Further studies in populations with larger body sizes are thus required. Finally, the present study was based not on incident but on prevalent cases, meaning that the ORs of colorectal adenoma presented in this study did not necessarily indicate the risk of "developing" colorectal adenoma, but rather the risk of "having" colorectal adenoma at a point in time, and should therefore be interpreted with caution.

In summary, adiponectin may decrease the risk of colorectal neoplasia through mechanisms other than the indirect mechanism through insulin resistance. Taking recent evidence from basic research into account, we hypothesize that adiponectin may exert an anticarcinogenic effect on the large intestine by interfering with leptin, and that leptin could conversely exert a carcinogenic effect under conditions of a lower abundance of adiponectin. Our observations add to a growing body of evidence for the interactive effects of adiponectin and leptin in the early stage of colorectal tumorigenesis separate to their profound involvement in insulin resistance.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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ORIGINAL PAPER

Leisure-time physical activity and breast cancer risk by hormone receptor status: effective life periods and exercise intensity

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Abstract
Objective Physical activity may decrease breast cancer risk. However, it is unclear what intensity of exercise and during which life periods this effect on decreasing risk is efficiently expressed, and whether the associations differ by the estrogen-/progesterone- receptor (ER/PR) status of tumors. We investigated associations between age- and intensity-specific leisure-time physical activity and ER/PR-defined breast cancer risk.
Methods We conducted a hospital-based case–control study in Nagano, Japan. Subjects were 405 cases newly diagnosed (>99% known ER/PR) from 2001 to 2005, who were age-/area-matched with 405 controls. Activity was assessed with a self-reported questionnaire which considered intensity level (moderate and/or strenuous) at different ages (at 12 and 20 years, and in the previous 5 years). Odds ratios (ORs) and 95% confidence intervals were calculated using logistic regression.
Results Strenuous but not moderate physical activity at age 12 was inversely associated with pre- and post-menopausal breast cancer risk across ER/PR subtypes [overall $OR_{\geq 5\ days/week\ vs.\ none} = 0.24\ (0.14–0.43)$]. Moderate physical activity in the previous 5 years was significantly associated with a decrease in risk for post menopausal ER + PR + tumors only [$OR_{\geq 1\ day/week\ vs.\ none} = 0.35\ (0.18–0.67)$].
Conclusion Strenuous activity in teens and moderate activity after menopause may contribute to a reduction in breast cancer risk.

Keywords Breast cancer · Physical activity · Estrogen receptor · Progesterone receptor · Risk

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Abbreviations

BMI	Body mass index
CIs	Confidence intervals
ER	Estrogen receptor
FFQ	Food frequency questionnaire
IGF-1	Insulin-like growth factor 1
OC	Oral contraceptives
OR	Odds ratio
PMH	Postmenopausal hormones
PR	Progesterone receptor
SHBG	Sex hormone-binding globulin
SD	Standard deviation

Introduction

Breast cancer now ranks as the most frequent cancer in Japanese women [1]. Data from 13 population-based

Cancer Registries show a steep and continuous increase in incidence, with an age-standardized rate of 17 per 100,000 in 1975 versus 43 in 2003.

Physical activity has been hypothesized to decrease the risk of both premenopausal [2] and postmenopausal breast cancer, as suggested by an expert panel of the World Cancer Research Fund/American Institute for Cancer Research [3]. Although the biologic mechanisms underlying the inverse relationship between physical activity and breast cancer have yet to be explained, they may partly include decreased production [4] or bioavailability of endogenous sexual hormones [5–7] or of metabolic-related hormones and growth factors, such as estrogens, insulin [8], and insulin-like growth factors (IGF-1) [9], which may stimulate cellular proliferation/differentiation in the breast [10–13]. Other proposed mechanisms involve an improvement in the immunity [14] and balancing of oxidative stress [15–16].

To our knowledge, 13 epidemiological studies have investigated the relationship between physical activity and estrogen and/or progesterone receptor (ER and/or PR) defined breast cancer risk, namely eight cohort [2, 17–23] and five case–controls studies [24–28]. Results have been inconsistent, however, and only a few studies considered both age and the intensity of exercise [2, 17, 18, 22]. Of them, only one has reported in Asia [27]. Thus, epidemiological evidence is lacking for the association between physical activity and risk of ER/PR-defined breast cancer in consideration of both age and intensity of exercise, particularly in the Asian population.

Here, we conducted a hospital-based case–control study among 810 women in Nagano, Japan, >99% of whom had complete data on ER/PR tumor status.

Materials and methods

Subjects

The subjects of the study have been described in detail elsewhere [29]. Briefly, the study base consisted of Japanese women aged 20–74 years living in Nagano, Japan, who received hospital-based treatment or examination at Nagano Red Cross Hospital, Nagano Municipal Hospital, Nagano Matsushiro General Hospital, or Hokushin General Hospital from 2001 to 2005. Eligible cases were 412 patients with newly diagnosed and histologically confirmed primary invasive breast cancer who were under cancer treatment in the study base. Written informed consent was obtained from 405 patients (response rate 98%). Eligible control subjects were cancer-free women who underwent medical examination at two of the hospitals, Nagano Matsushiro General Hospital and Hokushin General Hospital. Although one subject declined participation (response rate >99%), 405 control subjects were selected and individually pair-matched with the 405 case subjects by age (within 3 years) and residential area. All 405 matched pairs provided written informed consent and were defined as the study subjects in the current analysis. The study protocol was approved by the Institutional Review Board of the National Cancer Center in 2000.

Exposure assessment for physical activity

Information on physical activity was collected from 2001 to 2005 using a self-reported questionnaire. In the questionnaire, we enquired about a leisure-time physical activity in each of three age periods in life, namely at ages 12 years, 20 years, and during the past 5 years.

With regard to intensity, participants were first asked whether they were engaged in "strenuous exercises", with running, basketball, and competitive swimming provided as examples, as well as "moderate activity", such as leisure-time volleyball, softball, walking, and cycling. Additionally, we created new intensity category to combine moderate with strenuous activity. If a subject answered with different frequencies across moderate and strenuous intensities, the higher frequency was applied.

With regard to frequency, the questionnaire asked how often they were physically active at these times, with the six frequency categories of less than once per month, 1–3 days per month, 1–2 days per week, 3–4 days per week, 5–6 days per week, and every day of the week. For analyses, frequency levels were subdivided into four categories [(1) None; (2) $\leqq$3 days per month; (3) 1–4 days per week; and (4) $\geq$5 days per week]. Results were evaluated in three intensity levels [(1) participation at all levels (i.e., strenuous and/or moderate); (2) strenuous; or (3) moderate] with four categories of frequency in three periods of life (ages 12, 20 years, and during the past 5 years).

To assess the validity of estimated physical activity, we compared the distribution of participation rates in recreational physical activity between 253 of our control subjects aged 45–64 and 5,109 Nagano residents aged 45–64 who comprised a subgroup in the 5-year follow-up survey of the Japanese Public Health Center–based prospective study (the JPHC Study), which had validated information on physical activity [30]. Results showed that the prevalence of physical activity in the present subjects was similar to that in this JPHC subsample cohort from the same prefecture, Nagano (37% in the current study and 38% in JPHC sub-cohort).

To assess dietary habits, participants were also asked to report the average frequency and/or portion size of each food item during the past year in a validated semi-quantitative food-frequency questionnaire (FFQ), which included 136 commonly consumed food items [31, 32]. Estimated

nutrient intakes based on the FFQ were calculated using food composition values from the standard tables of food composition in Japan [33].

Identification of breast cancer cases

Histologically confirmed incident cases of invasive breast cancer and information on the ER and PR status (±) of breast tumors were identified by linkage with medical records. Regarding ER and PR status, 291 cases (71.9% of total cases) were evaluated with an immunohistochemical assay and 111 cases (27.4%) with an enzyme-linked immunoassay. Three cases (0.7%) had no information on assay. For immunohistochemical assay, we defined a tumor as hormone receptor positive if any positive cell was found on examination of a specimen. For enzyme-linked immunoassay, a tumor was diagnosed as hormone receptor positive with ≥10 fmol/mg protein.

Data analysis

Baseline characteristics between cases and controls were compared using the Cochran-Mantel-Haenszel test or chi-square test.

To assess the overall association between physical activity at age 12 and 20 years, and in the preceding 5 years and breast cancer risk, we used a conditional logistic regression model to estimate odds ratios (ORs) and 95% confidence intervals (CIs). All analyses excluded women with incomplete data on leisure-time physical activity as a main exposure of interest and the corresponding paired subjects.

In the multivariable analyses with stratification by menopausal status, ORs were calculated by an unconditional logistic regression model to minimize the exclusion of subjects. We adjusted for age, area (urban or rural), body mass index [BMI; weight in kilograms divided by the square of height in meters] (<21, 21–23.9, 24–26.9, ≥27), alcohol drinking status (non-regular, regular), smoking status (never, current, past), educational level (junior/high school, junior college or technical school, university or higher), age at menarche (<12, 12, 13, 14, ≥15), age at first birth (nulliparous, <27, ≥27 years) and the number of live birth (nulliparous, 1–2, ≥3), menopausal status (pre- or postmenopause), total energy-adjusted isoflavone intake (quintiles) and total energy-adjusted vegetable consumption (quintiles). For postmenopausal women, use of non-contraceptive exogenous hormones (never, ever) was additionally included in the model.

Although we had no precise information on the duration of physical activity, approximate lifetime duration was classified into four categories, namely (1) no participation in three periods of life (ages 12, 20 years, and in the last 5 years); (2) participation in one life period (women who participated in any one of three periods of life); (3) participation in two life periods; or (4) participation in three life periods, and added as a co-variate in the model in further analyses.

Tests for trend were calculated using an ordinal variable for frequency level of physical activity and including it in the regression model as a linear variable.

