A Prospective Study of Dietary Polyunsaturated Fatty Acids and Colorectal Cancer Risk in Chinese Women

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Abstract

In animal models of colon cancer, n-3 polyunsaturated fatty acids (PUFA) have antineoplastic properties, whereas n-6 PUFAs may promote carcinogenesis. Prior epidemiologic studies have been inconsistent regarding the association of PUFAs and colorectal cancer. We prospectively evaluated the association between PUFA intake and colorectal cancer in a cohort of 73,242 Chinese women who were interviewed in person at the baseline survey for the Shanghai Women's Health Study. Dietary fatty acid consumption was derived using data collected from two food frequency questionnaires administered at baseline and 2 to 3 years later. The dietary total n-6 to n-3 PUFA ratio was strongly associated with colorectal cancer risk. Compared with women in the lowest quintile group, elevated relative risks (RR) were observed for the second [RR, 1.52; 95% confidence intervals (CI), 1.00-2.32], third (RR, 2.20; 95% CI, 1.41-3.45), fourth (RR, 1.65; 95% CI, 0.99-2.75), and fifth (RR, 1.95; 95% CI, 1.07-3.54) quintile groups. Arachidonic acid was associated with colorectal cancer risk with elevated RRs of $1.20_{Q2\text{-}Q1}$ (95% CI, 0.87-1.64), $1.44_{Q3\text{-}Q1}$ (95% CI, 1.05-1.98), $1.61_{Q4\text{-}Q1}$ (95% CI, 1.17-2.23), and $1.39_{Q5\text{-}Q1}$ (95% CI, 0.97-1.99; $P_{trend} = 0.03$) with increasing dietary quintile. In a subset of 150 cancer cases and 150 controls, we found a statistically significant trend between an increasing n-6 to n-3 PUFA ratio and increasing production of prostaglandin E_2 (PGE_2) as measured by urinary PGE_2 metabolites ($P = 0.03$). These results suggest that dietary PUFA and the ratio of n-6 to n-3 PUFA intake may be positively associated with colorectal cancer risk, and this association may be mediated in part through PGE_2 production. (Cancer Epidemiol Biomarkers Prev 2009;18(8):2283–91)

Introduction

Despite effective screening interventions, colorectal cancer remains a leading cause of cancer-related mortality (1). Because of the suboptimal rates of colorectal cancer screening, studies to identify modifiable life-style factors for primary prevention as well as safe and effective chemopreventive therapies are necessary to augment cancer control programs (2, 3). Long-chain n-3 polyunsaturated fatty acids (PUFA), principally found in marine fish oils, have consistently shown antineoplastic and anti-inflammatory effects in animal models and human cell lines (4, 5). However, research in humans has been inconsistent regarding the association of n-3 PUFAs with colorectal cancer risk (6, 7).

n-3 PUFAs, such as eicosapentanoic acid (EPA), and n-6 PUFAs, such as arachidonic acid, use the same biochemical pathway yet produce prostanoids with different physiologic effects. Arachidonic acid is a membrane phospholipid PUFA and the parent compound for multiple inflammatory eicosanoids (8). Arachidonic acid is released from cellular membranes through the action of phospholipase A_2 and metabolized to prostaglandin H_2 by cyclooxygenase-1 and cycloxygenase-2 enzymes. Free arachidonic acid can be converted into various series 2 and 4 prostaglandins, leukotrienes, and thromboxanes including prostaglandin E_2 (PGE_2), PGD_2, $PGF_2\alpha$, PGI_2, and TXA_2. Conversely, EPA is released from cellular membranes and converted via these same enzymes into series 3 prostanoids and series 5 leukotrienes. It is generally described that series 2 and 4 eicosanoids are more inflammatory than their series 3 and 5 counterparts, and the balance of such eicosanoids may be related to dietary intake of n-6 and n-3 PUFAs (9, 10).

One proposed mechanism for the protective effect of n-3 PUFAs in colorectal neoplasm is through competitive inhibition of proinflammatory series 2 prostanoids, such as PGE_2 (11). PGE_2 is the most abundant prostaglandin detected in colorectal neoplasms and is believed to contribute toward colorectal tumorigenesis through several signaling pathways including the up-regulation of β-catenin, activating the phosphatidylinositol-3-kinase and AKT-kinase oncogenes, and activating the RAS-mitogen-activated protein kinase pathway (12-16). We have recently shown, in a nested case-control study of women enrolled in the Shanghai Women's Health Study (SWHS), that a high urinary PGE_2 metabolite level is associated with a substantially elevated risk of colorectal cancer (17).

Given the significant role of PGE_2 in colorectal tumorigenesis and the role of arachidonic acid in PGE_2 synthesis, we hypothesized that diets lower in arachidonic acid and other n-6 PUFAs and higher in EPA and other n-3 PUFAs might be associated with a lower production of PGE_2, and thus, a lower risk of colorectal cancer. We analyzed data collected as part of the SWHS, a large, population-based

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cohort study of Chinese women to investigate these hypotheses.

Materials and Methods

Study Population. The SWHS is a population-based prospective cohort study which, from 1996 to 2000, enrolled 74,943 women ages 40 to 70 y from seven urban communities in Shanghai. Details of the study design have been previously published (18). Briefly, all study participants completed a baseline survey including information on dietary habits, reproductive history and hormone use, physical activity, disease history, smoking and alcohol history, occupational history, and family cancer history. The overall participation rate was 92.7%. For this study, we excluded participants who reported any prior history of cancer (n = 1,576) and subjects reporting implausible total energy intakes (n = 123), specifically caloric intake <500 and >3,500 kcal at the baseline survey.

