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Original Article

Role of serum polyunsaturated fatty acids in the development of colorectal cancer

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Abstract: We aimed to investigate the role of serum levels of polyunsaturated fatty acid (PUFA) in the development of colorectal cancer (CRC). Serum levels of n-3 and n-6 PUFA in 69 healthy control (Ctrl), 62 benign colorectal polys (CRP) and 100 CRC patients were detected by gas chromatograph. The adjusted odds ratio (OR) by quartiles of n-3 and n-6 PUFA were analyzed. During the process of Ctrl to CRP, total n-3 PUFA (OR=0.159, *P*<0.001), total n-6 PUFA (OR=0.190, *P*<0.001), C20:5 n-3 (OR=0.263, *P*=0.030), C22:6 n-3 (OR=0.125, *P*<0.001), and C18:2 n-6 (OR=0.299, *P*=0.025) were inversely associated with CRP risk. The ratio of total n-6 PUFA and total n-3 PUFA (OR=4.667, *P*=0.002), and the ratio of C20:4 n-6 and (C20:5 n-3+C22:6 n-3) (OR=6.000, *P*<0.001) were positively associated with CRP risk. During the process of CRP to CRC, total n-3 PUFA (OR=4.059, *P*=0.007), total n-6 PUFA (OR=8.146, *P*<0.001), C22:6 n-3 (OR=3.789, *P*=0.048), and C18:2 n-6 (OR=3.667, *P*=0.045) were positively associated with CRC risk. The ratio of C20:4 n-6 and (C20:5 n-3+C22:6 n-3) (OR=0.588, *P*=0.001) was inversely associated with CRC. In conclusion, our results found that the total n-3 PUFA, C22:6 n-3, the total n-6 PUFA, C18:2 n-6, and the ratio of C20:4 n-6 and (C20:5 n-3 +C22:6 n-3) played controversy role in the process of CRP and the process of CRC, and may provide nutritional intervention suggestions for the clinical practice.

Keywords: Polyunsaturated fatty acids, colorectal polys, colorectal cancer, odds ratio

Introduction

Colorectal cancer (CRC) is one of the most common cancers the world. The etiology of CRC is complex. It may evolve from genetic alterations in oncogenes or tumor suppressor genes. However, about 50%-80% of CRC patients are considered due to environmental factors, such as dietary habits which play important role in the development and progression of CRC [1, 2]. In some studies, the level of dietary fat had been demonstrated to be positively associated with CRC, however, there were also some studies indicated that the incidence of CRC is low in populations consuming large amounts of fish. The controversy effects depend mainly on the type of dietary fat.

n-3 polyunsaturated fatty acid (PUFA) is demonstrated to have an inverse association with the risk of CRC [3-5]. However, the results of the association are inconsistent. Some other studies found that the n-3 showed null or positive association [6-8]. n-6 PUFA is demonstrated to have a positive association with the risk of CRC [5, 9], but also some studies found null or positive association [10-13]. The association of n-3 PUFA, n-6 PUFA with the risk of CRC is inconsistent. In addition, most of the studies focused on the risk of healthy control (Ctrl) and CRC patients [5, 14, 15]. As we known, the natural history of CRC is long in humans, it was improper to use of CRC incidence as the end point in clinical intervention studies, and colorectal polys (CRP) should be required for the analysis.

In our study, we aimed to investigate the role of serum levels of PUFAs in the process of Ctrl to CRP, and CRP to CRC. Our study may identify the association of serum levels of PUFAs and

Table 1. Clinical characteristic of the samples in our study

Characteristic	Ctrl (n=69)	CRP (n=62)	CRC (n=100)
Age	49.7±0.9	54.5±2.0	59.0±1.1
Sex (Male/Female)	(38, 31)	(37, 25)	(64, 36)
Smoke (C/F/N, %)	(4.35, 18.84, 76.81)	(24.19, 37.10, 38.71)	(28.00, 21.00, 51.00)
Alcohol (C/F/N, %)	(27.54, 18.84, 53.62)	(54.84, 19.35, 25.81)	(42.00, 14.00, 44.00)
Body mass index	25.7±3.1	24.9±3.7	26.2±4.1
Total cholesterol	4.52 (4.03, 4.92)	4.57 (4.08, 5.02)	4.37 (3.81, 5.09)
LDL cholesterol	2.56 (1.87, 3.34)	2.82 (2.19, 3.58)	2.84 (2.61, 3.09)
HDL cholesterol	1.36 (1.18, 1.59)	1.19 (0.97, 1.31)	1.02 (0.90, 1.19)
Triglycerides	1.00 (0.81, 1.32)	1.36 (0.95, 1.78)	1.31 (0.98, 1.64)
Total energy intake (Kcal/d)	2039 (1521, 2588)	1982 (1376, 2607)	1996 (1262, 2733)
Total protein intake (g/d)	76 (60, 93)	73 (61, 88)	77 (57, 95)
Total fat intake (g/d)	42 (34, 47)	45 (38, 49)	51 (32, 68)
Total carbohydrate intake (g/d)	313 (197, 428)	327 (219, 446)	319 (201, 442)

Abbreviation: Ctrl: healthy controls; CRP: colorectal polys; CRC: colorectal cancer.

the risk of CRP and CRP, and may be helpful for the nutritional intervention in clinical practice.

