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Leisure-time physical activity and breast cancer risk defined by estrogen and progesterone receptor status—The Japan Public Health Center-based Prospective Study

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ABSTRACT

Objective. The study aims to investigate the association between leisure-time physical activity and breast cancer risk in consideration of tumor estrogen-receptor/progesterone-receptor status.

Methods. We conducted a population-based prospective cohort study among 53,578 women in the Japan Public Health Center-based Prospective Study. Leisure-time physical activity was assessed by self-reported questionnaires. A Cox proportional hazards regression model was used to derive relative risks and 95% confidence intervals.

Results. From 1990–1993 to the end of 2007, 652 cases were identified. The breast cancer rates (per 100,000 person-years) in the sedentary groups (≤3 days/month) was 84 in overall, 97 in premenopausal and 75 in postmenopausal women. We observed a statistically significant inverse association between leisure-time physical activity and breast cancer risk (relative risk$_{\geq 3 \text{ days/week vs. } \leq 3 \text{ days/month}}$ = 0.73; 95% confidence interval 0.54–1.00; p_{trend} 0.037), particularly in estrogen receptor+progesterone receptor+ (relative risk 0.43; 0.19–1.00; p_{trend} 0.022), and this inverse trend was apparent among postmenopausal women (relative risk 0.25; 0.06–1.06; p_{trend} 0.041). An inverse trend was also observed between daily total physical activity and postmenopausal estrogen receptor+progesterone receptor+ risk (p = 0.046). Among body mass index ≥25 kg/m^2 group, leisure-time physical activity was associated with decreased risk (relative risk$_{\geq 1 \text{ day/week vs. } \leq 3 \text{ days/month}}$ = 0.65; 0.43–0.97; p_{trend} 0.033).

Conclusion. Active participation in leisure-time physical activity may contribute to a decrease in breast cancer risk, particularly for postmenopausal estrogen receptor+progesterone receptor+ tumors.

Introduction

The latest report of the World Cancer Research Fund (World Cancer Resarch Fund/American Institute for Cancer Resarch, 2007) states that physical activity (PA) probably contributes to a decrease in the risk of breast cancer. The biological mechanisms underlying this inverse association have yet to be confirmed but may partly include the decreased production or bioavailability of endogenous female hormones (McTiernan et al., 2004), or of metabolic-related hormones and growth factors, such as estrogens, insulin (Regensteiner et al., 1991) and insulin-like growth factors (Raastad et al., 2000), which may stimulate cellular proliferation/differentiation in the breast (Bernstein and Ross, 1993; Hankinson et al., 1998). Other proposed mechanisms include an improvement in immune function (Shephard et al., 1995).

Owing to the possible involvement of hormone-related mechanisms, the association has been evaluated with consideration to the estrogen- and progesterone-receptor (ER/PR) status of tumors (Adams et al., 2006; Bardia et al., 2006; Bernstein et al., 2005; Britton et al., 2002; Chlebowski et al., 2007; Dallal et al., 2007; Enger et al., 2000; Lee et al., 2001; Leitzmann et al., 2008; Peters et al., 2009; Schmidt et al., 2008). The majority of studies were conducted among Western populations, however, and the results have been inconsistent.

In Japan, the incidence rate of breast cancer has increased steeply over the last three decades, and this cancer is currently the most

Abbreviations: CIs, confidence intervals; BMI, body mass index; DTPA, daily total physical activity; EFH, exogenous female hormone; ER, estrogen receptor; PA, physical activity; PHC, public health center; PR, progesterone receptor; FFQ, food frequency questionnaire; LPA, leisure-time physical activity; METs, metabolic equivalents; RR, relative risk; SD, standard deviation.

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common cancer (Matsuda et al., 2010). Among Asian populations, however, few epidemiological studies have prospectively evaluated the association in consideration of ER/PR (Suzuki et al., 2010).

We hypothesized that PA may be associated with a decreased risk of breast cancer partly through hormone-related mechanisms, on the basis that PA may lead to a decrease in body fat (Sternfeld et al., 2005), the main source of endogenous estrogen after menopause (Cleland et al., 1985). Here, we evaluated the association between PA and ER/PR-defined breast cancer risk in 53,578 Japanese women in the Japan Public Health Center-based Prospective Study (JPHC).

Methods

Study participants

The JPHC was launched in 1990 to evaluate the association between lifestyle factors, cancer, and cardiovascular disease among the Japanese population. Details have been provided elsewhere (Tsugane and Sobue, 2001). The target population was all Japanese residents aged 40–69 years enrolled in the residential registries of 11 public health centers (PHCs). Two cohorts were enrolled (cohort I, Iwate-Ninohe, Akita-Yokote, Nagano-Saku, Okinawa-Chubu, and Tokyo-Kastushika; and cohort II, Ibaraki-Mito, Niigata-Nagaoka, Kochi-Chuohigashi, Nagasaki-Kamigoto, Okinawa-Miyako, and Osaka-Suita). Initially, 71,698 women were invited. Kastushika (cohort I) could not be included due to a lack of information on cancer incidence ($n=4,178$). We excluded women who did not possess Japanese nationality, moved before the start of follow-up, were not aged 40–69 years, or who had duplicate data ($n=146$).

Of the remainder, 55,838 completed the baseline questionnaires (response rate 83%). All eligible subjects were sent 5-year (1995–1998; response rate 80%) and 10-year follow-up questionnaires (2000–2003; response rate 78%). We excluded women with a self-reported history of cancer before the start of follow-up ($n=1,509$). To investigate the impact of leisure-time physical activity (LPA) on breast cancer risk, we excluded women with missing information on LPA ($n=751$). Age-area-adjusted analysis was conducted in 53,578 women.

Further, we then excluded women who had missing or unreliable information on height, BMI, BMI at age 20 years (<14 or ≥40), alcohol intake, smoking, or use of exogenous female hormones (EFH) ($n=13,804$), as well as those with a family history of breast cancer ($n=210$) and women who reported unreasonable estimates of total energy intake (±3SD) ($n=395$). Finally, 39,169 women were included in multivariable-adjusted analysis. We also performed sub-analyses to evaluate the impact of daily total physical activity (DTPA) in cohort II only because baseline information on DTPA was available.

Exposure measurement

The main exposure of interest was participation frequency in LPA. We inquired about the frequency of participation in non-occupational LPA, such as sports and exercise, at the baseline and 5-year follow-up surveys. In both questionnaires, we asked 'How many times did you participate in sports and PA other than during working hours,' with five predefined categories of almost never exercise: 1–3 days per month, 1–2 days per week, 3–4 days per week, and almost daily.

In cohort II, we evaluated the impact of DTPA on breast cancer risk. DTPA was measured as metabolic equivalents (METs-hours/day). Calculation in METs has been explained elsewhere (Inoue et al., 2008). The same methods were used in the baseline and 5-year follow-up surveys because they contained common questions on sleeping time, heavy physical work or strenuous exercise, standing or walking time, and sitting time.

Although LPA was not directly validated, the validity and reproducibility of the total METs/day score for the 5-year follow-up questionnaire was previously evaluated using 4-day, 24-hour PA records as an objective standard in 108 volunteer subjects in the cohort. In brief, correlations between the 5-year follow-up questionnaire and 4-day, 24-h record showed reasonable validity, with a Spearman rank correlation coefficient of 0.35 in women (Inoue et al., 2008). Reproducibility for the 5-year follow-up questionnaire was also supported, with a Spearman rank correlation coefficient of 0.68 (Imai et al., 2010).

Ascertainment of cases and follow-up

Breast cancer cases were identified by active patient notification from major local hospitals and data linkage with population-based cancer registries, with permission from the local governments responsible for the registries. Cases were defined as codes C500–509 (World Health Organization, 2000). Diagnosis was microscopically verified for 97% of all case patients. ER/PR status was evaluated by either immunohistochemical assay or enzyme-linked immunoassay. The cut-off point for positive receptor status was defined by clinical estimation at the treating hospital or by the assay method of the clinical laboratory. In most but not all cases, hormone receptor-positivity was defined as the presence of ≥10 fmol/mg protein in enzyme-linked immunoassay or by the finding of any positive cells in a specimen in immunohistochemical assay.

Follow-up was started on the date of administration of the baseline questionnaire and continued until the date of diagnosis of breast cancer, date of death, date of moving, or end of follow-up (December 31, 2007), whichever occurred first. Date of death or moving was verified through linkage with the death or residential registry at the respective PHC.

Statistical analysis

We used time-dependent multivariable Cox proportional hazards regression models to evaluate relative risks (RRs) and 95% confidence intervals (CIs) using age as the time scale (Korn et al., 1997). Women were subdivided into three categories by LPA [≤3 days/month, 1–2 days/week, ≥3 days/week]. The multivariable adjusted model included height, recent BMI, BMI at age 20 years, smoking status, age at menarche, age at first birth, parity, age at menopause, use of EFH, alcohol intake and isoflavone intake. These factors were based on the self-administered baseline questionnaires and were updated with the follow-up surveys, if available. If they could not be properly adjusted due to the small number of ER/PR-defined cases, these covariates were excluded, as mentioned in the footnotes in Table 2. For DTPA, women were subdivided according to tertile. Trend tests were conducted by creating a continuous variable in the rank order of each category. Additional analyses were conducted with stratification by menopausal and BMI status. All analyses were performed using the SAS statistical package version 9.1 (SAS Institute, Cary, NC). All statistical tests were two-sided, and statistical significance was defined as $p<.05$.

Results

After an average 14.5 years of follow-up, 652 breast cancer cases were diagnosed among 53,578 women. Information on ER/PR status was available for 299, showing 135 cases of ER+PR+, 64 of ER+PR−, and 83 of ER−PR−. Although height and BMI did not appear to differ by LPA level, women who tended to participate were more likely to be older and not to use EFH (Table 1).

