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Abnormalities in Fatty Acids in Plasma, Erythrocytes and Adipose Tissue in Japanese Patients with Colorectal Cancer

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Abstract. *Aim: In previous animal studies, we confirmed that linoleic acid (LNA) enhanced colon carcinogenesis, whereas eicosapentaenoic acid (EPA) had protective effects in azoxymethane-induced colon tumorigenesis. In regard to the protective effects of marine n-3 polyunsaturated fatty acids (PUFAs) on colorectal cancer however, evidence from epidemiological studies is inconsistent. Materials and Methods: In the present study we investigated the fatty acid composition in plasma, red blood cells (RBCs) and adipose tissue from Japanese patients with colorectal cancer, or benign disease. Results: Sixty-one patients with histologically-confirmed colorectal cancer and 42 patients with non-malignant disease were recruited for this study. The fatty acid composition of the total phospholipid (PL) fraction of plasma and washed RBCs was determined by gas chromatography. The fatty acid composition of the triacylglycerol (TAG) fraction of subcutaneous adipose tissue was determined in a similar manner. The EPA proportion in the plasma and RBC PL fractions was significantly lower in patients with cancer than in the controls ($p<0.05$). Similarly, the LNA proportion in the RBC PL fraction was lower in patients with cancer, but no changes were found in the plasma PL fraction. Arachidonic acid was the only PUFA in the adipose TAG fraction that exhibited significant differences, with higher levels in the patients with cancer than in the controls. Conclusion: Our findings suggest that patients with cancer have abnormalities in PUFAs in the plasma PL, erythrocyte PL, and adipose TAG fractions. Further investigation is needed to clarify the differences in the results between the various fractions.*

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Cancer risk is linked to diet (1) and dietary fat, in particular, may play an important role in the etiology of colonic, breast, and prostatic cancer (2-5). Although the roles of individual fatty acids in human cancer risk have been poorly-investigated overall, the research that has been undertaken has focused on n-6 and n-3 polyunsaturated fatty acids (PUFAs) (6-8).

n-3 PUFAs have different biological properties from n-6 PUFAs. In general, experimental animal studies with rats have shown that the n-6 PUFA dietary linoleic acid (LNA) enhanced chemically-induced colonic carcinogenesis, whereas n-3 PUFAs reduced it (9, 10). However, the role of dietary LNA and n-3 PUFAs in human colonic cancer development remains controversial. Essentially all of the epidemiological studies undertaken in Western countries to determine the effects of n-3 PUFAs on colon cancer risk have two disadvantages in common: very high total fat and relatively low n-3 PUFA intake. Small changes in n-3 PUFA intake against a high background level of total fat may make it difficult to detect any benefits of n-3 PUFA consumption.

Compared with Western countries, Japan has a low incidence of colonic cancer, but a high incidence of gastric cancer (11, 12). However, dietary habits and lifestyle have changed greatly in Japan over the last few decades, and the incidence of colonic cancer is rapidly approaching that of Western countries (13-15). Mortality from colorectal cancer increased more than five-fold between 1970 and 2010 in Japan, and colorectal cancer is now the third cause of cancer-

related deaths among women and the first among men, accounting for an estimated 45,000 annual deaths among both sexes (16). In Japan, fat consumption increased rapidly between the end of the Second World War and 1975 (to account for 22.3% of total energy), and thereafter has been stable at around 25% of total energy with intakes of fish oil and long-chain n-3 PUFAs in Japan being a few times higher than those in Western countries (17, 18). In this context, it seems important to investigate the association between dietary fatty acid and colorectal cancer in Japanese people.

The fatty acid composition of serum lipids has been considered a reliable index reflecting dietary intake of fatty acids over a period of weeks or months (19, 20). The rate of fatty acid changes in red blood cells (RBCs) is slower than that of plasma lipids (21, 22), and the fatty acid composition of adipose tissue has been shown to be a valid index for the habitual dietary fatty acid composition over the preceding two and half years among adults (23). The results of fatty acid analysis of these lipid fractions with longer turnover periods may be more relevant to colonic causing cancer than dietary histories of a few days or weeks, which are assessed by traditional dietary methods.

