27.0)		49 (22.2)		
Drinking status, n (%)					
Drinkers	46 (62.2)		123 (55.7)		NS
Ex-drinkers	1 (1.4)		6 (2.7)		
Nondrinkers	27 (36.5)		92 (41.6)		
Smoking status, n (%)					
Smokers	22 (29.7)		53 (24.0)		NS
Ex-smokers	24 (32.4)		59 (26.7)		
Nonsmokers	28 (37.8)		109 (49.3)		
Dietary intake					
Meat (g/1,000 kcal)	17.3	10.5	17.2	11.5	NS
Fish (g/1,000 kcal)	22.2	12.0	20.7	11.3	NS
Other seafood (g/1,000 kcal)	16.5	10.2	19.8	14.3	<0.05
Green-yellow vegetables (g/1,000 kcal)	34.4	23.9	32.5	20.7	NS
Energy from fat (%)	23.4	6.3	23.8	7.0	NS
Total fat (g/1,000 kcal)	26.0	7.0	26.4	7.8	NS
SFAs* (g/1,000 kcal)	12.3	2.3	13.2	3.5	<0.05
MUFAs* (g/1,000 kcal)	17.1	3.5	18.0	4.9	NS
PUFAs* (g/1,000 kcal)	14.2	3.2	14.9	4.3	NS
n-6 PUFAs* (g/1,000 kcal)	11.4	2.9	12.0	3.8	NS
n-3 PUFAs* (g/1,000 kcal)	2.5	0.5	2.6	0.6	NS
n-3 HUFAs* (g/1,000 kcal)	0.84	0.35	0.84	0.33	NS

Abbreviation: NS, not significant.
*Dietary intakes of each fatty acid group were explained in Materials and Methods and mainly composed of the selected 13 fatty acids as follows: SFAs = 14:0 + 16:0 + 18:0; MUFAs = 16:1n-7 + 18:1n-9; PUFAs = n-6 PUFAs + n-3 PUFAs; n-6 PUFAs = 18:2n-6 + 18:3n-6 + 20:3n-6 + 20:4n-6 (AA); n-3 PUFAs = 18:3n-3 + n-3 HUFAs; and n-3 HUFAs = 20:5n-3 (EPA) + 22:5n-3 + 22:6n-3 (DHA).

(23, 29).[5] We applied 17:0 (heptadecanoic acid) as an internal standard. Each fatty acid was identified with the use of commercial standards of known retention time, and integration of the peak areas was done with the GC solution version 2 software. The laboratory for measuring fatty acids was completely blinded to links between biomaterials and any information of study subjects.

In line with our previous studies, we selected 13 fatty acids as described below (23, 24, 40), predominating in both dietary intakes and erythrocyte contents. Fatty acid compositions of erythrocyte membranes were determined as molar percentage (mol%) of total fatty acid concentrations (mmol/L) for each because it was difficult to accurately determine the numbers of erythrocytes in 50 μL and evaluate the concentrations because erythrocytes vary in size and surface area (29).[5] Intra-assay coefficients of variation (CV) was based on the analysis of a series of 10 samples and then all were extracted and analyzed within 1 day. The intra-assay CV was <4.0%, except a minor group of 14:0, 18:3n-6, and 18:3n-3 (≤0.5% of total fatty acids for each) (29).[5] Interassay CVs were based on the replicate analyses of pooled erythrocytes over a period of 10 days, and a total of 100 samples (10 samples/day × 10 days) were measured. The interassay CVs were also <4.0%, except for a minor group of 14:0, 18:3n-6, and 18:3n-3 (29).[5]

Selected Fatty Acids and Grouping. We here selected the following 13 fatty acids: 14:0 (myristic acid), 16:0 (palmitic acid), 16:1n-7 (palmitoleic acid), 18:0 (stearic acid), 18:1n-9 (oleic acid), 18:2n-6 (linoleic acid), 18:3n-6 (γ-linolenic acid), 18:3n-3 (α-linolenic acid), 20:3n-6 (dihomo-γ-linolenic acid), 20:4n-6 (AA), 20:5n-3 (EPA), 22:5n-3 (docosapentaenoic acid), and 22:6n-3 (DHA). We use both (*a*) a series of standard systematic notation to specify fatty acid chain length, number of double bonds, and position and configuration of double bond nearest the methyl end (n-end) of the chain and (*b*) a series of established common names to refer to fatty acid chains in parentheses (21). To facilitate understanding chemical characteristics of each fatty acid, the former series of names were here used. The specification begins with two numbers separated by a colon; these numbers refer to the number of carbons in the chain and the number of double bonds, respectively; the next number, preceded by ''n,'' indicates the distance of first double bond from the n-end of the chain. About geometric isomerism, all of them have *cis* type of the double bond nearest the n-end of the chain. We used both systematic and common names in Table 2, but the latter were omitted in Tables 3 and 4.

We calculated the compositions of fatty acids in erythrocyte membranes and summarized the data into the following seven groups, with the use of total fatty acids as the denominator: SFAs (14:0 + 16:0 + 18:0), MUFAs (16:1n-7 + 18:1n-9), PUFAs (n-6 PUFAs + n-3 PUFAs), n-6 PUFAs (18:2n-6 + 18:3n-6 + 20:3n-6 + AA), n-3 PUFAs (18:3n-3 + n-3 HUFAs), and n-3 HUFAs (EPA + 22:5n-3 + DHA). We also defined the ratios of specific fatty acid as follows: the ratios of 18:0/18:1n-9 (as saturated index$_{n-9}$), 16:0/16:1n-7 (as saturated index$_{n-7}$), SFAs/PUFAs, SFAs/n-6 PUFAs, SFAs/n-3 PUFAs, SFAs/n-3 HUFAs, n-6 PUFAs/n-3 PUFAs, n-6 PUFAs/n-3 HUFAs, AA/EPA, and AA/DHA. The saturated indices are indicators of membrane fluidity; its reciprocal can be considered to be and index of the activity of the rate-limiting enzyme Δ9 desaturase that transforms SFAs into the corresponding MUFAs (17-19, 21). The ratio of n-6 PUFAs/n-3 PUFAs has been suggested to be important for human health (41).

