Soy Intake and Other Lifestyle Determinants of Serum Estrogen Levels among Postmenopausal Chinese Women in Singapore[1]

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Abstract

Endogenous estrogen levels are strongly associated with breast cancer risk, but its determinants are not well understood. We conducted a cross-sectional study of 144 healthy postmenopausal women, participants in a population-based prospective investigation of diet and cancer risk among Chinese in Singapore. The relationships between plasma levels of estrone (E_1), estradiol (E_2), and androstenedione and dietary intake of soy and other food groups were investigated. Data on diet and other lifestyle factors were obtained from a structured questionnaire with a validated dietary component that was administered in-person to all participants. Few dietary factors emerged as determinants of plasma estrogen levels in this population. An exception was soy, which was significantly associated with plasma E_1 levels. Specifically, E_1 levels were 15% lower among individuals in the highest quartile of soy protein intake compared with those in the lower three quartiles of intake ($P = 0.047$). E_1 levels did not differ between individuals in the lower three quartiles of soy protein intake. Similar patterns of differences in E_1 levels emerged in analyses by intake of isoflavones and total soy products. These findings on soy were independent of the four nondietary factors (see below) that significantly influenced estrogen levels. Both E_1 and E_2 levels increased with high body mass index [BMI (weight/height^2)]; the respective levels were 41% (two-sided $P = 0.02$) and 17% higher ($P = 0.34$) among women in the highest BMI category (BMI $\geq$ 24) compared with those in the lowest category (BMI $<$ 20). After adjustment for BMI and age, women with a late age at menarche (age 17 years or older) showed significantly lower E_1 (30% lower; $P = 0.02$) and E_2 levels (24% lower; $P = 0.02$) compared with women with earlier age at menarche (before age 17 years) and who were nulliparous or had a late age at first live birth (after age 31 years). Current smokers showed significantly higher E_2 levels (28%) than nonsmokers ($P = 0.04$). These findings are discussed in relation to the recent doubling of breast cancer incidence among Chinese women in Singapore.

Introduction

There is overwhelming evidence that estrogen levels are a critical determinant of breast cancer risk (1, 2). In a pooled analysis of prospective studies, postmenopausal women who subsequently developed breast cancer had a 15% higher mean concentration of serum E_2[3] than women who did not (3). These results are confirmed in a recent, expanded analysis (4). Low-risk traditional women in Asia have been shown to have lower urinary and blood estrogen levels than high-risk Caucasian women (5). However, after migration to the west, Asians residing in the United States do not display lower endogenous estrogen levels than whites (6, 7), consistent with their known elevation in breast cancer risk compared with native Asians (8).

Reasons for the higher endogenous estrogen levels in women who develop breast cancer compared with those who do not and in whites compared with native Asians have not been identified. Cross-sectional studies of healthy women have sought to determine whether established breast cancer risk factors such as early age at menarche, nulliparity, high postmenopausal body size, and alcohol use may be mediated by endogenous hormones (9–14). The most consistent finding is a positive association between blood estrogen levels and body size, measured by either BMI (weight/height^2; Refs. 9, 11, and 13) or height (14). Although high intake of dietary fat, low intake of dietary fiber, and low intake of dietary soy have been associated with an increased risk of breast cancer in some studies, the collective evidence is discrepant, and an etiological role of diet in breast cancer remains controversial (15). Dietary fat (10, 12, 16, 17), fiber (10, 16), and soy (18, 19) have not shown consistent influences on blood estrogen levels in cross-sectional studies.

Between the 1970s and 1990s, the largest increases in breast cancer incidence worldwide have occurred in Asia; rates more than doubled in Singapore and Japan (20). With these alarming increases in breast cancer incidence among traditionally low-risk populations, it is a priority to identify lifestyle determinants of endogenous estrogen levels so that modifiable preventive measures may be developed. Little data exist on the determinants of endogenous hormone levels in Asian populations. In this report, we describe the distribution of serum E_1,

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[3] The abbreviations used are: E_2, estradiol; E_1, estrone; BMI, body mass index; CI, confidence interval.

E_2, and androstenedione levels among postmenopausal Singaporean Chinese women. We investigated the associations of hormone levels with menstrual and reproductive factors, cigarette smoking, and dietary factors including fat, fiber, and soy intake.

