2003; Tsubono *et al*, 2003). The average frequency was reported in six categories for cohort I: <1 day per month, 1–3 days per month, 1–2 days per week, 3–4 days per week, 5–6 days per week, and everyday. Subjects consuming alcoholic beverages at least once a week were also asked about the types of drinks consumed and the average consumption. Subjects in cohort II were also asked about their drinking status, never-, ex-, or current drinkers. Ex- and current drinkers provided information on the average frequency, the types of drinks consumed and average daily consumption. The average frequency of consumption was divided into the following categories, to each of which a score was assigned: 1.5 for 1–2 days per week, 3.5 for 3–4 days per week, and 6 for 5–6 days per week and everyday in the cohort I questionnaire, and 1.5 for 1–2 days per week, 3.5 for 3–4 days per week, and 6 for almost everyday in the cohort II questionnaire. The amount of ethanol by type of beverage was calculated as follows: 180 ml of sake (rice wine) was regarded as 23 g of ethanol, 180 ml of shochu or awamori (white spirits) as 36 g, 633 ml of beer as 23 g, 30 ml of whiskey or brandy as 10 g, and 60 ml wine as 6 g. Finally, the weekly ethanol intake was estimated by multiplying the amount by the score. In the present analysis, alcohol drinking was classified into six categories: nondrinkers (<1 day per month), occasional drinkers (1–3 days per week), and four categories of regular drinkers (1–149, 150–299, 300–449, and ⩾450 g per week).

Follow-up and analysis

Subjects were followed from the baseline survey until December 31, 2001. Residence status, including survival, was confirmed annually through the residential registry kept in each municipality of the areas where the study subjects resided. Among the study subjects, 6.5% moved away and 0.06% were lost to follow-up during the study period. Information on the cause of each death was supplemented by checking against death certificate files with permission, and the cause of death was defined according to the International Classification of Disease, 10th Version (ICD-10) (WHO, 1990).

Cancers were identified by active patient notification from the local major hospitals in the study area and approved data linkage with the population-based cancer registries. Death certificates were used as a supplementary information source. Cases were coded using the International Classification of Diseases for Oncology, Third Edition (ICD-O-3) (WHO, 2000). In 2.2% of cancer cases, information was available only from death certificates (DCO). The earliest date of diagnosis was used in cases with multiple primary cancers at different times.

Person-years were accrued from baseline survey until the following end points: for cancer incidence – the date of occurrence of cancer, the date of emigration from the study area, the date of death, or the end of the study period, whichever came first; for total cancer deaths, the date of emigration from the study area, the date of death, or the end of the study period, whichever came first. Persons who were lost to follow-up were censored at the last confirmed date of presence in the study area.

The study outcomes were defined as newly occurring cancers of any site and all cancer deaths during the study period. Hazard ratios (HR) and their 95% confidence intervals (95% CI) were used to describe the relative risk cancer deaths associated with the alcohol categories at baseline (nondrinkers, occasional drinkers, 1–149 g of ethanol per week, 150–299, 300–449, and ⩾450 g per week), with 'occasional drinkers' representing the reference category. In men, stratified analyses were further conducted to evaluate whether alcohol effects and cancer risk varied with smoking status. Interaction terms were generated by multiplying the ordinal smoking categories by ordinal alcohol drinking categories. The HRs were further estimated separately for alcohol-related cancers, namely cancer of the oral cavity, pharynx, larynx, oesophagus, and liver (IARC, 1988; WHO, 2003) (ICD-0-3: C00-C10, C12-C15, C22, C32), and for cancers not considered to be alcohol-related. The Cox proportional hazards model was used to control for such potential confounding factors as age at baseline

Table 1 Baseline characteristics of the study subjects according to alcohol drinking category

		Alcohol drinking category					
				Weekly ethanol intake (g per week)			
	Total	Nondrinkers	Occasional drinkers	1–149	150–299	300–449	⩾450
Men (n = 35 007)							
Number of subjects	35 007	7009	3555	7853	7039	4899	4652
Proportion (%)		20.0	10.2	22.4	20.1	14.0	13.3
Age (years) ±s.d.	49.1±5.9	49.8±6.0	48.6±5.9	48.7±6.0	49.0±5.9	49.4±5.8	49.1±5.9
Smoking status (%)							
Never	24.7	29.5	33.0	30.2	21.3	16.9	15.5
Former	21.1	19.8	19.0	22.7	21.9	21.6	20.1
Current	54.2	50.7	48.0	47.1	56.8	61.5	64.4
Green vegetable intake (%)							
Almost everyday	23.5	23.8	24.1	22.4	23.2	24.4	23.6
Leisure-time physical activity (%)							
⩾1–2 times per week	18.0	15.2	18.7	21.0	18.5	17.9	15.6
Women (n = 38 274)							
Number of subjects	38 274	29 356	4329	3584	593	191	221
Proportion (%)		76.7	11.3	9.4	1.5	0.5	0.6
Age (years) ±s.d.	49.4±5.9	49.8±5.9	47.8±5.7	48.0±5.6	47.9±5.7	47.2±5.4	47.3±5.5
Smoking status (%)							
Never	92.4	94.8	90.4	85.6	60.6	52.9	49.3
Former	1.4	1.0	2.1	2.4	6.3	6.8	3.6
Current	6.2	4.2	7.5	12.0	33.2	40.3	47.1
Green vegetable intake (%)							
Almost everyday	31.1	31.6	28.2	31.1	27.9	22.2	27.9
Leisure-time physical activity (%)							
⩾1–2 times per week	15.8	15.0	18.1	19.3	16.9	14.7	13.2

(continuous), study area (nine PHC areas), smoking status (pack-years (0, 1–19, 20–29, 30–39, ⩾40)), green vegetable intake (⩽3–4 times per week, everyday), and leisure-time physical activity (⩽1–3 times per month, ⩾1–2 times per week). These variables are either known or suspected risk factors for cancer or had been found to be associated with cancer risk in previous studies (Tsugane *et al*, 1999; Hara *et al*, 2002).

To express the impact of alcohol drinking on the risk of overall cancer, the population-attributable fraction (PAF) (%) was estimated as pd × (HR−1/HR), where pd is the proportion of cases exposed to the risk factors. This formula is considered more valid than the popular formula Pe × (RR−1)/(Pe × (RR−1) + 1), where Pe is the proportion of the source population exposed to the risk factor, when a confounding variable exists (Rockhill *et al*, 1998). Confidence intervals (95%) for the adjusted PAF were estimated using the formula of Greenland (1999). Stata version 8 special edition software (Stata Corporation, 2003) was used to perform the statistical analyses.

RESULTS

During the 721 302.5 person-years of follow-up (average: 9.8 years) for the 73 281 subjects (35 007 men and 38 274 women), a total of 3403 newly diagnosed cancers (1904 men and 1499 women) and 1208 cancer deaths (758 men and 450 women) were included in the analyses. With regard to cancer incidence, gastric cancer was the commonest cancer in men ($n=533$, 28.0%), followed by colon ($n=281$) and lung cancers ($n=230$); in women, breast cancer was commonest ($n=314$, 20.9%), followed by gastric cancer ($n=203$), and colon cancers ($n=170$). For mortality, lung cancer was the commonest cause of cancer death in men ($n=167$, 22.0%), followed by gastric ($n=148$) and liver cancer ($n=74$); in women, gastric cancer was the commonest cause of cancer death ($n=58$, 12.9%), followed by lung ($n=56$), and breast cancer ($n=40$).

At baseline, 70% of men were regular drinkers and 48% consumed alcohol 3–4 times per week or more; in women, 77% were nondrinkers and 12% regular drinkers. The average frequency of alcohol consumption among regular drinkers was 5.2 days per week in men and 3.6 days per week in women. Both in men and women (Table 1), the proportion of current smokers was increased in the higher ethanol intake groups, in which leisure-time physical activities were less frequent. However, no consistent trend in green vegetable intake was observed across the alcohol categories.

The adjusted cancer HRs by alcohol category are presented in Table 2, together with the ratios for cancer mortality. In men, the lowest risk was observed in occasional drinkers, compared to

Table 2 Hazard ratios (HR)[a] and 95% confidence interval (95% CI) of cancer incidence and deaths according to alcohol-drinking status

				Weekly ethanol intake (g per week)				
		Nondrinkers	Occasional drinkers	1–149	150–299	300–449	⩾450	*P* for trend
Men ($n=35\,007$)								
Cancer incidence	Person-years	68 161.0	35 151.9	74 007.4	67 934.7	48 173.5	46 194.9	
Total ($n=1904$)								
No. of cases		360	138	353	359	339	355	
HR (95% CI)		1.10 (0.90–1.34)	1.00 (reference)	1.18 (0.96–1.44)	1.17 (0.96–1.44)	1.43 (1.17–1.75)	1.61 (1.32–1.97)	<0.001
PAF% (95% CI)						5.4 (1.4–9.1)	7.1 (2.9–11.0)	
Excluding first 5 years ($n=1172$)								
No. of cases		214	81	217	234	206	220	
HR (95% CI)		1.11 (0.86–1.44)	1.00 (reference)	1.25 (0.97–1.62)	1.29 (0.99–1.67)	1.44 (1.10–1.87)	1.64 (1.26–2.12)	0.002
PAF% (95% CI)						5.4 (0.3–10.2)	7.3 (1.9–12.4)	
Cancer deaths	Person-years	69 175.7	35 547.7	75 042.2	69 039.4	49 194.1	47 259.3	
Total ($n=758$)								
No. of cases		161	59	138	119	133	148	
HR (95% CI)		1.10 (0.81–1.49)	1.00 (reference)	1.06 0.77–1.44)	0.92 (0.67–1.26)	1.33 (0.97–1.83)	1.58 (1.16–2.15)	<0.001
PAF% (95% CI)						4.4 (−1.7–10.1)	7.1 (0.4–13.4)	
Excluding first 5 years ($n=533$)								
No. of cases		107	35	106	82	96	107	
HR (95% CI)		1.25 (0.85–1.85)	1.00 (reference)	1.39 (0.94–2.06)	1.08 (0.72–1.62)	1.62 (1.09–2.42)	1.90 (1.28–2.81)	0.009
PAF% (95% CI)						6.9 (−1.1–14.3)	9.5 (0.6–17.6)	
Women ($n=38\,274$)								
Cancer incidence	Person-years	293 866.0	43 116.9	34 966.7	5757.5	1803.8	2168.2	
Total ($n=1499$)								
No. of cases		1170	178	118	20	6	7	
HR (95% CI)		0.94 (0.80–1.11)	1.00 (reference)	0.80 (0.63–1.01)	0.68 (0.42–1.11)	0.73 (0.32–1.66)	0.68 (0.32–1.46)	0.659
Excluding first 5 years ($n=908$)								
No. of cases		707	115	68	12	3	3	
HR (95% CI)		0.88 (0.72–1.08)	1.00 (reference)	0.70 (0.51–0.95)	0.59 (0.31–1.13)	0.59 (0.19–1.88)	0.47 (0.15–1.49)	0.351
Cancer deaths	Person-years	298 259.9	43 775.0	35 434.0	5827.6	1821.6	2187.6	
Total ($n=450$)								
No. of cases		368	43	28	6	3	2	
HR (95% CI)		1.08 (0.79–1.49)	1.00 (reference)	0.79 (0.49–1.27)	0.54 (0.19–1.52)	1.27 (0.39–4.15)	0.68 (0.16–2.86)	0.896
Excluding first 5 years ($n=325$)								
No. of cases		273	31	17	3	1	0	
HR (95% CI)		1.14 (0.78–1.66)	1.00 (reference)	0.69 (0.38–1.25)	0.20 (0.03–1.51)	0.69 (0.09–5.09)	—	0.289

