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Serum levels of *n*-3 PUFA and colorectal cancer risk in Chinese population

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Abstract

Circulating *n*-3 PUFA, which integrate endogenous and exogenous *n*-3 PUFA, can be better used to investigate the relationship between *n*-3 PUFA and disease. However, studies examining the associations between circulating *n*-3 PUFA and colorectal cancer (CRC) risk were limited, and the results remained inconclusive. This case–control study aimed to examine the association between serum *n*-3 PUFA and CRC risk in Chinese population. A total of 680 CRC cases and 680 sex- and age-matched (5-year interval) controls were included. Fatty acids were assayed by GC. OR and 95 % CI were calculated using multivariable logistic regression after adjustment for potential confounders. Higher level of serum α-linolenic acid (ALA), docosapentaenoic acid (DPA), DHA, long-chain *n*-3 PUFA and total *n*-3 PUFA were associated with lower odds of CRC. The adjusted OR and 95 % CI were 0·34 (0·24, 0·49, $P_{\text{for trend}} < 0·001$) for ALA, 0·57 (0·40, 0·80, $P_{\text{for trend}} < 0·001$) for DPA, 0·48 (0·34, 0·68, $P_{\text{for trend}} < 0·001$) for DHA, 0·39 (0·27, 0·56, $P_{\text{for trend}} < 0·001$) for long-chain *n*-3 PUFA and 0·31 (0·22, 0·45, $P_{\text{for trend}} < 0·001$) for total *n*-3 PUFA comparing the highest with the lowest quartile. However, there was no statistically significant association between EPA and odds of CRC. Analysis stratified by sex showed that ALA, DHA, long-chain *n*-3 PUFA and total *n*-3 PUFA were inversely associated with odds of CRC in both sexes. This study indicated that serum ALA, DPA, DHA, long-chain *n*-3 PUFA and total *n*-3 PUFA were inversely associated with odds of having CRC in Chinese population.

Key words: **DHA: *n*-3 PUFA: Biomarker: Serum: Colorectal cancer**

Colorectal cancer (CRC) is ranked as one of the most common cancers globally. In 2020, there were more than 1·9 million new CRC cases and 0·9 million deaths worldwide[1]. In China, there were 0·555 million new CRC cases which ranks second among all cancers and approximately 0·286 million CRC patients died in 2020[2].

The cause of CRC is complex and includes lifestyle and dietary risk factors, such as alcohol drinking, high red and processed meat intake, low fibre intake, and lower physical activity[3]. There is an ongoing interest in the associations of fatty acids, especially *n*-3 PUFA with CRC risk. *n*-3 PUFA include α-18:3 *n*-3 (α-linolenic acid, ALA), long-chain *n*-3 PUFA such as 20:5 *n*-3 (EPA), 22:5 *n*-3 (docosapentaenoic acid, DPA) and 22:6 *n*-3 (DHA). The potential protective mechanisms of *n*-3 PUFA against CRC risk include inhibiting the production of eicosanoids derived from arachidonic acid, which may result in decreased cell proliferation and increased cell apoptosis[4–7]. Moreover, *n*-3 PUFA could inhibit the mammalian target of rapamycin signalling pathway which plays an essential role in cell growth, proliferation and angiogenesis[8,9].

Previous studies mainly focused on investigating the association between dietary intake of *n*-3 PUFA and CRC risk[10–13]. However, self-report dietary questionnaires used to assess dietary *n*-3 PUFA intake might have recall bias and measurement error[14]. Additionally, *n*-3 PUFA can be obtained not only through dietary intake but also from endogenous synthesis. ALA, an essential *n*-3 PUFA from plants, is converted to long-chain *n*-3 PUFA by chain elongation and desaturation in the body. However, bioconversion of ALA to EPA and DHA is limited, and marine fish is the main dietary source of EPA and DHA. DPA is present in fairly low levels in most fish oils[15], and circulating DPA is largely derived from endogenous metabolism. Therefore, circulating *n*-3 PUFA, which integrate endogenous and exogenous *n*-3 PUFA, can be better used to investigate

Abbreviations: ALA, α-linolenic acid; CRC, colorectal cancer; DPA, docosapentaenoic acid.

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the association between *n*-3 PUFA and disease. The alterations of fatty acids and their metabolites can be observed in tumour microenvironment, and significant differences were found in circulating *n*-3 PUFA, including ALA, EPA, and DPA, between CRC cases and controls[16].

So far, only seven epidemiological studies[17–23] have explored the associations between circulating levels of *n*-3 PUFA and CRC risk, and the results remained inconclusive. Some studies did not find significant associations of circulating ALA[19,21–23], EPA[17–22], DPA[19–21,23], DHA[20–22], long-chain *n*-3 PUFA[20,21] and total *n*-3 PUFA[19,21] with CRC risk. However, it was reported that plasma[19] or erythrocyte[21,23] DHA was inversely associated with CRC risk. Erythrocyte EPA and total *n*-3 PUFA were inversely associated with CRC risk[23]. Serum level of ALA, DPA, DHA, total *n*-3 PUFA[17] and the whole blood level of long-chain *n*-3 PUFA[18] were found to be significantly inversely related to CRC risk only among males. A meta-analysis including the above-mentioned five studies[17–20,22] indicated that blood levels of *n*-3 PUFA was inversely associated with CRC risk[24]. Additionally, of seven studies[17–23], four were conducted in Western countries[18–20,23]. To date, no study has reported the relationship between serum levels of *n*-3 PUFA and CRC risk among Chinese population. In consideration of the ethnic, genetic and dietary pattern differences, more studies are needed to perform in Chinese population.