Statistical Methods. Body mass index (BMI; kg/m^2) was calculated from height (m) and weight (kg) data. Student's t test and χ^2 test were done comprising cases and controls for each variable. In control subjects, partial Spearman's correlation coefficients between fatty acids in dietary intakes (g/1,000 kcal)

and erythrocyte compositions (mol%) were adjusted for age, sex, BMI, and season of sample collection. Partial Pearson's correlation coefficients between pairs of fatty acids in erythrocyte membranes were adjusted for the same variables described above. Odds ratio (OR) and the 95% confidence interval (95% CI) were calculated, using conditional logistic regression models, after adjustment for BMI (continuous), habitual exercise, drinking and smoking habits, green-yellow vegetable intake (g/1,000 kcal), and family history of colorectal cancer in parents and/or siblings (yes or no) due to control for the effects of potential confounding factors. With reference to previously published reports, including our latest results for colorectal cancer risk (12, 42), adjustment was made for possible confounding factors. In Japan, users of nonsteroidal anti-inflammatory drugs are very few. Cases were categorized according to the tertile levels of fatty acids in erythrocyte membranes among control subjects, and the ORs for the middle and the highest to the lowest tertile were estimated. Tests for trends in each variable were conducted by assigning the weight median values in control subjects. All statistical analyses were conducted with SAS version 9.1 (SAS Institute, Inc., Cary, NC), and $P < 0.05$ was considered statistical significant.

Results

Table 1 shows characteristics of the subjects and their dietary consumption of foods, fat, and fatty acids. In both case and control subjects, the average age was 58.0 to 58.4 ± 9.6 years (33-80 years), and female subjects were ~40%. The percentage of family history of colorectal cancer in parents and/or siblings was higher in case than in control subjects ($P < 0.05$), whereas dietary intakes of seafood and SFAs were lower in cases ($P < 0.05$ for both). However, the two groups did not differ in variables about BMI, lifestyle-related factors (habitual exercise and drinking and smoking habits), and dietary intakes of foods, fat, and fatty acids.

Table 2 shows mean percentage (mol%) of fatty acid compositions of erythrocyte membranes in case and control subjects. Of the 13 selected fatty acids, nine fatty acids were most abundant in erythrocyte membranes in the following order: 16:0, 18:0, 18:1n-9, 18:2n-6, 20:4n-6, 22:6n-3, 20:5n-3, 22:5n-3, and 16:1n-7. Compared with control subjects, cases had higher percentages of 16:0, 16:1n-7, and SFAs and the ratio of SFAs/PUFAs and lower percentages of 18:3n-6 and PUFAs and saturation index$_{n-9}$ ($P < 0.05$ to < 0.001 for all).

In control subjects, dietary intakes (g/1,000 kcal) of fish were positively associated with compositions of 16:0 and EPA in erythrocyte membranes [partial Spearman's correlation coefficients (r) = 0.17 and 0.18; $P < 0.05$] and negatively with those of n-6 PUFAs and AA ($r = -0.19$ and -0.14; $P < 0.05$). Intake of n-3 HUFAs had associations with erythrocyte compositions of 16:0, EPA, n-6 PUFAs, and 18:2n-6 ($r = 0.16$, 0.20, -0.20, and -0.16; $P < 0.05$ for all). That of n-6 PUFAs was associated with erythrocyte compositions of n-6 PUFAs, 18:2n-6, SFAs, and 16:0 ($r = 0.22$, 0.23, -0.18, and -0.19; $P < 0.01$ for all), and the ratio of n-6 PUFAs/n-3 PUFAs in diet was positively linked to the corresponding ratio in erythrocyte membranes ($r = 0.17$; $P < 0.05$). Consumption rates for fat, SFAs, and MUFAs were also related to the compositions of

Table 2. Percentage fatty acid compositions (mol%) in erythrocyte membranes in case and control subjects

Fatty acid	Common name	Case subjects (n = 74)		Control subjects (n = 221)		P, χ^2 or t test
		Mean	SD	Mean	SD	
SFAs*		53.0	1.8	52.1	2.2	<0.01
14:0	Myristic acid	0.8	0.3	0.7	0.2	NS
16:0	Palmitic acid	30.9	1.4	30.2	1.8	<0.001
18:0	Stearic acid	21.3	1.1	21.1	1.1	NS
MUFAs†		18.5	1.3	18.4	1.7	NS
16:1n-7	Palmitoleic acid	1.2	0.3	1.1	0.4	<0.05
18:1n-9	Oleic acid	17.3	1.3	17.3	1.5	NS
PUFAs‡		28.5	2.1	29.5	3.1	<0.01
n-6 PUFAs§		20.0	1.7	20.7	2.0	<0.05
18:2n-6	Linoleic acid	10.0	1.1	10.3	1.3	NS
18:3n-6	γ-Linolenic acid	0.08	0.03	0.09	0.12	<0.05
20:3n-6	Dihomo-γ-linolenic acid	0.78	0.18	0.75	0.18	NS
20:4n-6	Arachidonic acid (AA)	9.2	1.3	9.5	1.5	NS
n-3 PUFAs‖		8.5	1.6	8.8	2.0	NS
18:3n-3	α-Linolenic acid	0.4	0.2	0.4	0.3	NS
n-3 HUFAs¶		8.1	1.6	8.4	2.0	NS
20:5n-3	Eicosapentaenoic acid (EPA)	1.4	0.5	1.5	0.6	NS
22:5n-3	Docosapentaenoic acid	1.4	0.4	1.4	0.3	NS
22:6n-3	Docosahexaenoic acid (DHA)	5.3	1.0	5.5	1.3	NS
Saturation index $_{n-7}$	(16:0/16:1n-7)	27.03	7.88	31.22	13.02	<0.01
Saturation index $_{n-9}$	(18:0/18:1n-9)	1.24	0.14	1.23	0.14	NS
SFAs/PUFAs		1.87	0.21	1.80	0.28	<0.05
SFAs/n-6 PUFAs		2.67	0.29	2.55	0.34	<0.01
SFAs/n-3 PUFAs		6.53	1.74	6.32	1.97	NS
SFAs/n-3 HUFAs		6.93	2.06	6.67	2.14	NS
n-6 PUFAs/n-3PUFAs		2.46	0.62	2.47	0.61	NS
n-6 PUFAs/n-3HUFAs		2.61	0.73	2.61	0.67	NS
AA/EPA**		7.77	5.56	7.35	3.31	NS
AA/DHA ††		1.79	0.45	1.78	0.39	NS

