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

Members of the Japan Public Health Center–based prospective study group included the following: S. Tsugane (principal investigator), M. Inoue, T. Sobue, and T. Hanaoka, Research Center for Cancer Prevention and Screening, National Cancer Center, Tokyo; J. Ogata, S. Baba, T. Mannami, A. Okayama, and Y. Kokubo, National Cardiovascular Center, Suita; K. Miyakawa, F. Saito, A. Koizumi, Y. Sano, I. Hashimoto, T. Ikuta, and Y. Tanaba, Iwate Prefectural Ninohe Public Health Center, Ninohe; Y. Miyajima, N. Suzuki, S. Nagasawa, Y. Furusugi, and N. Nagai, Akita Prefectural Yokote Public Health Center, Yokote; H. Sanada, Y. Hatayama, F. Kobayashi, H. Uchino, Y. Shirai, T. Kondo, R. Sasaki, Y. Watanabe, Y. Miyagawa, Y. Kobayashi, and M. Machida, Nagano Prefectural Saku Public Health Center, Saku; Y. Kishimoto, E. Takara, T. Fukuyama, M. Kinjo, M. Irei, and H. Sakiyama, Okinawa Prefectural Chubu Public Health Center, Okinawa; K. Imoto, H. Yazawa, T. Seo, A. Seiko, F. Ito, F. Shoji, and R. Saito, Katsushika Public Health Center, Tokyo; A. Murata, K. Minato, K. Motegi, and T. Fujieda, Ibaraki Prefectural Mito Public Health Center, Mito; T. Abe, M. Katagiri, M. Suzuki, and K. Matsui, Niigata Prefectural Kashiwazaki and Nagaoka Public Health Center, Kashiwazaki and Nagaoka; M. Doi, A. Terao, Y. Ishikawa, and T. Tagami, Kochi Prefectural Chuo-Higashi Public Health Center, Tosayamada; H. Doi, M. Urata, N. Okamoto, F. Ide, and H. Sueta, Nagasaki Prefectural Kamigoto Public Health Center, Arikawa; H. Sakiyama, N. Onga, H. Takaesu, and M. Uehara, Okinawa Prefectural Miyako Public Health Center, Hirara; F. Horii, I. Asano, H. Yamaguchi, K. Aoki, S. Maruyama, M. Ichii, and M. Takano, Osaka Prefectural Suita Public Health Center, Suita; S. Matsushima and S. Natsukawa, Saku General Hospital, Usuda; M. Akabane, Tokyo University of Agriculture, Tokyo; M. Konishi, K. Okada, and I. Saito, Ehime University, Toon; H. Iso, Osaka University, Suita; Y. Honda, K. Yamagishi, S. Sakurai, and N. Tsuchiya, Tsukuba University, Tsukuba; H. Sugimura, Hamamatsu University, Hamamatsu; Y. Tsubono, Tohoku University, Sendai; M. Kabuto, National Institute for Environmental Studies, Tsukuba; S. Tominaga, Aichi Cancer Center Research Institute, Nagoya; M. Iida, W. Ajiki, and A. Ioka, Osaka Medical Center for Cancer and Cardiovascular Disease, Osaka; S. Sato, Osaka Medical Center for Health Science and Promotion, Osaka; N. Yasuda, Kochi University, Nankoku; K. Nakamura, Niigata University, Niigata; S. Kono, Kyushu University, Fukuoka; K. Suzuki, Research Institute for Brain and Blood Vessels Akita, Akita; Y. Takashima and M. Yoshida, Kyorin University, Mitaka; E. Maruyama, Kobe University, Kobe; M. Yamaguchi, Y. Matsumura, S. Sasaki, and S. Watanabe, National Institute of Health and Nutrition, Tokyo; T. Kadowaki, Tokyo University, Tokyo; M. Noda and T. Mizoue, International Medical Center of Japan, Tokyo; Y. Kawaguchi, Tokyo Medical and Dental University, Tokyo; and H. Shimizu, Sakihae Institute, Gifu.

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Reprint requests

Address requests for reprints to: Norie Sawada, MD, PhD, Epidemiology and Prevention Division, Research Center for Cancer Prevention and Screening, National Cancer Center, 5-1-1 Tsukiji, Chuo-ku, Tokyo, 104-0045 Japan. e-mail: nsawada@ncc.go.jp; **fax: (81) 3-3547-8578.**

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Conflicts of interest

The authors disclose no conflicts.

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Original Contribution

Association Between Plasma 25-Hydroxyvitamin D and Colorectal Adenoma According to Dietary Calcium Intake and Vitamin D Receptor Polymorphism

Taiki Yamaji*, Motoki Iwasaki, Shizuka Sasazuki, Hiromi Sakamoto, Teruhiko Yoshida, and Shoichiro Tsugane

* Correspondence to Dr. Taiki Yamaji, Epidemiology and Prevention Division, Research Center for Cancer Prevention and Screening, National Cancer Center, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan (e-mail: tyamaji@ncc.go.jp).

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The anticarcinogenic potential of vitamin D might be mediated by not only calcium metabolism but also other mechanisms initiated by vitamin D receptor (VDR). The authors measured plasma 25-hydroxyvitamin D in healthy volunteer examinees who underwent total colonoscopy in Tokyo, Japan, 2004–2005, and evaluated its influence on colorectal adenoma, both alone and in interaction with *VDR* polymorphisms, which correspond to the *Fok*I and *Taq*I restriction sites. The main analysis of plasma 25-hydroxyvitamin D included 737 cases and 703 controls. Compared with the lowest quintile of plasma 25-hydroxyvitamin D, only the highest was related to a significantly decreased odds ratio of colorectal adenoma (odds ratio = 0.64, 95% confidence interval: 0.45, 0.92). In contrast, all but the lowest quintile of dietary calcium intake presented similarly reduced odds ratios (odds ratio for the highest = 0.67, 95% confidence interval: 0.47, 0.95). Of note, the association between plasma 25-hydroxyvitamin D levels and colorectal adenoma was modified by the *Taq*I polymorphism of the *VDR* gene ($P_{interaction}$ = 0.03) but not by dietary calcium intake ($P_{interaction}$ = 0.93). These observations highlight the importance of vitamin D in colorectal tumorigenesis. Vitamin D might protect against colorectal neoplasia, mainly through mechanisms other than the indirect mechanism via calcium metabolism.

adenoma; calcium; case-control studies; intestine, large; Japan; polymorphism, single nucleotide; vitamin D

Abbreviations: CI, confidence interval; OR, odds ratio; SNP, single nucleotide polymorphism; VDR, vitamin D receptor.

Accumulating evidence has indicated that adequate levels of vitamin D may confer protection against the risk of colorectal cancer and adenoma, a well-established precursor lesion of colorectal cancer (1, 2). Recent meta-analyses of vitamin D intake and colorectal neoplasia have generally shown a weak inverse association (2, 3), while those of serum/plasma 25-hydroxyvitamin D, the predominant form of vitamin D in the circulation, have fairly consistently demonstrated a significant inverse association (3–5). This discrepancy in the magnitude of the association may reflect the fact that vitamin D in the body is derived from not only the diet but also the skin, where a substantial amount of pre-vitamin D can be synthesized from 7-dehydrocholesterol through stimulation by solar ultraviolet B radiation (6).

The primary role of vitamin D is the maintenance of calcium homeostasis, the disruption of which is also related to colorectal carcinogenesis (2, 7). Vitamin D exerts its effects on calcium metabolism through binding to vitamin D receptor (VDR), a member of the nuclear receptor superfamily, which regulates the transcription of genes involved in calcium absorption from the small intestine. The VDR gene (*VDR*) has a number of single nucleotide polymorphisms (SNPs), including rs2228570 (previously rs10735810) and rs731236. These 2 polymorphisms, which correspond to the *Fok*I and *Taq*I restriction sites, respectively, have been intensively explored over the last decade for their possible association with colorectal tumorigenesis (8, 9). The *Fok*I polymorphism exists at exon 2 of the *VDR* gene, and the *Taq*I polymorphism exists at exon 9. Given their distinctly separate locations, it is likely that the *Fok*I and *Taq*I polymorphisms are differently related to the development of colorectal neoplasia, if indeed they are related (9).

Although several epidemiologic studies have investigated the association between circulating vitamin D levels and colorectal neoplasia in conjunction with total/dietary calcium intake (10–15), few have done so in consideration of *VDR* polymorphisms (13, 14), despite the fact that the anticarcinogenic potential of vitamin D might be mediated by not only calcium metabolism but also other mechanisms initiated by VDR. Here, we measured plasma concentrations of 25-hydroxyvitamin D in 1,520 middle-aged and elderly Japanese and evaluated its influence on colorectal adenoma, both alone and in interaction with dietary calcium intake and the *Fok*I and *Taq*I polymorphisms of the *VDR* gene.