Materials and methods

Study population

The study was approved by the Ethics Committee of the Chinese People's Liberation Army General Hospital (Beijing, China). All patients provided informed written consent for the study sample collection, as well as permission for their use in research.

231 serum samples included 69 Ctrl people, 62 benign CRP patients, 100 CRC patients were collected for detection. Serum samples were collected before any treatment, such as surgery, chemotherapy or radiation therapy. Ctrl people were detected based on based on their negative results including blood biomarker test, X-ray, ultrasound, CT examination, fecal occult-blood testing, and colonoscopy. CRP and CRC patients were diagnosed according to combined clinical criteria, including imaging data, serum tumor markers, and further confirmed by histopathological analysis. All the patients had no history of CRC. All study populations are Han Chinese in origin and lived in northern inland cities, and without extra PUFAs intake. Body mass index (BMI) was calculated as weight (kilograms)/height (square meters). Smoke and alcohol drinking are reported as current (C), former (F), and never (N) status. Clinical characteristics were shown in **Table 1**.

Serum collection

10 mL of peripheral blood samples were collected in tubes containing separating gel and clot activator in the morning after 12 hours fast. After centrifuging at 3400 rpm for 7 minutes, the supernatant was transferred into new tubes, and the serum was aliquoted and stored at -80°C until detection. No freeze thawing was allowed prior to polyunsaturated fatty acids and cytokine detection.

Measurement of serum PUFA

The procedure of measuring the serum levels of PUFAs After thawing, 200 μL fasting serum sample was collected and transferred to a glass methylation tube. 5 μg C23:0 which served as intern control, 1 mL of hexane and 1 mL of 14% BF3/MeOH reagent were added into the methylation tube. Then the mixture was blanketed with nitrogen, and heated to 100°C for 45 minutes. After cooled to room temperature, 1 mL H_2O was added to the tube. After centrifugation at 1200 r/min for 5 minutes, the upper hexane layer was transferred to a new tube and concentrated by nitrogen. Total Fatty acid methyl esters were carried out on GC-2010 Plus Gas Chromatograph (Chiyoda-ku, Tokyo, Japan) with a Omegawax™ 250 column (Supelco, Belletonte, PA) 30 m ×0.25 mm ×0.25 um film thickness. Column temperature Program was 210°C and held 45 min. The concentrations of polyunsaturated fatty acids were expressed as a percentage [16]. The total n-3