Overall, we observed a statistically significant inverse association between LPA and breast cancer risk [multivariable-adjusted $RR_{\geq 3\ days/week\ vs.\ \leq 3\ days/week}=0.73$; 95% CI 0.54–1.00; p_{trend} 0.038]. In particular, the observed inverse association was apparent for ER+PR+ tumors (corresponding $RR_{ER+PR+}=0.43$ (0.19–1.00) p_{trend} 0.022), but not for others (Table 2). Without updating exposure information (i.e. by using the baseline information only), the corresponding result for ER+PR+ was no longer statistically significant [0.64 (0.29–1.38) $p_{trend}=0.13$ (text only)], although the point estimates of RRs were less than 1 at either baseline alone or with updated information. Further analyses without adjustment of recent BMI or BMI at 20 years old gave similar results.

In analyses stratified by menopausal status, LPA participation was marginally inversely associated with overall breast cancer risk among premenopausal women, although null association was observed after considering ER/PR tumor status. Among postmenopausal women, in contrast, LPA was associated with a decreased risk of ER+PR+ tumors using repeated exposure information (i.e. both baseline and 5-year follow-up surveys) [multivariable-adjusted $RR_{\geq 3\ days/week\ vs.\ \leq 3\ days/month}=0.25$ (0.06–1.06) p_{trend} 0.041; Table 2].

Table 1
Subject characteristics according to category of participation in leisure-time activity in the Japan Public Health Center-based Prospective Study (1990/1993–).

Characteristic	Frequency of participation in leisure-time physical activity		
	≤3 days/month	1–2 days/week	≥3 days/week
At baseline survey (%)	81.4	9.7	8.9
At 5-year follow-up survey (%)	78.1	10.8	11.2
Age at baseline survey, y, mean (SD)	51.1 (7.8)	50.5 (7.9)	54.2 (8.2)
Body mass index at age 20, kg/m^2, mean (SD)	21.5 (2.6)	21.2 (2.4)	21.6 (2.7)
Body mass index at baseline, kg/m^2, mean (SD)	23.3 (3.1)	23.2 (2.9)	23.5 (3.2)
Height, cm, mean (SD)	152.2 (5.4)	153.4 (5.3)	152.1 (5.7)
Age at menarche, y, mean (SD)	14.5 (1.8)	14.3 (1.8)	14.9 (1.9)
Age at first birth, y, mean (SD)[a]	24.9 (3.4)	25.1 (3.1)	25.0 (3.5)
Number of children, *n*, mean (SD)	2.6 (1.5)	2.6 (1.4)	2.7 (1.6)
Age at menopause, y, mean (SD)	48.3 (4.7)	48.4 (4.8)	48.7 (4.5)
Use of exogenous hormones at baseline (ever), %	12.6	12.5	11.7
Alcohol drinking status at baseline (ever), %	22.4	29.9	23.1
Smoking status at baseline (ever), %	8.0	7.6	7.2
Intake of isoflavones, mg, mean[b]	36.2	39.0	42.9

BMI = body mass index, SD = standard deviation.
[a] Based on information among parous women.
[b] Standardized according to food frequency questionnaires.

In cohort II, the impact of DTPA on breast cancer risk showed no overall association (multivariable-adjusted $RR_{\text{tertile3 vs. tertile1 METs/day score}} = 1.03$ (0.75–1.41) p_{trend} 0.86; Table 3). On consideration of menopausal and ER/PR status, however, we observed a substantial inverse trend between DTPA and ER+PR+ tumors among Postmenopausal women (age-area adjusted $RR_{\text{tertile3 vs. tertile1 METs/day score}} = 0.43$ (0.17–1.08) p_{trend} 0.046; Table 3).

On stratification by BMI (<25 or ≥25 kg/m^2), no association between LPA and breast cancer risk was seen among women with BMI <25 kg/m^2. Among overweight women (BMI ≥25 kg/m^2), however, participation in LPA was associated with a decreased risk of breast cancer risk overall ($RR_{\geq 1\text{ day/week vs. }\leq 3\text{ days/month}} = 0.65$ (0.43–0.97) p_{trend} 0.033; Table 4).

Discussion

This is the first large prospective cohort study to evaluate the association between LPA and breast cancer risk in consideration of ER/PR status in a Japanese population. Overall, LPA showed a substantial inverse association with breast cancer risk after adjustment for all covariates. Among premenopausal women, LPA was marginally associated with a decreased risk overall but not for specific ER/PR tumors. Among postmenopausal women, LPA was associated with a decreased risk for ER+PR+ tumors. Although there was no overall association between DTPA and breast cancer risk, we observed a considerable inverse trend between DTPA and postmenopausal ER+PR+ tumors in a JPHC sub-cohort. Further, on stratification by BMI, we observed a substantial inverse association between LPA and breast cancer risk among overweight women.

Our observed favorable impact of LPA against breast cancer risk was consistent with previous results for overall (Bardia et al., 2006) and ER+ tumors (Bernstein et al., 2005), although a cohort study suggested an inverse association for ER− but not ER+ tumors (Dallal et al., 2007).

Among premenopausal women, the marginal inverse trend of an association of LPA with breast cancer risk was found for overall tumors but not for any tumor subtypes. PA has been reported to exert a protective effect on risk for overall tumors (Maruti et al., 2008) and irrespective of hormone receptor positivity (Enger et al., 2000) (Adams et al., 2006) (Suzuki et al., 2010). The observed weak inverse trend might be due to the fact that our follow-up period did not cover the entire premenopausal period because follow-up started at around age 40.

Unlike previous results (McTiernan et al., 2003) (Lee et al., 2001), we found no inverse trend among postmenopausal women. For ER+PR+ tumors, however, a substantial inverse trend was found, in line with some (Chlebowski et al., 2007; Peters et al., 2009; Schmidt et al., 2008) but not all previous studies (Lee et al., 2001) (Leitzmann et al., 2008). A protective effect of PA on both ER+PR+ and ER+PR− tumors has also reported (Bardia et al., 2006).

Among overweight women, a substantial decreased in risk with LPA was observed overall. Similarly, a weak inverse trend was also observed for ER+PR+ tumors. In other studies, however, an inverse association was observed among a low-BMI group (Leitzmann et al., 2008), particularly for ER+PR+ tumors (Enger et al., 2000). These inconsistent results indicate the need for further careful evaluation.

Unlike LPA, our sub-analyses for DTPA (average 9.2 person-years of follow-up) did not show any overall favorable impact, which was consistent with our previous analysis with an average 7.5 person-years of follow-up (from 1995–1999 to 2004) (Inoue et al., 2008). In contrast, our corresponding present results for the postmenopausal ER+PR+ tumors showed a substantial inverse trend with DTPA. Although these results could not be clearly explained and might not exclude the possible involvement of non-hormone-related mechanisms, the observed results for postmenopausal ER+PR+ tumors might support the idea that PA is associated with a decreased risk of breast cancer partly through hormone-related mechanisms. After menopause, exercise may lead to a decrease in adipose tissue (Sternfeld et al., 2005), a major source of endogenous estrogen derived from the peripheral conversion of androgens to estrogens (Cleland et al., 1985) or to an increase in sex hormone-binding globulin (van Gils et al., 2009), the main protein carrier of estradiols, or both. A lack of association of DTPA with overall breast cancer risk in the present and a previous JPHC study (Inoue et al., 2008) might be explained without consideration of menopausal and ER/PR status. Further study with regard to menopausal status, ER/PR status or type of PA is required.

Strengths of our study include its prospective population-based cohort study design and large study size, adjustment for a broad range of potential confounders, and availability of repeated measurements for exposure as well as some covariates, which can change during long follow-up. Time-dependent analyses may reduce the misclassification of exposure and improve statistical efficiency. The study design, with a long follow-up period and repeated exposure measurements, might have aided detection of this inverse association.

Our main limitation was that ER/PR status was available for only about 46% of cases. The major reason for an unknown ER/PR status was likely that data collection began in 2002, while data during follow-up from 1990 to 2002 were obtained by retrospective review of medical records or pathology reports. Potential bias due to this relatively large number of cases with unknown ER/PR status should be

Table 2
Relative risks (RRs) and 95% confidence intervals (CIs) for the association between leisure-time activity and breast cancer risk among Japanese women in the Japan Public Health Center-based Prospective Study, 1990–2007.