Abbreviation: NS, not significant.
*SFAs = 14:0 + 16:0 + 18:0.
†MUFAs = 16:1n-7 + 18:1n-9.
‡PUFAs = n-6 PUFAs + n-3 PUFAs.
§n-6 PUFAs = 18:2n-6 + 18:3n-6 + 20:3n-6 + 20:4n-6 (AA).
‖n-3 PUFAs = 18:3n-3 + n-3 HUFAs.
¶n-3 HUFAs = 20:5n-3 (EPA) + 22:5n-3 + 22:6n-3 (DHA).
**20:4n-6/20:5n-3.
†† 20:4n-6/22:6n-3.

Table 3. Partial Pearson's correlation coefficients (*r*) between each fatty acid compositions (mol%) in erythrocyte membranes in control subjects (*n* = 221)

	SFAs			MUFAs			PUFAs								
								n-6 PUFAs			n-3 PUFAs	n-3 HUFAs			
		16:0	18:0		16:1n-7	18:1n-9			18: 2n-6	20:4n-6 (AA)			20:5n-3 (EPA)	22: 5n-3	22:6n-3 (DHA)
SFAs*	1.00														
16:0	0.86	1.00													
18:00	0.52	NS	1.00												
MUFAs†	0.28	0.44	−0.22	1.00											
16:1n-7	0.45	0.46	NS	0.61	1.00										
18:1n-9	0.20	0.38	−0.26	0.98	0.44	1.00									
PUFAs‡	−0.84	−0.84	−0.24	−0.75	−0.65	−0.69	1.00								
n-6 PUFAs§	−0.62	−0.68	NS	−0.63	−0.50	−0.59	0.78	1.00							
18:2n-6	−0.16	−0.27	0.15	−0.39	−0.21	−0.39	0.33	0.66	1.00						
20:4n-6 (AA)	−0.67	−0.65	−0.22	−0.51	−0.51	−0.45	0.75	0.72	NS	1.00					
n-3 PUFAs‖	−0.72	−0.66	−0.29	−0.57	−0.54	−0.51	0.81	0.28	NS	0.48	1.00				
n-3 HUFAs¶	−0.72	−0.64	−0.33	−0.54	−0.54	−0.47	0.80	0.27	−0.16	0.51	0.99	1.00			
20:5n-3 (EPA)	−0.35	−0.31	−0.17	−0.39	−0.35	−0.35	0.46	NS	−0.23	0.15	0.79	0.80	1.00		
22:5n-3	−0.70	−0.63	−0.30	−0.51	−0.48	−0.45	0.77	0.31	NS	0.46	0.90	0.91	0.66	1.00	
22:6n-3 (DHA)	−0.79	−0.70	−0.35	−0.54	−0.56	−0.46	0.84	0.37	NS	0.60	0.95	0.96	0.62	0.86	1.00

NOTE: Partial Pearson's correlation (*r*) is adjusted for age, sex, BMI, and season of data collection: $P < 0.05$ for −0.17, −0.16, and 0.15; $P < 0.01$ for −0.24, −0.23, −0.22, −0.21, and −0.20; and $P < 0.0001$ for others.
Abbreviation: NS, not significant.
*SFAs = 14:0 + 16:0 + 18: 0.
†MUFAs = 16:1n-7 + 18:1n-9.
‡PUFAs = n-6 PUFAs + n-3 PUFAs.
§n-6 PUFAs = 18:2n-6 + 18:3n-6 + 20:3n-6 + 20:4n-6 (AA).
‖n-3 PUFAs = 18:3n-3 + n-3 HUFAs.
¶n-3 HUFAs = 20:5n-3 (EPA) + 22:6n-3 (DHA).

16:0 ($r = -0.14$ and -0.16 for SFAs and MUFAs; $P < 0.05$), n-6 PUFAs ($r = 0.19$, 0.19, and 0.14, in that order; $P < 0.05$), and AA ($r = 0.16$ for SFAs; $P < 0.05$), respectively. No significant relationship was observed for meat consumption. Table 3 shows partial Pearson's correlation coefficients between fatty acid contents in erythrocyte membranes in control subjects. Excepting 18:2n-6 and EPA, the contents of PUFAs, n-6 PUFAs, AA, n-3 PUFAs, n-3HUFAs, 22:5n-3, and DHA had strong negative associations with values for SFAs, especially 16:0, ($r = -0.62$ to -0.84; $P < 0.0001$ for all) and also with those for 16:1n-7, 18:1n-9, and MUFAs ($r = -0.45$ to -0.75; $P < 0.0001$ for all). As PUFAs, compositions of 18:2n-6 and EPA had significant correlation coefficients with PUFAs ($r = 0.33$ and 0.46; $P < 0.0001$, respectively). The composition of 18:2n-6 was negatively associated with that of EPA, and, in contrast, that of AA had positive links to those of 22:5n-3, DHA, and n-3 HUFAs ($r = -0.23$, 0.46, 0.60, and 0.51; $P < 0.0001$ for all).