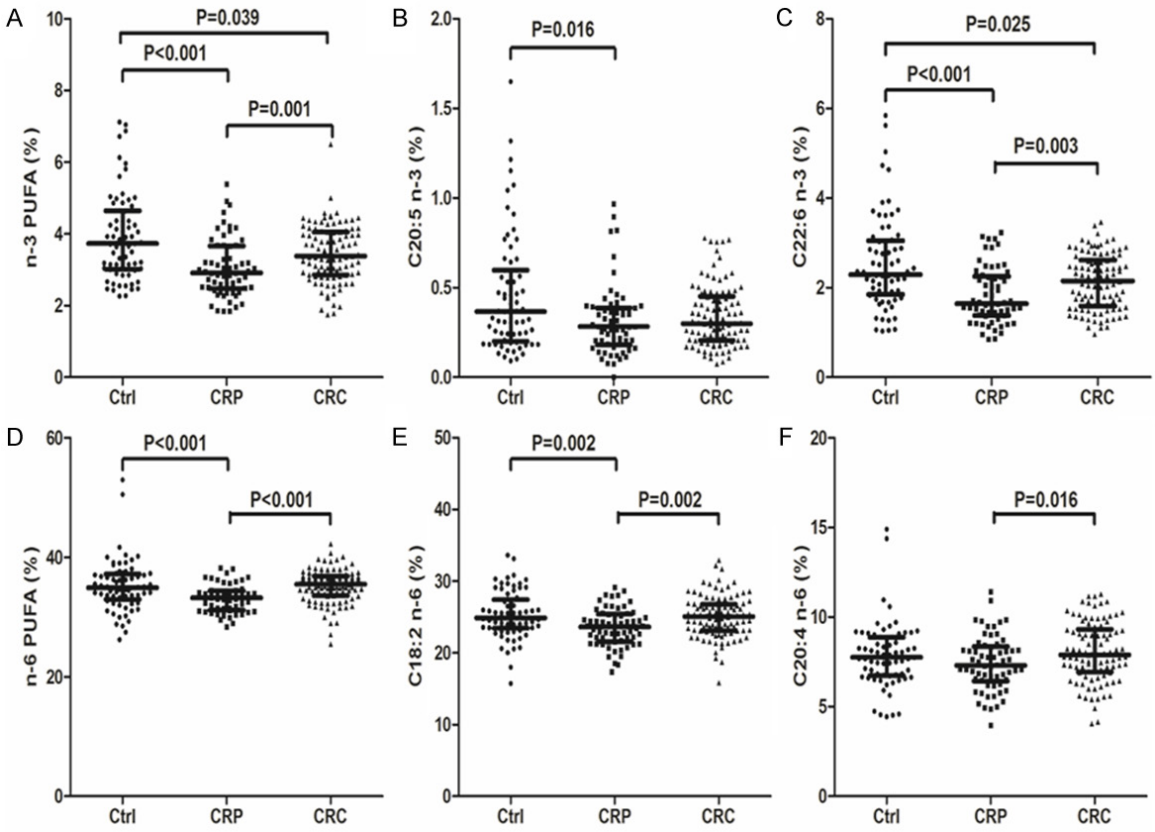

Figure 1. Comparison of the serum levels of n-3 PUFA (including total n-3 PUFA, C18:3 n-3, C20:5 n-3, C22:5 n-3 and C22:6 n-3) and n-6 PUFA (including total n-6 PUFA, C18:2 n-6, C18:3 n-6, C20:3 n-6, C20:4 n-6 and C22:5 n-6) in the Ctrl, CRP and CRC group. Abbreviation: Ctrl: Healthy controls; CRP: Colorectal polys; CRC: Colorectal cancer.

PUFA included C18:3 n-3 (α-linolenic acid), C20:5 n-3 (eicosapentaenoic acid), C22:5 n-3 (docosapentaenoic acid) and C22:6 n-3 (docosahexaenoic acid). The total n-6 PUFA included C18:2 n-6 (linoleic acid), C18:3 n-6 (γ-linolenic acid), C20:3 n-6 (Dihomo-γ-linolenic acid), C20:4 n-6 (arachidonic acid) and C22:5 n-6 (docosapentaenoic acid).

Statistical analysis

The serum levels of n-3 and n-6 PUFAs between groups were compared by one-way analysis of variance with the Bonferroni correction. Conditional logistic regression models were used to calculate the odds ratios (OR) and 95% confidence interval (CI) for the incidence of CRP-Ctrl and CRC-CRP study design for the serum levels of PUFAs. The models were adjusted for age, sex, smoke, alcohol drinking, body mass index (BMI), total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, total energy, protein, fat and carbohydrate intake. OR were calculated for the second quartile (Q2), third quartile (Q3), and highest quartile (Q4) versus the lowest quartile (Q1). To test for linear trends in odds ratios over quartiles, we coded each quartile as 0, 1, 2, or 3 and incorporated these data into the logistic model as a single variable. *P* values for the trend were estimated by creating a continuous variable using the median value within quartiles. All statistical analyses were performed on SAS 9.2 statistical package (SAS Institute, Inc. Cary, USA) with a statistical significance level set at $P<0.05$.

Results

Comparison of n-3 PUFA and n-6 PUFA in the different groups

The percentage of n-3 PUFA (including C18:3 n-3, C20:5 n-3, C22:5 n-3 and C22:6 n-3) and n-6 PUFA (including C18:2 n-6, C18:3 n-6, C20:3 n-6, C20:4 n-6 and C22:5 n-6) in serum of Ctrl, CRP and CRC group were compared.