Type of tumor	All					Premenopausal women[b]					Postmenopausal women[c]				
	Participation frequency in leisure-time physical activity					Participation frequency in leisure-time physical activity					Participation frequency in leisure-time physical activity				
		≤3 days/ month	1–2 days/ week	≥3 days/ week			≤3 days/ month	1–2 days/ week	≥3 days/ week			≤3 days/ month	1–2 days/ week	≥3 days/ week	
	Cases/*n*	Ref.	RR (95% CI)	RR (95% CI)	p_{trend}	Cases/*n*	Ref.	RR (95% CI)	RR (95% CI)	p_{trend}	Cases/*n*	Ref.	RR (95% CI)	RR (95% CI)	p_{trend}
Cases		529	59	64			254	25	21			275	34	43	
Total person-years		627669	73985	78439			260618	33986	24129			367051	39999	54310	
Model[a]	652/53,578	1.00 (ref.)	0.98 (0.75–1.29)	0.83 (0.64–1.08)	0.19	300/21,799	1.00 (ref.)	0.76 (0.50–1.15)	0.70 (0.45–1.10)	0.06	352/31,779	1.00 (ref.)	1.16 (0.81–1.66)	0.98 (0.71–1.36)	0.89
		389	45	45			200	23	17			189	22	28	
Model[d]	479/39,169	1.00 (ref.)	0.86 (0.63–1.18)	0.73 (0.54–1.00)	0.037	240/17,332	1.00 (ref.)	0.82 (0.53–1.27)	0.66 (0.40–1.09)	0.074	239/21837	1.00 (ref.)	0.88 (0.56–1.38)	0.78 (0.52–1.17)	0.21
ER+PR+		115	10	10			55	3	4			60	7	6	
Model[a]	135/53,578	1.00 (ref.)	0.83 (0.43–1.58)	0.61 (0.32–1.18)	0.12	62/21,799	1.00 (ref.)	0.48 (0.15–1.54)	0.61 (0.22–1.70)	0.19	73/31,779	1.00 (ref.)	1.13 (0.51–2.47)	0.67 (0.29–1.54)	0.44
		89	6	6			48	3	4			41	3	2	
Model[d]	101/39,169	1.00 (ref.)	0.55 (0.24–1.26)	0.43 (0.19–1.00)	0.022	55/17,332	1.00 (ref.)	0.54 (0.17–1.74)	0.64 (0.23–1.78)	0.25	46/21,837	1.00 (ref.)	0.62 (0.19–2.01)	0.25 (0.06–1.06)	0.041
ER+PR−		50	6	8			25	4	2			25	2	6	
Model[a]	64/53,578	1.00 (ref.)	1.21 (0.52–2.82)	1.18 (0.55–2.50)	0.60	31/21,799	1.00 (ref.)	1.45 (0.50–4.20)	0.73 (0.17–3.11)	0.91	33/31,779	1.00 (ref.)	0.83 (0.20–3.50)	1.60 (0.65–3.94)	0.37
		33	5	8			19	4	2			14	1	6	
Model[e]	46/39,169	1.00 (ref.)	1.28 (0.49–3.32)	1.93 (0.87–4.26)	0.11	25/17,332	1.00 (ref.)	2.04 (0.68–6.16)	0.90 (0.20–3.94)	0.74	21/21,837	1.00 (ref.)	0.56 (0.07–4.56)	3.12 (1.15–8.50)	0.049
ER−PR−		66	6	11			28	1	4			38	5	7	
Model[a]	83/53,578	1.00 (ref.)	0.91 (0.39–2.11)	1.30 (0.68–2.47)	0.51	33/21,799	1.00 (ref.)	0.34 (0.045–2.47)	1.35 (0.47–3.89)	0.92	50/31,779	1.00 (ref.)	1.35 (0.53–3.45)	1.34 (0.59–3.02)	0.41
		49	4	8			22	3				27	4	5	
Model[e]	61/39,169	1.00 (ref.)	0.67 (0.24–1.88)	1.06 (0.49–2.26)	0.92	25/17,332	1.00 (ref.)	0.55 (0.16–1.86)[f]		0.34	36/21,837	1.00 (ref.)	1.32 (0.46–3.82)	1.07 (0.41–2.82)	0.79
Unknown		285	35	33			137	15	9			148	20	24	
Model[a]	353/53,578	1.00 (ref.)	0.99 (0.70–1.42)	0.75 (0.52–1.07)	0.15	161/21,799	1.00 (ref.)	0.74 (0.43–1.28)	0.53 (0.27–1.04)	0.038	192/31,779	1.00 (ref.)	1.22 (0.76–1.95)	0.96 (0.62–1.48)	0.97
		210	28	22			104	14	7			106	14	15	
Model[d]	260/39,169	1.00 (ref.)	0.94 (0.63–1.40)	0.64 (0.41–1.00)	0.06	125/17,332	1.00 (ref.)	0.85 (0.48–1.51)	0.51 (0.23–1.10)	0.08	135/21,837	1.00 (ref.)	0.95 (0.54–1.67)	0.72 (0.41–1.24)	0.25

[a] Cox proportional hazards models was adjusted for age (time-scales) and area (10).
[b] For premenopausal women, multivariable Cox proportional hazards models were adjusted for all covariates (footnote d or e), except age at menopause.
[c] For postmenopausal women, multivariable Cox proportional hazards models were adjusted for all covariates (footnote d or e) and age at menopause (≤44, 45–54, ≥55 years).
[d] Multivariable Cox proportional hazards models were adjusted for age (time-scales), area (10), height (continuous), recent BMI (continuous), BMI at age 20 years (continuous), smoking status (never, ever), age at menarche (≤13, 14, 15, ≥16 years, or missing), age at first birth (nulliparous, <26 years, ≥26 years, or missing), parity (nulliparous, 1–2 times, 3 times, and ≥4 times, or missing), age at menopause (pre, ≤44, 45–54, ≥55 years), use of exogenous female hormones (ever, never), alcohol intake (non-/past-/occasional drinkers, regular drinkers ≤150 or >150 ethanol g/week), and energy-adjusted intake of isoflavones (continuous) and daily total physical activity (tertile of METs or missing).
[e] Multivariable Cox proportional hazards models were adjusted for age (time-scales), area (10), height (continuous), recent BMI (continuous), BMI at age 20 years (continuous), smoking status (never, ever), age at menarche (≤13, 14, 15, ≥16 years, or missing), age at menopause (pre, ≤44, 45–54, ≥55 years), use of exogenous female hormones (ever, never), alcohol intake (non-/past-/occasional drinkers, regular drinkers), and energy-adjusted intake of isoflavones (continuous) and daily total physical activity (tertile of MET or missing).
[f] Participation frequency in leisure-time physical activity was categorized (≤ 3 days/month vs. ≥1 day/week).

Table 3
Relative risks (RRs) and 95% confidence intervals (CIs) for the association between daily total physical activity (DTPA) level and breast cancer risk among Japanese women in the Japan Public Health Center-based Prospective Study (Cohort II), 1990–2007.

	All					Premenopausal women[b]					Postmenopausal women[c]				
	DTPA (METs/day score)					DTPA (METs/day score)					DTPA (METs/day score)				
	Cases/*n*	Tertile 1 Ref.	Tertile 2 RR (95% CI)	Tertile 3 RR (95% CI)	p_{trend}	Cases/*n*	Tertile 1 Ref.	Tertile 2 RR (95% CI)	Tertile 3 RR (95% CI)	p_{trend}	Cases/*n*	Tertile 1 Ref.	Tertile 2 RR (95% CI)	Tertile 3 RR (95% CI)	p_{trend}
Total person-years		128960	143178	152199			46084	57485	53928			82875	85694	98270	
All		106	92	96			41	44	43			65	48	53	
Model[a]	294/31917	1.00 (ref.)	1.08 (0.82–1.43)	0.90 (0.68–1.19)	0.48	128/11953	1.00 (ref.)	1.07 (0.70–1.65)	0.86 (0.56–1.32)	0.48	166/19964	1.00 (ref.)	1.07 (0.73–1.55)	0.93 (0.65–1.34)	0.72
		82	70	76			35	40	35			47	30	41	
Model[d]	228/23977	1.00 (ref.)	1.13 (0.82–1.56)	1.03 (0.75–1.41)	0.86	110/9979	1.00 (ref.)	1.24 (0.78–1.97)	0.89 (0.55–1.43)	0.61	118/13998	1.00 (ref.)	1.02 (0.64–1.63)	1.11 (0.72–1.70)	0.65
ER+PR+		22	32	43			4	5	5			18	5	6	
Model[a]	43/31917	1.00 (ref.)	0.61 (0.29–1.30)	0.57 (0.27–1.17)	0.11	14/11953	1.00 (ref.)	1.35 (0.36–5.04)	1.19 (0.31–4.56)	0.81	29/19964	1.00 (ref.)	0.42 (0.16–1.13)	0.43 (0.17–1.08)	0.046
ER+PR−		7	10	5			4	4	3			3	6	2	
Model[a]	22/31917	1.00 (ref.)	1.94 (0.73–5.18)	0.79 (0.25–2.50)	0.74	11/11953	1.00 (ref.)	1.03 (0.26–4.12)	0.59 (0.13–2.64)	0.49	11/19964	1.00 (ref.)	3.87 (0.89–16.91)	0.98 (0.16–5.91)	0.88
ER−PR−		4	8	9			2	3	2			2	5	7	
Model[a]	21/31917	1.00 (ref.)	2.38 (0.71–7.93)	2.36 (0.72–7.70)	0.17	7/11953	1.00 (ref.)	1.58 (0.26–9.46)	0.90 (0.13–6.37)	0.90	14/19964	1.00 (ref.)	3.20 (0.62–16.55)	4.17 (0.86–20.14)	0.07
Unknown		71	64	70			31	32	32			40	32	38	
Model[a]	205/31917	1.00 (ref.)	1.10 (0.79–1.55)	0.95 (0.68–1.32)	0.73	95/11953	1.00 (ref.)	1.01 (0.62–1.66)	0.83 (0.50–1.36)	0.44	110/19964	1.00 (ref.)	1.14 (0.72–1.82)	1.03 (0.66–1.61)	0.90

[a] Cox proportional hazards models was adjusted for age (time-scales) and area (10).
[b] For premenopausal women, multivariable Cox proportional hazards models were adjusted for all following covariates (d or e) except age at menopause.
[c] For postmenopausal women, multivariable Cox proportional hazards models were adjusted for all following covariates (d or e) and age at menopause (≤44, 45–54, ≥55 years).
[d] Multivariable Cox proportional hazards models were adjusted for age (time-scales), area (10), height (continuous), recent BMI (continuous), BMI at age 20 years (continuous), smoking status (never, ever), age at menarche (≤13, 14, 15, ≥16 years, or missing), age at first birth (nulliparous, <26 years, ≥26 years, or missing), parity (nulliparous, 1–2 times, 3 times, and ≥4 times, or missing), age at menopause (pre, ≤44, 45–54, ≥55 years), use of exogenous female hormones (ever, never), alcohol intake (non-/past-/occasional drinkers, regular drinkers ≤150 or >150 ethanol g/week), and energy-adjusted intake of isoflavones (continuous) and participation frequency in leisure-time physical activity (≤3 days/month, 1–2 days/week, ≥3 days/week).

Table 4
Relative risks (RRs) and 95% confidence intervals (CIs) for the association between leisure-time physical activity and hormone receptor status-defined breast cancer risk stratified by BMI in the Japan Public Health Center-based Prospective Study 1990–2007.