Outcome Assessment. The cohort was followed with a combination of biennial in-home interviews and annual record linkage with the Shanghai Cancer Registry and Shanghai Vital Statistics database. The in-person follow-up rate for the first, second, and third follow-up surveys were 99.8%, 98.7%, and 96.7%, respectively. Cancer registry matches identified colorectal cancer cases which were subsequently verified through medical charts from the diagnostic hospital. For this study, we included all incident colorectal cancers (N = 396) diagnosed from baseline enrollment to June 2007.

Exposure Assessment. At baseline, participants completed a comprehensive dietary assessment questionnaire. A second dietary assessment was completed during the first follow-up survey from 2000 to 2002. Data were obtained regarding usual dietary intake over the past 12 mo. Individual nutrient intakes for antioxidants (vitamins A, C, and E, selenium, carotenoids, and retinoids), fats, fatty acids, and other nutrients were calculated by the product of the amount of each food consumed by the nutrient content of the specific food based on the Chinese Food Composition Table (19). To improve the validity of assessing usual dietary intake, we calculated the mean reported dietary intake for specific nutrients based on the baseline and first follow-up dietary questionnaires. This mean value was then included as the dietary exposure value within all of the analyses. For participants who were diagnosed with either cancer or diabetes mellitus during the period between the baseline food frequency questionnaire and the second follow-up food frequency questionnaire, only the baseline-reported fatty acid intake was used for the data analysis given the concern that some women may have changed their dietary habits after the diagnosis of these diseases. Total n-3 PUFA were calculated by combining 18:3 (linolenic acid), 20:5 (EPA), 22:5 (docosapentaenoic acid), and 22:6 (docosahexaenoic acid; DHA) PUFAs, and total n-6 PUFA were based on 18:2 (linoleic acid) and 20:4 (arachidonic acid) fatty acids. Total n-3 highly unsaturated fatty acids (HUFA), which are fatty acids with 20 or greater carbon molecules, were calculated by combining EPA, docosapentaenoic acid, and DHA. The ratio of total n-6 PUFA to total n-3 PUFA was determined by dividing the sum of the reported dietary intake of linoleic acid and arachidonic acid by the sum of the reported dietary intake of linolenic acid, EPA, docosapentaenoic acid, and DHA.

Urinary PGE-M Determination. As part of the SWHS, spot urine samples were collected at baseline for ~65,754 (87.7%) of cohort members. In a nested case-control study involving 150 cases and 150 controls (17), urinary PGE_2 metabolic (PGE-M; 11-α-hydroxy-9,15-dioxo-2,3,4,5-tetranorprostane-1,20-dioic acid) level was measured using a liquid chromatography/tandem mass spectrometric method previously described by Murphey et al. (20) to quantify endogenous PGE_2 production. Briefly, 0.75 mL of urine per subject was titrated to a pH of 3 using 1 mol/L of HCl and then 0.5 mL of methyloxime HCl. Methoximated PGE-M was extracted and applied to a C-18 Sep-Pak (Waters Associates) and eluted with 5 mL of ethyl acetate. An internal standard of $^2H_6$ *O*-methyloxime PGE-M was added. Liquid chromatography was done on a Zobrax Eclipse XDB-C18 column attached to a Thermo Finnigan Surveyor MS Pump (Thermo Finnigan). For endogenous PGE-M, the predominant product ion m/z 336 representing $[M\text{-}(OCH_3 + H_2O)]^-$ and the analogous ion m/z 339 representing $[M\text{-}OC(^2H_3 + H_2O)]$, for the deuterated internal standard, were monitored in the selected reaction monitoring mode. Quantification of endogenous PGE-M used the ratio of the mass chromatogram peak areas of the m/z 336 and m/z 339 ions. Urine creatinine was also measured (Sigma) and values of PGE-M were reported as nanograms per milligram of creatinine. Laboratory staff was blinded to case status of the urine samples and the identity of the quality control samples included in the study.

Statistical Analysis. Dietary PUFAs were categorized into quintiles based on the overall distribution of nutrient intakes of the cohort. Dietary intake levels of fatty acids and red meat consumption were adjusted for energy intake using the residual method (21). Baseline characteristics were compared according to dietary PUFA quintile. For categorical variables, we used the stratified Cochran-Mantel-Haenszel test to compare age-adjusted proportions (22). ANOVA was used to compare age-adjusted means for continuous variables.

Cox proportional hazards analysis was used to estimate the relative risks (RR) and 95% confidence intervals (95% CI) for the association of colorectal cancer risk with dietary fatty acid consumption. Covariates for inclusion within the model were selected from those associated with both fatty acid intake and colorectal cancer risk, and only variables that appreciably affected point estimates, defined as a >10% change, were included as potential confounders. The multivariate model was adjusted for age at cohort entry (continuous), total energy intake in kilocalories (continuous), smoking status (ever, never), alcohol use (ever, never), regular physical activity in past 5 y (regularly was defined as least weekly, for >3 mo, continuously), energy-adjusted total red meat consumption in grams per day (continuous), menopausal status (postmenopausal, premenopausal, or perimenopausal), use of hormone replacement therapy (ever, never), use of a multivitamin (ever, never), and regular use of aspirin (defined as using aspirin at least thrice a week for >2 consecutive mo in the past 12 mo). For models in which the independent variable was an n-6 PUFA (linoleic acid, arachidonic acid, or total n-6 PUFA), we also adjusted for total n-3 PUFA intake and the n-6 to n-3 PUFA ratio. For models

in which the independent variable was an n-3 PUFA (α-linolenic acid, n-3 HUFA, or total n-3 PUFA), we included total n-6 PUFA intake and total n-6 to n-3 PUFA ratio as covariates. In the models with total fish intake and the ratio of total n-6 to n-3 PUFA as the independent variable, we included both total n-6 PUFA intake and total n-3 PUFA intake as covariates. To test for a possible interaction between total n-6 PUFA and total n-3 PUFA, we included an interaction term of these two variables within the fully adjusted model. The interaction term was the product of the total dietary intake of n-6 PUFA as a continuous variable, multiplied by the total dietary intake of n-3 PUFA as a continuous variable. A second interaction term was constructed as the product of intake of n-6 PUFA as a categorical variable (quintiles) multiplied by total n-3 PUFA intake as a categorical variable (quintiles). Our level of significance for the interaction term was set at 0.05. To evaluate the shape of the association between the ratio of total n-6 PUFA to total n-3 PUFA with colorectal cancer risk, nonlinear terms were included in the models using the restricted cubic spline function with four knots (23).

