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Original Contribution

Types of Fish Consumed and Fish Preparation Methods in Relation to Pancreatic Cancer Incidence

The VITAL Cohort Study

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The associations of types of fish and fish preparation methods with pancreatic cancer risk remain unknown. The authors conducted a prospective cohort study in western Washington State among 66,616 adults, aged 50–76 years, who participated in the VITamins And Lifestyle cohort study. Diet was assessed by a food frequency questionnaire. Pancreatic cancer cases were identified by linkage to the Surveillance, Epidemiology, and End Results cancer registry. During an average follow-up of 6.8 years, 151 participants developed pancreatic cancer (adenocarcinoma). Long-chain (*n*-3) polyunsaturated fatty acids (LC-PUFAs) and nonfried fish intake were inversely associated with pancreatic cancer incidence. When the highest and lowest tertiles of exposure were compared, the multivariable-adjusted hazard ratio of pancreatic cancer was 0.62 (95% confidence interval: 0.40, 0.98) ($P_{trend} = 0.08$) for LC-PUFAs and 0.55 (95% confidence interval: 0.34, 0.88) ($P_{trend} = 0.045$) for nonfried fish. Docosahexaenoic acid showed a greater inverse association with pancreatic cancer than eicosapentaenoic acid. No statistically significant associations were observed with fried fish and shellfish consumption. The potential health impact of fish consumption may depend on the types of fish consumed and fish preparation methods. LC-PUFAs, particularly docosahexaenoic acid, and nonfried fish, but not shellfish or fried fish, may be beneficial in the primary prevention of pancreatic cancer.

cohort study; pancreatic cancer; preparation method; type of fish

Abbreviations: CI, confidence interval; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; LC-PUFA, long-chain (*n*-3) polyunsaturated fatty acid; NSAID, nonsteroidal antiinflammatory drug; SEER, Surveillance, Epidemiology, and End Results; VITAL, VITamins And Lifestyle.

Because of the poor prognosis and short survival period, it is extremely important to identify factors that may lead to prevention of pancreatic cancer (1). Extensive research efforts have provided us with insight into the role of diet, a modifiable exposure, in the development of pancreatic cancer (2). Various dietary factors have been investigated as potential predictors of this disease. Particularly, long-chain (*n*-3) polyunsaturated fatty acids (LC-PUFAs) abundant in fish, including eicosapentaenoic acid (EPA, 20:5*n*-3), docosapentaenoic acid (DPA, 22:5*n*-3), and docosahexaenoic acid (DHA, 22:6*n*-3), were hypothesized to be beneficial against pancreatic cancer because of the antiinflammatory properties of these nutrients (3), and chronic inflammation may play a role in pancreatic carcinogenesis (4, 5). However, the associations between fish or LC-PUFA intake and risk of pancreatic cancer remain unclear. To date, 7 cohort studies examined fish consumption in relation to incidence or mortality of pancreatic cancer (6–12). None found a statistically significant association between fish intake and pancreatic cancer risk. The pooled relative risk for pancreatic cancer was 0.98 (95% confidence interval (CI): 0.86, 1.12) when comparing the

highest with the lowest category of total fish consumption (13). Of note, all prior cohort studies focused on total fish rather than type of fish or preparation methods (e.g., fried vs. nonfried fish). Because frying, especially deep frying, may substantially reduce LC-PUFA content (14) and generate unexpected chemicals such as heterocyclic amines and benzo(*a*)pyrene (15) that may promote pancreatic carcinogenesis (16, 17), combining fried and nonfried fish may attenuate or mask any possible association between fish consumption and pancreatic cancer. Therefore, we prospectively examined different types of fish consumption and LC-PUFA intake in relation to pancreatic cancer incidence in the VITamins And Lifestyle (VITAL) cohort study, which has detailed information on fish consumption and comprehensive assessment of LC-PUFA exposures, including dietary and supplemental sources.

MATERIALS AND METHODS

Study population and design

The VITAL cohort was established in 2000–2002, with the primary aim to investigate associations of dietary supplement use with cancer risk. Details of the study design and methods have been described elsewhere (18). Briefly, participants were men and women aged 50–76 years at entry, living in a 13-county area covered by the Seattle-Puget Sound Surveillance, Epidemiology, and End Results (SEER) cancer registry, who completed a 24-page baseline questionnaire. A total of 364,418 baseline questionnaires were mailed, followed by a postcard reminder after 2 weeks. Of these, 77,719 were returned and deemed eligible (18, 19). For the present study, we excluded participants with a positive ($n = 49$) or missing ($n = 213$) history of pancreatic cancer at baseline. We also excluded those who had missing data on family history of pancreatic cancer ($n = 922$), education ($n = 1,284$), alcohol consumption ($n = 1,215$), and height and weight ($n = 2,565$). In addition, participants were excluded from the nutrient and food analysis if they did not complete all pages of the food frequency section (at least 5 items per page), if their total calorie intake was below 800 kcal for men or 600 kcal for women, or their calorie intake was above 5,000 kcal for men or above 4,000 kcal for women ($n = 4,847$). Moreover, 8 cases of pancreatic neuroendocrine tumors that occurred during follow-up were excluded because the biology of this tumor is different from that of pancreatic adenocarcinoma. After these exclusions, 66,616 participants remained in the analysis.