Type of tumor	BMI <25 (n=38,959)				BMI ≥25 (n=14,619)			
	Cases	Leisure-time physical activity			Cases	Leisure-time physical activity		
		≤3 days/month	≥1 day/week			≤3 days/month	≥1 day/week	
		Ref.	RR (95% CI)	p_{trend}		Ref.	RR (95% CI)	p_{trend}
Person-years		454047	110033			173623	42391	
		359	94			170	29	
All[a]	453/38959	1.00 (ref.)	1.02 (0.81–1.28)	0.90	199/14619	1.00 (ref.)	0.65 (0.43–0.97)	0.033
		75	15			40	5	
ER+PR+[a]	90/38959	1.00 (ref.)	0.84 (0.48–1.48)	0.55	45/14619	1.00 (ref.)	0.50 (0.20–1.27)	0.14
		32	12			18	2	
ER+PR−[a]	44/38959	1.00 (ref.)	1.61 (0.82–3.16)	0.17	20/14619	1.00 (ref.)	0.51 (0.12–2.23)	0.37
		51	14			15	3	
ER−PR−[a]	65/38959	1.00 (ref.)	1.11 (0.61–2.01)	0.74	18/14619	1.00 (ref.)	0.93 (0.27–3.27)	0.91
		192	49			93	19	
Unknown[a]	241/38959	1.00 (ref.)	0.92 (0.67–1.26)	0.6	112/14619	1.00 (ref.)	0.72 (0.43–1.18)	0.19

[a] Cox proportional hazards models were adjusted for age (time-scales), area (10).

considered. Nevertheless, RR for unknown tumors was similar to that for overall tumors, suggesting that there was little bias in our results. Further, our information on LPA included frequency only and not intensity or duration. Finally, we are unable to rule out the possibility of a chance finding, measurement error in exposure information due to self-reporting, and residual confounding due to unmeasured/unknown information.

Conclusion

LPA was associated with a decreased risk of breast cancer in overall and postmenopausal ER+PR+ tumors. Among overweight women, a substantially decreased risk with LPA was observed. We also observed a substantial inverse trend between DTPA and postmenopausal ER+PR+ tumors, although DTPA was not associated with overall breast cancer risk. Active participation in LPA might represent a useful public health message against breast cancer, particularly among elderly women, given that the majority of breast tumors occurring after menopause are ER+PR+ tumors.

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Conflict of interest statement
The authors declare that there are no conflicts of interest.

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Appendix

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Zinc and heme iron intakes and risk of colorectal cancer: a population-based prospective cohort study in Japan[1–3]

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ABSTRACT

Background: Food sources and intakes of zinc and heme iron may differ between Western and Asian populations. However, all of the studies on the association between zinc and heme iron intakes and colorectal cancer have been conducted in Western populations.

Objective: We investigated the association between zinc and heme iron intakes and colorectal cancer risk in a Japanese general population.

Design: We conducted a large, population-based prospective study in 39,721 men and 45,376 women aged 45–74 y. Heme iron and zinc intakes were measured by using a validated food-frequency questionnaire in either 1995 or 1998.

Results: During as many as 808,053 person-years of follow-up until the end of 2006, 1284 colorectal cancer cases were identified. In multivariate-adjusted models, zinc and heme iron intakes were not associated with colorectal cancer in either men or women. In comparison with the lowest quartile, the HRs (95% CIs) for developing colorectal cancer in the fourth quartile of zinc and heme iron intakes were 0.77 (0.58, 1.03; *P*-trend = 0.2) and 1.06 (0.79, 1.42; *P*-trend = 0.6), respectively, for men and 1.05 (0.77, 1.44; *P*-trend = 0.4) and 0.88 (0.61, 1.29; *P*-trend = 0.4), respectively, for women.

Conclusion: Our results in a Japanese population with lower intakes and different major food sources of zinc and heme iron in comparison with those of Western populations suggest that zinc and heme iron intakes are not associated with colorectal cancer. *Am J Clin Nutr* 2012;96:864–73.

INTRODUCTION

A recent joint report by the World Cancer Research Fund and the American Institute for Cancer Research concluded that there was "convincing" evidence to support a positive association between colorectal cancer (CRC) and intakes of both red and processed meats (1). Red meat is rich in zinc and heme iron. Zinc is an antioxidant and is involved in various cellular functions, including DNA repair and apoptosis (2), whereas heme iron is a prooxidant and may contribute to colorectal carcinogenesis by promoting free radical production and lipid peroxidation (3–5).

Only 3 prospective studies (6–8) and one case-control study (9) have examined an association of dietary zinc intake with CRC risk, whereas several studies have reported an association between heme iron intake and CRC risk (6–8, 10–13). However, all of these studies were conducted in Western populations, and we know of no data reported for prospective cohort studies in Asian general populations. Food sources of zinc and heme iron might differ between Western and Asian populations, because Asian populations tend to consume more fish (14) and poultry (15) and less red meat (16) than do Western populations. Therefore, similar studies in Asian populations are important to confirm the generalizability of these associations.

In addition, zinc and heme iron concentrations may be modified by alcohol consumption. For example, serum zinc concentrations in alcohol-drinking patients are reportedly lower than those in nondrinkers (17), and this difference may be due to an ethanol-induced increase in urinary zinc excretion (18). In addition, alcohol consumption is known to disrupt iron homeostasis (19–22). Some previous studies have reported that associations of CRC with zinc or heme iron intake are more pronounced among alcohol drinkers (6, 7, 10).

Vitamins B-6 and B-12, which are plentiful in foods rich in zinc and heme iron, such as meat and fish, may also affect the association between zinc and heme iron intakes and CRC risk. We previously reported that vitamins B-6 and B-12 are associated with decreased and increased risk of CRC, respectively (23). Discrepancies between the results of previous studies may be due to confounding or effect modification of vitamin B-6 or vitamin B-12, which was not taken into account previously. Therefore, more studies that consider vitamins B-6 and B-12 as confounding factors are needed.

Here, we investigated the association between zinc and heme iron intakes and CRC risk in a population-based, prospective cohort study in Japan. Our hypothesis was that a higher intake of

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zinc would decrease CRC risk, whereas higher heme iron intake would increase CRC risk. We also considered the effects of vitamin B-6 and B-12 intakes on CRC risk and tested whether any associations depended on alcohol consumption.

SUBJECTS AND METHODS

Study population

The Japan Public Health Center–based Prospective Study was initiated in 1990 for cohort I and in 1993 for cohort II. Participants were all registered Japanese inhabitants of 11 public health center areas and were aged 40–69 y (cohort I: 40–59 y; cohort II: 40–69 y) at the beginning of each cohort's baseline survey. Details of the study design have been described previously (24). The institutional review board of the National Cancer Center, Tokyo, Japan, approved the study. The participants in the present study were subjects in the Japan Public Health Center study who responded to a 5-y follow-up questionnaire during the period from 1995 to 1999 at ages 45–74 y. The present study used the 5-y follow-up survey as a baseline because this survey included a detailed self-administered food-frequency questionnaire (FFQ). The participants from 2 public health center areas (Tokyo and Osaka) were excluded from the present analysis because the selection criteria of participants differed from those in other public health center areas, which left 116,896 participants as the study population. After the exclusion of non-Japanese participants (n = 51), late reports of emigration occurring before the starting point (n = 168), ineligibility due to incorrect birth date (n = 4), and duplicate enrollments (n = 4), we established a population-based cohort of 116,669 participants. After the exclusion of 1626 participants who had died, moved out of the study area, or were lost to follow-up before the starting point, 115,043 eligible participants remained. From these, 91,245 responded to the questionnaire, yielding a response rate of 79.3%. We excluded participants who had been diagnosed with or reported as having CRC before the starting point (n = 605) or who reported extreme total energy intakes (upper 2.5% or lower 2.5%; n = 5543). The final analysis included 85,097 participants (39,721 men and 45,376 women).

Questionnaire

We asked participants to reply to a lifestyle questionnaire that covered sociodemographic characteristics, medical history, smoking and drinking habits, and diet. We designed the FFQ to estimate dietary intake from 138 food items and validated it for the estimation of various nutrients and food groups (25). The participants were asked to estimate how often they consumed the individual food items (frequency of intake) and to estimate representative relative portion sizes compared with standard portions during the previous year (26). The FFQ contained questions on frequency (never, 1–3 times/mo, 1–2 times/wk, 3–4 times/wk, 5–6 times/wk, once per day, 2–3 times/d, 4–6 times/d, or ≥7 times/d) and portion sizes relative to a standard portion [small (50% smaller), medium (same as standard), and large (50% larger)]. Daily food intake was calculated by multiplying frequency by standard portion and relative size for each food item in the FFQ. Daily intakes of zinc and iron were calculated by using the Fifth Revised and Enlarged Edition of the Standard Tables of Food Composition in Japan (27). Heme iron intake was calculated by multiplying type-specific percentages of heme iron by total iron content (mg/g) to yield heme iron contents for the reported intake of 16 meat items (7 food groups) and 19 fish and shellfish items (one food group); the percentages of heme iron used for the various types of meat, fish, and shellfish were as follows: 69% for beef; 39% for pork, ham, bacon, and luncheon meat; 26% for chicken, fish, and shellfish (10, 12); and 21% for liver (12). We did not collect information on the use of iron and zinc supplements, because zinc and iron supplementation is reported to be low in Japan (<0.5% in both men and women, and 0.2% in men and 2.4% in women, respectively) (28). Intakes of food and nutrients were log-transformed and adjusted for total energy intake by means of the residual model (29).

The validity of the energy-adjusted zinc or iron 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 intakes of zinc and iron from the questionnaire and from dietary records were 0.50 and 0.44 (cohort I) and 0.44 and 0.54 (cohort II), respectively, for men and 0.35 and 0.38 (cohort I) and 0.40 and 0.55 (cohort II), respectively, for women (30). We also calculated Spearman's correlation coefficients between the energy-adjusted heme iron intakes from the questionnaire and from dietary records in cohorts I and II: 0.26 and 0.28 for men and 0.11 and 0.27 for women, respectively (A Hara, unpublished data, 2012).

Follow-up and identification of CRC cases

We followed participants 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 the Family Registry Law, respectively, and the registries are thought to be complete. During the follow-up period in the present study, 9425 (11.1%) participants died, 3695 (4.4%) moved out of the study area, and 308 (0.4%) were lost to follow-up.

We identified incidence data for CRC by active patient notification from major local hospitals in the study area and from data linkage with population-based cancer registries. We coded CRC cases according to the *International Classification of Diseases for Oncology, third edition* (31) (C18–C20). We conducted analyses of site-specific cancers: C18 for colon cancer (C18.0–C18.5 for proximal colon cancer and C18.6–C18.7 for distal colon cancer) and C19 and C20 for rectal cancer. In our cancer registry system, the proportion of cases for which information was available from death certificates only was 2.7%.

Statistical analysis

We calculated person-years of follow-up for each participant from the starting point to the date of CRC diagnosis, date of emigration from the study area, date of death, or end of the follow-up (31 December 2006), whichever came first. We censored

participants lost to follow-up at the last confirmed date of presence in the study area.