[a]Adjusted for age at baseline (continuous), study area (9 PHC area), pack-years of smoking (0, 1–19, 20–29, 30–39, ⩾40), green vegetable intake (⩽3–4 times per week, almost everyday), and leisure-time physical activity (⩽1–3 times per month, ⩾1–2 times per week).

whom a significant increase in risks of cancer occurrence was observed as ethanol intake increased among regular drinkers (1–149 g per week: HR = 1.18, 150–299 g per week: HR = 1.17, 300–449 g per week: HR = 1.43, ⩾450 g per week: HR = 1.61, *P* for trend <0.001). This trend did not change when cases where the cancers occurred within the first five years of the study were excluded. For total cancer mortality, a similar trend was observed. Among males, 12.5% of the cancers in the study period were attributable to heavy drinking (⩾300 g of ethanol per week): 5.4% for the consumption of 300–449 g of ethanol per week and 7.1% for the consumption of ⩾450 g per week. Similar results were obtained for cancer mortality. Unlike men, cancer risk was the

Table 3 Hazard ratios (HR)[a] and 95% confidence interval (95% CI) of cancer incidence and deaths attributed to alcohol-related cancers and nonalcohol-related cancers[b] according to the alcohol drinking status in men (*n* = 35 007)

			Weekly ethanol intake (g per week)					
	Nondrinkers	**Occasional drinkers**	**1–149**	**150–299**	**300–449**	**⩾450**	***P* for trend**	***P* for interaction**
Cancer incidence								
Never-smokers (n = 315)								
No. of cases	78	42	75	54	37	30		
HR (95% CI)	0.90 (0.62–1.31)	1.00 (reference)	0.87 (0.60–1.28)	0.86 (0.57–1.29)	1.03 (0.66–1.62)	1.02 (0.64–1.64)	0.370	
Current smokers (n = 1163)								0.018
No. of cases	196	58	202	226	224	257		
HR (95% CI)	1.39 (1.03–1.88)	1.00 (reference)	1.69 (1.25–2.28)	1.64 (1.22–2.20)	1.93 (1.43–2.60)	2.32 (1.72–3.11)	<0.001	
Alcohol-related cancers (*n* = 250)								
No. of cases	29	8	38	49	52	74		
HR (95% CI)	1.57 (0.72–3.45)	1.00 (reference)	2.28 (1.06–4.90)	2.95 (1.39–6.28)	4.03 (1.90–8.56)	6.16 (2.95–12.8)	<0.001	
Never smokers (n = 34)								
No. of cases	7	2	8	5	6	6		
HR (95% CI)	1.88 (0.39–9.09)	1.00 (reference)	2.09 (0.44–9.95)	1.91 (0.37–9.95)	3.42 (0.65–17.9)	4.70 (0.93–23.7)	0.109	
Current smokers (n = 163)								
No. of cases	14	5	19	36	33	56		
HR (95% CI)	1.12 (0.40–3.15)	1.00 (reference)	1.95 (0.73–5.24)	3.23 (1.26–8.28)	3.72 (1.44–9.62)	6.41 (2.55–16.1)	<0.001	
Nonalcohol-related cancers (*n* = 1654)								
No. of cases	331	130	315	310	287	281		
HR (95% CI)	1.07 (0.87–1.31)	1.00 (reference)	1.11 (0.90–1.36)	1.06 (0.86–1.31)	1.27 (1.03–1.57)	1.34 (1.11–1.66)	0.004	
Never smokers (n = 282)								
No. of cases	71	40	67	49	31	24		
HR (95% CI)	0.85 (0.57–1.26)	1.00 (reference)	0.81 (0.55–1.20)	0.80 (0.53–1.23)	0.92 (0.57–1.48)	0.85 (0.51–1.42)	0.733	
Current smokers (n = 1000)								
No. of cases	182	53	183	190	191	201		
HR (95% CI)	1.41 (1.03–1.93)	1.00 (reference)	1.66 (1.21–2.27)	1.48 (1.08–2.03)	1.76 (1.28–2.41)	1.94 (1.42–2.66)	0.039	
Cancer deaths								
Never-smokers (n = 124)								
No. of cases	36	25	27	19	7	10		
HR (95% CI)	0.67 (0.40–1.12)	1.00 (reference)	0.53 (0.31–0.92)	0.49 (0.27–0.91)	0.33 (0.14–0.78)	0.55 (0.26–1.16)	0.634	
Current smokers (n = 484)								<0.001
No. of cases	81	23	83	84	99	114		
HR (95% CI)	1.43 (0.89–2.31)	1.00 (reference)	1.68 (1.04–2.69)	1.52 (0.94–2.44)	2.15 (1.35–3.44)	2.57 (1.62–4.09)	<0.001	
Alcohol-related cancers (*n* = 143)								
No. of cases	19	5	25	28	31	35		
HR (95% CI)	1.58 (0.59–4.25)	1.00 (reference)	2.26 (0.86–5.92)	2.55 (0.98–6.67)	3.86 (1.48–10.0)	4.89 (1.90–12.5)	0.003	
Never smokers (n = 22)								
No. of cases	7	2	4	4	2	3		
HR (95% CI)	1.76 (0.36–8.54)	1.00 (reference)	1.01 (0.18–5.59)	1.45 (0.26–8.04)	1.29 (0.18–9.37)	2.35 (0.38–14.4)	0.280	
Current smokers (n = 89)								
No. of cases	6	3	14	20	20	26		
HR (95% CI)	0.81 (0.20–3.26)	1.00 (reference)	2.17 (0.62–7.57)	2.66 (0.78–9.07)	3.45 (1.01–11.8)	4.78 (1.43–15.9)	0.014	
Nonalcohol-related cancers (*n* = 615)								
No. of cases	142	54	113	91	102	113		
HR (95% CI)	1.06 (0.77–1.45)	1.00 (reference)	0.94 (0.68–1.31)	0.76 (0.54–1.08)	1.09 (0.78–1.53)	1.27 (0.91–1.77)	0.010	
Never smokers (n = 102)								
No. of cases	29	23	23	15	5	7		
HR (95% CI)	0.57 (0.33–1.00)	1.00 (reference)	0.49 (0.27–0.88)	0.41 (0.21–0.80)	0.26 (0.10–0.68)	0.41 (0.17–0.96)	0.287	
Current smokers (n = 395)								
No. of cases	75	20	69	64	79	88		
HR (95% CI)	1.53 (0.92–2.54)	1.00 (reference)	1.60 (0.96–2.67)	1.34 (0.80–2.25)	1.96 (1.18–3.25)	2.25 (1.36–3.72)	0.008	

[a]Adjusted for age at baseline (continuous), study area (9 PHC area), daily cigarette consumption (number of cigarettes, continuous, current smoker only), green vegetable intake (⩽3–4 times per week, almost everyday), and leisure-time physical activity (⩽1–3 times per month, ⩾1–2 times per week). [b]Alcohol-related cancers consist of cancer of the oral cavity, pharynx, oesophagus, liver, and larynx. Nonalcohol-related cancers consist of all other cancers not considered to be alcohol related.

highest among occasional drinkers in women, but none of the risk values reached statistical significance.

The HRs for cancer in men were estimated separately by smoking status at baseline for each alcohol-drinking category (Table 3). For cancer incidence, no risk fluctuation was observed among never-smokers, whereas current smokers exhibited a constantly elevated risk compared with occasional drinkers (1–149 g per week: HR = 1.69, 150–299 g per week: HR = 1.64, 300–449 g per week: HR = 1.93, ⩾450 g per week: HR = 2.32, P for trend <0.001). Similar trends were observed for cancer mortality, with a somewhat decreased risk tendency among never-smokers. A statistically significant interaction between alcohol and smoking status applied to the risks of both total cancer incidence ($P=0.018$) and mortality ($P<0.001$).

The HRs for cancer in men were also separately determined for alcohol-related and other cancers (Table 3). As expected, increased risks were more evident for alcohol-related cancers than those for other cancers. In a further analysis restricted to alcohol-related cancers by smoking status, increased risks for the high-alcohol categories were also observed among never smokers, though less than among current smokers.

DISCUSSION

In this cohort study, the lowest risk of cancer was observed among male occasional drinkers, and a linear positive association with increasing ethanol intake was seen, with up to a 61% excess cancer risk among subjects with an ethanol intake of ⩾450 g. The positive association was similar for both the cancer incidence and mortality, but was more striking among current smokers and for alcohol-related cancers. Considerable interaction between smoking and alcohol drinking was observed. On the other hand, no clear association between alcohol drinking and cancer was found in women, probably because few of them were regular drinkers. Among males, nearly 13% of the cancers were considered attributable to heavy drinking (⩾300 g of ethanol per week).

We assigned occasional drinkers to the reference category since nondrinkers were a mixture of never- and ex-drinkers, both of which contained subjects who are unable to drink due to a deficiency in the key enzyme for alcohol metabolism, common in the Japanese population. This might have complicated the interpretation of the results for nondrinkers. We were unable to assess the risk of never- and ex-drinkers separately, since the baseline questionnaire for cohort I did not discriminate abstainers from nondrinkers. Additional analyses using cohort II subjects, however, found that only 2% of all male subjects were ex-drinkers, and also no marked difference in the association among nondrinkers whether ex-drinkers were included or excluded. If this was also the case in cohort I, it seems unlikely that 'never-drinking' would be associated with an increased risk of cancer, though the possible residual confounding effects cannot be ruled out. Approximately half of the Japanese individuals were found to have a deficient phenotype for aldehyde dehydrogenase-2, a key enzyme in the conversion of acetaldehyde to acetate (Agarwal *et al*, 1981; Shibuya and Yoshida, 1988), resulting in higher levels of acetaldehyde exposure, which is considered to be carcinogenic (IARC, 1988). The fraction of cancer risk attributable to alcohol might therefore be greater among Japanese than among Western drinkers.