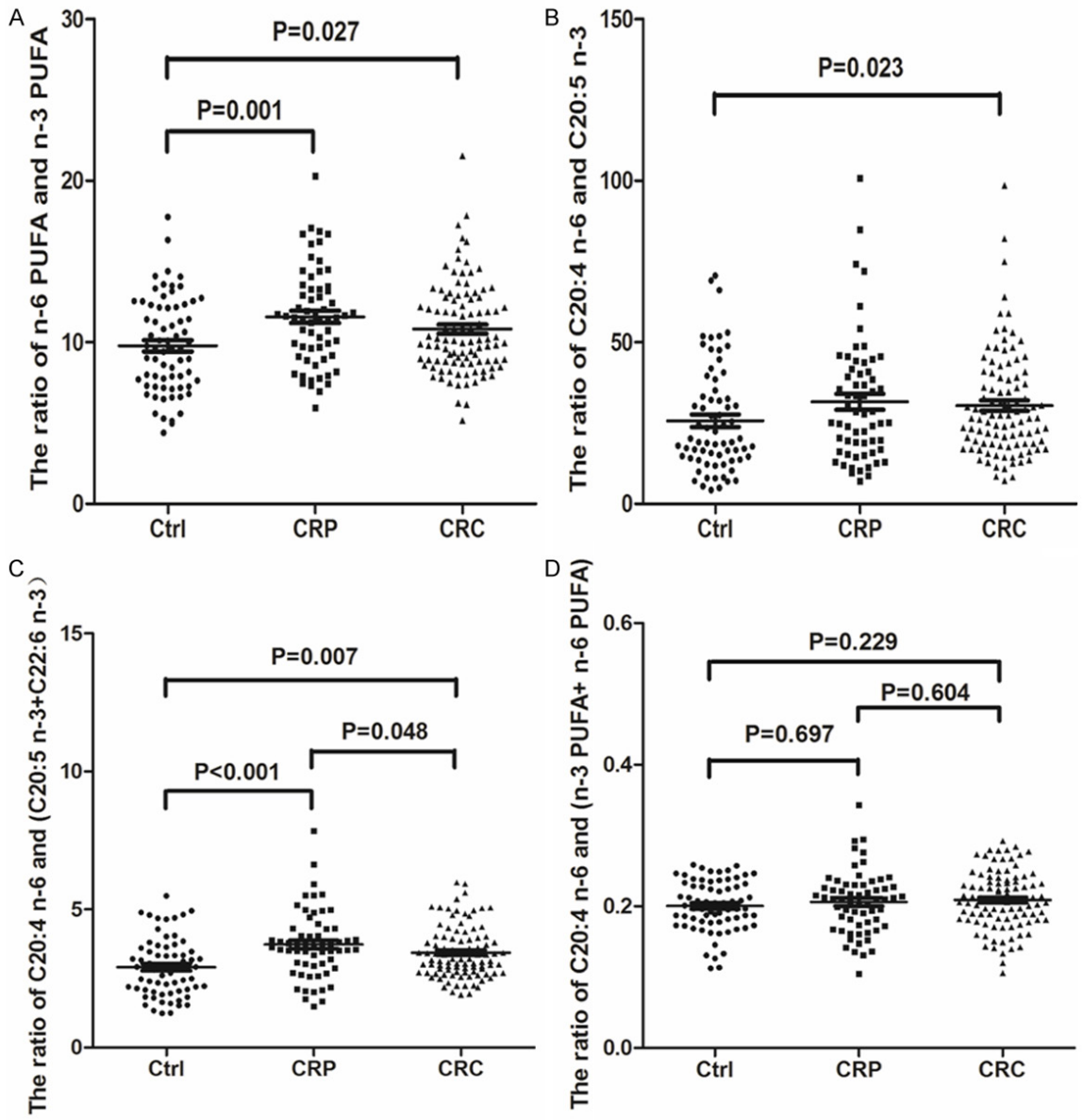

Figure 2. Comparison of the ratio of total n-6 PUFA and total n-3 PUFA, the ratio of C20:4 n-6 and C20:5 n-3, the ratio of C20:4 n-6 and (C20:5 n-3+C22:6 n-3) and the ratio of C20:4 n-6 and (total n-6 PUFA+ total n-3 PUFA) in the Ctrl, CRP and CRC groups. Abbreviation: Ctrl: Healthy controls; CRP: Colorectal polys; CRC: Colorectal cancer.

Compared to the Ctrl group, n-3 PUFA (P<0.001), C20:5 n-3 (P=0.016), C22:6 n-3 (P<0.001), n-6 PUFA (P<0.001) and C18:2 n-6 (P=0.002) in the CRP group showed significantly reduced as shown in **Figure 1**. The other kinds of PUFAs showed no significant difference in the CRP group when compared to the Ctrl group. Compared to the CRP group, n-3 PUFA (P=0.001), C22:6 n-3 (P=0.003), n-6 PUFA (P<0.001), C18:2 n-6 (P=0.002) and C20:4 n-6 (P=0.016) showed significantly increased in the CRC group, as also shown in **Figure 1**. The other kinds of PUFAs showed no significant difference in the CRC group when compared to the CRP group.

The ratio of total n-6 PUFA and total n-3 PUFA, ratio of 20:4 n-6 and C20:5 n-3, ratio of C20:4 n-6 and (C20:5 n-3+C22:6 n-3) and the ratio of C20:4 n-6 and (total n-6 PUFA+ total n-3 PUFA) in the Ctrl, CRP and CRC groups were also compared, as shown in **Figure 2**. Compared to the Ctrl group, the ratio of n-6 PUFA and n-3 PUFA (P=0.001) and ratio of C20:4 n-6 and (C20:5