To examine the role of fatty acids, especially n-6 [LNA and arachidonic acid (ARA)] and n-3 fatty acids (alpha-linolenic acid and long-chain n-3 PUFAs), in colorectal cancer, we investigated the fatty acid composition in plasma, RBCs, and adipose tissue from 103 patients treated for colorectal cancer or benign disease at the Kansai Medical University in Japan.

Materials and Methods

Participants. Sixty-one patients (39 men, 22 women; age range 43-84 years) with histologically-confirmed colorectal cancer at the Kansai Medical University Hospital were enrolled in the study when the following criteria were met: no body weight loss (self-reported), no dietary restriction due to intestinal obstruction, no serious concomitant disease (diabetes, hepatic or renal dysfunction, or hyperlipidemia requiring drug treatment), and no history of other malignancy. Tumor sites were classified as proximal colon (including the appendix, cecum, and ascending and transverse colon), distal colon (descending and sigmoid colon), and rectum.

Tumors were staged according to the Astler-Coller modification (24) of the Dukes' classification after surgery, with review of the pathological specimen and occurrence of distant metastasis. According to this classification, tumors confined to the submucosa are considered Dukes' A, and those involving the *muscularis propria* and extending to the serosa or pericolic fat are classified as Dukes' B. Dukes' C tumors are found in patients with B lesions and positive lymph nodes, and patients with Dukes' D tumors have distant metastasis, regardless of the status of local disease or local lymph nodes.

To serve as control patients, 42 patients (31 men, 11 women; age range=40-81 years) who underwent surgery at the Kansai Medical University for non-malignant disease, such as inguinal hernia, were recruited. Patients were excluded if they were receiving any n-3 PUFA supplements or statins, had hyperlipidemia likely to require drug therapy, had any significant hematological or biochemical abnormalities, or had cholesterol cholecystolithiasis. Patients with cholesterol cholecystolithiasis were excluded because we previously showed a significantly lower proportion of eicosapentaenoic acid (EPA) in the plasma and RBC phospholipid (PL) fractions compared with patients with other non-malignant conditions (25).

The protocol of the present study was approved by the Ethics Committee of the Kansai Medical University. Written informed consent for participation and use of blood samples and subcutaneous fat for research purposes was obtained from all patients.

Collection of samples. Blood samples obtained after an overnight (12-14 h) fast were collected and placed in plastic tubes. Plasma and packed RBCs were obtained from EDTA-anticoagulated blood. Plasma was then transferred to smaller tubes for storage at –80˚C until analysis. RBCs were washed twice with ice-cold saline and frozen at –80˚C until analysis. A sample of adipose tissue was removed from abdominal subcutaneous fat during surgery. No samples were obtained before any type of therapy such as chemotherapy or radiation. After rinsing with saline, samples were immediately frozen on dry ice and stored at –80˚C until analysis. The laboratory was blinded to the sample origin (cancer or control case).

Fatty acid analysis. The fatty acid composition of the total PL fraction of plasma and washed RBCs was determined as follows. Total lipids were extracted using the method of Bligh and Dyer (26). The total PL fraction was separated by thin-layer chromatography and, after transmethylation with HCl-methanol, the fatty acid composition was analyzed by gas chromatography (GC14A; Shimadzu Corporation, Kyoto, Japan) with a capillary column DB-225 (length, 30 m; internal diameter, 0.25 mm; film, 0.25 μm; J&M Scientific, Folsom, CA, USA). The column temperature was maintained at 170˚C for 1 min, raised to 220˚C at a rate of 4˚C/min, and kept at this temperature for 22 min. The entire system was controlled using gas chromatography software, CLSS-GC10 ver. 1.3 (Shimadzu Corporation). The fatty acid composition of the triacylglycerol (TAG) fraction of subcutaneous adipose tissue was determined in a similar manner, except the TAG fraction which was separated by thin-layer chromatography. We adopted the area percentage of each fatty acid over that of all detected fatty acids as the measurement value. The limit of detection (LOD) and limit of quantification (LOQ) were defined to be the lowest peak area with a signal-to-noise ratio >3 for LOD and 10 for LOQ.