Previous studies have indicated that the effect of alcohol drinking does not appear to be due to any specific type of alcoholic beverage, but rather due to ethanol itself. In our study, most male regular drinkers drank two or more types of alcohol; 36% of the total ethanol intake among these subjects was from Japanese sake (rice wine), 33% from Japanese hard liquor, 24% from beer and 7% from whisky, whereas <1% was from wine and other alcoholic beverages.

Our results indicate that the combination of alcohol drinking and smoking is associated with a particularly increased risk of cancer and presumably makes a major contribution to both incidence and mortality of the overall cancer risk, while no such tendency was detected among never-smokers. Except for our previous work, studies of the effect of interaction between drinking and smoking on the total cancer risk in the Japanese are sparse (Tsugane *et al*, 1999; Hara *et al*, 2002). CYP2E1, the expression of which is induced by alcohol, metabolises procarcinogens, such as *N*-nitroso compounds, present in tobacco smoke and foods (Anderson *et al*, 1994); it also catalyses the conversion of alcohol to acetaldehyde. Animal experiments have suggested that carcinogens in tobacco smoke are metabolised more slowly among drinkers (Van de Wiel *et al*, 1993; Anderson *et al*, 1994). Although interaction between alcohol and smoking may greatly contribute to the risk of both cancer incidence and mortality, alcohol may also be an independent risk factor, at least for alcohol-related cancers.

The major strengths of our study were its prospective design, its high response rate, and the negligible proportion of losses to follow-up. The collection of alcohol details before cancer diagnosis precluded the exposure recall bias inherent in case–control studies. However, misclassification of the self-reported alcohol due to modified alcohol-drinking behaviour during the study period is possible. However, these would probably be nondifferential and may underestimate the true relative risk. Although the quality of the cancer registry system was satisfactory during the study period, there was some geographical variation by study area, so this was adjusted for in the analysis. We also confirmed that the quality of the registry was not affected by drinking status at baseline, so underreporting of cancer should also have been nondifferential.

Our cohort study found that an increased ethanol intake substantially elevates the risk of total cancer, but this effect appeared to be largely due to interactions with smoking. For cancer prevention therefore, combined cessation of smoking and alcohol drinking is important for the reduction of cancer risk.

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Appendix

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Active and passive smoking and breast cancer risk in middle-aged Japanese women

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To examine the hypothesis that tobacco smoke is associated with the risk of female breast cancer, we estimated the relative risks of active and passive smoke in middle-aged Japanese women in a population-based prospective study. The cohort consisted of residents in 4 public health center areas, aged 40 to 59 years. A self-administered questionnaire survey was conducted in 1990. This analysis included 21,805 subjects, 180 of whom had developed breast cancer by December 31, 1999. When the reference was defined as never-active smokers without passive smoking, adjusted relative risks (RRs) were 1.9 (95% confidence interval [CI] = 1.0–3.6) in current active smokers, 1.2 (95% CI = 0.4–4.0) in ex-active smokers and 1.2 (95% CI = 0.8–1.6) in never-active smokers with passive smoking. The elevated risk for ever-smokers was clearly observed in premenopausal women at baseline (RR = 3.9, 95% CI = 1.5–9.9) but not in postmenopausal women (RR = 1.1, 95% CI = 0.5–2.5). In never-active smokers, the adjusted RR for passive smoking, residential or occupational/public tobacco smoke exposure was 1.1 (95% CI = 0.8–1.6). In premenopausal women, passive smoking increased the risk (RR = 2.6; 95% CI = 1.3–5.2) but not in postmenopausal women (RR = 0.7; 95% CI = 0.4–1.0). We conclude that tobacco smoking increases the risk of female breast cancer in premenopausal women.

Key words: breast neoplasms; smoking; passive smoking; cohort study

Because most established risk factors for female breast cancer cannot be modified, the etiological role of tobacco smoking has been of interest in the public health field. As shown in a recent general comment by WHO's Executive Director, the link between smoking and breast cancer has been elusive; some studies have suggested a positive link, others found no relationship and a few have suggested that smoking has protective effects.[1] A positive association has been observed in some previous case-control studies.[2–7] In contrast, little relationship has been reported by cohort studies.[8–11] Theoretically, a cohort study provides better evidence compared to a case-control study, but the limitations, *e.g.*, reference category and misclassification of smoking habits, in recent cohort studies are still under dispute.[12–15]

Tobacco smoke is well known to contain numerous possible carcinogens.[16] Although they do not directly contact mammary cells, many studies utilizing biomarkers have demonstrated that tobacco-related carcinogens reach human breast tissue.[17–19] On the other hand, antiestrogenic effects of tobacco smoke have been suggested by many published observations.[20–23] Thus, the exposure may decrease the breast cancer risk, especially in postmenopausal women.[24,25]

The objective of our study was to examine the hypothesis that tobacco smoking is associated with the risk of female breast cancer. We estimated the risks of active and passive smoking among middle-aged Japanese women in a population-based cohort study. The influence of tobacco smoke as a breast cancer risk was elucidated by menopausal status at the baseline survey of the study.

Material and methods

Study cohort

The study cohort is part of the Japan Public Health Center (JPHC)-based prospective study on cancer and cardiovascular diseases (JPHC Study, cohort I) established on January 1, 1990. The study population was defined as Japanese residents aged 40–59 years, 27,063 men and 27,435 women, in 14 administrative districts in 4 PHC areas across Japan.[26] After the initiation of the study, 37 women were found to be ineligible and were excluded, leaving 27,398 women eligible for the study. Study procedures were approved by the ethics committee of the National Cancer Center, Tokyo, Japan.

Baseline survey

A self-administered questionnaire was distributed mostly by hand and partly by mail to the subjects in 1990. They were asked about their personal and familial medical histories, smoking habit, alcohol consumption, dietary habits and other lifestyle factors. A total of 22,482 women responded to the survey (82.1% response rate). Although the date of questionnaire completion ranged from January 1990 to May 1992, 54% responded between February 1990 and March 1990. Only 4% of questionnaires were completed after October 1990. The questions on active smoking consisted of current and former smoking status, age at initiation of smoking, average number of cigarettes smoked per day and age at cessation of smoking for former smokers. Questions on passive smoking were in 2 parts: a) "Have you lived with any regular smokers?" and age at exposure ($\leq$ 20 years old, $>$ 20 years old, both) and b) " In places outside the home, *e.g.*, at work, how often are you exposed to environmental tobacco smoke $\geq$ 1 hr/day?" (almost never, 1 to 3 days/month, 1 to 4 days/week, almost everyday).

Follow-up and identification of breast cancer

We followed the subjects from recruitment until December 31, 1999. In Japan, all death certificates are submitted to a local government office and forwarded to the PHC in the area of residence. Mortality data are then sent to the Ministry of Health, Labour and Welfare and coded for inclusion in the National Vital Statistics. The registration of deaths in Japan is required by the Family Registration Law and is theoretically complete. Therefore, all deaths of the subjects were based upon death certificates from each PHC, when they remained in the original area. Changes in residence status were identified annually through the residential registry in each area. Collection of cancer incidence data and migration data was described in a previous report.[27] Briefly, on January 1, 1990, a specific cancer registry for the JPHC Study was

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established to collect cancer incidence data on the study subjects living within the study area *via* voluntary reports from local major hospitals, on-site visits to the hospitals and records from the prefecture-wide population-based cancer registry, if available (Akita and Nagano Prefectures do not have a prefecture-wide cancer registry). Cancer incidence data were collected only for subjects who were living within the study area. Site of origin and histologic type were coded using the International Classification of Disease for Oncology, second edition (ICD-O-2). By December 31, 1999, 226 new breast cancer cases had been identified. Twelve carcinoma *in situ* were not included among these breast cancer cases. A diagnosis of breast cancer was histologically confirmed in 97% of the cases. The incidence/mortality ratio in the cancer registration was 5.4, and no cases were ascertained by death certificate alone [Death Certificate Only (DCO)]. In 1.1% of cases the subjects' death certificates were used as a supplementary information source for the registry [Death Certificate Notification (DCN)]. The estimated completeness of the registration was 91.8%, which suggested that the completeness for this cohort was reasonably high.[28,29]

Migration data were obtained from residential registries. Among non-case study subjects, 1,837 (6.7%) moved out of the study area and 34 (0.1%) were lost to follow-up within the study period.

Data analysis

From the 22,482 subjects, we excluded 612 more (including 12 breast cancer cases) with a past history of cancer in any site. Consequently, after excluding still another 53 subjects who submitted incomplete information on active or passive smoking status, a total of 21,805 subjects, 180 of whom developed breast cancer, were included in this analysis. Person-years of follow-up were counted from the date of questionnaire completion until the dates of a diagnosis of breast cancer, migration out of the study areas, death or the end of the study (December 31, 1999), whichever came first.

The relative risk (RR) and 95% confidence interval (CI) were estimated by the Cox proportional hazards model, adjusting for age and area according to the SAS PHREG procedure (SAS Institute, Inc., Cary, NC). For further adjustment, we incorporated additional possible confounders into the model; education level (≥ high school and < high school), employment status (employed and unemployed), body mass index (< 22, < 25, and ≥ 25), family history of breast cancer in mother or sisters, history of past benign breast disease, age at menarche, number of births (0, ≥ 1), menopausal status (pre and post), hormone use and alcohol consumption per week (< once/week, < 250 g/week, ≥ 250 g/week). Concerning body mass index and the number of births, influence on the estimates was similar between the categorical and continuous variables. Height, weight, fruit and vegetable intake and physical activity had little influence on the estimates and thus were omitted from the adjustment in the final analysis. Breast-feeding was not incorporated in the adjustment factors because it was not included in the questionnaire. We coded current occupations recorded in an open-end column in the questionnaire according to a major occupational category (Standard Occupational Classification for Japan, the third revision of 1997, Statistic Bureau, The Ministry of Public Management). The occupational categories consisted of professionals and technicians; managers; clerks; shop and market sales workers; service workers; security workers; agricultural, forestry and fishery workers; transport and communication workers; assemblers and manual laborers; workers unclassified and unemployed. Most agricultural, forestry and fishery workers were farmers. In the analysis concerning active smoking, passive smoking was defined as a history of exposure to residential sidestream smoke in any period or exposure to sidestream smoke (almost everyday) in any occupational and/or public setting.

After excluding from the analyses 6 cases whose pathological information was uncertain, we obtained results similar to those presented.