About dietary intake of meat, fish, fat, and each fatty acid, no association with colorectal cancer risk was found (data not shown). Although dietary intakes of seafood and SFAs were significantly lower in cases, wide distributions of the two variables were noted in controls. The ORs for colorectal cancer according to each fatty acid in erythrocyte membranes are shown in Table 4. The composition of 16:0 and SFAs was significantly associated with colorectal cancer risk. Compared with the lowest tertile, ORs of the middle and the highest tertile of 16:0 and SFAs were 3.29 and 6.46 (95% CIs, 1.32-8.19 and 2.41-17.26; $P_{trend} < 0.0005$) for 16:0 and 4.80 and 8.20 (95% CIs, 1.84-12.52 and 2.86-23.52; $P_{trend} < 0.0001$) for SFAs. For the composition of 16:1n-7, the ORs were 8.23 and 5.99 (95% CIs, 2.94-23.03 and 2.00-17.93; $P_{trend} < 0.01$) for the middle and the highest tertile. In contrast, the analyses suggested significant inverse associations between the risk and the compositions of DHA, AA, n-6 PUFAs, and PUFAs. For the composition of DHA, the OR was 0.36 (95% CI, 0.14-0.93 for the highest tertile; $P_{trend} < 0.05$), and those for AA and n-6 PUFAs were also 0.42 and 0.24 (95% CIs, 0.18-0.95 and 0.10-0.59; $P_{trend} < 0.05$ and < 0.005), respectively. For the composition of PUFAs, overall, the OR was 0.15 (95% CI, 0.05-0.46; $P_{trend} < 0.005$).

The saturation $index_{n-7}$ was also related to the colorectal cancer risk (ORs, 2.16 and 0.28; 95% CIs, 1.10-4.26 and 0.10-0.76 for the middle and the highest tertile; $P_{trend} < 0.01$), but the saturation $index_{n-9}$ had no association. The ratio of SFAs/PUFAs showed a strong positive link (ORs, 9.65 and 9.45; 95% CIs, 3.08-30.23 and 2.84-31.43 for the middle and the highest tertile; $P_{trend} < 0.005$). Ratios of SFAs/n-6 PUFAs and SFAs/n-3 HUFAs had also positive associations with the risk (ORs, 3.79 and 4.67; 95% CIs, 1.50-9.56 and 1.80-12.10 for the middle and the highest tertile of the former; $P_{trend} < 0.005$; ORs, 3.77 and 2.83; 95% CIs, 1.61-8.84 and 1.08-7.37 for the latter, not significant for trend).

Discussion

In the present study, dietary intakes of meat, fish, fat, and each fatty acid were not associated with colorectal cancer risk. However, we could show significant positive associations between risk and the content of SFAs, especially 16:0, in erythrocyte membranes, as well as significant inverse relationships with PUFAs. The decreased risk was related to higher erythrocyte compositions of DHA and AA, which were a representative n-3 PUFAs and n-6 PUFAs, in that order. The ratio of SFAs/PUFAs in erythrocyte membranes, therefore, had a very strong positive association with the risk. Contrary to expectation, however, a competitive relationship between n-3 PUFAs and n-6 PUFAs for risk was not found. About the saturation $index_{n-9}$, no significant association was observed for the risk.

Compared with the traditional Japanese diet, meat consumption has remarkably increased since 1950 (9). The ratios of meat/seafood (including processed meat and fish, respectively) are 1.3 for energy and 2.2 for fat intake, possibly increasing the likelihood of colorectal cancer development

(43, 44). In the Japanese, Mediterranean, and American diets, the amount of fat intake (energy intake, %) and the ratios of SFAs:MUFAs:PUFAs are 40 to 50 g (20-25%) and 1:1:1, 70 to 80 g (30-35%) and 2:5:2, and 80 to 90 g (35-40%) and 2:2:1, in that order, and this diversity may be closely related to their disease prevalence, including cancers in several sites (45). The latest cancer registry data, however, show that Japanese have high cancer incidence rates in the colon and rectum and, indeed, Japan has one of the highest colorectal cancer incidences in the world (46). The particularly high risk among Japanese may be

Table 4. ORs for colorectal cancer and the 95% CIs according to tertile of fatty acid compositions (mol%) in erythrocyte membranes

