TABLE 2. Hazard ratios for total cancer incidence according to daily total physical activity level (n = 79,771), Japan Public Health Center-based Prospective Study, 1995–2004

Quartile of physical activity level (quartile of METs*/day score)	No. of subjects	Person-years of follow-up	Total					Excluding cases diagnosed within first 3 years				
			No. of cases	HR1*,†	95% CI*	HR2‡	95% CI	No. of cases	HR1	95% CI	HR2	95% CI
Men (n = 37,898)					(n = 2,704)					(n = 1,804)		
Lowest	12,966	92,421	921	1.00	Reference	1.00	Reference	604	1.00	Reference	1.00	Reference
Second	7,822	57,957	575	1.00	0.90, 1.10	1.00	0.90, 1.11	381	0.98	0.86, 1.11	0.98	0.86, 1.11
Third	7,579	56,512	574	0.96	0.86, 1.06	0.96	0.86, 1.07	386	0.95	0.83, 1.08	0.95	0.83, 1.08
Highest	9,531	72,841	634	0.87	0.79, 0.96	0.87	0.78, 0.96	433	0.86	0.76, 0.97	0.86	0.76, 0.98
p for trend					0.006		0.005			0.015		0.017
Per 1-MET increase				0.99	0.99, 0.998	0.99	0.99, 0.998		0.99	0.99, 0.999	0.99	0.99, 0.999
Per 10-MET increase				0.93	0.88, 0.99	0.93	0.88, 0.99		0.93	0.87, 0.996	0.93	0.87, 0.997
Women (n = 41,873)					(n = 1,630)					(n = 1,056)		
Lowest	13,277	99,385	569	1.00	Reference	1.00	Reference	368	1.00	Reference	1.00	Reference
Second	10,838	83,644	428	0.92	0.81, 1.04	0.93	0.82, 1.05	290	0.94	0.81, 1.10	0.94	0.81, 1.10
Third	9,663	74,073	350	0.84	0.73, 0.96	0.84	0.73, 0.96	222	0.80	0.68, 0.95	0.79	0.67, 0.94
Highest	8,095	62,284	283	0.83	0.72, 0.96	0.84	0.73, 0.97	176	0.78	0.65, 0.93	0.78	0.65, 0.94
p for trend					0.004		0.007			0.002		0.002
Per 1-MET increase				0.99	0.98, 0.997	0.99	0.98, 0.997		0.98	0.97, 0.995	0.98	0.97, 0.995
Per 10-MET increase				0.89	0.82, 0.97	0.90	0.82, 0.98		0.85	0.77, 0.95	0.85	0.77, 0.95

* MET(s), metabolic equivalent(s); HR, hazard ratio; CI, confidence interval.

† Adjusted for age (stratified, 5-year categories) and area (stratified, 10 public health center areas).

‡ Adjusted for age (stratified, 5-year categories), area (stratified, 10 public health center areas), total energy intake (stratified, quintiles), history of diabetes (no, yes), smoking status (never smoking, past smoking, or 1–19, 20–29, or ≥30 cigarettes/day), alcohol intake status (almost none, occasional, or regular), body mass index (weight (kg)/height (m)^2: <20, 20–<27, or ≥27), and leisure-time sports or physical exercise (<1, 1–2, or ≥3–4 days/week).

in 1995–1999 at age 45–74 years. Initially, at baseline, 133,323 subjects were identified as being in the study population. After excluding 241 persons with non-Japanese nationality (n = 51), duplicate enrollment (n = 4), a late report of emigration occurring before the start of the follow-up period (n = 180), or ineligibility due to an incorrect birth date (n = 6), a population-based cohort of 133,082 subjects was established. After exclusion of the 13,663 persons who had died, moved out of the study area, or been lost to follow-up before the starting point, the remaining 119,419 subjects were considered eligible for the present study. A total of 96,566 subjects responded to the questionnaire, yielding a response rate of 81 percent.

Questionnaire

The questionnaire included items on demographic factors, personal medical history, physical activity, smoking and alcohol drinking, other lifestyle factors, and diet (via a validated food frequency questionnaire containing questions on 138 food items and 14 supplementary questions (13)). Persons who had been diagnosed with cancer before the starting point (n = 2,153) or who had missing data for physical activity-related factors (n = 6,346) or other factors included in the multivariate model (n = 8,296) were excluded. Finally, 79,771 eligible subjects (37,898 men and 41,873 women) were included in the analysis.

Follow-up

Subjects were followed from the starting point until December 31, 2004. Residence status, including survival, was confirmed through the residential registry. Inspection of the resident registry is available to anyone under the resident registration law. Among the study subjects, 5,271 died, 3,166 moved out of the study area, one withdrew from the study, and 239 (0.3 percent) were lost to follow-up within the follow-up period. Information on the cause of death for deceased subjects was obtained from death certificates (provided by the Ministry of Health, Labour, and Welfare with the permission of the Ministry of Internal Affairs and Communications), on which cause of death is defined according to the *International Classification of Diseases*, Tenth Revision (14). Resident registration and death registration are required by law in Japan, and the registries are believed to be complete.

Incident cancers were identified through notification from the major hospitals in the study area and through data linkage with population-based cancer registries. Death certificates were used as a supplementary information source. The site and histology of each case were coded using the *International Classification of Diseases for Oncology*, Third Edition (15). In our cancer registry system, the proportion of cases for which information was available from death certificates only was 3.7 percent. For the present analysis, the

TABLE 3. Hazard ratios for total cancer incidence according to daily total physical activity level and body mass index or frequency of leisure-time sports or physical exercise (n = 79,771), Japan Public Health Center-based Prospective Study, 1995–2004

Quartile of physical activity level (quartile of METs*/day score)	No. of subjects	Person-years of follow-up	Total			Excluding cases diagnosed within the first 3 years		
			No. of cases	HR*,†	95% CI*	No. of cases	HR†	95% CI
Men (n = 37,898)								
Age (years)								
<60								
Lowest	8,239	61,181	364	1.00	Reference	259	1.00	Reference
Second	5,063	38,860	239	1.00	0.85, 1.18	174	1.00	0.83, 1.22
Third	4,709	36,624	219	0.94	0.79, 1.12	161	0.94	0.77, 1.15
Highest	6,301	49,823	269	0.86	0.73, 1.01	202	0.87	0.72, 1.06
p for trend					0.049			0.135
≥60								
Lowest	4,727	31,240	557	1.00	Reference	345	1.00	Reference
Second	2,759	19,096	336	0.99	0.86, 1.14	207	0.96	0.80, 1.14
Third	2,870	19,887	355	0.97	0.85, 1.11	225	0.96	0.81, 1.14
Highest	3,230	23,018	365	0.87	0.76, 1.00	231	0.85	0.72, 1.01
p for trend					0.051			0.064
p for interaction					0.505			0.976
Body mass index‡								
<20								
Lowest	2,316	15,737	196	1.00	Reference	121	1.00	Reference
Second	1,409	10,180	118	0.93	0.73, 1.17	69	0.85	0.63, 1.16
Third	1,407	10,194	131	0.97	0.77, 1.22	89	1.02	0.77, 1.36
Highest	1,772	13,162	126	0.79	0.63, 1.00	71	0.69	0.51, 0.94
p for trend					0.063			0.031
20–<27								
Lowest	9,081	65,122	632	1.00	Reference	420	1.00	Reference
Second	5,493	40,888	386	0.97	0.86, 1.11	264	0.99	0.83, 1.14
Third	5,325	39,896	397	0.96	0.85, 1.09	263	0.92	0.79, 1.08
Highest	6,779	52,341	451	0.87	0.77, 0.98	324	0.89	0.77, 1.04
p for trend					0.026			0.118
≥27								
Lowest	1,569	11,562	93	1.00	Reference	63	1.00	Reference
Second	920	6,889	71	1.16	0.84, 1.62	48	1.23	0.83, 1.84
Third	847	6,422	46	0.84	0.58, 1.22	34	0.94	0.60, 1.46
Highest	980	7,339	57	0.93	0.66, 1.32	38	0.96	0.63, 1.47
p for trend					0.501			0.713
p for interaction					0.515			0.797
Frequency of leisure-time sports or physical exercise (days/week)								
<1								
Lowest	10,378	74,547	723	1.00	Reference	479	1.00	Reference
Second	6,077	45,423	453	1.02	0.91, 1.15	309	1.01	0.88, 1.17
Third	5,704	42,999	443	1.00	0.88, 1.12	303	0.98	0.85, 1.14
Highest	7,497	57,786	499	0.88	0.79, 0.99	343	0.87	0.75, 1.00
p for trend					0.032			0.044
≥1								
Lowest	2,588	17,875	198	1.00	Reference	125	1.00	Reference
Second	1,745	12,534	122	0.90	0.72, 1.14	72	0.84	0.63, 1.13
Third	1,875	13,513	131	0.84	0.67, 1.06	83	0.84	0.63, 1.12
Highest	2,034	15,055	135	0.78	0.62, 0.99	90	0.82	0.62, 1.09
p for trend					0.034			0.190
p for interaction					0.766			0.566

