CLINICAL—LIVER

Consumption of n-3 Fatty Acids and Fish Reduces Risk of Hepatocellular Carcinoma

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BACKGROUND & AIMS: Fish is a rich source of n-3 polyunsaturated fatty acids (PUFAs), such as eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), and docosahexaenoic acid (DHA). Although consumption of fish and n-3 PUFA has been reported to protect against the development of some types of cancer, little is known about its association with hepatocellular carcinoma (HCC). **METHODS:** We investigated the association between fish and n-3 PUFA consumption and HCC incidence (n = 398) in a population-based prospective cohort study of 90,296 Japanese subjects (aged, 45–74 y). Hazard ratios and 95% confidence intervals (CIs) for the highest vs the lowest quintile were estimated from multivariable adjusted Cox proportional hazards regression models. We also conducted subanalyses of subjects with known hepatitis B virus (HBV) or hepatitis C virus (HCV) status, and of subjects who were anti-HCV and/or hepatitis B surface antigen positive. All tests of statistical significance were 2-sided. **RESULTS:** Among all subjects, consumption of n-3 PUFA-rich fish and individual n-3 PUFAs was associated inversely with HCC, in a dose-dependent manner. Hazard ratios for the highest vs lowest quintiles were 0.64 (95% CI, 0.42–0.96) for n-3 PUFA-rich fish, 0.56 (95% CI, 0.36–0.85) for EPA, 0.64 (95% CI, 0.41–0.98) for DPA, and 0.56 (95% CI, 0.35–0.87) for DHA. These inverse associations were similar irrespective of HCV or HBV status. **CONCLUSIONS: Consumption of n-3 PUFA-rich fish or n-3 PUFAs, particularly EPA, DPA, and DHA, appears to protect against the development of HCC, even among subjects with HBV and/or HCV infection.**

Keywords: Diet; Liver Cancer; Cancer Prevention; Omega-3 Fatty Acid.

The most important risk factor in the development of hepatocellular carcinoma (HCC) in human beings is chronic infection with hepatitis B virus (HBV) or hepatitis C virus (HCV).[1] The markedly poor prognosis of HCC, with a 5-year survival rate in Japan of less than 20%,[2] emphasizes the need for effective preventive measures, particularly in hepatitis virus carriers. Although dietary factors also might be risk factors, the role of diet in the etiology of HCC remains unclear, except with regard to alcohol consumption and aflatoxin contamination.[3]

A recent prospective study showed an inverse association between white meat, including fish, and liver cancer.[4] Inverse associations with the consumption of white meat or fish were observed in some studies,[5–8] but were not confirmed in others.[9–11] Moreover, except for 2 case-control studies,[5,7] most previous epidemiologic studies of white meat or fish and HCC did not consider HCV or HBV infection status.

Fish is a rich source of n-3 polyunsaturated fatty acids (PUFAs), such as eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), and docosahexaenoic acid (DHA), and several studies have documented a protective effect of dietary n-3 PUFA on the development of several cancers.[12,13] However, less is known about the influence of n-3 PUFA on HCC.

Here, we investigated the presence of an association between fish and n-3 PUFA consumption and HCC in a large-scale, population-based, cohort study in Japan, with consideration for HCV and HBV infection status.

Materials and Methods

Study Population

The Japan Public Health Center–based prospective study was launched in 1990. The study design has been described in detail previously.[14] The study population was defined as all residents of 11 public health center (PHC) areas across Japan who were aged 40–69 years at the start of the respective baseline survey (n = 140,420). In the present analysis, we excluded one PHC area (Tokyo) because data on cancer incidence were not available, as well as some subjects from a second PHC

Abbreviations used in this paper: ALA, alpha-linolenic acid; ALT, alanine aminotransferase; CI, confidence interval; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; FFQ, food frequency questionnaire; HBsAg, hepatitis B virus antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HR, hazard ratio; PHC, public health center; PUFAs, polyunsaturated fatty acids.

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(Osaka) area for whom different definitions were used (n = 16,841). The study was approved by the Institutional Review Board of the National Cancer Center (Tokyo, Japan).

Baseline Survey

Cohort participants responded to a self-administered questionnaire at baseline in 1990 (cohort I) and 1993–1994 (cohort II). A 5-year follow-up survey was conducted in 1995 (cohort I) and 1998 (cohort II). The 5-year follow-up survey included more comprehensive information on food intake frequency than the baseline survey, and accordingly was used as baseline for the present study. We initially identified 113,378 participants as the study population at the baseline survey. The questionnaire also included information on personal medical history, smoking and drinking habits, diet, and other lifestyle factors. After exclusion of 205 participants who were found to be ineligible because of non-Japanese nationality ($n = 44$), late report of emigration that occurred before the start of the follow-up period ($n = 155$), incorrect birth date ($n = 3$), and duplicate registration ($n = 3$), the remaining 113,171 participants were considered eligible for the present study. Completed questionnaires were received from 94,999 subjects (response rate, 84%). Further, subjects who had been diagnosed with cancer before the starting point were excluded from analysis (n = 3022).