All participants gave informed consent. The study design and data analyses were approved by the institutional review boards of the University of North Carolina at Chapel Hill and the Fred Hutchinson Cancer Research Center.

Diet assessment

In the VITAL cohort study, data were self-reported by using a 24-page gender-specific, optically scanned questionnaire that covered 3 content areas: supplement use, diet, and health history and risk factors (18).

Diet was assessed by using a 120-item food frequency questionnaire that included adjustment questions on types of foods and preparation techniques. The food frequency questionnaire was adapted from the one used in the Women's Health Initiative and other studies (20–22). Participants were asked about their usual frequency of intake of 120 foods and beverages over the past year. The medium serving size for each food or food group was gender specific and given as a reference, and participants were asked to indicate the amount of food consumption as small, medium, or large for adjustment of nutrient data. Fish intake was measured on the basis of questions ascertaining the frequency of consumption of the following: 1) fried fish, fish sandwiches, and fried shellfish (e.g., shrimp and oysters); 2) shellfish, not fried (e.g., shrimp, lobster, crab, and oysters); 3) white fish (broiled or baked) (e.g., sole, halibut, and cod); 4) dark fish (broiled or baked) (e.g., salmon and fresh tuna); and 5) canned tuna, tuna salad, and tuna casserole. Because frying may generate unexpected chemicals related to pancreatic carcinogenesis (16, 17) and shellfish likely contain some carcinogenetic toxins (23), we classified fish consumption into 3 groups in the analyses: 1) fried fish (including fried fish and fish sandwiches and fried shellfish); 2) nonfried fish; and 3) shellfish (not fried). Nutrient values including LC-PUFAs were computed by using the Minnesota Nutrient Data System based on the frequencies of consumption of each food and portion size (24). Participants were also asked to report the number of days per week for how many years of use of fish oil supplements during the 10 years prior to baseline. Total LC-PUFA intake was calculated from both dietary and supplemental sources.

The overall measurement properties of the food frequency questionnaire were evaluated previously (22, 25). The Pearson correlation coefficients between the food frequency questionnaire and 8 days of dietary recalls and food records were 0.63 for saturated fat, 0.64 for monounsaturated fat, and 0.54 for polyunsaturated fat (22). However, the correlation coefficient for LC-PUFAs was not reported in the evaluation studies. Also, the measurements for types of fish and fish preparation have not been specifically evaluated.

Outcome identification

Participants were followed for pancreatic cancers occurring from baseline through 2008, by linking the cohort to the Seattle-Puget Sound SEER registry. Registry cases were captured through all hospitals in the area; through offices of pathologists, oncologists, and radiotherapists; and from state death certificates. Pancreatic cancer cases were identified in the cohort by using matching algorithms on personal identifiers and human review (18). In this study, pancreatic cancer referred to adenocarcinoma and neuroendocrine tumors. All incident cases of pancreatic adenocarcinoma were defined on the basis of *International Classification of Diseases for Oncology*, Third Edition (ICD-O-3), codes C250–C259, C25.0–C25.3, or C25.7–C25.9. We excluded endocrine pancreatic tumors (C25.4) in the analyses because the etiology of these cancers is thought to be different. For each participant, the person-time was calculated depending on the earliest date of the following events:

Table 1. Baseline Characteristics of Participants According to Tertile of LC-PUFA Intake, VITAL Cohort Study, 2000–2008[a]

Characteristic	Tertile of LC-PUFA									Total (n = 66,616)		
	1 (n = 22,206)			2 (n = 22,205)			3 (n = 22,205)					
	Mean (SD)	Median (25th–75th Percentile)	%	Mean (SD)	Median (25th–75th Percentile)	%	Mean (SD)	Median (25th–75th Percentile)	%	Mean (SD)	Median (25th–75th Percentile)	%
LC-PUFA (EPA + DPA + DHA), g/day[b]	0.06 (0.03)			0.20 (0.05)			0.56 (0.32)			0.27 (0.28)		
EPA	0.02 (0.01)			0.06 (0.02)			0.20 (0.14)			0.09 (0.11)		
DHA	0.04 (0.02)			0.12 (0.03)			0.32 (0.19)			0.16 (0.16)		
Age, years	61.9 (7.5)			61.6 (7.4)			61.6 (7.2)			61.7 (7.4)		
Body mass index[c]	27.5 (5.5)			27.5 (5.2)			27.3 (4.8)			27.4 (5.2)		
Female			62.6			52.0			36.9			50.5
White			94.4			93.9			92.6			93.6
Education												
High school graduate or less			25.8			17.6			12.8			18.7
Some college			41.6			38.1			34.1			37.9
College or advanced degree			32.6			44.3			53.1			43.3
Physical activity, MET hours/week		4.0 (0.7–11.6)			5.9 (1.4–15.0)			8.8 (2.6–19.6)			6.1 (1.3–15.5)	
Smoking status												
Never			47.7			47.6			47.0			47.5
Former (quit >10 years)			33.4			37.3			40.2			37.0
Former (quit ≤10 years)			8.3			7.3			6.6			7.4
Current			10.6			7.8			6.2			8.2
Alcohol consumption, g/day		0.7 (0.0–5.8)			2.0 (0.0–10.6)			4.2 (0.1–13.9)			1.6 (0.0–10.6)	
Diabetes			7.0			6.6			6.1			6.6
Family history of pancreatic cancer			2.8			3.1			2.9			2.9
NSAID use			24.7			26.0			29.5			26.7
Diet intakes												
Nonfried fish, servings/week	0.64 (0.42)			1.25 (0.71)			2.52 (1.69)			1.47 (1.34)		
Shellfish, servings/week	0.16 (0.13)			0.29 (0.29)			0.51 (0.68)			0.32 (0.46)		
Fried fish, servings/week	0.18 (0.15)			0.34 (0.32)			0.60 (0.82)			0.37 (0.54)		
Fruit, servings/day	1.4 (1.3)			1.7 (1.4)			2.0 (1.5)			1.7 (1.4)		
Vegetables, servings/day	1.9 (1.3)			2.3 (1.3)			2.7 (1.5)			2.3 (1.4)		