Table continues

TABLE 3. Continued

Quartile of physical activity level (quartile of METs/day score)	No. of subjects	Person-years of follow-up	Total			Excluding cases diagnosed within the first 3 years		
			No. of cases	HR†	95% CI	No. of cases	HR†	95% CI
Women (n = 41,873)								
Age (years)								
<60								
Lowest	7,946	61,385	279	1.00	Reference	184	1.00	Reference
Second	7,053	55,628	261	1.03	0.87, 1.22	184	1.09	0.88, 1.33
Third	6,271	48,932	202	0.90	0.75, 1.08	131	0.86	0.69, 1.08
Highest	5,501	43,242	188	0.95	0.79, 1.15	120	0.91	0.72, 1.14
p for trend					0.419			0.241
≥60								
Lowest	5,331	38,000	290	1.00	Reference	184	1.00	Reference
Second	3,785	28,016	167	0.81	0.67, 0.98	106	0.78	0.61, 0.996
Third	3,392	25,141	148	0.77	0.63, 0.95	91	0.72	0.56, 0.93
Highest	2,594	19,042	95	0.71	0.56, 0.90	56	0.63	0.47, 0.86
p for trend					0.001			0.001
p for interaction					0.667			0.396
Body mass index								
<20								
Lowest	2,896	20,823	116	1.00	Reference	72	1.00	Reference
Second	2,383	17,909	86	0.92	0.68, 1.22	64	1.08	0.76, 1.54
Third	2,096	15,459	69	0.87	0.64, 1.18	45	0.92	0.63, 1.36
Highest	1,598	12,009	47	0.76	0.54, 1.09	35	0.92	0.60, 1.40
p for trend					0.119			0.623
20–<27								
Lowest	8,467	63,889	370	1.00	Reference	238	1.00	Reference
Second	7,117	55,220	283	0.91	0.78, 1.06	190	0.92	0.76, 1.12
Third	6,453	49,990	239	0.82	0.70, 0.97	149	0.76	0.62, 0.93
Highest	5,515	42,597	192	0.81	0.68, 0.97	116	0.73	0.58, 0.92
p for trend					0.009			0.002
≥27								
Lowest	1,914	14,673	83	1.00	Reference	58	1.00	Reference
Second	1,338	10,516	59	1.05	0.74, 1.48	36	0.94	0.61, 1.44
Third	1,114	8,624	42	0.82	0.56, 1.20	28	0.79	0.49, 1.25
Highest	982	7,678	44	0.96	0.65, 1.41	25	0.76	0.46, 1.25
p for trend					0.643			0.223
p for interaction					0.839			0.137
Frequency of leisure-time sports or physical exercise (days/week)								
<1								
Lowest	10,837	81,716	464	1.00	Reference	297	1.00	Reference
Second	8,773	68,595	354	0.95	0.83, 1.10	236	0.96	0.81, 1.14
Third	7,521	58,563	274	0.84	0.72, 0.98	174	0.80	0.66, 0.97
Highest	5,811	45,696	223	0.92	0.78, 1.08	139	0.87	0.70, 1.06
p for trend					0.140			0.065
≥1								
Lowest	2,440	17,670	105	1.00	Reference	71	1.00	Reference
Second	2,065	15,049	74	0.80	0.59, 1.09	54	0.85	0.59, 1.22
Third	2,142	15,510	76	0.81	0.59, 1.09	48	0.74	0.51, 1.08
Highest	2,284	16,587	60	0.61	0.44, 0.84	37	0.55	0.37, 0.83
p for trend					0.003			0.003
p for interaction					0.158			0.105

* METs, metabolic equivalents; HR, hazard ratio; CI, confidence interval.

† Adjusted for age (stratified, 5-year categories), area (stratified, 10 public health center areas), total energy intake (stratified, quintiles), history of diabetes (no, yes), smoking status (never smoking, past smoking, or 1–19, 20–29, or ≥30 cigarettes/day), alcohol intake status (almost none, occasional, or regular), body mass index (weight (kg)/height (m)^2; <20, 20–<27, or ≥27), and leisure-time sports or physical exercise (<1, 1–2, or ≥3–4 days/week).

‡ Weight (kg)/height (m)^2.

earliest date of diagnosis was used in cases with multiple primary cancers diagnosed at different times. A total of 4,334 newly diagnosed cancer cases were identified.

Physical activity levels

The main exposure of interest in the present study was daily total physical activity level. In our questionnaire (see Appendix), subjects were asked about the average amount of time spent per day in three types of physical activity: heavy physical work or strenuous exercise (none, <1 hour, or ≥1 hour), sitting (<3, 3–<8, or ≥8 hours), and standing or walking (<1, 1–<3, or ≥3 hours). The following values were assigned as time scores for each activity: heavy physical work or strenuous exercise—0 for none, 0.5 for <1 hour, and 3 for ≥1 hour; sitting—1.5 for <3 hours, 5.5 for 3–<8 hours, and 7.5 for ≥8 hours; standing or walking—0.5 for <1 hour, 2 for 1–<3 hours, and 8.5 for ≥3 hours. The midpoint of the time range for each category was assigned when minimum and maximum values were presented on the questionnaire, and arbitrary values considered to have the highest validity from the validation study were assigned for the highest category. MET-hours/day were estimated by multiplying the daily time score for each activity by the MET intensity of that activity (16): for heavy physical work or strenuous exercise, 4.5; for standing or walking, 2.0; for being sedentary, 1.5; and for sleep or other passive activity, 0.9. After data were summed across all activities, subjects were grouped by sex into four exposure levels according to quartile of total METs/day score. Because the question on MET calculation incorporated all activities, including occupation, housework, leisure-time sports, etc., a separate question on the frequency of leisure-time sports and physical exercise was not included in the estimation of total physical activity level.

The validity of the total METs/day score was assessed among 108 eligible samples (53 men and 55 women) derived from 110 original volunteer subjects from the cohort using 4-day, 24-hour physical activity records (Sunday or another day off plus three weekdays) in two different seasons (namely, harvesting and one other season in a single year). The mean number of total METs/day for physical activity obtained from the self-report was 33.5 in men and 33.4 in women, while the mean from the 24-hour physical activity record was 39.5 in men and 40.8 in women. Energy expenditure estimated in METs showed little difference by area. Spearman's rank correlation coefficient for the correlation between the total METs/day score and the physical activity records was 0.46 when the average of two seasons was taken (men, 0.53; women, 0.35).

Analysis

The number of person-years in the follow-up period was counted from the starting point (i.e., the date of response to the 5-year follow-up questionnaire) to the date of occurrence of any cancer, emigration from the study area, death, or the end of the study period, whichever came first. For subjects who withdrew from the study or were lost to follow-up, the date of withdrawal or the last confirmed date of presence in the study was used as the date of censoring.

Hazard ratios and 95 percent confidence intervals were used to characterize the relative risk of cancer occurrence associated with daily total physical activity level. Daily total physical activity was assessed in quartiles of total METs/day score. The median METs/day value for each quartile was used when the linear association was assessed. To investigate whether the effect on the outcome differed by type of physical activity, we also assessed risk by the frequency of leisure-time sports or physical exercise (≤1–3 days/month, 1–2 days/week, 3–4 days/week, or almost every day), in addition to the amount of time spent per day in heavy physical work or strenuous exercise (none, <1 hour, or ≥1 hour) and in standing or walking (<1, 1–<3, or ≥3 hours). Ordinal values were used to assess linear trends for these variables.

The Cox proportional hazards model was employed to control for potentially confounding factors, namely age at the starting point (5-year categories), area (10 public health center areas), history of diabetes (no, yes), smoking status (never smoking, past smoking, or 1–19, 20–29, or ≥30 cigarettes/day), alcohol intake status (almost none, occasional, or regular), body mass index (weight (kg)/height $(m)^2$; 14–<20, 20–<27, or ≥27), and total energy intake (in quintiles, estimated by semiquantitative food frequency questionnaire). These variables, obtained from the questionnaire, are either known or suspected risk factors for cancer that have been identified in previous studies. We treated age, area, and total energy intake as strata to allow for a different baseline hazard for each stratum. In testing of the proportional hazards assumption by Schoenfeld residuals and scaled Schoenfeld residuals, we found no violation of proportionality. In addition, we evaluated whether the effect of total physical activity was influenced by age, body mass index, or frequency of leisure-time sports or physical exercise using a test of interaction, by entering into the model multiplicative terms for interaction between the respective factors. Since the effect of total physical activity was significantly influenced by sex (p for interaction ≤ 0.001), all analysis were conducted by sex. All statistical analyses were performed using Stata 10 (Stata Corporation, College Station, Texas) (17).