Food Frequency Questionnaire

The food frequency questionnaire (FFQ) asked subjects about their usual intake of 138 food items in standard portions/units during the previous year, including 19 fish questions. The questionnaire contained 9 frequency categories (never, 1–3 times/mo, 1–2 times/wk, 3–4 times/wk, 5–6 times/wk, once/d, 2–3 times/d, 4–6 times/d, and ≥7 times/d). Nineteen items inquired about fish and shellfish intake, including salted fish, dried fish, canned tuna, salmon or trout, bonito or tuna, cod or flat fish, seabream, horse mackerel or sardine, mackerel pike or mackerel, dried small fish, salted roe, eel, squid, octopus, prawn, short-necked clam or crab shell, vivipara, *chikuwa* (fish paste product), and *kamaboko* (fish paste product). Standard portion sizes were specified for each food item in the 3 amount choices of small (50% smaller), medium (same as standard), and large (50% larger). Fish consumption in g/day was calculated by multiplying frequency by standard portion size for each food item. In our FFQ, dishes in which food was just a constituent were not included. We calculated the daily intake of all n-3 PUFAs combined and of individual PUFAs, namely α-linolenic acid (ALA), EPA, DPA, and DHA, using a fatty acid composition table of Japanese foods.[15] Furthermore, based on the value of n-3 PUFA per 100 g edible portion of fish, we also calculated the consumption of n-3 PUFA-rich fish (salmon or trout, sea bream, horse mackerel or sardine, mackerel pike or mackerel, and eel).[15] Intake of food and nutrients was log-transformed and adjusted for total energy intake by the residual model.[16] We also used the nutrient density method and obtained similar results.

We documented the validity of the FFQ in the assessment of fish, ALA, EPA, DPA, and DHA consumption in subsamples using 14- or 28-day dietary records. Based on 102 men and 113 women in cohort I, the Spearman correlation coefficients between energy-adjusted intake of fish,[17] n-3 PUFA, ALA, EPA, DPA, and DHA[18] from the questionnaire and from dietary records were 0.37, 0.21, 0.27, 0.38, 0.32, and 0.34 for men, and 0.32, 0.34, 0.25, 0.45, 0.39, and 0.37 for women, respectively. The percentage differences between the dietary records and the FFQ for fish were −16% for men, and −1% for women.[19] Thus, validities for fish and n-3 PUFAs were considered moderate.

Among the 91,977 subjects who responded to the questionnaire and had no past history of cancer, subjects who reported extreme total energy intake (upper or lower 1.0%) were excluded, leaving 90,296 subjects for analysis.

Blood Collection and Laboratory Assays

Subjects were asked to voluntarily provided 10 mL of blood during health checkups in 1993–1995, at which time plasma alanine aminotransferase (ALT) level was determined. Samples were divided into the plasma and buffy layers, and preserved at −80°C until analysis. Among subjects who provided blood (n = 33,329), plasma samples from a portion of the subjects (n = 17,497) were screened for anti-HCV using a third-generation immunoassay (Lumipulse II Ortho HCV; Ortho-Clinical Diagnostics K.K., Tokyo, Japan)[20] and for hepatitis B virus antigen (HBsAg) by reversed passive hemagglutination with a commercial kit (Institute of Immunology Co, Ltd, Tokyo, Japan).

Follow-up and Identification of Hepatocellular Carcinoma

Subjects were followed up from the baseline survey until December 31, 2008. Changes in residence status, including survival, were identified annually through the residential registry in the respective public health center area. Among study subjects, 2775 (3.1%) moved out of their study area and 318 (0.4%) were lost to follow-up evaluation during the study period.

Incidence data on HCC were identified by active patient notification from major local hospitals in the study area and data linkage with population-based cancer registries, with permission from the local governments responsible for the registries. Death certificates were used as a supplementary information source, with 10.6% of cases in our cancer registry system based on death certificate only. Cases were coded using the International Classification of Diseases for Oncology, 3rd ed (code C22.0).[21] During an average follow-up period of 11.2 years (1,008,595 person-years), a total of 398 cases of HCC were newly diagnosed among 90,296 subjects who had returned the baseline questionnaire. In one subgroup, a total of 74 cases of HCC were newly diagnosed among 17,497 subjects who had data on anti-HCV and HBsAg status and ALT level.

Statistical Analysis

Person-years of follow-up evaluation were calculated for each subject from the date of completion of the baseline questionnaire to the date of HCC diagnosis, date of emigration from the study area, or date of death, whichever occurred first; or if none of these occurred, follow-up evaluation was through the end of the study period (December 31, 2008). Subjects who were lost to follow-up evaluation were censored at the last confirmed date of presence in the study area. Hazard ratios (HRs) of HCC were calculated by quintiles of consumption of the respective food items or nutrients, with the lowest consumption category as the reference. HRs and 95% confidence intervals (CIs) were calculated by the Cox proportional hazards model, and adjusted for age at baseline survey (continuous), sex, and study area (10 PHC areas) according to the SAS PHREG procedure (version 9.1; SAS Institute, Inc, Cary, NC). For further adjustment, additional possible confounders were incorporated into the model, namely smoking status (never, former, current); alcohol intake (almost never, 1–3 times/mo, ≥1 times/wk); body mass index (continuous); past history of diabetes mellitus (yes or no); intake of coffee (almost never, 1–4 d/wk, ≥1 cups/d); and soy foods, vegetables, vegetable oil, protein, and iron (continuous). Because of a high correlation coefficient between

vegetable oil and ALA, vegetable oil was not adjusted for in the analysis of the association between ALA and HCC. In the subgroup analysis among subjects who had data on hepatitis virus, further adjustment was added for HCV and HBV infection status (positive or negative) and ALT level (<30 IU/L, 30 to <70 IU/L, ≥70 IU/L). These variables are either known or suspected risk factors for cancer or were associated previously with the risk of HCC. Trends were assessed by assignment of the median value in each category. All *P* values were 2-sided, and statistical significance was determined at a *P* value of less than .05.