Calculations for the correlation of urinary PGE-M levels with the baseline reported n-6 to n-3 PUFA ratio were done using data from a nested case-control study of 150 colorectal cancer cases and 150 controls participating in the SWHS. After excluding participants with urinary PGE-M levels of zero ($n = 4$), 296 participants were included in the analysis to evaluate the age-adjusted correlation between urinary PGE-M and dietary PUFAs. Urinary PGE-M data were skewed to the high value so we normalized the distribution by log transformation of urinary PGE-M data. We calculated Pearson's correlation coefficients between log-transformed urinary PGE-M levels and total n-6 to n-3 ratios. In addition, Pearson's correlation coefficients were calculated between log-transformed urinary PGE-M levels and the total n-6 to n-3 ratio stratified by time between urine sample collection and cancer diagnoses. All statistical analyses were conducted using SAS version 9.1 (SAS Institute). All values were two-sided and significant at $P = 0.05$.

Results

A total of 73,243 women were included in this analysis. Baseline characteristics stratified by dietary intake of total n-6 PUFA, total n-3 PUFA, and the n-6 to n-3 ratio are presented in Table 1. In general, individuals who consumed higher levels of total n-6 and total n-3 PUFA were more likely to report regular exercise, reported higher intakes of red meat, were more likely to use alcohol, and were more likely to use aspirin, multivitamins, or hormone replacement therapy. Participants reporting higher intakes of n-3 PUFA tended to be younger than those reporting lower intakes. Participants reporting higher consumption of n-6 PUFA relative to n-3 PUFA were older, had higher body mass indexes (BMI), were more likely to smoke, were more likely to engage in regular exercise, and consumed lower amounts of red meat (Table 1). Median intakes for each PUFA along with intraquartile ranges are presented in Table 2.

There were no associations or significant trends found with dietary intake of linoleic acid, total n-6 PUFA, α-linolenic acid, highly unsaturated n-3 PUFAs, or total n-3 PUFA

Table 1. Demographic characteristics stratified by quintile of PUFA intake

Characteristic	Q1	Q2	Q3	Q4	Q5	*P*
Total n-6 PUFA						
Mean age, y ± SD	52.8 ± 9.0	52.0 ± 9.0	52.1 ± 9.1	52.3 ± 9.0	53.1 ± 9.1	<0.0001
Mean BMI, kg/m^2 ± SD	24.5 ± 3.6	23.9 ± 3.4	23.8 ± 3.4	23.8 ± 3.3	24.0 ± 3.4	<0.0001
Currently smoking, *n* (%)	418 (3.2)	290 (2.2)	318 (2.4)	325 (2.5)	384 (2.9)	0.005
Regular alcohol use, *n* (%)	255 (2.5)	272 (2.6)	291 (2.8)	357 (3.4)	470 (4.7)	<0.0001
Regular physical activity, *n* (%)	4,448 (36.0)	4,736 (38.3)	4,952 (40.1)	5,453 (44.1)	6,089 (49.4)	<0.0001
Mean total red meat intake, g/d ± SD	45.9 ± 33.3	46.0 ± 31.6	48.6 ± 32.2	53.0 ± 35.3	60.3 ± 43.8	<0.0001
Postmenopausal, *n* (%)	7,498 (53.6)	6,883 (49.1)	6,867 (49.1)	7,087 (50.6)	7,505 (53.8)	0.34
Regular hormone replacement therapy use, *n* (%)	205 (1.7)	258 (2.1)	309 (2.5)	376 (3.1)	366 (3.2)	<0.0001
Regular multivitamins use, *n* (%)	580 (5.4)	888 (8.3)	1,058 (9.9)	1,230 (11.6)	1,433 (13.7)	<0.0001
Regular aspirin use, *n* (%)	227 (2.1)	234 (2.3)	293 (2.8)	310 (3.0)	416 (4.1)	<0.0001
Total n-3 PUFA						
Mean age, y ± SD	54.0 ± 9.1	52.6 ± 9.2	52.1 ± 9.0	51.9 ± 9.0	51.8 ± 8.9	<0.0001
Mean BMI, kg/m^2 ± SD	24.6 ± 3.6	23.9 ± 3.4	23.7 ± 3.3	23.8 ± 3.3	24.0 ± 3.3	<0.0001
Currently smoking, *n* (%)	455 (2.7)	338 (2.1)	281 (1.8)	335 (2.2)	326 (2.3)	0.0001
Regular alcohol use, *n* (%)	280 (2.6)	273 (2.6)	269 (2.6)	356 (3.4)	467 (4.8)	<0.0001
Regular physical activity, *n* (%)	4,758 (35.8)	4,781 (36.4)	5,073 (39.0)	5,305 (40.9)	5,761 (44.6)	<0.0001
Mean total red meat intake, g/d ± SD	42.0 ± 31.4	43.6 ± 29.6	47.9 ± 31.4	53.7 ± 33.7	66.7 ± 45.5	<0.0001
Postmenopausal, *n* (%)	8,234 (52.3)	7,156 (45.6)	6,882 (44.0)	6,864 (43.9)	6,704 (42.9)	0.01
Regular hormone replacement therapy use, *n* (%)	186 (1.6)	266 (2.3)	295 (2.6)	379 (3.3)	388 (3.5)	<0.0001
Regular multivitamins use, *n* (%)	493 (5.1)	846 (8.7)	1,012 (10.4)	1,272 (13.1)	1,566 (16.1)	<0.0001
Regular aspirin use, *n* (%)	233 (2.0)	233 (2.1)	294 (2.6)	337 (3.0)	383 (3.4)	<0.0001
n-6 to n-3 PUFA ratio						
Mean age, y ± SD	49.9 ± 8.3	51.4 ± 8.6	52.6 ± 8.8	53.6 ± 9.2	55.0 ± 9.4	<0.0001
Mean BMI, kg/m^2 ± SD	23.7 ± 3.3	23.9 ± 3.3	24.0 ± 3.4	24.2 ± 3.5	24.3 ± 3.6	<0.0001
Currently smoking, *n* (%)	302 (2.4)	283 (2.5)	324 (3.0)	352 (3.5)	474 (4.9)	<0.0001
Regular alcohol use, *n* (%)	362 (2.6)	266 (1.9)	310 (2.4)	335 (2.7)	372 (3.1)	0.21
Regular physical activity, *n* (%)	4,574 (30.3)	4,889 (33.7)	5,221 (37.4)	5,595 (41.2)	5,399 (41.6)	<0.0001
Mean total red meat intake, g/d ± SD	60.5 ± 40.3	56.4 ± 35.9	51.0 ± 33.4	46.2 ± 32.8	39.7 ± 32.9	<0.0001
Postmenopausal, *n* (%)	5,565 (43.2)	6,540 (51.7)	7,273 (58.7)	7,882 (65.5)	8,580 (73.7)	<0.0001
Regular hormone replacement therapy use, *n* (%)	322 (1.5)	369 (1.7)	321 (1.5)	299 (1.4)	203 (1.0)	<0.0001
Regular multivitamins use, *n* (%)	1,275 (5.3)	1,255 (5.6)	1,008 (4.6)	966 (4.5)	685 (3.3)	<0.0001
Regular aspirin use, *n* (%)	253 (1.6)	288 (1.8)	337 (2.2)	324 (2.1)	278 (1.8)	<0.0001