Results

Among the 21,805 women, the prevalence of current, ex- and never-active smokers was 5.7%, 1.7% and 92.6%, respectively. Among never-active smokers, 69% reported that they had been exposed to sidestream smoke (Table I). Table II compares known risk factors and possible confounders for breast cancer among 4 categories of smoking status. These factors included characteristics reported in the literature to be risk factors, and most of them served as adjustment factors in further statistical analyses. Table III shows RRs of incidence according to active smoking. Without taking account of passive smoking in the reference category, the adjusted RR for current active smokers was 1.7 (95% CI = 1.0–3.1). When the reference condition was defined as never-active smokers without passive smoking, a 2-fold risk was observed among current active smokers (adjusted RR = 1.9; 95% CI = 1.0–3.6). Stratified analyses by employment status showed the following adjusted RRs; 1.0 (95% CI = 0.5–2.0) for unemployed women with passive smoking, 0.8 (95% CI = 0.2–3.9) for unemployed women with active smoking, 1.2 (95% CI = 0.8–1.9) for employed women with passive smoking and 2.3 (95% CI = 1.1–4.8) for employed women with active smoking. After omitting the first 3 years after the study baseline to exclude possibly ill subjects, we observed similar results (data not shown).

In premenopausal women at baseline, ever-active smokers showed a 4-fold increased risk (adjusted RR = 3.9; 95% CI = 1.5–9.9); never-active smokers with passive smoking also exhibited a significantly increased risk (adjusted RR = 2.6; 95% CI = 1.3–5.2)compared to never-active smokers without passive smoking. Stratified analyses by employment status showed increased risk for active and passive smoking in both unemployed and employed women; adjusted RR = 4.4 (95% CI = 0.6–34.6) for unemployed women with passive smoking; 7.9 (95% CI = 0.7–90.8) for unemployed women with ever-active smoking, 2.3 (95% CI = 1.1–4.9) for employed women with passive smoking and 3.3 (95% CI = 1.2–9.4) for employed women with ever-active smoking.

In postmenopausal women at baseline, no significant increased risk was observed for ever-active smokers (adjusted RR = 1.1; 95% CI = 0.5–2.5). Stratified analyses by employment status showed the following adjusted RRs; 0.6 (95% CI = 0.3–1.3) for unemployed women with passive smoking, 0.3 (95% CI = 0.04–2.6) unemployed women with ever-active smoking, 0.7 (95% CI = 0.4–1.2) for employed women with passive smoking and 1.5 (95% CI = 0.6–3.9) for employed women with ever-active smoking. When ex-smokers were eliminated from the statistical model because of the small number of cases and person-years, the risk of smoking remained essentially unchanged (data not shown).

TABLE I – SMOKING STATUS IN FEMALE STUDY SUBJECTS: JPHC STUDY COHORT I

Passive smoking	Active smoking		
	Never-smokers (n = 20169)	Ex-smokers (n = 374)	Current smokers (n = 1238)
Residential passive smoking (%)[1]			
Never	6175 (31.0)	79 (21.4)	234 (19.1)
Ever			
Before age 20	2231 (11.2)	54 (14.6)	225 (18.4)
After age 20	6957 (35.0)	136 (36.8)	444 (36.3)
Both	4536 (22.8)	101 (27.3)	320 (26.2)
Passive smoking in occupational and/or public settings (%)[2]			
Almost never	13626 (68.0)	199 (53.6)	553 (44.8)
1–3 days/month	1534 (7.7)	29 (7.8)	76 (6.2)
1–4 days/week	1057 (5.3)	25 (6.7)	76 (6.2)
Almost everyday	3811 (19.0)	118 (31.8)	529 (42.9)

[1]Missing and unavailable answers were omitted from the calculation; 270 in never-smokers, 4 in ex-smokers, 15 in current smokers.– [2]Missing were omitted from the calculation; 141 in never-smokers, 3 in ex-smokers, 4 in current smokers.

TABLE II – DISTRIBUTION OF KNOWN RISK FACTORS AND POSSIBLE CONFOUNDERS FOR BREAST CANCER BY SMOKING STATUS: JPHC STUDY COHORT 1

	Never-smokers		Ex-smokers (n = 374)	Current smokers (n = 1238)	p for trend[1]
	Without passive smoking (n = 5660)	With passive smoking (n = 14533)			
Age (mean)	49.9	49.6	49.1	48.6	<0.0001
Occupation, farmer (%)[2]	1281 (23.4)	3,014 (21.2)	46 (12.5)	131 (10.9)	<0.0001
Occupation, unemployed (%)[2]	2850 (52.1)	6,423 (45.2)	164 (44.6)	494 (41.2)	<0.0001
Education (> high school, %)[2]	597 (10.9)	1,746 (12.4)	68 (18.7)	140 (11.8)	0.02
Height (mean)	151.1	151.8	152.3	152.2	<0.0001
Weight (mean)	54.3	54.2	55.8	54.2	0.58
Body mass index (mean)	23.7	23.5	24.1	23.3	<0.0001
Family history of breast cancer in mother or sisters (%)[2]	18 (0.3)	90 (0.6)	3 (0.8)	5 (0.4)	0.18
History of past benign breast disease (%)[2]	455 (8.0)	1,525 (10.5)	40 (10.7)	98 (7.9)	0.08
Age at menarche (mean)	14.7	14.6	14.4	14.8	0.30
Parous women (%)[2]	4,922 (93.3)	13,063 (95.2)	307 (89.5)	1,043 (90.7)	0.04
Age at first delivery among parous women (mean)	25.0	24.9	25.5	24.5	<0.0001
Number of deliveries among parous women (mean)	2.9	2.9	2.8	2.9	0.29
Menopausal status (postmenopausal, %)[2]	3,045 (55.2)	7,734 (54.2)	189 (51.9)	602 (49.4)	<0.001
Previous and/or current hormone use (%)[2]	1,114 (21.0)	2,786 (20.4)	82 (23.0)	258 (22.1)	0.58
Alcohol consumption per week (mean grams)	79.2	115.7	164.0	239.3	<0.0001

[1]p for trend was calculated by Cochran-Mantel-Haenszel test.– [2]Missing were omitted from the calculation; 619 in occupation, 743 in education, 53 in family history of breast cancer, 53 in history of past benign breast disease, 1,369 in child birth, 473 in menopausal status and 1,369 in hormone use.

TABLE III – RELATIVE RISK OF FEMALE BREAST CANCER ACCORDING TO ACTIVE SMOKING: 10-YEAR FOLLOW-UP IN JPHC STUDY COHORT 1

Exposure	Number of case	Person-years	RR[1] (95% CI)	RR[2] (95% CI)
Pre-and post-menopausal women at baseline:				
Never-smoker	162	187,063	1.0	1.0
Ex-smoker	4	3,344	1.4 (0.5 to 3.8)	1.1 (0.4 to 3.5)
Current smoker	14	10,901	1.5 (0.9 to 2.6)	1.7 (1.0 to 3.1)
Pre-and post-menopausal women at baseline:				
Never-smoker without passive smoking	40	52,884	1.0	1.0
Never-smoker with passive smoking	122	134,178	1.2 (0.8 to 1.7)	1.1 (0.8 to 1.6)
Ex-smoker	4	3,344	1.6 (0.6 to 4.5)	1.2 (0.4 to 4.0)
Current smoker	14	10,901	1.7 (0.9 to 3.1)	1.9 (1.0 to 3.6)
Premenopausal women at baseline:				
Never-smoker without passive smoking	9	22,982	1.0	1.0
Never-smoker with passive smoking	68	60,272	2.9 (1.4 to 5.8)	2.6 (1.3 to 5.2)
Current- + ex-smoker	11	6,907	4.1 (1.7 to 9.9)	3.9 (1.5 to 9.9)
Postmenopausal women at baseline:				
Never-smoker without passive smoking	31	28,583	1.0	1.0
Never-smoker with passive smoking	52	71,602	0.7 (0.4 to 1.0)	0.6 (0.4 to 1.0)
Current- + ex-smoker	7	7,056	0.9 (0.4 to 2.1)	1.1 (0.5 to 2.5)

[1]Relative risks adjusted for public health center (4 areas) and age (4 5-year age groups).– [2]Relative risks adjusted for public health center, age, employment status (employed and unemployed), education level (≥high school and <high school), body mass index (<22, 22 ≤<25 and ≥25), family history of breast cancer in mother or sisters, history of past benign breast disease, age at menarche, number of births (0 and ≥1), menopausal status (pre and post), hormone use and alcohol consumption per week (<once/week, <250 g/week and ≥250 g/week).

Table IV shows RRs of incidence according to passive smoking status. Adjusted RR for any passive smoking was 1.1 (95% CI = 0.8–1.6). In premenopausal women at baseline, those with any passive smoking revealed a significantly increased risk (adjusted RR = 2.6; 95% CI = 1.3–5.2), and exposure to sidestream smoke in occupational and/or public settings itself showed increased risk (adjusted RR = 2.3; 95% CI = 1.4–3.8). Concerning passive smoking in occupational and/or public settings in premenopausal women, a dose-dependent increase was found (adjusted RR = 1.0 for "almost none"; 0.6 [95% CI = 0.4–2.4] for "1 to 3 days/month", 2.2 [95% CI = 1.4–3.7] for "≥ 1 days/week", p for trend 0.002). Past exposure to sidestream smoke at home did not show an increased risk. Among postmenopausal women at baseline, RRs for passive smoking were 0.7 (95% CI = 0.4–1.0), and those exposed to sidestream smoke in an occupational and/or public setting showed a marginal decreased risk (adjusted RR = 0.5; 95% CI = 0.2–1.0).

Discussion

In the present population-based prospective study of middle-aged Japanese women, an increased risk for active premenopausal smoking women was observed, especially when the reference was defined as never-active smokers without exposure to sidestream smoke. A subgroup analysis revealed that only premenopausal women at the study baseline showed increased risks from passive smoking. These findings were independent of reproductive risk factors and other potential confounders. In previous case-control studies, the risk for active and passive smoking was equivalent,[3,4,6,7] which seems to be implausible. However, the estimated risk for active smoking was larger than that for passive smoking in our study.