We also analyzed the association between fish and n-3 PUFA intake and HCC in the 17,497 subjects for whom HCV and HBV infection status and ALT level was known, as well as in the 1303 subjects who were either or both anti-HCV or HBsAg positive.

Results

During an average follow-up period of 11.2 years, a total of 398 HCC cases were identified in total subjects. Baseline characteristics of subjects according to total fish consumption are shown in Table 1. Subjects with higher fish consumption tended to be older, smoke less, and drink less alcohol and coffee. Body mass index and soybean intake was not substantially different according to consumption. Intake of vegetables, iron, and fatty acid increased as fish intake increased. The proportion of subjects positive for anti-HCV, HBsAg, or both among quintiles of fish consumption was similar. The pattern of characteristics was similar according to intake of n-3 PUFA-rich fish (data not shown).

Spearman correlation coefficients for the associations between total fish, n-3 PUFA-rich fish, n-3 PUFA, EPA, DPA, and DHA were analyzed. There were strong correlations between fish and n-3 PUFA (r = 0.73), EPA (r = 0.85), DPA (r = 0.83), and DHA (r = 0.87) and between n-3 PUFA-rich fish and n-3 PUFA (r = 0.73), EPA (r = 0.86), DPA (r = 0.87), and DHA (r = 0.84).

Table 2 presents hazard ratios in relation to fish and n-3 PUFA consumption for HCC cases. Total fish consumption had a weak inverse association with the risk of HCC, with a multivariable HR for the highest vs lowest quintile of 0.64 (95% CI, 0.41–1.02; P_{trend} = .07). n-3 PUFA-rich fish consumption was dose-dependently associated with a decreased risk of HCC, with a multivariable HR for the highest vs lowest quintile of 0.64 (95% CI, 0.42–0.96; P_{trend} = .04). In addition, inverse associations were seen between EPA, DPA, DHA, and HCC, with multivariable HRs for the highest vs lowest quintile of 0.56 (95% CI, 0.36–0.85; P_{trend} = .01) for EPA, 0.64 (95% CI, 0.41–0.98; P_{trend} = .05) for DPA, and 0.56 (95% CI, 0.35–0.87; P_{trend} = .03) for DHA. n-3 PUFA and ALA did not show statistically significant inverse associations with HCC, with respective multivariable HRs for the highest vs lowest quintile of 0.63 (95% CI, 0.36–1.10) and 0.78 (95% CI, 0.48–1.28). No substantial change in results was seen on additional analyses for HCC stratified by sex, smoking status, or body mass index (data not shown). Furthermore, our analyses did not change when restricted to cases that occurred after the first 3 years of follow-up evaluation (122 cases excluded) and when cases identified by death certificate only were excluded (42 cases excluded) (data not shown). Moreover, when subjects with self-reported pre-existing liver diseases were excluded (133 cases excluded), the results were attenuated but not substantially changed. The prevalence of fish oil supplement use was 0.06%; no change was seen when these users were excluded.

Table 1. Subject Characteristics at Baseline According to Fish Consumption Among Japanese (n = 90,296) and Those Who Were Anti-HCV or HBsAg Positive (n = 1372) in the Japan Public Health Center–Based Prospective Study