Table 2. Association of n-3 PUFAs with the development of CRC

Nutrient	Q*	Value	CRP (no.)	Ctrl (no.)	OR*,†	95% CI*	*P* for trend	Q	Value	CRC (no.)	CRP (no.)	OR*,†	95% CI*	*P* for trend
Total n-3 PUFA	Q1	<2.66	23	11	1.000		<0.001	Q1	<2.70	17	23	1.000		0.007
	Q2	2.66-3.18	18	13	0.662	0.241, 1.823		Q2	2.70-3.19	23	18	1.729	0.717, 4.166	
	Q3	3.18-4.14	13	21	0.296	0.109, 0.803		Q3	3.19-3.95	30	11	3.690	1.452, 9.379	
	Q4	>4.14	8	24	0.159	0.054, 0.476		Q4	>3.95	30	10	4.059	1.568, 10.510	
C18:3 n-3	Q1	<0.33	21	16	1.000		0.803	Q1	<0.32	23	17	1.000		0.431
	Q2	0.33-0.43	9	19	0.586	0.220, 1.562		Q2	0.32-0.49	22	18	0.903	0.373, 2.196	
	Q3	0.43-0.57	12	21	0.707	0.284, 1.763		Q3	0.49-0.64	30	12	1.848	0.738, 4.624	
	Q4	>0.57	20	13	1.905	0.771, 4.706		Q4	>0.64	25	15	1.232	0.503, 3.018	
C20:5 n-3	Q1	<0.19	16	15	1.000		0.030	Q1	<0.20	24	17	1.000		0.293
	Q2	0.19-0.31	18	14	1.205	0.447, 3.250		Q2	0.20-0.29	24	16	1.063	0.438, 2.579	
	Q3	0.31-0.47	21	15	1.313	0.499, 3.452		Q3	0.29-0.42	23	19	0.857	0.360, 2.045	
	Q4	>0.47	7	25	0.263	0.088, 0.785		Q4	>0.42	29	10	2.054	0.794, 5.312	
C22:5 n-3	Q1	<0.32	15	17	1.000		0.965	Q1	<0.32	23	15	1.000		0.843
	Q2	0.32-0.41	17	19	1.014	0.391, 2.633		Q2	0.32-0.40	29	14	1.351	0.543, 3.360	
	Q3	0.41-0.49	13	14	1.052	0.377, 2.935		Q3	0.40-0.49	23	16	0.938	0.377, 2.332	
	Q4	>0.49	17	19	1.014	0.391, 2.633		Q4	>0.49	25	17	0.959	0.392, 2.349	
C22:6 n-3	Q1	<1.53	23	9	1.000		<0.001	Q1	<1.51	20	22	1.000		0.048
	Q2	1.53-2.08	19	15	0.496	0.178, 1.382		Q2	1.51-1.93	23	16	1.581	0.656, 3.811	
	Q3	2.08-2.62	12	20	0.235	0.082, 0.672		Q3	1.93-2.50	26	15	1.907	0.793, 4.587	
	Q4	>2.62	8	25	0.125	0.041, 0.379		Q4	>2.50	31	9	3.789	1.454, 9.874	

*Q: quartiles; OR: odds ratio; CI: confidence interval. †Odds ratio were derived from a conditional logistic analysis model adjusted for potential confounding factors, including age, sex, smoke, alcohol drinking, body mass index (BMI), total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, total energy, protein, fat and carbohydrate intake. Abbreviation: Ctrl: healthy controls; CRP: colorectal polys; CRC: colorectal cancer.

Table 3. Association of n-6 PUFAs with the development of CRC

Nutrient	Q*	Value	CRP (no.)	Ctrl (no.)	OR*,†	95% CI*	*P* for trend	Q	Value	CRC (no.)	CRP (no.)	OR*,†	95% CI*	*P* for trend
Total n-6 PUFA	Q1	<31.85	21	12	1.000		<0.001	Q1	<32.34	16	23	1.000		<0.001
	Q2	31.85-33.86	20	12	0.952	0.348, 2.609		Q2	32.34-34.46	16	26	0.885	0.363, 2.158	
	Q3	33.86-36.24	13	21	0.354	0.131, 0.953		Q3	34.46-36.45	34	7	6.982	2.483, 19.633	
	Q4	>36.24	8	24	0.190	0.065, 0.555		Q4	>36.45	34	6	8.146	2.774, 23.920	
C18:2 n-6	Q1	<22.52	21	11	1.000		0.025	Q1	<22.60	18	22	1.000		0.045
	Q2	22.52-24.28	15	19	0.414	0.153, 1.119		Q2	22.60-24.38	25	16	1.910	0.789, 4.623	
	Q3	24.28-26.07	14	18	0.407	0.148, 1.118		Q3	24.38-26.32	27	14	2.357	0.961, 5.781	
	Q4	>26.07	12	21	0.299	0.108, 0.828		Q4	>26.32	30	10	3.667	1.420, 9.470	
C18:3 n-6	Q1	<0.22	17	15	1.000		0.075	Q1	<0.20	30	13	1.000		0.487
	Q2	0.22-0.28	13	21	0.546	0.205, 1.455		Q2	0.20-0.28	22	17	0.567	0.226, 1.390	
	Q3	0.28-0.34	13	21	0.546	0.205, 1.455		Q3	0.28-0.34	24	13	0.800	0.313, 2.043	
	Q4	>0.34	19	12	1.397	0.513, 3.806		Q4	>0.34	24	19	0.547	0.226, 1.328	
C20:3 n-6	Q1	<1.30	16	25	1.000		0.411	Q1	<1.28	27	13	1.000		0.698
	Q2	1.30-1.58	12	12	1.563	0.565, 4.320		Q2	1.28-1.60	22	16	0.662	0.263, 1.667	
	Q3	1.58-1.91	20	15	2.083	0.832, 5.215		Q3	1.60-1.92	25	19	0.634	0.260, 1.544	
	Q4	>1.91	14	17	1.287	0.500, 3.313		Q4	>1.92	26	14	0.894	0.354, 2.260	
C20:4 n-6	Q1	<6.64	18	15	1.000		0.108	Q1	<6.70	21	19	1.000		0.203
	Q2	6.64-7.64	18	15	1.000	0.379, 2.653		Q2	6.70-7.73	24	17	1.277	0.531, 3.074	
	Q3	7.64-8.57	14	19	0.614	0.232, 1.624		Q3	7.73-9.03	25	16	1.414	0.585, 3.417	
	Q4	>8.57	12	20	0.500	0.186, 1.347		Q4	>9.03	30	10	2.714	1.053, 6.999	
C22:5 n-6	Q1	<0.13	15	17	1.000		0.064	Q1	<0.14	27	16	1.000		0.542
	Q2	0.13-0.18	9	23	0.443	0.157, 1.251		Q2	0.14-0.20	26	11	1.401	0.548, 3.578	
	Q3	0.18-0.25	17	17	1.133	0.431, 2.979		Q3	0.20-0.26	23	15	0.909	0.370, 2.229	
	Q4	>0.25	21	12	1.983	0.735, 5.351		Q4	>0.26	24	20	0.711	0.302, 1.675	