MATERIALS AND METHODS

Study population

The Colorectal Adenoma Study in Tokyo (16, 17), a case-control study conducted by the Research Center for Cancer Prevention and Screening, a branch of the National Cancer Center of Japan, was specifically designed to investigate environmental and genetic factors related to the early stage of colorectal carcinogenesis among healthy volunteer examinees of a colorectal cancer screening program. The Research Center conducts its cancer screening programs on a research basis and accordingly requires all examinees to provide written informed consent prior to admission to the use of data and materials collected through the screening programs to be used for medical research. This means that virtually no examinee refuses to participate in medical research initiated by the Research Center. Examinees who attend the Research Center are primarily self-referred, and more than 90% reside in Tokyo and its 6 neighboring prefectures, collectively called the Kanto region. The study protocol was approved by the institutional review board of the National Cancer Center.

Eligible subjects were defined in advance as men aged 50–79 years and women aged 40–79 years who underwent total colonoscopy from the anus to the cecum and who were without a history of colorectal adenoma, any malignant neoplasia, ulcerative colitis, Crohn's disease, familial adenomatous polyposis, carcinoid tumor, or colectomy. Of a consecutive series of 3,212 examinees undergoing magnifying colonoscopy with indigo carmine dye spraying between February 2004 and February 2005, 2,234 met these conditions. On the basis of the pit pattern of colorectal lesions, namely, the characteristics of mucosal crypts, 526 men and 256 women were determined to have at least 1 adenoma and were thus included as adenoma cases. Pit-pattern classification based on magnifying chromoendoscopy has been detailed elsewhere (18). Of the remaining 1,452 examinees, we identified 482 men and 721 women as potential controls who were also free from other benign lesions (e.g., hyperplastic polyps, inflammatory polyps, and diverticula). Because there were fewer potential controls than cases in men, all potential male controls were included in the study, whereas female controls were randomly selected from potential controls and frequency matched to the female cases in 5 age categories (40–49, 50–54, 55–59, 60–64, and ≥65 years of age) and 2 screening periods (first and second halves). The screening period was matched because standard operating procedures were improved during the first half period after the establishment of the Research Center, which might have influenced, for example, the accuracy of diagnosis. Finally, the study enrolled 526 cases and 482 controls in men and 256 cases and 256 controls in women. A total of 242 male and 104 female cases had adenomas of ≥5 mm in diameter and were referred to clinical hospitals for definitive diagnosis and treatment. Of 1,362 adenomatous lesions referred to the National Cancer Center in 2004–2008, 1,221 (90%), 53 (4%), and 88 (6%) were histologically confirmed as adenoma, early cancer, and nonneoplastic lesions, respectively (unpublished data).

Blood collection and laboratory procedures

Blood is collected from all examinees of the Research Center for research purposes almost without exception. Examinees were scheduled for blood collection prior to any cancer screening procedures on the first day of screening. Fasting venous blood was drawn into a vacutainer tube with ethylenediaminetetraacetic acid (EDTA). The vacutainer tubes were centrifuged to obtain the plasma and buffy coat layer, and the blood samples were preserved at −80°C until analysis. Plasma and buffy coat samples were available for all subjects of this study.

Plasma 25-hydroxyvitamin D concentrations were measured by a radioimmunoassay method by using a commercially available reagent (Kyowa Medex, Tokyo, Japan) with a minimum detection level of 6 ng/mL at an external laboratory (SRL, Tokyo, Japan). The laboratory reported intra- and interassay coefficients of variation of 5.96% and 5.31% for plasma 25-hydroxyvitamin D concentrations of 25.0 and 20.1 ng/mL, respectively. All laboratory personnel were blinded with respect to case and control status.

Genomic DNA was extracted from white blood cells in the buffy coat layer by using a FlexiGene DNA kit (Qiagen, Hilden, Germany) in our laboratory. More than 90% of buffy coat samples provided a sufficient amount of genomic DNA to perform genotyping. The *Fok*I and *Taq*I polymorphisms of the *VDR* gene were analyzed by using the TaqMan SNP genotyping assays (Applied Biosystems, Foster City, California). These analyses were carried out with blinding to case and control status.

Self-administered questionnaire

Prior to cancer screening, all examinees were encouraged to complete a self-administered questionnaire concerning lifestyle and socioeconomic characteristics, as well as personal and family medical history. Details of the questionnaire have been described elsewhere (16, 17). Although some examinees left individual items blank, no examinee refused to answer any substantial portion of the questionnaire.

The questionnaire also included a food frequency questionnaire of 145 food and beverage items with standard portions/units and 9 frequency categories. The amount of each food consumed per day in the past year was first calculated from the responses, and then total energy and nutrient intakes, including calcium, were estimated by reference to the *Standard Tables of Food Composition in Japan*, Fifth Revised Edition (19). The food frequency questionnaire of the present study was

essentially the same as that used in a large prospective cohort study among a Japanese population (20, 21). A validation study conducted among subsamples of cancer screening examinees revealed that the dietary calcium intake estimated from this food frequency questionnaire was relatively well correlated with that from 4-day dietary records, with deattenuated Spearman's correlation coefficients for energy-adjusted calcium intake of 0.64 and 0.61 for men and women, respectively (unpublished data).

Statistical analysis

Odds ratios and 95% confidence intervals of colorectal adenoma for plasma 25-hydroxyvitamin D, dietary calcium intake, and the *Fok*I and *Taq*I polymorphisms of the *VDR* gene were estimated by using an unconditional logistic regression model. Dietary calcium intake was energy adjusted for each sex by using a linear regression model with natural logarithm-transformed intakes of total energy and calcium as independent and dependent variables, respectively (22). Plasma 25-hydroxyvitamin D concentrations and dietary calcium intake were divided into sex-specific quintiles by cutoff points derived from the distribution among controls. Statistical adjustment was made in a manner similar to that in our previous studies of colorectal adenoma (16, 17). Model 1 controlled for sex, matching variables (i.e., age categories and screening periods), and season of blood collection (spring, summer, fall, and winter). Model 2 adjusted for the same variables as model 1 and additionally for cigarette smoking (never, ≤20, 21–40, and >40 pack-years), alcohol drinking (never, past, <150, 150–299, and ≥300 g/week), body mass index (<21.0, 21.0–22.9, 23.0–24.9, and ≥25.0 kg/m^2), family history of colorectal cancer (yes or no), and nonsteroidal anti-inflammatory drug use (yes or no). Model 2 also adjusted for attained adult height, an indicator of gross energy intake in childhood and adolescence, and average daily energy intake in the past year. These variables were divided into quintiles, the cutoff points of which were based on the sex-specific distribution among controls. Linear trends in the odds ratios of colorectal adenoma were examined by assigning ordinal values to quintiles of plasma 25-hydroxyvitamin D and dietary calcium intake.

We then investigated the influence of plasma 25-hydroxyvitamin D on colorectal adenoma in interaction with dietary calcium intake and the *Fok*I and *Taq*I polymorphisms of the *VDR* gene. Three genotypes of each *VDR* polymorphism were dichotomized on the basis of the dominant model, with the first homozygous for the major allele and the second heterozygous and homozygous for the minor allele combined. Similarly, quintiles of plasma 25-hydroxyvitamin D and dietary calcium intake were reduced to 2 levels, namely, lower and higher, on the basis of their association with colorectal adenoma. The likelihood ratio test with 1 df was used to evaluate whether dietary calcium intake and the *VDR* polymorphisms modified the association between plasma 25-hydroxyvitamin D and colorectal adenoma.

Of 1,443 subjects without extreme energy intakes (<800 or >4,200 kcal/day) or calcium supplement use, 3 subjects had missing information, 1 with regard to height and 2 for cigarette smoking. These were then excluded, and the analyses of plasma 25-hydroxyvitamin D and dietary calcium intake were conducted in the remaining 737 cases and 703 controls. Of note, we excluded calcium supplement users, who accounted for <4% of study subjects, and focused our analysis on dietary calcium intake. In the analyses of the *Fok*I and *Taq*I polymorphisms of the *VDR* gene, 7 and 8 subjects with an undetermined genotype were excluded, respectively, from 1,332 subjects with a sufficient amount of genomic DNA to perform genotyping, leaving 1,325 (684 cases, 641 controls) and 1,324 (684 cases, 640 controls), respectively, for inclusion. Two-sided P values less than 0.05 were regarded as statistically significant. All statistical analyses were carried out using SAS, version 9.1, software (SAS Institute, Inc., Cary, North Carolina).