We calculated HRs and 95% CIs for developing CRC for the categories of energy-adjusted intakes of zinc and heme iron 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 as follows: age (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, or ≥30); smoking status (never, past, or current); alcohol consumption (for men—none; drinker: <150, 150–299, 300–449, or ≥450 g ethanol/wk; for women—none; drinker: <150 or ≥150 g ethanol/wk); quartile of physical activity in metabolic equivalent task-hours/d; history of type 2 diabetes (yes or no); screening examinations for CRC (fecal occult blood test, barium enema, or colonoscopy); menopausal status (premenopausal or natural or induced postmenopausal) and use of exogenous female hormones (never or ever) in women; and quartiles of energy-adjusted intakes of calcium, magnesium, vitamin B-6, vitamin B-12, folic acid, vitamin D, n−3 PUFAs, and fiber.

We calculated *P* values for the analyses of linear trends by assigning ordinal values for categories of zinc and heme iron intakes and entering the values as continuous terms in the regression model. We also statistically evaluated the interactions between sex and zinc and heme iron intakes and between alcohol consumption and zinc and heme iron intakes with regard to the risk of CRC based on the likelihood ratio test with 1 df. The interaction was assessed by a product term consisting of a dichotomous variable for alcohol drinking and an ordinal variable for heme or zinc. We then created an interaction term by multiplying the dichotomous value for alcohol consumption by ordinal values for zinc or heme iron intake. All *P* values are 2-sided, and significance was determined at the $P < 0.05$ level. We performed all statistical analyses with SAS software, version 9.1 (SAS Institute Inc).

RESULTS

During 808,053 person-years of follow-up, we identified 1284 new CRC cases (786 for men, 498 for women).

The major sources of zinc in our population were grains (37%), red meat (15%), pulses (9.5%), dairy products (9.2%), and fish (9.1%). Dietary heme iron was derived mainly from various types of fish and shellfish (49%), beef (20%), and pork, ham, bacon, and luncheon meat (19%).

The characteristics of participants according to zinc and heme iron intakes are shown in **Tables 1** and **2** for men and women, respectively. Mean (±SE) zinc and heme iron intakes were 8.5 ± 0.007 and 0.50 ± 0.001 mg/d, respectively, in men and 7.9 ± 0.004 and 0.44 ± 0.001 mg/d, respectively, in women. Men and women with a high intake of zinc were less likely to be drinkers (alcohol consumption ≥1 g ethanol/wk), were more likely to have a history of type 2 diabetes, and generally consumed more of most of the foods and nutrients listed in Tables 1 and 2, compared with those with a low intake of zinc. Men with a higher zinc intake were less likely to be ever smokers, and women with a higher zinc intake were more likely to be postmenopausal. Both men and women whose heme iron intakes were higher were more likely to consume zinc, vitamin D, vitamin B-6, vitamin B-12, n−3 PUFAs, fish, and red meat and less likely to consume calcium and fiber compared with those whose heme iron intakes were lower. In men, individuals with a higher heme iron intake were less likely to be drinkers than those with a lower heme iron intake. The relations between magnesium, folate, and vegetable intakes and heme iron intake for men differed from the relations for women.

Associations between zinc and heme iron intakes and CRC risk in men and women are shown separately (**Tables 3** and **4**, respectively). In an age- and area-adjusted model, the quartile category of zinc intake was associated with decreased risk of colorectal, colon, and rectal cancer in men, whereas heme iron intake was not associated with CRC risk. However, in multivariate-adjusted models, zinc intake was not significantly associated with the risk of CRC among men; the HRs (95% CIs) for the highest quartile compared with the lowest quartile of zinc intake were 0.77 (0.58, 1.03) for colorectal, 0.76 (0.54, 1.07) for colon, and 0.80 (0.49, 1.32) for rectal cancer. Heme iron intake was not associated with CRC risk, whereas we found significantly higher HRs for vitamin B-12 intake and lower HRs for vitamin B-6 intake in the higher-intake categories in the same multivariate-adjusted model; the HRs (95% CIs) for the highest quartile compared with the lowest quartile were 1.52 (1.05, 2.20; *P*-trend = 0.01) and 0.68 (0.49, 0.94; *P*-trend = 0.009), respectively. Similar results were observed when we evaluated the risk of either proximal or distal colon cancer (data not shown). In women, there was no significant association between zinc and heme iron intakes and CRC risk in the age- and area-adjusted model or in the multivariate-adjusted model. Results were essentially unchanged when analyses were restricted to postmenopausal women (data not shown). There was no statistical interaction between sex and zinc or heme iron intakes with regard to the risk of CRC (all *P* values for interaction were >0.1).

The results of stratified analysis by alcohol intake among men are shown in **Table 5**. We found no significant interactions (all *P* values for interaction were >0.07), although a significant inverse association between zinc intake and CRC risk was observed only among drinkers; HRs (95% CIs) for the highest quartile compared with the lowest quartile of zinc intake were 0.63 (0.47, 0.85; *P*-trend = 0.001) for colorectal, 0.62 (0.43, 0.89; *P*-trend = 0.01) for colon, and 0.67 (0.39, 1.13; *P*-trend = 0.04) for rectal cancer. However, among drinkers, the dose-response was not clear; the HR for men who consumed more alcohol (≥450 g/wk) was similar to those who consumed less alcohol (<150 g/wk) in the highest quartile of zinc intake. No significant association was observed between heme iron intake and CRC risk in the analysis stratified by alcohol intake. These effects of alcohol intake could not be examined in women, because the number of women who consumed ≥150 g alcohol/wk was insufficient. The results among all nondrinkers in women were similar to the results among all women; the HRs (95% CI) for the highest quartile compared with the lowest quartile were 1.10 (0.77, 1.57; *P*-trend = 0.3) for zinc intake and 0.88 (0.58, 1.33; *P*-trend = 0.4) for heme iron intake.

DISCUSSION

To our knowledge, our study is the first large-scale prospective cohort study to evaluate the effect of heme iron and zinc intakes on CRC risk in Asia, where the dietary sources of zinc and heme iron differ from those in Western countries. Zinc and heme iron

TABLE 1
Characteristics of the study participants at the 5-y follow-up survey according to quartiles of energy-adjusted intakes of heme iron and zinc among men in the JPHC study (n = 39,721)[1]

Variable	Q1	Q2	Q3	Q4	P value[2]
Zinc intake					
No. of participants	9930	9930	9931	9930	
Heme iron intake (mg/d)[3]	0.37 ± 0.002[4]	0.45 ± 0.002	0.50 ± 0.002	0.66 ± 0.004	<0.0001
Zinc intake (mg/d)[3]	6.8 ± 0.008	8.2 ± 0.002	8.9 ± 0.002	10.1 ± 0.008	<0.0001
Age (y)	55.4 ± 0.08	56.3 ± 0.08	57.2 ± 0.08	57.8 ± 0.08	<0.0001
BMI (kg/m^2)	23.6 ± 0.03	23.5 ± 0.03	23.6 ± 0.03	23.6 ± 0.03	0.7
Ever smoker (%)	66.8	61.7	57.1	54.5	<0.0001
Alcohol drinker (%)[5]	93.8	79.1	64.8	51.8	<0.0001
METs (MET-h/d)	33.1 ± 0.07	33.0 ± 0.07	33.0 ± 0.07	32.4 ± 0.07	<0.0001
History of type 2 diabetes (%)	6.0	5.8	7.0	10.0	<0.0001
CRC screening, yes (%)[6]	27.9	33.9	34.5	32.8	<0.0001
Dietary intake[3]					
Total energy (kcal/d)	2187 ± 6.2	2133 ± 6.1	2119 ± 6.5	2230 ± 7.2	<0.0001
Calcium (mg/d)	365 ± 1.4	453 ± 1.6	516 ± 1.9	633 ± 2.8	<0.0001
Vitamin D (μg/d)	7.6 ± 0.05	9.5 ± 0.06	10.4 ± 0.06	11.4 ± 0.07	<0.0001
Magnesium (mg/d)	245 ± 0.5	276 ± 0.5	291 ± 0.5	313 ± 0.7	<0.0001
Vitamin B-6 (mg/d)	1.4 ± 0.004	1.5 ± 0.003	1.6 ± 0.003	1.7 ± 0.003	<0.0001
Vitamin B-12 (μg/d)	7.0 ± 0.04	8.4 ± 0.04	9.2 ± 0.04	10.8 ± 0.05	<0.0001
Folate (μg/d)	302 ± 1.1	356 ± 1.2	390 ± 1.3	437 ± 1.6	<0.0001
Fiber (g/d)	9.3 ± 0.04	11.6 ± 0.04	12.6 ± 0.04	13.3 ± 0.05	<0.0001
n−3 PUFAs (g/d)	2.4 ± 0.01	3.0 ± 0.01	3.3 ± 0.01	3.6 ± 0.01	<0.0001
Vegetables (g/d)	150 ± 1.0	188 ± 1.1	211 ± 1.3	228 ± 1.5	<0.0001
Fish (g/d)	73.2 ± 0.5	88.5 ± 0.5	93.7 ± 0.5	99.0 ± 0.6	<0.0001
Red meat (g/d)	36.3 ± 0.3	45.0 ± 0.3	52.6 ± 0.3	70.4 ± 0.5	<0.0001
Heme iron intake					
No. of participants	9930	9930	9931	9930	
Heme iron intake (mg/d)[3]	0.22 ± 0.0008	0.39 ± 0.0004	0.53 ± 0.0005	0.84 ± 0.003	<0.0001
Zinc intake (mg/d)[3]	7.9 ± 0.01	8.2 ± 0.01	8.6 ± 0.01	9.3 ± 0.01	<0.0001
Age (y)	56.5 ± 0.08	56.7 ± 0.08	56.7 ± 0.08	56.7 ± 0.08	0.2
BMI (kg/m^2)	23.4 ± 0.03	23.4 ± 0.03	23.7 ± 0.03	23.8 ± 0.03	<0.0001
Ever smoker (%)	61.5	60.0	59.3	59.2	0.02
Alcohol drinker (%)[5]	77.6	72.8	71.1	68.0	<0.0001
METs (MET-h/d)	33.5 ± 0.07	32.8 ± 0.07	32.6 ± 0.07	32.7 ± 0.07	<0.0001
History of type 2 diabetes (%)	6.0	7.0	8.3	7.5	<0.0001
CRC screening, yes (%)[6]	33.6	33.9	32.4	29.3	<0.0001
Dietary intake[3]					
Total energy (kcal/d)	2286 ± 6.0	2058 ± 6.0	2053 ± 6.3	2273 ± 7.4	<0.0001
Calcium (mg/d)	516 ± 2.8	499 ± 2.1	488 ± 2.0	465 ± 1.9	<0.0001
Vitamin D (μg/d)	6.7 ± 0.04	9.0 ± 0.05	10.6 ± 0.06	12.6 ± 0.08	<0.0001
Magnesium (mg/d)	274 ± 0.7	282 ± 0.6	284 ± 0.6	284 ± 0.6	<0.0001
Vitamin B-6 (mg/d)	1.4 ± 0.003	1.5 ± 0.003	1.6 ± 0.003	1.7 ± 0.004	<0.0001
Vitamin B-12 (μg/d)	5.6 ± 0.02	7.8 ± 0.03	9.5 ± 0.04	12.4 ± 0.06	<0.0001
Folate (μg/d)	349 ± 1.5	367 ± 1.4	377 ± 1.4	391 ± 1.4	<0.0001
Fiber (g/d)	11.8 ± 0.05	11.9 ± 0.05	11.8 ± 0.04	11.2 ± 0.04	<0.0001
n−3 PUFAs (g/d)	2.3 ± 0.008	2.9 ± 0.008	3.3 ± 0.009	3.9 ± 0.014	<0.0001
Vegetables (g/d)	184 ± 1.5	196 ± 1.3	198 ± 1.2	198 ± 1.1	<0.0001
Fish (g/d)	53.6 ± 0.3	79.5 ± 0.4	97.9 ± 0.5	123.2 ± 0.7	<0.0001
Red meat (g/d)	23.3 ± 0.2	39.5 ± 0.2	55.7 ± 0.3	85.8 ± 0.5	<0.0001