Table continues

Table 1. Continued

Characteristic	Tertile of LC-PUFA								Total ($n = 66,616$)		
	1 ($n = 22,206$)			2 ($n = 22,205$)			3 ($n = 22,205$)				
	Mean (SD)	Median (25th–75th Percentile)	%	Mean (SD)	Median (25th–75th Percentile)	%	Mean (SD)	Median (25th–75th Percentile)	%	Mean (SD)	Median (25th–75th Percentile)	%
Dairy products, servings/day	0.4 (0.4)			0.5 (0.5)			0.5 (0.5)			0.5 (0.5)		
Red/processed meat, servings/day	0.6 (0.5)			0.7 (0.6)			0.8 (0.6)			0.7 (0.6)		
Total calories, kcal/day	1,575.8 (662.0)			1,850.6 (718.6)			2,156.7 (819.5)			1,861.0 (773.6)		

Abbreviations: DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; LC-PUFA, long-chain (*n*-3) polyunsaturated fatty acid; MET, metabolic equivalent; NSAID, nonsteroidal antiinflammatory drug; SD, standard deviation; VITAL, VITamins And Lifestyle.

[a] *P* values for any difference across the tertiles were calculated by analysis of variance, the Kruskal-Wallis test, or the χ^2 test as appropriate. All *P* values were less than 0.001 except family history of pancreatic cancer ($P = 0.10$).

[b] LC-PUFA intake includes both dietary and supplemental sources.

[c] Body mass index: weight (kg)/height $(m)^2$.

withdrawal from the study (0.3%); death (6.8%); emigration out of the SEER catchment area (5.4%); or December 31, 2008 (87.5%), the last date of linkage to the SEER registry. Deaths were ascertained by linkage to the Washington State death file, and moves out of the area were determined through the National Change of Address System and by follow-up letters and telephone calls.

Statistical analysis

Comparison of baseline covariates was conducted by using analysis of variance or Kruskal-Wallis or χ^2 test as appropriate. Multivariable-adjusted hazards and 95% confidence intervals of the incidence of pancreatic cancer according to fish or LC-PUFA intake were estimated by using Cox proportional hazards models. To increase statistical power, we used tertiles rather than quartiles or quintiles of each exposure variable in Cox models with the lowest tertile as the reference group. The proportional hazards assumption was assessed by graphical methods and the "Supremum test" (25). Risk factors for pancreatic cancer in previous studies were evaluated for confounding and were included as covariates if the risk factor was determined to be a common cause of fish or LC-PUFA intake and pancreatic cancer risk or if inclusion of the factor in statistical models affected the parameter estimate by at least 10%. In model 1, we considered 4 demographic variables as confounders, including age, gender, ethnicity, and educational levels. In model 2, we further included major lifestyle variables and other dietary and nondietary confounders, including body mass index, physical activity, smoking status, alcohol consumption, family history of pancreatic cancer, diabetes mellitus, nonsteroidal antiinflammatory drug (NSAID) use, and dietary intakes of fruits, vegetables, dairy products, red/processed meat, and total calories. In addition, individual types of fish were mutually adjusted for other types of fish intake. We examined total LC-PUFAs (EPA + DHA + DPA) and EPA and DHA separately. Because DPA is an intermediary between EPA and DHA and the amount of DPA is relatively small, we did not examine DPA as a separate exposure. To further ensure that the comparison groups are comparable with multivariable adjustment, we calculated and adjusted for propensity scores instead of multiple covariates (26, 27). Furthermore, to determine if the missing data in covariates substantially biased our results, we performed a multiple imputation procedure using a regression switching approach (multiple imputation by chained equations) assuming that data were missing at random (28). *P* values for linear trend were computed by using the continuous variables while excluding values above the 98th percentile for each exposure in multivariable-adjusted models.

All analyses were performed by using SAS, version 9.2, software (SAS Institute, Inc., Cary, North Carolina). All *P* values reported are 2 sided, and those that were less than 0.05 were considered statistically significant.

RESULTS

During an average follow-up of 6.8 years, 151 new cases of pancreatic cancer (adenocarcinoma) were identified.

Table 1 gives demographic and other baseline characteristics of the study population by total LC-PUFA intake. Compared with those in the lowest tertile of LC-PUFA intake, participants in the highest tertile tended to be male; former or never smokers; college graduates; physically active; NSAID users; and consumers of greater amounts of fruits, vegetables, red or processed meats, alcohol, and calories. Although statistically different, there were no appreciable differences in age and body mass index across LC-PUFA intake groups.