RESULTS

Table 1 shows selected characteristics of controls according to plasma 25-hydroxyvitamin D level. Increasing levels of plasma 25-hydroxyvitamin D were associated with older age and a higher intake of dietary vitamin D, while other selected characteristics were not related to plasma 25-hydroxyvitamin D levels.

Plasma 25-hydroxyvitamin D levels were inversely associated with the prevalence of colorectal adenoma (Table 2), albeit in a nonlinear manner. Compared with the lowest quintile of plasma 25-hydroxyvitamin D, only the highest showed a statistically significant decrease in the adjusted odds ratio of colorectal adenoma (odds ratio (OR) = 0.64, 95% confidence interval (CI): 0.45, 0.92). A similar pattern was noted when the analysis was replicated in men and women separately ($P_{\text{interaction}} = 0.30$) (Web Table 1, the first of 3 Web tables posted on the *Journal*'s Web site (http://aje.oupjournals.org/)). Given the well-known seasonal variation in circulating levels of 25-hydroxyvitamin D, we also conducted a stratified analysis by season of blood collection, which revealed that the association between plasma 25-hydroxyvitamin D levels and colorectal adenoma was not modified by season of blood collection ($P_{\text{interaction}} = 0.55$) (Web Table 2). A nonlinear inverse association was also observed for dietary calcium intake, although this differed from that for plasma 25-hydroxyvitamin D: Using the first quintile of dietary calcium intake as reference, we found that the second showed a significant decrease in the adjusted odds ratio of colorectal adenoma (OR = 0.64, 95% CI: 0.45, 0.90), while the third to fifth showed no further decline. Again, we saw no apparent difference in the association by sex ($P_{\text{interaction}} = 0.70$) (Web Table 1). When mutually adjusted for plasma 25-hydroxyvitamin D and dietary calcium intake, the odds ratio for the highest quintile of plasma 25-hydroxyvitamin D was 0.66 (95% CI: 0.46, 0.95), whereas those for the second and fifth quintiles of dietary calcium intake were 0.65 (95% CI: 0.46, 0.92) and 0.69 (95% CI: 0.48, 0.99), respectively. The *Fok*I and *Taq*I polymorphisms of the *VDR* gene were not associated with the prevalence of colorectal adenoma (Table 2). Genotype frequencies among controls were in agreement with Hardy-Weinberg equilibrium for both *VDR* polymorphisms ($P = 0.79$ and 0.82 for *Fok*I and *Taq*I, respectively).

Table 1. Selected Characteristics of Controls According to Plasma 25-Hydroxyvitamin D Level, the Colorectal Adenoma Study in Tokyo, Japan, 2004–2005[a]

Characteristic	Plasma 25-Hydroxyvitamin D Level[b]									$P_{difference}$[c]
	Quintile 1 (Lowest)			Quintile 3 (Middle)			Quintile 5 (Highest)			
	No.	%	Median (IQR)	No.	%	Median (IQR)	No.	%	Median (IQR)	
Continuous variables										
Plasma 25-hydroxyvitamin D, ng/mL			16 (14–19)			24 (24–26)			32 (31–34)	<0.001
Age, years			57 (54–63)			60 (56–65)			61 (57–65)	0.005
Height, cm			165 (158–169)			163 (156–169)			162 (155–168)	0.60
Energy intake, kcal/day			1,855 (1,540–2,212)			1,829 (1,594–2,182)			1,894 (1,599–2,227)	0.96
Dietary vitamin D intake, μg/day			6.0 (4.3–7.7)			6.6 (4.7–8.4)			7.2 (4.9–10.0)	0.02
Dietary calcium intake, mg/day			542 (383–685)			565 (422–784)			590 (459–781)	0.15
Categorical variables										
Men	86	66.6		95	65.5		100	63.6		0.73
Ever smoker	64	49.6		74	51.0		70	44.5		0.79
Ever drinker	93	72.0		111	76.5		121	77.0		0.89
Overweight or obesity	33	25.5		32	22.0		30	19.1		0.71
NSAID user	12	9.3		13	8.9		8	5.1		0.53
Family history of colorectal cancer[d]	19	14.7		17	11.7		20	12.7		0.91

Abbreviations: IQR, interquartile range; NSAID, nonsteroidal antiinflammatory drug.

[a] Presenting characteristics of controls in quintiles 1, 3, and 5.

[b] Respective median (range) of each plasma 25-hydroxyvitamin D quintile by sex—for men, quintile 1: 18 ng/mL (1–20); quintile 3: 25 ng/mL (24–26); quintile 5: 33 ng/mL (≥31); for women, quintile 1: 15 ng/mL (1–17); quintile 3: 23 ng/mL (22–24); quintile 5: 30 ng/mL (≥28).

[c] Based on the Wilcoxon rank-sum test for median difference and the Fisher exact test for percentage difference.

[d] History of colorectal cancer in parents and siblings.

Among 737 cases, 325 had a largest adenoma of ≥5 mm in diameter (44.1%). Excluding 12 cases with missing information, 388 had the largest adenoma at the proximal colon (53.5%), 259 at the distal colon (35.7%), and 78 at the rectum (10.8%). We then investigated the association of plasma 25-hydroxyvitamin D and dietary calcium intake with the size and location of the largest adenoma using a multinomial logistic regression model (Table 3). The inverse association of plasma 25-hydroxyvitamin D and dietary calcium intake was even more striking in cases with a largest adenoma of ≥5 mm in diameter. By location of the largest adenoma, the inverse association of plasma 25-hydroxyvitamin D was most pronounced in cases of proximal colon adenoma, whereas that of dietary calcium intake was most prominent in rectal adenoma cases.

We further evaluated the association of plasma 25-hydroxyvitamin D and dietary calcium intake with colorectal adenoma stratified by major risk factors of colorectal adenoma, namely, smoking and drinking habits and body fatness. Although no interaction of dietary calcium intake with body fatness was seen, such an interaction was suggested for plasma 25-hydroxyvitamin D ($P_{interaction} = 0.05$), in which the odds ratio of colorectal adenoma for the highest compared with lowest quintile was statistically significant in subjects with a body mass index of <23 kg/m^2 but not in those of ≥23 kg/m^2 (Web Table 3). With respect to smoking and drinking habits, we did not see any effect modification for either plasma 25-hydroxyvitamin D or dietary calcium intake (data not shown).

Table 4 shows the association of plasma 25-hydroxyvitamin D with colorectal adenoma according to dietary calcium intake and *VDR* polymorphism. Although we saw no multiplicative interaction, higher levels of plasma 25-hydroxyvitamin D and dietary calcium intake combined were related to the greatest decrease in odds ratio of colorectal adenoma (OR = 0.49, 95% CI: 0.33, 0.72). With regard to the *VDR* polymorphisms examined, we observed a significant interaction with the *TaqI* polymorphism ($P_{interaction} = 0.03$), for which an inverse association of plasma 25-hydroxyvitamin D was more evident in heterozygotes and homozygotes for the minor allele combined ($P_{trend} = 0.001$) than in homozygotes for the major allele ($P_{trend} = 0.25$). When examined in heterozygotes or homozygotes for the minor allele of *TaqI*, the adjusted odds ratio of colorectal adenoma for higher compared with lower levels of plasma 25-hydroxyvitamin D was 0.32 (95% CI: 0.16, 0.65).

DISCUSSION

In this study, we found a nonlinear inverse association of plasma 25-hydroxyvitamin D and dietary calcium intake with colorectal adenoma. Moreover, we noted a significant

Table 2. Association of Plasma 25-Hydroxyvitamin D, Dietary Calcium Intake, and Vitamin D Receptor Polymorphisms With Colorectal Adenoma, the Colorectal Adenoma Study in Tokyo, Japan, 2004–2005

Variable	No. of Subjects		Model 1[a]		Model 2[b]	
	Cases	Controls	OR	95% CI	OR	95% CI
Plasma 25-hydroxyvitamin D[c]						
Quintile 1 (lowest)	145	129	1	Referent	1	Referent
Quintile 2	132	128	0.89	0.63, 1.26	0.86	0.60, 1.24
Quintile 3 (middle)	157	145	0.90	0.64, 1.26	0.91	0.64, 1.29
Quintile 4	175	144	1.01	0.72, 1.41	1.03	0.73, 1.46
Quintile 5 (highest)	128	157	0.66	0.47, 0.94	0.64	0.45, 0.92
P_{trend}				0.08		0.09
Dietary calcium intake[d]						
Quintile 1 (lowest)	201	140	1	Referent	1	Referent
Quintile 2	124	140	0.58	0.42, 0.81	0.64	0.45, 0.90
Quintile 3 (middle)	141	141	0.64	0.46, 0.88	0.78	0.55, 1.10
Quintile 4	142	140	0.63	0.45, 0.87	0.80	0.56, 1.13
Quintile 5 (highest)	129	142	0.55	0.39, 0.77	0.67	0.47, 0.95
P_{trend}				0.002		0.13
*Fok*I genotype[e,f]						
FF	274	260	1	Referent	1	Referent
Ff	324	294	1.06	0.83, 1.34	1.01	0.79, 1.29
ff	86	87	0.93	0.66, 1.32	0.91	0.63, 1.31
Ff/ff	410	381	1.03	0.82, 1.29	0.99	0.78, 1.25
*Taq*I genotype[e,f]						
TT	523	492	1	Referent	1	Referent
Tt	156	139	1.06	0.82, 1.39	1.06	0.81, 1.40
tt	5	9	0.56	0.18, 1.70	0.47	0.15, 1.51
Tt/tt	161	148	1.03	0.80, 1.34	1.03	0.79, 1.34

Abbreviations: CI, confidence interval; OR, odds ratio.