[1] CRC, colorectal cancer; JPHC, Japan Public Health Center–based; MET, metabolic equivalent task; Q, quartile.
[2] Derived by using ANOVA or the chi-square test.
[3] All mean total intakes of food and nutrition were energy adjusted.
[4] Mean ± SE (all such values).
[5] Alcohol consumption ≥1 g ethanol/wk.
[6] CRC screening included fecal occult blood test, barium enema, or colonoscopy.

intakes were not significantly associated with CRC risk. We found an inverse association between zinc intake and CRC risk among drinkers in men, although there were no significant interactions.

Food sources of zinc and heme iron vary among ethnic groups and cultures. In the Western diet, the major food sources of dietary zinc are red meat, poultry, dairy foods, whole grains, and fortified cereals (7, 8), whereas in our study's Japanese population, the main sources were grains, red meat, pulses, dairy products, and fish. Fish was also the main food source of heme iron in our study. To date, only 3 cohort studies (6–8) and one case-control study (9) have examined the association between

TABLE 2
Characteristics of the study participants at the 5-y follow-up survey according to quartiles of energy-adjusted intakes of heme iron and zinc among women in the JPHC study (n = 45,376)[1]

Variable	Q1	Q2	Q3	Q4	P value[2]
Zinc intake					
No. of participants	11,344	11,344	11,344	11,344	
Heme iron intake (mg/d)[3]	0.34 ± 0.002[4]	0.40 ± 0.002	0.45 ± 0.002	0.58 ± 0.003	<0.0001
Zinc intake (mg/d)[3]	6.9 ± 0.005	7.7 ± 0.001	8.1 ± 0.001	9.0 ± 0.006	<0.0001
Age (y)	55.9 ± 0.07	57.2 ± 0.07	57.3 ± 0.07	57.9 ± 0.07	<0.0001
BMI (kg/m^2)	23.4 ± 0.03	23.6 ± 0.03	23.6 ± 0.03	23.7 ± 0.03	<0.0001
Ever smoker (%)	8.0	4.4	3.8	4.7	<0.0001
Alcohol drinker (%)[5]	23.0	15.7	14.4	13.0	<0.0001
METs (MET-h/d)	32.1 ± 0.06	32.0 ± 0.06	32.0 ± 0.06	31.6 ± 0.06	<0.0001
History of type 2 diabetes (%)	2.3	3.2	3.8	5.5	<0.0001
CRC screening, yes (%)[6]	27.1	32.3	34.4	33.0	<0.0001
Postmenopausal status (%)	68.3	72.8	73.8	74.3	<0.0001
Ever hormone use (%)	12.4	12.4	12.9	14.1	<0.0001
Dietary intake[3]					
Total energy (kcal/d)	1873 ± 5.3	1812 ± 5.0	1822 ± 5.2	1908 ± 5.9	<0.0001
Calcium (mg/d)	432 ± 1.3	497 ± 1.5	557 ± 1.7	653 ± 2.5	<0.0001
Vitamin D (μg/d)	8.2 ± 0.06	9.4 ± 0.05	10.0 ± 0.05	10.6 ± 0.0	<0.0001
Magnesium (mg/d)	254 ± 0.4	269 ± 0.4	282 ± 0.4	298 ± 0.6	<0.0001
Vitamin B-6 (mg/d)	1.3 ± 0.003	1.4 ± 0.002	1.5 ± 0.002	1.5 ± 0.003	<0.0001
Vitamin B-12 (μg/d)	6.8 ± 0.03	7.9 ± 0.03	8.6 ± 0.04	10.0 ± 0.05	<0.0001
Folate (μg/d)	358 ± 1.2	388 ± 1.2	413 ± 1.3	442 ± 1.5	<0.0001
Fiber (g/d)	12.8 ± 0.04	13.2 ± 0.04	13.6 ± 0.04	13.6 ± 0.05	<0.0001
n−3 PUFAs (g/d)	2.8 ± 0.009	3.1 ± 0.009	3.2 ± 0.008	3.4 ± 0.010	<0.0001
Vegetables (g/d)	202 ± 1.1	223 ± 1.1	236 ± 1.2	240 ± 1.4	<0.0001
Fish (g/d)	75.7 ± 0.5	83.9 ± 0.4	86.7 ± 0.4	89.5 ± 0.5	<0.0001
Red meat (g/d)	34.4 ± 0.2	41.4 ± 0.3	46.0 ± 0.3	58.5 ± 0.4	<0.0001
Heme iron intake					
No. of participants	11,344	11,344	11,344	11,344	
Heme iron intake (mg/d)[3]	0.21 ± 0.0007	0.35 ± 0.0003	0.47 ± 0.0004	0.74 ± 0.002	<0.0001
Zinc intake (mg/d)[3]	7.6 ± 0.009	7.7 ± 0.007	7.9 ± 0.007	8.4 ± 0.008	<0.0001
Age (y)	57.1 ± 0.07	57.3 ± 0.07	57.0 ± 0.07	57.0 ± 0.07	0.003
BMI (kg/m^2)	23.4 ± 0.03	23.5 ± 0.03	23.6 ± 0.03	23.9 ± 0.03	<0.0001
Ever smoker (%)	4.6	4.5	5.4	6.4	<0.0001
Alcohol drinker (%)[5]	16.2	16.3	17.1	16.6	0.2
METs (MET-h/d)	32.2 ± 0.06	31.9 ± 0.06	31.8 ± 0.06	31.8 ± 0.06	<0.0001
History of type 2 diabetes (%)	3.3	3.7	4.0	3.8	0.04
CRC screening, yes (%)[6]	33.7	32.8	31.4	29.0	<0.0001
Postmenopausal status (%)	73.1	72.9	71.7	71.6	0.06
Ever hormone use (%)	12.7	12.3	13.5	13.2	0.001
Dietary intake[3]					
Total energy (kcal/d)	1969 ± 5.0	1734 ± 4.7	1756 ± 5.1	1957 ± 6.1	<0.0001
Calcium (mg/d)	591 ± 2.3	542 ± 1.8	522 ± 1.8	484 ± 1.7	<0.0001
Vitamin D (μg/d)	7.0 ± 0.04	9.0 ± 0.04	10.4 ± 0.05	11.8 ± 0.07	<0.0001
Magnesium (mg/d)	282 ± 0.5	278 ± 0.5	274 ± 0.5	269 ± 0.5	<0.0001
Vitamin B-6 (mg/d)	1.3 ± 0.003	1.4 ± 0.002	1.5 ± 0.002	1.6 ± 0.003	<0.0001
Vitamin B-12 (μg/d)	5.5 ± 0.02	7.4 ± 0.02	9.0 ± 0.03	11.3 ± 0.05	<0.0001
Folate (μg/d)	404 ± 1.4	402 ± 1.3	397 ± 1.3	398 ± 1.3	0.0004
Fiber (g/d)	14.5 ± 0.05	13.7 ± 0.04	13.0 ± 0.04	11.9 ± 0.04	<0.0001
n−3 PUFAs (g/d)	2.5 ± 0.007	3.0 ± 0.007	3.3 ± 0.008	3.7 ± 0.011	<0.0001
Vegetables (g/d)	238 ± 1.4	230 ± 1.2	223 ± 1.1	211 ± 1.1	<0.0001
Fish (g/d)	54.8 ± 0.3	76.5 ± 0.3	92.7 ± 0.4	111.7 ± 0.6	<0.0001
Red meat (g/d)	21.4 ± 0.1	35.1 ± 0.2	49.1 ± 0.2	74.7 ± 0.4	<0.0001

[1] CRC, colorectal cancer; JPHC, Japan Public Health Center–based; MET, metabolic equivalent task; Q, quartile.
[2] Derived by using ANOVA or the chi-square test.
[3] All mean total intakes of food and nutrition were energy adjusted.
[4] Mean ± SE (all such values).
[5] Alcohol consumption ≥1 g ethanol/wk.
[6] CRC screening included fecal occult blood test, barium enema, or colonoscopy.