Table 2 shows results of the associations between LC-PUFA intake and pancreatic cancer risk. LC-PUFA intake was inversely associated with incident pancreatic cancer. Comparing the highest tertile of LC-PUFA intake with the lowest tertile, we found that the multivariable-adjusted hazard ratio was 0.62 (95% CI: 0.40, 0.98) ($P_{\text{linear trend}} = 0.08$). The inverse association was stronger for the DHA intake (hazard ratio (HR) = 0.56, 95% CI: 0.35, 0.91) ($P_{\text{trend}} = 0.04$) than for the EPA intake (HR = 0.69, 95% CI: 0.44, 1.08) ($P_{\text{trend}} = 0.34$).

Table 3 presents findings on fish consumption and pancreatic cancer incidence. Relative to the lowest tertile, participants who consumed the highest tertile of nonfried fish had a statistically significant reduction in pancreatic cancer incidence (HR = 0.55, 95% CI: 0.34, 0.88) ($P_{\text{trend}} = 0.045$). No statistically significant associations were found with total fish, shellfish, and fried fish.

At baseline, we excluded approximately 14% of participants from the VITAL cohort mainly because of missing data, but no significant differences were observed in major demographic and lifestyle variables between the study population and the original VITAL cohort (data not shown). In addition, when we used the multiple imputation procedure for all missing values in a sensitivity analysis, the results remained and the hazard ratio was 0.54 (95% CI: 0.33, 0.89) for nonfried fish consumption and 0.64 (95% CI: 0.41, 0.995) for LC-PUFA intake comparing the highest tertile of exposure with the lowest. Also, in the main analyses, we focused on participants who had data available on both fish consumption and fish oil supplement use, so that we excluded some participants who had data on fish oil supplement use only. When we included those participants in the sensitivity analysis of examining LC-PUFA intake and pancreatic cancer risk, the results were essentially the same (data not shown). Approximately 10% of participants used fish oil supplements. To explore the impact of fish oil supplementation, we adjusted for fish oil supplement use and analyzed data in supplement nonusers only. Our findings were consistent (data not shown). In addition, considering that the LC-PUFA contents are different in dark and white meat nonfried fish, we examined these 2 types of fish separately; no appreciable differences were found between these 2 fish groups (data not shown). Moreover, the observed associations remained after controlling for propensity scores, which were derived from the same covariates in the multivariable analyses. For example, the hazard ratio was 0.52 (95% CI: 0.32, 0.84) for nonfried fish consumption and 0.59 (95% CI: 0.37, 0.94) for LC-PUFA intake when the highest tertile of exposure was compared with the lowest.

DISCUSSION

In this prospective cohort study, we found that intakes of LC-PUFAs, particularly DHA, were inversely related to incidence of pancreatic cancer. The association between fish consumption and incidence of pancreatic cancer varied depending on the type of fish consumed. Higher intake of nonfried fish, but not fried fish or shellfish, was associated with lower incidence of pancreatic cancer.

Seven prospective cohort studies have reported results on the association of fish intake with incidence of or mortality from pancreatic cancer (6–12). None of the previous studies found a significant association, and the combined association between fish consumption and pancreatic cancer was also statistically nonsignificant. Of note, these studies were designed to study either meat consumption or dietary fat intake but not specifically fish or LC-PUFAs. Thus, most of these studies focused on total fish rather than types of fish or preparation methods. The variation of potential health impacts of fish consumption from different cooking methods has not been well studied, but it has been suggested that broiled and baked fish, but not fried fish and fish sandwiches, may provide a cardioprotective benefit (29, 30). Although more research is needed, these studies on cardiovascular outcomes indicate that the potential effects of fish intake on human health may vary by preparation methods. Laboratory studies suggest that frying may modify the lipid profile through a decrease in LC-PUFA content (14). Deep frying (e.g., fried fast foods) may also cause the presence of *trans*-fatty acids and lipid oxidation products that may consequently increase the risk of carcinogenesis (31–33) by promoting systemic inflammation (34, 35) and oxidant stress (36, 37). In addition, frying may generate unexpected chemicals such as heterocyclic amines and benzo(*a*)pyrene (15), and these chemicals alone and in interaction with other factors may contribute to the development of pancreatic cancer (16, 17).

Consistent with prior cohort studies, the present study found little evidence for a significant association between total fish consumption and incidence of pancreatic cancer. However, a significant inverse association of nonfried fish intake with incident pancreatic cancer was observed in our study. Interestingly, we found that shellfish intake was modestly related to the elevated incidence of pancreatic cancer, although the association was statistically nonsignificant. It has been recently hypothesized that shellfish consumption may be a risk factor for colorectal and other digestive system cancers because shellfish accumulates diarrheic shellfish poisoning toxins such as okadaic acid (38). Moreover, a positive association between shellfish consumption and risk of colorectal cancer has been reported in a large cohort study (39). Hence, any potential pancreatic cancer risk reduction associated with nonfried fish is likely to be substantially attenuated or masked by combining nonfried fish with fried fish and/or shellfish.