Statistical analysis. Data are expressed as the mean±SD. The background characteristics of age and body mass index (BMI) were compared between patients with cancer and controls using a *t*-test (age and BMI) and that of sex was compared using the chi-square test. Fatty acids in various fractions were compared between the two patient groups using a *t*-test. Individual fatty acid, total saturated fatty acids, total monounsaturated fatty acids, n-3 PUFAs, n-6 PUFAs, the ratio of n-6/n-3 PUFAs, and total PUFAs were compared between proximal colon cancer, distal colon cancer, and rectal cancer using a one-way ANOVA and multiple comparisons with Bonferroni correction. All statistical analyses were performed with the statistical software SPSS, version 19.0 (IBM Japan, Tokyo, Japan), with *p*-values of less than 0.05 considered significant.

Results

Participants. The mean age of the patients with cancer was 66.5 years (range=43-84 years) and that of the controls was 65.7 years (range=40-81 years) (Table I). BMI was significantly lower in the patients with cancer than in the controls (p=0.04). There were no significant differences in the sex ratio between the groups.

Of the 61 colorectal tumors, 17 were located in the proximal colon, 16 in the distal colon, and 28 in the rectum (specifically, five in the cecum, seven in the ascending colon, five in the transverse colon, four in the descending colon, 12 in the sigmoid colon, and 28 in the rectum). According to Dukes' classification as modified by Astler and Coller, there were 9 (15%), 26 (43%), 20 (33%), and 6 (10%) patients with stage A, B, C, and D disease, respectively.

Fatty acid analysis among patients with colorectal cancer and controls. Plasma PLs: The mean fatty acid compositions of the plasma PL fraction in the colorectal cancer and control patients are shown in Table II. In both the colorectal cancer and control patient groups, saturated fatty acids accounted for the majority of the PL fraction (48.6±1.2% and 47.4±1.6%, respectively) compared with all other types of fatty acids (PUFAs: 36.7±3.0% and 38.4±1.5%, respectively). The proportions of total saturated fatty acids were significantly greater in the patients with cancer than in the controls (p<0.0001), which was mainly due to a higher proportion of palmitic acid. The proportions of PUFAs were significantly lower in the patients with cancer than in the control patients (p<0.001), as was the EPA proportion in the plasma PL fraction (p<0.01). There was no difference in the proportion of any n-6 PUFAs in the plasma PL fraction between the two patient groups. There was no significant difference in the n-6/n-3 ratio between the groups.

Erythrocyte PLs: The proportions of saturated and monounsaturated fatty acids and PUFAs in the erythrocyte PL fraction of the colorectal cancer and control groups were similar to those in the plasma PL fraction of both groups; there were no significant differences in the proportions of total saturated fatty acids, monounsaturated fatty acids, or PUFAs. The EPA proportions in the erythrocyte PL fraction of cancer and control patients were 1.9±0.7 and 2.2±0.9, respectively (p<0.05), whereas the LNA proportions were 8.1±1.2 and 8.6±1.4, respectively (p<0.05). There was no significant difference in the n-6/n-3 ratio between the groups.

Adipose TAG: The proportions of saturated and monounsaturated fatty acids and PUFAs in the adipose TAG fraction were quite different from those of the plasma and erythrocyte PL fractions. Monounsaturated fatty acids accounted for the largest proportion in the adipose TAG fraction in both the control and colorectal cancer groups, followed by saturated fatty acids. LNA was the most abundant PUFA. Very small amounts of long-chain n-3 PUFAs were found in the adipose TAG fraction, and α-linolenic acid (18:3 n-3) was the predominant n-3 fatty acid in the adipose TAG fraction of both patient groups. ARA was the only PUFA that differed significantly between the groups, with a higher level in patients with colorectal cancer than in controls.

Table I. *Characteristics of cases and controls participating in this study.*

	Controls n=61	Cases n=41	p-value†
Age (years at death)	65.0±8.9	66.5±9.5	0.40
Gender (male/female)	31/11	39/22	0.29
BMI	23.0±3.3	21.8±2.7	0.04

†Chi-square test for categorical variables and unpaired t-test for continuous variables.