Breast cancer risks differ based on menopausal status.[30] Thus, the risk factors and the magnitude of their risk may be different before and after menopause. The etiological roles of endogenous

TABLE IV – RELATIVE RISK OF FEMALE BREAST CANCER ACCORDING TO PASSIVE SMOKING IN FEMALE NEVER-SMOKERS; 10-YEAR FOLLOW-UP IN JPHC STUDY COHORT I

		Passive smoking		
	Never	(A) Past residential exposure (in any period)	(B) Occupational and/or public exposure (everyday)	(A) or (B)
All never-smokers				
Number of cases	40	114	37	122
Person-years	50,662	127,309	35,258	134,299
RR[1] (95% CI)	1.00	1.1 (0.8 to 1.5)	1.3 (0.9 to 1.8)	1.2 (0.8 to 1.7)
RR[2] (95% CI)	1.00	1.0 (0.7 to 1.4)	1.3 (0.9 to 1.9)	1.1 (0.8 to 1.6)
Premenopausal women at baseline:				
No. of cases	9	61	28	68
Person-years	22,263	56,896	17,884	60,320
RR[1] (95% CI)	1.00	1.7 (1.0 to 3.0)	2.1 (1.3 to 3.4)	2.9 (1.4 to 5.8)
RR[2] (95% CI)	1.00	1.6 (0.9 to 2.7)	2.3 (1.4 to 3.8)	2.6 (1.3 to 5.2)
Postmenopausal women at baseline:				
Number of cases	31	51	8	52
Person-years	27,345	68,364	16,625	71,674
RR[1] (95% CI)	1.00	0.7 (0.4 to 1.1)	0.5 (0.3 to 1.1)	0.6 (0.4 to 1.0)
RR[2] (95% CI)	1.00	0.7 (0.4 to 1.1)	0.4 (0.2 to 1.0)	0.7 (0.4 to 1.0)

[1]Relative risks adjusted for public health center (4 areas) and age 4 5-year age group).– [2]Relative risks adjusted for public health center, age, employment status (employed and unemployed), education level (≥high school and <high school), body mass index (<22, 22 ≤<25 and ≥25), family history of breast cancer in mother or sisters, history of past benign breast disease, age at menarche, number of births (0 and ≥4), menopausal status (pre and post), hormone use and alcohol consumption per week (<once/week, <250 g/week and ≥250 g/week).

hormones admit of no doubt, and a causal model of breast cancer suggested that hormones increased the breast cancer risk in adults by increasing cell proliferation and the number of target cells, and also heightened the risk of the retention of spontaneous somatic mutations.[31] Therefore, higher levels of estrogens in premenopausal women may act jointly with exogenous carcinogens in breast carcinogenesis. The carcinogenic effects of tobacco smoke may result from a balance between its carcinogenic and anti-estrogenic effects.[6] Therefore, premenopausal women are likely to be affected by tobacco carcinogens because their estrogen levels are higher, thereby possibly canceling out the anti-estrogenic effects of tobacco smoke.

Smoking was reported to be associated with a decrease in the incidence of endometrial neoplasia in postmenopausal women.[23] The net effect of tobacco smoke may be antiestrogenic in the endometrium. However, available evidence, excluding 1 prospective study in Japan,[32] indicates that smoking has no beneficial effects in the breast. We did not observe statistically significant beneficial effects in the present study. However, our data suggest that at least the carcinogenic effects of tobacco smoke are not present in postmenopausal women.

Active and passive smoking are influenced by socioeconomic status.[33,34] Occupation is in fact related to smoking habits especially in women; working women generally smoke more and are exposed to sidestream smoke more frequently. Indeed, smoking status differed among several occupation-related factors in this cohort. A stratified analysis by employment status revealed interesting findings. In postmenopausal women, increased risk was observed only in employed women, although the small numbers of cases in the subgroup analyses precluded firm conclusions. Their pack-years were comparable (employed 10 ± 11 and unemployed 13 ± 13). These findings suggest that there were unknown residual confounders or different smoking behavior in these 2 groups. Risks for passive smoking were not increased in either employed or unemployed postmenopausal women. However, in premenopausal women, risks for active or passive smoking were increased in both employed and unemployed women. These findings suggest that any tobacco smoke exposure elevated the risk in premenopausal women no matter what their occupation. Educational level can be a surrogate indicator of socioeconomic status and has been reported as one of the important risk factors for breast cancer. Although we incorporated employment status and educational level into our statistical models, unknown residual confounders concerning socioeconomic status might not necessarily have been excluded from our analysis.

In our study, past exposure to sidestream smoke at home showed different effects from those by the occupational/social exposure. Residential exposure was defined as "a smoker(s) who had lived with a subject", although the current occupational/social exposure was assessed semi-quantitatively by self-report. Intensity or duration of daily exposure could not be estimated for the residential exposure. Previous cohort studies in Japanese women also used the smoking status of husbands as an index of passive smoking and did not observe elevated risk.[32,35]

The limitations of previous case-control studies were that recall and selection bias would tend to produce spurious positive association.[11] On the other hand, the limitations of previous cohort studies including misclassification of exposure and reference category have also been pointed out.[12–15] However, a well-designed prospective study is known to provide persuasive evidence. Our prospective study design also has some advantages in estimating the risks of smoking. Although recall bias may exist with information concerning passive smoking in a case-control study, there was no recall bias in our study because of its prospective nature. Never-active smokers without passive smoking were assigned to the reference, allowing for more accurate classification of exposure. Nonresidential passive smoking, *i.e.*, occupational or public exposure to tobacco smoke, was taken into account in the analyses. Subgroup analyses concerning menopausal status were done because the combined analyses may dilute the risk estimation.

On the other hand, there are some admitted limitations. Because the exposure assessment was done at 1 point (at baseline), a misclassification of the exposure might have occurred, thereby diluting the effects if some smoking women had quit smoking during the follow-up period. Information on the menopausal status was obtained at baseline. Therefore, we did not examine the risks for pre- and post-menopausal cancer. The relatively small number of incidence cases precluded further subgroup analyses. Results of the subgroup analyses according to menopausal status in this report should be confirmed by continued follow-up.

Different effects of active or passive smoking regarding breast cancer risk had been shown in premenopausal and postmenopausal women.[7,36] In a recent study, the risk of breast cancer among smokers has been clearly reported to be elevated in premenopausal women.[36] Immature breast cells are suggested to have especially increased susceptibility to smoking-related carcinogens.[6] In our

study, 94% of subjects had delivered children, but the effect of smoking in strata defined by age of full-term birth could not be examined. On the other hand, in postmenopausal women, the risk of breast cancer among smokers has been reported not to be elevated.[36] These previous observations are consistent with our observations regarding both active and passive smoking. Race is also an important factor in the interpretation of our results. To our knowledge, this is the first prospective study to link active smoking to breast cancer risk in Asian women, although recent large-scale cohort studies in America did not detect any increased risk of breast cancer.[10,11] Genetic differences concerning important metabolic enzymes, for example, higher frequency of a variant allele of cytochrome P450 1A1 gene, were reported,[37] and endogenous estrogen levels and the number of estrogen receptors have been reported to differ between Japanese and Caucasians.[38,39] Thus, an association between smoking and breast cancer might appear more readily in Japanese. The incidence of breast cancer among premenopausal women (88/90,161 person-year) was almost the same as that among postmenopausal women (90/107,241 person-year), and the association observed in premenopausal women was strong. These might be why we observed an elevated risk due to tobacco smoking in the overall subjects.

In conclusion, tobacco smoking increases the risk of female breast cancer in premenopausal women. Both active and passive smoking are promising targets in the prevention of breast cancer.

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Fish, Long-Chain n-3 Polyunsaturated Fatty Acids, and Risk of Colorectal Cancer in Middle-Aged Japanese: The JPHC Study

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Abstract: *Although long-chain n-3 polyunsaturated fatty acids (Ln-3 PUFA), which are abundant in fish, have shown protective effects on colorectal cancer in laboratory studies, epidemiological studies to date have not been consistent. We evaluated the relationship of consumption of fish and Ln-3 PUFA to the colon and rectal cancer risk in the two cohorts of the Japan Public Health Center-based prospective study of 42,525 men and 46,133 women. Dietary and other exposure data were obtained between 1990 and 1994. Through December 1999, 705 cases of colon and rectal cancer were documented. When data from the two cohorts were pooled, multivariable relative risks (RRs) for the highest quartile compared with the lowest quartile of fish consumption were 1.07 (95% confidence interval, CI = 0.77–1.48) for colon cancer and 0.95 (95% CI = 0.63–1.43) for rectal cancer with no dose-risk trend. RRs for the highest quartile compared with the lowest quartile of eicosapentaenoic acid consumption were 1.05 (95% CI = 0.76–1.46) for colon cancer and 0.91 (95% CI = 0.60–1.38) for rectal cancer with no dose-risk trend. This study does not support the role of fish and Ln-3 PUFA in the etiology of colon and rectal cancer in this population whose fish consumption was high and the variation in Ln-3 PUFA consumption was large.*

Introduction

Colorectal cancer is the second most common cause of cancer incidence and mortality in more developed countries. An estimated 945,000 new cases are diagnosed worldwide each year, with 492,000 deaths (1). Lifestyle factors have been suggested to play important roles in its etiology and prevention (2).

From the results of in vitro and in vivo studies, along with those of animal studies, it is evident that long-chain n-3 polyunsaturated fatty acids (Ln-3 PUFA), which are abundant in fish, have protective effects on colorectal cancer (3–5). In contrast, previous epidemiological studies did not provide sufficient evidence for an association between fish intake and colorectal cancer (6,7). Furthermore, only a few studies have examined the association between Ln-3 PUFA and colorectal cancer. In a recent prospective study of Swedish women, none of the specific fatty acids examined were associated with colorectal cancer (8). However, Ln-3 PUFA intake was not so high in the subjects of the Swedish prospective study.

Japanese are characterized by high fish consumption and a large variation in Ln-3 PUFA consumption compared with Western populations (9,10). To further examine the association between fish and Ln-3 PUFA consumption and the risk of colorectal cancer, we conducted a population-based, prospective cohort study in Japan.

Methods

Study Cohort

The Japan Public Health Center-based prospective study on cancer and cardiovascular disease (JPHC study) started in 1990 for Cohort I and in 1993 for Cohort II. Cohort I consisted of five Public Health Center (PHC) areas (Iwate, Akita, Nagano, Okinawa, and Tokyo), and Cohort II consisted of six PHC areas (Ibaraki, Niigata, Kochi, Nagasaki, Okinawa, and Osaka) across Japan. We excluded the subjects in Tokyo and those in Osaka because different definitions of study population were applied. For the remaining nine PHC areas, study populations were defined to be all inhabitants in the study areas aged 40–59 yr old in Cohort I and 40–69 yr old in Cohort II at the beginning of each study (January 1, 1990, in Cohort I and January 1, 1993, in Cohort II). As a whole, a population-based cohort of 57,714 men (27,063 in Cohort I and 30,651 in Cohort II) and 59,182 women (27,435 in Cohort I and 31,747 in Cohort II) was established. After the initiation of the study, 123 men and 79 women were found to be ineligible and were thus excluded (49 persons of non-Japanese na-

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tionality, 151 with delayed reports of out-migration before the start of the follow-up, and 2 with mistakenly recorded birthdays), leaving 57,591 men and 59,103 women eligible for the study.

Exposure Data

A self-administered questionnaire, which included dietary habits, previous medical history, and other lifestyle factors, was distributed to all registered residents in 1990 for Cohort I and in 1993–1994 for Cohort II (11,12). Among the eligible subjects, 45,452 men (79%) and 49,924 women (84%) responded to the survey.