In addition, 3 prior cohort studies examined the association between LC-PUFA intake and pancreatic cancer risk, and 2 studies found no significant association with LC-PUFAs and pancreatic cancer (7, 12), while one study reported a significant positive association between DHA and

Table 2. Multivariable-adjusted Hazard Ratios of Incidence of Pancreatic Cancer by Intakes of Total LC-PUFA, EPA, and DHA, VITAL Cohort Study, 2000–2008[a]

	Range, g/day	No. of Participants at Risk	No. of Events	Model 1[b]		Model 2[c]	
				HR	95% CI	HR	95% CI
LC-PUFA							
Tertile 1	<0.123	22,206	68	1	Referent	1	Referent
Tertile 2	0.123–0.286	22,205	44	0.66	0.45, 0.97	0.74	0.50, 1.09
Tertile 3	≥0.287	22,205	39	0.59	0.39, 0.88	0.62	0.40, 0.98
Continuous				0.44	0.20, 1.01	0.45	0.18, 1.10
P_{trend}[d]					0.052		0.08
EPA							
Tertile 1	<0.037	22,206	64	1	Referent	1	Referent
Tertile 2	0.037–0.089	22,205	46	0.73	0.50, 1.08	0.82	0.55, 1.22
Tertile 3	≥0.090	22,205	41	0.65	0.44, 0.98	0.69	0.44, 1.08
Continuous				0.31	0.04, 2.25	0.36	0.04, 2.96
P_{trend}[d]					0.25		0.34
DHA							
Tertile 1	<0.072	22,204	63	1	Referent	1	Referent
Tertile 2	0.072–0.169	22,207	55	0.89	0.62, 1.28	0.99	0.68, 1.44
Tertile 3	≥0.170	22,205	33	0.53	0.35, 0.83	0.56	0.35, 0.91
Continuous				0.19	0.04, 0.83	0.18	0.03, 0.92
P_{trend}[d]					0.03		0.04

Abbreviations: CI, confidence interval; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; HR, hazard ratio; LC-PUFA, long-chain (*n*-3) polyunsaturated fatty acid; MET, metabolic equivalent; NSAID, nonsteroidal antiinflammatory drug; VITAL, VITamins And Lifestyle.

[a] All models were constructed by using Cox proportional hazards regression analysis.

[b] Model 1: adjusted for age (time variable), gender, ethnicity (white, nonwhite), and education (high school graduate or less, some college, college or advanced degree).

[c] Model 2: additionally adjusted for body mass index (<25, 25–29, ≥30 kg/m^2), physical activity (0, tertile of MET-hours/week), smoking status (never smokers, former smokers quit >10 years, former smokers quit ≤10 years, and current smokers), alcohol consumption (tertile), diabetes mellitus (yes or no), family history of pancreatic cancer (yes or no), NSAID use (yes or no), and dietary intakes (tertile) of fruits, vegetables, dairy products, red/processed meat, and calories.

[d] $P_{linear\ trend}$ was examined by using the continuous variable of exposure.

pancreatic cancer (40). Nevertheless, the pooled association between LC-PUFAs and pancreatic cancer was nonsignificant (13). Of note, none of these 3 studies specified if fish oil supplement use was accounted for in total LC-PUFA intake. The generally consistent associations between fish and LC-PUFAs in relation to pancreatic cancer risk may reflect that LC-PUFA intake assessed by a diet measurement instrument is more likely a surrogate marker of fish consumption given that LC-PUFA intake is calculated on the basis of the frequency of fish consumption. Thus, parallel associations between fish and dietary LC-PUFA intake with health outcomes are likely to be observed.

In the present study, we also found an inverse association between total LC-PUFA intake and pancreatic cancer incidence. Our findings are biologically plausible. Studies suggest that a number of cytokines, transcription factors, and proinflammatory enzymes are associated with pancreatic cancer; for example, tumor necrosis factor-alpha and interleukin-6 are increased in pancreatic cancer (41, 42). In addition, the expression of cyclooxygenase-2, an inducible isoform activated by cytokines and growth factors that produce predominately proinflammatory prostaglandins (43), is increased in pancreatic cancer, which further suggests a link between the inflammatory and oncogenic pathways (44). In addition, evidence from both experimental and observational studies suggests that LC-PUFAs exhibit antiinflammatory properties by competitively inhibiting the arachidonic aid (45–47). Thus, higher intake of LC-PUFAs may provide beneficial effects on pancreatic cancer development by reducing inflammation levels.

Further, we found that DHA was superior to EPA in relation to pancreatic cancer risk reduction. Laboratory studies indicated that DHA is more readily incorporated into tissue phospholipids than EPA and thus should be more effective in decreasing inflammation (48–50). Moreover, human studies have shown that a high concentration of DHA, but not EPA, in serum phospholipids and plasma may reduce the incidence of inflammatory-related chronic diseases (51, 52).