Comparison of fatty acid compositions between patients with proximal colon, distal colon, and rectal cancer. The numbers of patients with tumor of the proximal colon, distal colon, and rectum were 17, 16, and 28, respectively; the corresponding proportions of men were 76.4%, 50.0%, and 64.3%. There were no significant differences in the proportion of any fatty acid or their combinations in the plasma PL fraction between the three patient groups (Table III). Stearic acid (18:0) levels in RBC PL were significantly higher in patients with rectal cancer than in those with distal colonic cancer (p<0.05). Oleic acid (18:1 n-9) levels in adipose TAG were significantly higher in distal patients with colonic cancer than in those with cancer of the proximal colon (p<0.05).

Discussion

In this study, we investigated the fatty acid composition of plasma, RBCs, and adipose tissue in patients with colorectal cancer and control patients in order to determine whether dietary n-3 and n-6 PUFAs are reduced and increased respectively in patients with cancer, compared with controls with benign disease. Our findings suggest that the patients with cancer had abnormalities in PUFAs in the plasma PL, erythrocyte PL, and adipose TAG fractions.

In previous studies using purified ethyl esters of LNA, stearic acid, and EPA, we confirmed that LNA enhanced colon carcinogenesis (9), whereas EPA had protective effects on azoxymethane-induced colonic tumorigenesis (10). However, evidence from epidemiological studies on the protective effects of marine n-3 PUFAs on colorectal cancer is inconsistent (27-29). de Deckere (27) reviewed studies on the influence of fish and fish n-3 PUFAs on colorectal cancer

Table II. *Fatty acid compositions of phospholipids in the plasma and RBCs and composition of triglycerides in the adipose tissue. Data are the means±standard deviation.*