*Q: quartiles; OR: odds ratio; CI: confidence interval. †Odds ratio were derived from a conditional logistic analysis model adjusted for age, sex, smoke, alcohol drinking, body mass index (BMI), total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, total energy, protein, fat and carbohydrate intake. Abbreviation: Ctrl: healthy controls; CRP: colorectal polys; CRC: colorectal cancer.

Table 4. Association of n-6 and n-3 PUFA indicators with the development of CRC

Indicators	Q*	Value	CRP (no.)	Ctrl (no.)	OR*,†	95% CI*	*P* for trend	Q	Value	CRC (no.)	CRP (no.)	OR*,†	95% CI*	*P* for trend
Total n-6 PUFA/total n-3 PUFA	Q1	<7.95	9	24	1.000		0.002	Q1	<8.88	28	12	1.000		0.284
	Q2	7.95-10.61	14	18	2.074	0.736, 5.849		Q2	8.88-10.76	28	13	0.923	0.359, 2.371	
	Q3	10.61-12.62	18	15	3.200	1.145, 8.944		Q3	10.76-12.93	22	19	0.496	0.199, 1.237	
	Q4	>12.62	21	12	4.667	1.643, 13.256		Q4	>12.93	22	18	0.524	0.209, 1.314	
C20:4 n-6/(C20:5 n-3+C22:6 n-3)	Q1	<2.35	8	24	1.000		<0.001	Q1	<2.80	27	13	1.000		0.001
	Q2	2.35-3.32	11	23	1.435	0.489, 4.206		Q2	2.80-3.47	34	7	2.339	0.820, 6.674	
	Q3	3.32-3.90	21	11	5.727	1.940, 16.912		Q3	3.47-4.02	17	24	0.341	0.138, 0.845	
	Q4	>3.90	22	11	6.000	2.040, 17.649		Q4	>4.02	22	18	0.588	0.237, 1.460	
C20:4 n-6/C20:5 n-3	Q1	<15.01	11	20	1.000		0.070	Q1	<18.22	23	16	1.000		0.886
	Q2	15.01-24.10	15	19	1.435	0.528, 3.901		Q2	18.22-26.50	27	14	1.342	0.541, 3.325	
	Q3	24.10-39.33	17	15	2.061	0.749, 5.667		Q3	26.50-40.32	26	15	1.206	0.490, 2.967	
	Q4	>39.33	19	15	2.303	0.847, 6.259		Q4	>40.32	24	17	0.982	0.403, 2.393	
C20:4 n-6/(total n-6 PUFA+total n-3 PUFA)	Q1	<0.18	17	19	1.000		0.671	Q1	<0.18	22	16	1.000		0.804
	Q2	0.18-0.20	9	16	0.629	0.221, 1.790		Q2	0.18-0.21	31	15	1.503	0.616, 3.665	
	Q3	0.20-0.23	20	16	1.397	0.553, 3.532		Q3	0.21-0.23	21	13	1.175	0.457, 3.023	
	Q4	>0.23	16	18	0.993	0.388, 2.541		Q4	>0.23	26	18	1.051	0.435, 2.535	

*Q: quartiles; OR: odds ratio; CI: confidence interval. †Odds ratio were derived from a conditional logistic analysis model adjusted for potential confounding factors, including age, sex, smoke, alcohol drinking, body mass index (BMI), total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, total energy, protein, fat and carbohydrate intake. Abbreviation: Ctrl: healthy controls; CRP: colorectal polys; CRC: colorectal cancer.

n-3+C22:6 n-3) (*P*<0.001) and showed significantly increased in the CRP group, the other kinds of PUFAs indicators showed no significant difference in the CRP group when compared to the Ctrl group. Compared to the CRP group, the ratio of C20:4 n-6 and (C20:5 n-3+C22:6 n-3) (*P*=0.048) showed significantly reduced in the CRC group, the other kinds of PUFAs indicators showed no significant difference in the CRC group when compared to the CRP group.