[a] Model 1 was adjusted for sex, age, screening period, and season of blood collection.

[b] Model 2 was adjusted for the same variables as model 1 and additionally for cigarette smoking, alcohol drinking, body mass index, family history of colorectal cancer, nonsteroidal antiinflammatory drug use, daily energy intake, and height.

[c] Respective median (range) of each plasma 25-hydroxyvitamin D quintile by sex—for men, quintile 1: 18 ng/mL (1–20); quintile 3: 25 ng/mL (24–26); quintile 5: 33 ng/mL (≥31); for women, quintile 1: 15 ng/mL (1–17); quintile 3: 23 ng/mL (22–24); quintile 5: 30 ng/mL (≥28).

[d] Respective median (range) of each dietary calcium intake quintile by sex—for men, quintile 1: 288 mg/day (1–366); quintile 3: 514 mg/day (463–567); quintile 5: 867 mg/day (≥717); for women, quintile 1: 419 mg/day (1–498); quintile 3: 676 mg/day (613–742); quintile 5: 1,069 mg/day (≥881).

[e] The number of subjects providing sufficient genomic DNA to perform genotyping was 1,332.

[f] For *Fok*I and *Taq*I, 7 and 8 subjects with undetermined genotype were excluded, respectively.

interaction between plasma 25-hydroxyvitamin D and the *Taq*I polymorphism of the *VDR* gene. These findings underline the importance of vitamin D in colorectal carcinogenesis, at least in its early stage.

Circulating levels of 25-hydroxyvitamin D have been evaluated in at least 7 prospective studies of colorectal cancer and 6 observational studies of colorectal adenoma (best summarized by Gandini et al. (23)). However, only 2 of these were conducted in an Asian or, more specifically, Japanese population (6, 24). Although neither reported a straightforward overall association, the investigation of colorectal adenoma showed a nonlinear inverse association, similar to ours, but only in subjects who provided blood during the winter season (24). With respect to total/dietary calcium intake, we are aware of at least 4 observational studies of colorectal cancer in Asian populations (21, 25–27) but no study of colorectal adenoma in a similar population. Even when the lower consumption levels in Asian than Western populations were considered, all studies consistently reported an inverse association (21, 25–27).

A recent comprehensive review that estimated optimal concentrations of 25-hydroxyvitamin D for multiple health

Table 3. Association of Plasma 25-Hydroxyvitamin D and Dietary Calcium Intake With the Size and Location of the Largest Adenoma, the Colorectal Adenoma Study in Tokyo, Japan, 2004–2005

Variable	Size of Largest Adenoma						Location of Largest Adenoma[a]								
	≥5 mm in Diameter			<5 mm in Diameter			Proximal Colon			Distal Colon			Rectum		
	No. of Cases	OR[b]	95% CI	No. of Cases	OR[b]	95% CI	No. of Cases	OR[b]	95% CI	No. of Cases	OR[b]	95% CI	No. of Cases	OR[b]	95% CI
Plasma 25-hydroxyvitamin D[c]															
Quintile 1 (lowest)	70	1	Referent	75	1	Referent	75	1	Referent	53	1	Referent	17	1	Referent
Quintile 2	56	0.75	0.48, 1.17	76	0.97	0.64, 1.47	80	1.00	0.66, 1.51	40	0.74	0.45, 1.21	9	0.49	0.20, 1.16
Quintile 3 (middle)	67	0.81	0.52, 1.25	90	1.03	0.69, 1.55	74	0.82	0.54, 1.24	65	1.10	0.70, 1.74	18	0.87	0.41, 1.82
Quintile 4	79	0.94	0.61, 1.43	96	1.12	0.74, 1.67	93	1.03	0.68, 1.55	58	0.96	0.60, 1.54	18	0.88	0.42, 1.85
Quintile 5 (highest)	53	0.54	0.34, 0.86	75	0.74	0.49, 1.13	66	0.63	0.41, 0.96	43	0.62	0.38, 1.02	16	0.68	0.31, 1.46
P_{trend}			0.06			0.35			0.07			0.21			0.72
Dietary calcium intake[d]															
Quintile 1 (lowest)	101	1	Referent	100	1	Referent	96	1	Referent	75	1	Referent	29	1	Referent
Quintile 2	53	0.55	0.36, 0.84	71	0.76	0.51, 1.14	60	0.63	0.41, 0.95	48	0.72	0.46, 1.13	16	0.59	0.29, 1.17
Quintile 3 (middle)	67	0.74	0.49, 1.13	74	0.84	0.56, 1.26	73	0.78	0.51, 1.17	52	0.87	0.55, 1.37	12	0.47	0.22, 1.00
Quintile 4	54	0.60	0.39, 0.94	88	1.04	0.70, 1.56	83	0.90	0.60, 1.36	44	0.76	0.47, 1.22	13	0.55	0.26, 1.16
Quintile 5 (highest)	50	0.50	0.32, 0.79	79	0.88	0.58, 1.33	76	0.74	0.49, 1.13	40	0.66	0.41, 1.09	8	0.29	0.12, 0.70
P_{trend}			0.009			0.91			0.57			0.17			0.007

Abbreviations: CI, confidence interval; OR, odds ratio.

[a] Twelve cases had missing information on the location of the largest adenoma.

[b] Adjusted for sex, age, screening period, season of blood collection, cigarette smoking, alcohol drinking, body mass index, family history of colorectal cancer, nonsteroidal antiinflammatory drug use, daily energy intake, and height.

[c] Respective median (range) of each plasma 25-hydroxyvitamin D quintile by sex—for men, quintile 1: 18 ng/mL (1–20); quintile 3: 25 ng/mL (24–26); quintile 5: 33 ng/mL (≥31); for women, quintile 1: 15 ng/mL (1–17); quintile 3: 23 ng/mL (22–24); quintile 5: 30 ng/mL (≥28).

[d] Respective median (range) of each dietary calcium intake quintile by sex—for men, quintile 1: 288 mg/day (1–366); quintile 3: 514 mg/day (463–567); quintile 5: 867 mg/day (≥717); for women, quintile 1: 419 mg/day (1–498); quintile 3: 676 mg/day (613–742); quintile 5: 1,069 mg/day (≥881).

Table 4. Association of Plasma 25-Hydroxyvitamin D With Colorectal Adenoma According to Dietary Calcium Intake and Vitamin D Receptor Polymorphism, the Colorectal Adenoma Study in Tokyo, Japan, 2004–2005

Variable	Plasma 25-Hydroxyvitamin D								$P_{interaction}$
	Quintiles 1–4 (Lower)				Quintile 5 (Higher)				
	No. of Cases	No. of Controls	OR[a]	95% CI	No. of Cases	No. of Controls	OR[a]	95% CI	
Dietary calcium intake									0.93
Quintile 1 (lower)	169	113	1	Referent	32	27	0.69	0.38, 1.26	
Quintiles 2–5 (higher)	440	433	0.73	0.54, 0.98	96	130	0.49	0.33, 0.72	
*Fok*I genotype[b,c]									0.27
FF	228	212	1	Referent	46	48	0.85	0.53, 1.36	
Ff/ff	338	291	1.06	0.82, 1.38	72	90	0.65	0.44, 0.96	
*Taq*I genotype[b,c]									0.03
TT	423	388	1	Referent	100	104	0.80	0.57, 1.11	
Tt/tt	143	113	1.17	0.87, 1.57	18	35	0.43	0.23, 0.79	

Abbreviations: CI, confidence interval; OR, odds ratio.

[a] Adjusted for sex, age, screening period, season of blood collection, cigarette smoking, alcohol drinking, body mass index, family history of colorectal cancer, nonsteroidal antiinflammatory drug use, daily energy intake, and height.