	Total fish consumption				
	Lowest	Second	Third	Fourth	Highest
Median intake, *g*	35.0	60.6	82.8	109.9	160.6
Age ± SD, *y*	51.8 ± 8.2	51.4 ± 8.0	51.7 ± 7.8	52.2 ± 7.8	53.2 ± 7.7
Current smoker, *%*	27.1	25.4	23.6	20.6	18.6
Regular drinker (yes), *%*	22.3	22.7	21.4	20.5	18.1
Body mass index, mean ± SD, kg/m^2	23.7 ± 3.1	23.5 ± 3.1	23.5 ± 3.0	23.5 ± 3.0	23.6 ± 3.1
History of diabetes (yes), *%*	5.1	4.9	5.0	5.8	6.5
Coffee, daily, *%*	34.2	35.0	32.3	30.3	26.0
Soybean, mean ± SD, *g/d*	96.8 ± 119.8	91.2 ± 79.3	90.5 ± 72.9	89.6 ± 63.5	91.5 ± 65.9
Vegetables, mean ± SD, *g/d*	201.3 ± 165.5	217.1 ± 137.2	225.4 ± 132.5	232.9 ± 125.3	240.5 ± 131.4
Iron, mean ± SD, *mg/d*	8.7 ± 2.6	9.1 ± 2.2	9.4 ± 2.1	9.7 ± 2.0	10.8 ± 2.1
Vegetable oil, mean ± SD, *g/d*	9.0 ± 5.5	9.9 ± 4.4	10.4 ± 4.1	11.1 ± 3.8	12.5 ± 4.2
Fatty acid, mean ± SD, *g/d*	48.1 ± 19.4	50.1 ± 15.4	51.6 ± 14.2	53.2 ± 13.2	56.1 ± 13.6
n-3 PUFA, mean ± SD, *g/d*	2.3 ± 0.8	2.8 ± 0.7	3.2 ± 0.7	3.7 ± 0.1	4.7 ± 1.2
ALA, mean ± SD, *g/d*	1.92 ± 0.81	2.05 ± 0.66	2.15 ± 0.62	2.25 ± 0.58	2.39 ± 0.60
EPA, mean ± SD, *g/d*	0.16 ± 0.10	0.27 ± 0.09	0.37 ± 0.11	0.49 ± 0.16	0.78 ± 0.40
DPA, mean ± SD, *g/d*	0.04 ± 0.03	0.07 ± 0.02	0.10 ± 0.03	0.13 ± 0.04	0.20 ± 0.10
DHA, mean ± SD, *g/d*	0.30 ± 0.15	0.47 ± 0.13	0.62 ± 0.16	0.81 ± 0.22	1.25 ± 0.56
Infection status (n = 17,497)					
HCV(−)/HBV(−)	92.76	92.56	91.87	92.70	92.87
HCV(−)/HBV(+)	3.04	2.20	1.83	1.97	2.15
HCV(+)/HBV(−)	4.06	5.24	6.21	5.24	4.81
HCV(+)/HBV(+)	0.14	0	0.09	0.09	0.17

Table 2. Hazard Ratio and 95% Confidence Intervals for HCC According to Quintile of Intake of Fish and n-3 PUFA in the Japan Public Health Center–Based Prospective Study (n = 90,296)

	Lowest	Second	Third	Fourth	Highest	P_{trend}
Median fish intake, *g/d*	35.0	60.6	82.8	109.9	160.6	
Cases, n	92	79	78	74	75	
Person-years of follow-up evaluation	201,649	201,387	202,084	202,365	201,110	
Age, area, sex-adjusted HR (95% CI)	1	0.91 (0.68–1.24)	0.90 (0.66–1.22)	0.85 (0.62–1.16)	0.82 (0.60–1.13)	.19
Multivariate HR (95% CI)[a]	1	0.83 (0.59–1.17)	0.84 (0.59–1.20)	0.75 (0.51–1.11)	0.64 (0.41–1.02)	.07
n-3 PUFA-rich fish (median), g/d[b]	9.6	19.7	29.5	43.0	70.6	
Cases, n	89	83	79	71	76	
Person-years of follow-up evaluation	202,479	202,296	202,034	202,357	199,411	
Age, area, sex-adjusted HR (95% CI)	1	0.97 (0.72–1.31)	0.90 (0.67–1.23)	0.79 (0.57–1.08)	0.75 (0.55–1.02)	.03
Multivariate HR (95% CI)[a]	1	0.98 (0.70–1.37)	0.86 (0.61–1.23)	0.84 (0.58–1.21)	0.64 (0.42–0.96)	.04
n-3 PUFA (median), *g/d*[b]	1.95	2.65	3.18	3.77	4.80	
Cases, n	101	75	79	80	63	
Person-years of follow-up evaluation	200,491	200,103	201,864	203,023	203,115	
Age, area, sex-adjusted HR (95% CI)	1	0.84 (0.62–1.13)	0.97 (0.72–1.31)	1.01 (0.75–1.36)	0.73 (0.53–1.00)	.18
Multivariate HR (95% CI)[a]	1	0.77 (0.53–1.12)	0.99 (0.66–1.49)	1.02 (0.65–1.62)	0.63 (0.36–1.10)	.29
ALA (median), *g/d*[b]	1.25	1.68	1.98	2.31	2.84	
Cases, n	107	90	77	64	60	
Person-years of follow-up evaluation	199,727	199,879	201,557	203,044	204,388	
Age, area, sex-adjusted HR (95% CI)	1	0.97 (0.73–1.29)	0.97 (0.72–1.30)	0.90 (0.65–1.23)	0.95 (0.68–1.32)	.62
Multivariate HR (95% CI)[c]	1	0.84 (0.60–1.18)	0.78 (0.53–1.15)	0.75 (0.49–1.15)	0.78 (0.48–1.28)	.27
Median EPA, *g/d*[b]	0.14	0.26	0.36	0.48	0.74	
Cases, n	86	78	86	73	75	
Person-years of follow-up evaluation	201,446	200,959	200,759	202,205	203,226	
Age, area, sex-adjusted HR (95% CI)	1	0.88 (0.64–1.19)	0.94 (0.69–1.27)	0.76 (0.55–1.06)	0.70 (0.50–0.96)	.02
Multivariate HR (95% CI)[a]	1	0.76 (0.54–1.07)	0.85 (0.60–1.21)	0.73 (0.50–1.06)	0.56 (0.36–0.85)	.01
DPA (median), *g/d*[b]	0.04	0.07	0.09	0.12	0.19	
Cases, n	84	78	81	78	77	
Person-years of follow-up evaluation	204,239	201,463	200,839	200,190	201,864	
Age, area, sex-adjusted HR (95% CI)	1	0.93 (0.69–1.28)	0.95 (0.69–1.29)	0.88 (0.64–1.20)	0.76 (0.55–1.05)	.08
Multivariate HR (95% CI)[a]	1	0.84 (0.60–1.18)	0.91 (0.64–1.29)	0.85 (0.59–1.23)	0.64 (0.41–0.98)	.05
DHA (median), *g/d*[b]	0.28	0.46	0.61	0.8	1.19	
Cases, n	89	71	81	80	77	
Person-years of follow-up evaluation	202,203	200,834	200,568	202,231	202,759	
Age, area, sex-adjusted HR (95% CI)	1	0.79 (0.57–1.08)	0.87 (0.64–1.19)	0.82 (0.60–1.13)	0.71 (0.52–0.98)	.07
Multivariate HR (95% CI)[a]	1	0.73 (0.52–1.03)	0.77 (0.54–1.10)	0.77 (0.53–1.12)	0.56 (0.35–0.87)	.03