	OR (95% CI)* by tertiles			P_{trend}
	T1 (reference)	T2	T3	
SFAs[†]	<50.890	50.890-52.797	>52.797	
	1.00	4.80 (1.84-12.52)	8.20 (2.86-23.52)	<0.0001
14:0	<0.633	0.633-0.781	>0.781	
	1.00	1.16 (0.56-2.41)	1.66 (0.83-3.32)	0.13
16:0	<29.175	29.175-30.845	>30.845	
	1.00	3.29 (1.32-8.19)	6.46 (2.41-17.26)	<0.0005
18:0	<20.706	20.706-21.420	>21.420	
	1.00	0.58 (0.28-1.19)	0.84 (0.44-1.62)	0.68
MUFAs[‡]	<17.555	17.555-18.850	>18.850	
	1.00	2.07 (0.97-4.45)	1.93 (0.88-4.23)	0.15
16:1n-7	<0.887	0.887-1.251	>1.251	
	1.00	8.23 (2.94-23.03)	5.99 (2.00-17.93)	<0.01
18:1n-9	<16.661	16.661-17.670	>17.670	
	1.00	0.87 (0.44-1.74)	1.01 (0.51-1.99)	0.96
PUFAs[§]	<28.205	28.205-31.085	>31.085	
	1.00	1.13 (0.57-2.23)	0.15 (0.05-0.46)	<0.005
n-6 PUFAs[‖]	<19.743	19.743-21.506	>21.506	
	1.00	0.71 (0.38-1.33)	0.24 (0.10-0.59)	<0.005
18:2n-6	<9.734	9.734-10.892	>10.892	
	1.00	0.85 (0.44-1.62)	0.59 (0.28-1.23)	0.16
18:3n-6	<0.056	0.056-0.081	>0.081	
	1.00	1.52 (0.68-3.39)	2.54 (1.15-5.61)	<0.05
20:3n-6	<0.671	0.671-0.808	>0.808	
	1.00	1.15 (0.59-2.23)	1.10 (0.57-2.15)	0.88
20:4n-6 (AA)	<8.625	8.625-10.178	>10.178	
	1.00	0.91 (0.48-1.73)	0.42 (0.18-0.95)	<0.05
n-3 PUFAs[¶]	<7.975	7.975-9.745	>9.745	
	1.00	1.40 (0.70-2.82)	0.41 (0.15-1.09)	0.16
18:3n-3	<0.292	0.292-0.440	>0.440	
	1.00	0.80 (0.40-1.62)	1.18 (0.63-2.21)	0.51
n-3 HUFAs**	<7.639	7.639-9.221	>9.221	
	1.00	1.27 (0.63-2.53)	0.51 (0.21-1.25)	0.20
20:5n-3 (EPA)	<1.186	1.186-1.703	>1.703	
	1.00	1.04 (0.52-2.07)	0.69 (0.32-1.50)	0.32
22:5n-3	<1.237	1.237-1.508	>1.508	
	1.00	1.29 (0.63-2.65)	0.83 (0.34-2.05)	0.81
22:6n-3 (DHA)	<5.063	5.063-6.107	>6.107	
	1.00	1.17 (0.58-2.36)	0.36 (0.14-0.93)	<0.05
Saturation index $_{n-7}$[††]	<24.251	24.251-32.808	>32.808	
	1.00	2.16 (1.10-4.26)	0.28 (0.10-0.76)	<0.01
Saturation index $_{n-9}$[‡‡]	<1.177	1.177-1.272	>1.272	
	1.00	1.02 (0.51-2.04)	1.00 (0.50-1.99)	0.99
SFAs/PUFAs	<1.640	1.640-1.860	>1.860	
	1.00	9.65 (3.08-30.23)	9.45 (2.84-31.43)	<0.005
SFAs/n-6 PUFAs	<2.359	2.359-2.675	>2.675	
	1.00	3.79 (1.50-9.56)	4.67 (1.80-12.10)	<0.005
SFAs/n-3 HUFAs	<5.480	5.480-6.901	>6.901	
	1.00	3.77 (1.61-8.84)	2.83 (1.08-7.37)	0.14
n-6 PUFAs/n-3 PUFAs	<2.106	2.106-2.647	>2.647	
	1.00	1.29 (0.63-2.62)	0.84 (0.36-1.98)	0.74
n-6 PUFAs/n-3 HUFAs	<2.209	2.209-2.800	>2.800	
	1.00	1.22 (0.60-2.47)	0.89 (0.39-2.04)	0.81
AA/EPA[§§]	<5.511	5.511-8.035	>8.035	
	1.00	1.21 (0.62-2.35)	0.71 (0.32-1.57)	0.38
AA/DHA[‖‖]	<1.573	1.573-1.846	>1.846	
	1.00	0.83 (0.38-1.80)	1.23 (0.53-2.89)	0.46

*Adjusted for BMI, habitual exercise, drinking and smoking status, green-yellow vegetable intake, and family history of colorectal cancer.
[†]SFAs = 14:0 + 16:0 + 18:0.
[‡]MUFAs = 16:1n-7 + 18:1n-9.
[§]PUFAs = n-6 PUFAs + n-3 PUFAs.
[‖]n-6 PUFAs = 18:2n-6 + 18:3n-6 + 20:3n-6 + 20:4n-6 (AA).
[¶]n-3 PUFAs = 18:3n-3 + n-3 HUFAs.
**n-3 HUFAs = 20:5n-3 (EPA) + 22:5n-3 + 22:6n-3 (DHA).
[††]16:0/16:1n-7.
[‡‡]18:0/18:1n-9.
[§§]20:4n-6/20:5n-3.
[‖‖]20:4n-6/22:6n-3.

related to inherited genetic predispositions about distribution of body fat and metabolisms of fat and lipids (43, 47).

Most phospholipids are glycerol-3-phosphate derivatives and contain abundant SFAs (e.g., 16:0 and 18:0) at the α-position of glycerol, whereas both MUFAs (e.g., 18:1n-9) and PUFAs (e.g., 18:2n-6, AA, EPA, and DHA) are predominantly found at the β-position. In phospholipids of erythrocyte membranes, we showed that (*a*) PUFAs, including n-3 PUFAs and n-6 PUFAs, had stronger inverse associations with SFAs than with MUFAs; (*b*) EPA showed a weak negative link with 18:2n-6; and (*c*) 22:5n-3, DHA, and n-3 HUFAs were positively associated with AA. We here observed that not only the combination of EPA and 18:2n-6 but also that of PUFAs and SFAs might compete for incorporation into phospholipids in erythrocyte membranes. Moreover, dietary intakes of n-3 HUFAs and n-6 PUFAs were linked to the corresponding contents of EPA and n-6 PUFAs in erythrocyte membranes, whereas dietary n-3 HUFA intake exhibited a negative relation to the levels of AA and n-6 PUFAs.