[b] The number of subjects providing sufficient genomic DNA to perform genotyping was 1,332.

[c] For *Fok*I and *Taq*I, 7 and 8 subjects with undetermined genotype were excluded, respectively.

outcomes, including colorectal cancer, concluded that the most advantageous concentrations of 25-hydroxyvitamin D began at around 30 ng/mL for all endpoints assessed (28), with which our observations essentially agree. With regard to dietary calcium intake, a pooled analysis of 10 cohort studies reported a threshold effect of dietary calcium intake in which all quintiles above the lowest showed a similar decrease in the risk of colorectal cancer (7), which strongly supports our present results.

We saw no multiplicative interaction between plasma 25-hydroxyvitamin D and dietary calcium intake. Previous observational studies of primary colorectal cancer and adenoma have also failed to identify such interaction (10–15). Although these findings do not rule out the existence of biologic interaction, they may suggest that vitamin D exerts an anticarcinogenic effect on the large intestine itself, and that its influence on calcium homeostasis plays only a minor role in colorectal tumorigenesis.

Although not nonsynonymous, the *Taq*I polymorphism of the *VDR* gene appears to be in linkage disequilibrium with a series of polymorphisms in the 3′ end of the *VDR* gene (29), for example, the polyadenylated microsatellite in the 3′ untranslated region, the length of which likely determines messenger RNA stability and hence likely affects intracellular levels of VDR (30). To date, the 2 studies of colorectal neoplasia that have examined the *Taq*I polymorphism in conjunction with vitamin D, as measured by dietary intake (31) or circulating levels ((14); the results were shown in the text only), indicated the absence of any obvious interaction.

We investigated effect modification by the *VDR* gene using 2 traditional SNPs, although the gene spans approximately 100 kilobases and has numerous genetic polymorphisms. In fact, sequencing of the *VDR* gene in a Japanese population identified >20 SNPs with a minor allele frequency of >0.05, including *Fok*I and *Taq*I polymorphisms, at least some of which would serve as tag SNPs to capture the common variation in the gene (32). Further, recent genome-wide scans revealed several genes associated with circulating 25-hydroxyvitamin D concentrations (33, 34). Our findings, based on a limited number of SNPs in a single gene, provide at most an intriguing insight into the gene-environmental interaction in the vitamin D pathway.

The strengths of the present study include its measurement of plasma 25-hydroxyvitamin D concentrations, which may provide a relatively accurate classification of study subjects by vitamin D status. In addition, the provision of total colonoscopy to all study subjects likely decreased the possibility of misclassification between cases and controls. Conversely, a major limitation is its cross-sectional nature, and the observed associations might have been due to reverse causality. In contrast to colorectal cancer, however, colorectal adenoma likely does not affect circulating levels of vitamin D, because colorectal adenoma is an asymptomatic benign tumor. A second limitation is that adenoma cases were not histologically confirmed and necessarily included those with an early cancer or nonneoplastic lesion. However, our preliminary survey reported an accuracy of diagnosis based on magnifying chromoendoscopy of 90%, a result similar to those previously reported (35, 36), and the influence of any misclassification caused by the technique is therefore likely to have been minimal. Third, we were unable to analyze groups of cases and their frequency-matched controls in single batches, because single groups contained too many subjects to allow placement in the same batch. Although the impact of variability in assay performance was not reduced by simultaneously analyzing all subjects in a matching category, blood samples were at least analyzed irrespective of case and control status, reducing differential misclassification between cases and controls. Fourth, blinded control samples from the study population were not available and were therefore not

included in the measurement of plasma 25-hydroxyvitamin D; quality control for this measurement was performed by an external laboratory by using nonblinded controls. Accordingly, the reported intra- and interassay coefficients of variation would likely have underestimated the true underlying variations. Finally, we did not match cases and controls by season of examination or blood collection. If such matching had been conducted, we could have taken better account of the seasonal variation in plasma 25-hydroxyvitamin D concentrations.

In summary, we found that both plasma 25-hydroxyvitamin D and dietary calcium intake were inversely associated with the prevalence of colorectal adenoma, albeit in a nonlinear manner. We further noted that plasma 25-hydroxyvitamin D levels interacted with the *Taq*I polymorphism of the *VDR* gene but not with dietary calcium intake. These observations highlight the importance of vitamin D in colorectal carcinogenesis, at least in its early stage. Vitamin D might protect against colorectal cancer and adenoma, mainly through mechanisms other than the indirect mechanism via calcium metabolism.

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Isoflavone intake and risk of gastric cancer: a population-based prospective cohort study in Japan[1–3]

Azusa Hara, Shizuka Sasazuki, Manami Inoue, Motoki Iwasaki, Taichi Shimazu, Norie Sawada, Taiki Yamaji, and Shoichiro Tsugane for the Japan Public Health Center–Based Prospective Study Group

ABSTRACT

Background: Isoflavones are structurally similar to 17β-estradiol and may be able to prevent gastric cancer. However, there is contradictory evidence concerning the relation between the intake of soy food, which is rich in isoflavones, and gastric cancer. The association with gastric cancer might differ between isoflavones and soy foods, and research on the effects of isoflavone intake alone on gastric cancer is needed.

Objective: We investigated the association between isoflavone intake and the incidence of gastric cancer.

Design: We conducted a large, population-based prospective study of 39,569 men and 45,312 women aged 45–74 y. Dietary soy and isoflavone intakes were measured by using a validated food-frequency questionnaire in 1995 and 1998.

Results: During 806,550 person-years of follow-up, we identified 1249 new gastric cancer cases. Isoflavone intake was not associated with gastric cancer in either men or women. Compared with the lowest quartile, the HR and 95% CI for developing gastric cancer in the fourth quartile of isoflavone intake was 1.00 (0.81, 1.24) for men and 1.07 (0.77, 1.50) for women. In a stratified analysis by exogenous female hormones (women only), however, we found an increasing trend in risk of gastric cancer associated with higher isoflavone intakes among exogenous female hormone users (P-trend = 0.03) but not for nonusers (P-interaction = 0.04).

Conclusion: The current study does not support the hypothesis that higher intakes of isoflavones prevent gastric cancer in either men or women. *Am J Clin Nutr* 2012;95:147–54.

INTRODUCTION

Although its incidence and mortality rate have been declining over the years (1), GC[4] is still the most common cancer in Japan and the second leading cause of death from cancer globally. Prevention of GC is one of the most important elements for cancer control strategy both in Japan and around the world.

Sex-based discrepancies in GC are found throughout the world, and the incidence of GC in men is 2- to 3-fold that in women (2). This difference is consistent across international populations regardless of different prevalences of environmental risk factors, such as *Helicobacter pylori* infection, tobacco smoking, and different dietary patterns (1, 3). A possible explanation involves biologic differences related to sex hormones, such as estrogen (3).

Isoflavones are structurally similar to 17β-estradiol, have a particular affinity for the β-estrogen receptor (4), and may be able to prevent GC. Because isoflavones are phytoestrogenic compounds that are abundant in soybeans, soy products have been of considerable interest in the etiology of GC (5). However, evidence of the relation between soy food intake and GC is contradictory. Non-isoflavone aspects of soy food, such as salt intake and fermentation, might contribute to the different association with GC between soy food and isoflavones, because salt is a well-known risk factor for GC (6), and fermented soy foods may contain nitroso compounds, which have been reported to induce gastric carcinogenesis (7, 8). Therefore, the association of isoflavones with GC might be different from that of soy food, and further research on the effects of isoflavones alone on GC is needed. However, no large-scale prospective study to assess this association has been conducted.

Here, we investigated the association between isoflavone intake and risk of GC in a population-based, prospective, cohort study in Japan. Our hypothesis was that a higher intake of isoflavones would prevent GC because of their estrogen-like effects.

SUBJECTS AND METHODS

Study population

The JPHC-Based Prospective Study was started in 1990 for cohort I and in 1993 for cohort II. Subjects were all registered Japanese inhabitants in 11 public health center areas who were aged 40–69 y (cohort 1: 40–59 y; cohort 2: 40–69 y) at the beginning of each cohort's baseline survey. Details of the study

[1] From the Epidemiology and Prevention Division, Research Center for Cancer Prevention and Screening, National Cancer Center, Tokyo, Japan.

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[3] Address reprint requests and correspondence to S Sasazuki, Epidemiology and Prevention Division, Research Center for Cancer Prevention and Screening, National Cancer Center, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan. E-mail: ssasazuk@ncc.go.jp.

[4] Abbreviations used: EFH, exogenous female hormones; FFQ, food-frequency questionnaire; GC, gastric cancer; JPHC, Japan Public Health Center.