[a]Adjusted for age, area, sex, smoking status, alcohol frequency, body mass index, past history of diabetes mellitus, and intake of coffee, soy foods, vegetables, vegetable oil, protein, and iron.
[b]n-3 PUFA-rich fish included salmon or trout, sea bream, horse mackerel or sardine, mackerel pike or mackerel, and eel.
[c]Adjusted for age, area, sex, smoking status, alcohol frequency, body mass index, past history of diabetes mellitus, and intake of coffee, soy foods, vegetables, protein, and iron.

Fish consumption might reflect other lifestyle factors. In particular, subjects with higher fish consumption tended to drink less alcohol and coffee, and tended to have a past history of diabetes. Although we also assessed the effect of fish consumption according to alcohol, coffee drinking status, or history of diabetes, an inverse association between fish and n-3 PUFA-rich fish and HCC risk was shown in both regular (1–2 times/wk) and nonregular (<1 time/wk) alcohol drinkers, in both daily and nondaily coffee drinkers, and in both those with and without a history of diabetes (data not shown). Interaction between n-3 PUFA-rich fish and alcohol, coffee drinking status, or history of diabetes was not detected ($P_{interaction}$ =.25, .57, and .58, respectively).

To adjust for HCV and HBsAg status, we also analyzed the association between fish and n-3 PUFAs and HCC risk among subjects who had information on HCV and HBV infection status (Table 3). Although statistical significance was diminished because of a small sample size, similar results were seen, with multivariable HRs for the highest vs lowest tertile of 0.54 (95% CI, 0.23–1.24) for fish, 0.73 (95% CI, 0.35–1.53) for n-3 PUFA-rich fish, 0.51 (95% CI, 0.20–1.32) for n-3 PUFA, 0.70 (95% CI, 0.29–1.71) for ALA, 0.62 (95% CI, 0.28–1.39) for EPA, 0.80 (95% CI, 0.34–1.85) for DPA, and 0.63 (95% CI, 0.27–1.49) for DHA.

To clarify the association between fish and n-3 PUFAs and HCC risk among HBV- and/or HCV-infected subjects, we restricted analysis to subjects who were either or both anti-HCV or HBsAg positive (n = 1303) and anti-HCV positive (n = 911) (Table 4). Total fish consumption was not statistically significantly associated with the risk of HCC, with a multivariable HR for the highest vs lowest quintile of 0.52 (95% CI, 0.20–1.32; P_{trend} = .31), and the inverse association between total fish and HCC was strengthened when subjects were limited to those who were anti-HCV positive, with a multivariable HR for the highest vs lowest quintile of 0.30 (95% CI, 0.11–0.82; P_{trend} = .03). Higher n-3 PUFA-rich fish and n-3 PUFA consumption appeared to decrease the risk of HCC, but without statistical significance. Multivariable HRs for the highest vs lowest tertile among subjects who were either or both anti-HCV or HBsAg positive was 0.60 (95% CI, 0.25–1.40) for n-3 PUFA-rich fish and 0.41 (95% CI, 0.14–1.19) for n-3 PUFA, whereas the HR among subjects who were anti-HCV positive was 0.42 (95% CI, 0.16–1.12) for n-3 PUFA-rich fish and 0.44 (95% CI, 0.13–1.42) for n-3 PUFA. ALA, EPA, DPA, and DHA consumption also tended to be associated with a decreased risk of HCC among subjects who were either or both anti-HCV or HBsAg positive,

Table 3. Hazard Ratio and 95% Confidence Intervals for HCC According to Quintile of Intake of Fish and n-3 PUFA Among Japanese Men and Women Whose HCV and HBV Status Was Known in the Japan Public Health Center–Based Prospective Study (n = 17,497)

