Factors Associated with Serum Levels of Estradiol and Sex Hormone-binding Globulin among Premenopausal Japanese Women

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We measured serum levels of estradiol (E_2) and sex hormone-binding globulin (SHBG) among 50 healthy premenopausal Japanese women in 1994 in Gifu, Japan, to investigate the relationships between potential risk factors for breast cancer and hormone levels. Using a self-administered questionnaire, we collected data on body size, physical activity, and previous disease history, as well as menstrual and reproductive histories of the woman and her mother. Blood samples were drawn from each subject on the 11th and 22nd days of her menstrual cycle. Higher serum E_2 levels were observed for women with shorter menstrual cycles. Age as well as cycle length were included in the regression models to determine the associations between hormone levels and study variables. Body mass index (BMI) was inversely related to SHBG level measured at the 11th day of the cycle, after adjusting for age and cycle length (r = -0.33; p = 0.03). Women born in spring/summer had higher levels of E_2 on the 22nd day (p = 0.07) and higher levels of SHBG on both the 11th and 22nd days of the cycle (p = 0.01 and p = 0.06, respectively) than those born in other seasons. Physical activity at 13–15 years of age was inversely related to E_2 level on the 11th day of the cycle after controlling for age, cycle length, BMI, and birth month (r = -0.35; p = 0.04). *Key words*: body mass index, estradiol, Japanese, month of birth, sex hormone-binding globulin, physical activity, premenopause.
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Endogenous hormones, particularly estrogens, have been implicated in the etiology of breast cancer (*1*). Recently, numerous studies have focused not only on total estradiol (E_2) levels but also on the percentage of free E_2 (*2,3*). The level of sex hormone-binding globulin (SHBG), which is a major determinant of biologically available E_2, has also been suggested to affect breast cancer risk (*4,5*). However, measurements of serum levels of E_2 or SHBG have produced inconsistent results. Epidemiological evidence for their etiological roles is limited because most data are from case–control studies.

A recent large-scale prospective study found that levels of total E_2, percent free E_2, and percent E_2 bound to SHBG were related to the risk of developing breast cancer among postmenopausal women (*6*).

It is possible that the known breast cancer risk factors may be mediated by an estrogen mechanism. The relationships between the hormone profile and some of the risk factors, such as early age at menarche, nulliparity, and obesity have been investigated among premenopausal and postmenopausal women (*7–11*), but results have been inconsistent. Therefore, we evaluated serum levels of E_2 and SHBG in healthy Japanese premenopausal women with respect to aseries of breast cancer risk factors. We also explored factors that some epidemiological studies have suggested are associated with risk for breast cancer, such as physical activity (*12,13*), alcohol use (*14*), and birth month (*15,16*), to determine whether they affect hormone profiles of premenopausal Japanese women.

Subjects and Methods

Fifty healthy college women from 21 to 42 years old were studied in January, February, and March of 1994. Informed consent was obtained from each subject. Subjects were asked to complete a self-administered questionnaire including menstrual status, height and weight, smoking and drinking habits, physical activity, reproductive history, oral contraceptive and other hormone use, previous disease history, and information on maternal age at menarche and delivery. Alcohol consumption was estimated from frequency of drinking and usual serving size for six different types of liquors during the past year. Physical activity was assessed from the average hours per week spent in strenuous (jogging, tennis, bicycling on hills, swimming, and aerobics) and moderate (brisk walking, golfing, bowling, bicycling on level ground, and gardening) activities at 13–15 years of age, 16–18 years, and in the past year. Each activity level was assigned an intensity score according to a relative metabolic rate method. The hours at each level of activity, weighted by these intensity scores, were summed to score the physical activity level of individuals. The validity of this method was tested by Suzuki et al. for establishing a physical activity level during the past year. The Spearman correlation coefficient was 0.69 (Suzuki et al., unpublished data) for a comparison between energy expended as measured by calorimeter or the calorie counter method (*17*) and as estimated from questionnaire data.

No subject had a history of diabetes or endocrine diseases and none had ever taken oral contraceptives. There were no current users of other types of hormonal medication. One women had used hormonal medication about 2 years before the study, but only for a few months.