In general, the AA cascade is suggested to play critical roles in tumor development at several sites. Previous reports in United Kingdom and Spain, however, have shown to be lower erythrocyte composition of AA in colorectal cancer patients than in controls, but the findings were inconsistent (17-20). In Italian women, the higher composition of 18:2n-6 and n-6 PUFAs were associated with breast cancer risk, but that of AA had no association (21). In Russian women, the higher compositions of AA and 18:2n-6 were reported to be related to decreased breast cancer risk (48), but in Swedish postmenopausal women, no association was observed (49). For prostate cancer, n-6 PUFAs and 18:2n-6 have shown increased risk in American men (50, 51). Higher compositions of AA, 18:2n-6, and n-6 PUFAs in Jamaican men were found to be associated with both high level of serum prostate-specific antigen and risk (52). In the present study, higher compositions of AA and 18:2n-6 and n-6 PUFAs were related to decreased risk for colorectal cancer. However, our overall knowledge about associations between cancer risk in different body sites and the fatty acid compositions in erythrocyte membranes are very limited.

In epidemiologic studies about fish consumption, evidence of an inverse association with colorectal cancer risk has not been consistent (12-16, 53-55). In our recent report (507 incident cases and 2,535 controls) without biomarkers, no association was found for the colon and rectum (42). In the latest large-scale prospective Japanese cohort study with or without biomarkers, colorectal cancer risk was also observed inconsistent (13, 54). The study without biomarkers was found no association between the risk and dietary intakes of foods and nutrients based on a validated food frequency questionnaire, which had significant positive relations between dietary intakes of fish, DHA, and n-3 PUFAs and serum levels of the corresponding fatty acids (13, 54). In contrast, the same study with biomarkers has shown high serum contents of DHA and n-3 PUFAs to significantly decrease the incidence risk (54). Therefore, we conclude that measure of fatty acids in biomaterials is essential for accurate assessment of effect on risk. Erythrocytes are easily to collect from study subjects and the fatty acid contents are available for evaluating dietary intakes of fish, fat, and fatty acids during the recent 4 months (120 days half-life), without recall bias about dietary intakes of foods and nutrients.

In previous studies, erythrocyte compositions of 18:0 and 18:1n-9 were higher in colorectal cancer patients (17, 18), and those of MUFAs, 16:1n-7 and 18:1n-9 were related to increased risk of breast cancer (21). The saturation $\text{index}_{n\text{-}9}$ in erythrocyte membranes has been observed to be lower in colorectal cancer patients than in controls (20) and in Italian postmenopausal women was linked to decrease breast cancer risk, with activity of Δ9 desaturase suggested to play a modifying role, especially with dietary intakes of SFAs and PUFAs, because this enzyme is linked to fat content in the diet (21). In the present study, however, whereas SFAs and 16:0 had significant increased risks of colorectal cancer, no consistent association was found for the saturation $\text{index}_{n\text{-}9}$. There was no definite dose-response trend for the saturation $\text{index}_{n\text{-}7}$ because the risks in the middle and the highest to the lowest were opposite sides. Compared with other studies, including Asian populations (17-21, 49, 56), erythrocyte compositions of 16:0, 18:0, and SFAs in our study subjects were high, and a possible reason might be our method for measuring fatty acids in erythrocyte membranes (29).[5]

Potential limitations of the present study should be considered. This was a retrospective study. Colorectal cancer risk was not directly elucidated through a causal relationship with fatty acid compositions in erythrocyte membranes because disease status of the cancer may affect responses to dietary questionnaire or fatty acid compositions in erythrocyte membranes. The sample size was relatively small; therefore, we could not assess cancer risks in colon and rectum separately. However, we randomly selected the control subjects, who were individually matched for age, sex, and season of sample collection with a 1:3 case-control ratio to increase the statistical power. Our study design allowed exclusion of the influence of seasonal variation in both dietary intakes of fat and fatty acids and their biomarker levels. One methodologic issue is the selection of control subjects, but we applied cancer-free outpatients at the ACCH for this purpose because it is reasonable to assume our case subjects arose within this population base. About general lifestyles, an essential point with our control subjects is that they are representative of the general population randomly selected from the electoral roll in same area (32). Another potential source of bias is the medical background of control subjects. We here clarified that the majority did not have any specific medical conditions (57, 58) and excluded individuals with any diseases related to fat and/or lipid metabolisms. We therefore conclude that use of noncancer outpatients as references for the present study was acceptable. A blinded quality control for measuring compositions was not used during the period of laboratory work. The compositions of SFAs (e.g., 16:0) and PUFAs (e.g., AA, EPA, and DHA) might be relatively high and low, respectively, compared with previous reports, but we have no appropriate international biomaterial standards as control for measuring each fatty acid. Within a study, however, it is not a problem to compare fatty acid compositions in erythrocyte membranes.

In conclusion, using a new method for measuring fatty acids in biomaterials, we here could show clear associations between colorectal cancer risk and the composition of erythrocyte membranes. Higher erythrocyte content of SFAs, especially 16:0, and a high ratio of SFAs/PUFAs were strongly linked to increased risk, whereas PUFAs, including DHA and AA, seemed protective. Our findings do not support the prevailing paradigm about colorectal carcinogenesis via the AA cascade. Further research with large sample is needed to investigate the discrepancy about the role of AA.

Acknowledgments

We thank S. Inui, M. Watanabe, H. Achiwa, and T. Sato for their technical help for laboratory assays; H. Fujikura, K. Fukaya, Y. Kamori, K. Tomita, M. Sato, Y. Yamauchi, and C. Yoshida for their help in interviewing subjects and data collection/cleaning; and Dr. M.A. Moore for checking the English language.

References

1. James MJ, Gibson RA, Cleland LG. Dietary polyunsaturated fatty acids and inflammatory mediator production. Am J Clin Nutr 2000;71:343–8S.
2. Jones R, Adel-Alvarez LA, Alvarez OR, Broaddus R, Das S. Arachidonic acid and colorectal carcinogenesis. Mol Cell Biochem 2003;253:141–9.
3. Tokudome S, Yokoyama Y, Kamiya T, et al. Rationale and study design of dietary intervention in patients polypectomized for tumors of the colorectum. Jpn J Clin Oncol 2002;32:550–3.