Association of n-3 PUFAs with the development of CRC

As shown in **Table 2**, during the process of Ctrl to CRP, total n-3 PUFA was inversely associated with CRP risk, showing a 84.1 percent risk reduction when Q4 and Q1 were compared (OR=0.159, 95% CI: 0.054-0.476; *P* for trend <0.001). C20:5 n-3 and C22:6 n-3 were also inversely associated with CRP risk, showing separate 73.7 and 87.5 percent risk reduction (OR=0.263 and 0.125, 95% CI: 0.088-0.785 and 0.041-0.379; *P* for trend=0.030 and <0.001). C18:3 n-3 and C22:5 n-3 showed no significant association with the CRP risk. These results indicated that the total n-3 PUFA, C20:5 n-3 and C22:6 n-3 were protective factors for CRP.

During the process of CRP to CRC, total n-3 PUFA was positively associated with CRC risk, with a 4.059-fold increased risk of CRC when Q4 and Q1 were compared (OR=4.059, 95% CI: 1.568-10.510; *P* for trend=0.007). C22:6 n-3 were also positively associated with CRC risk, with a 3.789-fold increased risk of CRC (OR=3.789, 95% CI: 1.454-9.874; *P* for trend=0.048). C18:3 n-3, C20:5 n-3 and C22:5 n-3 showed no significant association with the CRC risk. These results indicated that the total n-3 PUFA and C22:6 n-3 were risk factors for CRC.

Our results found that the total n-3 PUFA and C22:6 n-3 played converse role in the process of CRP and the process of CRC. During the process of Ctrl to CRP, they were protective factors, but during the process of CRP to CRC, they were risk factors.

Association of n-6 PUFAs with the development of CRC

As shown in **Table 3**, during the process of Ctrl to CRP, total n-6 PUFA was inversely associated with CRP risk, showing a 81.0 percent risk reduction when Q4 and Q1 were compared (OR=0.190, 95% CI: 0.065-0.555; *P* for trend <0.001). C18:2 n-6 were also inversely associated with CRP risk, showing a 70.1 percent risk reduction (OR=0.299, 95% CI: 0.108-0.828; *P* for trend=0.025). C18:3 n-6, C20:3 n-6, C20:4 n-6 and C22:5 n-6 showed no significant association with the CRP risk. These results indicated that the total n-6 PUFA and C18:2 n-6 were protective factors for CRP.

During the process of CRP to CRC, total n-6 PUFA was positively associated with CRC risk, with a 8.146-fold increased risk of CRC when Q4 and Q1 were compared (OR=8.146, 95% CI: 2.774-23.920; *P* for trend <0.001). C18:2 n-6 were also positively associated with CRC risk, with a 3.667-fold increased risk of CRC (OR=3.667, 95% CI: 1.420-9.470; *P* for trend=0.045). C18:3 n-6, C20:3 n-6, C20:4 n-6 and C22:5 n-6 showed no significant association with the CRC risk. These results indicated that the total n-6 PUFA and C18:2 n-6 were risk factors for the CRC.

Our results found that the total n-6 PUFA and C18:2 n-6 played converse role in the process of CRP and the process of CRC. During the process of Ctrl to CRP, they were protective factors, but during the process of CRP to CRC, they were risk factors.

Association of n-6 PUFA and n-3 PUFA indicators with the development of CRC

As shown in **Table 4**, during the process of Ctrl to CRP, the ratio of total n-6 PUFA and total n-3 PUFA was positively associated with CRP risk, with a 4.667-fold increased risk of CRP risk when Q4 and Q1 were compared (OR=4.667, 95% CI: 1.643-13.256; *P* for trend=0.002). The ratio of C20:4 n-6 and (C20:5 n-3+C22:6 n-3) also was positively associated with CRP risk, with a 6.000-fold increased risk of CRP risk (OR=6.000, 95% CI: 2.040-17.649; *P* for trend <0.001). The other n-6 PUFA and n-3 PUFA indicators showed no significant association with CRP risk.

During the process of CRP to CRC, the ratio of C20:4 n-6 and (C20:5 n-3+C22:6 n-3) was inversely associated with CRC, showing a 41.2 percent risk reduction (OR=0.588, 95% CI: 0.209-1.314; *P* for trend=0.001). The other n-6

PUFA and n-3 PUFA indicators showed no significant association with CRC risk.

Our results found that the ratio of C20:4 n-6 and (C20:5 n-3+C22:6 n-3) played converse role in the process of CRP and the process of CRC. During the process of Ctrl to CRP, they were risk factors, but during the process of CRP to CRC, they were protective factors.