The average frequency of consumption of 44 food items by Cohort I and 52 food items by Cohort II was reported during the previous month. The Cohort I questionnaire included four items on fish ("fresh fish," "dried and salted fish," "fish roe," and "fermented fish products") and used four categories: almost never, 1–2 days/wk, 3–4 days/wk, and almost daily. The Cohort II questionnaire included six items on fish ("fresh fish," "dried and salted fish," "fermented fish products," "small fry," "fish paste," and "canned fish") and used five categories: never, less than 1 day/wk, 1–2 days/wk, 3–4 days/wk, and almost daily. Information on fish oil supplements was not asked in this study.

Based on 14- to 28-day diet record data, we developed a food composition table that corresponded to the fish items listed in the questionnaire and determined the portion size for each fish item based on the observed median values (13,14). To calculate the amount of fish and Ln-3 PUFA intakes, four items were used in Cohort I, with a portion size of 100 g for fresh fish (9.7 mg of Ln-3 PUFA) in men and 86 g for fresh fish (8.6 mg of Ln-3 PUFA) in women, 20 g for dried and salted fish (3.2 mg of Ln-3 PUFA) in men and 10 g for dried and salted fish (1.7 mg of Ln-3 PUFA) in women, 20 g for fish roe (4.1 mg of Ln-3 PUFA) in men and 17 g for fish roe (3.7 mg of Ln-3 PUFA) in women, and 20 g for fermented fish products (0.9 mg of Ln-3 PUFA) in men and 10 g for fermented fish products (0.5 mg of Ln-3 PUFA) in women. Six items were used in Cohort II, with a portion size of 100 g for fresh fish (7.6 mg of Ln-3 PUFA) in men and 75 g for fresh fish (4.8 mg of Ln-3 PUFA) in women, 48 g for dried and salted fish (5.3 mg of Ln-3 PUFA) in men and 40 g for dried and salted fish (3.7 mg of Ln-3 PUFA) in women, 10 g for fermented fish products (0.4 mg of Ln-3 PUFA) in men and 10 g for fermented fish products (0.4 mg of Ln-3 PUFA) in women, 8 g for small fry (0.2 mg of Ln-3 PUFA) in men and 7 g for small fry (0.2 mg of Ln-3 PUFA) in women, 25 g for fish paste (0.4 mg of Ln-3 PUFA) in men and 23 g for fish paste (0.3 mg of Ln-3 PUFA) in women, and 24 g for canned fish (1.2 mg of Ln-3 PUFA) in men and 20 g for canned fish (0.6 mg of Ln-3 PUFA) in women.

The validity was assessed among subsamples with 14- to 28-day diet record and blood sample. Spearman correlation coefficients between the diet record and the questionnaires of Cohort I (94 men and 107 women) and Cohort II (176 men and 178 women), respectively, were 0.49 in men and 0.45 in women of Cohort I and 0.33 in men and 0.44 in women of Cohort II for the amount of fish intake (g/day), 0.55 in men and 0.54 in women of Cohort I and 0.38 in men and 0.47 in women of Cohort II for calculated eicosapentaenoic acid (EPA) intake (g/day), 0.52 in men and 0.48 in women of Cohort I and 0.44 in men and 0.54 in women of Cohort II for calculated docosahexaenoic acid (DHA) intake (g/day), and 0.50 in men and 0.36 in women of Cohort I and 0.61 in men and 0.61 in women of Cohort II for the ratio of n-3 to n-6 PUFA (Ln-3/n-6 PUFA). These correlation coefficients were improved after adjusting for within-person variability, treating each weekly dietary record as a unit of observation (10). Spearman correlation coefficients between the serum phospholipid level (percent of total fatty acid) and the questionnaire in men of Cohort I ($n = 83$) were 0.54 for EPA and 0.41 for DHA. The average intakes of fish according to quartiles, which was estimated from diet records, were 81.3, 121.3, 150.3, and 195.7 g in men and 66.8, 93.0, 118.4, and 150.2 g in women of Cohort I. The fish intakes averaged 86.1, 115.8, 140.3, and 196.4 g in men and 68.3, 88.1, 106.5, and 131.5 g in women of Cohort II against those from questionnaires: 14.2, 29.9, 56.7, and 92.2 g in men and 13.4, 25.1, 47.2, and 74.3 g in women of Cohort I and 24.6, 44.5, 66.8, and 109.8 g in men and 20.9, 35.3, 49.8, and 80.9 g in women of Cohort II in the same subjects.

Among 45,452 men and 49,924 women who responded to the questionnaire, 687 men and 1,363 women who reported a past history of cancer and 2,240 men and 2,428 women who reported extreme total energy intake (upper 2.5% or lower 2.5%) were excluded, leaving 42,525 men and 46,133 women for the analysis.

Follow-Up

Colorectal cancer incidence data were collected through two data sources, one from local hospitals and the other from population-based cancer registries. Death certificates were used to supplement the information on cancer incidence.

The cancer incidence data and migration data gathered were described in an early report (15). Up to December 31, 1999, 764 new colorectal cancer cases were identified. For multiple primary cancers of the colon or rectum, only the earliest diagnosis was used. Among these incident cases, 705 were pathologically confirmed as adenocarcinoma (M: 8140, 8210, 8211, 8240, 8243, 8260, 8261, 8262, and 8263 according to the *International Classification of Disease for Oncology*, 2nd ed.) (16). Among non-case study subjects, 5.0% moved out of the study area, and 0.04% was lost to follow-up within the study period.

Statistical Analysis

We computed the colorectal cancer incidence rate for a quartile category of fish or Ln-3 PUFA consumption by dividing the number of colorectal cancer cases by person-years of follow-up. Person-years of follow-up were counted from the start of the study periods of January 1, 1990, for Cohort I

and January 1, 1993, for Cohort II until the date of diagnosis of colorectal cancer, death, moving away from a PHC area, or the end of follow-up (December 31, 1999), whichever occurred first. We employed the Cox proportional-hazards regression model to estimate the relative risk (RRs) using the SAS PHREG procedure (17). RRs were adjusted for the following variables: age (5-yr age groups); PHC areas; smoking status (never, former, current); body mass index, BMI (<19, 19, and <23, ≥23 and <27, ≥27); alcohol consumption (none, occasional, <150 g/wk, ≥150 g/wk); leisure time physical activity (less than once a month and once a month or more); use of supplements for vitamin A, C, or E (yes or no); total energy intake and cereal, vegetable, and meat intake (in quartiles); and family history of colorectal cancer (yes or no).

First, we estimated the RRs for colon cancer and rectal cancer in each cohort because the questionnaire makeup was different for Cohort I and Cohort II, respectively. Second, we pooled the results obtained from the two cohorts and calculated the pooled RRs by the use of a fixed-effects model weighting the two RRs by the inverse of the standard error (18). Tests of heterogeneity were used to evaluate whether associations differed between men and women and between Cohorts I and II. For all the associations, no statistically significant heterogeneity was seen. We repeated all the analyses after excluding the 125 colorectal cancer cases diagnosed in the first 2 yr of follow-up (76 men and 49 women). The *P* values for the test of linear trend were two sided.

Results

The distribution of the baseline characteristics according to quartile of fish intake is shown in Table 1. For Cohorts I and II, men with high fish intake were found to have higher alcohol consumption, whereas women with high fish intake were less likely to be current smokers. Participants with high fish consumption also reported a higher intake of total energy, meat, vegetable, and fruit. However, no appreciable difference was observed in cereal consumption. Fish intake was not linearly associated with age, family history of colorectal cancer, BMI, leisure time physical activity, or vitamin supplement use.

RRs of colon and rectal cancer for quartiles of fish and Ln-3 PUFA consumption from each cohort and from combined cohorts are listed in Table 2 for men and in Table 3 for women. Because area- and age-adjusted and fully adjusted RRs were not substantially changed, we present fully adjusted results. For both men and women, intakes of fish, EPA, and DHA were not associated with colon cancer or rectal cancer for Cohorts I and II. No such association was found either when cohorts were combined. In combined cohorts, adjusted RRs for the highest quartile of fish consumption compared with the lowest quartile in men and women, respectively, were 1.07 (95% confidence interval, CI = 0.72–1.58) and 1.05 (95% CI = 0.61–1.82) for colon cancer and 1.31 (95% CI = 0.78–2.22) and 0.69 (95% CI = 0.35–1.36) for rectal cancer with no dose-risk trend. Adjusted RRs for the highest quartile of EPA consumption compared with the lowest quartile in men and women, respectively, were 1.06 (95% CI = 0.71–1.58) and 1.04 (95% CI = 0.60–1.80) for colon cancer and 1.37 (95% CI = 0.81–2.32) and 0.57 (95% CI = 0.29–1.15) for rectal cancer with no dose-risk trend. Ln-3/n-6 PUFA was also not associated with colon cancer or rectal cancer for each cohort and combined cohorts. In combined cohorts, adjusted RRs for the highest quartile of the Ln-3/n-6 PUFA compared with the lowest quartile in men and women, respectively, were 1.08 (95% CI = 0.74–1.56) and 0.92 (95% CI = 0.55–1.54) for colon cancer and 1.26 (95% CI = 0.77–2.05) and 0.61 (95% CI = 0.31–1.19) for rectal cancer with no dose-risk trend.

The null relation between fish, EPA, DHA, and the Ln-3/n-6 PUFA and colon cancer and rectal cancer was consistent when the data of men and women were combined and the data of Cohorts I and II were combined. Adjusted RRs for the highest quartile of fish consumption compared with the lowest quartile were 1.07 (95% CI = 0.77–1.48) for colon cancer and 0.95 (95% CI = 0.63–1.43) for rectal cancer. RRs for the highest quartile of EPA consumption compared with the lowest quartile were 1.05 (95% CI = 0.76–1.46) for colon cancer and 0.91 (95% CI = 0.60–1.38) for rectal cancer with no dose-risk trend.

We next excluded the 125 colorectal cancer cases diagnosed during the first 2 yr of follow-up. None of the results changed substantially (data not shown).

Discussion

In our population-based cohorts of Japanese men and women with high and varied consumption of fish, intake of fish was not associated with any decreased incidence of colon or rectal cancer. Furthermore, we did not obtain evidence of any appreciable benefit from Ln-3 PUFA, such as EPA and DHA, or the Ln-3/n-6 PUFA.

Although the association of total fish consumption with colon and/or rectal cancer incidence has been considered in many previous epidemiological studies, results have not been consistent. Of 15 case-control studies that evaluated the association of fish intake with colon and/or rectal cancer risk, 3 studies found an inverse association (19–21), 2 studies showed results that were inconsistent between men and women (22,23), and 10 studies found no substantial association (24–33). We identified nine prospective studies that evaluated the association of fish intake with colon and/or rectal cancer risk. Our findings are in general agreement with those of eight prospective studies, which show no substantial association with risk of colon and/or rectal cancer (34–41). However, the New York University Women's Health Study showed a significant inverse association for fish intake (42).