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design were described previously (9). The institutional review board of the National Cancer Center, Tokyo, Japan, approved the study. The participants in the current study were subjects in the JPHC study who responded to a 5-y follow-up questionnaire in 1995–1999 at the age of 45–74 y. This follow-up survey was used as the starting point in the current study. The subjects from 2 public health center areas (Katsushika in Tokyo prefecture and Suita in Osaka prefecture) were excluded from the current analysis because the selection of subjects was different from that in other public health center areas, which left 116,896 subjects as the study population. After the exclusion of subjects with a non-Japanese nationality (n = 51), a late report of emigration occurring before the starting point (n = 168), or ineligibility due to incorrect birth date (n = 4) or duplicate enrollment (n = 4), we established a population-based cohort of 116,669 subjects. After the exclusion of 1625 subjects who had died, moved out of the study area, or were lost to follow-up before the starting point, 115,044 eligible subjects remained. Of these, 91,246 responded to the questionnaire, which yielded a response rate of 78.2%.

Questionnaire

We asked the subjects to reply to a lifestyle questionnaire that covered sociodemographic characteristics, medical history, smoking and drinking habits, diet, and other characteristics. We designed the FFQ to estimate dietary intake from 138 food items and validated it for the estimation of various nutrients and food groups (10). The participants were asked about how often they consumed the individual food items (frequency of intake) and to estimate representative relative sizes compared with standard portions during the previous year (11). Of the 138 food items, 8 items (standard portion size) dealt specifically with consumption of soy and isoflavones: miso soup (150 g), soymilk (200 g), tofu for miso soup (20 g), tofu for other dishes (75 g), *yushidofu* (predrained tofu; 150 g), *koyadofu* (freeze-dried tofu; 60 g), *aburaage* (deep-fried tofu; 2 g), and *natto* (fermented soybeans; 50 g). These 8 items contributed 95.9% of the total genistein and daidzein intakes in the estimates from dietary records in our validation study (12). We defined fermented soy food as miso (for miso soup) and *natto*, whereas nonfermented soy food was defined as soymilk, tofu for miso soup, tofu for other dishes, *yushidofu*, *koyadofu*, *and aburaage* (13). We then estimated genistein and daidzein intakes from either fermented or nonfermented foods. For miso soup, the FFQ included questions on the frequency of consumption (almost never, 1–3 d/mo, 1–2 d/wk, 3–4 d/wk, 5–6 d/wk, or daily) and on the daily amount consumed (number of bowls: <1, 1, 2, 3, 4, 5, 6, 7–9, or ≥ 10). For soymilk, the FFQ included questions on 10 frequency categories only: almost never, 1–3 times/mo, 1–2 times/wk, 3–4 times/wk, 5–6 times/wk, 1 glass/d, 2–3 glasses/d, 4–6 glasses/d, 7–9 glasses/d, or ≥ 9 glasses/d. For other soy foods, the FFQ contained questions on frequency (almost never, 1–3 times/mo, 1–2 times/wk, 3–4 times/wk, 5–6 times/wk, 1 time/d, 2–3 times/d, 4–6 times/d, or ≥ 7 times/d) and sizes relative to a standard portion [small (50% smaller than standard), medium (same as standard), or large (50% larger than standard)].

The daily intake of each food item was calculated by multiplying the frequency by the standard portion and, if available, the relative portion size for each item in the FFQ. We calculated daily intakes of isoflavones (genistein and daidzein) using values in a specially developed food-composition table of Japanese foods (14), which contained measured values of soy foods (15, 16). This allowed for the effect of food processing on isoflavone content, including fermentation, to be taken into consideration when intakes were estimated. We did not collect information on the use of isoflavone supplements. Intake of food and nutrients was log transformed and adjusted for total energy intake by using the residual model (17). Because the estimates of genistein and daidzein intakes were highly correlated (Spearman's rank correlation coefficient = 0.997), the results for genistein are provided as representative for isoflavones.

The validity of the energy-adjusted genistein intake assessed from the 5-y FFQ was evaluated in a subsample with consecutive 14- or 28-d dietary records. Spearman's correlation coefficients between the energy-adjusted intake of genistein from the questionnaire and from dietary records was 0.65 (cohort I) and 0.48 (cohort II) for men and 0.55 (cohort I) and 0.45 (cohort II) for women (18–21). The reproducibility between the 2 questionnaires for energy-adjusted genistein intake assessed 1 y apart showed Spearman's correlation coefficients of 0.75 (men) and 0.69 (women) for cohort I and 0.51 (men) and 0.41 (women) for cohort II (18–21).

We excluded subjects with a diagnosis of GC or who reported having GC before the starting point (n = 746), who had missing data regarding isoflavone intake (n = 1115), or who reported extreme total energy intakes (upper: 2.5%; lower: 2.5%) (n = 4504). The final analysis included 84,881 subjects (39,569 men and 45,312 women).

Follow-up and identification of GC cases

We followed subjects from the 5-y follow-up survey until 31 December 2006. We identified changes in residence status, including survival, annually through the residential registry in each area or, for those who had moved out of the area, through the municipal office of the area to which they had moved. Mortality data for persons in the residential registry are forwarded to the Ministry of Health, Labor, and Welfare and are coded for inclusion in the national Vital Statistics database. Residency registration and death registration are required by the Basic Residential Register Law and Family Registry Law, respectively, and the registries are thought to be complete. During the follow-up period in the current study, 9370 (11.0%) subjects died, 3675 (4.3%) moved out of the study area, and 305 (0.4%) were lost to follow-up.

We identified incident data for GC by active patient notification from major local hospitals in the study area and from data linkage with population-based cancer registries. We coded GC cases according to the International Classification of Diseases for Oncology, third edition (22) (C16.0–C16.9). Tumors located in the lower side of the stomach were classified as distal GC (noncardia; code C16.2–16.7) and in the upper side as proximal GC (cardia; code C16.0–16.1). Tumors that could not be classified because they were overlapping lesions (code C16.8) or because no information was available (code C16.9) were categorized as unclassified. Histologic classification was based on review of the record from the respective hospital as described previously (23) and divided into differentiated and undifferentiated types, corresponding to the intestinal type and diffuse type, respectively, in the Lauren classification (24). In our

cancer registry system, the proportion of cases for which information was available from death certificates was only 4.2%.

Statistical analysis

We calculated person-years of follow-up for each subject from the starting point to the date of GC diagnosis, date of emigration from the study area, date of death, or end of the follow-up (31December 2006), whichever came first. We censored subjects lost to follow-up at the last confirmed date of presence in the study area.

We calculated HRs and 95% CIs of developing GC for the categories of energy-adjusted consumption of isoflavones, isoflavones from fermented soy food, isoflavones from nonfermented soy food, miso soup, and soy food in quartiles for men and women separately, with the lowest consumption category as the reference. We used Cox proportional hazards models with adjustment for potential confounding variables, such as age (in y), public health center area, BMI (in kg/m^2: <18.4, 18.5–19.9, 20–22.4, 22.5–24.9, 25–29.9, and ≥30), smoking status (never, past, and current), alcohol consumption (none and <150, 150–299, 300–449, and ≥450 g ethanol/wk for men and none and <150 and ≥150 g ethanol/wk for women), family history of GC, menopausal status (premenopausal, natural, or induced postmenopausal) and use of EFHs in women (never, past, and current), quartiles of total energy intake, and energy-adjusted intake of salt, vegetable, fruit, and fish.

We calculated *P* values for the analysis of linear trends by assigning ordinal values for categories of isoflavone intake and entering the number as a continuous term in the regression model. We also statistically evaluated the interactions between EFH use [never compared with ever (past and current)] and isoflavone in the risk of GC based on the likelihood ratio test with 1 df. Ordinal values were assigned to 2 categories of EFH (never compared with ever) and to 4 categories of isoflavone. An interaction term was then created by multiplying ordinal values for EFH by those for isoflavone. All *P* values are 2-sided, and statistical significance was indicated at the $P < 0.05$ level. We performed all statistical analyses with SAS software (version 9.1; SAS Institute Inc).

RESULTS

During 806,550 person-years of follow-up, we identified 1249 new GC cases (899 for men and 350 for women). The characteristics of participants according to isoflavone intake are shown in **Table 1**. Those with higher intakes were older, less likely to be current smokers and regular drinkers, and more likely to be postmenopausal and to consume more salt, vegetables, fruit, and fish. BMI was also distributed differently by isoflavone intake.