RESULTS

During 599,117 person-years of follow-up (average follow-up period, 7.5 years) for the 79,771 subjects (37,898 men and 41,873 women), 4,334 newly diagnosed cases of cancer (2,704 in men and 1,630 in women), including skin cancer (n = 53; 1.2 percent), were identified and included in the analyses. In men, gastric cancer was the most common cancer (n = 621; 23.0 percent), followed by cancers of the lung (n = 388; 14.3 percent), colon (n = 328; 12.1 percent), and prostate (n = 279; 10.3 percent). In women, breast cancer was the most common (n = 294; 18.0 percent), followed by cancers of the stomach (n = 232; 14.2 percent), colon (n = 228; 14.0 percent), and lung (n = 144; 8.8 percent).

Characteristics of the study subjects according to physical activity level are shown in table 1. The median values in the lowest, second, third, and highest quartiles of total METs/day

TABLE 4. Hazard ratios* for total cancer incidence according to type of physical activity (n = 79,771), Japan Public Health Center-based Prospective Study, 1995–2004

	No. of subjects	Person-years of follow-up	Total			Excluding cases diagnosed within the first 3 years		
			No. of cases	HR†	95% CI†	No. of cases	HR	95% CI
Men (n = 37,898)								
Heavy physical work or strenuous exercise (hours/day)								
None	22,235	161,694	1,670	1.00	Reference	1,093	1.00	Reference
<1	5,165	38,119	324	0.95	0.84, 1.07	229	1.02	0.88, 1.18
≥1	10,498	79,918	710	0.89	0.81, 0.98	482	0.89	0.80, 1.00
p for trend					0.014			0.071
Standing or walking (hours/day)								
<1	8,243	59,839	564	1.00	Reference	369	1.00	Reference
1–<3	9,143	65,023	649	1.04	0.92, 1.17	425	1.04	0.90, 1.21
≥3	20,512	154,869	1,491	0.99	0.89, 1.11	1,010	0.99	0.87, 1.13
p for trend					0.787			0.764
Sitting (hours/day)								
<3	17,251	128,076	1,230	1.00	Reference	821	1.00	Reference
3–<8	17,472	128,067	1,247	0.97	0.89, 1.06	835	0.97	0.88, 1.08
≥8	3,175	23,588	227	1.02	0.87, 1.18	148	0.97	0.80, 1.16
p for trend					0.839			0.599
Leisure-time sports or physical exercise (days/week)								
<1	29,656	220,754	2,118	1.00	Reference	1,434	1.00	Reference
1–2	4,095	30,011	240	0.92	0.80, 1.05	155	0.87	0.74, 1.03
≥3–4	4,147	28,965	346	1.12	0.998, 1.26	215	1.09	0.94, 1.26
p for trend					0.158			0.519
Women (n = 41,873)								
Heavy physical work or strenuous exercise (hours/day)								
None	31,286	238,962	1,266	1.00	Reference	832	1.00	Reference
<1	4,097	30,583	138	0.91	0.76, 1.09	89	0.90	0.72, 1.12
≥1	6,490	49,840	226	0.93	0.80, 1.07	135	0.84	0.70, 1.01
p for trend					0.200			0.043
Standing or walking (hours/day)								
<1	6,077	45,688	259	1.00	Reference	164	1.00	Reference
1–<3	9,828	73,552	410	1.00	0.85, 1.18	266	1.02	0.84, 1.25
≥3	25,968	200,146	961	0.89	0.77, 1.04	626	0.90	0.75, 1.09
p for trend					0.054			0.128
Sitting (hours/day)								
<3	18,981	144,501	724	1.00	Reference	463	1.00	Reference
3–<8	20,184	153,659	785	0.98	0.88, 1.09	509	0.97	0.85, 1.11
≥8	2,708	21,226	121	1.05	0.86, 1.29	84	1.10	0.86, 1.41
p for trend					0.896			0.748
Leisure-time sports or physical exercise (days/week)								
<1	32,942	254,570	1,315	1.00	Reference	846	1.00	Reference
1–2	4,338	31,712	136	0.91	0.76, 1.09	85	0.91	0.73, 1.15
≥3–4	4,593	33,104	179	1.05	0.89, 1.23	125	1.20	0.99, 1.45
p for trend					0.883			0.160

* The model included age (stratified, 5-year categories), area (stratified, 10 public health center areas), total energy intake (stratified, quintiles), history of diabetes (no, yes), smoking status (never smoking, past smoking, or 1–19, 20–29, or ≥30 cigarettes/day), alcohol intake status (almost none, occasional, regular), body mass index (weight (kg)/height (m)^2; <20, 20–<27, or ≥27), heavy physical work or strenuous exercise (none, <1 hour, or ≥1 hour/day), sitting (<3, 3–<8, or ≥8 hours/day), standing or walking (<1, 1–<3, or ≥3 hours/day), and leisure-time sports or physical exercise (<1, 1–2, or ≥3–4 days/week).

† HR, hazard ratio; CI, confidence interval.

TABLE 5. Hazard ratios for incidence of cancer at specific sites according to daily total physical activity level (n = 79,771), Japan Public Health Center-based Prospective Study, 1995–2004

Site (*International Classification of Diseases for Oncology*, Third Edition, code)	Quartile of physical activity level (quartile of METs*/day score)	No. of subjects	Person-years of follow-up	No. of cases	Hazard ratio†	95% confidence interval
Men (n = 37,898)						
Stomach (C16)	Lowest	12,966	92,421	194	1.00	Reference
	Second	7,822	57,957	134	1.10	0.88, 1.37
	Third	7,579	56,512	136	1.10	0.88, 1.37
	Highest	9,531	72,841	157	1.04	0.84, 1.29
	p for trend					0.785
Colon (C18)	Lowest	12,966	92,421	131	1.00	Reference
	Second	7,822	57,957	72	0.83	0.62, 1.11
	Third	7,579	56,512	59	0.65	0.48, 0.89
	Highest	9,531	72,841	66	0.58	0.43, 0.79
	p for trend					<0.001
Rectum (C19–20)	Lowest	12,966	92,421	51	1.00	Reference
	Second	7,822	57,957	41	1.30	0.85, 1.97
	Third	7,579	56,512	35	1.11	0.72, 1.72
	Highest	9,531	72,841	35	0.88	0.57, 1.36
	p for trend					0.464
Liver (C22)	Lowest	12,966	92,421	82	1.00	Reference
	Second	7,822	57,957	32	0.69	0.45, 1.06
	Third	7,579	56,512	44	1.01	0.69, 1.49
	Highest	9,531	72,841	31	0.62	0.40, 0.96
	p for trend					0.062
Pancreas (C25)	Lowest	12,966	92,421	36	1.00	Reference
	Second	7,822	57,957	20	0.90	0.52, 1.57
	Third	7,579	56,512	15	0.67	0.36, 1.24
	Highest	9,531	72,841	16	0.55	0.30, 1.00
	p for trend					0.038
Lung (C34)	Lowest	12,966	92,421	108	1.00	Reference
	Second	7,822	57,957	81	1.22	0.91, 1.63
	Third	7,579	56,512	103	1.44	1.09, 1.90
	Highest	9,531	72,841	96	1.10	0.83, 1.45
	p for trend					0.494
Prostate (C61)	Lowest	12,966	92,421	77	1.00	Reference
	Second	7,822	57,957	68	1.39	1.00, 1.94
	Third	7,579	56,512	63	1.21	0.86, 1.69
	Highest	9,531	72,841	71	1.13	0.82, 1.57
	p for trend					0.644

Table continues

score were 25.45, 31.85, 34.25, and 42.65, respectively, in men and 26.10, 31.85, 34.25, and 42.65, respectively, in women. Men who were more physically active were more likely to report regular drinking, a higher frequency of leisure-time sports or physical exercise, and higher daily mean energy consumption and were less likely to report a history of diabetes mellitus and liver disease. No difference in body mass index was observed between groups by physical activity level. In women, similar trends were observed, except that the differences in the proportion of regular drinkers were not significant.