For the multivariable analyses with stratification by menopausal status, we excluded women with incomplete information on leisure-time physical activity and all these adjusted factors, such as BMI, alcohol drink, smoking status, education, age at menarche, parity, age at menopause and women who reported extreme total energy intake as estimated by food frequency questionnaires (FFQ; <800 or ≥4,000 kcal/day).

In evaluating moderate activity, all subjects who engaged in strenuous activity were excluded. We performed analyses stratified by menopausal status (pre-, postmenopausal) and levels of BMI (<21, 21–24, ≥25 kg/m^2) and to assess possible interactions of physical activity level with them because these factors changed hormonal status. The cross-product terms of these factors and levels of physical activity frequency in the preceding 5 years were introduced into the model. *P*-value for interaction was calculated by a likelihood ratio test, which compared models with and without the interaction terms.

To evaluate the association between physical activity and ER/PR-defined breast cancer risk and to test heterogeneity in the association of physical activity across response functions of each joint hormone receptor status, we used a polytomous logistic regression model. All analyses were performed using the SAS statistical package release 9.1 (SAS Institute, Cary, NC). All statistical tests were two-sided, and statistical significance was defined as $P < .05$.

Results

Baseline characteristics of case and control subjects are shown in Table 1. Mean age of all subjects was 53.8 years. Compared with breast cancer case subjects, control subjects were more likely to have a higher consumption of vegetables and to be non-smokers and university graduates. Case subjects tended to have not actively engaged in exercise at ages 12 and 20 years or in the past 5 years. All subjects tended to have a higher participation rate in daily physical activity (≥5 days/week) at age 12 years than at age 20 years or older.

At age 12 years, daily participation (≥5 days/week) in leisure-time activity was associated with a decreased risk of breast cancer [multivariable-adjusted OR for moderate

Table 1 Characteristics of 405 breast cancer cases and matched control subjects

	Controls		Cases		P^a
	(n = 405)		(n = 405)		
	Mean	±SD[b]	Mean	±SD[b]	
Age (years)	53.9	10.2	53.6	10.5	
Body mass index (kg/m^2)	22.9	±3.2	22.7	±3.4	0.07
Age at menarche (years)	13.2	±1.6	13.4	±1.7	0.31
Age at first birth (years)[c]	26.4	±3.1	26.9	±3.6	0.54
Age at menopause (years)[d]	49.3	±4.2	49.1	±4.4	0.14
Vegetable intake (g/day)[e]	314	169	271	143	<.0001
	n	(%)	n	(%)	
Premenopausal women	143	(35)	185	(46)	<.0001
Smoking status (ever)	31	(8)	84	(21)	<.0001
Alcohol drinking (regular drinker)	121	(30)	105	(26)	0.2
Education					
Junior high school	26	(6)	61	(15)	<.0001
High school	210	(52)	207	(51)	
Junior/technical college	84	(21)	115	(28)	
University	85	(21)	22	(6)	
Physical activity[f]					
At age 12 years					
No	183	(45)	241	(60)	<.0001
≦3 days/month	9	(2)	13	(3)	
1–4 day/week	55	(14)	66	(16)	
≥5 days/week	158	(39)	85	(21)	
At age 20 years[g]					
No	256	(63)	299	(74)	<.0001
≦3 days/month	47	(12)	51	(13)	
1–4 day/week	76	(19)	44	(11)	
≥5 days/week	26	(6)	10	(2)	
In the last 5 years[g]					
No	236	(58)	264	(65)	0.02
≦3 days/month	36	(9)	42	(10)	
1–4 day/week	99	(25)	72	(18)	
≥5 days/week	34	(8)	26	(6)	

mean ± standard deviation or n (%)

[a] P values were calculated using the Cochran-Mantel-Haenszel test with matched-pair strata

[b] *SD* standard deviation

[c] Parous women only

[d] postmenopausal women only

[e] Total energy-adjusted

[f] All levels of intensity (any participation either moderate or strenuous)

[g] subjects with unknown/missing data were excluded in Table 1

or strenuous was 0.38 (95% CI: 0.23–0.62; P_{trend} 0.0001)]. The observed inverse association was apparent for strenuous intensity [the corresponding OR = 0.24 (95% CI: 0.14–0.43; P_{trend} < .0001)], but no such decrease was seen for moderate intensity [OR = 0.91 (0.31–2.72; P_{trend} 0.94); Table 2].

At age 20 years, daily participation in leisure-time activity was not associated with a decreased risk of breast cancer either overall (i.e., moderate or strenuous intensity) or for strenuous intensity after adjustment for all co-variates, although a statistically significant inverse trend was observed for moderate intensity [$ORs_{most\ vs.\ least}$ for moderate = 0.33 (95% CI: 0.06–1.96; P_{trend} 0.041); Table 2].

With regard to daily participation in leisure-time activity in the preceding 5 years, a weak inverse trend was observed overall [$OR_{most\ vs.\ least}$ 0.60 (0.30–1.18; P_{trend} 0.11); Table 2]. According to intensity levels, no inverse trend was seen with strenuous intensity [$OR_{most\ vs.\ least}$ for strenuous = 1.04 (95% CI: 0.11–9.61; P_{trend} 0.42)], but we found a statistically significant inverse association with moderate intensity [for moderate = 0.45 (0.21–0.97; P_{trend} 0.008); Table 2].

After multivariable adjustment and stratification by menopausal status, strenuous leisure-time activity (≥5 days/week) at age 12 years was statistically significantly associated with a decreased risk of premenopausal breast cancer [$OR_{most\ vs.\ least}$ = 0.42 (95% CI: 0.22–0.82; P_{trend} 0.009)] and postmenopausal breast cancer [$OR_{most\ vs.\ least}$ = 0.19 (95% CI: 0.10–0.35; P_{trend} < .0001); $P_{interaction}$ 0.17; Table 3]. The observed inverse association of strenuous activity at age 12 years with risk was not eliminated in additional analyses with further exclusion of women not engaged in moderate activity in the last 5 years (data not shown), with further simultaneous adjustment for moderate physical activity at age 20 and in the last 5 years (Table 3), or with adjustment for approximate lifetime duration (data not shown). In contrast, moderate activity at age 12 years was not associated with breast cancer risk among women not engaged in strenuous activity even after multivariable adjustment and stratification by menopausal status (data not shown).

Regarding activity at age 20 years, we specifically focused on moderate intensity only because the inverse trend was confined to moderate intensity in Table 2.

After exclusion of participants with strenuous activity at age 20, moderate leisure-time physical activity at age 20 years was not associated with breast cancer risk among premenopausal women, but there was an inverse trend among postmenopausal women [$OR_{most\ vs.\ least}$ = 0.29 (95% CI: 0.05–1.57; P_{trend} 0.042); Table 3]. However, the observed association was attenuated after mutual adjustment for strenuous excise at age 12 and moderate excise in the preceding 5 years in overall analysis (P_{trend} = 0.27). The observed ORs were not heterogeneous across menopausal status [$P_{interaction}$ 0.94; Table 3]. Strenuous activity at age 20 was not associated with breast cancer risk in

Table 2 Odds ratio (ORs) and 95% confidence intervals (CIs) of breast cancer according to intensity levels of leisure-time physical activity by three life periods (age at 12 years, 20 years, and in the last 5 years) among Japanese women in Nagano

Level of intensity	Age at 12 years old				Age at 20 years old				In the last 5 years			
	N1	N2	OR (95% CI)	OR[a] (95% CI)	N1	N2	OR (95% CI)	OR[a] (95% CI)	N1	N2	OR (95% CI)	OR[a] (95% CI)
Moderate or strenuous			(405 Pairs)	(402 Pairs)			(404 Pairs)	(402 Pairs)			(404 Pairs)	(402 Pairs)
No (ref.)	241	183	1.00 (ref.)	1.00 (ref.)	299	255	1.00 (ref.)	1.00 (ref.)	264	236	1.00 (ref.)	1.00 (ref.)
≦3 days/month	13	9	0.88(0.36–2.14)	1.03(0.34–3.13)	51	47	0.96(0.63–1.45)	0.93(0.52–1.66)	42	36	1.05(0.66–1.67)	1.41(0.74–2.68)
1–4 day/week	66	55	0.85(0.56–1.30)	0.78(0.45–1.35)	44	76	0.50(0.33–0.75)	0.74(0.42–1.30)	72	98	0.65(0.46–0.93)	0.75(0.47–1.20)
≥5 days/week	85	158	0.37(0.26–0.53)	0.38(0.23–0.62)	10	26	0.33(0.15–0.71)	0.56(0.20–1.60)	26	34	0.70(0.41–1.19)	0.60(0.30–1.18)
P_{trend}			<.0001	0.0001			<.0001	0.16			0.019	0.11
Strenuous level			(401 Pairs)	(377 Pairs)[b]			(400 Pairs)	(378 Pairs)[b]			(394 Pairs)	(381 Pairs)[b]
No (ref.)	338	252	1.00 (ref.)	1.00 (ref.)	379	365	1.00 (ref.)	1.00 (ref.)	377	382	1.00 (ref.)	1.00 (ref.)
≦3 days/month	7	2	2.20 (0.43–11.15)	1.91(0.23–15.68)	6	1	6.00(0.72–49.79)	4.76(0.25–91.73)	6	2	3.00(0.61–14.86)	1.47(0.21–10.48)
1–4 day/week	10	19	0.33 (0.14–0.80)	0.37(0.12–1.10)	9	21	0.37(0.16–0.88)	0.78(0.23–2.63)	9	7	1.29(0.48–3.45)	2.22(0.47–10.42)
≥5 days/week	46	128	0.27(0.18–0.40)	0.24(0.14–0.43)	6	13	0.46(0.18–1.21)	2.25(0.54–9.46)	2	3	0.67(0.11–3.99)	1.04(0.11–9.61)
P_{trend}			<.0001	<.0001			0.020	0.47			0.65	0.42
Moderate level[c]			(213 Pairs)	(209 Pairs)			(341 Pairs)	(332 Pairs)			(361 Pairs)	(347 Pairs)
No (ref.)	155	157	1.00 (ref.)	1.00 (ref.)	265	237	1.00 (ref.)	1.00 (ref.)	252	217	1.00 (ref.)	1.00 (ref.)
≦3 days/month	4	4	1.02(0.25–4.13)	0.95(0.15–6.16)	40	46	0.81(0.52–1.26)	0.58(0.31–1.09)	34	32	0.96(0.58–1.59)	1.15(0.56–2.35)
1–4 day/week	33	32	1.05(0.61–1.79)	1.13(0.50–2.52)	32	48	0.60(0.37–0.98)	0.63(0.32–1.26)	55	83	0.56(0.38–0.84)	0.55(0.32–0.94)
≥5 days/week	21	20	1.08(0.53–2.20)	0.91(0.31–2.72)	4	10	0.31(0.08–1.16)	0.33(0.06–1.96)	20	29	0.62(0.34–1.12)	0.45(0.21–0.97)
P_{trend}			0.81	0.94			0.007	0.041			0.004	0.008