Associations of isoflavone, isoflavone from fermented soy food, isoflavone from nonfermented soy food, miso soup, and soy food for GC risk in men and women are shown separately for men (**Table 2**) and for women (**Table 3**). In an age- and area-adjusted model, no measurable associations were found between isoflavone, isoflavone from fermented soy food, isoflavone from nonfermented soy food, and soy food intakes and GC in either men or women, whereas the quartile category of miso soup intake was dose-dependently associated with an increased risk of GC in men and a decreased risk of GC in women (*P*-trend = 0.03 and 0.02, respectively); however, relations were not statistically significant in multivariate-adjusted models. Neither fermented soy food nor nonfermented soy food intake was associated with the risk of GC (data not shown). When isoflavone and soy food were respectively entered into the models as deciles of intakes, no substantial association was observed.

The results of stratified analysis by EFH use among women are shown in **Table 4**. We observed increased GC risks with isoflavone and soy food intakes among EFH ever users; compared with the lowest quartile, the HRs (and 95% CIs) of the second, third, and fourth quartiles of isoflavone intake were 1.25 (0.38, 4.06), 1.78 (0.58, 5.47), and 2.80 (0.93, 8.39) (*P*-trend = 0.03) and for soy food intake were 1.69 (0.48, 5.94), 3.20 (0.99, 10.3), and 3.76 (1.14, 12.4) (*P*-trend = 0.01). Among EFH never users, no association was observed between isoflavone and soy food intakes and GC risk, and a decreased GC risk with miso soup intake was observed. We found statistically significant interactions between isoflavone and soy food intakes and EFH (P = 0.04 and 0.02, respectively). Similar results were observed when we separately analyzed for isoflavone intakes from fermented and nonfermented soy food.

When cases were divided by histologic type, we observed no substantial association between isoflavone, miso soup, and soy food intakes and GC (data not shown). Stratified analyses by age, alcohol consumption, smoking status, salt intake, salted food (pickled vegetables, dried and salted fish, and salted fish roe) intake, and menopausal status also showed essentially the same results (data not shown). The association between daidzein intakes and GC risk was similar to that observed for genistein intake (data not shown).

DISCUSSION

In this large, population-based, prospective study, which was characterized by high soy food consumption, isoflavone intake overall was not found to be significantly associated with the risk of GC in either men or women. In a stratified analysis by EFH (women only), however, we found an increase in risk of GC associated with higher isoflavone intakes among EFH users. To our knowledge, this was the first large-scale prospective cohort study to examine the association of isoflavone intake with GC risk.

Two case-control studies have reported that isoflavone intake was not associated with GC. Nomura et al (25) showed no association between total isoflavone intake and gastric adenocarcinoma of the distal stomach among 300 cases and 446 population-based controls in Hawaii. Lagiou et al (26) reported that isoflavone intake was not associated with GC among 110 patients with incident stomach adenocarcinoma and 100 control patients in Greece. Our results, from a large population-based cohort study, support these previous case-control studies. As for the different exposure estimates, one small nested case-control study reported that high plasma concentrations of isoflavones were associated with a decreased risk of GC from 131 cases and 393 matched controls (27). Differences from our exposure estimates might explain the conflicting results. Alternatively, plasma concentrations of isoflavones might be better measurements of bioactive or bioavailable isoflavones, thus explaining the respective findings arising from the different approaches. The concentration of isoflavone in blood reflects individual differences in absorption and metabolism, in which intestinal microflora play an important

TABLE 1
Characteristics of the study subjects on the 5-y follow-up survey according to quartile of energy-adjusted intake of isoflavone (genistein) in the Japan Public Health Center–Based Prospective Study

	Quartile of energy-adjusted intake of isoflavone (genistein)									
	Men (n = 39,569)					Women (n = 45,312)				
	Lowest	Second	Third	Highest	P^1	Lowest	Second	Third	Highest	P^1
No. of subjects (%)	9892	9892	9893	9892		11,328	11,328	11,328	11,328	
Age (y)	56.2 ± 0.08[2]	56.4 ± 0.08	56.5 ± 0.08	57.5 ± 0.08	<0.0001	56.9 ± 0.08	56.7 ± 0.07	57.0 ± 0.07	57.7 ± 0.07	<0.0001
BMI ≥ 25 kg/m^2 (%)	28.7	27.9	27.5	28.3	<0.0001	28.9	27.7	28.4	29.8	<0.0001
Current smoker (%)	46.3	45.0	43.4	38.5	<0.0001	5.7	4.3	3.8	3.7	<0.0001
Regular drinker, ≥150 g ethanol/wk (%)	50.2	50.4	48.9	44.5	<0.0001	3.4	2.4	2.1	2.0	<0.0001
Family history of gastric cancer (%)	5.3	5.6	5.5	5.8	0.6	5.2	6.1	6.3	5.7	0.003
Postmenopausal status (%)	—	—	—	—		67.7	70.9	74.4	76.2	<0.0001
Exogenous female hormones, ever user (%)	—	—	—	—		12.3	12.4	13.4	13.6	<0.0001
Dietary intake[3]										
Energy (kcal/d)	2165 ± 6.8	2155 ± 6.4	2206 ± 6.7	2146 ± 6.4	<0.0001	1848 ± 5.6	1857 ± 5.4	1888 ± 5.4	1824 ± 5.1	<0.0001
NaCl deducted from Na content (g/d)	10.1 ± 0.04	11.8 ± 0.03	12.7 ± 0.04	13.4 ± 0.04	<0.0001	10.3 ± 0.1	11.6 ± 0.1	12.1 ± 0.03	12.7 ± 0.03	<0.0001
Pickled vegetables (g/d)	24.8 ± 0.4	30.3 ± 0.4	32.5 ± 0.4	36.2 ± 0.4	<0.0001	30.8 ± 0.4	35.5 ± 0.4	37.8 ± 0.4	39.7 ± 0.4	<0.0001
Dried and salted fish (g/d)	15.4 ± 0.2	17.0 ± 0.2	18.6 ± 0.2	20.0 ± 0.3	<0.0001	16.3 ± 0.2	17.4 ± 0.2	18.6 ± 0.2	18.9 ± 0.2	<0.0001
Salted fish roe (g/d)	1.0 ± 0.04	1.6 ± 0.03	2.0 ± 0.04	2.0 ± 0.03	<0.0001	1.1 ± 0.03	1.7 ± 0.04	1.9 ± 0.03	1.9 ± 0.03	<0.0001
Vegetables (g/d)	167 ± 1.3	188 ± 1.2	200 ± 1.2	221 ± 1.4	<0.0001	201 ± 1.2	223 ± 1.2	233 ± 1.1	245 ± 1.3	<0.0001
Fruit (g/d)	148 ± 1.5	168 ± 1.5	178 ± 1.4	190 ± 1.5	<0.0001	220 ± 1.8	232 ± 1.5	237 ± 1.5	240 ± 1.5	<0.0001
Fish (g/d)	81.9 ± 0.6	86.7 ± 0.5	92.1 ± 0.5	93.0 ± 0.5	<0.0001	79.7 ± 0.5	83.7 ± 0.4	86.1 ± 0.4	86.1 ± 0.5	<0.0001
Miso soup (mL/d)	144 ± 1.1	257 ± 1.5	297 ± 1.7	316 ± 1.9	<0.0001	124 ± 0.9	212 ± 1.3	245 ± 1.4	264 ± 1.5	<0.0001
Soy food (g/d)[4]	34.0 ± 0.1	63.3 ± 0.2	90.4 ± 0.3	163.6 ± 1.2	<0.0001	34.2 ± 0.1	63.0 ± 0.2	89.1 ± 0.3	164.1 ± 1.1	<0.0001
Daidzein (mg/d)	5.6 ± 0.02	11.0 ± 0.01	16.4 ± 0.02	29.7 ± 0.1	<0.0001	5.6 ± 0.01	10.9 ± 0.01	16.3 ± 0.02	29.1 ± 0.1	<0.0001
Genistein (mg/d)	8.8 ± 0.03	17.2 ± 0.02	26.2 ± 0.03	48.8 ± 0.2	<0.0001	8.9 ± 0.02	17.3 ± 0.02	26.2 ± 0.03	48.1 ± 0.2	<0.0001
Genistein from fermented soy food (mg/d)[5]	4.5 ± 0.03	9.6 ± 0.04	15.1 ± 0.06	27.2 ± 0.2	<0.0001	4.3 ± 0.03	9.2 ± 0.04	14.8 ± 0.06	25.9 ± 0.2	<0.0001
Genistein from nonfermented soy food (mg/d)[6]	4.3 ± 0.03	7.6 ± 0.04	11.1 ± 0.06	21.6 ± 0.2	<0.0001	4.6 ± 0.02	8.1 ± 0.04	11.4 ± 0.06	22.2 ± 0.2	<0.0001