There was little evidence of a lower risk of colon and/or rectal cancer with higher intakes of n-3 PUFA. In two case-control studies, one found no substantial association with risk of colon and/or rectal cancer (43), whereas the other study found an inverse association with the consumption of

Table 1. Characteristics of Subjects According to Total Fish Consumption

	Quartiles of Total Fish Consumption							
	Cohort I				Cohort II			
	1 (low)	2	3	4 (high)	1 (low)	2	3	4 (high)
Number of men	5,226	4,345	4,710	5,064	5,665	5,924	5,784	5,807
Age (yr)[a]	49.1 ± 5.9	48.7 ± 6.0	49.5 ± 5.9	50.5 ± 5.9	53.8 ± 9.1	52.3 ± 8.6	53.9 ± 8.7	55.2 ± 8.4
Family history of colorectal cancer (%)	0.7	1.4	1.0	0.8	1.2	1.6	1.4	1.4
Body mass index (kg/m^2)	23.8 ± 2.9	23.3 ± 2.7	23.5 ± 2.7	23.3 ± 2.7	23.5 ± 3.0	23.5 ± 2.9	23.5 ± 2.9	23.5 ± 2.9
Leisure time physical activity (>1 day/month, %)	33.9	35.8	34.5	31.8	31.4	35.4	34.1	29.8
Smoking status (%)								
Never	26.7	21.3	24.9	23.1	26.4	23.9	24.1	23.7
Past	24.6	20.7	23.7	22.0	21.6	23.6	25.4	25.7
Current	48.7	58.0	51.5	54.9	52.0	52.5	50.4	50.6
Alcohol consumption (%)								
None	27.2	18.2	20.9	16.8	32.2	24.4	24.3	22.2
<1 day/week	16.3	9.8	10.5	6.6	11.0	8.6	7.8	6.8
≤150 g/week	19.0	16.9	18.6	15.5	15.8	18.0	16.8	14.6
>150 g/week	37.4	55.2	50.1	61.1	41.0	48.9	51.1	56.3
Vitamin supplement user (%)	5.7	4.5	5.1	6.2	12.5	13.2	13.2	13.1
Total energy intake (MJ)	7.6 ± 2.0	9.0 ± 2.2	8.9 ± 2.1	9.9 ± 2.2	6.3 ± 1.8	7.0 ± 1.8	7.3 ± 1.8	7.8 ± 1.9
Cereal consumption (g)	344.2 ± 109.2	394.1 ± 124.4	377.9 ± 122.9	393.9 ± 126.1	303.2 ± 97.3	319.0 ± 99.8	324.7 ± 102.2	323.9 ± 105.2
Meat consumption (g)	41.8 ± 22.1	43.6 ± 20.8	47.0 ± 23.4	53.0 ± 28.9	20.7 ± 13.7	26.5 ± 13.9	28.1 ± 15.6	29.3 ± 19.4
Vegetable consumption (g)	136.5 ± 88.4	161.3 ± 84.6	177.2 ± 87.0	207.5 ± 100.4	46.6 ± 71.3	57.2 ± 61.5	62.2 ± 67.5	73.8 ± 91.5
Fruit consumption (g)	70.3 ± 85.5	90.0 ± 84.8	94.2 ± 89.4	117.1 ± 122.4	45.0 ± 63.7	55.7 ± 61.6	61.6 ± 67.0	71.1 ± 82.4
Number of women	5,427	4,639	5,459	5,236	6,100	6,584	6,346	6,342
Age (yr)	49.3 ± 5.9	49.3 ± 5.9	49.4 ± 5.8	50.5 ± 5.7	55.2 ± 9.1	53.0 ± 9.1	54.0 ± 8.6	55.5 ± 8.2
Family history of colorectal cancer (%)	0.9	1.0	1.1	0.9	0.7	1.6	1.5	1.3
Body mass index (kg/m^2)	23.8 ± 3.2	23.4 ± 3.1	23.4 ± 3.0	23.5 ± 3.1	23.6 ± 3.4	23.4 ± 3.1	23.5 ± 3.2	23.6 ± 3.3
Leisure time physical activity (>1 day/month, %)	19.8	22	22.3	20.7	24	26.4	26.6	25.7
Smoking status (%)								
Never	91.3	92.3	93.7	94.1	92.2	92.5	93.9	94.0
Past	2.0	1.8	1.6	1.3	1.1	1.0	1.2	1.0
Current	6.7	5.9	4.7	4.6	6.6	6.5	4.9	5.0
Alcohol consumption (%)								
None	83.4	74.6	75.3	76.7	85.6	80.9	81.1	83.1
<1 day/week	10.0	13.0	13.5	11.5	7.1	9.5	8.8	7.0
≤150 g/week	4.9	9.2	9.4	9.0	5.1	7.3	7.6	7.2
>150 g/week	1.7	3.1	1.9	2.9	2.2	2.2	2.5	2.8
Vitamin supplement user (%)	8.5	7.1	7.6	7.7	14.6	15.1	17.2	17.1
Total energy intake (MJ)	5.2 ± 1.1	5.7 ± 1.2	5.9 ± 1.2	6.3 ± 1.2	3.9 ± 0.9	4.4 ± 0.9	4.6 ± 0.9	5.0 ± 0.9
Cereal consumption (g)	218.3 ± 60.7	231.5 ± 64.6	236.9 ± 64.1	235.1 ± 66.3	196.0 ± 51.5	202.1 ± 52.3	205.3 ± 52.7	209.0 ± 54.5
Meat consumption (g)	35.5 ± 19.0	37.4 ± 19.3	38.5 ± 19.7	40.7 ± 24.1	16.9 ± 11.8	21.9 ± 12.1	23.5 ± 13.1	25.3 ± 15.9
Vegetable consumption (g)	148.4 ± 82.1	173.4 ± 81.6	188.6 ± 75.4	207.8 ± 81.7	45.9 ± 59.4	55.5 ± 53.7	62.7 ± 59.2	73.5 ± 74.3
Fruit consumption (g)	98.6 ± 87.3	126.5 ± 90.8	137.3 ± 85.2	148.4 ± 98.6	59.9 ± 62.8	75.4 ± 59.1	85.3 ± 62.7	95.8 ± 66.7

a: Values are reported as means with standard deviations.

Table 2. Multivariate Relative Risks (RR) of Colon and Rectal Cancer According to Fish and N-3 PUFA Consumption: JPHC Study Cohort I Men (1990–1999) and II Men (1993–1999) Combined[a]

	Colon Cancer					Rectal Cancer				
	1 (low)	2	3	4 (high)	Test for Trend *P* Value	1 (low)	2	3	4 (high)	Test for Trend *P* Value
Fish										
Total person-years	84,576	78,034	80,777	85,184						
Total number of cases	63	68	76	93		40	36	29	49	
Median intake (g), Cohort I	21.4	34.2	58.6	104.3						
Median intake (g), Cohort II	15.1	36.4	63.1	111.7						
RR[b] (95% CI), Cohort I	1.00	0.90 (0.54–1.50)	0.99 (0.61–1.61)	0.74 (0.44–1.27)	0.36	1.00	0.68 (0.32–1.44)	0.53 (0.25–1.15)	0.88 (0.42–1.84)	0.75
RR[b] (95% CI), Cohort II	1.00	1.45 (0.82–2.57)	1.46 (0.83–2.57)	1.59 (0.90–2.81)	0.14	1.00	1.72 (0.84–3.53)	1.35 (0.63–2.90)	1.96 (0.94–4.10)	0.14
RR[b] (95% CI), Pooled	1.00	1.11 (0.76–1.63)	1.17 (0.81–1.69)	1.07 (0.72–1.58)	0.73	1.00	1.11 (0.66–1.86)	0.85 (0.50–1.46)	1.31 (0.78–2.22)	0.39
EPA										
Total person-years	81,751	81,744	81,714	83,363						
Total number of cases	61	70	79	90		41	36	26	51	
Median intake (g), Cohort I	0.09	0.17	0.27	0.46						
Median intake (g), Cohort II	0.04	0.11	0.17	0.31						
RR[b] (95% CI), Cohort I	1.00	0.79 (0.47–1.32)	1.01 (0.61–1.69)	0.71 (0.40–1.25)	0.40	1.00	0.74 (0.36–1.52)	0.48 (0.21–1.10)	0.98 (0.45–2.12)	0.99
RR[b] (95% CI), Cohort II	1.00	1.62 (0.92–2.85)	1.46 (0.83–2.59)	1.58 (0.90–2.79)	0.20	1.00	1.37 (0.67–2.78)	1.12 (0.53–2.39)	1.83 (0.90–3.72)	0.14
RR[b] (95% CI), Pooled	1.00	1.09 (0.75–1.60)	1.19 (0.81–1.74)	1.06 (0.71–1.58)	0.74	1.00	1.01 (0.61–1.68)	0.76 (0.43–1.33)	1.37 (0.81–2.32)	0.28
DHA										
Total person-years	81,956	81,639	82,025	82,952						
Total number of cases	65	67	77	91		43	35	28	48	
Median intake (g), Cohort I	0.18	0.28	0.43	0.71						
Median intake (g), Cohort II	0.09	0.20	0.32	0.56						
RR[b] (95% CI), Cohort I	1.00	0.81 (0.49–1.33)	0.89 (0.54–1.48)	0.70 (0.41–1.21)	0.28	1.00	0.73 (0.36–1.50)	0.54 (0.25–1.19)	0.88 (0.41–1.89)	0.76
RR[b] (95% CI), Cohort II	1.00	1.22 (0.69–2.16)	1.38 (0.79–2.40)	1.40 (0.80–2.46)	0.23	1.00	1.12 (0.56–2.25)	0.98 (0.47–2.05)	1.49 (0.74–3.00)	0.33
RR[b] (95% CI), Pooled	1.00	0.97 (0.66–1.41)	1.09 (0.75–1.58)	0.98 (0.66–1.46)	0.93	1.00	0.91 (0.55–1.50)	0.74 (0.43–1.27)	1.17 (0.70–1.96)	0.61
Ln-3/n-6 PUFA										
Total person-years	82,037	81,840	81,962	82,733						
Total number of cases	61	68	78	93		40	31	34	49	
Median intake (g), Cohort I	0.05	0.09	0.12	0.19						
Median intake (g), Cohort II	0.04	0.08	0.12	0.21						
RR[b] (95% CI), Cohort I	1.00	0.94 (0.58–1.52)	1.08 (0.67–1.75)	0.84 (0.51–1.40)	0.61	1.00	1.02 (0.52–2.03)	0.74 (0.35–1.57)	1.17 (0.58–2.38)	0.79
RR[b] (95% CI), Cohort II	1.00	1.22 (0.70–2.13)	1.24 (0.72–2.14)	1.41 (0.83–2.42)	0.23	1.00	0.73 (0.34–1.57)	1.25 (0.64–2.44)	1.34 (0.69–2.63)	0.23
RR[b] (95% CI), Pooled	1.00	1.05 (0.73–1.51)	1.15 (0.80–1.64)	1.08 (0.74–1.56)	0.65	1.00	0.88 (0.53–1.47)	0.99 (0.60–1.64)	1.26 (0.77–2.05)	0.29

a: Abbreviations are as follows: EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; Ln-3/n-6 PUFA, ratio of long-chain n-3 polyunsaturated fatty acid (EPA+DHA) to n-6 polyunsaturated fatty acid (sum of n-6 polyunsaturated fatty acids).

b: Adjusted for age, area, family history of colorectal cancer, BMI, physical activity, smoking status, alcohol intake, use of vitamin supplement, total energy, cereal, vegetable, and meat intake.