Conditional logistic regression models were matched for age and residential area (urban or rural)

[a] Multivariable conditional logistic regression models were matched for age and residential area (urban or rural) and adjusted for body mass index (<21, 21–23.9, 24–26.9, ≥27), alcohol drinking status (non-regular, regular), smoking status (never, current, past), education (junior/high school, junior/technical college, university), age at menarche (<12, 12, 13, 14, ≥15), age at first birth (nulliparous, <27, ≥27 years) and number of live birth (nulliparous, 1–2, ≥3), menopausal status (pre, postmenopause), total energy-adjusted isoflavone intake (quintiles), total energy-adjusted vegetable consumption (quintiles) and mutually adjusted for the corresponding intensity physical activity in the different life periods (i.e., at ages 12, 20 and in the last 5 years)

[b] The analyses were additionally adjusted for moderate level of activity in the corresponding life periods

[c] The analyses were confined to women who did not participate in a strenuous level of activity

N1 = cases; N2 = controls

Table 3 Multivariable-adjusted odds ratios (ORs) and 95% confidence intervals (CIs) between age-specific leisure-time physical activity and breast cancer risk by menopausal status among Japanese women in Nagano

	Premenopausal women						Postmenopausal women[a]						$P_{interaction}$
	Cases	Controls	OR	(95% CI)	OR	(95% CI)[e]	Cases	Controls	OR	(95% CI)	OR	(95% CI)[e]	Pre versus post
At age 12 years: strenuous[b,c]				($n = 315$)		($n = 272$)				($n = 455$)		($n = 411$)	
No or ≦3 days/month (ref.)	149	87	1.00	(ref.)	1.00	(ref.)	182	163	1.00	(ref.)	1.00	(ref.)	0.17
1–4 days/week	2	3	0.42	(0.05–3.43)	0.56	(0.05–6.47)	7	14	0.46	(0.16–1.30)	0.61	(0.16–2.29)	
≥5 days/week	27	47	0.42	(0.22–0.82)	0.37	(0.17–0.83)	15	74	0.19	(0.10–0.35)	0.22	(0.11–0.44)	
P_{trend}				0.009		<0.01				<.0001		<.0001	
At age 20 years: moderate[d]				($n = 288$)		($n = 272$)				($n = 433$)		($n = 411$)	
No (ref.)	122	79	1.00	(ref.)	1.00	(ref.)	159	172	1.00	(ref.)	1.00	(ref.)	0.94
≦3 days/month	23	19	0.85	(0.39–1.85)	0.68	(0.28–1.65)	20	25	0.79	(0.40–1.56)	0.71	(0.30–1.67)	
1–4 days/week	19	22	0.58	(0.26–1.30)	0.57	(0.23–1.41)	15	31	0.58	(0.28–1.22)	0.73	(0.31–1.70)	
≥5 days/week	2	2	1.28	(0.13–12.64)	2.70	(0.14–52.71)	2	9	0.29	(0.05–1.57)	0.47	(0.07–3.10)	
P_{trend}				0.27		0.33				0.042		0.27	
In the last 5 years: moderate[d]				($n = 300$)		($n = 272$)				($n = 441$)		($n = 411$)	
No (ref.)	109	96	1.00	(ref.)	1.00	(ref.)	139	133	1.00	(ref.)	1.00	(ref.)	0.036
≦3 days/month	21	10	2.31	(0.88–6.05)	1.60	(0.54–4.71)	14	23	0.55	(0.24–1.26)	0.64	(0.26–1.56)	
1–4 day/week	27	24	1.04	(0.51–2.14)	1.13	(0.50–2.57)	30	64	0.43	(0.24–0.75)	0.46	(0.24–0.86)	
≥5 days/week	8	5	1.16	(0.30–4.54)	0.97	(0.23–4.15)	13	25	0.41	(0.19–0.92)	0.38	(0.16–0.89)	
P_{trend}				0.67		0.85				0.0009		0.0019	

Multivariable unconditional logistic regression models were adjusted for age (continuous), residential area (urban or rural), body mass index (<21, 21–23.9, 24–26.9, ≥27), alcohol drinking status (non-regular, regular), smoking status (never, current, past), education (junior/high school, junior/technical college, university), age at menarche (<12, 12, 13, 14, ≥15), age at first birth (nulliparous, <27, ≥27 years) and number of live birth (nulliparous, 1–2, ≥3), menopausal status (pre, postmenopause), total energy-adjusted isoflavone intake (quintiles) and total energy-adjusted vegetable consumption (quintiles)

[a] For postmenopausal women, adjusted for use of exogenous hormones

[b] For strenous activity, additionally adjusted for moderate activity (No, ≦3 days/month, 1–4 days/week and ≥5 days/week)

[c] For stratified analyses by menopausal status, frequency levels were subdivided into three categories (No or ≦3 days/month, 1–4 days/week and ≥5 days/week)

[d] For moderate activity, participants in strenuous activity were further excluded

[e] Mutual adjustments for physical activity at age 12 (strenuous, moderate), at age 20 (moderate), and in the last 5 years (moderate) among 730 women (296 premenopausal and 434 postmenopausal) with further exclusion of women who engaged in strenous exercise at age 20 and in the last 5 years

overall, premenopausal, or postmenopausal women after adjustment for moderate activity (text only).

For activity in the preceding 5 years, we focused on moderate intensity only because the observed inverse association was confined to moderate intensity in Table 2.

A statistically significant inverse trend was observed for the association between moderate activity in the preceding 5 years and breast cancer risk among postmenopausal [$OR_{most\ vs.\ least}$ = 0.41 (0.19–0.92; P_{trend} 0.0009)] but not premenopausal women [1.16 (0.30–4.54; P_{trend} 0.67)]. The observed results were heterogeneous across menopausal status ($P_{interaction}$ 0.036; Table 3). To evaluate the impact of moderate activity in the preceding 5 years independently of strenuous activity at age 12 years, further corresponding analysis was performed among postmenopausal women by excluding participants in strenuous activity at age 12 years. Results also supported a statistically significant inverse trend [$OR_{most\ vs.\ least}$ = 0.41 (0.14–1.19; P_{trend} 0.048) text only].

According to the levels of BMI (<21, 21–24, ≧25 kg/m^2), the observed associations between leisure-time physical activity and breast cancer risk were not modified in overall ($P_{interaction}$ 0.81; Appendix) as well as postmenopausal women ($P_{interaction}$ 0.98; Appendix).

We also examined the risk of ER/PR-defined breast cancer associated with physical activity. Among the 388 invasive breast cancer cases in multivariable analyses, information on joint ER/PR status was available for 385 (99% of total cases). Of these, 216 cases were ER + PR + (56% of all known cases), 69 were ER + PR– (18%), 12 were ER–PR + and 88 were ER–PR– (23%). The number of case patients with ER–PR + tumors was too small to analyze separately.

Strenuous leisure-time activity at age 12 years was associated with a statistically significant decrease in the risk of breast cancer irrespective of the ER/PR status of tumors. Compared with the least active women, women who engaged in physical activity(≧1 day/week) had a statistically significant decrease in risk for all ER/PR-defined tumors [$ORs_{most\ vs.\ least,\ for\ ER+PR+}$ 0.35 (0.22–0.58; P_{trend} < .0001), $_{ER+PR-}$ 0.21 (0.08–0.52; P_{trend} 0.0002), $_{ER-PR-}$ 0.25 (0.12–0.52; P_{trend} < .0001), all $P_{heterogeneities\ vs.\ ER+PR+}$ ≥ 0.27; Table 4].

At age 20 years and in the preceding 5 years, we focused on moderate intensity among postmenopausal women only. Moderate activity at age 20 years was not associated with breast cancer risk for ER + PR + and ER + PR– tumors. For ER–PR– tumors, we observed an inverse association [$OR_{most\ vs.\ least}$ 0.16 (0.03–0.81; P_{trend} 0.084); Table 4], although these ORs were not heterogeneous across ER/PR status [all $P_{heterogeneities\ vs.\ ER+PR+}$ ≧ 0.48; Table 4].

Compared to the least active women, engagement in leisure-time activity with moderate intensity in the preceding 5 years (≥1 day/week) was associated with a statistically significant decrease in risk for postmenopausal ER + PR + tumors [OR_{ER+PR+} 0.35 (0.18–0.67; P_{trend} 0.004)], but not for ER + PR– [0.42 (0.17–1.05; P_{trend} 0.14)] or ER–PR– tumors [0.63 (0.30–1.35; P_{trend} 0.44); Table 4]. These ORs were not heterogeneous across ER/PR status [all $P_{heterogeneities\ vs.\ ER+PR+}$ ≥ 0.14; Table 4].

Discussion

In this hospital-based case–control study with consideration to both age and exercise intensity, we found a statistically significant decrease in the risk of breast cancer with increased frequency of participation in strenuous leisure-time physical activity at age 12 years, and in moderate physical activity at age 20 years and in the previous 5 years.