In this context, this case–control study was conducted among Chinese population to assess whether the risk of CRC was associated with serum levels of *n*-3 PUFA.

Methods

Study subjects

This ongoing case–control study began in July 2010 and was conducted in Sun Yat-sen University Cancer Center, China. The ascertainment and selection of CRC case and control subjects have been previously described[10]. CRC patients aged 30–75 years were recruited if they were histologically confirmed and diagnosed no more than 3 months. Patients who were not able to speak or understand Mandarin/Cantonese or had a history of other cancers were excluded. Participants with familial adenomatous polyposis or hereditary non-polyposis CRC were also excluded. Between July 2010 and May 2021, a total of 3174 cases were enrolled and 2833 eligible cases completed the interview, yielding a response rate of 89·26 %. Among them, 1303 blood samples were successfully collected. Due to high cost and time-consuming of measuring circulating fatty acids, not all blood samples were tested for serum fatty acids. A total of 680 case subjects were selected by random sampling from 1303 with blood samples and included in the final measurement and analysis.

Controls were selected from two groups. The first control group was recruited from the inpatients admitted to Departments of Vascular Surgery, Ophthalmology, Otorhinolaryngology and Plastic and Reconstructive Surgery during the same time period as the cases. They were admitted for a wide spectrum of non-neoplastic conditions, such as sudden deafness, chronic otitis media, chronic sinusitis, vocal cord polyp, varicose veins, deafness, giddiness, cholesteatoma of middle ear, nasal polyp and nasal septum deviation, etc. So far, no evidence has been found that these conditions were obviously related to a dietary cause that may affect circulating *n*-3 PUFA. The second control group was residents in the same community as the cases and recruited through written invitations, advertisements or referrals. Eligibility criteria for controls were the same as described for the cases except that they were not diagnosed with CRC. Eventually, after frequency-matching with cases on sex and age (5-year interval), 680 control subjects (591 hospitals-based controls and 89 community-based controls) with available blood samples were included in our analysis.

The procedures for this study were approved by the ethical committee of the School of Public Health, Sun Yat-sen University (approval number: 2019-018) and were conducted according to the principles of the Declaration of Helsinki. All participants signed written informed consent forms to participate in the study before investigation.

Data collection

To collect the demographics, lifestyles, fish oil supplement use, and family history of cancer of study participants, face-to-face surveys were performed by trained interviewers with a structured questionnaire. Reproductive history was also obtained among females. Relevant diagnosis, pathological findings and tumour node metastasis stage were abstracted from the patients' medical records. We calculated BMI by using standard method (weight (kg)/ height (m^2)). Occupational activity was categorised into non-working, sedentary, low intensity, moderate intensity and vigorous intensity. We also collected information on frequency (d/week) and duration (h/d) for household and leisure-time activity. The mean metabolic equivalent (MET) task-hours value of each activity was calculated by referring to the Compendium of Physical Activities[25,26].

An 81-item validated FFQ[27] was used to assess dietary intake. Information on frequency of intake and commonly used portion size during the past 12 months before diagnosis for cases or interview for controls was collected and was used to estimate the daily intake of each food item. Red and processed meat consisted of pork, beef, lamb, organ meat, sausage, ham, bacon and hot dogs. The energy intake was calculated according to the 2002 China Food Composition Table[28]. Photographs of commonly consumed foods with usual intake portions were provided to help estimate the amounts of food consumed. The FFQ has been used in previous studies[10,29].

Blood collection and serum n-3 PUFA measurements

Venous blood samples were drawn in the morning of the second day of hospitalisation after 12 h fasting and before any treatment or examination. Within 2 h after collection, the blood samples were centrifuged at the speed of 3000 rpm for 15 min at 4°C. All serum samples were then stored at −80°C for further measurement.

Serum fatty acids were assayed by GC. Lipids of serum samples (50 μl) were extracted using a method previously described[30]. Total lipids were extracted by chloroform

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methanol (2:1, v/v). After extraction, samples were evaporated to dryness with nitrogen at 25°C. After methyl esterification by 14 weight percentage boron trifluoride methanol, *n*-3 PUFA were measured by a gas chromatograph 7890A (Agilent Technologies) equipped with a methyl polysiloxane capillary column (60-m × 0·25-mm inside diameter; 0·15-μm thickness; DB-23, Agilent Technologies).

The temperature in front injector and detector was 250°C and 280°C, respectively. The flow rate of nitrogen carrier gas was kept at 0·8 ml/min. A total of 1 μl derivatised sample was injected at a 5:1 split ratio. The initial temperature of column was held at 50°C for 1 min and then increased to 170°C at the rate of 25°C/min and held at this temperature for 10 min, and finally increased to 230°C at the rate of 2·2°C/min and kept at this temperature for 17 min. A sample was injected into the column every hour. Peak retention time were identified by injecting known standards using ChemStation software (Agilent Technologies) for analysis. The individual fatty acids of samples were separated and identified by the comparison of their respective retention time with those of fatty acids methyl ester standard. According to the retention time, we manually integrated each peak to calculate peak area. Peak area normalisation method was used for calculating relative content of compositions. The serum level of each specific fatty acid was expressed as the percentages of all detected fatty acids. The intra-day and inter-day repeatability were measured using the same serum sample extracts in every forty samples being tested. In between, the quality-control samples after extraction were kept at–20°C. The precision was expressed with the CV for corresponding peak areas. The intra-day CVs of quality-control samples were 9·47 % for ALA, 6·64 % for EPA, 12·94 % for DPA and 3·92 % for DHA. The inter-day CVs of quality-control samples were 14·46 %, 14·67 %, 13·61 % and 9·68 % for ALA, EPA, DPA and DHA, respectively.

