Table 1 Population characteristics according to supplement use categories, Japan Public Health Center-based Prospective Study

		Men (*n* = 28,903)					Women (*n* = 33,726)				
		Never use	Past use	Recent use	Consistent use	*P*	Never use	Past use	Recent use	Consistent use	*P*
No. (%)		23,535 (81.4)	3161 (10.9)	1026 (3.6)	1181 (4.1)		25,525 (75.7)	4672 (13.9)	1567 (4.6)	1962 (5.8)	
Age in years (mean (SE*))		55.7 (0.05)	57.9 (0.1)	57.1 (0.2)	58.2 (0.2)	<.0001	56.1 (0.05)	57.9 (0.1)	56.7 (0.2)	57.8 (0.2)	<.0001
Body mass index ≥ 25 kg/m^2 (%)		29.1	26.9	27.1	25.9	0.0049	29.0	31.0	26.3	23.6	<.0001
Smoking status (%)	Former smoker	16.6	18.6	19.5	21.3	<.0001	0.7	1.2	1.7	1.4	<.0001
	Current smoker	47.8	44.8	41.4	42.2		4.3	5.7	4.7	5.2	
Regular drinker, ≥ 150 g ethanol/wk (%)		50.5	46.1	46.8	45.5	<.0001	2.4	2.6	3.1	3.6	0.002
Mean MET*-hours/d	(mean (SE))	33.1 (0.05)	32.9 (0.1)	33.3 (0.2)	32.7 (0.2)	0.06	32.2 (0.04)	31.9 (0.09)	32.3 (0.2)	32.0 (0.1)	0.003
Medication (%)	Hypertension	16.7	21.9	17.0	21.5	<.0001	17.8	24.4	18.1	20.0	<.0001
	Hyperlipidemia	3.0	4.3	3.9	4.9	<.0001	6.0	7.8	7.5	9.3	<.0001
	Diabetes	3.2	4.2	2.8	4.5	0.001	2.0	2.8	2.2	1.5	0.0005
	Others	12.2	17.1	15.4	15.1	<.0001	10.9	13.1	16.3	15.9	<.0001
History (%)	Angina	0.9	1.3	1.1	1.6	0.03	1.0	1.2	1.5	1.2	0.2
	Diabetes	5.8	6.6	5.8	7.5	0.04	3.0	3.4	3.3	2.5	0.2
	Gastric ulcer	5.0	5.4	5.0	4.9	0.8	2.1	2.3	2.6	2.9	0.07
	Duodenal ulcer	2.5	2.0	1.9	3.5	0.02	1.2	1.2	1.4	1.3	0.9
	Gastric polyp	2.2	2.3	2.6	3.3	0.09	2.6	2.4	3.3	3.7	0.004
	Colonic polyp	4.1	4.3	4.9	5.8	0.03	1.8	1.7	2.0	3.3	<.0001
	Hepatitis	1.7	1.9	2.3	3.2	0.0004	0.7	0.9	1.2	1.2	0.005
Screening examination (%)		83.3	84.0	87.0	88.0	<.0001	86.0	86.3	88.8	89.9	<.0001
Total energy intake (kcal/day) (mean (SE))		2206 (4.2)	2200 (12.1)	2222 (20.4)	2228 (18.1)	0.5	1886 (3.6)	1879 (8.6)	1938 (14.5)	1925 (12.9)	0.0001
Energy-adjusted	Salt intake (g/d)	12.2 (0.03)	12.3 (0.07)	12.4 (0.1)	12.3 (0.1)	0.5	12.0 (0.08)	11.9 (0.05)	11.7 (0.09)	11.7 (0.08)	0.5
food intake	Soy food (g/d)	86 (0.5)	93 (1.6)	89 (2.3)	89 (1.8)	0.0001	86 (0.5)	94 (1.2)	86 (1.6)	89 (1.6)	<.0001
(mean (SE))	Green vegetables (g/d)	38 (0.2)	38 (0.6)	41 (1.2)	40 (1.0)	0.01	48 (0.2)	47 (0.6)	50 (1.1)	49 (0.8)	0.055
	Fruits (g/d)	172 (1.0)	187 (2.9)	189 (4.8)	204 (4.7)	<.0001	239 (1.1)	242 (2.5)	242 (4.0)	254 (3.9)	0.001
	Fish (g/d)	91 (0.4)	91 (1.0)	92 (1.8)	92 (1.5)	0.7	87 (0.3)	86 (0.7)	86 (1.2)	86 (1.0)	0.4
	Red meat (g/d)	52 (0.3)	51 (0.7)	53 (1.3)	51 (1.0)	0.5	46 (0.2)	48 (0.5)	45 (0.8)	46 (0.7)	0.0009
Energy-adjusted	α-tocopherol (mg/d)	6.6 (0.02)	6.8 (0.05)	7.1 (0.08)	7.1 (0.07)	<.0001	7.3 (0.01)	7.5 (0.03)	7.5 (0.05)	7.6 (0.05)	<.0001
nutrition intake	Vitamin B_1 (mg/d)	1.05 (0.003)	1.10 (0.008)	1.08 (0.01)	1.11 (0.01)	<.0001	1.08 (0.002)	1.12 (0.006)	1.11 (0.009)	1.11 (0.008)	<.0001
(mean (SE))	Vitamin B_2 (mg/d)	1.41 (0.003)	1.45 (0.009)	1.50 (0.02)	1.52 (0.01)	<.0001	1.43 (0.003)	1.46 (0.007)	1.51 (0.01)	1.54 (0.01)	<.0001
	Niacin (mg/d)	20.1 (0.04)	20.1 (0.1)	20.6 (0.2)	20.4 (0.1)	0.002	18.1 (0.03)	18.1 (0.07)	18.2 (0.1)	18.3 (0.09)	0.08
	Vitamin B_6 (mg/d)	1.56 (0.002)	1.58 (0.006)	1.60 (0.01)	1.60 (0.01)	<.0001	1.46 (0.002)	1.47 (0.004)	1.48 (0.008)	1.49 (0.007)	<.0001
	Vitamin B_{12} (μ g/d)	9.1 (0.03)	9.2 (0.09)	9.3 (0.1)	9.4 (0.1)	0.02	8.6 (0.03)	8.7 (0.06)	8.7 (0.1)	8.6 (0.09)	0.2
	Folate (μ g/d)	377 (0.9)	385 (2.7)	401 (4.9)	399 (4.1)	<.0001	409 (0.9)	413 (2.2)	422 (3.8)	426 (3.1)	<.0001
	Pantothenic acid (mg/d)	6.7 (0.01)	6.8 (0.03)	6.9 (0.05)	7.1 (0.05)	<.0001	6.6 (0.008)	6.7 (0.02)	6.8 (0.03)	6.9 (0.03)	<.0001
	Vitamin C (mg/d)	118 (0.4)	122 (1.2)	128 (2.2)	130 (1.9)	<.0001	151 (0.5)	149 (1.1)	154 (1.7)	158 (1.6)	<.0001
	Vitamin D (mg/d)	10.1 (0.04)	10.0 (0.1)	10.4 (0.2)	10.3 (0.2)	0.2	10.0 (0.04)	9.9 (0.09)	9.9 (0.1)	9.9 (0.1)	0.4

Never use, neither past nor recent use; Past use, past use but not recent use; Recent use, recent use but not past use; Consistent use, both past and recent use.
*SE, standard error; MET, metabolic equivalent task.