The observed decrease in risk with strenuous activity at age 12 was not heterogeneous across menopausal, BMI, and receptor statuses. At age 20 years, there was an inverse trend between moderate activity and breast cancer risk among postmenopausal women. Leisure-time activity in the last 5 years with moderate intensity was associated with a decrease in risk among postmenopausal women, especially for ER + PR + tumors.

In early life, the impact of strenuous activity on risk reduction for breast cancer appears to be obvious. Further simultaneous adjustment for moderate activity at age 20 and in last 5 years did not eliminate the inverse association in our result. This suggested that the impact of strenuous activity at age 12 years on risk was independent of other lifetime physical activity.

Our results are consistent with those of two large prospective cohort studies, the Nurses' Health Study II [2] and the Women's Health Initiative Cohort Study [18], which also suggested a significant association between 'past participation in strenuous exercise' during age 12–22 years [2] and age 35 years [18], respectively, and a decreased risk of breast cancer. Two case–control studies showed similar results, albeit with borderline significance [25, 27].

In adulthood, the inverse association against breast cancer with increased physical activity we saw is partly supported by some [17–19, 22, 24, 27, 28] but not all [2, 20, 21, 25] previous studies. Further, our finding that exercise by postmenopausal women need not be strenuous to confer a decrease in risk is supported by two large cohort studies, the Women's Health Study [17] and the Women's Health Initiative Cohort Study [18], although not by all [22].

In line with previous recommendations [34], our results might indicate that the optimal intensity of exercise to

Table 4 Odds ratios (ORs) and 95% confidence intervals (CIs) of ER/PR-defined breast cancer according to levels of age-specific leisure-time physical activity among Japanese women in Nagano

Period of life and intensity	Controls	ER + PR + tumor			ER + PR− tumor			ER−PR− tumor			$P_{heterogeneity}$
		Cases	OR	(95% CI)	Cases	OR	(95% CI)	Cases	OR	(95% CI)	
At age 12 years: strenuous level											
(All = 770)				(212 Cases)			(68 Cases)			(87 Cases)	
No (ref.)	248	178	1.00	(ref.)	61	1.00	(ref.)	76	1.00	(ref.)	
≦3 days/month	2	2	2.13	(0.22–20.47)	1	2.27	(0.12–44.92)	1	3.46	(0.24–50.48)	
≥1 day/week	138	32	0.35	(0.22–0.58)	6	0.21	(0.08–0.52)	10	0.25	(0.12–0.52)	$P = 0.27$ $_{ER+PR+ \text{ vs. } ER+PR-}$
P_{trend}				<.0001			0.0002			<.0001	$P = 0.44$ $_{ER+PR+ \text{ vs. } ER-PR-}$
At age 20 years: moderate level[a]											
(Postmenopausal = 433)				(90 Cases)			(49 Cases)			(54 Cases)	
No (ref.)	172	73	1.00	(ref.)	40	1.00	(ref.)	44	1.00	(ref.)	
≦3 days/month	25	8	0.62	(0.25–1.55)	3	0.47	(0.12–1.79)	8	1.07	(0.41–2.78)	
≥1 day/week	40	9	0.63	(0.27–1.48)	6	0.70	(0.23–2.12)	2	0.16	(0.03–0.81)	$P = 0.90$ $_{ER+PR+ \text{ vs. } ER+PR-}$
P_{trend}				0.20			0.40			0.084	$P = 0.48$ $_{ER+PR+ \text{ vs. } ER-PR-}$
In the last 5 years: moderate level[a]											
(Postmenopausal = 441)				(94 Cases)			(45 Cases)			(54 Cases)	
No (ref.)	133	70	1.00	(ref.)	31	1.00	(ref.)	35	1.00	(ref.)	
≦3 days/month	23	5	0.37	(0.12–1.14)	5	1.26	(0.35–4.52)	4	0.74	(0.21–2.69)	
≥1 day/week	89	19	0.35	(0.18–0.67)	9	0.42	(0.17–1.05)	15	0.63	(0.30–1.35)	$P = 0.53$ $_{ER+PR+ \text{ vs. } ER+PR-}$
P_{trend}				0.004			0.14			0.44	$P = 0.14$ $_{ER+PR+ \text{ vs. } ER-PR-}$

Polytomous logistic regression adjusted for age (<40, ≥40, ≥50, ≥60), residential area (urban or rural), body mass index (<21, 21–23.9, 24–26.9, ≥27), alcohol drinking status (non-regular, regular), smoking status (never, current, past), education (junior/high school, junior/technical college, university), age at menarche (<12, 12, 13, 14, ≥15), age at first birth (nulliparous, <27, ≥27 years) and number of live birth (nulliparous, 1–2, ≥3), menopausal status (pre, postmenopausal), total energy-adjusted isoflavone intake (quintiles) and total energy-adjusted vegetable consumption (quintiles)

Number of cases was too small to analyze for ER−PR+ tumors and unknown tumors

[a] We excluded women who engaged in a strenuous level of activity and additionally adjusted for use of exogenous hormones (ever, never)

lower breast cancer risk differs across women's life stages. One possibility may be that aging brings a change in the physiological sensitivity to exercise, which might affect the balance between the anti- and pro-oxidative effects of exercise in the developing breast tumor [15, 35]. In general, however, participation of women in physical activity tends to decline with age [2], and the observed association might be emphasized by the high participation in strenuous exercise among young women and moderate participation among elderly women. In fact, the number of participants in strenuous activities in the last 5 years in our study was markedly low ($n = 29$), and our results for the impact of strenuous activity in the last 5 years should accordingly be interpreted with care.

In subgroup analyses, the substantial decrease in risk among postmenopausal women who participated in physical activity in the preceding 5 years is consistent with some [27, 28, 36, 37] but not all [21] previous studies. Although previous studies suggested relatively strong risk reductions among lean premenopausal women [2], PMH never users [23], and women without a family history of breast cancer [26], the present and others studies suggest that the association between physical activity and breast cancer risk is not modified by BMI [19, 21, 24], or family history of breast cancer [18, 19, 21].

With regard to ER/PR status, we observed that physical activity at age 12 years was associated with a lower risk of breast cancer for nearly all subtypes of tumors. Similar to our results, weak inverse associations for both ER + and ER– [2] have been suggested, as well as a substantially lower risk for ER–PR–, with increased levels of exercise at ages 13–19 years [27] or among premenopausal women [24]. Nevertheless, a case–control study among women aged 22–44 years suggested a heterogeneity in risk between ER + PR + and ER–PR– tumors [25].

Although a marginal inverse trend was seen for the association between moderate activity at age 20 years and risk for ER–PR– tumors among postmenopausal women, this may have been a chance finding due to the small number of cases ($n = 2$) in the highest category.

Among postmenopausal women, our results showed an inverse association of leisure-time activity in the last 5 years with the development of ER + PR + tumors, with marginal heterogeneity in risk between ER + PR + and ER–PR– tumors. Although some previous studies did not support either an apparent inverse association of physical activity with the risk of ER + PR + tumors [17, 23] or any substantial difference in risk across ER- or PR-defined tumors [20, 22, 27], the inverse association for ER + PR + tumors in our study and a recent German study [28] may support the partial involvement of an ER-mediated estrogen mechanism among postmenopausal women. At the same time, our and other results for ER + PR– tumors [19, 24] could not exclude the possible involvement of an ER-mediated but estrogen-independent pathway.

The mechanism of the inverse association between physical activity and breast cancer risk has not been precisely identified. Nevertheless, several biologically plausible explanations for our results have been proposed.

Our result for the inverse association between activity and the development of ER + PR + tumors may be explained by estrogen-related mechanisms, because physical activity may decrease lifetime exposure to estrogens. At a young age, physical activity may alter menstrual characteristics by affecting ovulatory cycles and delaying menarche [6, 7]. After menopause, physical activity may contribute to a decrease in body weight/adipose tissue [4], a major source of endogenous estrogen derived from peripheral conversion of androgens to estrogens [38]. Further, physical activity may be associated with increased levels of sex hormone–binding globulin (SHBG; the main protein carrier of estradiols [5, 39, 40]) and decreased levels of insulin [8] and bio-active IGF-1 concentrations [9], which may inhibit the hepatic synthesis and release of SHBG [41]. Non-estrogen-related mechanisms are also proposed, including favorable immune function [14, 42] and an increase in antioxidant enzymes [15]. Further, given suggestions that IGF-1 may induce lower PR expression in breast tumors through ligand-independent (i.e., non-classical estrogen-mediated) ER activity [43], the result for PR– tumors might be explained by an alternative IGF-1 signaling pathway.

Our results for a substantial inverse association between participation in physical activity at age 12 and 20 years irrespective of ER/PR tumors indicate that physical activity at a young age may exert its affect partly through non-estrogen-related mechanisms. A previous in vivo study suggested that prepubertal physical activity may decrease neoplastic transformation and ER-α/ER-β ratio, as well as increases in the expression of several tumor suppressor genes, such as BRCA1 and p53, in the rat mammary gland [44].

The major strengths of the present study include its high response rate [total participation of cases (98%) and controls (99%)] and high availability of ER/PR status of breast tumors (>99%), which minimized potential selection bias. Detailed information on physical activity, potential confounders, and effect modifiers in our questionnaire allowed us to investigate the association between physical activity and breast cancer with regard to the intensity, frequency, and timing of PA, as well as menopausal status and ER/PR status of tumors within one study.

Several potential limitations of the study also warrant consideration. First, because we used a hospital-based case–control design [45], selection bias should be considered due to the non-random sampling of control subjects, who were

selected from among outpatient visitors receiving medical check-ups at two of the four hospitals. The distribution of risk factors for breast cancer may have differed from that in the general population because of greater health consciousness.

However, a similar prevalence of exercise between our subjects and a sub-cohort of Nagano residents from the Japan Public Health Center Cohort Study (response rate 80%) have been observed, suggesting that the likelihood of serious selection bias in the present study (37% in the current study and 38% in the sub-cohort) was likely low.