Associations between daily total physical activity level by total METs/day score and total cancer incidence are shown

TABLE 5. Continued

Site (*International Classification of Diseases for Oncology*, Third Edition, code)	Quartile of physical activity level (quartile of METs/day score)	No. of subjects	Person-years of follow-up	No. of cases	Hazard ratio†	95% confidence interval
Women (n = 41,873)						
Stomach (C16)	Lowest	13,277	99,385	91	1.00	Reference
	Second	10,838	83,644	53	0.74	0.52, 1.04
	Third	9,663	74,073	54	0.78	0.55, 1.10
	Highest	8,095	62,284	34	0.63	0.42, 0.94
	p for trend					0.020
Colon (C18)	Lowest	13,277	99,385	83	1.00	Reference
	Second	10,838	83,644	58	0.87	0.62, 1.22
	Third	9,663	74,073	48	0.74	0.52, 1.07
	Highest	8,095	62,284	39	0.82	0.56, 1.21
	p for trend					0.198
Rectum (C19–20)	Lowest	13,277	99,385	24	1.00	Reference
	Second	10,838	83,644	24	1.26	0.71, 2.23
	Third	9,663	74,073	16	1.05	0.55, 2.00
	Highest	8,095	62,284	22	1.79	0.99, 3.23
	p for trend					0.077
Liver (C22)	Lowest	13,277	99,385	29	1.00	Reference
	Second	10,838	83,644	19	0.96	0.52, 1.78
	Third	9,663	74,073	19	0.99	0.53, 1.84
	Highest	8,095	62,284	7	0.54	0.23, 1.29
	p for trend					0.248
Pancreas (C25)	Lowest	13,277	99,385	19	1.00	Reference
	Second	10,838	83,644	15	0.98	0.50, 1.95
	Third	9,663	74,073	11	0.83	0.39, 1.76
	Highest	8,095	62,284	13	1.29	0.62, 2.67
	p for trend					0.601
Lung (C34)	Lowest	13,277	99,385	50	1.00	Reference
	Second	10,838	83,644	37	0.90	0.58, 1.38
	Third	9,663	74,073	31	0.90	0.57, 1.42
	Highest	8,095	62,284	26	0.92	0.56, 1.49
	p for trend					0.686
Breast (C50)	Lowest	13,277	99,385	85	1.00	Reference
	Second	10,838	83,644	91	1.24	0.92, 1.66
	Third	9,663	74,073	67	1.02	0.74, 1.40
	Highest	8,095	62,284	51	0.91	0.64, 1.29
	p for trend					0.529

* METs, metabolic equivalents.

† Adjusted for age (stratified, 5-year categories), area (stratified, 10 public health center areas), total energy intake (stratified, quintiles), history of diabetes (no, yes), smoking status (never smoker, past smoker, or 1–19, 20–29, or ≥30 cigarettes/day), alcohol intake status (almost none, occasional, or regular), body mass index (weight (kg)/height (m)^2; <20, 20–<27, or ≥27), and leisure-time sports or physical exercise (<1, 1–2, or ≥3–4 days/week).

in table 2. Upon multivariate adjustment, compared with subjects in the lowest quartile, increased daily total physical activity was significantly associated with a decreased risk of cancer incidence in both men and women. In men, hazard ratios in the second, third, and highest quartiles were 1.00 (95 percent confidence interval (CI): 0.90, 1.11), 0.96 (95 percent CI: 0.86, 1.07), and 0.87 (95 percent CI: 0.78, 0.96), respectively (p for trend = 0.005); in women, they were 0.93

(95 percent CI: 0.82, 1.05), 0.84 (95 percent CI: 0.73, 0.96), and 0.84 (95 percent CI: 0.73, 0.97), respectively (p for trend = 0.007). Our estimates also showed that the risk decreased by 7 percent in men and 10 percent in women with each 10-MET/day increase in physical activity level. The results did not differ substantially after exclusion of early cancer cases—those occurring within 3 years of the starting point—or after further exclusion of subjects with very low physical activity levels (<23 METs/day; 2 percent of subjects), considered to result from poor physical condition. On further estimation of the population attributable fraction (18) from our results, 4.5 percent of cases in men and 5.5 percent of cases in women were considered to have been preventable if the persons in the lowest physical activity category had increased their activity to a higher level.

In both sexes, the degree of risk decrease was attenuated among persons with increasing body mass index. In contrast, it was strengthened among the elderly and among persons who regularly engaged in leisure-time sports or physical exercise; this relation appeared more clearly in women. No significant interaction was observed for age, obesity status, or frequency of leisure-time sports and physical exercise (table 3). No particularly significant associations were identified in analysis by type of physical activity (table 4).

Results from analyses of specific cancer sites are shown in table 5. Significantly decreased risks were observed for colon, liver, and pancreatic cancer in men and for stomach cancer in women. In additional analyses for these cancers stratified by age, body mass index, and frequency of leisure-time sports or physical exercise, larger risk reductions were observed in persons with a lower body mass index, persons with frequent leisure-time sports or physical exercise, and the elderly for female stomach cancer and in persons with lower body mass index and persons with infrequent leisure-time sports or physical exercise for male colon cancer. For male liver and pancreatic cancers, we did not detect any significant difference or tendency in risk between stratified groups. In the analysis of breast cancer, the null association was not influenced by menopausal status.

DISCUSSION

The health benefits of physical activity are well established for certain cancer sites (1, 19), but the extent to which the grand sum of these effects influences total cancer incidence has not been clarified. Of course, any such association depends to some degree on the background population, namely the site distribution of cancers which are strongly or weakly associated with physical activity. According to recent statistics, in Japan the cancer sites with the highest incidence rates are the stomach, followed by the lung, colon, liver, and prostate, for men and the breast, followed by the stomach, colon, uterus, and lung, for women (20). In this large-scale, population-based cohort study of Japanese men and women, we found a significant inverse association between daily total physical activity level and total cancer incidence. To reduce the potential for spurious associations from reverse causation, we excluded all subjects with a history of cancer at the starting point. Moreover, exclusion of early cases (those occurring within 3 years of the starting point) had no substantial effect on the results.

To our knowledge, only two studies have assessed the association between physical activity and total risk of cancer (2, 3); both were carried out in relatively small populations. One, which targeted men only, observed a reduced risk with increased physical activity (2), while the second observed an increased risk with increased nonrecreational physical inactivity (3). Our findings, obtained with a substantially larger sample, accord with those of these previous studies.

Our results showed basically similar risk reductions in men and women. Shephard and Shek (21) suggested that differences between the sexes in benefits associated with regular physical activity are due to the difference in hormonal conditions, which may lead to the failure to adapt activity questionnaires to traditional patterns of physical activity in females. Methodologically, it is commonly noted that men are more likely to be physically active in their jobs and women are more likely to be involved in housework (22). In our questionnaire, rank correlation coefficients for correlation with the 24-hour physical activity record were higher in men than in women. This may have partly resulted from the failure of our questionnaire to suitably account for housework. This type of measurement error may have led to underestimation of the association. Nevertheless, in the present study, a stronger effect of total physical activity among persons who engaged in regular leisure-time sports or physical exercise than among those who did not appears to have been more clearly observed in women. The larger proportion of strenuous work as a fraction of total physical activity in men than in women may be one reason for this discrepancy between men and women.

Our findings also showed that the effect of physical activity was diminished among subjects with a high body mass index, which is accordant with a previous report (3). To a substantial degree, physical activity may affect the risk of cancer by reducing weight and body mass index. We therefore suggest that the effect of physical activity appears less clear in persons with a high body mass index.

By site, our results showed inverse associations for colon, liver, and pancreatic cancer in men and for stomach cancer in women. In our population, we observed a positive association with a high body mass index for colon cancer only (23) and little association for pancreatic cancer (24). A recent evaluation found no association for stomach or liver cancer (1). In addition, nonalcoholic fatty liver disease, an increasingly recognized cause of chronic liver disease across the world, appears to be most strongly associated with central obesity and insulin resistance, and hepatocellular carcinoma has been postulated to arise through the development and progression of nonalcoholic fatty liver disease (25, 26). In the Japanese population, however, most cases of hepatocellular carcinoma are associated with hepatitis virus infection, and attribution to other factors may be small. Therefore, the effect of physical activity on these cancers, if any, appears to be operating not only via any improvement in obesity and related factors but also via other mechanisms.

Discussions on the possible mechanisms by which physical activity protects against cancer remain inconclusive. Various mechanisms have been plausibly associated with various cancers, such as alterations in sex hormones or insulin and

insulin-like growth factors, immune modulation, alterations in free radical generation, changes in body fatness, and direct effects on cancer (1, 19, 27–32). Hyperinsulinemia produces an increase in circulating insulin-like growth factor 1, which is thought to play a major role in promoting carcinogenesis, and a decrease in insulin-like growth factor-binding proteins (33). Exercise increases insulin sensitivity and decreases fasting insulin and C-peptide levels (34), which may improve insulin resistance. Exercise-induced changes in the activity of macrophages, natural killer cells, lymphokine-activated killer cells, neutrophils, and regulating cytokines suggest that immunomodulation may contribute to the protective value of exercise (35). Strenuous physical exercise enhances oxygen free radical production, and the increased number of reactive oxygen species that are generated potentially results in damage to lipids, protein, and DNA. The antioxidant defense systems have co-evolved to counteract oxidative damage from oxygen free radicals (24, 36, 37). Moderate physical activity may be of benefit as a means of slowing or stopping the loss of antioxidants, whereas severe exercise might overwhelm the antioxidant system, potentially leading to damage and increased cell mutagenesis (37). Other mechanisms include a decrease in gut transit time, which has beneficial effects on bile content and secretion (1, 38), and have been proposed by site (1).