Four *n*-3 PUFA, including ALA, EPA, DPA and DHA, were measured. We calculated the content of long-chain *n*-3 PUFA (EPA + DPA + DHA) and total *n*-3 PUFA (the sum of ALA, EPA, DPA and DHA).

Statistical analysis

SPSS 26.0 (SPSS Inc.) was used to conduct statistical analysis. *P*-values ≤ 0·05 was considered statistically significant. All data were expressed as mean and standard deviation or median (interquartile range 25th and 75th percentiles, IQR). The χ^2-test was used to compare categorical variables between cases and controls, and *t* test or Wilcoxon rank-sum test was used for continuous variables. Dietary red meat and processed meat intake were adjusted for total energy intake using the residual method[31].

Serum levels of *n*-3 PUFA were converted into categorical variables by using quartiles (Q1–Q4) according to serum levels of each fatty acid in control subjects, separately for men and women. Multivariable logistic regression models were used to estimate the associations between serum *n*-3 PUFA and CRC risk, and the associations were expressed as OR and 95 % CI. The lowest quartile group served as the reference group. The following potential confounding variables were included in the multivariable model: age (years), sex (male/female), residence (rural/urban), socio-economic factors (including marital status, educational level, occupation and income), occupational activity, household and leisure-time activities, regular smoking, passive smoking, alcohol drinking, first-degree relative with cancer, BMI, total energy intake, red and processed meat intake, serum SFA, serum MUFA, and serum *n*-6 PUFA. Linear trend was tested by entering the median of each quartile as a continuous variable in the regression models.

A sex-stratified analysis was performed. Multiplicative models were used to evaluate the interaction effect between sex and serum *n*-3 PUFA in relation to the odds of having CRC by including the product term in multivariable logistic regression models. Analysis by tumour site (colon cancer and rectal cancer) was also conducted. To test for heterogeneity in the OR by tumour subsite, we regarded colon/rectal status as the dependent variable (outcome) in the logistic regression models. We had greater than 99 % power to detect OR of 0·34, 0·48, 0·39 and 0·31 for the association of serum ALA, DHA, long-chain *n*-3 PUFA and total *n*-3 PUFA with CRC risk. Our sample gave us 70·88 % and 98·50 % power to detect the OR of 0·57 and 0·48 for the association of serum EPA and DPA with CRC risk at $P < 0·05$ (two-tailed).

Results

The characteristics of CRC case and control subjects are presented in Table 1. The proportions of cases who had been married, had low incomes, had a history of cancer in first-degree relatives and had lower education were significantly higher than those in the control subjects. Meanwhile, cases had a higher frequency of regular and passive smoking, engaged in fewer occupational activities, and had higher consumption of red and processed meat. More than 80 % of cases (565, 83·09 %) were in tumour node metastasis stage 0/I–III. Among female subgroup, more cases had earlier menarche age.

As shown in Table 2, serum SFA were significantly higher in cases compared with controls. Cases had significantly lower serum levels of ALA, EPA, DPA, DHA, long-chain *n*-3 PUFA, total *n*-3 PUFA and total *n*-6 PUFA compared with control subjects.

Table 3 shows the results of the associations between serum levels of *n*-3 PUFA and the risk of CRC. Compared with the lowest quartile, the odds of having CRC in the highest quartile were 70 % lower for serum ALA, 43 % lower for EPA, 41 % lower for DPA, 62 % lower for DHA, 68 % lower for long-chain *n*-3 PUFA and 74 % lower for total *n*-3 PUFA. Additionally adjusting for serum SFA, MUFA and *n*-6 PUFA did not change the results of the associations between serum levels of *n*-3 PUFA and the risk of CRC, except that the association between serum EPA and odds of having CRC attenuated to statistically insignificant (adjusted $OR_{Q4\ v.\ Q1} = 0·72$, 95 % CI = 0·50, 1·04, $P_{for\ trend} = 0·060$).

Analysis stratified by sex found that serum ALA, DHA, long-chain *n*-3 PUFA and total *n*-3 PUFA were inversely related to odds of CRC among both males and females. However, serum EPA and DPA displayed significant and inverse associations with odds of CRC mainly among females. The adjusted OR values for