Second, information on physical activity was obtained using a self-administered non-validated questionnaire completed after the diagnosis of breast cancer had been made and used retrospectively. Information on other types of activity, such as household and occupational activity, was not available. Thus, the possibility of differential misclassification of exposure due to recall bias or underestimation of physical activity cannot be precluded [46].

Third, the early symptoms of cancer may bring about changes in recent activity levels and other lifestyle factors among patients with cancer.

Fourth, the measurement of receptor status in the current study included two assay methods with different cut-off points for distinguishing receptor positivity. This may have led to misclassification of ER/PR status, which would in turn have biased our results toward a finding of no difference between receptor groups.

Further, given the observational case–control design of the study, the possibility of unmeasured or residual confounding should be considered. With particular regard to education, the percentage of subjects who had advanced to university was 21% in the controls versus 6% in the cases, as presented in Table 1. Although we adjusted for education level in the model, the presence of selection bias could generally be inferred, on the basis that the higher participation in strenuous leisure-time physical activity among controls at ages 12 or 20 (such as running, basketball, and competitive swimming) might be due to the better education, in general, and higher socio-economic status. After multivariable adjustment which included education, however, the magnitude of our estimate was similar to that of other population-based case–control [24, 27] and prospective cohort studies [21].

In summary, we observed substantial decreases in the risk of breast cancer among women who participated in leisure-time physical activity. Participation in leisure-time physical activity with strenuous intensity at age 12 years was associated with reduced risk, whereas that with moderate intensity was not. On participation at age 20 years, the inverse association was not observed in overall or premenopausal women, but was observed among postmenopausal women with moderate activity. In late adult life, moderate physical activity was associated with a decrease in risk of postmenopausal breast cancer, especially for ER + PR + tumors. Our results suggest the potential involvement of both classical estrogen-dependent and -independent mechanisms. From a public health point of view, participation in leisure-time physical activity should be encouraged in all age groups. If confirmed, our results would be useful in the development of preventive strategies against breast cancer and offer valuable insights into the biologic mechanisms by which physical activity influences breast cancer risk.

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Appendix

See Table 5.

Table 5 Multivariable-adjusted odds ratios (ORs) and 95% confidence intervals (CIs) between age-specific leisure-time physical activity and breast cancer risk by levels of body mass index among Japanese women in Nagano

	BMI (<21)				BMI (21–25)				BMI (≧25)				$P_{interaction}$
	Cases	Controls	OR	(95% CI)	Cases	Controls	OR	(95% CI)	Cases	Controls	OR	(95% CI)	
At age 12 years: strenuous[a]			(n = 770)										
No (ref.)	113	62	1.00	(ref.)	147	128	0.76	(0.49–1.16)	67	58	0.75	(0.45–1.26)	0.41
≦3 days/month or 1–4 days/week	2	4	0.35	(0.06–2.11)	8	8	0.67	(0.21–2.07)	3	7	0.29	(0.07–1.26)	
≥5 days/week	9	35	0.16	(0.07–0.39)	22	58	0.29	(0.15–0.55)	11	28	0.28	(0.12–0.63)	

Table 5 continued

	BMI (<21)				BMI (21–25)				BMI (≧25)				$P_{interaction}$
	Cases	Controls	OR	(95% CI)	Cases	Controls	OR	(95% CI)	Cases	Controls	OR	(95% CI)	
In the last 5 years: moderate[b]			(n = 741)										
No (ref.)	79	62	1.00	(ref.)	108	107	0.88	(0.54–1.42)	61	60	0.83	(0.48–1.44)	0.81
≦3 days/month	12	5	1.61	(0.50–5.17)	17	22	0.77	(0.34–1.72)	6	6	0.79	(0.21–2.94)	
≥1 day/week	25	35	0.53	(0.27–1.04)	40	57	0.62	(0.35–1.12)	13	26	0.45	(0.20–1.01)	
(Postmenopausal women only)[c]			(n = 441)										
No (ref.)	32	30	1.00	(ref.)	64	59	0.98	(0.50–1.94)	43	44	0.81	(0.39–1.69)	0.98
≦3 days/month	3	2	0.79	(0.10–6.29)	8	16	0.51	(0.16–1.59)	3	5	0.39	(0.07–2.21)	
≥1 day/week	11	23	0.35	(0.13–0.93)	22	45	0.42	(0.19–0.94)	10	21	0.38	(0.14–1.07)	

Multivariable unconditional logistic regression models were adjusted for age (continuous), residential area (urban or rural), alcohol drinking status (non-regular, regular), smoking status (never, current, past), education (junior/high school, junior/technical college, university), age at menarche (<12, 12, 13, 14, ≥15), age at first birth (nulliparous, <27, ≥27 years) and number of live birth (nulliparous, 1–2, ≥3), menopausal status (pre, postmenopause), total energy-adjusted isoflavone intake (quintiles) and total energy-adjusted vegetable consumption (quintiles)

[a] For strenous activity at age 12 years, we additionally adjusted for moderate activity (no, <1–3 days/month, 1–4 days/week and ≥5 days/week). Because of the small number of participants in the category (<3 days/month), frequency of activity was differently subdivided from the analyses for moderate activity in the last 5 years

[b] For moderate activity, we further excluded participants in strenuous activity

[c] For postmenopausal women, adjusted for use of exogenous hormones

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Alcohol consumption-associated breast cancer incidence and potential effect modifiers: the Japan Public Health Center-based Prospective Study

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Epidemiological studies have evaluated whether the impact of alcohol intake on breast cancer risk is modified by use of exogenous estrogens, folate intake, body weight and smoking status, but results have been inconsistent. Further, effect modification by intake of isoflavones and alcohol-induced facial flushing, which are prevalent in Asian populations, have not been investigated. We investigated the association between alcohol intake and breast cancer risk and whether the association is modified by these factors among 50,757 premenopausal and postmenopausal women (aged 40–69 years) in the population-based Japan Public Health Center-based Prospective Study. Alcohol consumption and other related factors were assessed using self-reported questionnaires. Through to the end of 2006, 572 patients were identified. Relative risks (RRs) and 95% confidence intervals (CIs) were estimated by hazard ratios derived from Cox proportional hazards regression models. Compared with never-drinkers, regular alcohol drinkers (>150 g of ethanol/week) had a higher risk of the development of breast cancer; the multivariable-adjusted RRs were 1.75 (95% CI = 1.16–2.65; p_{trend} = 0.035) for overall, 1.78 (95% CI = 1.09–2.90) for premenopausal and 1.21 (95% CI = 0.53–2.75) for postmenopausal women. There was no statistical evidence for effect modification by menopausal status, use of exogenous estrogens, intakes of isoflavone and folate, body weight, alcohol-induced facial flushing or smoking (All $p_{interactions} \geq 0.15$). Excessive alcohol intake was associated with an increase in the risk of breast cancer in this population. There was no statistical evidence for effect modification.

Alcohol-related carcinogenesis of the breast has been identified in a number of animal and epidemiological studies, and confirmed by the International Agency for Research on Cancer (IARC) working group,[1,2] the Second Expert Report from the World Cancer Research Fund,[3] and meta-analyses.[4,5] Apparently consistent with this, the steep increase in the incidence of breast cancer in Japan over the last 3 decades[6] has coincided with an upward trend in alcohol consumption,[7] although other explanations may include increased height, early menarche, low birthrate or late first birth.[8–11] Nevertheless, a recent qualitative review[12] of 11 epidemiological studies conducted in Japanese women suggested that the association remains inconclusive, albeit that one prospective study in 35,844 women, including 151 cases, reported a positive association.[13]

Proposed mechanisms of ethanol-associated breast carcinogenesis include both hormone-dependent[14–19] as well as hormone-independent carcinogenic pathways, such as the induction of carcinogenesis, mutagenesis and DNA damage by acetaldehyde, a toxic ethanol metabolite; reactive oxygen species and an effect on one carbon metabolism, which is involved with folate level and vitamin B intake.[20–24]

Although the results of a meta-analysis[5] supported both pathways by showing a positive association for estrogen

Key words: alcohol, breast cancer, risk, isoflavones, prospective cohort, estrogen receptor

Abbreviations: ALDH2: aldehyde dehydrogenase 2; BMI: body mass index; CIs: confidence intervals; ER: estrogen receptor; FFQ: food frequency questionnaire; OC: oral contraceptives; PMH: postmenopausal hormones; PR: progesterone receptor; RE: risk estimate; SD: standard deviation

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receptor-positive (ER+) progesterone receptor-positive (PR+) and ER+PR− tumors, the summarized results appeared to differ across Western and Asian populations, likely due to the availability of only 2 hospital-based case-control studies in Asia.[25,26]

Epidemiological studies have evaluated whether the impact of alcohol intake on breast cancer risk may vary by folate intake[27–30] or use of exogenous hormone replacement therapy.[31–33] To our knowledge, however, no large prospective study has investigated whether the association between alcohol intake and breast cancer risk is modified by the intake of dietary isoflavones, which are plant-derived estrogen-like compounds with estrogenic and/or anti-estrogenic activity,[34] although these agents have attracted attention as substitutes for exogenous hormones.

In addition, alcohol-induced facial flushing due to acetaldehyde accumulation is more prevalent among oriental than Western populations,[35] and is considered a predictor of aldehyde dehydrogenase 2 (ALDH2)-deficiency,[36,37] since ALDH2 activity is responsible for detoxifying acetaldehyde in ethanol metabolism. It has been hypothesized that the effect of alcohol on carcinogenesis could be modified by genetic variation in ALDH2 in esophageal cancer,[38] but the presence of such an effect in breast cancer remains unclear.[39]

Here, we prospectively evaluated the association between alcohol consumption and breast cancer risk in consideration of the ER/PR status of tumors, and investigated whether the association is modified by menopausal status, use of exogenous estrogens, the intake of isoflavones and folate, body weight, alcohol-induced facial flushing and smoking status among 50,757 Japanese women in the Japan Public Health Center-based Prospective Study (JPHC Study).