Table 2 Hazard ratios for total cancer and cardiovascular disease according to supplement use categories

		Total					Excluding cases within 5 years		
	Person-years	No. of cases	HR*1 (95% CI*)	P	HR2 (95% CI)	P	No. of cases	HR2 (95% CI)	P
Men									
			Total cancer						
Never use	220,948	2152	1.00 (reference)		1.00 (reference)		1210	1.00 (reference)	
Past use	28,863	324	0.98 (0.87-1.10)	0.8	0.98 (0.87-1.10)	0.8	167	0.95 (0.80-1.11)	0.5
Recent use	9603	102	1.00 (0.82-1.22)	0.97	1.01 (0.83-1.23)	0.9	59	1.05 (0.80-1.36)	0.7
Consistent use	10,863	139	1.11 (0.94-1.32)	0.2	1.10 (0.93-1.31)	0.3	75	1.13 (0.89-1.43)	0.3
			Cardiovascular disease						
Never use	203,013	934	1.00 (reference)		1.00 (reference)		490	1.00 (reference)	
Past use	26,639	125	0.91 (0.75-1.09)	0.3	0.89 (0.73-1.07)	0.2	61	0.86 (0.66-1.12)	0.3
Recent use	8889	31	0.71 (0.50-1.02)	0.06	0.72 (0.51-1.04)	0.08	15	0.66 (0.39-1.10)	0.1
Consistent use	10,059	53	1.03 (0.78-1.36)	0.8	1.02 (0.77-1.35)	0.9	28	1.04 (0.71-1.53)	0.8
Women									
			Total cancer						
Never use	248,659	1299	1.00 (reference)		1.00 (reference)		698	1.00 (reference)	
Past use	44,237	287	1.19 (1.04-1.35)	0.01	1.17 (1.02-1.33)	0.02	157	1.21 (1.01-1.44)	0.04
Recent use	15,217	101	1.25 (1.02-1.53)	0.03	1.24 (1.01-1.52)	0.04	56	1.26 (0.96-1.66)	0.1
Consistent use	18,892	97	0.94 (0.76-1.16)	0.6	0.92 (0.75-1.13)	0.4	47	0.82 (0.61-1.11)	0.2
			Cardiovascular disease						
Never use	227,570	530	1.00 (reference)		1.00 (reference)		262	1.00 (reference)	
Past use	40,586	116	1.11 (0.91-1.36)	0.3	1.08 (0.88-1.32)	0.5	63	1.24 (0.94-1.64)	0.1
Recent use	13,918	43	1.30 (0.95-1.77)	0.1	1.32 (0.97-1.81)	0.08	20	1.26 (0.80-1.99)	0.3
Consistent use	17,309	26	0.60 (0.40-0.89)	0.01	0.60 (0.41-0.89)	0.01	14	0.70 (0.41-1.21)	0.2

Never use, neither past nor recent use; Past use, past use but not recent use; Recent use, recent use but not past use; Consistent use, both past and recent use.
*HR, hazard ratio; CI, confidence interval.
HR1: Adjusted for age and public health center area.
HR2: Further adjusted for body mass index, smoking status, ethanol intake, occupation, daily total physical activity level, green vegetable intake, total energy intake, medication, and screening examination.

These statistically significant findings remained unchanged when we further adjusted dietary vitamin B_2, B_6, B_{12}, folate, α-tocopherol, vitamin C, and vitamin D intake separately and simultaneously (data not shown).

Age, smoking status, alcohol intake, and dietary intake of vitamin B_2, B_6, B_{12}, folate, α-tocopherol, vitamin C, and vitamin D did not significantly interact with any of the above results (for all interactions, $P > 0.5$).

Discussion

In this prospective cohort study in an Asian population, we found that vitamin supplement use has little effect on the risk of total cancer or CVD in men. In women, however, past and recent use of vitamin supplements may be associated with higher risk of cancer, whereas consistent use may be associated with lower risk of CVD.

Several observational studies have examined the association between vitamin supplements and the risk of cancer and CVD incidence, but results have varied [11-29], partly because vitamin supplement use is an inconsistent behavior in individuals [13,30]. In our study, we found that only 4.1% of men and 5.8% of women continued to use vitamin supplements from the first to the second survey. Although some studies have found reduced incidence and mortality risk of cancer and CVD with a long duration of vitamin supplement use [13,14,27,28,31-34], to our knowledge, only limited data are available to clarify the consistency of vitamin supplement use over two surveys [13,31]. One prospective cohort study in the United States investigated consistency for vitamin supplement use through two surveys among 145,260 subjects, observing 797 incident cases of colorectal cancer, and found that multivitamin supplement use in the first survey and in both surveys was associated with reduced risk of colorectal cancer, whereas multivitamin supplement use in the second survey had no association with the disease [13]. Another study, in which 3490 deaths were observed among 11,178 study subjects in the United States, found that use of vitamin E supplements at two points within a relatively short period (baseline and study inception 3 years earlier) was associated with reduced risk of coronary heart disease mortality, whereas use at one point did

not show significant association in multivariate analysis [31].

In the present study, the inverse associations for CVD, especially for ischemic brain infarction, was observed with consistent supplement use in women. It is known that homocysteine may promote atherogenesis by damaging the vascular matrix, increasing the proliferation of endothelial cells, and facilitating oxidative injury to vascular walls [56-58] and may be related to CVD [59,60]. Although several large trials of homocysteine-lowering B-vitamin therapy have all failed to demonstrate a reduction in coronary heart disease risk, some studies have shown possible evidence for stroke [9,10]. It has also been reported that B vitamins are important enzymatic cofactors in the synthesis of methionine from homocysteine and that a deficiency in any of them raises homocysteine concentrations in the blood [61,62]. In the present study, when we adjusted for several kinds of dietary B vitamins (vitamin B_2, vitamin B_6, vitamin B_{12}, and folate), similar results were observed. Moreover, the most common vitamin supplement in the second survey was B vitamins in men and women in the present study (36.1% and 25.0%, respectively, among vitamin supplement users). Therefore, the inverse association between the consistent use of vitamin supplement and risk of CVD in women, especially ischemic brain infarction, might be caused by supplementation with B vitamins.

Alternatively, past and recent use of vitamin supplements was associated with higher risk of cancer in women. Women with past use tended to have unhealthy characteristics, such as a higher BMI, a greater likelihood of smoking, and medication use (hypertension and diabetes). Recent use in women may have been prompted by symptoms of ill health because women with recent use had a higher proportion of disease histories (e.g., gastric and colonic polyps) despite their younger age and had a significantly higher proportion of medication use except for hypertension, hyperlipidemia, and diabetes. Furthermore, the association of cancer with recent use was not significant when we estimated the HR after excluding women diagnosed as having cancer within 5 years of baseline, though that might be partly caused by the decreased number of cases. Elevated risk may be partly explained by characteristics of the women that were not measured or could not be controlled for in our study. Moreover, it might be partly caused by a pro-oxidant effect of supplementation with vitamin C [63-65], producing DNA damage and increasing the risk of cancer, because use of vitamin C in the second survey was associated with increased risk of total cancer among women. Furthermore, high-dose antioxidant supplementation might cause an increased risk of cancer among a high-risk group; in addition, two large, randomized clinical trials in which high doses of β-carotene were used, the Beta-Carotene And Retinol Efficacy Trial (CARET) in the United States and the Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) trial in Finland, found that β-carotene, alone or in combination with vitamin E or retinyl palmitate, increased the incidence of lung cancers compared with placebo among high-risk groups, such as heavy smokers and those with a history of exposure to asbestos [6,7].

In the present study, vitamin supplement use was associated with the risk of total cancer or CVD in women but not in men. The characteristics of subjects with each vitamin supplement pattern were different between men and women, suggesting that these characteristics and unmeasured or residual confounders might cause the sex-based difference in results.

Our study has a potential limitation due to the differences in questionnaires regarding vitamin supplement use between the first and second surveys, and these differences might cause misclassification of vitamin supplement use prevalence, which was lower in the second survey than that in the first survey. Short-time vitamin supplement use of <1 year was regarded as vitamin supplement use in the first survey. In the second survey, recategorized self-reported categories of vitamin supplementation were used to improve sensitivity in identifying vitamin supplement use [43] and vitamin supplement use was defined by vitamin supplements being taken ≥ 1 time/week for a year or longer. Information of duration was not available in the first survey. In addition, the possibility of selection bias needs to be considered when generalizing the present findings because 15% of the eligible subjects did not reply in the second survey. In our previous report, risks of mortality for all causes, all cancers, and CVD were higher among non-responders to the first survey compared with responders and elevated risk for cancer was observed only in the first 2 years of follow-up, whereas that for stroke was relatively stable for the entire period [66].

The strength of this study was its prospective design, which enabled us to avoid exposure recall bias. We selected subjects from the general population, we kept the sample size large, the response rate for the surveys was acceptable given its setting, and the loss to follow-up was negligible. In addition, the registries of cancer, stroke, and myocardial infarction were of sufficient quality to reduce the misclassification of outcomes. To our knowledge, this is the first prospective cohort study to examine associations between vitamin supplement use pattern and risk of cancer and CVD incidence simultaneously.

Conclusions

Allowing for the methodologic issues, our results from a population-based prospective cohort study in Japan

suggest that vitamin supplement use pattern has an impact on the subsequent risk of total cancer and CVD in women but not men. Elevated risk of cancer among women who were past and recent users of vitamin supplements may be partly explained by preexisting diseases or unhealthy background, which could not be completely controlled for in our study. Although consistent use of vitamin supplements for women might possibly reduce the risk of CVD, further research with detailed long-term data regarding components, doses, and patterns of vitamin supplement use is needed to confirm the generalizability of our findings.