[1] ANOVA or chi-square-test.
[2] Mean ± SE (all such values).
[3] All mean total intakes of food and nutrition are energy adjusted.
[4] Total of fermented and nonfermented soy food.
[5] The consumption of miso (for miso soup) and *natto*.
[6] The consumption of soymilk, tofu for miso soup, tofu for other dishes, *yushidofu*, *koyadofu*, and *aburaage*.

role (28). In particular, most likely because of differences in intestinal bacteria, only 30–50% of adults have the capacity to metabolize daidzein into equol—a compound known to have stronger estrogenic activity than daidzein (29). This might be relevant because the effect of isoflavones may be modulated by endogenous concentration of estrogens. However, the evidence was insufficient, both in the association between serum isoflavone concentrations and GC risk and that between isoflavone intake and GC risk. Moreover, our validation study, which used a subsample of the cohort, yielded satisfactorily high correlation coefficients for genistein estimates from dietary records measured repeatedly for 1 y, a fasting serum sample, and a single FFQ (dietary records compared with serum: 0.33; dietary records compared with. FFQ: 0.59) (12). Furthermore, we previously reported an association between plasma isoflavone concentrations and breast, prostate, and lung cancer risk from nested case-control studies within the JPHC Study (30–32) and found results similar to those we previously obtained in the JPHC Study using an FFQ (18, 20, 33). Further large prospective studies are needed to confirm the relation between isoflavones and GC risk.

As for soy food intake, several studies have examined the association with the risk of GC, but results have been varied: some epidemiologic studies reported that soy products significantly decrease the risk of GC (5, 34, 35), whereas others reported an increased risk of GC (6, 36) or no significant association (6, 36–38). A recent meta-analysis reported that a high intake of fermented soy foods is associated with an increased GC risk, whereas a high intake of nonfermented soy foods is associated with a decreased GC risk (13). However, because the possible confounding effects of salt, vegetable, fruit, and other dietary factors had not been considered in the soy product analysis in most studies included in the meta-analysis, the effects of these uncontrolled factors cannot be ruled out (5, 35). In the current study, we adjusted for these dietary factors and found no association between isoflavone, miso soup, and soy food intakes and the risk of GC.

We observed an increased risk of isoflavone and soy food intakes for GC among women with ever EFH use, although no association was found for isoflavone and soy food intakes among women with never EFH use. Such a differential association between isoflavone or soy food intake and GC by EFH status has not been documented previously. Our previous study showed that

TABLE 2
HRs and 95% CIs of gastric cancer according to quartile of energy-adjusted intake of isoflavone (genistein), miso soup, and soy food among men[1]

			All gastric cancer			Upper third, including cardia		Distal	
Quartiles	Median	Person-years	No. of cases	HR1 (95% CI)[2]	HR2 (95% CI)[3]	No. of cases	HR2 (95% CI)[3]	No. of cases	HR2 (95% CI)[3]
Isoflavone (genistein) (mg/d)									
First	9.2	90,530	187	1.00 (reference)	1.00 (reference)	12	1.00 (reference)	121	1.00 (reference)
Second	17.2	92,407	219	1.01 (0.83, 1.23)	1.01 (0.82, 1.23)	32	2.28 (1.15, 4.52)	145	0.98 (0.76, 1.26)
Third	25.9	93,569	234	0.98 (0.80, 1.20)	0.99 (0.81, 1.23)	27	1.83 (0.89, 3.77)	167	1.02 (0.79, 1.31)
Fourth	42.3	92,078	259	0.98 (0.80, 1.20)	1.00 (0.81, 1.24)	33	2.00 (0.97, 4.12)	176	0.97 (0.74, 1.26)
P-trend				0.8	0.96		0.2		0.9
Isoflavone (genistein) from fermented soy food (g/d)[4]									
First	3.1	89,125	169	1.00 (reference)	1.00 (reference)	11	1.00 (reference)	106	1.00 (reference)
Second	8.3	92,699	201	1.04 (0.84, 1.29)	1.01 (0.82, 1.26)	22	1.63 (0.76, 3.49)	145	1.09 (0.83, 1.42)
Third	14.4	94,270	253	1.15 (0.92, 1.43)	1.13 (0.90, 1.41)	40	2.74 (1.28, 5.84)	163	1.02 (0.77, 1.35)
Fourth	26.7	92,490	276	1.09 (0.87, 1.36)	1.09 (0.86, 1.38)	31	1.95 (0.87, 4.35)	195	1.07 (0.80, 1.43)
P-trend				0.4	0.4		0.1		0.8
Isoflavone (genistein) from nonfermented soy food (g/d)[5]									
First	2.8	91,629	219	1.00 (reference)	1.00 (reference)	26	1.00 (reference)	145	1.00 (reference)
Second	6.1	92,384	244	1.05 (0.87, 1.26)	1.08 (0.89, 1.30)	21	0.81 (0.45, 1.45)	173	1.15 (0.92, 1.44)
Third	10.2	92,541	224	0.94 (0.78, 1.14)	0.97 (0.80, 1.18)	32	1.22 (0.71, 2.08)	150	0.99 (0.78, 1.25)
Fourth	20.2	92,031	212	0.91 (0.75, 1.10)	0.94 (0.77, 1.14)	25	0.95 (0.54, 1.69)	141	0.94 (0.74, 1.20)
P-trend				0.2	0.3		0.8		0.4
Miso soup (mL/d)									
First	63	88,482	177	1.00 (reference)	1.00 (reference)	19	1.00 (reference)	109	1.00 (reference)
Second	175	90,957	208	1.03 (0.84, 1.26)	1.02 (0.83, 1.26)	19	0.81 (0.43, 1.56)	145	1.14 (0.89, 1.47)
Third	294	94,149	232	1.08 (0.88, 1.33)	1.08 (0.87, 1.33)	29	1.10 (0.59, 2.05)	164	1.18 (0.91, 1.53)
Fourth	449	94,997	282	1.22 (1.00, 1.49)	1.17 (0.94, 1.47)	37	1.18 (0.61, 2.27)	191	1.22 (0.92, 1.61)
P-trend				0.03	0.1		0.4		0.2
Soy food (g/d)[6]									
First	33.4	89,909	192	1.00 (reference)	1.00 (reference)	14	1.00 (reference)	130	1.00 (reference)
Second	59.3	92,407	237	1.05 (0.87, 1.28)	1.06 (0.87, 1.29)	32	1.95 (1.02, 3.73)	152	0.95 (0.75, 1.21)
Third	86.1	93,669	241	1.01 (0.83, 1.23)	1.03 (0.84, 1.26)	28	1.64 (0.83, 3.24)	174	1.02 (0.80, 1.31)
Fourth	140.6	92,601	229	1.00 (0.81, 1.22)	1.02 (0.82, 1.25)	30	1.82 (0.92, 3.60)	153	0.95 (0.73, 1.22)
P-trend				0.8	0.99		0.2		0.8

[1] Cox proportional hazards models were used.
[2] HR adjusted for age and public center area.
[3] HR further adjusted for BMI, smoking status, ethanol intake, family history of gastric cancer, vegetable intake, fruit intake, fish intake, salt intake, and total energy intake.
[4] The consumption of miso (for miso soup) and *natto*.
[5] The consumption of soymilk, tofu for miso soup, tofu for other dishes, *yushidofu*, *koyadofu*, and *aburaage*.
[6] Total of fermented and nonfermented soy food.

EFH users had an increased risk of the differentiated type of GC compared with never users among postmenopausal women (39), although some studies reported that EFH reduced the risk of GC (40). It has been shown that the biologic behavior of isoflavones may be modulated by an individual's endogenous concentration of estrogens. In vitro studies have shown that isoflavones can act primarily as estrogen agonists in a low-estrogen environment, whereas they can act as estrogen antagonists in a high-estrogen environment (41). Therefore, it is possible that isoflavones worked as antagonists with a high-estrogen environment among EFH users. Meanwhile, compared with never EFH users, EFH users were more likely to have higher proportions of smoking, regular drinking, family history of GC, and screening examination for GC (data not shown), which suggests that an elevated risk among EFH users may be partly explained by characteristics that were not measured or could not be totally adjusted for in our study. Further studies are needed to confirm these findings.

The strength of the study was its prospective design, which enabled us to avoid exposure recall bias. We selected subjects from the general population, we kept the sample size large, the response rate for the surveys was acceptable for studies of settings such as this, and the loss to follow-up was negligible. Participants were recruited from the Japanese population, which has a relatively higher isoflavone intake than Western populations. Isoflavone intake was measured by a questionnaire with a reasonably high level of validity and reproducibility. In addition, the registry of cancer was of sufficient quality to reduce the misclassification of the outcome.