Table 3. Multivariate Relative Risks (RR) of Colon and Rectal Cancer According to Fish and N-3 PUFA Consumption: JPHC Study Cohort I Women (1990–1999) and II Women (1993–1999) Combined[a]

	Colon Cancer					Rectal Cancer				
	1 (low)	2	3	4 (high)	Test for Trend *P* Value	1 (low)	2	3	4 (high)	Test for Trend *P* Value
Fish										
Total person-years	91,779	87,154	93,992	92,577						
Total number of cases	37	34	39	46		29	18	27	21	
Median intake (g), Cohort I	18.4	26.3	48.8	91.8						
Median intake (g), Cohort II	12.3	29.6	49.5	84.3						
RR[b] (95% CI), Cohort I	1.00	0.95 (0.48–1.89)	0.81 (0.40–1.65)	1.10 (0.53–2.25)	0.83	1.00	0.48 (0.17–1.32)	0.99 (0.42–2.35)	0.59 (0.21–1.64)	0.63
RR[b] (95% CI), Cohort II	1.00	1.23 (0.59–2.60)	1.46 (0.70–3.06)	1.00 (0.44–2.31)	0.85	1.00	0.87 (0.39–1.96)	0.79 (0.34–1.85)	0.79 (0.32–1.92)	0.57
RR[b] (95% CI), Pooled	1.00	1.07 (0.65–1.78)	1.08 (0.65–1.79)	1.05 (0.61–1.82)	0.77	1.00	0.69 (0.37–1.30)	0.88 (0.48–1.62)	0.69 (0.35–1.36)	0.46
EPA										
Total person-years	91,349	90,565	91,441	92,147						
Total number of cases	37	37	36	46		30	18	28	19	
Median intake (g), Cohort I	0.08	0.14	0.24	0.41						
Median intake (g), Cohort II	0.03	0.07	0.11	0.20						
RR[b] (95% CI), Cohort I	1.00	0.93 (0.47–1.84)	0.80 (0.38–1.68)	1.03 (0.49–2.16)	0.94	1.00	0.46 (0.18–1.24)	0.92 (0.37–2.25)	0.55 (0.20–1.58)	0.55
RR[b] (95% CI), Cohort II	1.00	1.31 (0.63–2.76)	1.29 (0.61–2.72)	1.06 (0.47–2.39)	0.88	1.00	0.78 (0.34–1.77)	0.88 (0.40–1.96)	0.59 (0.23–1.49)	0.34
RR[b] (95% CI), Pooled	1.00	1.09 (0.66–1.80)	1.02 (0.60–1.71)	1.04 (0.60–1.80)	0.87	1.00	0.63 (0.33–1.18)	0.90 (0.49–1.63)	0.57 (0.29–1.15)	0.27
DHA										
Total person-years	91,175	90,914	91,175	92,238						
Total number of cases	37	33	41	45		29	19	27	20	
Median intake (g), Cohort I	0.15	0.26	0.38	0.64						
Median intake (g), Cohort II	0.07	0.13	0.21	0.35						
RR[b] (95% CI), Cohort I	1.00	0.96 (0.48–1.89)	1.15 (0.57–2.30)	1.17 (0.56–2.45)	0.56	1.00	0.99 (0.40–2.46)	1.30 (0.52–3.27)	0.98 (0.34–2.79)	0.89
RR[b] (95% CI), Cohort II	1.00	1.02 (0.48–2.17)	1.28 (0.61–2.67)	0.99 (0.44–2.22)	0.88	1.00	0.47 (0.19–1.14)	0.80 (0.37–1.76)	0.49 (0.19–1.24)	0.27
RR[b] (95% CI), Pooled	1.00	0.98 (0.59–1.63)	1.21 (0.73–2.00)	1.08 (0.63–1.87)	0.59	1.00	0.67 (0.36–1.27)	0.98 (0.54–1.78)	0.66 (0.33–1.33)	0.47
Ln-3/n-6 PUFA										
Total person-years	91,224	90,198	91,199	92,162						
Total number of cases	35	39	41	41		28	23	25	19	
Median intake (g), Cohort I	0.05	0.09	0.12	0.19						
Median intake (g), Cohort II	0.03	0.06	0.09	0.15						
RR[b] (95% CI), Cohort I	1.00	1.42 (0.74–2.72)	1.03 (0.51–2.08)	1.27 (0.64–2.55)	0.78	1.00	0.72 (0.30–1.73)	0.84 (0.35–2.00)	0.80 (0.32–1.99)	0.72
RR[b] (95% CI), Cohort II	1.00	0.64 (0.29–1.39)	1.29 (0.67–2.46)	0.61 (0.28–1.32)	0.55	1.00	1.07 (0.50–2.27)	1.05 (0.49–2.27)	0.44 (0.17–1.19)	0.16
RR[b] (95% CI), Pooled	1.00	1.02 (0.62–1.68)	1.16 (0.72–1.87)	0.92 (0.55–1.54)	0.84	1.00	0.90 (0.51–1.60)	0.95 (0.57–1.69)	0.61 (0.31–1.19)	0.20

a: Abbreviations are as follows: EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; Ln-3/n-6 PUFA, ratio of long-chain n-3 polyunsaturated fatty acid (EPA+DHA) to n-6 polyunsaturated fatty acid (sum of n-6 polyunsaturated fatty acids).

b: Adjusted for age, area, family history of colorectal cancer, BMI, physical activity, smoking status, alcohol intake, use of vitamin supplement, total energy, cereal, vegetable, and meat intake.

n-3 PUFA and a positive association with Ln-6/n-3 PUFA (44). Our findings are in general agreement with three prospective studies that show no substantial association with risk of colon and/or rectal cancer (8,41,45).

It is reported that the protective effects of n-3 PUFA consumption are seen only in areas where fish consumption is high (46). In a recent case-control study, in which participants' fish consumption was relatively high, the protective effects of n-3 PUFA intake were found (44). Although mean daily intakes of fish and Ln-3 PUFA in the present study were higher than those in this case-control study, intakes of fish and Ln-3 PUFA were not associated with a decreased incidence of colon or rectal cancer.

Our questionnaires included only a few fish items, and the estimated portion size might have been small because it was determined based on the observed median values on all fish items from diet record data. Therefore, fish and Ln-3 PUFA intake calculated from the questionnaires were underestimated. However, the correlations between fish and Ln-3 PUFA intake estimated from the questionnaires and those from the 28-day dietary record were reasonably high; also, the correlations between fish and Ln-3 PUFA intake estimated from the questionnaires and Ln-3 PUFA levels of serum phospholipid level appeared high. Thus, the questionnaire appeared to be useful in terms of ranking of subjects, although not so much for absolute intakes. In fact, we earlier observed associations between some dietary factors and the risk of cancer (15,47,48). Therefore, it is unlikely that failure to observe a protective association was due to the crude designs of our questionnaires. However, we could not examine the risk among persons with very low intakes of fish and Ln-3 PUFA because the questionnaire used in the present study complicated the estimation of very low amounts of fish and Ln-3 PUFA intake.

The makeup of Cohort I and Cohort II questionnaires was different in terms of the number of items in the fish and frequency categories. Therefore, the estimated fish and Ln-3 PUFA consumption was different between Cohorts I and II. However, correlations between fish and Ln-3 PUFA intake estimated from the questionnaires and those from the 28-day dietary record were relatively high in both Cohort I and Cohort II.

Our questionnaires did not ask for information on fish oil supplements. However, prevalence of the use of this supplement was only 1.9% among a subgroup of the cohorts who participated in the validation study of the food-frequency questionnaire (Kobayashi, unpublished data). Therefore, the lack of items on fish oil supplements in our questionnaire would not materially distort our observations.

The hypothesis that fish and Ln-3 PUFA might lower the risk of colorectal cancer has been reported (2,49,50). It is known that cyclooxygenase (COX)-1 and COX-2 are among the targets of nonsteroidal anti-inflammatory agents (NSAIDs), and that treatment with NSAIDs is associated with a decrease in COX-2 activity in colon tumors. Ln-3 PUFA inhibits COX-2 and the oxidative metabolism of arachidonic acid to prostaglandin E_2 (PGE_2) (51,52). A recent study reported that retinoid X receptors, a family of nuclear receptors implicated in cancer chemoprevention, are preferentially activated by n-3 PUFA in mouse and human colonocytes (53). On another note, it has been reported that a diet rich in n-6 PUFA increases the frequency of etheno-DNA adducts, which are highly miscoding lesions in mammalian cells and are thought to initiate the carcinogenic process through specific point mutations (54,55). It has also been reported that an increase in n-3 PUFA intake can inhibit the metabolism of n-6 PUFA (5). However, our findings were not consistent with this hypothesis.

It is reported that the shift toward a Western diet usually involves a decrease in n-3 PUFA intake and an increase in n-6 PUFA intake (56). In addition, several animal and human studies reported that reductions in epithelial cell proliferation rates, mammary tumorigenesis, and PGE_2 biosynthesis can best be achieved with a relatively high intake ratio of n-3 to n-6 PUFA (n-3/n-6 PUFA) (57–61). When we calculated the n-3/n-6 PUFA, we did not take into account linolenic acid; hence, the median intake of Ln-3/n-6 PUFA was lower, but the variation was wider than in other studies (43,44). Therefore, it was expected that the possible association between the Ln-3/n-6 PUFA and colon and/or rectal cancer incidence has been clearly shown. Nevertheless, we found no association of the Ln-3/n-6 PUFA with colon and/or rectal cancer.

In conclusion, in a prospective cohort study of middle-aged Japanese, fish and Ln-3 PUFA intake were not associated with colon and/or rectal cancer risk, even though there was a large variation in fish consumption among subjects.

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