Table 1. Characteristics and selected risk factors of study subjects

Variables	Cases (*n* 680)			Controls (*n* 680)			*P**
		n	%		*n*	%	
Age (years)							0·456
Mean	53·69			53·25			
SD	10·71			10·60			
Sex (*n* (%))							0·745
Males		350	51·47		344	50·59	
Females		330	48·53		336	49·41	
Marital status (*n* (%))							0·013
Married		645	94·85		622	91·47	
Unmarried/divorces/widowed		35	5·15		58	8·53	
Residence (*n* (%))							0·060
Urban		455	66·91		487	71·62	
Rural		225	33·09		193	28·38	
Educational level (*n* (%))							< 0·001
Primary school or below		194	28·53		177	26·03	
Junior high school		200	29·41		166	24·41	
Senior high school/secondary technical school		174	25·59		157	23·09	
College or above		112	16·47		180	26·47	
Occupation (*n* (%))							0·242
Administrator/other white-collar worker		112	16·47		131	19·26	
Blue-collar worker		192	28·24		170	25·00	
Farmer/other		376	55·29		379	55·74	
Income (Yuan/month) (*n* (%))							0·002
< 2000		98	14·41		133	19·56	
2001–5000		207	30·44		171	25·15	
5001–8000		208	30·59		176	25·88	
> 8001		167	24·56		200	29·41	
BMI (kg/m^2)							0·067
Mean	23·28			23·60			
SD	3·31			3·16			
Regular smoker (*n* (%))		184	27·06		112	16·47	< 0·001
Passive smoker (*n* (%))		185	27·21		129	18·97	< 0·001
Regular drinker (*n* (%))		104	15·29		110	16·18	0·655
First-degree relative with cancer (*n* (%))		98	14·41		69	10·15	0·017
Fish oil supplement user (*n* (%))		24	3·53		27	3·97	0·669
Occupational activity (*n* (%))							0·019
Non-working		113	16·62		114	16·76	
Sedentary		180	26·47		221	32·50	
Light occupation		187	27·50		164	24·12	
Moderate occupation		96	14·12		66	9·71	
Heavy-activity occupation		104	15·29		115	16·91	
Household and leisure-time activities (MET-h/week)							0·081
Mean	32·47			35·28			
SD	28·44			31·09			
Energy intake (kcal/d)							0·906
Median	1483·93			1465·65			
IQR	629·15			532·24			
Red and processed meat (g/d)†							0·044
Median	109·20			99·51			
IQR	69·21			61·46			
Age at menarche (years)‡							< 0·001
Mean	14·72			15·35			
SD	2·16			2·10			
Menopausal status (*n* (%))‡							0·604
Premenopausal		98	29·70		106	31·55	
Postmenopausal		232	70·30		230	68·45	
TNM stage							
0/I		107	15·74				
II		236	34·71				
III		222	32·65				
IV		104	15·29				
Unknown		11	1·61				
Site of cancer							
Ascending colon		89	13·09				
Hepatic flexure of colon		38	5·59				
Transverse colon		39	5·74				
Ileocecal colon		11	1·62				

Table 1. (*Continued*)

Variables	Cases (*n* 680)		Controls (*n* 680)		
	n	%	*n*	%	*P**
Splenic flexure of colon	10	1·47			
Descending colon	35	5·15			
Sigmoid colon	174	25·59			
Descending–sigmoid junction colon	9	1·32			
Multiple primary cancer	6	0·88			
Colon, NOS	2	0·29			
Rectosigmoid junction	23	3·38			
Rectum, NOS	244	35·88			

MET, metabolic equivalent task; IQR, interquartile range 25th and 75th percentiles; TNM, tumour node metastasis; NOS, not otherwise specified.
Normally distributed mean values and standard deviations; non-normally distributed medians and interquartile range 25th and 75th percentiles; numbers and percentages.
* Continuous variables were evaluated using *t* test or Wilcoxon rank-sum test. Categorical variables were evaluated using χ^2 test. $P < 0·05$: significant.
† The consumption was adjusted for total energy intake by the regression residual method.
‡ Among female subgroup.

Table 2. Serum levels of detected fatty acids among colorectal cancer cases and controls

	Cases (*n* 680)		Controls (*n* 680)		
	Median	IQR	Median	IQR	*P**
C18:3 *n*-3 (ALA) (%)†	0·29	0·16	0·36	0·20	< 0·001
C20:5 *n*-3 (EPA) (%)†	0·43	0·16	0·46	0·20	< 0·001
C22:5 *n*-3 (DPA) (%)†	0·27	0·14	0·30	0·16	< 0·001
C22:6 *n*-3 (DHA) (%)†	1·16	0·56	1·31	0·62	< 0·001
LC *n*-3 PUFA (%)†	1·90	0·62	2·12	0·76	< 0·001
Total *n*-3 PUFA (%)†	2·23	0·63	2·52	0·81	< 0·001
C14:0 (%)†	0·15	0·16	0·13	0·09	0·190
C16:0 (%)†	33·90	3·05	32·10	3·68	< 0·001
C18:0 (%)†	16·49	2·86	15·44	2·80	< 0·001
C20:0 (%)†	0·28	0·10	0·26	0·09	< 0·001
C22:0 (%)†	0·26	0·20	0·34	0·25	< 0·001
C24:0 (%)†	0·30	0·17	0·34	0·18	< 0·001
Total SFA (%)†	51·37	5·22	48·59	6·43	< 0·001
C14:1 (%)†	0·16	0·13	0·13	0·09	< 0·001
C16:1 (%)†	0·96	0·60	1·02	0·71	0·337
C18:1 (%)†	13·17	2·90	13·20	3·13	0·054
C20:1 (%)†	0·22	0·16	0·18	0·10	0·038
C22:1 (%)†	0·56	0·52	0·46	0·47	< 0·001
C24:1 (%)†	0·56	0·26	0·48	0·24	< 0·001
Total MUFA (%)†	16·00	3·05	15·80	3·54	0·180
C18:2 *n*-6 (%)†	21·48	4·22	23·55	4·91	< 0·001
C18:3 *n*-6 (%)†	0·26	0·20	0·33	0·23	< 0·001
C20:4 *n*-6 (%)†	3·95	1·32	4·51	1·46	< 0·001
Total *n*-6 PUFA (%)†	25·73	4·71	28·45	4·95	< 0·001

IQR, interquartile range 25th and 75th percentiles; ALA, α-linolenic acid; DPA, docosapentaenoic acid; LC *n*-3 PUFA, long-chain *n*-3 PUFA.
Non-normally distributed medians and interquartile range 25th and 75th percentiles.
* Wilcoxon rank-sum test comparing the median levels between cases and controls.
† Among total detected serum fatty acids. The serum level of fatty acid was expressed as the percentages of all detected fatty acids.

the fourth quartile (*v.* the first quartile) were 0·56 (95 % CI = 0·32, 0·98) ($P_{interaction} = 0·069$) for EPA and 0·22 (95 % CI = 0·12, 0·40) ($P_{interaction} < 0·001$) for DPA, respectively (Table 4).