4. Gerster H. Can adults adequately convert α-linolenic acid(18:3n-3) to eicosapentaenoic acid (20:5n-3) and docosahexaenoic acid (22:6n-3)? Int J Vitam Nutr Res 1998;68:159–73.
5. Arab L. Biomarkers of fat and fatty acid intake. J Nutr 2003;133:925–32S.
6. Kramer HJ, van den Ham WA, Slob W, Pieters MN. Fish oil fatty acids and human platelets: dose-dependent decrease in dienoic and increase in trienoic thromboxane generation. Biochem Pharmacol 1996;52:1211–7.
7. Busstra MC, Siezen CL, Grubben MJ, et al. Tissue levels of fish fatty acids and risk of colorectal adenomas: a case-control study (Netherlands). Cancer Causes Control 2003;14:269–76.
8. Zhang J, Temme EH, Kesteloot H. Fish consumption is inversely associated with male lung cancer mortality in countries with high levels of cigarette smoking or animal fat consumption. Int J Epidemiol 2000;29:615–21.
9. Kuriki K, Tokudome S, Tajima K. Association between type II diabetes and colon cancer among Japanese with reference to changes in food intake. Asian Pac J Cancer Prev 2004;5:28–35.
10. Hill MJ. Bile acids and colorectal cancer: hypothesis. Eur J Cancer Prev 1991; 1:69–74.
11. Pickering JS, Lupton JR, Chapkin RS. Dietary fat, fiber, and carcinogen alter fecal diacylglycerol composition and mass. Cancer Res 1995;55:2293–8.
12. World Cancer Research Fund/American Institute for Cancer Research. Food, nutrition and the prevention of cancer: a global perspective. Washington (DC): AICR; 1997. p. 216–51.
13. Kojima M, Wakai K, Tamakoshi K, et al. Diet and colorectal cancer mortality: results from the Japan Collaborative Cohort Study. Nutr Cancer 2004;50:23–32.
14. Norat T, Bingham S, Ferrari P, et al. Meat, fish, and colorectal cancer risk: the European Prospective Investigation into cancer and nutrition. J Natl Cancer Inst 2005;97:906–16.
15. Lin J, Zhang SM, Cook NR, Lee IM, Buring JE. Dietary fat and fatty acids and risk of colorectal cancer in women. Am J Epidemiol 2004;160:1011–22.
16. Senesse P, Boutron-Ruault MC, Faivre J, et al. Foods as risk factors for colorectal adenomas: a case-control study in Burgundy (France). Nutr Cancer 2002;44:7–15.
17. Baro L, Hermoso JC, Nunez MC, Jimenez-Rios JA, Gil A. Abnormalities in plasma and red blood cell fatty acid profiles of patients with colorectal cancer. Br J Cancer 1998;77:1978–83.
18. Neoptolemos JP, Clayton H, Heagerty AM, et al. Dietary fat in relation to fatty acid composition of red cells and adipose tissue in colorectal cancer. Br J Cancer 1988;58:575–9.
19. Neoptolemos JP, Thomas BS. Erythrocyte membrane stearic acid: oleic acid ratios in colorectal cancer using tube capillary column gas liquid chromatography. Ann Clin Biochem 1990;27:38–43.
20. Kelly SB, Miller J, Wood CB, Williamson RC, Habib NA. Erythrocyte stearic acid desaturation in patients with colorectal carcinoma. Dis Colon Rectum 1990;33:1026–30.
21. Pala V, Krogh V, Muti P, et al. Erythrocyte membrane fatty acids and subsequent breast cancer: a prospective Italian study. J Natl Cancer Inst 2001;93:1088–95.
22. Hunter D. Biochemical indicators of dietary intake. In: Willett W, editor. Nutritional epidemiology. New York: Oxford University Press; 1990. p. 186–216.
23. Kuriki K, Nagaya T, Imaeda N, et al. Discrepancies in dietary intakes and plasma concentrations of fatty acids according to age among Japanese female dietitians. Eur J Clin Nutr 2002;56:524–31.
24. Kuriki K, Nagaya T, Tokudome Y, et al. Plasma concentrations of (n-3) highly unsaturated fatty acids are good biomarkers of relative dietary fatty acid intakes: a cross-sectional study. J Nutr 2003;133:3643–50.
25. Farquhar JW, Ahrens EH, Jr. Effects of dietary fats on human erythrocyte fatty acid patterns. J Clin Invest 1963;42:675–85.
26. Dougherty RM, Galli C, Ferro-Luzzi A, Iacono JM. Lipid and phospholipid fatty acid composition of plasma, red blood cells, and platelets and how they are affected by dietary lipids: a study of normal subjects from Italy, Finland, and the USA. Am J Clin Nutr 1987;45:443–55.
27. Feunekes GI, Van Staveren WA, De Vries JH, Burema J, Hautvast JG. Relative and biomarker-based validity of a food-frequency questionnaire estimating intake of fats and cholesterol. Am J Clin Nutr 1993;58:489–96.
28. Vidgren HM, Agren JJ, Schwab U, Rissanen T, Hanninen O, Uusitupa MI. Incorporation of n-3 fatty acids into plasma lipid fractions, and erythrocyte membranes and platelets during dietary supplementation with fish, fish oil, and docosahexaenoic acid-rich oil among healthy young men. Lipids 1997; 32:697–705.
29. Kuriki K, Tajima K, Tokudome S. Accelerated solvent extraction for quantitative measurement of fatty acids in plasma and erythrocytes. Lipids. In press 2006.
30. Tajima K, Hirose K, Inoue M, et al. A model of practical cancer prevention for out-patients visiting a hospital: the Hospital-based Epidemiologic Research Program at Aichi Cancer Center (HERPACC). Asian Pac J Cancer Prev 2000;1:35–47.
31. Tokudome Y, Kuriki K, Imaeda N, et al. Seasonal variation in consumption and plasma concentrations of fatty acids in Japanese female dietitians. Eur J Epidemiol 2003;18:945–53.