zinc intake and CRC risk, and all of these studies involved Western populations. The results indicated a decreased risk of CRC of zinc intake in 2 studies from the United States (6, 8) and one from Australia (9) but not in a study from Sweden (7). In the present study, we observed no significant association between zinc intake and CRC risk in the general population of Japan,

TABLE 3
HRs and 95% CIs for colorectal cancer risk according to quartiles of intakes of zinc and heme iron among men[1]

	Q1	Q2	Q3	Q4	*P*-trend
Zinc intake					
Median (mg/d)	7.05	8.19	8.9	9.83	
Person-years	91,139	93,391	93,237	92,355	
Colorectal cancer					
No. of cases	230	182	185	189	
Age- and area-adjusted HR (95% CI)	1.00 (reference)	0.71 (0.58, 0.86)	0.68 (0.56, 0.83)	0.70 (0.57, 0.85)	0.0004
Multivariate-adjusted HR (95% CI)[2]	1.00 (reference)	0.79 (0.63, 0.98)	0.80 (0.63, 1.03)	0.77 (0.58, 1.03)	0.2
Colon cancer					
No. of cases	157	116	130	124	
Age- and area-adjusted HR (95% CI)	1.00 (reference)	0.66 (0.52, 0.84)	0.71 (0.56, 0.90)	0.68 (0.54, 0.87)	0.005
Multivariate-adjusted HR (95% CI)[2]	1.00 (reference)	0.74 (0.57, 0.97)	0.84 (0.62, 1.13)	0.76 (0.54, 1.07)	0.3
Rectal cancer					
No. of cases	73	66	55	65	
Age- and area-adjusted HR (95% CI)	1.00 (reference)	0.81 (0.58, 1.13)	0.63 (0.44, 0.90)	0.73 (0.52, 1.02)	0.03
Multivariate-adjusted HR (95% CI)[2]	1.00 (reference)	0.87 (0.60, 1.28)	0.72 (0.46, 1.12)	0.80 (0.49, 1.32)	0.3
Heme iron intake					
Median (mg/d)	0.24	0.39	0.53	0.77	
Person-years	94,132	92,107	91,799	92,084	
Colorectal cancer					
No. of cases	214	174	194	204	
Age- and area-adjusted HR (95% CI)	1.00 (reference)	0.85 (0.69, 1.03)	0.99 (0.81, 1.21)	1.06 (0.87, 1.29)	0.3
Multivariate-adjusted HR (95% CI)[2]	1.00 (reference)	0.87 (0.69, 1.08)	1.00 (0.78, 1.28)	1.06 (0.79, 1.42)	0.6
Colon cancer					
No. of cases	149	111	132	135	
Age- and area-adjusted HR (95% CI)	1.00 (reference)	0.78 (0.61, 1.00)	0.98 (0.78, 1.25)	1.02 (0.80, 1.29)	0.6
Multivariate-adjusted HR (95% CI)[2]	1.00 (reference)	0.77 (0.59, 1.01)	0.97 (0.72, 1.30)	1.02 (0.71, 1.46)	0.7
Rectal cancer					
No. of cases	65	63	62	69	
Age- and area-adjusted HR (95% CI)	1.00 (reference)	0.99 (0.70, 1.40)	1.01 (0.71, 1.44)	1.15 (0.82, 1.63)	0.4
Multivariate-adjusted HR (95% CI)[2]	1.00 (reference)	1.11 (0.75, 1.63)	1.09 (0.70, 1.71)	1.17 (0.69, 1.98)	0.6

[1] Cox proportional hazards models were used. Q, quartile.
[2] Adjusted for age, area, BMI, smoking status, ethanol intake, metabolic equivalent tasks, history of type 2 diabetes, screening for colorectal cancer, and intakes of energy-adjusted magnesium, vitamin B-6, vitamin B-12, folate, calcium, vitamin D, n−3 PUFAs, and fiber. Zinc and heme iron intakes were simultaneously included in the model.

where the major food sources of zinc differ from those in Western countries and where zinc intake is lower than that in Western countries; the intake ranges of the third quintiles in Western studies were 9.7–10.3 mg/d (7) to 11.5–14.8 mg/d (6).

Six cohort studies reported an association between heme iron intake and CRC risk, but the association remains controversial (6–8, 10–12). A recent meta-analysis of 5 of these studies suggests a modest positive association between heme iron intake and colon cancer risk (highest quintile compared with lowest quintile; HR: 1.18; 95% CI: 1.06, 1.32) (13). The controversy may be due to confounding or effect modification of vitamin B-6 or vitamin B-12, which was not taken into account previously. These vitamins are present in some of the same foods as heme iron, such as meat and fish (27) (the correlation coefficients between vitamins B-6 and B-12 and heme iron in this study were 0.40 and 0.58 in men and 0.31 and 0.54 in women, respectively). In addition, we previously reported that higher vitamin B-6 intake is associated with a decreased risk of CRC, whereas high vitamin B-12 intake tends to increase CRC risk (*P*-trend = 0.05) in men (23). Vitamins B-6 and B-12 are coenzymes in one-carbon metabolism, which is critical for the synthesis and methylation of DNA (32, 33). Low dietary intake of these nutrients may result in colon carcinogenesis via the induction of aberrations in DNA methylation and synthesis (34, 35). However, high concentrations of vitamin B-12 may also induce hypermethylation in this pathway. Associations of vitamin B-12 with DNA methylation have been observed in rats (36, 37) and humans (38, 39), although the idea that DNA methylation is a cause of CRC remains speculative. In the present study, we found significantly higher CRC risk associated with high vitamin B-12 intake, lower CRC risk associated with high vitamin B-6 intake, and no association of CRC risk with heme iron intake in the multivariate model. These findings suggest that heme iron intake may only partly explain the apparent increased risk of CRC and that other factors present in foods along with heme iron, such as vitamin B-12, might be involved in colorectal carcinogenesis. In addition, differences in other constituents of the major food sources of heme iron between Western and Japanese populations might have produced the different associations with CRC risk observed in previous studies and the present study. Major sources of heme iron in Western populations are red and processed meats. Other constituents of red and processed meats, such as nitrate and heterocyclic amines, reportedly increase CRC risk (11). In contrast, fish and shellfish were the major sources of heme iron in the Japanese population. These foods also include nutrients such as n−3 PUFAs and vitamin D, which protect against CRC (40, 41).

TABLE 4
HRs and 95% CIs for colorectal cancer risk according to quartiles of intakes of zinc and heme iron among women[1]

	Q1	Q2	Q3	Q4	*P*-trend
Zinc intake					
Median (mg/d)	7.04	7.67	8.14	8.83	
Person-years	107,466	109,520	110,258	110,687	
Colorectal cancer					
No. of cases	107	110	143	138	
Age- and area-adjusted HR (95% CI)	1.00 (reference)	0.93 (0.71, 1.22)	1.19 (0.92, 1.53)	1.10 (0.85, 1.43)	0.2
Multivariate-adjusted HR (95% CI)[2]	1.00 (reference)	0.92 (0.70, 1.22)	1.16 (0.88, 1.54)	1.05 (0.77, 1.44)	0.4
Colon cancer					
No. of cases	71	76	105	99	
Age- and area-adjusted HR (95% CI)	1.00 (reference)	0.96 (0.69, 1.33)	1.30 (0.96, 1.77)	1.18 (0.87, 1.61)	0.1
Multivariate-adjusted HR (95% CI)[2]	1.00 (reference)	0.97 (0.69, 1.36)	1.33 (0.95, 1.86)	1.19 (0.81, 1.74)	0.2
Rectal cancer					
No. of cases	36	34	38	39	
Age- and area-adjusted HR (95% CI)	1.00 (reference)	0.88 (0.55, 1.40)	0.95 (0.60, 1.51)	0.95 (0.60, 1.51)	0.9
Multivariate-adjusted HR (95% CI)[2]	1.00 (reference)	0.83 (0.51, 1.35)	0.85 (0.51, 1.41)	0.81 (0.46, 1.43)	0.5
Heme iron intake					
Median (mg/d)	0.23	0.35	0.47	0.67	
Person-years	110,299	108,752	109,056	109,823	
Colorectal cancer					
No. of cases	128	127	120	123	
Age- and area-adjusted HR (95% CI)	1.00 (reference)	1.02 (0.80, 1.30)	1.01 (0.78, 1.29)	1.05 (0.81, 1.35)	0.8
Multivariate-adjusted HR (95% CI)[2]	1.00 (reference)	0.96 (0.73, 1.26)	0.89 (0.65, 1.22)	0.88 (0.61, 1.29)	0.4
Colon cancer					
No. of cases	88	95	80	88	
Age- and area-adjusted HR (95% CI)	1.00 (reference)	1.11 (0.83, 1.48)	0.98 (0.72, 1.33)	1.10 (0.82, 1.48)	0.7
Multivariate-adjusted HR (95% CI)[2]	1.00 (reference)	1.05 (0.76, 1.44)	0.88 (0.60, 1.28)	0.94 (0.60, 1.46)	0.6
Rectal cancer					
No. of cases	40	32	40	35	
Age- and area-adjusted HR (95% CI)	1.00 (reference)	0.83 (0.52, 1.32)	1.05 (0.68, 1.64)	0.93 (0.58, 1.48)	0.99
Multivariate-adjusted HR (95% CI)[2]	1.00 (reference)	0.77 (0.47, 1.29)	0.93 (0.53, 1.63)	0.78 (0.39, 1.58)	0.6

[1] Cox proportional hazards models were used. Q, quartile.
[2] Adjusted for age, area, BMI, smoking status, ethanol intake, metabolic equivalent tasks, history of type 2 diabetes, screening for colorectal cancer, menopausal status, use of exogenous female hormones, and intakes of energy-adjusted magnesium, vitamin B-6, vitamin B-12, folate, calcium, vitamin D, n−3 PUFAs, and fiber. Zinc and heme iron intakes were simultaneously included in the model.

In the present study, we observed a significant inverse association between high zinc intake and CRC risk among drinkers in men. However, there was no significant interaction and the dose-response was not clear, which suggests that the inverse association between zinc intake and CRC risk among drinkers in men might be a random finding. Alternatively, the study might be underpowered to detect the significant interaction between alcohol consumption and zinc intakes with regard to CRC risk. Further research in a large population is needed to investigate the interaction with alcohol consumption.