Among the 680 cases, 403 were diagnosed with colon cancer and 277 with rectal cancer. Subgroup analysis by cancer site showed that serum ALA, DPA, DHA, long-chain *n*-3 PUFA and total *n*-3 PUFA were inversely associated with the odds of having both colon and rectal cancer, except for EPA (Table 5).

Discussion

The purpose of this study was to examine the association between serum level of *n*-3 PUFA and the risk of CRC among Chinese population with a relatively larger sample size. The results showed that serum total *n*-3 PUFA, ALA, DPA and DHA were statistically significantly inversely associated with CRC risk. In contrast, serum level of EPA displayed no significant association with the risk of CRC.

Previous studies[32–34] showed that there were significant differences between CRC cases and controls in circulating *n*-3 PUFA, which indicated that it is necessary to further examine the association of circulating *n*-3 PUFA and CRC risk. However, few studies have examined the association between circulating ALA and CRC risk. A nested case–control study from Japan[17] found a significant inverse association between serum ALA and CRC risk among males, which is in line with the present

Table 3. Association between serum *n*-3 PUFA and colorectal cancer risk

		Q2		Q3		Q4		
Serum *n*-3 PUFA	Q1	OR	95 % CI	OR	95 % CI	OR	95 % CI	$P_{for\ trend}$
C18:3 *n*-3 (ALA)								
Cases/controls	310/170	163/170		117/170		90/170		
Crude OR	1·00	0·53	0·40, 0·70	0·38	0·28, 0·51	0·29	0·21, 0·40	< 0·001
Adjusted OR1*	1·00	0·50	0·37, 0·68	0·38	0·28, 0·52	0·30	0·22, 0·42	< 0·001
Adjusted OR2†	1·00	0·60	0·44, 0·82	0·45	0·32, 0·63	0·34	0·24, 0·49	< 0·001
C20:5 *n*-3 (EPA)								
Cases/controls	197/170	207/170		169/170		107/170		
Crude OR	1·00	1·05	0·79, 1·40	0·86	0·64, 1·15	0·54	0·40, 0·75	< 0·001
Adjusted OR1*	1·00	0·99	0·73, 1·34	0·86	0·63, 1·17	0·57	0·41, 0·80	0·001
Adjusted OR2†	1·00	1·31	0·94, 1·81	1·17	0·84, 1·64	0·72	0·50, 1·04	0·060
C22:5 *n*-3 (DPA)								
Cases/controls	231/170	172/170		142/170		135/170		
Crude OR	1·00	0·75	0·56, 1·00	0·62	0·46, 0·83	0·58	0·43, 0·79	< 0·001
Adjusted OR1*	1·00	0·72	0·53, 0·98	0·60	0·44, 0·82	0·59	0·43, 0·82	< 0·001
Adjusted OR2†	1·00	0·76	0·55, 1·05	0·65	0·46, 0·90	0·57	0·40, 0·80	< 0·001
C22:6 *n*-3 (DHA)								
Cases/controls	253/170	187/170		134/170		106/170		
Crude OR	1·00	0·74	0·56, 0·98	0·53	0·39, 0·71	0·42	0·31, 0·57	< 0·001
Adjusted OR1*	1·00	0·71	0·52, 0·96	0·55	0·40, 0·76	0·38	0·28, 0·53	< 0·001
Adjusted OR2†	1·00	0·82	0·60, 1·14	0·70	0·50, 0·98	0·48	0·34, 0·68	< 0·001
LC *n*-3 PUFA								
Cases/controls	259/170	191/170		144/170		86/170		
Crude OR	1·00	0·74	0·56, 0·98	0·56	0·41, 0·75	0·33	0·24, 0·46	< 0·001
Adjusted OR1*	1·00	0·73	0·54, 0·98	0·54	0·40, 0·74	0·32	0·23, 0·46	< 0·001
Adjusted OR2†	1·00	0·89	0·65, 1·23	0·71	0·51, 0·99	0·39	0·27, 0·56	< 0·001
Total *n*-3 PUFA								
Cases/controls	303/170	166/170		134/170		77/170		
Crude OR	1·00	0·55	0·41, 0·73	0·44	0·33, 0·59	0·25	0·18, 0·35	< 0·001
Adjusted OR1*	1·00	0·56	0·42, 0·76	0·45	0·33, 0·61	0·26	0·18, 0·37	< 0·001
Adjusted OR2†	1·00	0·71	0·52, 0·98	0·57	0·41, 0·80	0·31	0·22, 0·45	< 0·001