32. Inoue M, Tajima K, Hirose K, et al. Epidemiological features of first-visit outpatients in Japan: comparison with general population and variation by sex, age, and season. J Clin Epidemiol 1997;50:69–77.
33. Tokudome S, Ikeda M, Tokudome Y, et al. Development of data-based semi-quantitative food frequency questionnaire for dietary studies in middle-aged Japanese. Jpn J Clin Oncol 1998;28:679–87.
34. Tokudome S, Goto C, Imaeda N, et al. Development of a data-based short food frequency questionnaire for assessing nutrient intake by middle-aged Japanese. Asian Pac J Cancer Prev 2004;5:40–3.
35. Tokudome Y, Goto C, Imaeda N, et al. Relative validity of a short food frequency questionnaire for assessing nutrient intake versus three-day weighed diet records in middle-aged Japanese. J Epidemiol 2005;15:135–45.
36. Goto C, Tokudome Y, Imaeda N, et al. Validation study of fatty acid consumption assessed with a short food frequency questionnaire against plasma concentration in middle-ages Japanese people. Scand J Nutr 2006;50:77–82.
37. Resources Council, Science and Technology Agency, Japan. Standard Tables of Food Composition in Japan, 4th ed. Tokyo: Printing Office, Ministry of Finance; 1982.
38. Resources Council, Science and Technology Agency, Japan. Follow-up of Standard Tables of Food Composition in Japan, 4th ed. Tokyo: Ishiyaku Shuppan; 1994.
39. Rose HG, Oklander M. Improved procedure for the extraction of lipids from human erythrocytes. J Lipid Res 1965;6:428–31.
40. Nagaya T, Nakaya K, Takahashi A, Yoshida I, Okamoto Y. Relationships between serum saturated fatty acids and serum total cholesterol and HDL-cholesterol in humans. Ann Clin Biochem 1994;31:240–4.
41. Lands WEM, Hamazaki T, Yamazaki K, et al. Changing dietary patterns. Am J Clin Nutr 1990;51:991–3.
42. Wakai K, Hirose K, Matsuo K, et al. Dietary risk factors for colon and rectal cancers: a comparative case-control study. J Epidemiol 2006;16:125–35.
43. Kuriki K, Hamajima N, Chiba H, et al. Increased risk of colorectal cancer due to interactions between meat consumption and the CD36 gene A52C polymorphism among Japanese. Nutr Cancer 2005;51:170–7.
44. Tokudome Y, Imaeda N, Ikeda M, et al. Foods contributing to absolute intake and variance in intake of fat, fatty acids and cholesterol in middle-aged Japanese. J Epidemiol 1999;9:78–90.
45. Tokudome S, Nagaya T, Okuyama H, et al. Japanese versus Mediterranean diets and cancer. Asian Pac J Cancer Prev 2000;1:61–6.
46. Parkin DM, Whelan SL, Ferlay J, Teppo L, Thomas DB. Cancer incidence in five continents. Volume VIII. No.155. Lyon: IARC; 2002.
47. WHO Expert Consultation. Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. Lancet 2004;363:157–63.
48. Zaridze DG, Chevchenko VE, Levtshuk AA, Lifanova YE, Maximovitch DM. Fatty acid composition of phospholipids in erythrocyte membranes and risk of breast cancer. Int J Cancer 1990;45:807–10.
49. Wirfalt E, Vessby B, Mattisson I, et al. No relations between breast cancer risk and fatty acids of erythrocyte membranes in postmenopausal women of the Malmo Diet Cancer cohort (Sweden). Eur J Clin Nutr 2004;58:761–70.
50. Godley PA, Campbell MK, Gallagher P, et al. Biomarkers of essential fatty acid consumption and risk of prostatic carcinoma. Cancer Epidemiol Biomarkers Prev 1996;5:889–95.
51. Newcomer LM, King IB, Wicklund KG, Stanford JL. The association of fatty acids with prostate cancer risk. Prostate 2001;47:262–8.
52. Ritch CR, Brendler CB, Wan RL, Pickett KE, Sokoloff MH. Relationship of erythrocyte membrane polyunsaturated fatty acids and prostate-specific antigen levels in Jamaican men. BJU Int 2004;93:1211–5.
53. Oh K, Willett WC, Fuchs CS, Giovannucci E. Dietary marine n-3 fatty acids in relation to risk of distal colorectal adenoma in women. Cancer Epidemiol Biomarkers Prev 2005;14:835–41.
54. Kojima M, Wakai K, Tokudome S, et al. Serum levels of polyunsaturated fatty acids and risk of colorectal cancer: a prospective study. Am J Epidemiol 2006;161:462–71.
55. Wakai K, Ito Y, Kojima M, et al. Intake frequency of fish and serum levels of long-chain n-3 fatty acids: a cross-sectional study within the Japan Collaborative Cohort Study. J Epidemiol 2005;15:211–8.
56. Fan WX, Parker R, Parpia B, et al. Erythrocyte fatty acids, plasma lipids, and cardiovascular disease in rural China. Am J Clin Nutr 1990;52:1027–36.
57. Hamajima N, Hirose K, Inoue M, et al. Age-specific risk factors of breast cancer estimated by a case-control study in Japan. J Epidemiol 1995;5: 99–105.
58. Yang CX, Matsuo K, Ito H, et al. Gene-environment interactions between alcohol drinking and the MTHFR C677T polymorphism impact on esophageal cancer risk: results of a case-control study in Japan. Carcinogenesis 2005;26:1285–90.

Risk of Colorectal Cancer Is Linked to Erythrocyte Compositions of Fatty Acids as Biomarkers for Dietary Intakes of Fish, Fat, and Fatty Acids

Kiyonori Kuriki, Kenji Wakai, Kaoru Hirose, et al.

Cancer Epidemiol Biomarkers Prev 2006;15:1791-1798.