Several other strengths of our study need to be highlighted in addition to the prospective study design and the large

Table 3. Multivariable-adjusted Hazard Ratios of Incidence of Pancreatic Cancer by Intakes of Fish, VITAL Cohort Study, 2000–2008[a]

	Range, servings/week	No. of Participants at Risk	No. of Events	Model 1[b]		Model 2[c]	
				HR	95% CI	HR	95% CI
Total fish							
Tertile 1	<1.225	22,218	58	1	Referent	1	Referent
Tertile 2	1.225–2.283	22,223	53	0.99	0.69, 1.44	1.09	0.75–1.60
Tertile 3	≥2.284	22,093	40	0.79	0.53, 1.18	0.83	0.54–1.28
Continuous				0.88	0.78, 1.01	0.89	0.77–1.02
P_{trend}[d]					0.06		0.08
Nonfried fish							
Tertile 1	<0.791	22,133	68	1	Referent	1	Referent
Tertile 2	0.791–1.490	22,476	44	0.66	0.45, 0.97	0.65	0.43, 0.98
Tertile 3	≥1.491	21,840	37	0.59	0.39, 0.88	0.55	0.34, 0.88
Continuous				0.83	0.70, 0.995	0.81	0.66, 0.996
P_{trend}[d]					0.04		0.045
Shellfish							
Tertile 1	<0.175	34,004	73	1	Referent	1	Referent
Tertile 2	0.175–0.289	12,423	31	1.30	0.85, 1.99	1.42	0.90, 2.26
Tertile 3	≥0.290	17,843	42	1.22	0.83, 1.79	1.45	0.94, 2.23
Continuous				1.07	0.77, 1.49	1.17	0.89, 1.55
P_{trend}[d]					0.70		0.27
Fried fish							
Tertile 1	<0.175	33,574	75	1	Referent	1	Referent
Tertile 2	0.175–0.289	10,940	26	1.10	0.70, 1.73	1.04	0.64, 1.69
Tertile 3	≥0.290	20,234	46	0.93	0.64, 1.36	0.91	0.60, 1.38
Continuous				0.74	0.50, 1.11	0.71	0.45, 1.12
P_{trend}[d]					0.14		0.14

Abbreviations: CI, confidence interval; HR, hazard ratio; NSAID, nonsteroidal antiinflammatory drug; VITAL, VITamins And Lifestyle.

[a] All models were constructed by using Cox proportional hazards regression analysis.

[b] Model 1: adjusted for age (time variable), gender, ethnicity (white, nonwhite), and education (high school graduate or less, some college, college or advanced degree).

[c] Model 2: additionally adjusted for body mass index (<25, 25–29, ≥30 kg/m^2), physical activity (0, tertile of nonzero values), smoking status (never smokers, former smokers quit >10 years, former smokers quit ≤10 years, and current smokers), alcohol consumption (tertile), diabetes mellitus (yes or no), family history of pancreatic cancer (yes or no), NSAID use (yes or no), and dietary intakes (tertile) of fruits, vegetables, dairy products, red/processed meat, and calories; further adjusted for fried fish and shellfish (for nonfried fish) or fried fish and nonfried fish (for shellfish), or nonfried fish and shellfish (for fried fish).

[d] $P_{linear\ trend}$ was examined by using the continuous variable of exposure.

cohort study population. Detailed information on fish consumption measured by a validated food frequency questionnaire enables us to specify types of fish in the analysis, which may be crucial in studies of fish and chronic diseases. Also, we used a comprehensive instrument that captured long-term use of fish oil supplements so that we are able to more accurately estimate total LC-PUFA exposure. This is especially important because fish oil supplementation is common now in the United States. The reliability and validity of the measures of 10-year average use have been evaluated (53). Notably, the assessment of average intake during the 10 years before baseline allowed us to more closely investigate supplement use over the relevant period of cancer development. In addition, pancreatic cancer cases were ascertained by using a comprehensive linkage system with the SEER registry, which we have estimated to be almost 100% complete, suggesting that the possibility of case misclassification should be small.

A few limitations of this study should also be considered. As with other observational studies, the possibility of uncontrolled or residual confounding cannot be completely excluded even though we did extensive adjustment in the analysis. Second, despite the large cohort study population, our capability of investigating potential effect modifiers (e.g., gender, NSAID use) is limited by the relatively small number of incident cases. Third, measurement error of diet assessment by food frequency questionnaire is inevitable. However, because of the prospective design, the

measurement error in our study would be expected to be nondifferential and therefore would have attenuated the results. Fourth, diabetes cases were self-reported, which might somehow confound our results. Finally, the great majority of participants are Caucasians so that generalizability may be limited.

In conclusion, findings from this prospective cohort study suggest that LC-PUFA, particularly DHA, and nonfried fish intake may be beneficial in the primary prevention of pancreatic cancer. Results from this study also indicate that the potential beneficial effect of fish consumption may depend on the types of fish consumed, with no benefits observed for shellfish or fried fish. Because a randomized placebo controlled clinical trial on fish and pancreatic cancer may not be feasible, additional prospective studies are needed to confirm our findings and to further investigate the impact of different types of fish consumption and fish preparation methods on pancreatic cancer development.