	Lowest	Middle	Highest	P_{trend}
Median fish intake, *g/d*	43.6	80.1	131.5	
Cases, n	24	30	20	
Person-years of follow-up evaluation	57,973	57,696	58,186	
Age, area, sex-adjusted HR (95% CI)	1	1.29 (0.75–2.22)	0.73 (0.40–1.34)	.36
Multivariate HR (95% CI)[a]	1	1.42 (0.73–2.76)	0.54 (0.23–1.24)	.24
Median n-3 PUFA-rich fish, *g/d*[b]	12.8	29.1	57.5	
Cases, n	25	23	26	
Person-years of follow-up evaluation	58,381	57,489	57,984	
Age, area, sex-adjusted HR (95% CI)	1	0.91 (0.51–1.61)	0.84 (0.48–1.46)	.53
Multivariate HR (95% CI)[a]	1	1.30 (0.66–2.57)	0.73 (0.35–1.53)	.42
Median n-3 PUFA, *g/d*[b]	2.27	3.05	4.12	
Cases, n	25	24	25	
Person-years of follow-up evaluation	57,776	57,683	58,115	
Age, area, sex-adjusted HR (95% CI)	1	1.02 (0.58–1.79)	0.84 (0.48–1.46)	.52
Multivariate HR (95% CI)[a]	1	0.67 (0.32–1.39)	0.51 (0.20–1.32)	.17
Median ALA, *g/d*[b]	1.49	2.02	2.63	
Cases, n	32	23	19	
Person-years of follow-up evaluation	57,211	57,962	58,401	
Age, area, sex-adjusted HR (95% CI)	1	0.96 (0.56–1.65)	0.97 (0.54–1.77)	.92
Multivariate HR (95% CI)[c]	1	0.75 (0.37–1.54)	0.70 (0.29–1.71)	.43
Median EPA, *g/d*[b]	0.17	0.33	0.58	
Cases, n	23	27	24	
Person-years of follow-up evaluation	57,994	57,447	58,133	
Age, area, sex-adjusted HR (95% CI)	1	0.95 (0.53–1.69)	0.71 (0.39–1.30)	.24
Multivariate HR (95% CI)[a]	1	1.39 (0.71–2.74)	0.62 (0.28–1.39)	.15
Median DPA, *g/d*[b]	0.05	0.09	0.15	
Cases, n	20	30	24	
Person-years of follow-up evaluation	58,066	57,585	57,923	
Age, area, sex-adjusted HR (95% CI)	1	1.30 (0.73–2.31)	0.89 (0.48–1.64)	.55
Multivariate HR (95% CI)[a]	1	1.72 (0.86–3.43)	0.80 (0.34–1.85)	.38
Median DHA, *g/d*[b]	0.32	0.57	0.96	
Cases, n	22	26	26	
Person-years of follow-up evaluation	57,926	57,460	58,187	
Age, area, sex-adjusted HR (95% CI)	1	1.03 (0.58–1.85)	0.88 (0.48–1.59)	.62
Multivariate HR (95% CI)[a]	1	1.15 (0.58–2.29)	0.63 (0.27–1.49)	.24

[a]Adjusted for age, area, sex, HCV, HBsAg, ALT level, smoking status, alcohol frequency, body mass index, past history of diabetes mellitus, and intake of coffee, soy foods, vegetables, vegetable oil, protein, and iron.
[b]n-3 PUFA-rich fish included salmon or trout, sea bream, horse mackerel or sardine, mackerel pike or mackerel, and eel.
[c]Adjusted for age, area, sex, HCV, HBsAg, ALT level, smoking status, alcohol frequency, body mass index, past history of diabetes mellitus, and intake of coffee, soy foods, vegetables, protein, and iron.

albeit without statistical significance (highest vs lowest: multivariable HR, 0.69; 95% CI, 0.26–1.86; HR, 0.55; 95% CI, 0.22–1.39; HR, 0.55; 95% CI, 0.21–1.42, and HR, 0.59; 95% CI, 0.22–1.57, respectively). When subjects were restricted to those who were anti-HCV positive, a dose-dependent inverse association was seen, with multivariable HRs for the highest vs lowest tertile of 0.33 (95% CI, 0.12–0.92; $P_{trend} = .03$) for EPA, 0.30 (95% CI, 0.10–0.88; $P_{trend} = .02$) for DHA, and 0.37 (95% CI, 0.13–1.05; $P_{trend} = .06$) for DPA. ALA showed no association with HCC.

Discussion

Here, we investigated the relationship between fish and n-3 PUFA consumption and the risk of HCC in a population-based prospective study in Japan. Results showed a decreased risk in those with a higher consumption of n-3 PUFA-rich fish and n-3 PUFAs, particularly EPA, DPA, and DHA. Of particular note was the inverse association even when analysis was confined to subjects who were also either or both HCV and HBV positive.