		Plasma			RBC			TAG		
		Controls	Cases	*p*-Value	Controls	Cases	*p*-Value	Controls	Cases	*p*-Value
Saturated fatty acids		47.44±1.59	48.57±1.16	0.00006	45.04±1.29	45.18±0.97	0.55	29.18±3.27	28.85±3.54	0.63
14:0	Myristic acid	0.23±0.08	0.25±0.08	0.24	0.24±0.08	0.24±0.07	0.93	2.10±0.58	2.03±0.60	0.56
16:0	Palmitic acid	29.69±1.73	30.70±1.54	0.002	24.70±1.29	25.14±1.35	0.095	21.34±2.52	21.66±2.17	0.49
18:0	Stearic acid	14.81±1.26	15.07±1.59	0.39	13.74±1.18	13.51±1.11	0.32	5.26±1.48	4.74±1.81	0.13
20:0	Arachidic acid	0.50±0.11	0.49±0.11	0.43	0.35±0.05	0.33±0.05	0.02	0.21±0.08	0.20±0.08	0.42
22:0	Behenic acid	1.16±0.34	1.12±0.22	0.54	1.47±0.24	1.42±0.19	0.23	0.09±0.24	0.06±0.18	0.42
24:0	Lignoceric acid	1.04±0.25	0.93±0.18	0.01	4.54±0.56	4.53±0.56	0.91	N.D.	N.D.	
Monounsaturated fatty acids		14.14±1.76	14.70±2.59	0.22	21.04±1.28	21.38±1.33	0.19	53.28±3.20	53.87±3.31	0.37
16:1n-7	Palmitoleic acid	0.50±0.20	0.62±0.41	0.097	0.35±0.20	0.36±0.18	0.82	4.70±1.78	4.71±1.68	0.96
18:1n-9	Oleic acid	8.67±1.35	9.11±2.31	0.27	13.57±1.05	13.61±1.23	0.88	43.90±2.48	44.54±2.94	0.26
18:1n-7	Vaccenic acid	2.10±0.41	2.08±0.35	0.78	1.63±0.22	1.67±0.18	0.32	3.47±0.78	3.41±0.51	0.64
20:1n-9	Gondoic acid	0.19±0.12	0.15±0.05	0.04	0.24±0.07	0.22±0.05	0.17	0.96±0.20	0.95±0.27	0.95
22:1n-9	Erucic acid	0.03±0.04	0.04±0.04	0.27	0.05±0.07	0.06±0.07	0.47	0.08±0.11	0.08±0.16	0.99
24:1n-9	Nervonic acid	2.65±0.73	2.71±0.58	0.65	5.19±0.63	5.46±0.63	0.04	N.D.	N.D.	
n-3 polyunsaturated fatty acids		12.05±2.85	11.06±2.29	0.053	12.35±1.92	11.88±1.55	0.17	1.973±0.56	1.90±0.47	0.46
18:3n-3	α-Linolenic acid	0.17±0.06	0.17±0.09	0.87	0.11±0.05	0.10±0.06	0.87	0.97±0.27	0.92±0.24	0.32
20:5n-3	Eicosapentaenoic acid	3.04±1.65	2.27±1.14	0.006	2.23±0.85	1.86±0.71	0.02	0.10±0.07	0.12±0.19	0.62
22:5n-3	Docosapentaenoic acid	1.00±0.22	0.99±0.26	0.80	2.12±0.27	2.11±0.30	0.93	0.25±0.22	0.28±0.18	0.37
22:6n-3	Docosahexaenoic acid	7.84±1.59	7.63±1.43	0.49	7.90±1.06	7.81±0.90	0.64	0.66±0.39	0.58±0.32	0.28
n-6 polyunsaturated fatty acids		26.37±2.68	25.67±3.29	0.25	21.57±2.24	21.57±1.86	0.99	15.56±2.45	15.38±1.97	0.68
18:2n-6	Linoleic acid	15.92±2.48	15.08±3.19	0.16	8.64±1.38	8.12±1.22	0.045	14.39±2.27	14.19±1.89	0.62
18:3n-6	γ-Linolenic acid	0.03±0.05	0.05±0.11	0.30	0.02±0.09	0.05±0.18	0.28	0.40±0.44	0.39±0.49	0.89
20:2n-6	Eicosadienoic acid	0.34±0.08	0.35±0.12	0.50	0.25±0.13	0.23±0.07	0.26	0.32±0.16	0.32±0.15	0.92
20:3n-6	Dihomo-γ-linolenic acid	1.83±0.53	1.94±0.54	0.29	1.04±0.24	1.07±0.21	0.49	0.23±0.14	0.24±0.11	0.75
20:4n-6	Arachidonic acid	8.13±1.30	8.08±1.36	0.87	10.13±1.24	10.44±1.18	0.19	0.20±0.07	0.23±0.08	0.04
22:4n-6	Docosatetraenoic acid	0.12±0.07	0.15±0.08	0.06	1.27±0.36	1.39±0.34	0.08	0.01±0.04	0.02±0.05	0.71
22:5n-6	Docosapentaenoic acid	N.D.	N.D.		0.22±0.08	0.26±0.13	0.09	0.00±0.01	0.00±0.01	0.82
n-6/n-3		2.35±0.76	2.44±0.69	0.52	1.82±0.49	1.86±0.37	0.61	8.47±2.51	8.61±2.40	0.77
PUFA		38.42±1.54	36.73±2.96	0.00096	33.92±1.20	33.44±1.27	0.06	17.53±2.59	17.28±2.15	0.59

p-Values were determined with paired *t*-test. ND=Almost all the data were below limit of quantification. PUFA: Polyunsaturated fatty acid; RBC: red blood cell; TAG: triacylglycerol.

risk (population, case–control, and prospective studies) and reported that four population studies exhibited an inverse but non-significant correlation of colorectal cancer mortality rates with fish consumption. In three out of 11 case–control studies, high fish consumption was found to be significantly protective, whereas no effect was found in the remaining studies. In one study it was shown that a high intake of seafood was associated with a significantly lowered risk of colon cancer in men, but a significantly increased risk in women. All prospective studies reported from Western countries have shown no association between colorectal cancer mortality and intake of long-chain n-3 PUFAs. MacLean *et al*. (30) systematically reviewed the effects of n-3 PUFAs on cancer risk; only one (31) out of nine studies from seven different cohorts showed there to be a weak inverse association between colorectal cancer risk in women and the intake of fish and shell-fish; no association between n-3 PUFA intake and the incidence of colorectal cancer was observed in the remaining eight studies. Furthermore, Nkondjock *et al*. (32) reviewed studies of the relation between dietary fatty acid intake or concentrations of specific fatty acids in adipose, RBC, plasma and colorectal cancer risk. They concluded that the evidence relating docosahexaenoic acid (DHA), n-3/n-6 fatty acid ratio, α-linolenic acid, and LNA to colorectal cancer was unconvincing.