Two blood samples were drawn from each subject in the mornings of the 11th and 22nd days of her menstrual cycle. Day 1 of the menstrual cycle was defined as the day of onset of menstrual bleeding. After centrifugation, the serum was separated and stored at -80°C until assayed. Hormone assays were carried out in one batch in one laboratory. Serum levels of total E_2 and SHBG were measured radioimmunologically with kits purchased from Diagnostic Products Corporation (Los Angeles, CA). The intra-assay coefficients of variation, based on control pools, were 9.8% for E_2 and 5.3% for SHBG.

Hormone concentration was log-transformed for statistical analysis to reduce departures from the normal distribution. To assess the association between the logarithm of E_2 or SHBG level and the study variables, we calculated the Pearson correlation coefficients. The variables examined in this way were height, weight, body mass index [BMI; weight (kg)/height (m^2)], age at menarche, age at start of regular cycle, birth order, and ages at menarche and delivery for the subject's mother. Although we restricted the sampling days according to the menstrual cycle, higher plasma E_2 levels were observed for women with shorter cycles. Therefore, adjustments for cycle length and age were performed by regressing the logarithms of hormones and study variables separately on these potential confounders. The correlation coefficients between the two sets of residuals were then calculated. The relationships between categorical variables [i.e., length of the menstrual cycle (≤27, 28–29, 30, 31–35 days, and irregular), smoking status, and

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month of birth] and the log of hormone values were analyzed by the multiple regression technique. All statistical analyses were performed using PC-SAS, Ver. 6.04 (SAS Institute, Cary, NC) (*18*).

Results

The E_2 level on the 11th day of the cycle was strongly associated with the reported length of the menstrual cycle. The trend of decreasing E_2 level with increasing cycle length was statistically significant on the 11th day of the cycle (Table 1).

BMI was significantly correlated with a decreasing level of SHBG on the 11th day of the cycle (r = -0.33; p = 0.03) (Table 2). BMI also tended to be related to a decreasing level of SHBG on the 22nd day of the cycle, but it did not achieve a statistical significance (r = -0.26; p = 0.09). Birth in a month between March and August was significantly associated with increasing level of SHBG on the 11th day (p = 0.01) (Table 3).

The relationship between physical activity at 13–15 years of age and a decreasing level of E_2 on the 11th day of the cycle was statistically significant after additional adjustments for BMI and birth month (r = -0.35; p = 0.04).

There were no significant associations between E_2 level at days 11 or 22 and the variables for parity, smoking, alcohol consumption, maternal age at delivery, and ages at menarche and start of regular cycle.

Table 1. Geometric mean concentrations (95% confidence intervals) of serum estradiol (E_2) and sex hormone-binding globulin (SHBG) by age and cycle length

Variables	Number of subjects	Follicular phase[a] E_2 (pg/ml)	Follicular phase[a] SHBG (nmol/l)	Luteal phase[b] E_2 (pg/ml)	Luteal phase[b] SHBG (nmol/l)
Age					
≤29	26	58.1 (48.2–69.9)	67.9 (57.8–79.7)	90.4 (73.2–111.7)	76.4 (65.4–89.2)
30–34	14	54.8 (43.8–68.7)	78.6 (63.3–97.6)	85.1 (66.1–109.7)	85.1 (71.6–101.2)
≥35	10	89.6 (56.5–141.8)	74.5 (54.1–102.6)	68.7 (44.6–105.9)	79.8 (58.2–109.5)
		$p_1{}^c$ = 0.06; $p_2{}^d$ = 0.03	p_1 = 0.58; p_2 = 0.57	p_1 = 0.43; p_2 = 0.20	p_1 = 0.73; p_2 = 0.78
Cycle length (days)					
25	3	145.1 (85.8–245.5)	90.2 (55.5–146.7)	63.9 (33.9–120.6)	98.7 (63.3–153.8)
26	1	65.0 (26.1–161.6)	72.9 (31.4–169.2)	74.1 (24.7–222.6)	80.5 (37.3–173.6)
27	1	88.8 (35.7–220.7)	75.2 (32.4–174.5)	59.5 (19.8–178.7)	72.3 (33.5–155.9)
28	12	91.0 (69.9–118.3)	86.0 (67.5–109.7)	95.6 (69.6–131.3)	97.7 (78.3–122.0)
29	2	66.1 (34.7–125.8)	56.9 (31.4–103.2)	129.8 (59.6–282.4)	65.2 (37.9–112.3)
30	16	48.1 (38.3–60.4)	61.2 (49.6–75.6)	66.4 (50.5–87.5)	66.8 (55.1–80.9)
34	2	64.9 (34.1–123.5)	44.9 (24.8–81.4)	76.7 (35.2–166.9)	55.6 (32.3–95.7)
35	2	49.2 (25.8–93.6)	87.1 (48.0–157.9)	134.3 (61.7–292.3)	102.7 (59.7–176.9)
Irregular[e]	11	47.2 (35.9–62.1)	77.4 (60.0–99.7)	100.4 (72.1–139.9)	81.8 (64.9–103.1)
		p_1 <0.01; p_2 <0.01	p_1 = 0.38; p_2 = 0.14	p_1 = 0.42; p_2 = 0.59	p_1 = 0.26; p_2 = 0.19