A recent prospective study in the United States also reported that the consumption of white meat, including fish, was significantly inversely associated with the risk of HCC (HR for the highest vs lowest quintile of 0.52, $P_{trend} < .001$), but this study lacked information about HBV and HCV.[4] In a previous study of the association between fish intake and HCC, results from a prospective study in Japan showed a significantly decreased risk of HCC mortality in the second category (3–4 times/wk), albeit in univariate analysis.[6] In a case-control study in China, liver cancer mortality was associated with a curvilinear reduction of fish intake.[8] Another case-control study in China also showed that the frequent intake of fresh fish (≥3 times/week) decreased risk of HCC, with an odds ratio after adjustment for confounding factors,

Table 4. Hazard Ratio and 95% Confidence Intervals for HCC According to Quintile of Intake of Fish and n-3 PUFA Among Japanese Men and Women Who Were anti-HCV or HBsAg Positive (n = 1303) and anti-HCV Positive (n = 911) in the Japan Public Health Center–Based Prospective Study

	Subjects who were anti-HCV or HBsAg positive (n = 1303)				Subjects who were anti-HCV positive (n = 911)			
	Lowest	Middle	Highest	P_{trend}	Lowest	Middle	Highest	P_{trend}
Median fish intake, *g/d*	41.0	76.7	126.4		48.1	80.6	131.1	
Cases, n	19	25	17		20	17	13	
Person-years of follow-up evaluation	4137	4073	4138		2809	2837	2831	
Age, area, sex-adjusted HR (95% CI)	1	1.44 (0.79–2.63)	0.80 (0.41–1.55)	.58	1	0.98 (0.51–1.88)	0.63 (0.31–1.29)	.22
Multivariate HR (95% CI)[a]	1	1.50 (0.71–3.15)	0.52 (0.20–1.32)	.31	1	1.15 (0.51–2.59)	0.30 (0.11–0.82)	.03
Median n-3 PUFA-rich fish, *g/d*[b]	12.8	29.5	58.0		15.1	31.7	59.3	
Cases, n	21	22	18		17	20	13	
Person-years of follow-up evaluation	4197	4048	4104		2872	2796	2809	
Age, area, sex-adjusted HR (95% CI)	1	1.11 (0.60–2.01)	0.70 (0.37–1.33)	.29	1	1.38 (0.71–2.67)	0.66 (0.32–1.37)	.31
Multivariate HR (95% CI)[a]	1	1.28 (0.61–2.69)	0.60 (0.25–1.40)	.27	1	1.40 (0.62–3.17)	0.42 (0.16–1.12)	.10
Median n-3 PUFA, *g/d*[b]	2.18	3.00	4.02		2.22	3.02	4.05	
Cases, n	20	21	20		19	15	16	
Person-years of follow-up evaluation	4137	4089	4123		2840	2820	2816	
Age, area, sex-adjusted HR (95% CI)	1	1.07 (0.58–2.00)	0.79 (0.42–1.49)	.46	1	0.82 (0.41–1.62)	0.68 (0.35–1.34)	.27
Multivariate HR (95% CI)[a]	1	0.59 (0.26–1.35)	0.41 (0.14–1.19)	.10	1	0.42 (0.17–1.07)	0.44 (0.13–1.42)	.16
Median ALA, *g/d*[b]	1.35	1.89	2.46		1.31	1.81	2.36	
Cases, n	24	20	17		22	12	16	
Person-years of follow-up evaluation	4003	4141	4205		2742	2858	2877	
Age, area, sex-adjusted HR (95% CI)	1	0.92 (0.51–1.68)	0.90 (0.47–1.73)	.75	1	0.60 (0.30–1.23)	0.88 (0.45–1.74)	.62
Multivariate HR (95% CI)[c]	1	0.85 (0.38–1.89)	0.69 (0.26–1.86)	.46	1	0.61 (0.24–1.53)	1.00 (0.35–2.83)	.97
Median EPA, *g/d*[b]	0.82	0.34	0.59		0.24	0.39	0.64	
Cases, n	18	25	18		18	19	13	
Person-years of follow-up evaluation	4221	4029	4098		2871	2780	2826	
Age, area, sex-adjusted HR (95% CI)	1	1.34 (0.71–2.51)	0.71 (0.36–1.41)	.22	1	1.15 (0.60–2.23)	0.60 (0.29–1.24)	.14
Multivariate HR (95% CI)[a]	1	1.35 (0.63–2.88)	0.55 (0.22–1.39)	.12	1	1.00 (0.45–2.21)	0.33 (0.12–0.92)	.03
Median DPA, *g/d*[b]	0.05	0.09	0.15		0.07	0.10	0.16	
Cases, n	18	24	19		15	22	13	
Person-years of follow-up evaluation	4211	4060	4078		2887	2781	2809	
Age, area, sex-adjusted HR (95% CI)	1	1.24 (0.66–2.31)	0.81 (0.41–1.57)	.43	1	1.49 (0.76–2.91)	0.77 (0.36–1.63)	.37
Multivariate HR (95% CI)[a]	1	1.45 (0.68–3.09)	0.55 (0.21–1.42)	.13	1	1.22 (0.56–2.69)	0.30 (0.10–0.88)	.02
Median DHA, *g/d*[b]	0.34	0.59	0.98		0.43	0.65	1.05	
Cases, n	16	25	20		16	18	16	
Person-years of follow-up evaluation	4223	4023	4102		2864	2803	2810	
Age, area, sex-adjusted HR (95% CI)	1	1.53 (0.80–2.92)	0.90 (0.45–1.77)	.56	1	1.22 (0.61–2.43)	0.86 (0.42–1.74)	.59
Multivariate HR (95% CI)[a]	1	1.33 (0.61–2.88)	0.59 (0.22–1.57)	.22	1	0.74 (0.32–1.71)	0.37 (0.13–1.05)	.06