The strength of this study was its prospective design, which enabled us to avoid exposure recall bias. Participants were selected from the general population, the sample size was large, the response rate for the surveys was acceptable for studies of settings such as this, and the loss to follow-up was negligible. In addition, the cancer registry was of sufficient quality to reduce misclassification of the outcomes.

Several limitations of the study warrant mention. First, we assessed zinc and iron intakes by using an FFQ, and heme iron content values were calculated on the basis of type-specific percentages of total iron content. In addition, in our FFQ, we included only 2 types of shellfish that were rich in zinc. Therefore, there may have been some misclassification of zinc and heme iron intakes. Especially for heme iron intake in women, the lower validity of the FFQ (r = 0.11–0.27 for women) may have resulted in the misclassification of individual intakes. The lack of association between heme iron intake and CRC risk among women in the present study may have been due partly to the poor validity of the FFQ for heme iron intake. However, no previous study has reported the validation of heme iron intake from an FFQ. Two studies showed the validation of iron intake (8) or major food sources of dietary heme iron, such as red meat and processed meat (8, 10), but none of the other studies showed the validation of heme iron intake (6, 7, 9, 11, 12). Therefore, we could not compare our validation of heme iron intake to validations in previous studies. Second, we did not collect information on zinc and iron supplement use. However, a survey of supplement use in Japan from 2000 to 2002 showed that the prevalence of zinc and iron supplementation is low (<0.5% in both men and women and 0.2% in men and 2.4% in women, respectively) (28), and thus we considered intake from supplements to be negligible. Third, although we measured and adjusted for possible confounding variables to the extent possible, the possibility of unmeasured confounding variables cannot be totally disregarded. Also, some of the significant findings may have been due to chance.

In conclusion, in this large-scale, population-based prospective cohort study in middle-aged Japanese men and women, whose

TABLE 5
HRs and 95% CIs for colorectal cancer risk among men according to quartiles of zinc and heme iron intakes and alcohol consumption[1]

	Q1		Q2		Q3		Q4			
	No. of cases	HR (95% CI)[2]	No. of cases	HR (95% CI)[2]	No. of cases	HR (95% CI)[2]	No. of cases	HR (95% CI)[2]	*P*-trend	*P*-interaction
Zinc intake										
Colorectal cancer										
Nondrinker	14	1.00 (ref)	30	0.69 (0.36, 1.33)	66	0.86 (0.46, 1.61)	80	0.75 (0.39, 1.46)	0.8	0.1
Drinker[3] (overall)	216	1.00 (ref)	150	0.74 (0.59, 0.92)	113	0.62 (0.48, 0.81)	101	0.63 (0.47, 0.85)	0.001	
<150 g/wk	12	1.00 (ref)	29	0.71 (0.35, 1.43)	46	0.66 (0.33, 1.33)	47	0.58 (0.27, 1.23)	0.2	
150–299 g/wk	33	1.00 (ref)	49	0.83 (0.52, 1.32)	33	0.75 (0.44, 1.29)	32	1.08 (0.59, 1.96)	0.9	
300–449 g/wk	51	1.00 (ref)	45	0.91 (0.59, 1.41)	24	0.77 (0.44, 1.36)	17	0.87 (0.44, 1.70)	0.7	
≥450 g/wk	120	1.00 (ref)	27	0.74 (0.47, 1.19)	10	0.67 (0.33, 1.38)	5	0.55 (0.21, 1.47)	0.1	
Colon cancer										
Nondrinker	7	1.00 (ref)	22	1.05 (0.44, 2.51)	48	1.32 (0.57, 3.07)	53	1.08 (0.44, 2.63)	0.9	0.07
Drinker[3] (overall)	150	1.00 (ref)	92	0.65 (0.49, 0.86)	79	0.63 (0.46, 0.86)	68	0.62 (0.43, 0.89)	0.01	
<150 g/wk	10	1.00 (ref)	19	0.57 (0.26, 1.26)	29	0.48 (0.22, 1.07)	29	0.41 (0.17, 0.99)	0.08	
150–299 g/wk	20	1.00 (ref)	27	0.69 (0.38, 1.26)	28	0.94 (0.49, 1.80)	21	1.00 (0.47, 2.10)	0.7	
300–449 g/wk	37	1.00 (ref)	30	0.87 (0.52, 1.48)	15	0.66 (0.33, 1.33)	15	1.11 (0.52, 2.38)	0.9	
≥450 g/wk	83	1.00 (ref)	16	0.57 (0.31, 1.02)	7	0.61 (0.26, 1.44)	3	0.45 (0.13, 1.56)	0.08	
Rectal cancer										
Nondrinker	7	1.00 (ref)	8	0.30 (0.10, 0.88)	18	0.36 (0.13, 0.96)	27	0.37 (0.13, 1.06)	0.4	1.0
Drinker[3] (overall)	66	1.00 (ref)	58	0.93 (0.63, 1.37)	34	0.61 (0.38, 0.98)	33	0.67 (0.39, 1.13)	0.04	
<150 g/wk	2	1.00 (ref)	10	1.45 (0.31, 6.87)	17	1.54 (0.33, 7.28)	18	1.42 (0.28, 7.23)	0.9	
150–299 g/wk	13	1.00 (ref)	22	1.04 (0.50, 2.17)	5	0.36 (0.12, 1.10)	11	1.32 (0.48, 3.67)	0.8	
300–449 g/wk	14	1.00 (ref)	15	0.99 (0.44, 2.24)	9	1.04 (0.37, 2.87)	2	0.31 (0.06, 1.62)	0.3	
≥450 g/wk	37	1.00 (ref)	11	1.27 (0.58, 2.79)	3	0.78 (0.21, 2.95)	2	0.89 (0.18, 4.54)	0.9	
Heme iron intake										
Colorectal cancer										
Nondrinker	45	1.00 (ref)	42	0.88 (0.55, 1.40)	46	0.91 (0.54, 1.53)	57	1.10 (0.60, 2.04)	0.7	0.7
Drinker[3] (overall)	166	1.00 (ref)	127	0.83 (0.64, 1.07)	145	1.02 (0.77, 1.36)	142	1.04 (0.74, 1.47)	0.6	
<150 g/wk	25	1.00 (ref)	27	0.87 (0.48, 1.57)	44	1.30 (0.70, 2.41)	38	1.13 (0.53, 2.38)	0.5	
150–299 g/wk	33	1.00 (ref)	39	1.04 (0.62, 1.74)	39	1.04 (0.57, 1.87)	36	0.92 (0.44, 1.91)	0.8	
300–449 g/wk	47	1.00 (ref)	23	0.56 (0.33, 0.98)	31	0.91 (0.50, 1.66)	36	1.11 (0.54, 2.30)	0.6	
≥450 g/wk	61	1.00 (ref)	38	0.94 (0.60, 1.49)	31	1.01 (0.59, 1.72)	32	1.13 (0.60, 2.13)	0.8	
Colon cancer										
Nondrinker	33	1.00 (ref)	28	0.87 (0.50, 1.52)	32	1.03 (0.56, 1.91)	37	1.30 (0.63, 2.72)	0.5	0.4
Drinker[3] (overall)	114	1.00 (ref)	80	0.72 (0.53, 0.99)	98	0.97 (0.69, 1.37)	97	1.01 (0.66, 1.53)	0.7	
<150 g/wk	19	1.00 (ref)	16	0.67 (0.33, 1.39)	28	1.14 (0.54, 2.40)	24	1.01 (0.41, 2.51)	0.7	
150–299 g/wk	21	1.00 (ref)	24	0.92 (0.48, 1.76)	27	0.98 (0.47, 2.03)	24	0.80 (0.32, 1.95)	0.7	
300–449 g/wk	33	1.00 (ref)	15	0.49 (0.25, 0.96)	21	0.84 (0.41, 1.73)	28	1.09 (0.46, 2.59)	0.8	
≥450 g/wk	41	1.00 (ref)	25	0.88 (0.51, 1.53)	22	1.08 (0.57, 2.04)	21	1.30 (0.60, 2.82)	0.5	
Rectal cancer										
Nondrinker	12	1.00 (ref)	14	0.91 (0.37, 2.21)	14	0.71 (0.26, 1.95)	20	0.78 (0.25, 2.44)	0.7	0.5
Drinker[3] (overall)	52	1.00 (ref)	47	1.08 (0.70, 1.68)	47	1.13 (0.68, 1.88)	45	1.13 (0.61, 2.08)	0.7	
<150 g/wk	6	1.00 (ref)	11	1.51 (0.51, 4.48)	16	1.90 (0.59, 6.10)	14	1.60 (0.41, 6.25)	0.6	
150–299 g/wk	12	1.00 (ref)	15	1.30 (0.56, 3.05)	12	1.13 (0.40, 3.18)	12	1.20 (0.34, 4.21)	0.9	
300–449 g/wk	14	1.00 (ref)	8	0.86 (0.32, 2.34)	10	1.23 (0.40, 3.74)	8	1.13 (0.27, 4.68)	0.7	
≥450 g/wk	20	1.00 (ref)	13	1.05 (0.48, 2.31)	9	0.82 (0.31, 2.17)	11	0.81 (0.26, 2.55)	0.7	

[1] Cox proportional hazards models were used. Q, quartile; ref, reference.
[2] Adjusted for age, public health center area, BMI, smoking status, metabolic equivalent tasks, history of type 2 diabetes, screening for colorectal cancer, and intakes of energy-adjusted magnesium, vitamin B-6, vitamin B-12, folate, calcium, vitamin D, n−3 PUFAs, and fiber. Heme iron and zinc intakes were simultaneously included in the model.
[3] Alcohol consumption ≥1 g ethanol/wk.

sources of zinc and heme iron intakes differed from those of Western populations and whose intakes of zinc and heme iron were moderate by Western standards, we found no substantial association between dietary zinc and heme iron intakes and CRC.

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CLINICAL—LIVER

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

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

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

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

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

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

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

Materials and Methods

Study Population

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

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

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

Baseline Survey

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

Food Frequency Questionnaire

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

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

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

Blood Collection and Laboratory Assays

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

Follow-up and Identification of Hepatocellular Carcinoma

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

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

Statistical Analysis

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