The major strength of the present study was its prospective design, which enabled us to avoid exposure recall bias. Study subjects were selected from the general population, the sample was large, the response rate to the questionnaire (81 percent) was acceptable for study settings such as this, and the loss to follow-up (0.3 percent) was negligible. Further, the number of exclusions due to missing data on physical activity (7 percent) was not particularly large. Although a difference in the characteristics of subjects with and without missing information had the potential to influence the results, no such difference was seen. In addition, the cancer registry in the study population was of sufficient quality to reduce the possibility of misclassification of the outcome.

In addition to those mentioned above, however, several methodological limitations can be identified. In particular, since assessment of physical activity was based on self-reports, misclassification may have been unavoidable. Nevertheless, because the data were collected before diagnosis, any imprecision is likely to have resulted in underestimation of the association. Changes in physical activity over time may also have caused misclassification, which might have led to underestimation of the association. In addition, some types of cancers or health conditions related to them may have caused low levels of physical activity from the starting point of the study; therefore, we cannot deny the possibility of spurious associations. Further, although adjustment was made for lifestyle factors possibly associated with cancer, unmeasured confounders may not have been controlled. Finally, our results may not be generalizable to populations with a different general lifestyle or a different degree of leanness from the Japanese.

Allowing for these methodological issues, our results suggest that increased daily total physical activity may be beneficial in preventing the development of cancer among Japanese men and women, who are characterized as relatively lean. Further research on the generalizability of our results to other relatively lean populations is warranted.

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APPENDIX

Questions related to physical activity in the 5-year follow-up survey of the Japan Public Health Center-based Prospective Study:

How long on average do you engage in the following activities each day?

Heavy physical work or strenuous exercise	None	<1 hour	≥1 hour
Sitting	<3 hours	3–<8 hours	≥8 hours
Standing or walking	<1 hour	1–<3 hours	≥3 hours

How often do you participate in sports or physical exercise?

Almost never	≤1–3 days a month	1–2 days a week	3–4 days a week	Almost every day

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ORIGINAL REPORT

Plasma Isoflavone Level and Subsequent Risk of Breast Cancer Among Japanese Women: A Nested Case-Control Study From the Japan Public Health Center-Based Prospective Study Group

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ABSTRACT

Purpose
Because they have large variations in consumption, Asian countries are suitable settings for studies of the effect of relatively high-dose isoflavone intake on breast cancer risk. Nevertheless, no prospective study from Asia has assessed blood or urine levels as biomarkers of isoflavone intake.

Patients and Methods
A total of 24,226 women ages 40 to 69 years in the Japan Public Health Center–based prospective study who responded to the baseline questionnaire and provided blood in 1990 to 1995 were observed to December 2002. During a mean 10.6 years of follow-up, 144 patients newly diagnosed with breast cancer were identified. Two matched controls for each patient were selected from the cohort. Isoflavone levels were assessed by plasma level and food frequency questionnaire, and the odds ratio of breast cancer according to isoflavone level was estimated using a conditional logistic regression model.

Results
We found a statistically significant inverse association between plasma genistein and risk of breast cancer, but no association for plasma daidzein. Adjusted odds ratios for the highest versus lowest quartile of plasma level were 0.34 for genistein (95% CI, 0.16 to 0.74; *P* for trend, .02) and 0.71 for daidzein (95% CI, 0.35 to 1.44; *P* for trend, .54). Median plasma genistein values in the control group were 31.9 ng/mL for the lowest and 353.9 ng/mL for the highest quartile groups. Regarding dietary intake of isoflavones, nonsignificant inverse associations were observed for both genistein and daidzein.

Conclusion
This nested case-control study found an inverse association between plasma genistein and the risk of breast cancer in Japan.

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INTRODUCTION

Soy foods, a traditional staple dish in Asian countries, are a primary source of isoflavones, such as genistein and daidzein, which are classified as phytoestrogens. Because breast cancer risk is substantially lower in Asian than Western countries,[1] the contribution of a high isoflavone intake to low breast cancer risk has been hypothesized.[2] This hypothesis has been supported by in vitro studies at high genistein concentrations and in the majority of animal studies, which together have demonstrated various anticancer effects of isoflavones acting via both estrogen-dependent and -independent mechanisms.[3,4] Estrogen-dependent mechanisms arise through the mediation of estrogen receptor α and β, owing to the similar chemical structure of isoflavones to the human estrogen hormone and their binding affinity to estrogen receptors.[4,5] For this reason, they have been hypothesized to behave like selective estrogen receptor modulators. In contradiction to potential protective effects, however, genistein exhibits estrogenic properties at low concentrations, which could theoretically enhance breast cancer risk.[3,4] In fact, some animal studies have reported that genistein stimulates tumor development and growth.[6,7] Although a recent meta-analysis found that soy intake was associated with a

small reduction in breast cancer risk, the authors concluded that in view of these risk-enhancing effects, recommendations for high-dose isoflavone supplementation to prevent breast cancer or its recurrence were premature.[8] Phytoestrogen supplements, however, are commercially marketed for use by postmenopausal women as natural and safe alternatives to hormone replacement therapy. The effect of relatively high-dose isoflavone on breast cancer risk is now of concern.

Because they have large variations in consumption among individuals, Asian countries serve as suitable venues for studies of the effect of relatively high-dose isoflavone intake on breast cancer risk. Despite this advantage, only a few epidemiological studies on soy or isoflavone intake and breast cancer risk from Asia have been reported.[9] In particular, no prospective study on isoflavone levels in blood or urine samples has been reported, notwithstanding that, because they are partly determined by individual differences in absorption and metabolism, blood or urine levels might better reflect interperson differences than dietary assessment. The three nested case-control studies which have investigated this association in Western populations have been inconsistent, with one reporting an inverse association with plasma genistein in the Netherlands,[10] the second showing no association with urinary genistein in the Netherlands,[11] and the third finding a positive association with urine and serum phytoestrogens in the United Kingdom.[12] This inconsistency might be in part explained by the apparently small variation in isoflavone levels in Western countries. For example, studies in the Netherlands, which has a high incidence of breast cancer (age-standardized rate per 100,000 world population, 86.7 in 2002),[13] reported a median genistein intake of 0.14 mg/d in women ages 49 to 70 years,[14] and a median plasma genistein level of 4.89 ng/mL in the control group of a nested-case control study.[10] In contrast, a study in Japan, where the incidence of breast cancer is low (age-standardized rate per 100,000 world population, 32.7 in 2002),[13] reported a median genistein intake of 22.3 mg/d and median serum level of 90.2 ng/mL.[15] This substantial variation in isoflavone levels suggests that the Japanese population represents an ideal setting for determining whether an association exists at relatively high levels achievable from dietary intake only.

Herein, to clarify the effect of relatively high-dose isoflavone exposure on breast cancer risk, we conducted a nested case-control study within a large-scale population-based prospective study in Japan.

PATIENTS AND METHODS

Study Population

The Japan Public Health Center–based prospective study, which began in 1990 for cohort I and in 1993 for cohort II, included 140,420 subjects (68,722 men and 71,698 women) living in the municipalities supervised by 11 public health centers (PHC). Details of the study design have been described elsewhere.[16] The study protocol was approved by the institutional review board of the National Cancer Center, Tokyo, Japan.

The study population comprised registered Japanese inhabitants living in each PHC area, ages 40 to 59 years in cohort I and 40 to 69 years in cohort II. In this analysis, one PHC area was excluded since data on cancer incidence were not available. We thus defined a population-based cohort of 67,426 women (27,389 in cohort I and 40,037 in cohort II) after the exclusion of ineligible subjects (n = 95).

Questionnaire Survey

A baseline survey was conducted from 1990 to 1994. A total of 55,891 women (83%) returned the questionnaire, which contained questions concerning demographic characteristics, medical history, menstrual and reproductive history, anthropometric factors, physical activity, smoking and drinking habits, and diet.

Blood Collection

Subjects voluntarily provided 10 mL of blood during health check-ups from 1990 to 1995. Blood samples were divided into plasma and buffy layers and stored at −80°C until analysis. Among respondents to the baseline questionnaire, a total of 24,996 women (45%) donated blood.

Follow-Up

All registered subjects were observed from the start of the study period to December 31, 2002. Data on residential relocation were obtained from residential registries. Among study subjects (n = 24,996), 1,289 subjects (5.2%) moved out of the study area and 5 (0.02%) were lost to follow-up within the study at-risk period.