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REFERENCES

1. Li D, Xie K, Wolff R, et al. Pancreatic cancer. *Lancet*. 2004;363(9414):1049–1057.
2. Johnson J, de Mejia EG. Dietary factors and pancreatic cancer: the role of food bioactive compounds. *Mol Nutr Food Res*. 2011;55(1):58–73.
3. Swamy MV, Citineni B, Patlolla JM, et al. Prevention and treatment of pancreatic cancer by curcumin in combination with omega-3 fatty acids. *Nutr Cancer*. 2008;60(suppl 1): 81–89.
4. Lowenfels AB, Maisonneuve P, Cavallini G, et al. Pancreatitis and the risk of pancreatic cancer. International Pancreatitis Study Group. *N Engl J Med*. 1993;328(20): 1433–1437.
5. Rothwell PM, Fowkes FG, Belch JF, et al. Effect of daily aspirin on long-term risk of death due to cancer: analysis of individual patient data from randomised trials. *Lancet*. 2011;377(9759):31–41.
6. Zheng W, McLaughlin JK, Gridley G, et al. A cohort study of smoking, alcohol consumption, and dietary factors for pancreatic cancer (United States). *Cancer Causes Control*. 1993;4(5):477–482.
7. Stolzenberg-Solomon RZ, Pietinen P, Taylor PR, et al. Prospective study of diet and pancreatic cancer in male smokers. *Am J Epidemiol*. 2002;155(9):783–792.
8. Michaud DS, Giovannucci E, Willett WC, et al. Dietary meat, dairy products, fat, and cholesterol and pancreatic cancer risk in a prospective study. *Am J Epidemiol*. 2003;157(12): 1115–1125.
9. Nothlings U, Wilkens LR, Murphy SP, et al. Meat and fat intake as risk factors for pancreatic cancer: the Multiethnic Cohort Study. *J Natl Cancer Inst*. 2005; 97(19):1458–1465.
10. Larsson SC, Hakanson N, Permert J, et al. Meat, fish, poultry and egg consumption in relation to risk of pancreatic cancer: a prospective study. *Int J Cancer*. 2006;118(11):2866–2870.
11. Lin Y, Kikuchi S, Tamakoshi A, et al. Dietary habits and pancreatic cancer risk in a cohort of middle-aged and elderly Japanese. *Nutr Cancer*. 2006;56(1):40–94.
12. Heinen MM, Verhage BA, Goldbohm RA, et al. Meat and fat intake and pancreatic cancer risk in the Netherlands Cohort Study. *Int J Cancer*. 2009;125(5):1118–1126.
13. Qin B, Xun P, He K. Fish or long-chain n-3 PUFA intake is not associated with pancreatic cancer risk in a meta-analysis and systematic review. *J Nutr*. 2012;142(6):1067–1073.
14. Echarte M, Zulet MA, Astiasaran I. Oxidation process affecting fatty acids and cholesterol in fried and roasted salmon. *J Agric Food Chem*. 2001;49(11):5662–5667.
15. Skog K. Cooking procedures and food mutagens: a literature review. *Food Chem Toxicol*. 1993;31(9):655–675.
16. Anderson KE, Kadlubar FF, Kulldorff M, et al. Dietary intake of heterocyclic amines and benzo(*a*)pyrene: associations with pancreatic cancer. *Cancer Epidemiol Biomarkers Prev*. 2005;14(9):2261–2265.
17. Stolzenberg-Solomon RZ, Cross AJ, Silverman DT, et al. Meat and meat-mutagen intake and pancreatic cancer risk in the NIH-AARP cohort. *Cancer Epidemiol Biomarkers Prev*. 2007;16(12):2664–2675.
18. White E, Patterson RE, Kristal AR, et al. VITamins And Lifestyle cohort study: study design and characteristics of supplement users. *Am J Epidemiol*. 2004;159(1):83–93.
19. Satia JA, Littman A, Slatore CG, et al. Associations of herbal and specialty supplements with lung and colorectal cancer risk in the VITamins and Lifestyle study. *Cancer Epidemiol Biomarkers Prev*. 2009;18(5):1419–1428.
20. Kristal AR, Feng Z, Coates RJ, et al. Associations of race/ethnicity, education, and dietary intervention with the validity and reliability of a food frequency questionnaire: the Women's Health Trial Feasibility Study in Minority Populations. *Am J Epidemiol*. 1997;146(10):856–869.
21. Kristal AR, Patterson RE, Neuhouser ML, et al. Olestra Postmarketing Surveillance Study: design and baseline results from the sentinel site. *J Am Diet Assoc*. 1998;98(11): 1290–1296.
22. Patterson RE, Kristal AR, Tinker LF, et al. Measurement characteristics of the Women's Health Initiative food frequency questionnaire. *Ann Epidemiol*. 1999;9(3): 178–187.