[a]Adjusted for age, area, sex, ALT level, smoking status, alcohol frequency, body mass index, past history of diabetes mellitus, and intake of coffee, soy foods, vegetables, vegetable oil, protein, and iron.
[b]n-3 PUFA-rich fish included salmon or trout, sea bream, horse mackerel or sardine, mackerel pike or mackerel, and eel.
[c]Adjusted for age, area, sex, ALT level, smoking status, alcohol frequency, body mass index, past history of diabetes mellitus, and intake of coffee, soy foods, vegetables, protein, and iron.

including HBV, of 0.32.[5] In contrast, several case-control studies showed no association between HCC and fermented fish in Thailand[10] or fish in Japan[11] or Italy.[9] Further, although adjusted by HBV and HCV, fish intake showed no association with HCC in a case-control study in Italy.[7] This inconsistency may be owing to errors in exposure measurement and limited variation in fish. Given that Japanese consume large quantities of fish, the inverse association between fish and HCC in our study might have been clarified by the comprehensive questionnaire and wide range of consumption.

Although fish are the principal source of n-3 PUFAs, we are unaware of any study of the association between n-3 PUFA intake and HCC. In the present study, we also observed that consumption of n-3 PUFAs, particularly EPA, DPA, and DHA, was associated inversely with HCC. In clinical trials, dietary supplementation with n-3 PUFAs for 1–3 months was associated with a decreased release of interleukin-1β and interleukin-6.[22–25] Given that HCC is an inflammation-related cancer that has a background of chronic inflammation, triggered by exposure to hepatitis virus infection or toxic compounds, such as ethanol,[26,27] the anti-inflammatory properties of n-3 PUFAs might decrease the risk of HCC. Of note, we showed that the risk of HCC was decreased with greater consumption of fish and n-3 PUFAs in subjects who were either or both anti-HCV or HBsAg positive. The intake of n-3 PUFA-rich fish might reduce the risk of HCC through the anti-inflammatory effects of n-3 PUFAs on chronic hepatitis.

Another possibility is that fish and n-3 PUFAs also might be associated with HCC through an improvement in insulin sensitivity. Given that recent epidemiologic data have suggested that diabetes and obesity are associated with an increased risk of HCC,[28–31] insulin resistance is now recognized as an independent risk factor for the development of HCC.[29] Animal experiments indicate that the intake of n-3 fatty acids from fish oils has a beneficial effect on insulin sensitivity in rats,[32] but not in human beings.[33–35] High concentration of n-3 PUFAs in human skeletal muscle cells have been associated with improved insulin sensitivity.[36] n-3 PUFAs from fish therefore might improve insulin resistance. In addition, a clinical study has shown the induction of plasma adiponectin in response to a daily intake of EPA and DHA.[37] Thus, the induction of adiponectin also might contribute to the

beneficial effect of n-3 PUFA on systemic insulin sensitivity. However, there was no difference in association between fish and n-3 PUFAs and HCC in participants with and without self-reported diabetes.

In contrast, ALA, which is another component of n-3 PUFAs, was weakly or not associated with HCC, although ALA might be converted to EPA and DHA. Other than fish, the other source of n-3 PUFA in this study population was vegetable oil, in which ALA is the only n-3 PUFA (EPA, DPA, and DHA are not included in vegetable oil). On adjustment for vegetable oil, results were not changed substantially. Therefore, EPA, DPA, or DHA among n-3 PUFA from fish might play particularly important roles as factors that lower the risk of HCC.

The strengths of the present study were its prospective design and negligible proportion of loss to follow-up evaluation (0.4%). Information on fish consumption was collected before the subsequent diagnosis of HCC, thereby diminishing the probability of the recall bias that is inherent to case-control studies. Another strength was that virus infection status was available at baseline, allowing us to clarify the association between n-3 PUFAs and HCC in a high-risk population, albeit the sample size was small. Further, dietary information was ascertained using a validated FFQ and the validity of fish and n-3 PUFAs intake was moderate.

Several limitations also warrant mention. First, because we estimated the consumption of fish and associated nutrients from self-reports and at one time point only, some measurement error in the assessment of consumption is inevitable. If present, however, this probably was nondifferential and likely would have lead to the underestimation of results. Second, we had no information on the clinical severity of hepatitis or on the treatment of subjects with hepatitis virus infection before or during the study period. If infected subjects had received treatment, the occurrence of HCC might have been decreased. However, this might have lead to the underestimation of HCC occurrence, which also would have biased the results toward the null. Finally, our study subjects were a middle-aged population, and caution accordingly is required in generalizing the present results to the young and elderly.

In conclusion, our large prospective study indicated that high consumption of n-3 PUFA-rich fish and n-3 PUFAs was associated with a reduced risk of HCC, even among a high-risk population. Given that the prognosis for HCC is extremely poor, our results would, if confirmed, have important implications for public health. Greater consumption of n-3 PUFA-rich fish and n-3 PUFAs may modify the development of HCC among HBV- and/or HCV-infected subjects.

Appendix

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Conflicts of interest

The authors disclose no conflicts.

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