Discussion

In our study, n-3 PUFA and C22:6 n-3 were positively associated with CRC risk. The results were opposite to some of the previous studies. In review of eight studies about n-3 PUFA supplementation in patients with previous sporadic colorectal adenomas, six of eight studies showed a 13-70% reduction in mucosal epithelial cell proliferation index compared to the placebo group, the other two studies showed no change in proliferation index [17]. The increased consumption of dietary C22:6 n-3 may result in increased incorporation in immune cell membranes [18], compete with arachidonic acid as a substrate for cyclooxygenase (COX) to result in inhibit the production of prostaglandin E2 and leukotriene B4 [19]. They can also influence the lipid raft composition and signaling properties of immune cells [20]. Although n-3 PUFA were demonstrated to be protective factor for gastrointestinal inflammation, however, recent studies provide controversial results [21]. It seemed that n-3 PUFA supplementation may depress immune environment through alterations in cytokine production, T-cell proliferation, and T-cell-mediated cytotoxicity. In addition, C22:6 n-3 can also exclusively suppress T regulatory function [22]. Other authors also found that the exaggerated inflammation and carcinogenesis induced by dietary C22:6 n-3 was associated with altered $CD8^+$ T-cell populations, $CD69^+$ activation, FoxP3 expression, and the frequency of $FoxP3^+$ $CD25^+$ $CD4^+$ Treg cells expressing L-selectin. These findings implicated that high doses of DHA consumed may promote impaired immune function [23]. In our study, the n-3 PUFA and C22:6 n-3 were demonstrated to be risk factor for the CRC when compared to the CRP. An analysis including 5 studies about Chinese people, one study of the 5 found that n-3 PUFA showed no significant association with the CRC [24], three studies showed significantly positive association with the CRC [6-8]. One showed significantly reverse association with the CRC [25]. The positive association between high intake of marine n-3 PUFA and rectal cancer risk may be related to at least one PARP codon 762 Ala allele [6].

In contrast to n-3 PUFA, n-6 PUFA was generally accepted as an increased risk of CRC. Animal studies showed that n-6 PUFA may enhance the risk of colorectal carcinogenesis [26]. However, in other studies, the results were not consistent. Some studies found there were positive association with the CRC [5, 27], some studies showed no association [15, 28], or inverse association [10, 29]. In our study, total n-6 PUFA and C18:2 n-6 were inversely associated with CRP risk. Our results indicated that the n-6 PUFA and C18:2 n-6 were protective factors for CRP risk. But n-6 PUFA and C18:2 n-6 were positively associated with CRC risk. Our results implied that the n-6 PUFA and C18:2 n-6 may play converse role in the process of CRP and CRC. In the previous studies, some authors found that n-6 PUFA can prevent or reduce the severity of autoimmune disease, and the desaturated/elongated metabolites are protective. n-6 PUFA are clinically useful in human autoimmune-inflammatory disorders [30]. C20:4 n-6 which had methylene interrupted double bonds may inhibit growth and perform cytotoxic effects because of peroxidation products that are generated during lipid peroxidation and COX activity [10].

The controversy role of n-3 PUFA and n-6 PUFA in CRP and CRC may be because the membrane phospholipids. When the n-6 PUFA concentration was low, it can serve as parts of the membrane phospholipids of the immune system to be protective factor, however, when the concentration was high, its derived eicosanoids such as PGE2 may be immunosuppressive. Studies had shown that n-3 PUFA in membrane can compete with n-6 PUFA as the substrates of cyclooxygenase and lipoxygenase enzymes. It can also decrease the production of n-6 PUFA derived eicosanoids such as PGE2 which is required for normal T cell function, however, when high concentration, it was immunosuppressive. In addition, when the n-3 PUFA concentration was low, it can bind with the PPAR-γ to regulate the IL-8, iNOS and MMP-1 to inhibit the cell proliferation, it can also increase the ROS to increase the cell apoptosis. When the concentration was high, it can incorporate into the member phospholipids to alter their fluidity

to inhibit the T-cell proliferation. The lipid rafts are crucial for T-cell activation, as are fences and pickets and protein-protein interactions that take part in the formation of the immunological synapse as a highly organized structure at the T-cell contact site to the antigen-presenting cell. n-3 PUFA treatment alters lipid rafts in altering the protein composition of the inner membrane lipid leaflet and inhibits T-cell responses. In addition, ROS which are the cellular consequences of oxidative stress may cause DNA oxidation, resulting in damage to all four bases and in the deoxy-ribose-molecule triggering the appearance of genetic mutations and initiating colorectal carcinogenesis [31].

In conclusion, our results demonstrated that the total n-3 PUFA, C22:6 n-3, the total n-6 PUFA, C18:2 n-6, and the ratio of C20:4 n-6 and (C20:5 n-3+C22:6 n-3) played controversy role in the process of CRP and the process of CRC, and may provide nutritional intervention suggestions for the clinical practice.

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Disclosure of conflict of interest

None.

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