Table 2. Median intake of selected PUFAs

	Median (±IQR)
Linoleic acid (18:2), g/d	5.86 (4.35-7.81)
Arachidonic acid (20:4), g/d	0.05 (0.03-0.07)
Total n-6 PUFA (18:2+20:4), g/d	5.91 (4.39-7.87)
α-Linolenic acid (18:3), g/d	0.87 (0.63-1.17)
Total n-3 HUFA (20:5 + 22:5 + 22:6), g/d	0.07 (0.03-0.14)
Total n-3 PUFA (18:3 + 20:5 + 22:5 + 22:6), g/d	0.97 (0.70-1.31)
Total fish intake, g/d	38.5 (21.1-66.0)
Total n-6 to n-3 ratio	6.21 (5.48-6.99)

NOTE: HUFA are fatty acids with 20 or more carbons.
Abbreviation: IQR, intraquartile range.

and colorectal cancer risk (Table 3). Dietary arachidonic acid was associated with colorectal cancer risk and this relationship seemed to be dose-dependent ($RR_{Q2\text{-}Q1}$, 1.20; 95% CI, 0.87-1.64; $RR_{Q3\text{-}Q1}$, 1.44; 95% CI, 1.05-1.98; $RR_{Q4\text{-}Q1}$, 1.61; 95% CI, 1.05-2.23; $RR_{Q5\text{-}Q1}$, 1.39; 95% CI, 0.97-1.99; $P_{trend} = 0.03$). The ratio of n-6 to n-3 was strongly associated with colorectal cancer risk ($RR_{Q2\text{-}Q1}$, 1.52; 95% CI, 1.00-2.32; $RR_{Q3\text{-}Q1}$, 2.20; 95% CI, 1.41-3.45; $RR_{Q4\text{-}Q1}$, 1.65; 95% CI, 0.99-2.75; $RR_{Q5\text{-}Q1}$, 1.95; 95% CI, 1.07-3.54; $P_{trend} = 0.19$). There was a statistically significant interaction between total n-6 PUFA and total n-3 PUFA intake and colorectal cancer risk ($P_{interaction} = 0.03$) when fatty acid intake was included within the model as a continuous variable. No statistically significant interaction was found ($P_{interaction} = 0.44$) when PUFA intake was include within the model as a categorical variable. We found an increased risk for colorectal cancer associated with total fish intake which was only statistically significant in the fourth quintile when compared with the first quintile (RR, 1.42; 95% CI, 1.01-2.00).

After excluding cases diagnosed during the first 2 years of follow-up, fish intake was no longer statistically significantly associated with an increased risk of colorectal cancer (Table 4). The pattern of the associations with the n-6 to n-3 ratio and all individual fatty acids, however, remained unchanged, although the trend test for the association of arachidonic acid with colorectal cancer risk was no longer statistically significant. Elevated RRs were found for women with a high ratio of n-6 to n-3 regardless of the anatomic location of the cancer (colon or rectum).

Table 3. Association between PUFA intake and colorectal cancer risk in the SWHS (1996-2007)