ALA, α-linolenic acid; DPA, docosapentaenoic acid; LC *n*-3 PUFA, long-chain *n*-3 PUFA.
OR and 95 % CI.
* OR1 adjusted for age, sex, residence, occupation, educational level, marital status, income, occupational activity, household and leisure-time activities, regular smoking, passive smoking, alcohol drinking, first-degree relative with cancer, BMI, total energy intake, and red and processed meat intake.
† OR2 additionally adjusted for serum SFA, serum MUFA and serum *n*-6 PUFA.

study. A Mendelian randomisation study also reported that circulating ALA was inversely associated with CRC risk[35]. However, some studies[19,21–23] did not find significant associations of circulating ALA with CRC risk. ALA cannot be synthesised endogenously in human body and therefore must be provided exogenously in the diet. The potential explanations for differences in results are likely to be associated with the difference of ALA intake across different populations. We previously[10] reported that mean ALA intake was 1·0 g/d among Chinese men and women. Average ALA intake was 1·90 g/d among Japanese people aged 35–66 years[36], whereas ALA intake was 0·76 g/d among Australia people aged 45–64 years[37]. Additionally, the relatively higher level of serum ALA in our study (case group: 0·29 %, control group: 0·36 %) compared with that in other studies (both cases and non-case group were 0·15 %)[19] may help explain the reduction of CRC risk with serum ALA in the present study. Additionally, relatively larger sample size in our study might contribute to find a significant association and narrower 95 % CI. For example, the previous study had wider 95 % CI (OR 0·39, 95 % CI 0·16, 0·91) due to smaller sample size (161 cases)[17]. Other studies did not find significant association between ALA and CRC risk which might be related to the smaller sample size, with OR (95 CI%) of 0·96 (0·69, 1·33) (395 cases)[19], and 1·18 (0·63, 2·21) (74 cases)[21], and 1·70 (0·84, 3·43) (350 cases)[22].

The inverse associations of serum DPA, DHA and long-chain *n*-3 PUFA with likelihood of having CRC observed in our study was in line with a nested case–control study from Japan[17]. However, some studies did not observe statistically significant associations of circulating DPA[19–21,23], DHA[20–22,35] and long-chain *n*-3 PUFA[20,21] with CRC risk. Our observation of the inverse association between total *n*-3 PUFA and odds of having CRC was consistent with a meta-analysis[24] and a nested case–control study from the European Prospective Investigation into Cancer and Nutrition (EPIC)[23]. Consistent with some previous studies[17–23,35], our study did not observe a statistically significant association between serum EPA and odds of having CRC. However, in the above-mentioned nested case–control study, erythrocyte EPA was found to be inversely related with CRC risk[23]. In a Mendelian randomisation study with individuals of European ancestry, circulating EPA and DPA were found to be positively associated with CRC risk[35]. One possible explanation for our observation of the inverse association between total and individual long-chain *n*-3 PUFA and odds of having CRC might be related to higher dietary *n*-3 PUFA intake due to higher consumption of fish in eastern compared with Western populations. Based on the national nutritional surveys, average fish

Table 4. Association between serum *n*-3 PUFA and colorectal cancer risk stratified by sex

	Males			Females			
Serum *n*-3 PUFA	Cases/controls	Adjusted OR*	95 % CI	Cases/controls	Adjusted OR†	95 % CI	$P_{interaction}$
C18:3 *n*-3 (ALA)							0·128
Q1	173/86	1·00		137/84	1·00		
Q2	70/86	0·36	0·22, 0·58	93/84	1·01	0·63, 1·61	
Q3	61/86	0·34	0·21, 0·57	56/84	0·60	0·36, 1·00	
Q4	46/86	0·23	0·14, 0·40	44/84	0·51	0·30, 0·89	
$P_{for\ trend}$		< 0·001			0·005		
C20:5 *n*-3 (EPA)							0·069
Q1	85/86	1·00		112/84	1·00		
Q2	101/86	1·50	0·92, 2·45	106/84	1·14	0·70, 1·86	
Q3	105/86	1·79	1·10, 2·92	64/84	0·75	0·44, 1·26	
Q4	59/86	0·96	0·56, 1·65	48/84	0·56	0·32, 0·98	
$P_{for\ trend}$		0·836			0·021		
C22:5 *n*-3 (DPA)							< 0·001
Q1	100/86	1·00		131/84	1·00		
Q2	73/86	0·79	0·48, 1·30	99/84	0·77	0·48, 1·23	
Q3	77/86	0·98	0·59, 1·61	65/84	0·49	0·30, 0·82	
Q4	100/86	1·13	0·68, 1·87	35/84	0·22	0·12, 0·40	
$P_{for\ trend}$		0·471			< 0·001		
C22:6 *n*-3 (DHA)							0·624
Q1	128/86	1·00		125/84	1·00		
Q2	90/86	1·00	0·63, 1·61	97/84	0·70	0·43, 1·14	
Q3	71/86	0·93	0·56, 1·53	63/84	0·56	0·34, 0·94	
Q4	61/86	0·59	0·35, 0·98	45/84	0·38	0·21, 0·66	
$P_{for\ trend}$		0·031			< 0·001		
LC *n*-3 PUFA							0·113
Q1	119/86	1·00		140/84	1·00		
Q2	91/86	0·97	0·61, 1·57	100/84	0·84	0·52, 1·36	
Q3	86/86	1·07	0·65, 1·74	58/84	0·44	0·26, 0·74	
Q4	54/86	0·56	0·33, 0·95	32/84	0·26	0·14, 0·46	
$P_{for\ trend}$		0·037			< 0·001		
Total *n*-3 PUFA							0·037
Q1	144/86	1·00		159/84	1·00		
Q2	72/86	0·66	0·41, 1·07	94/84	0·82	0·51, 1·31	
Q3	87/86	0·84	0·52, 1·36	47/84	0·37	0·22, 0·62	
Q4	47/86	0·40	0·23, 0·69	30/84	0·24	0·14, 0·43	
$P_{for\ trend}$		0·002			< 0·001		