[a]The 11th day of the menstrual cycle.
[b]The 22nd day of the menstrual cycle.
[c]For F values.
[d]For trend.
[e]Not included in calculation for trend.

Table 2. Correlation coefficients[a] of serum estradiol (E_2) and sex hormone-binding globulin (SHBG) with demographic characteristics, reproductive factors, and physical activity

Variables	Follicular phase[b] $E_2{}^d$ (pg/ml)	Follicular phase[b] SHBG[d] (nmol/l)	Luteal phase[c] E_2 (pg/ml)	Luteal phase[c] SHBG (nmol/l)
Height (cm)	0.06	0.07	-0.14	0.13
Weight (kg)	-0.12	-0.24	-0.16	-0.15
Body mass index	-0.19	-0.33*	-0.11	-0.26
Age at menarche (years)	-0.04	-0.03	0.05	0.00
Age at start of regular cycle (years)	-0.10	-0.03	0.06	-0.04
Number of births	0.06	-0.10	-0.06	0.00
Birth order	0.17	0.15	-0.20	0.13
Maternal age at menarche (years)	-0.00	-0.07	-0.18	-0.26
Maternal age at birth (years)	-0.10	0.00	-0.27	-0.02
Physical activity score[e]				
13–15 years old	-0.35*	0.00	0.13	0.09
16–18 years old	0.05	-0.12	0.16	-0.06
In the past year	0.13	-0.07	0.07	-0.03

[a]Adjusted for age and cycle length.
[b]The 11th day of the menstrual cycle.
[c]The 22nd day of the menstrual cycle.
[d]Transformed into logarithm.
[e]Adjusted for age, cycle length, body mass index, and birth month.
*p<0.05.

Discussion

We found that higher physical activity at 13–15 years of age was related to decreased E_2 levels on the 11th day of the cycle in adulthood. The previous studies of E_2 level and physical activity have yielded conflicting results. Decreased levels of E_2 in athletic women have been reported (*19,20*), but one study shows no change in E_2 level after 1 year of moderate aerobic training (*21*). Physical activity at 11–13 years of age may lower the E_2 level later in life, as its intensity level is relatively high at 13–15 years of age (estimated mean weekly energy expenditures were 3525, 2437, and 729 kcal/week for 13–15 years old, 16–18 years old, and the past year, respectively). The lack of association of E_2 levels with physical activity at 16–18 years of age and the past year may be due to a relatively low level of activity at these periods. We cannot deny a possibility that physical activity may have a profound effect on menstrual activity when a regular menstrual cycle should be established.

Physical activity has been reported to be associated with a lower risk of breast cancer by some investigators (*12,13,22*). These authors have suggested that physical activity should reduce the risk of breast cancer by reducing the number of ovulations that occur during the women's lifetime. Strenuous activity is associated with reduced frequency of ovulation or reduced length of luteal phase (*23,24*). The length of the menstrual cycle in the present study was not significantly associated with physical activity (r = 0.06, 0.15, and 0.01 for 13–15 years, 16–18 years, and the past year, respectively, after controlling for age and BMI). The finding of lower E_2 levels with higher physical activity at 13–15 years of age is consistent with physical activity reducing the risk of breast cancer.

The inverse association of BMI with SHBG level in premenopausal women has been reported by Apter et al. (*7*) and Ingram et al. (*9*). We confirmed these results in the present study.