	All cases (event = 396)					P_{trend}
	Q1	Q2	Q3	Q4	Q5	
Linoleic acid						
Adjusted RR (95% CI)*	1.00	0.94 (0.66-1.34)	1.03 (0.70-1.49)	1.24 (0.83-1.87)	1.07 (0.62-1.84)	0.61
Cases	78	67	73	90	88	
Median, g/d	4.24	4.71	5.54	6.74	9.49	
Arachidonic acid						
Adjusted RR (95% CI)*	1.00	1.20 (0.87-1.64)	1.44 (1.05-1.98)	1.61 (1.17-2.23)	1.39 (0.97-1.99)	0.03
Cases	84	79	84	84	65	
Median, g/d	0.02	0.03	0.05	0.06	0.09	
Total n-6 PUFA						
Adjusted RR (95% CI)*	1.00	0.90 (0.63-1.28)	1.00 (0.69-1.45)	1.16 (0.78-1.74)	1.01 (0.59-1.73)	0.73
Cases	80	66	74	88	88	
Median, g/d	4.28	4.75	5.59	6.80	9.56	
α-Linolenic acid						
Adjusted RR (95% CI)†	1.00	1.09 (0.77-1.55)	1.13 (0.76-1.67)	1.14 (0.73-1.78)	1.16 (0.66-2.06)	0.76
Cases	82	77	77	77	83	
Median, g/d	0.58	0.69	0.83	1.01	1.44	
Total n-3 HUFA‡						
Adjusted RR (95% CI)†	1.00	0.85 (0.61-1.17)	1.22 (0.89-1.68)	1.22 (0.87-1.71)	1.11 (0.77-1.61)	0.38
Cases	94	70	89	78	65	
Median, g/d	0.03	0.04	0.07	0.12	0.25	
Total n-3 PUFA						
Adjusted RR (95% CI)†	1.00	1.21 (0.85-1.73)	1.11 (0.73-1.69)	1.28 (0.80-2.05)	1.41 (0.77-2.57)	0.37
Cases	84	83	70	77	82	
Median, g/d	0.64	0.76	0.93	1.13	1.61	
Total fish intake						
Adjusted RR (95% CI)§	1.00	1.02 (0.74-1.41)	1.17 (0.84-1.64)	1.42 (1.01-2.00)	1.28 (0.87-1.90)	0.13
Cases	86	77	78	85	70	
Median, g/d	14.91	23.02	35.86	56.12	104.52	
Ratio n-6/n-3§						
Adjusted RR (95% CI)§	1.00	1.52 (1.00-2.32)	2.20 (1.41-3.45)	1.65 (0.99-2.75)	1.95 (1.07-3.54)	0.19
Cases	44	68	104	82	98	
Median	4.84	5.64	6.21	6.80	7.89	
Median n-6 PUFA, g/d	5.48	6.00	6.19	6.21	5.73	
Median n-3 PUFA, g/d	1.17	1.06	1.00	0.91	0.71	

*Adjusted for age, energy intake (kcal), total energy-adjusted n-3 PUFA intake (g/d), energy-adjusted ratio of total n-6 PUFA to n-3 PUFA intake, BMI (kg/m^2), current smoker, alcohol use, regular physical activity in past 5 y, total energy-adjusted red meat intake (g/d), menopausal status, hormone replacement therapy use, multivitamin use, and aspirin use.
†Adjusted for age, energy intake (kcal), total energy-adjusted n-6 PUFA intake (g/d), energy-adjusted ratio of total n-6 PUFA to n-3 PUFA intake, BMI (kg/m^2), current smoker, alcohol use, regular physical activity in past 5 y, total energy-adjusted red meat intake (g/d), menopausal status, hormone replacement therapy use, multivitamin use, and aspirin use.
‡HUFAs (EPA + DPA + DHA).
§Adjusted for age, energy intake (kcal), total energy-adjusted n-6 PUFA intake (g/d), total energy-adjusted n-3 PUFA intake (g/d), BMI (kg/m^2), current smoker, alcohol use, regular physical activity in past 5 y, total energy-adjusted red meat intake (g/d), menopausal status, hormone replacement therapy use, multivitamin use, and aspirin use.

Table 4. Association between PUFA intake and colorectal cancer risk in the SWHS stratified by cancer site