Acknowledgements
This study was supported by National Cancer Center Research and Development Fund, Grants-in-Aid for Cancer Research, and by the Third Term Comprehensive 10-Year Strategy for Cancer Control (H21-Sanjigan-Ippan-003) from the Ministry of Health, Labor, and Welfare of Japan.
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Authors' contributions
We thank all staff members in each study area for their painstaking efforts to conduct the survey and follow-up. The authors' responsibilities were as follows: ST (principal investigator); M. Inoue, conducted the study, managed the cancer data collection; HI, managed the CVD data collection; AH, analyzed and interpreted the data and prepared the manuscript; SS, M Iwasaki, TS, NS, TY, and JI helped to conduct the study. All authors provided critical suggestions for revision of the manuscript. All authors read and approved the final manuscript. AH received a research resident fellowship from the Foundation for Promotion of Cancer Research (Japan) for the 3rd term Comprehensive 10-year Strategy for Cancer Control.

Competing interests
The authors declare that they have no competing interests.

Received: 28 December 2010 Accepted: 8 July 2011
Published: 8 July 2011

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Pre-publication history
The pre-publication history for this paper can be accessed here:
http://www.biomedcentral.com/1471-2458/11/540/prepub

doi:10.1186/1471-2458-11-540
Cite this article as: Hara *et al.*: Use of vitamin supplements and risk of total cancer and cardiovascular disease among the Japanese general population: A population-based survey. *BMC Public Health* 2011 **11**:540.

Iwasaki et al. BMC Medicine 2011, 9:16
http://www.biomedcentral.com/1741-7015/9/16

BMC Medicine

RESEARCH ARTICLE Open Access

Comparison of postmenopausal endogenous sex hormones among Japanese, Japanese Brazilians, and non-Japanese Brazilians

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Abstract

Background: Differences in sex hormone levels among populations might contribute to the variation in breast cancer incidence across countries. Previous studies have shown higher breast cancer incidence and mortality among Japanese Brazilians than among Japanese. To clarify the difference in hormone levels among populations, we compared postmenopausal endogenous sex hormone levels among Japanese living in Japan, Japanese Brazilians living in the state of São Paulo, and non-Japanese Brazilians living in the state of São Paulo.

Methods: A cross-sectional study was conducted using a control group of case-control studies in Nagano, Japan, and São Paulo, Brazil. Participants were postmenopausal women older than 55 years of age who provided blood samples. We measured estradiol, estrone, androstenedione, dehydroepiandrosterone sulfate (DHEAS), testosterone and free testosterone by radioimmunoassay; bioavailable estradiol by the ammonium sulfate precipitation method; and sex hormone-binding globulin (SHBG) by immunoradiometric assay. A total of 363 women were included for the present analyses, comprising 185 Japanese, 44 Japanese Brazilians and 134 non-Japanese Brazilians.

Results: Japanese Brazilians had significantly higher levels of estradiol, bioavailable estradiol, estrone, testosterone and free testosterone levels, and lower SHBG levels, than Japanese. Japanese Brazilians also had significantly higher levels of bioavailable estradiol, estrone and DHEAS and lower levels of SHBG and androstenedione than non-Japanese Brazilians. Levels of estradiol, testosterone and free testosterone, however, did not differ between Japanese Brazilians and non-Japanese Brazilians. These differences were observed even after adjustment for known breast cancer risk factors. We also found an increase in estrogen and androgen levels with increasing body mass index, but no association for most of the other known risk factors.

Conclusions: We found higher levels of estrogens and androgens in Japanese Brazilians than in Japanese and levels similar to or higher than in non-Japanese Brazilians. Our findings may help explain the increase in the incidence and mortality rate of breast cancer among Japanese Brazilians.

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Background

The incidence and mortality rate of breast cancer vary considerably across countries and regions [1]. Although Japan has a lower risk for female breast cancer than Western countries, the incidence has gradually increased over the past 30 years [2,3]. The incidence and mortality rates in Japanese immigrants living in the United States and Brazil have approximated those in the host country [4-8]. For example, the mortality rate of first-generation Japanese immigrants to São Paulo, Brazil, increased from 1979 to 2001, with rates being intermediate between Japanese living in Japan and Brazilians living in the state of São Paulo [6].

Many epidemiologic studies have indicated that endogenous sex hormones, particularly estrogens, play an important role in the etiology of breast cancer [9]. A pooled analysis of nine prospective studies showed that higher estrogens and their androgen precursors were associated with a higher risk of breast cancer in postmenopausal women [9]. Differences in sex hormone levels among populations might therefore contribute to the variation in breast cancer incidence across countries and regions. Clarification of the difference in sex hormone levels among populations and their determinants might help our understanding of the etiology and prevention of breast cancer.

A relatively large number of epidemiological studies have examined sex hormone levels among ethnic groups and factors associated with sex hormone levels [10-16]. To our knowledge, however, no study has investigated sex hormone levels among Japanese Brazilians. In addition, although previous studies consistently showed that body weight and obesity were associated with higher estrogen levels in postmenopausal women [10-12,15], findings regarding other factors that influence circulating sex hormone levels have been inconsistent [10-14,16].

We have conducted a cross-sectional study using a control group of case-control studies in Nagano, Japan, and São Paulo, Brazil. The present study compared postmenopausal endogenous sex hormone levels among Japanese living in Japan, Japanese Brazilians living in São Paulo and non-Japanese Brazilians living in São Paulo, and examined factors associated with these levels.

Methods

Study participants

Participants were postmenopausal women who were enrolled as controls in multicenter, hospital-based, case-control studies of breast cancer. In addition to determining lifestyle factors and genetic susceptibility to the risk of breast cancer, the protocols of these studies were also designed to compare potential risk factors among Japanese living in Nagano, Japan, and Japanese Brazilians and non-Japanese Brazilians living in the state of São Paulo, Brazil. Details of this study have been described previously [17]. The study protocol was approved by Comissão Nacional de Ética em Pesquisa, Brasília, Brazil, and by the institutional review board of the National Cancer Center, Tokyo, Japan.

Briefly, eligible cases were a consecutive series of female patients ages 20 to 74 years with newly diagnosed and histologically confirmed invasive breast cancer. Inhabitants of the state of São Paulo were recruited and asked their ethnicity. Japanese and their descendants were defined as Japanese Brazilians, and Caucasian, black and mixed ethnicity populations were defined as non-Japanese Brazilians. A total of 405 individuals (98%) participated in Nagano, and 83 Japanese Brazilians (91%) and 389 non-Japanese Brazilians (99%) participated in São Paulo. In the study in Nagano, eligible controls were selected from among medical checkup examinees in two of the four hospitals and were confirmed not to have cancer. One control was matched for each case by age (within 3 years) and by residential area. Among potential controls, one examinee refused to participate and two refused to provide blood samples. In the study in São Paulo, eligible controls were preferentially selected from among cancer-free patients who visited the same hospital as the index cases. One control was matched for each case by age (within 5 years) and by ethnicity. Among potential controls, 22 patients refused to participate (participation rate, 96%). Consequently, we obtained written, informed consent from a total of 877 matched pairs (405 for Japanese, 83 for Japanese Brazilians and 389 for non-Japanese Brazilians).

Of 877 controls, we selected postmenopausal women over 55 years of age who provided blood samples and reported an energy intake between 500 and 4,000 kcal. Menopausal status was determined by self-report, and energy intake was assessed using a food frequency questionnaire (FFQ). The present study included a total of 382 women comprising 185 Japanese, 46 Japanese Brazilians and 151 non-Japanese Brazilians.

Data collection

Participants in Nagano were asked to complete a self-administered questionnaire, while in-person interviews were conducted in São Paulo by trained interviewers using a structured questionnaire. The two questionnaires contained closely similar questions concerning demographic characteristics, medical history, family history of cancer, menstrual and reproductive history, anthropometric factors, physical activity, smoking habits and dietary factors assessed by FFQ.

Participants in Nagano provided blood samples at the time they returned their self-administered questionnaire, and those in São Paulo provided blood samples at the time of the interview. Blood samples were divided into

serum aliquots in Nagano and into plasma aliquots and buffy coat layers in São Paulo. All blood samples were shipped to the Epidemiology and Prevention Division, Research Center for Cancer Prevention and Screening, National Cancer Center, Tokyo, Japan, and stored at -80°C until analysis.