The Japanese diet has been changing to become more similar to a Western style diet, especially among the younger generation, and fish consumption has gradually been decreasing. However, greatly larger quantities of fish are still

Table III. *Comparison of fatty acid compositions between patients with canser of the proximal colon, distal colon, and rectum. Data are the means±standard deviation.*

		Plasma			RBC			TAG		
		Proximal (n=17)	Distal (n=16)	Rectum (n=28)	Proximal (n=17)	Distal (n=16)	Rectum (n=28)	Proximal (n=17)	Distal (n=16)	Rectum (n=28)
Saturated fatty acids		48.73±1.11	48.51±1.55	48.51±0.96	45.53±0.78	44.85±0.97	45.14±1.02	29.70±3.39	28.82±3.04	28.36±3.88
14:0	Myristic acid	0.25±0.08	0.26±0.08	0.25±0.09	0.24±0.04	0.24±0.09	0.23±0.08	2.22±0.57	1.93±0.37	1.97±0.70
16:0	Palmitic acid	30.69±1.66	30.91±1.76	30.59±1.37	25.27±0.84	25.68±1.52	24.77±1.43	21.83±1.70	21.21±1.93	21.81±2.56
18:0	Stearic acid	15.21±2.03	14.85±1.69	15.10±1.23	13.67±0.80	12.85±1.14	13.79±1.13 *	5.18±2.23	5.21±1.65	4.21±1.51
20:0	Arachidic acid	0.50±0.10	0.48±0.11	0.48±0.13	0.33±0.05	0.31±0.05	0.33±0.05	0.20±0.08	0.22±0.08	0.18±0.09
22:0	Behenic acid	1.14±0.25	1.10±0.25	1.13±0.19	1.45±0.19	1.40±0.20	1.42±0.19	0.04±0.13	0.11±0.29	0.03±0.10
24:0	Lignoceric acid	0.93±0.19	0.89±0.17	0.96±0.18	4.57±0.57	4.37±0.43	4.60±0.62	N.D.	N.D.	N.D.
Monounsaturated fatty acids		14.75±3.44	14.56±1.58	14.75±2.55	21.23±1.49	21.61±1.16	21.34±1.34	52.60±3.78	54.29±2.61	54.40±3.27
16:1n-7	Palmitoleic acid	0.65±0.47	0.58±0.36	0.61±0.40	0.36±0.16	0.36±0.21	0.37±0.17	4.89±1.36	4.10±1.69	4.95±1.81
18:1n-9	Oleic acid	9.04±3.18	9.08±1.50	9.17±2.14	13.42±1.27	13.97±1.30	13.51±1.17	43.09±2.63	45.71±2.48	§44.75±3.09
18:1n-7	Vaccenic acid	2.09±0.34	2.08±0.35	2.07±0.37	1.71±0.22	1.71±0.19	1.62±0.15	3.43±0.61	3.29±0.46	3.46±0.47
20:1n-9	Gondoic acid	0.15±0.06	0.14±0.05	0.16±0.04	0.22±0.05	0.19±0.04	0.23±0.06	0.96±0.36	0.96±0.21	0.94±0.24
22:1n-9	Erucic acid	0.05±0.05	0.03±0.04	0.04±0.04	0.07±0.06	0.03±0.05	0.08±0.07	0.05±0.11	0.05±0.09	0.11±0.22
24:1n-9	Nervonic acid	2.78±0.59	2.65±0.75	2.70±0.48	5.44±0.62	5.35±0.57	5.53±0.68	N.D.	N.D.	N.D.
n-3 polyunsaturated fatty acids		11.27±2.07	10.52±2.42	11.24±2.36	11.97±1.41	11.42±1.17	12.09±1.79	1.96±0.50	1.75±0.51	1.94±0.43
18:3n-3	α-Linolenic acid	0.15±0.08	0.18±0.10	0.17±0.10	0.09±0.04	0.13±0.09	0.09±0.05	0.98±0.25	0.90±0.26	0.89±0.23
20:5n-3	Eicosapentaenoic acid	2.59±1.22	1.98±0.79	2.24±1.24	1.92±0.71	1.70±0.38	1.92±0.85	0.10±0.06	0.08±0.07	0.15±0.27
22:5n-3	Docosapentaenoic acid	1.01±0.33	0.96±0.23	1.00±0.23	2.12±0.23	2.03±0.28	2.15±0.34	0.24±0.17	0.27±0.20	0.31±0.18
22:6n-3	Docosahexaenoic acid	7.52±1.20	7.40±1.77	7.83±1.37	7.85±0.86	7.56±0.93	7.92±0.90	0.64±0.37	0.50±0.33	0.59±0.28
n-6 polyunsaturated fatty acids		25.25±3.78	26.41±2.82	25.50±3.27	21.27±2.26	22.11±1.61	21.43±1.72	15.74±2.15	15.14±1.89	15.30±1.95
18:2n-6	Linoleic acid	15.09±3.89	15.83±2.78	14.65±2.98	8.07±1.46	8.53±1.30	7.91±0.99	14.61±2.21	14.04±1.83	14.02±1.75
18:3n-6	γ-Linolenic acid	0.04±0.07	0.10±0.19	0.04±0.06	0.06±0.10	0.09±0.33	0.03±0.07	0.37±0.43	0.35±0.39	0.43±0.58
20:2n-6	Eicosadienoic acid	0.36±0.14	0.37±0.12	0.34±0.11	0.23±0.05	0.24±0.10	0.23±0.07	0.31±0.13	0.30±0.14	0.33±0.17
20:3n-6	Dihomo-γ-linolenic acid	1.82±0.50	1.94±0.40	2.01±0.63	1.07±0.24	1.01±0.16	1.10±0.22	0.23±0.09	0.22±0.11	0.26±0.12
20:4n-6	Arachidonic acid	7.79±1.41	8.03±1.17	8.29±1.43	10.14±1.41	10.54±1.11	10.57±1.06	0.21±0.06	0.21±0.07	0.25±0.09
22:4n-6	Docosatetraenoic acid	0.14±0.10	0.14±0.06	0.17±0.08	1.44±0.39	1.39±0.22	1.36±0.37	0.01±0.04	0.02±0.04	0.02±0.05
22:5n-6	Docosapentaenoic acid	N.D.	N.D.	N.D.	0.26±0.14	0.31±0.11	0.24±0.14	0.00±0.00	0.00±0.00	0.00±0.01
n-6/n-3		2.32±0.60	2.65±0.70	2.40±0.73	1.82±0.38	1.97±0.33	1.82±0.38	8.40±1.89	9.33±2.92	8.32±2.34
PUFA		36.52±3.93	36.93±2.04	36.74±2.83	33.24±1.57	33.53±1.20	33.52±1.12	17.70±2.38	16.89±2.13	17.24±2.04