	Analysis of colorectal cancer cases omitting the first 24 mo of observation (event = 332)					P_{trend}
	Q1	Q2	Q3	Q4	Q5	
Colorectal cancer						
Linoleic acid						
Adjusted RR (95% CI)*	1.00	0.94 (0.62-1.41)	1.04 (0.67-1.62)	1.38 (0.85-2.24)	1.20 (0.62-2.34)	0.42
Cases	70	59	60	74	69	
Arachidonic acid						
Adjusted RR (95% CI)*	1.00	1.20 (0.84-1.71)	1.48 (1.04-2.12)	1.44 (0.99-2.10)	0.96 (0.62-1.50)	0.86
Cases	77	72	74	65	44	
Total n-6 PUFA						
Adjusted RR (95% CI)*	1.00	0.88 (0.58-1.31)	1.00 (0.65-1.55)	1.25 (0.77-2.02)	1.10 (0.57-2.12)	0.52
Cases	72	58	61	72	69	
α-Linolenic acid						
Adjusted RR (95% CI)†	1.00	0.96 (0.64-1.44)	1.07 (0.68-1.69)	1.24 (0.74-2.09)	1.03 (0.52-2.05)	0.90
Cases	74	63	64	68	63	
Total n-3 HUFA‡						
Adjusted RR (95% CI)†	1.00	0.69 (0.47-1.00)	1.08 (0.75-1.54)	0.99 (0.67-1.46)	0.95 (0.62-1.45)	0.71
Cases	88	56	76	62	50	
Total n-3 PUFA						
Adjusted RR (95% CI)†	1.00	1.07 (0.71-1.62)	1.14 (0.70-1.84)	1.27 (0.73-2.20)	1.16 (0.56-2.39)	0.82
Cases	77	68	61	66	60	
Total fish intake						
Adjusted RR (95% CI)§	1.00	0.97 (0.67-1.39)	1.18 (0.81-1.71)	1.26 (0.86-1.86)	1.05 (0.66-1.65)	0.71
Cases	69	60	65	62	45	
Ratio n-6/n-3§						
Adjusted RR (95% CI)§	1.00	1.57 (0.96-2.57)	2.28 (1.35-3.86)	1.84 (1.01-3.43)	1.88 (0.93-3.80)	0.37
Cases	34	56	89	73	80	
	Analysis of colon cancer cases omitting the first 24 mo of observation (event = 200)					P_{trend}
	Q1	Q2	Q3†	Q4	Q5	
Colon cancer						
Linoleic acid						
Adjusted RR (95% CI)*	1.00	0.53 (0.31-0.93)	0.84 (0.50-1.42)	1.08 (0.63-1.86)	1.03 (0.53-2.00)	0.39
Cases	50	24	36	44	46	
Arachidonic acid						
Adjusted RR (95% CI)*	1.00	1.23 (0.79-1.91)	1.34 (0.85-2.11)	1.06 (0.64-1.76)	1.14 (0.67-1.94)	0.81
Cases	50	46	43	30	31	
Total n-6 PUFA						
Adjusted RR (95% CI)*	1.00	0.49 (0.28-0.86)	0.85 (0.50-1.45)	0.93 (0.52-1.67)	0.88 (0.40-1.92)	0.47
Cases	51	23	38	42	46	
α-Linolenic acid						
Adjusted RR (95% CI)†	1.00	0.99 (0.58-1.69)	1.02 (0.55-1.89)	1.62 (0.83-3.14)	1.40 (0.58-3.37)	0.31
Cases	47	34	32	45	42	
Total n-3 HUFA‡						
Adjusted RR (95% CI)†	1.00	0.57 (0.36-0.91)	0.73 (0.46-1.17)	0.80 (0.49-1.31)	0.79 (0.46-1.34)	0.96
Cases	64	33	36	37	30	
Total n-3-PUFA						
Adjusted RR (95% CI)†	1.00	1.10 (0.64-1.88)	1.15 (0.62-2.15)	1.57 (0.78-3.15)	1.51 (0.61-3.74)	0.35
Cases	50	35	33	43	39	
Total fish intake						
Adjusted RR (95% CI)§	1.00	0.99 (0.64-1.53)	0.86 (0.53-1.39)	0.90 (0.54-1.50)	0.94 (0.53-1.65)	0.83
Cases	48	42	32	30	28	
Ratio n-6/n-3§						
Adjusted RR (95% CI)§	1.00	1.34 (0.72-2.51)	1.81 (0.93-3.52)	1.68 (0.80-3.54)	2.02 (0.85-4.80)	0.17
Cases	22	32	47	45	54	
	Analysis of rectal cancer cases omitting the first 24 mo of observation (event = 132)					P_{trend}
	Q1	Q2	Q3†	Q4	Q5	
Rectal cancer						
Linoleic acid						
Adjusted RR (95% CI)*	1.00	2.03 (1.05-3.94)	1.63 (0.74-3.58)	2.46 (1.02-5.94)	2.05 (0.61-6.86)	0.83
Cases	20	35	24	30	23	
Arachidonic acid						
Adjusted RR (95% CI)*	1.00	1.16 (0.64-2.10)	1.73 (0.98-3.07)	2.07 (1.16-3.69)	0.68 (0.31-1.52)	0.98
Cases	27	26	31	35	13	
Total n-6 PUFA						
Adjusted RR (95% CI)*	1.00	1.87 (0.98-3.58)	1.41 (0.65-3.07)	2.19 (0.92-5.18)	1.37 (0.55-3.41)	0.90
Cases	21	35	23	30	23	

(Continued on the following page)

Table 4. Association between PUFA intake and colorectal cancer risk in the SWHS stratified by cancer site (Cont'd)

	Analysis of rectal cancer cases omitting the first 24 mo of observation (event = 132)					P_{trend}
	Q1	Q2	Q3†	Q4	Q5	
α-Linolenic acid						
Adjusted RR (95% CI)†	1.00	0.91 (0.49-1.72)	1.09 (0.54-2.17)	0.83 (0.37-1.87)	0.64 (0.22-1.89)	0.27
Cases	27	29	32	23	21	
Total n-3 HUFA‡						
Adjusted RR (95% CI)†	1.00	1.02 (0.53-1.93)	2.01 (1.11-3.65)	1.50 (0.78-2.89)	1.39 (0.70-2.85)	0.62
Cases	24	23	40	25	20	
Total n-3 PUFA						
Adjusted RR (95% CI)†	1.00	1.03 (0.54-1.96)	1.08 (0.51-2.27)	0.90 (0.37-2.17)	0.75 (0.23-2.41)	0.42
Cases	27	33	28	23	21	
Total fish intake						
Adjusted RR (95% CI)§	1.00	0.94 (0.49-1.81)	1.89 (1.04-3.45)	2.07 (1.10-3.87)	1.32 (0.61-2.84)	0.41
Cases	21	18	33	32	17	
Ratio n-6/n-3§						
Adjusted RR (95% CI)§	1.00	2.00 (0.89-4.49)	3.15 (1.32-7.53)	2.12 (0.77-5.83)	1.62 (0.48-5.50)	0.80
Cases	12	24	42	28	26	

*Adjusted for age, energy intake (kcal), total energy-adjusted n-3 PUFA intake (g/d), energy-adjusted ratio of total n-6 PUFA to n-3 PUFA intake, BMI (kg/m^2), current smoker, alcohol use, regular physical activity in past 5 y, total energy-adjusted red meat intake (g/d), menopausal status, hormone replacement therapy use, multivitamin use, and aspirin use.

†Adjusted for age, energy intake (kcal), total energy-adjusted n-6 PUFA intake (g/d), energy-adjusted ratio of total n-6 PUFA to n-3 PUFA intake, BMI (kg/m^2), current smoker, alcohol use, regular physical activity in past 5 y, total energy-adjusted red meat intake (g/d), menopausal status, hormone replacement therapy use, multivitamin use. and aspirin use.

‡HUFA = (EPA + DPA + DHA).

§Adjusted for age, energy intake (kcal), total energy-adjusted n-6 PUFA intake (g/d), total energy-adjusted n-3 PUFA intake (g/d), BMI (kg/m^2), current smoker, alcohol use, regular physical activity in past 5 y, total energy-adjusted red meat intake (g/d), menopausal status, hormone replacement therapy use, multivitamin use, and aspirin use.