Laboratory analysis

We used a radioimmunoassay method to measure estradiol, estrone, androstenedione, dehydroepiandrosterone sulfate (DHEAS), testosterone and free testosterone in serum for the Nagano participants and in plasma for the São Paulo participants. The following kits were used: estradiol (DSL-4800 Ultra-Sensitive Estradiol Radioimmunoassay Kit; Diagnostic System Laboratories, Inc., Webster, TX, USA), estrone (DSL-8700 Estrone Radioimmunoassay Kit; Diagnostic System Laboratories, Inc.), androstenedione (DPC · Androstenedione; Diagnostic Products Corporation, Llanberis, UK), DHEAS (DPC · DHEA-S Kit, Diagnostic Products Corporation), testosterone (DPC · Testosterone Kit; Diagnostic Products Corporation) and free testosterone (DPC · Free Testosterone Kit; Diagnostic Products Corporation). Bioavailable estradiol (free and albumin-bound estradiol) was measured using the ammonium sulfate precipitation method. Sex hormone-binding globulin (SHBG) was measured by immunoradiometric assay (IRMA) using Spectria SHBG IRMA (Orion Diagnostica, Espoo, Finland). The kit for estrone was applicable to serum samples only, although other kits or methods were applicable to both serum and plasma samples. We therefore measured estrone levels in both serum and plasma from the same women over 50 years of age (n = 38) and calibrated estrone levels in plasma on the basis of a regression function, although the two levels were highly correlated (correlation coefficient = 0.94) and the percentage difference was relatively small (mean = -4%; 95% confidence interval, -9% to 1%). Lower detection limits (LODs) were 5 pg/mL for estradiol, 15 pg/mL for estrone, 6.25 nM/L for SHBG, 0.1 ng/mL for androstenedione, 5 μg/dL for DHEAS, 0.05 ng/mL for testosterone and 0.4 pg/mL for free testosterone. Measurement values below the LOD were assigned half the value of the LOD if measurable values below the LOD were not available. The intra-assay coefficients of variation were 6.5% for estradiol at a mean concentration of 24.9 pg/mL (n = 12), 10.6% for bioavailable estradiol at a mean concentration of 48.1% (n = 10), 5.6% for estrone at a mean concentration of 101.7 pg/mL (n = 10), 4.7% for SHBG at a mean concentration of 104.6 nM/L (n = 10), 9.4% for androstenedione at a mean concentration of 1.33 ng/mL (n = 10), 5.2% for DHEAS at a mean concentration of 75 μg/dL (n = 10), 4.5% for testosterone at a mean concentration of 0.83 ng/mL (n = 10) and 11.6% for free testosterone at a mean concentration of 5.4 pg/mL (n = 10). Interassay coefficients of variation were 9.7% for estradiol at a mean concentration of 28.0 pg/mL (n = 8), 11.9% for bioavailable estradiol at a mean concentration of 52.3% (n = 9), 11.1% for estrone at a mean concentration of 90.1 pg/mL (n = 8), 5.5% for SHBG at a mean concentration of 124.0 nM/L (n = 10), 9.8% for androstenedione at a mean concentration of 1.10 ng/mL (n = 20), 5.3% for DHEAS at a mean concentration of 92.5 μg/dL (n = 20), 7.7% for testosterone at a mean concentration of 0.90 ng/mL (n = 20) and 9.0% for free testosterone at a mean concentration of 6.4 pg/mL (n = 10). All hormone assays were performed by a commercial laboratory (Mitsubishi Kagaku Bio-Clinical Laboratories, Tokyo, Japan).

Statistical analysis

We excluded a total of 19 participants with estrone values >125 pg/mL, estradiol values >75 pg/mL or testosterone values >125 ng/dL (indicating postmenopausal hormone use), leaving 185 Japanese, 44 Japanese Brazilians and 134 non-Japanese Brazilians for inclusion in the present analyses.

All hormone values were natural log-transformed to produce approximately normal distributions. Geometric mean hormone levels according to the three populations, known breast cancer risk factors and lifestyle factors were calculated using multivariate regression analysis. The following variables were used for adjustment: age, ethnic group, age at first menarche, age at menopause, number of births, age at first birth, height, body mass index (BMI), smoking status, alcohol drinking habits and physical activity during the past 5 years. Analysis of covariance was used to test for differences in mean hormone levels across the three populations, known breast cancer risk factors and lifestyle factors. For comparisons among the three populations, Japanese Brazilians living in São Paulo were used as the reference group. Linear trends for mean hormone levels were tested in the multivariate regression model using categories of each factor as ordinal or continuous variables. All P values reported are two-sided, and the statistical significance level was set at $P < 0.05$. All statistical analyses were performed using SAS software version 9.1 (SAS Institute, Inc., Cary, NC, USA).

Results

The characteristics of the study populations are presented in Table 1. Japanese participants had a later menarche, fewer births and lower BMI, and they smoked less, drank more and were physically more active than the other two populations. On the other hand, non-Japanese Brazilians had earlier ages at menopause and at first birth, more births and greater BMI,

Table 1 Characteristics of study populations

	Japanese living in Nagano, Japan	Japanese Brazilians living in São Paulo, Brazil	Non-Japanese Brazilians living in São Paulo, Brazil	*P* for difference
Number of participants	185	44	134	
Mean age (±SE), yr	62.8 (0.40)	63.8 (0.82)	63.6 (0.47)	0.37
P[a]	0.31	Reference	0.84	
Family history of breast cancer, *n* (%)	17 (9.2)	5 (11.4)	14 (10.5)	0.92
P[a]	0.68	Reference	0.98	
History of benign breast disease, *n* (%)	11 (6.0)	4 (9.1)	8 (6.0)	0.46
P[a]	0.65	Reference	0.62	
Mean age at first menarche (±SE), yr	13.9 (0.12)	13.2 (0.26)	13.3 (0.15)	<0.01
P[a]	<0.01	Reference	0.75	
Mean age at menopause (±SE), yr	50.0 (0.34)	50.8 (0.69)	48.2 (0.40)	<0.01
P[a]	0.29	Reference	<0.01	
Nulliparous, *n* (%)	17 (9.2)	5 (11.4)	15 (11.2)	0.55
P[a]	0.58	Reference	0.81	
Number of births (more than four births), *n* (%)[b]	6 (3.6)	12 (30.8)	57 (47.9)	<0.01
P[a]	<0.01	Reference	<0.01	
Mean age at first birth (±SE), yr[b]	26.2 (0.34)	26.5 (0.72)	23.6 (0.41)	<0.01
P[a]	0.65	Reference	<0.01	
Breast feeding (yes), *n* (%)[b]	154 (93.3)	35 (89.7)	107 (89.9)	0.72
P[a]	0.27	Reference	0.61	
Mean height (±SE), cm	152.9 (0.43)	151.8 (0.89)	157.1 (0.52)	<0.01
P[a]	0.29	Reference	<0.01	
Mean body mass index (±SE), kg/m^2	23.4 (0.28)	24.7 (0.57)	27.0 (0.34)	<0.01
P[a]	0.04	Reference	<0.01	
Smoking (ever smoker), *n* (%)	6 (3.3)	7 (15.9)	38 (28.4)	<0.01
P[a]	<0.01	Reference	<0.01	
Alcohol drinking (drinker), *n* (%)	67 (36.2)	5 (11.4)	25 (18.7)	<0.01
P[a]	<0.01	Reference	0.63	
Physical activity in past 5 years (yes), *n* (%)	85 (46.5)	19 (43.2)	26 (19.4)	<0.01
P[a]	<0.01	Reference	<0.01	

[a]*P* values for comparison with Japanese Brazilians living in São Paulo, Brazil; [b]Among parous women only.

and they smoked more and were taller and physically less active than the other two populations. Japanese Brazilians had an earlier menarche, more births and greater BMI, and they smoked more, drank less and were physically less active than Japanese, but they had later ages at menopause and first birth, fewer births and lower BMI, and they smoked less and were shorter and physically more active than non-Japanese Brazilians.

Because of an insufficient amount of sampled blood, we did not measure the levels of the following hormones: estradiol for 17 participants; bioavailable estradiol, estrone or SHBG for two participants each; or androstenedione for one participant. The proportion of participants with levels below the LOD were 0.9% for estradiol, 3.6% for estrone, 0% for bioavailable estradiol and SHBG, 0.6% for androstenedione and DHEAS, 24% for testosterone and 69% for free testosterone.

Adjusted hormone levels varied significantly across the three populations for all hormones (Table 2). Japanese Brazilians had significantly higher levels of estradiol, bioavailable estradiol, estrone, testosterone and free testosterone, and lower SHBG levels, than Japanese, whereas levels of androstenedione and DHEAS did not differ between the two populations (Table 2). Similar results were seen for analyses stratified by BMI (under and over 25), except for androstenedione level, which did not differ between Japanese Brazilians and Japanese whose BMI was under 25, but androstenedione level was significantly lower among Japanese Brazilians than among Japanese whose BMI was over 25 (Table 3).