Comparison was made between proximal colon cancer, distal colon cancer, and rectal cancer using one-way ANOVA and multiple comparisons with Bonferroni correction. *$p<0.05$ compared to distal. §$p<0.05$ compared to proximal. ND=Almost all the data were below limit of quantification. PUFA: Polyunsaturated fatty acid; RBC: red blood cell; TAG: triacylglycerol.

consumed in Japan than in Western countries (33, 34). In our previous study (25), total n-3 PUFAs, alpha-linolenate, EPA, and DHA levels in the plasma PL fraction were 3.1-, 2.6-, 4.4-, and 2.6-fold higher respectively, than those reported from the United States (35). Nine case–control (14, 15, 28, 36-41) and two cohort (42, 43) studies have addressed the association between fish intake and colorectal cancer in Japan. The results of six (15, 28, 36-39) out of the nine case–control studies suggested a protective effect of fish consumption on colorectal cancer, but two cohort studies showed no association. In the present study, the EPA proportions in the plasma and erythrocyte PL fractions of patients with cancer were significantly lower than those of controls. Considering these results in Japan, where more fish is consumed than in Western countries, fish consumption may contribute to reduce the risk of colorectal cancer in Western countries. The higher total fat consumption in the Western countries may dilute the effects of long-chain n-3 PUFA to undetectable levels.