Selection of Patients and Controls

Incidence data on breast cancer were collected for the Japan Public Health Center cancer registry through two data sources—major local hospitals and population-based cancer registries. Death certificates were used to supplement information on cancer incidence. Site of origin and histologic type were coded by members of our study group (Appendix A1, online only) using the International Classification of Diseases for Oncology, third edition, code C500-509. Up to the end of the study period, 144 new breast cancer cases (97 in cohort I and 47 in cohort II) were identified among the 24,226 women (9,689 in cohort I and 14,537 in cohort II) who had returned the baseline questionnaire, reported no history of breast cancer or ovarian cystoma, and provided blood samples. Diagnosis was microscopically verified in 98% of patients, and based on death certificates only in 0.7%. The mortality/incidence ratio was 0.14.

For each patient, two controls were selected using incidence density sampling from subjects who were not diagnosed with breast cancer during the follow-up period when the patient was diagnosed. Control selection was done without reference to incidence of other cancer sites. Controls were matched with each patient for age (within 3 years), PHC area, area (city or town and village), date of blood collection (within 90 days), time of day of blood collection (within 3 hours), fasting time at blood collection (within 3 hours), and baseline menopausal status.

Assessment of Dietary Intake

Dietary intakes of genistein and daidzein were assessed by a food frequency questionnaire of 44 items for cohort I and 52 for cohort II. Isoflavone intake was defined for this study as the sum of genistein and daidzein intake. We documented the questionnaire assessment of isoflavone intake to be reasonably valid (details in Appendix A1).[15,17]

Laboratory Assay

Plasma levels of isoflavone were analyzed using high-performance liquid chromatography with a coulometric array detector in accordance with the modified methods of Gamache and Acworth.[18] Concentrations of genistein and daidzein were determined by linear regression of the peak height for each standard, and adjusted according to the recovery rate of the internal plasma standard. The regression coefficient of peak height and concentration calculated for isoflavones revealed a linearity range of 0 to 0.75 μg/mL, with correlation coefficient values higher than 0.938. Voltametric response for the standard solution displayed coefficients of variation of 8% for intra- and 11% for interday variation. Recovery rates of isoflavones in plasma samples ranged between approximately 73% and 98%. Detection limits were 2.2 ng/mL for genistein and 2.7 ng/mL for daidzein. Laboratory personnel were blinded to case-control status when performing the analyses.

Statistical Analysis

Comparison of baseline characteristics, as well as plasma levels and dietary intake of isoflavones, between cases and controls was evaluated by the Mantel-Haenszel test using matched-set strata. Spearman's correlation coefficients were calculated among plasma levels and dietary intakes of isoflavone

among control subjects. Using a conditional logistic regression model, we calculated odds ratios (ORs) and 95% CIs of breast cancer for plasma levels and dietary intake of isoflavone divided into quartiles based on control distribution. The ORs were adjusted for number of births and age at first birth as potential confounders. The adjusted ORs were calculated based on a total of 405 subjects with complete information for covariates. Linear trends for ORs were tested in the conditional logistic regression model using the exposure categories as ordinal variables. All P values reported are two sided, and significance level was set at $P < .05$. All statistical analyses were performed with SAS software, version 9.1 (SAS Institute Inc, Cary, NC).

RESULTS

Case subjects and controls had significantly different distribution for number of births (Table 1). Other characteristics, such as age at menarche, age at first birth, body mass index (BMI), alcohol consumption, or dietary intake did not substantially differ between the two groups.

Plasma genistein was significantly lower among cases than controls whereas plasma daidzein values were similar (Table 2). No significant differences between the groups were seen for dietary genistein, daidzein, or isoflavone intake. Median isoflavone intake in the control group was 34.8 mg/d (36.1 in cohort I and 29.9 mg/d in cohort II). Genistein and daidzein were highly correlated for both plasma level ($r = 0.72$) and dietary intake ($r = 0.99$). Correlation coefficients between plasma and dietary levels were relatively low for both genistein ($r = 0.23$) and daidzein ($r = 0.31$).

We found a statistically significant inverse association between plasma genistein and the risk of breast cancer (P for trend, .02), but no statistically significant association for plasma daidzein (P for trend, .54; Table 3). Adjusted ORs for the highest versus lowest quartile of plasma level were 0.34 for genistein (95% CI, 0.16 to 0.74; $P \leq .01$) and 0.71 for daidzein (95% CI, 0.35 to 1.44; $P = .34$). Moreover, the results did not change substantially after adjustment for dietary intake of isoflavone or other potential confounders such as age at menarche, menopausal status at baseline, age at menopause, height, BMI, and alcohol consumption. Further, exclusion of cases diagnosed before the first 3 years of follow-up did not substantially change the results, nor did the exclusion of subjects who used vitamin supplements or who provided a nonfasting blood sample (ie, within 6 hours after a meal). Regarding dietary intake, we observed inverse associations for both genistein and daidzein but neither was statistically significant (Table 3). In addition, adjusted ORs by isoflavone intake were closely similar to those by genistein intake (data not shown).

A stratified analysis according to baseline menopausal status showed no remarkable difference between two strata for either genistein and daidzein, regardless of whether the values were assessed by plasma or questionnaire, although the inverse association between plasma genistein and risk of breast cancer tended to be more stable in postmenopausal than premenopausal women (Table 4).

Table 1. Characteristics of Patients and Matched Control Subjects at Baseline

Characteristic	Patients (n = 144) No.	Patients (n = 144) %	Controls (n = 288) No.	Controls (n = 288) %	P*
Mean age, years	51.7		51.8		
Standard deviation	7.1		7.1		—
Family history of breast cancer	2	1.4	2	0.7	.48
Premenopausal women	59	42	118	42	—
Postmenopausal women					
Natural menopause	70	50	140	50	—
Surgical menopause	10	7.2	20	7.2	—
Mean age at menopause, years	50.0		49.8		.76
SE†	0.38		0.27		
Mean age at menarche, years	14.6		14.8		.33
SE†	0.15		0.10		
Mean No. of births	2.3		2.8		.01
SE†	0.12		0.09		
Mean age at first birth, years	25.7		25.0		.22
SE†	0.30		0.21		
Use of exogenous female hormones (current use)	4	3.0	2	0.8	.10
Mean height, cm	151.7		151.4		.70
SE†	0.46		0.33		
Mean body mass index, kg/m²	23.4		23.5		.49
SE†	0.25		0.18		
Smoking (current smoker)	5	3.5	17	5.9	.23
Alcohol drinking (regular drinker)	18	13	26	9.1	.28
Leisure-time physical activity (≥ once per week)	30	21	57	20	.42
Vitamin supplement user	33	24	61	23	.65
Green tea intake (≥ five cups per day)	36	25	71	25	.42
Mean total energy intake, kcal/d	1,269.4		1,271.0		.41
SE‡	26.5		19.2		
Mean fish and shellfish intake, g/d	45.4		45.7		.75
SE‡	2.5		1.8		
Mean meat intake, g/d	30.5		28.5		.15
SE‡	1.7		1.2		
Mean vegetable intake, g/d	121.2		115.9		.20
SE‡	5.7		4.1		
Mean fruit intake, g/d	104.8		99.4		.79
SE‡	5.9		4.3		

*P for Mantel-Haenszel test with matched-set strata.
†Adjusted for age.
‡Adjusted for age and cohort.

DISCUSSION

In this study, we found a statistically significant inverse association between plasma genistein and the risk of breast cancer, but no association for plasma daidzein. This finding suggests that genistein may

Table 2. Plasma Levels and Dietary Intake of Isoflavone in Patients and Matched Controls

Parameter	Patients (n = 144) Median	Patients (n = 144) Interquartile Range	Controls (n = 288) Median	Controls (n = 288) Interquartile Range	P*
Plasma level					
Genistein, ng/mL	131.8	67.9-202.6	144.5	78.8-255.6	.046
Daidzein, ng/mL	16.7	7.0-34.0	17.9	5.5-40.8	.45
Dietary intake					
Genistein, mg/d	19.9	16.6-24.0	21.7	16.8-26.1	.37
Daidzein, mg/d	12.5	10.1-14.8	13.3	10.3-16.3	.36
Isoflavone, mg/d†	32.5	26.8-38.7	34.8	27.0-42.4	.36

*P for Mantel-Haenszel test with matched-set strata.
†Isoflavone intake = sum of genistein and daidzein intake.