ALA, α-linolenic acid; DPA, docosapentaenoic acid; LC *n*-3 PUFA, long-chain *n*-3 PUFA.
OR and 95 % CI.
* Adjusted for age, residence, occupation, educational level, marital status, income, occupational activity, household and leisure-time activities, regular smoking, passive smoking, alcohol drinking, first-degree relative with cancer, BMI, total energy intake, red and processed meat intake, serum SFA, serum MUFA and serum *n*-6 PUFA.
† Additionally adjusted for age at menarche.

intake was 92–108 g/d among Japanese middle-aged men in 2002[37]. Mean fresh fish intake was 77·51 g/d among Chinese population aged 30–75 years[38]. However, average fish intake was 37·1 g/d and 23·7 g/d from the EPIC in France[20] and among Australian population aged 18–59 years[39]. Meanwhile, the sample size in our study is larger than that of a case–control study from Japan with seventy-four cases[21] and one study from France with 461 cases[20]. Both of these studies[20,21] did not find significant association between long-chain *n*-3 PUFA and CRC risk which might be due to smaller sample size. Moreover, the relatively higher level of serum total *n*-3 PUFA and relatively large sample size in our study compared with previous studies[19,21] may help explain our observed reduction of the odds of having CRC with serum total *n*-3 PUFA.

The potential beneficial effects of *n*-3 PUFA against the risk of CRC are biologically plausible. *n*-3 PUFA might reduce CRC risk by inhibiting the COX-2 enzyme and the production of eicosanoids that are derived from arachidonic acid[4–7]. A clinical trial of fish oil supplementation observed reduced proliferation in the rectal mucosa of patients diagnosed with sporadic colorectal adenomas[40]. There are other mechanisms by which *n*-3 PUFA may decrease CRC risk, including the inhibition of ornithine decarboxylase, decreased bile acid excretion and NF-κB activity, the alteration of protein kinase C activity, the activation of peroxisome proliferator-activated receptor α and γ, and the reduced nitric oxide production[4,6,7,41]. Recent experimental animal study has indicated that *n*-3 PUFA elicit anti-CRC effect through regulating the DNA methylation process[42] and modulating profiles of eicosanoid metabolites[43]. Moreover, the mammalian target of rapamycin signalling pathway plays a key role in physiological and pathological processes of CRC[44]. *n*-3 PUFA have been found to down-regulate mammalian target of rapamycin signalling pathway in CRC cells[45–47] and contribute to the suppression of tumour initiation and progression[48] and the reduction of proliferation in colon cancer cell lines[49,50].

In our study, significantly inverse associations of serum EPA and DPA with odds of having CRC were only observed among females. No clear explanation exists for this point. It has been reported that female sex hormones might play a role in the aetiology of CRC[51,52]. Several studies suggested that women have

Table 5. Association between serum *n*-3 PUFA and colorectal cancer risk stratified by cancer site

	Colon cancer (*n* 403)			Rectal cancer (*n* 277)			
Serum *n*-3 PUFA	Cases/controls	Adjusted OR*	95 % CI	Cases/controls	Adjusted OR*	95 % CI	$P_{heterogeneity}$
C18:3 *n*-3 (ALA)							0·318
Q1	188/170	1·00		122/170	1·00		
Q2	102/170	0·65	0·46, 0·94	61/170	0·56	0·37, 0·84	
Q3	59/170	0·37	0·25, 0·56	58/170	0·56	0·37, 0·86	
Q4	54/170	0·36	0·24, 0·55	36/170	0·31	0·19, 0·50	
$P_{for\ trend}$		< 0·001			< 0·001		
C20:5 *n*-3 (EPA)							0·515
Q1	126/170	1·00		71/170	1·00		
Q2	118/170	1·17	0·80, 1·72	89/170	1·64	1·07, 2·51	
Q3	98/170	1·06	0·72, 1·56	71/170	1·38	0·89, 2·14	
Q4	61/170	0·65	0·42, 1·00	46/170	0·85	0·52, 1·38	
$P_{for\ trend}$		0·043			0·310		
C22:5 *n*-3 (DPA)							0·190
Q1	150/170	1·00		81/170	1·00		
Q2	98/170	0·67	0·46, 0·97	74/170	0·89	0·58, 1·37	
Q3	77/170	0·48	0·33, 0·72	65/170	0·88	0·57, 1·37	
Q4	78/170	0·52	0·35, 0·77	57/170	0·63	0·40, 0·99	
$P_{for\ trend}$		< 0·001			0·013		
C22:6 *n*-3 (DHA)							0·593
Q1	159/170	1·00		94/170	1·00		
Q2	104/170	0·70	0·48, 1·02	83/170	0·97	0·64, 1·46	
Q3	83/170	0·69	0·47, 1·02	51/170	0·67	0·43, 1·06	
Q4	57/170	0·40	0·27, 0·62	49/170	0·54	0·34, 0·86	
$P_{for\ trend}$		< 0·001			0·003		
LC *n*-3 PUFA							0·310
Q1	166/170	1·00		93/170	1·00		
Q2	106/170	0·77	0·53, 1·10	85/170	1·15	0·76, 1·73	
Q3	87/170	0·68	0·46, 0·99	57/170	0·72	0·46, 1·13	
Q4	44/170	0·31	0·20, 0·48	42/170	0·50	0·31, 0·81	
$P_{for\ trend}$		< 0·001			0·001		
Total *n*-3 PUFA							0·191
Q1	193/170	1·00		110/170	1·00		
Q2	98/170	0·62	0·43, 0·89	68/170	0·89	0·58, 1·34	
Q3	68/170	0·47	0·32, 0·70	66/170	0·77	0·50, 1·17	
Q4	44/170	0·28	0·18, 0·44	33/170	0·35	0·21, 0·57	
$P_{for\ trend}$		< 0·001			< 0·001		