However, the association with dietary arachidonic acid, total n-6, linoleic acid, and fish intake were more apparent for rectal cancer than colon cancer.

To evaluate the shape of the dose-response relationship between the n-6 and n-3 ratio, we included nonlinear terms using the restricted cubic spline function with four knots (Fig. 1). The total n-6 to n-3 ratio showed a strong nonlinear association with colorectal cancer risk (P for nonlinearity = 0.02).

The total n-6 to n-3 PUFA ratio was positively correlated to urinary levels of PGE-M ($r = 0.12$, $P = 0.03$). When stratified by case status, colorectal cases ($n = 151$) had a borderline significant positive correlation ($r = 0.15$, $P = 0.07$) and no correlation was found for controls ($n = 145$; $r = 0.05$, $P = 0.53$). Cases with urinary PGE-M measurements were stratified into quartiles based on the duration of time between the collection of the spot urine sample and the time to the diagnosis of colorectal cancer (Table 5). The correlation coefficient was most strong in individuals who had >43 months between the collection of the spot urine sample and the diagnosis of colorectal cancer ($r = 0.47$, $P = 0.003$). The correlation was nonsignificant when urine collection preceded the diagnosis of colorectal cancer by <43 months.

Discussion

In this prospective cohort study, we found a strong positive association between the ratio of dietary n-6 to n-3 PUFA and colorectal cancer risk. We found a similarly strong association between dietary arachidonic acid intake and colorectal cancer risk. This association was independent of the ratio of total n-n-6 to n-3 PUFA, suggesting that both absolute intake of arachidonic acid as well as the relative dietary intake of n-6 PUFAs with respect to n-3 PUFA may contribute toward the risk of colorectal cancer.

The ratio of total n-6 to n-3 PUFAs in colorectal cancer cases was found to be positively correlated with urinary levels of PGE-M, a biomarker that has previously been shown to strongly relate to colorectal cancer risk (17), lending support to the findings observed in the study of an association between dietary PUFA ratios and colorectal cancer risk. Of interest, this correlation was only significant when the time between urine collection and cancer diagnosis was nearly 4 years or more. These findings are intriguing and suggest that dietary fatty acid intake could alter the production of inflammatory prostanoids and consequently the risk of colorectal cancer but that this protective effect may only be at earlier stages of colon carcinogenesis.

Prior studies have produced inconsistent results regarding the association of arachidonic acid with colorectal cancer risk. Although some studies show an increased risk associated with increasing arachidonic acid intake (24, 25), most have reported null associations (26-30). We found that increasing dietary intake of arachidonic acid was associated with colorectal cancer, and this effect was independent of the dietary ratio of n-6 to n-3 PUFA. We found no association between n-3 PUFA intake and colorectal neoplasm risk, which is in concordance with most prior studies (26, 28, 29, 31-34); nevertheless, some studies have reported a protective effect specifically for EPA (27, 30, 35).

With regards to the ratio of total n-6 to n-3 PUFAs, two prior studies had produced results similar to our study with a positive association between colorectal cancer risk and an increasing n-6 to n-3 PUFA ratio (24, 30); however, most prior studies have found no association between the n-6 and n-3 PUFA ratio and colorectal cancer (25, 28, 31-33, 36). A potential reason for these discrepant findings could result from the nonlinear relationship between the dietary PUFA ratio and colorectal cancer risk as shown in Fig. 1. In this cohort, colorectal cancer risk increased

sharply with an increasing dietary ratio of n-6 to n-3 PUFA and then seemed to plateau. The median ratio of dietary n-6 PUFA to n-3 PUFA intake was much lower in this cohort of Chinese women (6.2:1) than that which is typically reported in Western societies (15:1-16.7:1; ref. 37), and as such, the lack of apparent association in prior studies of Western cohorts may be due to baseline dietary PUFA ratios which are too high above a potential threshold level to see an effect. In two human studies done by Bartram et al. (38, 39), volunteers were supplemented with different dosages of fish oil supplements titrated to different n-6 to n-3 PUFA ratios. Rectal epithelial cell proliferation and PGE_2 production was suppressed with a ratio of 2.5:1 but not at a ratio of 4:1. Although both of these dietary ratios were well below those in our cohort, it is possible that the beneficial effect of a low n-6 to n-3 PUFA ratio is only apparent below an absolute threshold.

Interestingly, we found that the total dietary ratio of n-6 PUFA to n-3 PUFA was associated with a greater risk for rectal cancers than colon cancers. Few studies have investigated site-specific cancer and the dietary ratio of n-6 to n-3 PUFAs. In the only other study we were able to identify that investigated the PUFA ratio to site-specific cancer, there were no major site-specific differences in the dietary ratio of EPA and DHA to total n-6 PUFA and risk of colon or rectal cancer (31). Additionally, in our study, we found an increased risk of rectal cancer associated with increased fish consumption. Most prior studies have not found any site-specific effect of fish consumption on rectal cancer (27, 31, 33, 40-42). One possible explanation for the inconsistent findings among studies of fish consumption and colorectal cancer risk could be related to the relative percentage of marine fish that is contributing toward total fish intake, which may differ between populations. This is important as EPA and DHA come predominately from cold water marine fish, and farm-raised as well as lake fish have substantially lower concentrations of n-3 PUFAs (43). In addition, lake and farm-raised fish may be exposed to different regional environmental contaminants that could affect cancer risk.