Table 3. ORs and 95% CIs of Breast Cancer According to Plasma Level and Dietary Intake of Isoflavone

Parameter	Quartile				*P* for trend
	1	2	3	4	
Plasma level					
Median genistein, ng/mL	31.9	108.1	190.8	353.9	
No. of patients	41	37	45	21	
No. of controls	72	72	72	72	
OR	1.00	0.84	1.04	0.46	.07
95% CI	Reference	0.47 to 1.51	0.57 to 1.91	0.23 to 0.91	
Adjusted OR*	1.00	0.69	0.87	0.34	.02
95% CI	Reference	0.36 to 1.32	0.45 to 1.67	0.16 to 0.74	
Median daidzein, ng/mL	0	12.0	27.0	53.7	
No. of patients	30	45	44	25	
No. of controls	72	72	72	72	
OR	1.00	1.50	1.44	0.79	.59
95% CI	Reference	0.85 to 2.64	0.80 to 2.61	0.41 to 1.54	
Adjusted OR*	1.00	1.30	1.51	0.71	.54
95% CI	Reference	0.70 to 2.42	0.80 to 2.86	0.35 to 1.44	
Dietary intake					
Median genistein, mg/d	15.7	18.5	22.9	27.3	
No. of patients	42	36	37	29	
No. of controls	69	75	71	73	
OR	1.00	0.78	0.83	0.58	.15
95% CI	Reference	0.46 to 1.35	0.47 to 1.48	0.30 to 1.12	
Adjusted OR*	1.00	0.81	0.92	0.58	.21
95% CI	Reference	0.46 to 1.45	0.50 to 1.70	0.29 to 1.18	
Median daidzein, mg/d	9.4	11.4	14.1	17.1	
No. of patients	40	39	35	30	
No. of controls	70	74	72	72	
OR	1.00	0.91	0.82	0.65	.21
95% CI	Reference	0.52 to 1.58	0.46 to 1.47	0.33 to 1.27	
Adjusted OR*	1.00	0.96	0.94	0.67	.34
95% CI	Reference	0.54 to 1.74	0.50 to 1.74	0.33 to 1.39	

Abbreviation: OR, odds ratio.
*Adjusted for number of births (0, 1, 2, 3, 4, 5+) and age at first birth (−21, 22-25, 26-29, 30+, nulliparous). Adjusted ORs were calculated based on a total of 405 subjects with complete information of covariates.

play a more important role in the etiology of breast cancer than daidzein. Our findings are in general agreement with those of a recent nested case-control study in the Netherlands,[10] albeit that our inverse association occurred at substantially higher plasma concentrations. For example, median plasma genistein values in the control group of the Netherlands study were 3.75 ng/mL for premenopausal and 4.89 ng/mL for postmenopausal women.[10] In contrast, the median value in our control group was 144.5 ng/mL, and only 3.2% of control subjects was under 5 ng/mL. This apparently high level is not surprising considering that the median value of 353.9 ng/mL in our highest plasma genistein quartile group, which had a significantly lower risk of breast cancer than the lowest group, corresponded to a median dietary intake of 28.5 mg/d for genistein and 46.5 mg/d for isoflavone, as estimated by the validation study data. Although some in vivo and in vitro studies have shown risk-enhancing effects of genistein, our study suggests that relatively high-dose isoflavones exposure achievable from dietary intake alone is associated with a decreased rather than increased risk.

We observed an approximately 65% reduction in breast cancer risk in the highest plasma genistein quartile group but no decrease in the other quartiles, indicating that only the highest group benefited from risk reduction. The apparent lack of a dose-response relationship might imply the presence of a threshold level of effect. Interestingly, this idea contradicts findings in Western populations, in whom inverse associations are seen despite materially low levels of isoflavones. Given the differences in hormonal milieu between the two populations, the potential protective effect of isoflavones in breast cancer might act differently between Western and Asian populations: sex hormone levels are higher in Western than Asian women,[19] for example, as is the prevalence of obesity.[20,21] In this regard, a case-control study in Shanghai found that the inverse association between urinary isoflavone level and breast cancer risk was stronger among women in the high BMI, waist-hip ratio, and estradiol level groups and in the low sex hormone-binding globulin level group than in the respectively converse low and high groups.[22] Alternatively, the apparent lack of a dose-response relationship might merely reflect uncontrolled confounding by other dietary characteristics or risk-lowering behaviors.

The reason for a role for genistein but not daidzein in the etiology of breast cancer is unclear, but several possibilities can be speculated. Genistein possesses stronger binding affinity for estrogen receptor than daidzein.[5] Further, a pharmacokinetic study showed higher plasma levels and a 1.5-fold longer half-life for genistein than daidzein

Table 4. ORs and 95% CIs of Breast Cancer According to Plasma Level and Dietary Intake of Isoflavone By Baseline Menopausal Status

Parameter	Quartile 1	2	3	4	*P* for trend
Premenopausal women					
Plasma genistein, ng/mL					
No. of patients	24	14	19	2	
No. of controls	41	28	25	24	
Adjusted OR*	1.00	0.76	1.75	0.14	.20
95% CI	Reference	0.31 to 1.86	0.68 to 4.50	0.03 to 0.69	
Plasma daidzein, ng/mL					
No. of patients	17	21	15	6	
No. of controls	27	45	23	23	
Adjusted OR*	1.00	0.80	1.27	0.49	.48
95% CI	Reference	0.34 to 1.88	0.48 to 3.38	0.15 to 1.57	
Dietary genistein intake, mg/d					
No. of patients	21	16	14	8	
No. of controls	35	31	32	20	
Adjusted OR*	1.00	0.92	0.86	0.62	.43
95% CI	Reference	0.41 to 2.05	0.34 to 2.18	0.21 to 1.84	
Dietary daidzein intake, mg/d					
No. of patients	20	17	14	8	
No. of controls	36	30	32	20	
Adjusted OR*	1.00	1.07	0.93	0.67	.53
95% CI	Reference	0.46 to 2.51	0.37 to 2.34	0.22 to 2.03	
Postmenopausal women					
Plasma genistein, ng/mL					
No. of patients	17	23	25	15	
No. of controls	28	41	46	45	
Adjusted OR*	1.00	0.54	0.57	0.36	.10
95% CI	Reference	0.18 to 1.62	0.20 to 1.65	0.12 to 1.12	
Plasma daidzein, ng/mL					
No. of patients	13	23	27	17	
No. of controls	40	27	47	46	
Adjusted OR*	1.00	2.86	2.06	1.16	.95
95% CI	Reference	1.03 to 7.98	0.82 to 5.17	0.43 to 3.15	
Dietary genistein intake, mg/d					
No. of patients	20	20	22	18	
No. of controls	33	42	35	50	
Adjusted OR*	1.00	0.73	0.93	0.52	.31
95% CI	Reference	0.30 to 1.77	0.38 to 2.27	0.19 to 1.42	
Dietary daidzein intake, mg/d					
No. of patients	19	22	20	19	
No. of controls	33	42	36	49	
Adjusted OR*	1.00	0.89	0.93	0.64	.43
95% CI	Reference	0.38 to 2.10	0.38 to 2.29	0.23 to 1.72	

Abbreviation: OR, odds ratio.
*Adjusted for number of births (0, 1, 2, 3, 4, 5+) and age at first birth (−21, 22-25, 26-29, 30+, nulliparous).

after ingestion of baked soybean powder containing closely similar amounts of the two.[23] Moreover, the absence of an association for plasma daidzein might be attributable to misclassification arising from the metabolization of this compound. Daidzein can be metabolized by intestinal bacteria to equol and O-desmethylangolites; because approximately only 30% to 50% of individuals are capable of equol production, probably due to differences in gut microflora, daidzein-to-equol metabolizers may have lower plasma daidzein levels than nonmetabolizers.[24] Equol has been suggested to have greater biologic activity than daidzein,[24] and an inverse association between equol level and breast cancer risk has been reported.[25] Here, the lowest plasma daidzein quartile group might conversely have had a lower breast cancer risk than the higher groups due to its inclusion of equol metabolizers, and such misclassification, if present, would lead to a null result.

Our study has several methodological advantages over previous studies of isoflavones and the risk of breast cancer. First, the direct measurement of plasma isoflavone levels provides not only an index of intake but also of the absorption and metabolism of isoflavone, an understanding of which is important to elucidating the mechanisms by which isoflavones might influence breast cancer development. Indirect measurement by dietary intake of genistein is likely a major reason for the present smaller and nonsignificant risk reduction of breast cancer than by plasma genistein. Exposure assessment using

blood samples is therefore likely a more sophisticated means of detecting an association. Second, two case-control studies in Australia and China showed an inverse association between urinary isoflavones and breast cancer risk.[25,26] In view of the retrospective design of these studies, however, blood or urine levels of isoflavones in breast cancer cases might have been influenced by metabolic changes after the breast cancer was detected or by altered eating habits among case subjects. In our nested case-control study within a prospective cohort, in contrast, blood samples were collected before cancer diagnosis, obviating any potential bias due to the presence of cancer. Third, cases and controls were selected from the same cohort, thereby avoiding the selection bias inherent to case-control studies.