Epidemiology

Materials and methods

Study population

The JPHC Study was launched in 1990–1994, to evaluate the association between lifestyle factors and cancer and cardiovascular disease in a Japanese population, as described previously.[40] Conduct of the study was approved by the Institutional Review Board of the National Cancer Center, Tokyo, Japan. In brief, the target population was all Japanese residents aged 40–69 years enrolled with the residential registries of areas served by 11 public health centers. Subjects were categorized by area into 2 cohorts, namely Cohort I (Iwate-Ninohe, Akita-Yokote, Nagano-Saku, Okinawa-Chubu, Tokyo-Katsushikaku) and Cohort II (Ibaraki-Mito, Nigata-Nagaoka, Kochi-Chuohigashi, Nagasaki-Kamigoto, Okinawa-Miyako, Osaka-Suita) at the baseline survey. Initially, 140,420 subjects were invited to join the JPHC cohort, including 71,698 female subjects. Data from one public health center (Tokyo-Katsushika) were not included in the present analysis (n = 4,178) because information on cancer incidence was not available.

Of invitees, 55,907 women completed a baseline questionnaire, giving a response rate of 82.8%. In 1995–1998, we sent a new questionnaire for the 5-year follow-up survey to all eligible cohort members (response rate 79.4%). Further follow-up questionnaire for the 10-year follow-up survey gave a response rate of 77.4%.

We excluded ineligible subjects, including women who moved before the start of follow-up or women with missing information on the date of moving out of the study area or date of death (n = 69); those with a self-reported history of cancer before the start of follow-up (n = 1,509) and those without information on alcohol intake at baseline (n = 1,856). Further, we excluded women without information on weight, height, smoking, leisure-time physical activity or who reported unreasonably high or low estimates of total energy intake (±3 SD) (n = 1,716). Finally, 50,757 were included in the analyses.

Exposure measurement

Alcohol consumption was assessed using a self-administered food frequency questionnaire (FFQ) and calculated as previously described.[41,42]

At baseline survey, 2 validated FFQs were used in Cohort I[43] and II.[41] In Cohort I, subjects were asked the average frequency of intake in 6 frequency categories (almost never, 1–3 days per month, 1–2 days per week, 3–4 days per week, 5–6 days per week and every day). In Cohort II, we first classified alcohol drinking status (never-drinkers, ex-drinkers and current drinkers) and then asked about frequency among ex-drinkers or current drinkers (1–3 days per month, 1–2 days per week, 3–4 days per week and almost daily). For the calculation of alcohol consumption, we assigned a score to each category of frequency, namely 1.5 for 1–2 days per week, 3.5 for 3–4 days per week, 5.5 for 5–6 days per week and 7 for every day in Cohort I at baseline survey. In Cohort II, scores were assigned as 1.5 for 1–2 days per week, 3.5 for 3–4 days per week and 6 for almost daily. Types of alcoholic beverages in the questionnaires and the amount of ethanol used in the calculations were as follows: sake (23 g of ethanol/180 ml), shochu or awamori (36 g of ethanol/180 ml), beer (23 g of ethanol/633 ml), whiskey or brandy (10 g of ethanol/30 ml) and wine (6 g of ethanol/60 ml). We also asked about average daily consumption for each type of alcoholic beverage.

At the 5-year and 10-year follow-up surveys, alcohol drinking was evaluated by a similar validated FFQ, which included 6 frequency categories (*i.e.*, almost never, 1–3 days per month, 1–2 days per week, 3–4 days per week, 5–6 days per week and daily) as well as questions about the average daily consumption of each of 5 types of alcohol, namely sake, shochu or awamori, beer, whiskey and wine. Among women who drank at least 1 day per week, weekly ethanol consumption was quantitatively assessed by multiplying the amount of ethanol by the frequency of alcohol drinking.[41] In the calculations of alcohol consumption, the score for each category of frequency and the amount of ethanol in each type of alcohol beverage was applied in the same way as in baseline survey.

Validity of this FFQ-based estimated alcohol consumption was evaluated in a subsample of the JPHC cohort who completed dietary records (DRs). Spearman rank correlation coefficients between the FFQs and DRs were 0.44 (107 women) in Cohort I[43] and 0.40 (178 women) in Cohort II for the baseline survey,[41] and 0.51 (113 women) in Cohort I[44] and 0.48 (176 women) in Cohort II[45] for the 5-year follow-up survey.

Dietary assessment was also done using validated FFQs, which included the 44 or 52 food items in the baseline survey[46] and 138 food items in the 5-year follow-up survey.[44] Subjects were asked to report the average frequency of consumption of each food item during the preceding 1 year. For seasonal fruits and vegetables products, we asked about the frequency of consumption when they were in season and calculated total consumption by taking into account seasonal length. The estimated nutrient intakes were calculated based on the fourth[47] and the fifth revised editions of the Standard Tables of Food Composition in Japan.[48] Nutritional covariates, except alcohol intake, were adjusted for total caloric intake using the residual method.[49] The questionnaires were also used to obtain information on anthropometric and reproductive characteristics, and other lifestyle-related factors, such as physical activity, smoking status and menopausal status.

Ascertainment of breast cancer cases and follow-up of the cohort

Incident cases of breast cancer were identified through linkage with the JPHC Cancer Registry, which is administered by the National Cancer Center (NCC) in collaboration with major local hospitals and the Regional Cancer Registry in each study area. Cases of breast cancer were defined as codes C500–509 according to the Third Edition of the International Classification of Diseases for Oncology.[50] Ten breast cancer cases (1.7% of total cases) were identified through information on death certificates (*i.e.*, Death Certificate Notification, DCN), of which 6 (1.0% of total cases) had no information on diagnosis (*i.e.*, Death Certificate Only; DCO). Diagnosis was microscopically verified in 97% of all case patients. ER and PR status were evaluated either by enzyme-linked immunoassay or immunohistochemical assay. Receptor status (*i.e.*, positive or negative) was determined using either the cut-off point for the assay method at the clinical laboratory performing the assay or by clinical estimation at the hospital treating the case patient.

Date of death during follow-up was verified through linkage with the Death Registries at the PHCS, which are required by the Ministry of Health, Labor and Welfare. Date of migration from the study area was verified through linkage with the Residence Registries at the regional PHCs in Japan. Follow-up started on the date of administration of the baseline questionnaire, and subjects were censored on the date of death, date of migration out of the study area or end of the follow-up (December 31, 2006), whichever occurred first.

Statistical analysis

To reduce misclassification of exposure and improve statistical efficiency, we used time-dependent multivariable Cox proportional hazards regression models to estimate relative risks (RRs) and 95% confidence intervals (CIs) using age as the time scale.[51] The proportional hazards assumptions were satisfied as evaluated by Kaplan–Meier curves.[52] Participants were subdivided into 6 categories by frequency of alcohol drinking [non-drinkers (past drinkers); non-drinkers (never-drinkers); occasional drinkers (*i.e.*, 1–3 days/month); regular drinkers (1–2 times/week); regular drinkers (3–4 times/week) and regular drinkers (≥5 times/week)]. For the amount of alcohol consumption, subjects were classified into 5 groups [*i.e.*, non-drinkers (past drinkers); non-drinkers (never-drinkers); occasional drinkers (non-quantitative estimation); regular drinkers (≤150 g ethanol/week) and regular drinkers (>150 g ethanol/week)]. The multivariable adjusted model included height (continuous, cm), BMI (continuous, kg/m^2), smoking status (never, ever) and leisure-time physical activity (no, 1–3 days/month, 1–2 days/week, 3–4 days/week, every day). We also adjusted for age at menarche (≤13, 14, 15, ≥16 years, missing), age at first birth (nulliparous, <26, ≥26 years, missing), parity (nulliparous, 1–2, 3, ≥4 children, missing), menopausal status or age (premenopausal, <48, 48–53, ≥54 years, missing), use of exogenous hormones (never, ever, missing) and energy-adjusted isoflavone intake (quintiles). Trend tests were conducted by creating a continuous variable in order of the rank of each alcohol drinking category and by including it in the regression model among all subjects except past drinkers.

We also calculated the population attributable fraction (PAF) of overall breast cancer for regular drinkers (>150 g ethanol/week) in this population. PAF was calculated as pd×{RR-1}/RR; where pd = proportion of exposed cases,[53] 95% CI of adjusted PAF was estimated according to the formula of Greenland.[54]

Among 37,681 women with quantitative information on alcohol consumption, we performed analyses stratified by menopausal status (pre or post), use of exogenous estrogens (never, ever), intakes of dietary isoflavones and folate [upper or lower intake; approximate median (30.5 mg/day for isoflavones; 351 mg/day for folate)], BMI (<25 or ≥25 kg/m^2), alcohol-induced facial flushing (yes or no), and smoking status (never, ever) and assessed possible interactions of alcohol intake with these factors in relation to breast cancer.

The cross-product terms of these factors and alcohol consumption (continuous) were introduced into the Cox proportional hazards regression model. The *p*-value for interaction was calculated by a likelihood ratio test which compared models with and without the interaction terms.

All analyses were performed using the PROC PHREG procedure of the SAS statistical package version 9.1 (SAS Institute, Cary, NC). All statistical tests were 2-sided, and statistical significance was defined as $p < 0.05$.

Results

After an average 13.8 years of follow-up, corresponding to 698,081 person-years, 572 cases of invasive breast cancer were diagnosed among 50,757 women. Mean age at recruitment was 52 years, while mean age at diagnosis was 50 years. Among the 572 cases, information on ER status was available for 275 (48% of total cases) and on PR for 262 (46% of total cases). Of these, 176 cases were ER+ (64% of all known ER cases) and 99 were ER−, whereas 129 cases were PR+ and 133 were PR−.

At baseline, ~79% of women were non-drinkers and 12.5% were regular drinkers (Table 1). During a long follow-up period (an average 13.8 years of follow-up), alcohol drinking habit in this population was slightly changed over time.