ALA, α-linolenic acid; DPA, docosapentaenoic acid; LC *n*-3 PUFA: long-chain *n*-3 PUFA.
* Adjusted for age, sex, residence, occupation, educational level, marital status, income, occupational activity, household and leisure-time activities, regular smoking, passive smoking, alcohol drinking, first-degree relative with cancer, BMI, total energy intake, red and processed meat intake, serum SFA, serum MUFA and serum *n*-6 PUFA.

higher endogenously synthesis of EPA and DHA than men[53,54]. Previous studies suggested that human can convert ALA to DHA in the liver predominantly[55–57]. After initiation of oral ethinyl estradiol treatment, conversion of EPA to DHA increased[54]. Further research is needed to clarify this issue.

The risk of CRC might differ by cancer type[58,59]. Some studies showed that plasma DHA displayed inversely significant associations with rectal cancer risk[19] and plasma ALA was inversely associated with colon cancer risk[22]. But several studies did not find significant associations of plasma ALA[19], EPA[19,22], DPA[19], DHA[22] and total *n*-3 PUFA[19] with the risk of colon or rectal cancer. Plasma EPA, DPA, DHA and long-chain *n*-3 PUFA were not associated with colon cancer risk[20]. The present study also showed no significant differences between serum *n*-3 PUFA and the risks of colon and rectal cancer.

Our study had some strengths. First, this is the first study to comprehensively investigate the association between serum *n*-3 PUFA and CRC risk among Chinese people. Second, the methods commonly used to measure fatty acids include HPLC, GC–MS and GC. Due to the high cost and professional maintenance of GC-MS and HPLC, GC is therefore the most commonly used method for the analysis of medium- and long-chain fatty acids[60]. Third, the relatively large number of CRC cases allowed subgroup analyses by sex and tumour site. Fourth, detailed information on potential CRC risk factors was collected and could be adjusted in the multivariable models.

The limitations of our study also need to be acknowledged. Firstly, all CRC patients recruited from Sun Yat-sen University Cancer Center might lead to selection bias. However, as the largest cancer centre in Southern China, the CRC patients admitted in this cancer centre have similar clinical characteristics to those of other big hospitals in Guangdong province or in other parts of mainland China[61,62], which might help to minimise selection bias. Secondly, measurement errors might be present in the process of detecting serum fatty acids. To reduce this bias, laboratory technician was blind to the diagnostic status of study subjects to ensure consistency in the sample preparation. Additionally, all samples were assayed by the same technician and quality controls were also applied. Thirdly, serum samples used in our study were stored at –80°C for 5–12 years. Iso *et al.*[63] measured serum fatty acids in 1990 and 1998 using

thirty-one duplicated serum aliquots frozen at −80°C in 1990. The results showed that no changes were seen for *n*-3 PUFA (12·8 % *v.* 12·3 %, $P = 0{\cdot}140$). This indicated that serum level of *n*-3 PUFA might be a stable biomarker. Fourthly, although a wide range of possible confounding factors were adjusted in the models, potential unmeasured confounders cannot be excluded. Fifthly, we had only serum samples available for fatty acids analyses. Fatty acids content can be measured in erythrocyte which reflect dietary intake over a longer period[60]. However, previous studies indicated good correlations between serum and erythrocyte *n*-3 PUFA[64]. Sixthly, one of the major drawbacks of fatty acids measured from serum and not the putative target tissue, in this case the colon and rectum, is the associated time factor. Serum fatty acids reflect what has been consumed over a period of weeks to days, whereas tissue fatty acid levels may reflect what has been consumer in months to years. However, measuring adipose tissue level of *n*-3 PUFA is more invasive, and fatty acids profiles might differ across sites within a person[60]. Furthermore, longitudinal intra-subjects analysis illustrated that serum novel circulating long-chain fatty acids levels are relatively stable over the short term of up to 90 weeks in both late-stage CRC patients and healthy controls[65]. Seventhly, due to the case–control study design, CRC cases might either change their diets or *n*-3 PUFA metabolism might be affected by cancer itself. Therefore, the association between *n*-3 PUFA and CRC risk should be interpreted with caution, and further prospective studies are needed among Chinese population.

In summary, our findings suggest the protective effect of serum ALA, DPA, DHA, long-chain *n*-3 PUFA and total *n*-3 PUFA on the risk of CRC in Chinese population. Our study indicates that serum *n*-3 PUFA might play a critical role in the incidence of CRC and may have significant implications for directing scientific diets of CRC prevention. In the light of existing evidence, we recommend to have an increased consumption of *n*-3 PUFA-rich food such as ALA-rich oil or marine fish to improve health. Further prospective studies are still needed to clarify the relationship between serum *n*-3 PUFA and the risk of CRC.

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