Table 2 Adjusted geometric mean hormone levels in three populations[a]

	Japanese living in Nagano, Japan	Japanese Brazilians living in São Paulo, Brazil	Non-Japanese Brazilians living in São Paulo, Brazil	*P* for difference
Estradiol, pg/mL				
Age-adjusted	9.0	13.8	15.5	<0.01
(95% CI)	(8.6 to 9.4)	(12.5 to 15.3)	(14.6 to 16.5)	
P[a]	<0.01	Reference	0.052	
Multivariate[b]	9.7	14.3	15.5	<0.01
(95% CI)	(8.7 to 10.9)	(12.5 to 16.4)	(14.0 to 17.1)	
P[a]	<0.01	Reference	0.28	
Bioavailable estradiol, %				
Age-adjusted	23.1	30.6	22.9	<0.01
(95% CI)	(22.1 to 24.1)	(28.0 to 33.4)	(21.7 to 24.1)	
P[a]	<0.01	Reference	<0.01	
Multivariate[b]	23.7	30.2	20.6	<0.01
(95% CI)	(21.6 to 26.0)	(27.0 to 33.8)	(19.0 to 22.3)	
P[a]	<0.01	Reference	<0.01	
Estrone, pg/mL				
Age-adjusted	23.0	40.3	34.1	<0.01
(95% CI)	(22.0 to 24.0)	(36.8 to 44.1)	(32.4 to 35.9)	
P[a]	<0.01	Reference	<0.01	
Multivariate[b]	23.8	41.1	33.3	<0.01
(95% CI)	(21.5 to 26.3)	(36.5 to 46.3)	(30.6 to 36.4)	
P[a]	<0.01	Reference	<0.01	
Sex hormone-binding globulin, nM/L				
Age-adjusted	74.1	54.3	60.2	<0.01
(95% CI)	(69.4 to 79.1)	(47.5 to 62.0)	(55.8 to 65.1)	
P[a]	<0.01	Reference	0.18	
Multivariate[b]	68.4	53.0	70.7	0.01
(95% CI)	(59.5 to 78.5)	(44.9 to 62.4)	(62.6 to 79.7)	
P[a]	<0.01	Reference	<0.01	
Androstenedione, ng/mL				
Age-adjusted	0.65	0.56	1.04	<0.01
(95% CI)	(0.60 to 0.70)	(0.47 to 0.66)	(0.95 to 1.15)	
P[a]	0.12	Reference	<0.01	
Multivariate[b]	0.73	0.60	1.00	<0.01
(95% CI)	(0.61 to 0.88)	(0.48 to 0.76)	(0.85 to 1.18)	
P[a]	0.06	Reference	<0.01	
DHEAS, μg/dL				
Age-adjusted	50.6	58.0	44.5	0.03
(95% CI)	(46.3 to 55.4)	(48.2 to 69.8)	(40.0 to 49.4)	
P[a]	0.19	Reference	0.01	
Multivariate[b]	57.2	63.1	46.7	0.04
(95% CI)	(46.6 to 70.2)	(49.4 to 80.6)	(39.2 to 55.8)	
P[a]	0.38	Reference	0.02	
Testosterone, ng/mL				
Age-adjusted	0.02	0.11	0.18	<0.01
(95% CI)	(0.02 to 0.03)	(0.07 to 0.17)	(0.14 to 0.24)	
P[a]	<0.01	Reference	0.06	
Multivariate[b]	0.03	0.10	0.14	<0.01
(95% CI)	(0.02 to 0.04)	(0.06 to 0.20)	(0.09 to 0.22)	
P[a]	<0.01	Reference	0.38	
Free testosterone, pg/mL				

Table 2 Adjusted geometric mean hormone levels in three populations[a] *(Continued)*

Age-adjusted	0.21	0.39	0.44	<0.01
(95% CI)	(0.19 to 0.23)	(0.33 to 0.46)	(0.40 to 0.48)	
P^a	<0.01	Reference	0.18	
Multivariate[b]	0.22	0.39	0.39	<0.01
(95% CI)	(0.19 to 0.26)	(0.32 to 0.47)	(0.34 to 0.45)	
P^a	<0.01	Reference	0.92	

DHEAS, dehyroepianrosterone sulfate; 95% CI, 95% confidence interval; [a]*P* values for comparison with Japanese Brazilians living in São Paulo, Brazil; [b]Adjusted for age (continuous), age at first menarche (continuous), age at menopause (continuous), number of births (0, 1, 2 or 3, 4+), age at first birth (≤22, 23 to 26, ≥27 yr, nulliparous), height (continuous), body mass index (continuous), smoking (never smokers, past smokers, current smokers), alcohol drinking (nondrinkers, occasional drinker, regular drinkers), and physical activity in past 5 years (no, ≤2 days/wk, ≥3 days/wk).

Japanese Brazilians had significantly higher levels of bioavailable estradiol, estrone and DHEAS, and lower levels of SHBG and androstenedione, than non-Japanese Brazilians. Levels of estradiol, testosterone and free testosterone, however, did not differ between Japanese Brazilians and non-Japanese Brazilians (Table 2). Similar results were obtained when analyses were stratified by BMI (under and over 25), except for estrone and DHEAS. Levels of estrone were significantly higher among Japanese Brazilians than among non-Japanese Brazilians in individuals with a BMI under 25, but estrone levels did not differ between the two populations in individuals whose BMI was over 25, while DHEAS level did not differ regardless of BMI (under or over 25) (Table 3).

We further examined associations between endogenous sex hormone levels and known breast cancer risk factors or lifestyle factors (Table 4). BMI was significantly associated with higher estradiol, bioavailable estradiol, estrone, androstenedione, testosterone and free testosterone levels, as well as lower SHBG levels, but was not associated with DHEAS levels. Stratified analyses by study site (that is, the study in Nagano vs. the study in São Paulo) showed similar results for the two study sites. No statistically significant associations were observed between sex hormone levels and family history of breast cancer, history of benign breast disease, age at first menarche, age at menopause, parity, number of births, age at first birth, breast-feeding, height, smoking, alcohol drinking or physical activity during the past 5 years except for the following. We found a significantly higher level of SHBG among women who had a later age at menopause and among shorter women. We also observed a significantly higher level of DHEAS among women who had more births and a significantly lower level of testosterone among physically more active women. In stratified analyses by study site, however, we did not observe any findings which were consistent between the sites.

Discussion

In this cross-sectional study among postmenopausal Japanese, Japanese Brazilian and non-Japanese Brazilian women, we found significant differences in endogenous sex hormones among the three populations even after adjustment for known breast cancer risk factors. In particular, levels of estrogen and androgen in Japanese Brazilians were higher than levels in Japanese and were similar to or higher than levels in non-Japanese Brazilians. This pattern was observed for women with BMI values under and over 25. We also confirmed an increase in estrogen and androgen levels and a decrease in SHBG levels with increasing BMI.

As an initial comment, several methodological limitations of this study should be considered. First, our findings might be subject to the difference in study methods between Japan and Brazil, albeit that the two studies were conducted under a similar protocol. For example, we used serum samples for Japanese and plasma samples for both Japanese Brazilians and non-Japanese Brazilians. In this regard, we measured estrone levels in both serum and plasma from the same participants (n = 38). Although both levels were highly correlated (correlation coefficient = 0.94) and the percentage difference was relatively small (mean = -4%; 95% confidence interval, -9% to 1%), we used corrected values for the present study because the kit for estrone was applicable to serum samples only. Concurrently, we compared estrone levels among the three populations using crude values and observed the same results. The difference in blood samples is therefore unlikely to have affected the difference in sex hormone levels between the two populations. Given that blood collection methods also differed between the Japan and Brazil study sites, in addition to the types of blood samples used, we cannot exclude the possibility that our findings were affected by these differences. Another example is the difference in questionnaire data and data collection methods between Japan and Brazil. If such differences led to exposure misclassification, this might explain the observed absence of associations between sex hormone levels and known breast cancer risk factors or lifestyle factors. Second, although at least more than 96% of participants had detectable levels of estradiol, estrone, bioavailable estradiol, SHBG, androstenedione and DHEAS, the

Table 3 Adjusted geometric mean hormone levels[a] of three populations with stratification by body mass index[b]