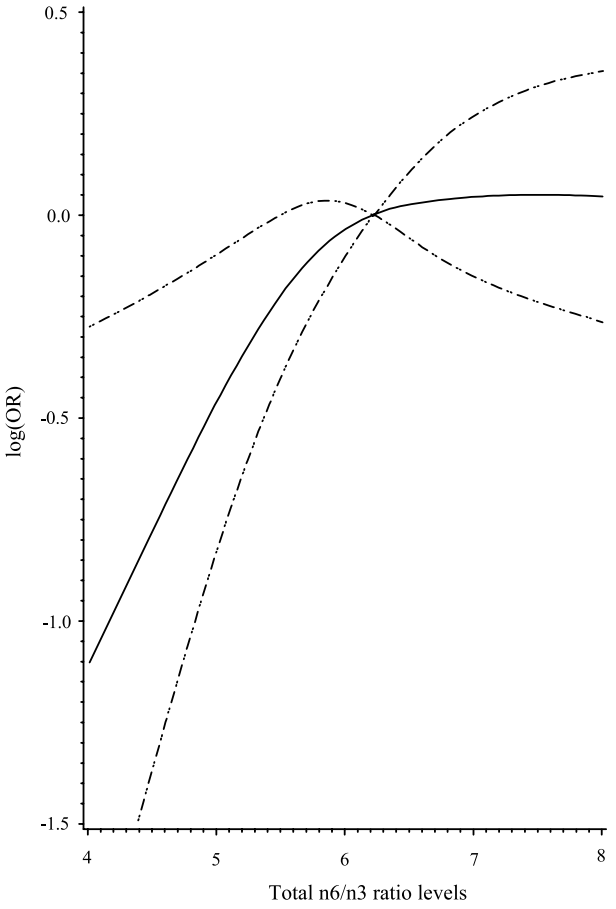

Figure 1. The association between dietary ratio of total n-6 to n-3 PUFAs and subsequent risk of colorectal cancer. *Solid line,* log(OR); *dashed lines,* 95% CI. The range presented for the total n-6 to n-3 level spans the 10th to the 90th percentiles.

Table 5. Age-adjusted correlation coefficient between total n-6 to n-3 ratio and urinary PGE-M stratified by interval time between urine collection and cancer diagnosis

Time to cancer (mo)	*n*	Correlation coefficient	*P*
<20.5	38	−0.09	0.49
20.5-35.3	38	0.11	0.75
35.4-43.3	38	0.07	0.77
>43.3	37	0.47	0.01

We found that increasing dietary linoleic acid consumption was associated with an increased risk of rectal cancers. Terry et al. (34), in a study including 159 rectal cancer cases, found a possible association of linoleic acid intake and rectal cancer risk with RRs of 1.59 (95% CI, 0.95-2.65), 2.02 (95% CI, 1.21-3.35), and 1.53 (95% CI, 0.87-2.69) for the second, third, and fourth quartiles, respectively, when compared with the lowest intake quartile. Our total number of rectal cancer cases was small and these estimates could be unstable; nevertheless, this finding should be explored as linoleic acid is the most frequently consumed essential dietary PUFA and is heavily consumed in Western societies (44).

In our study, we found a direct correlation between the dietary n-6 to n-3 PUFA ratio and PGE_2 production, which was evident only in the participants who developed colorectal cancer. This finding might be explained by the observation that cyclooxygenase-2 is overexpressed in almost 90% of colorectal adenocarcinomas and the rapid metabolism of arachidonic acid into inflammatory prostanoids is believed to play an important role in inducing and promoting colorectal tumorigenesis (45-47). Fernández-Bañares et al. (48) found that the tissue ratio of n-6 PUFA to n-3 PUFA increases in a stepwise manner between benign adenomas, *in situ* carcinoma adenomas, and Dukes' stage B cancer, with the highest ratio found in Dukes' stages C and D cancer mucosa. Of interest, the correlation between dietary fatty acid exposure and PGE_2 was strongest in individuals who developed colorectal cancer >3 years after providing the sample for urinary PGE-M determination. We had hypothesized that dietary intake of PUFA would have a stronger association with PGE_2 production with increasing proximity to cancer diagnosis as cyclooxygenase-2 expression is considerably greater in adenocarcinoma tissue compared with benign adenomas and normal mucosa (45); however, we found the opposite to occur. This would suggest that PGE_2 production is more reliant on PUFA

exposure during the adenoma phase, possibly becoming uncoupled to dietary intake once the lesion has become very advanced. If these findings are replicated, they might help to inform the timing of potential chemopreventive intervention using fish oil.

A potential limitation of our study is the use of self-report of dietary information to determine fatty acid levels. Several studies have evaluated the validity of food frequency questionnaires in assessing usual fatty acid intake. Biomarkers of fatty acid consumption include either lipid content of platelet or RBC membranes, which reflects intake over the proceeding 2 to 18 days (49-52), or adipose tissue biopsies, which would reflect fatty acid consumption over an estimated period of 1 to 3 years (53). Godley et al. (54) found a statistically significant correlation between reported fish consumption and EPA composition in RBC membranes. Fatty acid levels in adipose tissue were also found to be correlated with EPA levels ($r = 0.47$; ref. 55), PUFA ($r = 0.50$; ref. 55), and trans fatty acids ($r = 0.51$) estimated from food frequency questionnaires (56). We averaged the fatty acid intake between the baseline response and the follow-up response to further improve the accuracy of our exposure measurement. In addition, we found a statistically significant correlation between the n-6 to n-3 PUFA ratio and urinary PGE-M, which provides assurance for the validity of food frequency questionnaire in assessing dietary intake of fatty acids.

In conclusion, an increasing dietary ratio of n-6 PUFA to n-3 PUFA was associated with an increased risk of colorectal cancer. This association seemed to be nonlinear and may be dependent on both the absolute dietary content of arachidonic acid as well as the dietary concentrations relative to n-3 PUFAs. The dietary ratio of n-6 to n-3 PUFA was directly correlated with increasing urinary PGE-M, a valid biomarker of endogenous PGE_2 production. These results suggest that the ratio of n-6 to n-3 PUFA intake may be positively associated with colorectal cancer risk, and this association may be mediated in part through the increase in PGE_2 production. These findings are intriguing and warrant further investigation in future studies.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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