In present study, 85.2% of the cohort had a repeat measure at the 5-year follow-up survey. Of these women, 6.4% had stopped drinking, 7.9% had started drinking and 79.7% stayed in the same category as at baseline (Spearman rank correlation coefficient among women with quantitative information; r = 0.89); 82.9% of the cohort had information at the 10-year follow-up survey. Of these, 5.9% had stopped drinking, 7.9% had started drinking and 77% stayed in the same category as at baseline survey (Spearman rank correlation coefficient; r = 0.74).

Although height and relative body weight did not appear to differ by level of alcohol consumption, women with high alcohol consumption were more likely to be younger and ever-smokers and less likely to have a facial flushing response on alcohol drinking compared to non-drinkers. At baseline, alcohol consumption was inversely correlated with the intake of isoflavones (r = −0.11) and folate (r = −0.07) among women with quantitative information on alcohol.

In the present cohort, distribution of alcoholic beverages by type was sake 36%, shochu or awamori 30%, beer 26%, whiskey 8% and others 0.2% at baseline survey; sake 33%, shochu 36%, beer 25%, whiskey 5% and wine 1% at the 5-year follow-up survey and sake 29%, shouchu 39%, beer 26%, whiskey 4% and wine 1% at the 10-year follow-up survey.

Increased frequency of alcohol drinking was marginally positively associated with the development of breast cancer. Compared with never-drinkers, women who drank with a frequency of ≥5 times/week had an ~56% higher risk of breast cancer, with a multivariable-adjusted RR of 1.56 (95% CI = 1.09–2.23; Table 2).

The amount of alcohol consumption was also associated with an elevated risk of overall breast cancer. Regular drinkers (>150 g ethanol/week; = ~2 drinks/day) had an ~75% higher risk of overall breast cancer than never-drinkers (95% CI = 16–165%; p_{trend} = 0.035; Table 2). The corresponding risk estimate was 1.78 (95% CI = 1.09–2.90) among premenopausal and 1.21 (95% CI = 0.53–2.75; p_{trend} <0.05) among postmenopausal women (text only). The PAF of overall breast cancer incidence that was attributable to regular drinkers (>150 g ethanol/week) was 1.93% (95% CI = 1.76–2.12).

Among the 37,681 women with quantitative information on alcohol consumption, an increase in consumption of 10 g of ethanol/day (continuous) was associated with a 6% (95% CI = 1–13; p_{trend} = 0.047) increase in risk of overall breast cancer after adjustment for all covariates. Because we did not have complete information on past drinkers in Cohort I at baseline, a further analysis was conducted among regular drinkers only. Results showed a non-statistically significant 8% increase in risk (95% CI = −4 to 18; p_{trend} = 0.24: text only).

In consideration of the ER/PR status of breast tumors, the corresponding results were 1.08 (95% CI = 0.99–1.18; p_{trend} = 0.098) for all ER+; 0.88 (95% CI = 0.57–1.37; p_{trend} = 0.58) for all ER−; 1.06 (95% CI = 0.95–1.19; p_{trend} = 0.28) for ER+PR+; 1.17 (95% CI = 0.93–1.48; p_{trend} = 0.17) for ER+PR−; 0.89 (95% CI = 0.53–1.49; p_{trend} = 0.66) for ER−PR− and 1.07 (95% CI = 0.99–1.15; p_{trend} = 0.11) for unknown ER/PR tumors (text only). Although we did not observe an increased risk for the development of all ER− tumors and ER−PR− tumors among regular drinkers compared with non-drinkers, past drinkers had a statistically significant increased risk over never-drinkers; multivariable-adjusted RRs = 2.39 (95% CI = 1.42–4.05) for all ER− tumors, and 2.08 (95% CI = 1.12–3.87) for ER−PR− tumors (Table 2).

In the analyses stratified by menopausal status, the observed risk estimates were not statistically heterogeneous across menopausal status ($p_{interaction}$ = 0.46 for overall; Table 3). Therefore, further analyses stratified by other factors were performed without stratification by menopausal status and were conducted for overall and all ER+ tumors.

No evidence was seen for statistical interaction between alcohol intake and the use of exogenous estrogens in relation to breast cancer risk among postmenopausal women ($p_{interaction}$ = 0.24 for overall; 0.87 for ER+; Table 3).

There was no statistical significant interaction between isoflavone intake (< or ≥30.5 mg/day) and alcohol drinking in relation to breast cancer risk ($p_{interaction}$ = 0.15 for overall; 0.23 for ER+; Table 3).

There was no evidence for effect modification by folate intake for overall ($p_{interactions}$ = 0.25) or for all ER+ tumors ($p_{interactions}$ = 0.60), although there was a statistically significant positive association among women with low folate intake (<351 mg/day); the multivariable-adjusted RR for an increase in consumption of 10 g of ethanol/day was 1.08 (95% CI = 1.02–1.16; p_{trend} 0.012) for overall.

The observed association between alcohol intake and breast cancer risk was not modified with regard to BMI (<25, ≥25) ($p_{interaction}$ = 0.68 for overall; 0.96 for ER+), alcohol-induced facial flushing ($p_{interaction}$ = 0.29 for overall; 0.45 for ER+) or smoking status ($p_{interaction}$ = 0.39 for overall; 0.16 for ER+). Additional stratified analyses with changes to the cut-off point of BMI for Asian population (*e.g.*, 22, 23 or 24)[55] produced similar results to those in Table 3 (data not shown).

Discussion

In this large population-based prospective cohort study among Japanese women, we found a statistically significantly

Table 1. Distribution of alcohol consumption and age-standardized[1] characteristics according to category of alcohol consumption among 50,757 women in the Japan Public Health Center-based Prospective Study, Cohort I (1990-) and Cohort II (1993-)

	Category of alcohol consumption				
			Regular drinkers, g of ethanol/week		
Characteristic	Non-drinkers	Occasional	≤150	151–299	≥300
Alcohol consumption: at baseline survey	78.8%	8.6%	10.0%	1.6%	0.9%
Ethanol g/week at baseline, Mean (SD)			57.0 (38)	212.8 (45)	496.4 (183)
Alcohol consumption: at 10-year follow-up	74.3%	12.3%	8.6%	2.5%	2.3%
Height at baseline, cm, Mean (SD)	151.8 (5.4)	152.3 (5.2)	153.0 (5.1)	153.0 (5.4)	152.2 (5.4)
Body mass index at baseline, kg/m^2, Mean (SD)	23.5 (3.2)	23.5 (3.0)	22.9 (2.9)	23.1 (3.2)	23.4 (3.3)
Age at baseline, year, Mean (SD)	52.8 (8.1)	48.7 (6.7)	49.2 (7.4)	48.8 (7.1)	48.2 (6.2)
Age at menarche, year, Mean (SD)	14.7 (1.7)	14.5 (1.6)	14.4 (1.6)	14.6 (1.7)	15.0 (1.8)
Age at first birth, year, Mean (SD)[2]	24.9 (3.5)	25.0 (3.4)	25.0 (3.4)	24.4 (3.5)	24.0 (4.0)
Number of children, *n* Mean (SD)	2.6 (1.6)	2.5 (1.4)	2.3 (1.3)	2.4 (1.6)	2.5 (1.8)
Age at menopause, year, Mean (SD)	48.3 (4.3)	48.3 (4.5)	48.5 (4.3)	47.6 (5.0)	48.1 (4.8)
Use of exogenous hormones at baseline (ever), %	11.5	14.7	12.5	14.7	13.5
Smoking status (ever), %	5.7	11.5	15.8	37.9	46.1
Facial flushing response, %	37.1	41.1	33.8	31.3	34.3
Intake of Isoflaveones, mg/day, Mean	37.0	36.3	32.3	31.5	24.8
Intake of folate, mg/day, Mean	375	381	361	339	293

[1]Age-standardized according to the distribution of person-time of follow-up. [2]On the basis of the information among parous women. SD, standard deviation.

positive association between alcohol consumption and the development of breast cancer, as has also been reported in Western populations. The observed positive association was not modified by menopausal status, use of exogenous estrogens, intake of dietary isoflavones and folate, BMI level, alcohol-induced facial flushing or smoking status.

Similar to Western populations, alcohol consumption was positively associated with the development of breast cancer among a Japanese population, although the types of alcoholic beverages consumed by this cohort were largely different from those in Western populations. We found a 6% increase in the risk of breast cancer per 10 g increment of ethanol/day among this Japanese population after adjustment for all covariates. The corresponding results in Western populations reported a statistically significant 9%[56] and 12%[57] increase in the risk in a pooled analysis of 7 cohort studies[56] and the Million Women Study,[57] respectively. In the category of regular drinkers >150 g/week, the PAF of overall breast cancer incidence in this population (1.9%) was relatively lower than that in Western population as (~2.6%).[56] This may be partly due to the lower prevalence of regular drinkers in this population.

The biological plausibility of our main result has been supported by a number of proposed mechanisms of ethanol-associated breast carcinogenesis at the molecular, hormonal and other carcinogenic pathway levels.[23] Although these mechanisms have not been precisely determined, the involvement of ER-mediated[14,15,58] estrogen-dependent[16–19] mechanisms, and hormone-independent mechanisms[20–23] has been suggested.

For ER+ tumors, our results suggested no statistically significant association between alcohol intake and the development of breast cancer, albeit that a weak positive trend was indicated. In the current study, 54% of breast cancer cases did not have information on the ER/PR status of breast tumors and the results of unknown tumors showed a statistically significant positive association in the highest category. The observed null association for ER+ tumors should be carefully evaluated by further follow-up.

Our result for ER− tumors among past drinkers did not eliminate the possible involvement of hormone-independent mechanisms, including carcinogenic effects by acetaldehyde, the most toxic metabolite of ethanol[20]; and chromosomal[21] or DNA damage[22] due to alcohol-induced reactive oxygen species and/or lipid peroxidation,[23] as previously suggested.[28]

Consistent with the statement of the Second Expert Report from the World Cancer Research Fund,[3] our results suggest that the association between alcohol intake and breast cancer risk is not heterogeneous across menopausal status.