	Japanese living in Nagano, Japan	Japanese Brazilians living in São Paulo, Brazil	Non-Japanese Brazilians living in São Paulo, Brazil	*P* for difference
Estradiol, pg/mL				
Low (BMI < 25)	9.5	14.2	15.0	<0.01
P[c]	<0.01	Reference	0.60	
High (BMI ≥25)	8.2	12.2	14.5	<0.01
P[c]	<0.01	Reference	0.06	
Bioavailable estradiol, %				
Low (BMI <25)	22.4	28.7	17.9	<0.01
P[c]	<0.01	Reference	<0.01	
High (BMI ≥ 25)	25.6	32.5	23.4	<0.01
P[c]	<0.01	Reference	<0.01	
Estrone, pg/mL				
Low (BMI < 25)	22.5	40.4	32.1	<0.01
P[c]	<0.01	Reference	<0.01	
High (BMI ≥25)	23.2	38.4	34.2	<0.01
P[c]	<0.01	Reference	0.19	
Sex hormone-binding globulin, nM/L				
Low (BMI < 25)	76.6	62.8	85.8	0.03
P[c]	0.04	Reference	<0.01	
High (BMI ≥25)	59.6	43.8	59.5	0.03
P[c]	0.02	Reference	0.02	
Androstenedione, ng/mL				
Low (BMI < 25)	0.64	0.63	0.91	0.03
P[c]	0.90	Reference	0.02	
High (BMI ≥25)	0.76	0.51	1.05	<0.01
P[c]	0.03	Reference	<0.01	
DHEAS, μg/dL				
Low (BMI < 25)	51.9	64.7	48.7	0.21
P[c]	0.13	Reference	0.11	
High (BMI ≥25)	54.6	52.2	43.4	0.29
P[c]	0.81	Reference	0.32	
Testosterone, ng/mL				
Low (BMI < 25)	0.01	0.07	0.13	<0.01
P[c]	<0.01	Reference	0.27	
High (BMI ≥25)	0.04	0.15	0.18	<0.01
P[c]	<0.01	Reference	0.69	
Free testosterone, pg/mL				
Low (BMI < 25)	0.18	0.32	0.31	<0.01
P[c]	<0.01	Reference	0.90	
High (BMI ≥25)	0.26	0.46	0.48	<0.01
P[c]	<0.01	Reference	0.85	

BMI, body mass index; DHEAS, dehyroepianrosterone sulfate; [a]Adjusted for age (continuous), age at first menarche (continuous), age at menopause (continuous), number of births (0, 1, 2 or 3, 4+), age at first birth (≤22, 23 to 26, ≥27, nulliparous), height (continuous), BMI (continuous), smoking (never smokers, past smokers, current smokers), alcohol drinking (nondrinkers, occasional drinkers, regular drinkers) and physical activity in the past 5 years (no, ≤2 days/wk, ≥3 days/wk); [b]The total participants in the low and high BMI groups were 199 and 156, respectively; [c]*P* values for comparison with Japanese Brazilians living in São Paulo, Brazil.

proportion of participants with levels below the LOD was relatively high for testosterone (24%) and free testosterone (69%). Our findings for testosterone and free testosterone should therefore be interpreted cautiously. Third, since our study included only a small number of Japanese Brazilians (n = 44), the findings might be due to chance and should be interpreted with caution.

We found higher circulating levels of estrogen and androgen in Japanese Brazilians than in Japanese, which were not accounted for by differences in the prevalence

Table 4 Adjusted geometric mean hormone levels by breast cancer risk factors and lifestyle-factors[a]

Breast cancer risk and lifestyle factors	Participants, *n*	Estradiol, pg/mL	Bioavailable estradiol, %	Estrone, pg/mL	Sex hormone-binding globulin, nM/L	Androstenedione, ng/mL	DHEAS, µg/dL	Testosterone, ng/mL	Free testosterone, pg/mL
Family history of breast cancer									
No	327	13.9	22.7	32.6	66.2	0.84	52.7	0.09	0.34
Yes	36	13.8	21.2	31.6	74.6	0.80	51.4	0.05	0.36
P for difference		0.90	0.18	0.57	0.12	0.66	0.83	0.08	0.40
History of benign breast disease									
No	339	13.9	22.6	32.5	66.9	0.84	52.9	0.09	0.34
Yes	23	14.3	22.0	33.5	69.0	0.78	52.1	0.08	0.31
P for difference		0.69	0.68	0.67	0.75	0.61	0.92	0.72	0.38
Age at first menarche, yr									
<12	101	13.7	22.9	31.6	66.7	0.83	49.5	0.08	0.33
13 or 14	166	13.9	22.2	32.4	65.2	0.83	54.8	0.09	0.34
15+	96	13.9	22.6	33.6	69.7	0.85	53.3	0.08	0.35
P for trend		0.81	0.81	0.18	0.51	0.78	0.43	0.99	0.60
P for trend[b]		0.70	0.47	0.30	0.24	0.68	0.29	0.83	0.39
Age at menopause, yr									
<48	116	14.0	23.0	32.6	64.5	0.89	57.0	0.08	0.34
49 to 51	108	14.0	22.0	33.1	70.2	0.78	51.6	0.09	0.34
52+	139	13.6	22.5	32.1	67.0	0.80	48.5	0.09	0.33
P for trend		0.47	0.65	0.68	0.57	0.20	0.05	0.66	0.75
P for trend[b]		0.80	0.06	0.93	0.02	0.32	0.51	0.59	1.00
Parity									
Parous	326	13.8	22.0	32.3	67.5	0.80	48.4	0.08	0.33
Nulliparous	37	13.7	23.3	32.9	67.2	0.87	58.0	0.10	0.34
P for difference		0.89	0.28	0.73	0.95	0.42	0.11	0.51	0.86
Number of births[c]									
1	32	13.7	20.6	32.8	69.6	0.77	43.7	0.10	0.30
2 or 3	219	13.4	22.2	31.6	67.8	0.79	43.9	0.08	0.32
4+	75	14.7	22.3	33.2	65.8	0.86	56.0	0.08	0.35
P for trend		0.27	0.26	0.71	0.55	0.38	0.046	0.76	0.20
Age at first birth[c], yr									
<22	79	13.2	21.3	31.5	70.9	0.80	44.0	0.09	0.31
23 to 26.9	138	13.9	21.5	33.1	68.1	0.78	46.7	0.07	0.33
27+	109	14.7	22.3	33.1	64.3	0.84	52.2	0.10	0.32
P for trend		0.09	0.29	0.52	0.16	0.47	0.11	0.40	0.89
P for trend[b]		0.10	0.32	0.53	0.37	0.58	0.39	0.47	0.81
Breast-feeding[c]									
No	27	14.3	23.2	33.5	63.4	0.82	46.9	0.09	0.33
Yes	296	13.7	21.9	32.2	67.6	0.81	47.2	0.08	0.32

Table 4 Adjusted geometric mean hormone levels by breast cancer risk factors and lifestyle-factors[a] *(Continued)*

P for difference		0.59	0.33	0.53	0.47	0.87	0.96	0.85	0.87
Height, cm									
<150.9	107	13.8	22.3	32.2	69.4	0.84	54.7	0.09	0.34
151 to 156.9	126	14.3	22.1	33.4	67.2	0.81	51.9	0.08	0.34
157+	124	13.7	23.2	32.2	63.8	0.85	51.7	0.09	0.34
P for trend		0.83	0.31	0.99	0.16	0.91	0.54	0.71	0.86
P for trend[b]		0.62	0.07	0.65	0.01	0.33	0.96	0.47	0.72
BMI, kg/m^2									
<24.9	199	13.3	20.9	31.1	75.3	0.77	51.1	0.07	0.30
25 to 29.9	116	14.5	24.2	32.2	60.2	0.79	48.4	0.09	0.34
30+	40	15.5	26.4	38.4	51.2	1.15	65.3	0.16	0.50
P for trend		0.01	<0.01	<0.01	<0.01	0.01	0.21	0.01	<0.01
P for trend[b]		<0.01	<0.01	<0.01	<0.01	<0.01	0.13	0.01	<0.01
Smoking									
Never smoker	310	13.2	24.3	32.0	62.9	0.80	53.5	0.09	0.35
Past smoker	37	13.6	23.7	32.4	62.3	0.77	51.4	0.06	0.38
Current smoker	14	14.9	20.0	33.2	76.3	0.94	52.8	0.12	0.29
P for difference		0.48	0.06	0.91	0.28	0.55	0.95	0.43	0.28
Alcohol drinking									
Nondrinker	266	14.0	22.0	32.7	69.9	0.85	49.4	0.10	0.34
Occasional drinker	39	14.1	23.5	32.4	63.7	0.82	59.1	0.08	0.34
Regular drinker	58	13.5	22.2	32.4	67.1	0.83	49.8	0.08	0.34
P for difference		0.76	0.48	0.97	0.42	0.89	0.29	0.48	0.98
Physical activity in past 5 years									
No	231	14.0	22.5	32.8	66.7	0.84	52.2	0.11	0.34
≤2 days/wk	63	13.8	22.1	32.1	67.5	0.79	50.6	0.05	0.33
≥3 days/wk	68	13.5	23.3	32.1	66.8	0.85	55.8	0.07	0.35
P for trend		0.46	0.48	0.58	0.95	0.97	0.56	0.02	0.60

DHEAS, dehyroepianrosterone sulfate; BMI, body mass index; [a]Adjusted for age (continuous), ethnic group (Japanese, Japanese Brazilians, non-Japanese Brazilians (Caucasian, mixed, Black), age at first menarche (continuous), age at menopause (continuous), number of births (0, 1, 2 or 3, 4+), age at first birth (≤22, 23 to 26, ≥27 yr, nulliparous), height (continuous), BMI (continuous), smoking (never smokers, past smokers, current smokers), alcohol drinking (nondrinkers, occasional drinkers, regular drinkers) and physical activity in the past 5 years (no, ≤2 days/wk, ≥3 days/wk); [b]Continuous variables; [c]Among parous women only.

of known breast cancer risk factors. This hormonal profile in Japanese Brazilians is consistent with the higher incidence and mortality rate of breast cancer in this population [4-6]. For instance, the age-adjusted incidence per 100,000 population for breast cancer among first-generation Japanese Brazilians from 1969 to 1978 was 24, while the incidences among Japanese from 1973 to 1977 were 12.7 in Osaka and 17.5 in Miyagi [4]. The standard mortality ratio for breast cancer among first-generation Japanese Brazilians from 1999 to 2001 on the basis of age-specific rates for Japanese in 2000 was 139 [5].