LNA administration enhanced colonic carcinogenesis in experimental animal models (9), but this relationship has not been clearly demonstrated in epidemiological studies. Zock and Katan (44) investigated the relationship between LNA intake and age-adjusted colorectal cancer mortality in 16 cohorts. The mean intake of LNA ranged from 8 g/day in Japan, Rome, and eastern Finland to 22 g/day in Belgrade,

but age-adjusted mortality from colorectal cancer was not associated with LNA intake. In addition, a meta-analysis of 16 case–control studies showed no relationship between LNA intake and the risk of colorectal cancer. Changes in plasma fatty acid composition in the adenoma–carcinoma sequence were assessed in Spain (45). A significant decrease in LNA concentration in the plasma PL fraction was noted in patients with cancer compared with healthy controls. Baró *et al*. (46) investigated fatty acid profiles in the plasma and RBC PL fractions in patients with colorectal cancer and age-matched controls. Patients with colorectal cancer had significantly lower levels of LNA in plasma PLs than did controls, but no differences were found in erythrocyte PLs between the two groups. In the present study, the proportion of LNA in patients with cancer was significantly lowered only in the RBC PL fractions ($p<0.05$), and not in the plasma PL fraction, compared with levels in the control patients (Table II). There are two possible explanations for the decreased LNA levels: a lower dietary intake or increased metabolism of LNA. ARA is the precursor of important eicosanoids involved in cancer growth and metastasis (47, 48). Indeed, ARA levels have been reported to be increased in colonic cancer tissue compared with normal mucosa (49). In spite of the decrease in LNA in plasma and erythrocyte PL in patients with colonic cancer in this study, the levels of ARA in the plasma and erythrocyte PL fractions were very similar to those of controls. This might be due to partial reversion of delta-6-fatty acid desaturase inhibition in the liver; excess amounts of LNA inhibit the enzymatic system (50). Therefore, if LNA is decreased in plasma and/or erythrocytes of patients with colorectal cancer, the activity of the enzymatic system may be increased as discussed by Baró *et al*. (46).

Despite lower LNA levels in the plasma and erythrocyte PL fractions in the patients with colorectal cancer in the present study, ARA levels were very similar to those of the control patients. The levels of ARA in patients with cancer might be a product of both increased synthesis of ARA and also due to its increased consumption for eicosanoid formation.

In countries with a low overall risk for colorectal cancer, rectal cancer accounts for the largest proportion of all colorectal carcinomas (51). By contrast, the proportion of colonic cancer of all colorectal carcinomas is generally greater than 60% in Europe and North America (52). It has been suggested that the risk factors for colorectal cancer could vary according to cancer site. In Japan, the proportion of colonic cancer to colorectal cancer was 50% in 1975, which has increased to 64% since 2000 (53); in addition, we found a subsite shift of colonic cancer to the proximal site, as in Western countries (54). There were no significant differences in the proportion of any PUFAs in any fractions between carcinomas in different sites in the present study. Our findings are consistent with the results of a population-based study in the United States (29), as well as a population-based prospective cohort study of Swedish women (55).

Originally, we hypothesized that the difference in n-3 PUFAs of adipose tissue between patients with cancer and controls may be larger than any differences in plasma and RBCs, because the adipose tissue is a valid index for habitual dietary fatty acid consumption over the preceding two and half years (23). However, there were no significant differences in any n-3 PUFAs. This might be due to the fact that the levels of these n-3 PUFAs were very low in adipose tissue, and consequently the range was too narrow to detect any differences.

Several limitations of the present study should be considered. Firstly, the number of participants in each group was small in comparison to previous studies. Secondly, it is impossible to detect the causal relationship between low levels of plasma and RBC EPA and the risk of colorectal cancer because of the cross-sectional nature of the study. Thirdly, we had no information regarding dietary intake of fatty acids.

In conclusion, our findings suggest that there may be changes in PUFA biochemistry in plasma PL, erythrocyte PL, and adipose TAG fractions in human carcinogenesis. However, further investigation is needed to clarify the differences in these various fractions and their putative role(s).

Contributors

M. Okuno, H. Takada, T. Ogura, H. Kitade, T. Matsuura, R. Yoshida, T. Hijikawa, M. Kwon, S. Arita, M. Itomura, K. Hamazaki, and T. Hamazaki designed and performed the study; M. Okuno, H. Takada, T. Hamazaki, and K. Hamazaki wrote this article.

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Conflicts of Interest Statement

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