In line with the results of the European Prospective Investigation cohort,[59] we observed no evidence of effect modification by ever-use of exogenous estrogens among postmenopausal women. However, effect modification has been suggested by several previous studies,[31–33] which had a

Table 2. Relative risks (RRs) and 95% confidence intervals (CIs) for the association between alcohol consumption and breast cancer risk over 698,081 person-years among 50,757 women in the Japan Public Health Center-based Prospective Study, 1990–2006

Drinking status	Non-drinkers (Past-drinkers)	Non-drinkers (Never-drinkers)	Occasional drinkers	Regular drinkers, per week: 1–2 times	Regular drinkers, per week: ≥3–4 times	Regular drinkers, per week: ≥5–7 times	
Person-years	35,827	513,328	62,016	30,909	23,590	32,411	
No. of cases	68	373	56	25	14	36	
	RR (95% CI)	Ref.	RR (95% CI)	RR (95% CI)	RR (95% CI)	RR (95% CI)	p_{trend}[2]
Age-area adjusted	1.50 (1.16–1.95)	1.00 (ref.)	1.38 (1.04–1.83)	1.45 (0.96–2.18)	0.91 (0.53–1.56)	1.77 (1.24–2.51)	0.002
Multivariable-adjusted[1]	1.41 (1.08–1.83)	1.00 (ref.)	1.17 (0.88–1.57)	1.25 (0.82–1.88)	0.78 (0.45–1.34)	1.56 (1.09–2.23)	0.056
	(Past-drinkers) RR (95% CI)	(Never-drinkers) Ref.	Occasional RR (95% CI)	1–2 times RR (95% CI)	≥ 3–7 times RR (95% CI)	p_{trend}[2]	
Age-area adjusted	1.50 (1.16–1.95)	1.00 (ref.)	1.38 (1.04–1.83)	1.45 (0.96–2.18)	1.40 (1.03–1.90)	0.004	
Multivariable-adjusted[1]	1.40 (1.08–1.82)	1.00 (ref.)	1.17 (0.08–1.56)	1.24 (0.82–1.87)	1.21 (0.89–1.65)	0.10	

Drinking status	Non-drinkers (Past-drinkers)	Non-drinkers (Never-drinkers)	Occasional drinkers	Regular drinkers, g of ethanol per week: 1–150 g	Regular drinkers, g of ethanol per week: >150 g	
Person-years	35,827	513,328	62,016	67,197	19,713	
No. of cases	68	373	56	49	26	
	RR (95% CI)	Ref.	RR (95% CI)	RR (95% CI)	RR (95% CI)	p_{trend}[2]
Age-area adjusted	1.50 (1.16–1.95)	1.00 (ref.)	1.38 (1.04–1.83)	1.23 (0.90–1.67)	1.96 (1.31–2.94)	0.0007
Multivariable-adjusted[1]	1.40 (1.08–1.82)	1.00 (ref.)	1.17 (0.88–1.56)	1.06 (0.78–1.44)	1.75 (1.16–2.65)	0.035
Multivariable-adjusted[3]	1.41 (1.09–1.83)	1.00 (ref.)	1.17 (0.88–1.56)	1.06 (0.78–1.44)	1.76 (1.16–2.67)	0.036
	19	116	16	18	7	
All ER+ (176 cases)[1]	1.18 (0.72–1.93)	1.00 (ref.)	1.06 (0.62–1.80)	1.37 (0.82–2.29)	1.58 (0.72–3.48)	0.16
	13	76	10	8	6	
ER+PR+ (113 cases)[1]	1.28 (0.71–2.32)	1.00 (ref.)	1.04 (0.53–2.03)	0.98 (0.46–2.09)	2.09 (0.88–4.97)	0.27
	5	36	6	9		
ER+PR− (56 cases)[1]	0.92 (0.35–2.39)	1.00 (ref.)	1.25 (0.52–3.02)	1.51 (0.70–3.27)[4]		0.41
	19	58	13	9		
All ER− (99 cases)[1]	2.39 (1.42–4.05)	1.00 (ref.)	1.76 (0.95–3.24)	1.03 (0.50–2.13)[4]		0.26
	13	47	10	7		
ER−PR− (77 cases)[1]	2.08 (1.12–3.87)	1.00 (ref.)	1.77 (0.88–3.55)	1.09 (0.48–2.46)[4]		0.43

[1]Multivariable models were adjusted for age time-scales, area (10), height (continuous, cm), BMI (continuous, kg/m^2), smoking status (never, ever), leisure-time physical activity (no or 1–3 days/month, >1 day/week, 3–4 days/week, every day), 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, 3, >4 times or missing), age at menopause (premenopausal, <48, 48–53, ≥54 years or missing), use of exogenous female hormones (ever, never or missing), energy-adjusted intake of isoflavones (quintiles). [2]Among all subjects except past drinkers. [3]Additionally adjusted with energy-adjusted intake of folate (quintiles). [4]Among all regular drinkers including >150 g of ethanol per week.

Table 3. Multivariable relative risks (RRs)[1] and 95% confidence intervals (CIs) for the association between an increase in consumption of 10 g of ethanol/day and breast cancer risk with stratification by several epidemiological factors among 37,681 women[2] in the Japan Public Health Center-based Prospective Study, 1990–2006

	All tumors (412 cases)					All ER+ tumors (127 cases)			
Epidemiological factor	No. of subjects	No of cases	RR (95%CI)	p_{trend}	p_{inter}	No. of cases	RR (95%CI)	p_{trend}	p_{inter}
Menopausal status									
Premenopausal	15,120	194	1.05 (0.98–1.14)	0.18	0.46	63	1.06 (0.92–1.21)	0.42	0.70
Postmenopausal	22,561	218	1.01 (0.87–1.18)	0.86		64	1.05 (0.92–1.20)	0.46	
Use of exogenous estrogen among postmenopausal women[3]									
Never users	19,345	166	1.03(0.91–1.16)	0.65	0.24	47	1.05 (0.91–1.20)	0.52	0.87
Ever users	3,216	52	0.74 (0.33–1.65)	0.46		17	1.10 (0.64–1.88)	0.74	
Isoflavone intake, mg/day									
Low (<30.5)	18,530	191	1.10 (1.04–1.17)	0.002	0.15	55	1.12 (1.03–1.23)	0.01	0.23
High (≥30.5)	19,151	221	0.94 (0.77–1.16)	0.58		72	0.98 (0.74–1.29)	0.88	
Folate intake, mg/day									
Low (<351)	18,165	182	1.08 (1.02–1.16)	0.012	0.25	60	1.09 (0.96–1.24)	0.17	0.60
High (≥351)	19,516	230	1.01 (0.90–1.14)	0.81		67	1.04 (0.94–1.15)	0.48	
Body mass index, kg/m^2									
Low (<25)	26,582	265	1.09 (0.98–1.20)	0.11	0.68	81	1.07 (0.88–1.29)	0.49	0.96
High (≥25)	11,099	147	1.05 (0.98–1.13)	0.20		46	1.06 (0.97–1.15)	0.18	
Facial flushing response									
No	23,878	256	1.09 (0.99–1.21)	0.07	0.29	75	1.10 (0.94–1.29)	0.24	0.45
Yes	13,803	156	1.04 (0.96–1.13)	0.33		52	1.05 (0.95–1.16)	0.33	
Smoking status									
Never	34,318	375	1.09 (1.01–1.17)	0.026	0.39	113	1.14 (1.04–1.26)	0.007	0.16
Ever	3,363	37	1.04 (0.95–1.14)	0.43		14	0.99 (0.78–1.24)	0.91	

[1]Multivariable Cox proportional hazards models with age as the time scales were adjusted for area (10), height (continuous), body mass index (continuous, kg/m^2), smoking status (never, ever), leisure-time physical activity (no or 1–3 days/month, >1 day/week, 3–4 days/week, every day), use of exogenous female hormones (never, ever) and energy-adjusted isoflavones (quintiles), except the stratified factor in each analysis. [2]The above analyses excluded past/occasional drinkers and were restricted to those with complete information on all stratification factors. [3]Multivariable Cox proportional hazards models with age as the time scales were adjusted for area (10), height (continuous), body mass index (continuous, kg/m^2), smoking status (never, ever) and energy-adjusted isoflavones (quintiles).

relatively high prevalence of exogenous estrogen ever-users (~20–45%),[31–33] *versus* our 11% at baseline. Further investigation of this effect is warranted.

To our knowledge, this is the first large prospective study to investigate the interaction between alcohol intake and intake of dietary isoflavones in relation to ER-defined breast cancer risk. We found no evidence of statistical interaction between alcohol consumption and isoflavone intake in relation to overall, ER+ or ER+PR+ breast cancer risk, at least with regard to dietary sources of isoflavones from natural food products within the range of the general Japanese diet. A recent randomized trial[60] suggested that isoflavone supplementation did not modify breast density, and did not act in a hormone replacement therapy-like manner.

Several[27,61–64] studies have suggested the importance of adequate dietary folate intake for the prevention of breast cancer, particularly among heavy drinkers, although we did not found any effect modification by folate intake as well as other previous studies.[30,59,65] Further investigation for evaluation of effect modification is required, particularly among ER− tumors, because although the hypothesis includes ER− tumors, the present study was unable to evaluate this possibility due to the lack of any clear association between quantitative alcohol consumption and the development of ER− tumors.

Consistent with previous studies, the observed positive association between alcohol intake and overall breast cancer risk was not modified by BMI.[32,59] Our results for ER+ tumors in overweight women (BMI ≥ 25) do not support a previous report that the positive association between alcohol consumption and the development of ER+ tumors was confined to women with BMI <25.[66]

Our results showed that the effect of alcohol on breast carcinogenesis was not modified by alcohol-related facial flushing, despite the likely role of this response as a predictor