We also found higher circulating levels of bioavailable estradiol and estrone in Japanese Brazilians than in non-Japanese Brazilians, although levels of estradiol, testosterone and free testosterone did not significantly differ between the two populations. In the Multiethnic Cohort Study, Japanese Americans had significantly higher

estradiol levels than Caucasians and a slightly higher risk factor-adjusted incidence of breast cancer [10,18]. Although previous studies have shown lower incidence and mortality rates of breast cancer among Japanese Brazilians than among non-Japanese Brazilians [4-6], our findings suggest that the recent incidence and mortality rates among Japanese Brazilians might be similar to or higher than those of non-Japanese Brazilians.

The significant difference in sex hormone levels between Japanese Brazilians and Japanese might be determined by long-term exposure to environmental and lifestyle factors in Brazil. These differences were observed even after adjustment for known breast cancer risk factors, including BMI, which is a major determinant of estrogen levels in postmenopausal women. Although diet is one environmental factor that substantially differs between Japan and Brazil, the present study did not take into account dietary factors because we used different FFQ in the case-control studies in Nagano and São Paulo. Given that the report from the World Cancer Research Fund and American Institute for Cancer Research in 2007 showed no convincing or probable dietary risk factors for breast cancer [19], however, the difference in sex hormone levels between the two populations might not be explained by dietary factors only.

We observed an increase in estrogen and androgen levels and a decrease in SHBG levels with increasing BMI. Our findings are in general agreement with those of previous studies, and these associations have been consistently observed among both Asian and Western populations [10-13,15]. On the other hand, the determinants of sex hormone levels in postmenopausal women have not been firmly established, notwithstanding a relatively large number of epidemiological studies [10-14,16]. In the present study, we found a higher level of SHBG among women who had a later age at menopause and among shorter women. We also observed a higher level of DHEAS among women who had more births and a lower level of testosterone among physically more active women. In addition to the lack of consistency in these findings between the two study sites (that is, the study in Nagano vs. the study in São Paulo), our findings are inconsistent with those of previous studies, which found no significant associations among age at menopause, height and SHBG level, for example, or number of births and DHEAS level [12-14]. Higher physical activity levels were associated with lower levels of both estrogen and androgen [11,16], while another study reported no such association [10]. Given this lack of consistency with previous studies, our findings might be explained by multiple comparisons.

Conclusions

We found that levels of estrogen and androgen in Japanese Brazilians were higher than those in Japanese and similar to or higher than levels in non-Japanese Brazilians. Our findings may explain the previously observed increase in the incidence and mortality rate of breast cancer among Japanese Brazilians.

Abbreviations

BMI: body mass index; DHEAS: dehydroepiandrosterone sulfate; FFQ: food frequency questionnaire; IRMA: immunoradiometric assay; LOD: lower detection limit; SHBG: sex hormone-binding globulin.

Acknowledgements

This study was supported by a Grant-in-Aid for Research on Risk of Chemical Substances from the Ministry of Health, Labour and Welfare of Japan, and by Grants-in-Aid for Scientific Research on Priority Areas (17015049) and for Young Scientists (B) (17790378 and 19790415) from the Ministry of Education, Culture, Sports, Science, and Technology of Japan and the Japan Society for the Promotion of Science, and Foundation for Promotion of Cancer Research in Japan. We are grateful to the participants of the "São Paulo-Japan Breast Cancer Study Group": T. Hanaoka, M. Kobayashi, J. Ishihara, S. Ikeda, and C. Nishimoto (Research Center for Cancer Prevention and Screening, National Cancer Center, Tokyo); C. I. Yamaguchi, C. M. Kunieda, and S. S. Sugama (Nikkei Disease Prevention Center, São Paulo); C. K. Taniguchi and J. A. Marques (Departamento de Ginecologia, Hospital Pérola Byington, São Paulo); M. R. Eichhorn (Departamento de Nutrição, Hospital Pérola Byington, São Paulo); M. M. Netto, H. Iyeyasu, S. M. T. Carvalho, J. B. D. Collins, and C. E. M. Fontes (Departamento de Mastologia, Hospital A.C. Camargo, São Paulo); L. P. Kowalski and J. M. F. Toyota (Departamento de Cirurgia de Cabeça e Pescoço e Otorrinolaringologia, A. C. Camargo Hospital, São Paulo); E. M. Barbosa (Departamento de Mastologia, Instituto Brasileiro de Controle ao Câncer, São Paulo); O. Ferraro (Departamento de Mastologia, Hospital do Servidor Público Estadual Francisco Morato de Oliveira, São Paulo); E. H. Hotta and D. A. Petti (Instituto de Ginecologia e Mastologia, Hospital Beneficencia Portuguesa); and S. Mendes (Instituto Brasileiro de Mastologia e Ginecologia, Hospital Beneficencia Portuguesa).

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Authors' contributions

MI made substantial contribution to the conception and design of the study, as well as the analysis and interpretation of data, and was involved in drafting the manuscript. YK, SY, HO, HN, RK, GSH, INN, MSM, JM, FML and RA made substantial contributions to the study conception and design and the acquisition of data and were involved in critically revising the manuscript for important intellectual content. ST made substantial contributions to the study conception and design, as well as the analysis and interpretation of data, and was involved in critically revising the manuscript for important intellectual content. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Received: 7 January 2011 Accepted: 16 February 2011
Published: 16 February 2011

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Pre-publication history

The pre-publication history for this paper can be accessed here:
http://www.biomedcentral.com/1741-7015/9/16/prepub

doi:10.1186/1741-7015-9-16
Cite this article as: Iwasaki *et al.*: Comparison of postmenopausal endogenous sex hormones among Japanese, Japanese Brazilians, and non-Japanese Brazilians. *BMC Medicine* 2011 9:16.

Breast Cancer Res Treat (2011) 126:497–505
DOI 10.1007/s10549-010-1109-3

EPIDEMIOLOGY

Fragment c gamma receptor gene polymorphisms and breast cancer risk in case–control studies in Japanese, Japanese Brazilians, and non-Japanese Brazilians

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Received: 3 June 2010 / Accepted: 29 July 2010 / Published online: 10 August 2010

Abstract Previous studies showing the presence of antibodies against tumor-associated antigens in healthy individuals suggest that antibody-dependent cell cytotoxicity (ADCC) might play a role in the development of breast cancer. We hypothesized that functional polymorphisms in fragment c gamma receptor (FcgR) genes were associated with breast cancer risk. We conducted hospital-based case–control studies of patients aged 20–74 years with invasive breast cancer, and matched controls from medical checkup examinees in Nagano, Japan and from cancer-free patients in São Paulo, Brazil. A total of 869 pairs (403 Japanese, 80 Japanese Brazilians and 386 non-Japanese Brazilians) were genotyped for two single nucleotide polymorphisms (SNPs): a histidine (H)/arginine (R) polymorphism at position 131 of FcgRIIa (FcgRIIa H131R) and a valine (V)/phenylalanine (F) polymorphism at position 158 of FcgRIIIa (FcgRIIIa F158V). We found no statistically significant association between either of the two SNPs and breast cancer risk regardless of population. In analyses of the three populations combined, adjusted odds ratio (OR) was 0.93 [95% confidence interval (CI) 0.66–1.32] for women with the R/R versus H/H genotype of the FcgRIIa H131R polymorphism and 1.04 (95% CI 0.69–1.57) for the

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V/V versus F/F genotype of the FcgRIIIa F158V polymorphism. On combination of the two SNPs, compared to women with both the R/R genotype of the FcgRIIa H131R polymorphism and F/F genotype of the FcgRIIIa F158V polymorphism, the adjusted OR for women with both the H/H and V/V genotype was 0.68 (95% CI 0.37–1.27). In conclusion, our findings suggest that ADCC might not play a major role in the etiology of breast cancer.