23. Candela M, Astiasarán I, Bello J Deep-fat frying modifies high-fat fish lipid fraction. *J Agric Food Chem*. 1998; 46(7):2793–2796.
24. Schakel SF, Buzzard IM, Gebhardt SE. Procedures for estimating nutrient values for food composition databases. *J Food Comp Anal*. 1997;10(2):102–114.
25. Lin DY, Wei LJ, Ying Z. Model-checking techniques based on cumulative residuals. *Biometrics*. 2002;58(1):1–12.
26. Greenland S Randomization, statistics, causal inference. *Epidemiology*. 1990;1(6):421–429.
27. Rosenbaum PR, Rubin DB. The central role of the propensity score in observational studies for causal effects. *Biometrika*. 1983;70(1):41–55.
28. Royston P. Multiple imputation of missing values. *Stata J*. 2004;4(3):227–241.
29. Mozaffarian D, Lemaitre RN, Kuller LH, et al. Cardiac benefits of fish consumption may depend on the type of fish meal consumed: the Cardiovascular Health Study. *Circulation*. 2003;107(10):1372–1377.
30. Mozaffarian D, Psaty BM, Rimm EB, et al. Fish intake and risk of incident atrial fibrillation. *Circulation*. 2004; 110(4):368–373.
31. Chavarro JE, Stampfer MJ, Campos H, et al. A prospective study of *trans*-fatty acid levels in blood and risk of prostate cancer. *Cancer Epidemiol Biomarkers Prev*. 2008;17(1): 95–101.
32. Theodoratou E, Campbell H, Tenesa A, et al. Modification of the associations between lifestyle, dietary factors and colorectal cancer risk by APC variants. *Carcinogenesis*. 2008;29(9):1774–1780.
33. Chajes V, Thiebaut AC, Rotival M, et al. Association between serum *trans*-monounsaturated fatty acids and breast cancer risk in the E3N-EPIC Study. *Am J Epidemiol*. 2008;167(11):1312–1320.
34. Han SN, Leka LS, Lichtenstein AH, et al. Effect of hydrogenated and saturated, relative to polyunsaturated, fat on immune and inflammatory responses of adults with moderate hypercholesterolemia. *J Lipid Res*. 2002;43(3):445–452.
35. Baer DJ, Judd JT, Clevidence BA, et al. Dietary fatty acids affect plasma markers of inflammation in healthy men fed controlled diets: a randomized crossover study. *Am J Clin Nutr*. 2004;79(6):969–973.
36. Kuhnt K, Wagner A, Kraft J, et al. Dietary supplementation with 11*trans*- and 12*trans*-18:1 and oxidative stress in humans. *Am J Clin Nutr*. 2006;84(5):981–988.
37. Tomey KM, Sowers MR, Li X, et al. Dietary fat subgroups, zinc, and vegetable components are related to urine F_{2a}-isoprostane concentration, a measure of oxidative stress, in midlife women. *J Nutr*. 2007;137(11):2412–2419.
38. Manerio E, Rodas VL, Costas E, et al. Shellfish consumption: a major risk factor for colorectal cancer. *Med Hypotheses*. 2008;70(2):409–412.
39. Lee SA, Shu XO, Yang G, et al. Animal origin foods and colorectal cancer risk: a report from the Shanghai Women's Health Study. *Nutr Cancer*. 2009;61(2):194–205.
40. Thiebaut AC, Jiao L, Silverman DT, et al. Dietary fatty acids and pancreatic cancer in the NIH-AARP diet and health study. *J Natl Cancer Inst*. 2009;101(14): 1001–1011.
41. Friess H, Guo XZ, Nan BC, et al. Growth factors and cytokines in pancreatic carcinogenesis. *Ann N Y Acad Sci*. 1999;880:110–121. (doi:10.1111/j.1749-6632.1999.tb09515.x).
42. Le X, Shi Q, Wang B, et al. Molecular regulation of constitutive expression of interleukin-8 in human pancreatic adenocarcinoma. *J Interferon Cytokine Res*. 2000;20(11): 935–946.
43. Maier JA, Hla T, Maciag T. Cyclooxygenase is an immediate-early gene induced by interleukin-1 in human endothelial cells. *J Biol Chem*. 1990;265(19):10805–10808.
44. Kokawa A, Kondo H, Gotoda T, et al. Increased expression of cyclooxygenase-2 in human pancreatic neoplasms and potential for chemoprevention by cyclooxygenase inhibitors. *Cancer*. 2001;91(2):333–338.
45. Calder PC. Polyunsaturated fatty acids and inflammatory processes: new twists in an old tale. *Biochimie*. 2009; 91(6):791–795.
46. Wall R, Ross RP, Fitzgerald GF, et al. Fatty acids from fish: the anti-inflammatory potential of long-chain omega-3 fatty acids. *Nutr Rev*. 2010;68(5):280–289.
47. He K, Liu K, Daviglus ML, et al. Associations of dietary long-chain n-3 polyunsaturated fatty acids and fish with biomarkers of inflammation and endothelial activation (from the Multi-Ethnic Study of Atherosclerosis [MESA]). *Am J Cardiol*. 2009;103(9):1238–1243.
48. De Caterina R, Cybulsky MI, Clinton SK, et al. The omega-3 fatty acid docosahexaenoate reduces cytokine-induced expression of proatherogenic and proinflammatory proteins in human endothelial cells. *Arterioscler Thromb*. 1994; 14(11):1829–1836.
49. De Caterina R, Liao JK, Libby P. Fatty acid modulation of endothelial activation. *Am J Clin Nutr*. 2000;71(1 suppl): 213S–223S.
50. Mori TA, Woodman RJ. The independent effects of eicosapentaenoic acid and docosahexaenoic acid on cardiovascular risk factors in humans. *Curr Opin Clin Nutr Metab Care*. 2006;9(2):95–104.
51. Kompauer I, Demmelmair H, Koletzko B, et al. Association of fatty acids in serum phospholipids with lung function and bronchial hyperresponsiveness in adults. *Eur J Epidemiol*. 2008;23(3):175–190.
52. Shahar E, Boland LL, Folsom AR, et al. Docosahexaenoic acid and smoking-related chronic obstructive pulmonary disease. The Atherosclerosis Risk in Communities Study Investigators. *Am J Respir Crit Care Med*. 1999;159(6): 1780–1785.
53. Satia-Abouta J, Patterson RE, King IB, et al. Reliability and validity of self-report of vitamin and mineral supplement use in the VITamins And Lifestyle study. *Am J Epidemiol*. 2003;157(10):944–954.