Several limitations of this study warrant mention. First, we measured plasma isoflavones only once for each individual. The consumption of soy foods is a personal dietary preference, and intake levels of most individuals are assumed to be relatively stable over time in Japan, as suggested by our validation study, which showed high reproducibility of repeated measurements of genistein intake by food frequency questionnaire (correlation coefficient = 0.72 for 1-year interval and 0.61 for 5-year interval).[15,17] By comparison, plasma isoflavone levels may reflect short-term rather than long-term intake: isoflavones have short half-lives in blood (eg, 6 to 8 hours),[23,27] and plasma levels are particularly affected by time elapsed since the last meal. To minimize the attenuation of risk estimates derived from random measurement errors, we matched fasting time between cases and controls. Second, despite a reasonably large cohort population (24,226 women) and long follow-up period (average, 10.6 years), the number of breast cancer cases was relatively small, reflecting the low incidence rate in Japan (age-standardized rate per 100,000 world population, 32.7 in 2002).[13] The interpretability of our results might therefore be limited, particularly in stratified analyses. Third, although our cohort subjects were selected from the general population, subjects were restricted to the 24,226 women respondents (43%) to the baseline questionnaire who provided blood samples. Although health check-up examinees in our previous report had a different socioeconomic status than nonexaminees and a more favorable lifestyle profile,[28] no apparent difference in isoflavone intake and breast cancer risk factors was found between subjects in the subcohort for this study and the original cohort; median isoflavone intake, for example, was 32.5 and 32.1 mg/d, respectively, and the average number of births was 2.8 and 2.7, respectively.[29] Nevertheless, any extrapolation of the results to the general population should be done cautiously, particularly in view of a previous report showing the difficulty of extrapolating relative risk estimates for a subcohort to an entire cohort. This difficulty might in fact be inherent to prospective studies in general.[30]

Allowing for these methodological issues, we found an inverse association between plasma genistein and the risk of breast cancer in a nested case-control study in Japan. This finding suggests a risk-reducing rather than a risk-enhancing effect of isoflavones on breast cancer, even at relatively high concentrations within the range achievable from dietary intake alone.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

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Appendix

The Appendix is included in the full-text version of this article, available online at www.jco.org. It is not included in the PDF version (via Adobe® Reader®).

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Plasma organochlorine levels and subsequent risk of breast cancer among Japanese women: A nested case–control study

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ABSTRACT

To our knowledge, no prospective study has examined the association between blood levels of organochlorines and breast cancer risk in Asian countries. Here, we tested the hypothesis that higher blood levels of organochlorines are associated with an increased risk of breast cancer in Japanese women. A total of 24,226 women subjects of the Japan Public Health Center-based Prospective Study aged 40 to 69 years who responded to the baseline questionnaire and provided

Abbreviations: β-HCH, β-hexachlorocyclohexane; BMI, body mass index; CI, confidence interval; DDT, dichlorodiphenyltrichloroethane; ER-, estrogen receptor-negative; ER+, estrogen receptor-positive; HCB, hexachlorobenzene; JPHC Study, Japan Public Health Center-based Prospective Study; OR, odds ratio; PHC, public health center; *p,p'*-DDE, *p,p'*-dichlorodiphenyldichloroethylene; *p,p'*-DDT, *p,p'*-dichlorodiphenyltrichloroethane.

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blood in 1990–1995 were followed to December 2002. During 10.7 years follow-up, 144 cases of breast cancer were newly diagnosed. Two matched-controls for each case were selected from the cohort. Plasma levels of *p,p'*-dichlorodiphenyltrichloroethane (*p,p'*-DDT), *p,p'*-dichlorodiphenyldichloroethylene (*p,p'*-DDE), hexachlorobenzene (HCB), and β-hexachlorocyclohexane (β-HCH) were measured. A conditional logistic regression was used to estimate the odds ratio (OR) of breast cancer according to cholesterol-adjusted organochlorine levels based on 139 matched pairs. We found no statistically significant positive association between plasma organochlorine level and breast cancer risk. Adjusted ORs for *p,p'*-DDT, HCB, and β-HCH were less than 1. For *p,p'*-DDE, adjusted OR for the highest versus lowest quartile was 1.48 (95% confidence interval 0.70–3.13; p for trend = 0.25). A stratified analysis by menopausal status showed positive associations for *p,p'*-DDT and *p,p'*-DDE in premenopausal but not postmenopausal women, although without statistical significance. Our data do not support the hypothesis that plasma levels of *p,p'*-DDT, *p,p'*-DDE, HCB, and β-HCH are associated with an overall increased risk of breast cancer among Japanese women.

1. Introduction

The incidence rate of breast cancer in Japan is higher than in other Asian countries but lower than in Western countries (Parkin et al., 2002). It has increased 2.2 times during the last 25 years and is now the most frequently diagnosed cancer among Japanese women (Tajima et al., 2004; Marugame et al., 2006). The variation in rates among countries and secular trends in rates may be partly explained by differences in the distribution of preventive and risk factors. However, a previous study reported that changes in four major risk factors, namely age at menarche, age at first birth, age at menopause, and parity, accounted for less than 40% of the increase trend in Japan (Nagata et al., 1997). Among unexplained breast cancer risks, attention has focused on the potential of some organochlorines to act as environmental estrogens. Because they have shown weakly estrogenic or antiestrogenic effects in experimental studies (Kelce et al., 1995; Soto et al., 1995; Steinmetz et al., 1996), a possible association between exposure to organochlorines and the risk of breast cancer has been hypothesized (Wolff and Toniolo, 1995). One abundant organochlorine contaminant is dichlorodiphenyltrichloroethane (DDT), which was used worldwide from World War II for insect control in forestry and agriculture and for vector control. Although most developed countries had banned its use by the early 1980s, it is still used for malaria control in some countries. In Japan, it was used widely following World War II until the beginning of the 1980s and its residue is still detected in the blood because of its lipid solubility and resistance to metabolism (Hanaoka et al., 2002).

A number of epidemiological studies have investigated the association between organochlorines and the risk of breast cancer (Wolff et al., 1993; Krieger et al., 1994; Lopez Carrillo et al., 1997; Schecter et al., 1997; Hoyer et al., 1998; Olaya Contreras et al., 1998; Dorgan et al., 1999; Helzlsouer et al., 1999; Aronson et al., 2000; Hoyer et al., 2000; Romieu et al., 2000; Ward et al., 2000; Wolff et al., 2000; Hoyer et al., 2001; Laden et al., 2001a,b; Gammon et al., 2002; Lopez Cervantes et al., 2004; Raaschou Nielsen et al., 2005; Cohn et al., 2007; Gatto et al., 2007). A recent meta-analysis based on 22 published studies revealed that *p,p'*-dichlorodiphenyldichloroethylene (*p,p'*-DDE) was not associated with an increased risk (Lopez Cervantes et al., 2004). Most of these studies were conducted in Western countries, however, and to our knowledge only a few studies have been reported from Asian countries, such as a small hospital-based case–control study based on 21 breast cancer patients in Vietnam (Schecter et al., 1997). It has been suggested that sex hormone levels are higher in Western than Asian women (Shimizu et al., 1990), and that thus the hormonal milieu might differ between them. In addition, the prevalence of obesity (Flegal et al., 2002; Yoshiike et al., 2002) and breast feeding (Collaborative Group on Hormonal Factors in Breast, 2002), which might affect blood organochlorine levels, also differs between the two populations. Examination of the association in Japanese women might therefore help us better understand the etiological role of organochlorine exposure in the development of breast cancer, and might also help explain the increasing trend in Japan as well.

To test the hypothesis that higher blood levels of organochlorines are associated with an increased risk of breast cancer in Japanese women, we conducted a nested case–control study within a large-scale population-based prospective study in Japan.

2. Method

2.1. *Study population*

The Japan Public Health Center-based Prospective Study (JPHC Study), which began in 1990 for Cohort I and in 1993 for Cohort II, included 140,420 subjects (68,722 men and 71,698 women) living in 29 municipalities supervised by 11 public health centers (PHC). The details of the study design have been described elsewhere (Watanabe et al., 2001). The study protocol was approved by the institutional review board of the National Cancer Center, Tokyo, Japan.

The study population was registered Japanese inhabitants living in several municipalities in each PHC area, aged 40–59 years in Cohort I and 40–69 years in Cohort II. In the present analysis, one PHC area was excluded since data on cancer incidence were not available. Thus, after exclusion of ineligible subjects ($n=95$), we defined a population-based cohort of 67,426 women.

2.2. *Questionnaire survey*

A baseline self-administered questionnaire survey was conducted in 1990–1994. The questionnaire was distributed mostly by hand and partly by mail. Incomplete responses were supplemented by telephone interview. A total of 55,891