Keywords Fragment c gamma receptor gene · Single nucleotide polymorphism · Breast cancer · Case–control study · Immigrants

Abbreviations

ADCC	Antibody-dependent cell cytotoxicity
CI	Confidence interval
FcgR	Fragment c gamma receptor
HER2	Human epidermal growth factor receptor 2
MUC1	Epithelial mucin
NK	Natural killer
OR	Odds ratio
SNP	Single nucleotide polymorphism

Introduction

Breast cancer is the most common malignancy in women in Japan and many other parts of the world [1, 2]. Although sex hormones, particularly estrogens, play an important role in the etiology of breast cancer [3], present knowledge has proved insufficient to allow the disease to be overcome, and the identification of other important etiological factors thus requires further study.

It has been hypothesized that the immune system recognizes malignant cells as foreign agents and eliminates them. Although several epidemiological studies have supported this hypothesis, only a few studies have investigated the role of the immune system in the etiology of breast cancer [4–6]. Natural killer (NK) cells are large granular lymphocytes that mediate innate immunity against pathogens and tumors. Natural cytotoxicity is believed to play an important role in host anti-cancer defense mechanisms. In their cohort study of 3,625 participants in Japan with 11-year follow-up, e.g., Imai and colleagues [5] showed that high cytotoxic activity of peripheral blood lymphocytes was associated with a decreased risk of total cancer, while Dewan et al. [6] recently reported that NK activities of peripheral blood mononuclear cells from breast cancer patients were significantly lower than those of healthy individuals.

NK cells are also capable of mediating antibody-dependent cell cytotoxicity (ADCC) against antibody-coated targets via the expression of a low-affinity receptor for IgG [fragment c gamma receptor (FcgR) III]. Many kinds of autoantibodies against tumor-associated antigens have been investigated, some of which are also detected in healthy control sera [7]. Anti-epithelial mucin (MUC1) antibodies, e.g., are frequently detected in healthy individuals, particularly in women during pregnancy and lactation [8, 9]. Notably, Forsman and colleagues [10] reported that serum from multiparous women, but not nulliparous women or men, contained antibodies which selectively mediated ADCC against established mammary carcinoma cell lines. Given that breast cancer risk is higher in nulliparous than multiparous women, this finding suggests that ADCC might play a role in the development of breast cancer.

Immune effector cells, including NK cells, recognize antibodies bound to target cells through FcgRs and elicit ADCC activity, which can be modulated by FcgR gene polymorphisms. At least two functional FcgR gene polymorphisms that may affect the killing function of immune effector cells have been identified: a histidine (H)/arginine (R) polymorphism at position 131 of FcgRIIa (FcgRIIa H131R) and a valine (V)/phenylalanine (F) polymorphism at position 158 of FcgRIIIa (FcgRIIIa F158V) [11–14]. The H allele of the FcgRIIa H131R polymorphism has higher binding efficiency to human IgG2 than the R allele, and confers enhanced phagocytic activity [13, 14]. The V allele of the FcgRIIIa F158V polymorphism has higher affinity for human IgG than the F allele and cells bearing this allele mediate ADCC more effectively than those with the F allele [11, 12]. In fact, ADCC is a potential anti-tumor mechanism behind targeted therapy with the humanized monoclonal antibody trastuzumab for human epidermal growth factor receptor 2 (HER2)-positive breast cancer [15]. FcgR gene polymorphisms have, therefore, been suggested to modulate the clinical efficacy of trastuzumab-based therapy in patients with metastatic HER2-positive breast cancer [16].

In addition to this putative effect of polymorphisms in the FcgR gene in modulating ADCC activity, roles in several other mechanisms in the immune system have been suggested, with FcgRs on leukocytes also modulating phagocytosis, clearance of immune complexes, superoxide generation, degranulation, cytokine production, and regulation of antibody production [17].

To better understand the role of the immune system in the etiology of breast cancer, we tested the hypothesis that polymorphisms in the FcgR gene are associated with the risk of breast cancer using data from hospital-based case–control studies in Nagano, Japan and São Paulo, Brazil.

Materials and methods

Study subjects

We conducted multicenter, hospital-based case–control studies of breast cancer in Japan and Brazil. In addition to determining lifestyle factors and genetic susceptibility to the risk of breast cancer, the protocols of these studies were also designed to compare potential risk factors among Japanese living in Nagano, Japan, and Japanese Brazilians and non-Japanese Brazilians living in São Paulo, Brazil [18, 19]. Eligible case patients were a consecutive series of female patients aged 20–74 years with newly diagnosed and histologically confirmed invasive breast cancer. Case patients were recruited between 2001 and 2005 at four hospitals in Nagano, and between 2001 and 2006 at eight hospitals in São Paulo. A total of 405 case patients (98%) participated in Nagano, and 83 Japanese Brazilian (91%) and 389 non-Japanese Brazilian case patients (99%) in São Paulo. In the Nagano study, eligible control subjects were selected from medical checkup examinees in two of the four hospitals who were confirmed not to have cancer. One control subject was matched for each case patient by age (within 3 years) and residential area during the study period. Among potential controls, one examinee declined participation and two declined the provision of blood samples. Consequently, we obtained written informed consent from 405 matched pairs. In the São Paulo study, eligible control subjects were preferentially selected from cancer-free patients who visited the same hospital as the index patients. One control was matched with each case by age (within 5 years) and ethnicity during the study period. Among potential control subjects, 22 patients declined participation (participation rate = 96%). Consequently, we obtained written informed consent from 472 matched pairs (83 for Japanese Brazilians and 389 for non-Japanese Brazilians). The study protocol was approved by CONEP (Comissão Nacional de Ética em Pesquisa), Brasília, Brazil and by the institutional review board of the National Cancer Center, Tokyo, Japan.

Data collection

Participants in Nagano were asked to complete a self-administered questionnaire, while those in São Paulo were given in-person interviews conducted by trained interviewers using a structured questionnaire. The two questionnaires contained closely similar questions concerning demographic characteristics, medical history, family history of cancer, menstrual and reproductive history, anthropometric factors, physical activity, and smoking habits.

Information on estrogen receptor (ER) and progesterone receptor (PR) status was obtained from medical records. Hormone receptor status was determined by either enzyme-linked immunoassay or immunohistochemical assay. Hormone receptor positivity values were determined either as specified by the laboratory that performed the assay or in accordance with the laboratory's written interpretation thereof, or both.

Participants in Nagano provided blood at the time they returned their self-administered questionnaire, and those in São Paulo at the time of interview. Blood samples were divided into serum aliquots in Nagano and into plasma aliquots and buffy layers in São Paulo. All blood samples were shipped to the Epidemiology and Prevention Division, Research Center for Cancer Prevention and Screening, National Cancer Center, Tokyo, Japan and stored at −80°C until analysis.

Genotyping

Genomic DNA samples were extracted from peripheral blood using Qiagen FlexiGene® DNA Kits (Qiagen K.K., Tokyo, Japan) according to the manufacturer's protocol. We genotyped two single nucleotide polymorphisms (SNPs), namely FcgRIIa H131R (rs1801274) and FcgRIIIa F158V (rs396991) by TaqMan® SNP Genotyping Assays (Applied Biosystems, Foster City, CA, USA). Cases and matched controls were analyzed in the same well by laboratory personnel who did not know the case–control status. The quality of genotyping was assessed by duplicate quality control samples ($n = 140$), with concordance rates of 100% for FcgRIIa H131R and 99% for FcgRIIIa F158V.

Statistical analysis

We excluded subjects whose DNA samples were not available, leaving a total of 869 pairs (403 Japanese, 80 Japanese Brazilians and 386 non-Japanese Brazilians). Comparison of baseline characteristics between cases and controls was evaluated by the Mantel–Haenszel test using matched-pair strata in each population. Genotype frequencies were tested for deviation from the Hardy–Weinberg equilibrium with the Chi-square test. Odds ratios (ORs) and 95% confidence intervals (CIs) of breast cancer for SNPs and their combination were calculated using a conditional logistic regression model. Stratified analyses according to menopausal status and parity were calculated using an unconditional logistic regression model. Tests for interaction were performed based on the difference between two likelihood ratios of the models with and without the interaction terms. Associations between SNPs and hormone receptor-defined breast cancer were assessed by an unconditional polytomous logistic regression model. The Wald