Several studies have suggested that exposure to pregnancy estrogens have an important role in the etiology of breast cancer (*25–27*). Some factors related to pregnancy estrogen levels, such as maternal age at deliv-

Table 3. Geometric mean concentrations[a] (95% confidence interval) of serum estradiol (E_2) and sex hormone-binding globulin (SHBG) by smoking, alcohol consumption, and month of birth

Variables	Number of subjects	Follicular phase[b] E_2 (pg/ml)	Follicular phase[b] SHBG (nmol/l)	Luteal phase[c] E_2 (pg/ml)	Luteal phase[c] SHBG (nmol/l)
Smoking					
Never	45	64.8 (54.9–76.5)	72.4 (61.8–84.9)	83.1 (67.9–101.8)	79.7 (68.9–92.3)
Ever	5	86.2 (56.6–131.3)	81.5 (54.5–122.1)	79.2 (47.3–132.4)	91.0 (62.7–132.0)
		p_1[d] = 0.22	p_1 = 0.59	p_1 = 0.86	p_1 = 0.51
Alcohol consumption (g/week)					
0	14	73.5 (56.6–95.5)	70.9 (55.3–90.8)	78.4 (57.2–107.3)	77.0 (61.1–97.1)
≤18.7	18	71.7 (55.8–92.0)	67.3 (53.1–85.2)	73.6 (54.6–99.3)	78.7 (63.1–98.1)
≥18.8	18	59.8 (47.5–75.3)	81.5 (65.4–101.4)	94.6 (71.7–124.8)	86.5 (70.5–106.1)
		p_1 = 0.40; p_2[e] = 0.23	p_1 = 0.44; p_2 = 0.39	p_1 = 0.41; p_2 = 0.36	p_1 = 0.70; p_2 = 0.45
Month of birth					
September to February	21	68.6 (56.2–83.6)	63.8 (53.9–75.5)	72.7 (57.9–91.4)	73.1 (62.2–86.1)
March to August	28	65.1 (51.3–82.5)	88.2 (72.0–108.0)	100.3 (76.3–131.8)	92.5 (76.1–112.3)
		p_1 = 0.73	p_1 = 0.01	p_1 = 0.07	p_1 = 0.06

[a]Adjusted for age and length of menstrual cycle.
[b]On the 11th day of the menstrual cycle.
[c]On the 22nd day of the menstrual cycle.
[d]For F values.
[e]For trend.

ery and first versus subsequent pregnancies (*28,29*), were also related to future breast cancer risk (*30,31*). In our data, birth order as well as maternal age at delivery were not significantly associated with levels of E_2 and SHBG. There is no obvious explanation for the observed association between birth month and SHBG levels. It may be the influence of prenatal or perinatal factors on SHBG metabolism (conceivably pregnancy estrogens or other pregnancy hormones may have seasonal variation), the result of unknown confounders, or due to chance.

Although we used the BMI as an index of obesity, there may be a residual confounding effect of obesity for the observed association between month of birth and SHBG level.

Among the well-known breast cancer risk factors, early age at menarche was associated with E_2 level in the study reported by Apter et al. (*7*). However, Ingram et al. (*9*) and Bernstein et al. (*10*) found no evidence of the effect of age at menarche on the estrogen levels, which was consistent with the result in the present study.

Positive association between alcohol use and breast cancer has been observed in many epidemiological studies (*32*). Although alcohol consumption was not related to E_2 level in the present study, the average and variance of alcohol consumption in the subjects were too small to detect a significant association. All suitable women in a college class participated in the present study, but their alcohol consumption [mean ± standard deviation (SD) of 26.6 ± 39.9 g/week] appeared to be relatively low as compared with other groups of college women. Our recent survey (1996) showed that the mean ± SD of alcohol consumption was 47.6 ± 99.4 g/week in 205 women at two other colleges.

It is difficult to compare the levels of E_2 among premenopausal women because the E_2 level fluctuates greatly during the menstrual cycle. We restricted the days of drawing blood samples during the menstrual cycle and took the cycle length into consideration because the E_2 level on the 11th day of the cycle was negatively related to the cycle length. As we could not obtain the exact date of the onset of subsequent menstruation from each woman, the reported cycle length was utilized for controlling its effect after being categorized.

The present study may be still biased due to the residual confounding effects of the cycle length. The cycle length has been indicated to be related to breast cancer risk (*33*). It would be better to obtain a large number of data and restrict the analyses to the subjects with the same cycle lengths. However, even if we have knowledge of the exact cycle lengths of the subjects and the exact day of the cycle that measurements are taken and we perform subanalyses according to cycle length, it is almost impossible to completely avoid the effects of confounding factors related to differences in cycle characteristics on E_2 levels among individuals without repeating the measurements through the menstrual cycle.

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