Patients and Methods

Study Population. The subjects were participants in the Singapore Chinese Health Study, a population-based, prospective investigation of diet and cancer risk. From April 1993 through December 1998, a total of 63,257 Chinese women and men ages 45–74 years enrolled in the study (only women are included in this report). We restricted study subjects to the two major dialect groups of Chinese in Singapore: (*a*) the Hokkiens, who originated from the southern Fujian province; and (*b*) the Cantonese, who came from the central Guangdong province. The subjects were residents of government housing estates; 86% of the Singapore population live in these facilities. At recruitment, a face-to-face interview was conducted in the subject's home by a trained interviewer who used a structured questionnaire. The questionnaire included a validated dietary component (including questions about coffee, tea, and alcoholic beverages) that assessed current intake patterns (21). Each subject was asked to estimate his or her usual intake frequencies and portion sizes for 165 food and beverage items during the past 12 months. The questionnaire also requested information on demographics, lifetime use of tobacco, menstrual and reproductive history (women only), medical history, and family history of cancer.

The Chinese population in Singapore (and elsewhere in Asia) is particularly suited for studies on effects of soy foods because this food group has been a staple in the traditional Asian diet. Six kinds of soy products (plain tofu, taupok, taukwa, foopei, foojook, and tofu far) and soybean drink were included in our questionnaire. In addition, as part of the development of a Singapore Food Composition Database, levels of daidzein, genistein, and glycitein were measured in these main types of soy foods consumed in Singapore, thus allowing us to compute intake of total isoflavones in individual subjects (22). Given that isoflavone is only one of many constituents in soy foods that may have potential "protective" health effects (23), we also calculated grams of soy intake in our analysis. Weights of all soy products are expressed in units of plain tofu equivalents and then summed. Because taupok is taukwa cooked with oil, and tofu far is tofu cooked with syrup, conversion factors are required only for taukwa, foopei, and foojook. Based on the fact that 100 g of cooked tofu, taukwa, foopei, and foojook contain 89, 69, 58, and 54 g of water, respectively, we determined that 1 g of taukwa is equivalent to 2.8 (31:11) g of tofu. Similarly, 1 g of foopei and foojook is equivalent to 3.8 g (42:11) and 4.2 g (46:11) of tofu, respectively. One hundred g of soybean drink contain 92 g of water, and thus 1 g of soybean drink is equivalent to 0.73 g (8:11) of tofu. Self-reported levels of usual soy consumption were validated by spot urinary measurements of isoflavone levels in a subset of participants in this cohort (24). Total intake of legumes, from soy and nonsoy (*e.g.*, canned baked beans) sources, was also calculated. However, because weight units of nonsoy legumes differ qualitatively from soy legumes because of the water content in some soy foods (as described above), we did not simply add gram weights of the two sources of legumes. Instead, we ranked all subjects by their intake of soy legumes and nonsoy legumes separately and then summed the two ranks to derive a unit-free index of total legumes. In other words, for the 144 women included in this analysis, the theoretical range (assuming no ties) of values for the index is 2–288.

In April 1994, 1 year after the initiation of the cohort study, we started collection of blood and single-void urine specimens from a random 3% sample of study enrollees. A 20-ml blood sample was obtained from each subject. One 10-ml plain tube was used for the preparation of serum, and one 10-ml heparin-containing tube was used for plasma. Immediately after blood collection, the tubes were put on ice during transport from the subjects' homes to the laboratory. All specimens were then kept at room temperature for 2 h before separation into their various components (plasma, serum, RBCs, and buffy coat). All specimens were subsequently stored in a liquid nitrogen tank at –180°C until analyzed. Most blood samples were collected in the morning, with no requirement that the subjects fast. However, we asked and recorded the time the subjects took their last meal. The present investigation included the first 149 women in this substudy, who were at least 50 years of age, without a history of cancer, and had experienced a natural or a surgical menopause (*i.e.*, had both ovaries removed or a simple hysterectomy). Five of the 149 women were excluded; four were current users of replacement hormones, and one had missing data in E_1. Thus, 144 women were included in this report. The Institutional Review Boards at the University of Southern California and the National University of Singapore have approved this study.

Laboratory Methods. Plasma aliquots from study subjects were shipped in sealed containers on dry ice to one of us (M. C. Y.) at the University of Southern California. These specimens were then delivered to the laboratory of F. Z. S., who used radioimmunoassays previously validated in his laboratory (25–28) to measure plasma levels of E_1, E_2, and androstenedione. Before quantification, the hormones were first extracted with hexane:ethyl acetate (3:2) and then separated from interfering metabolites by use of Celite column partition chromatography. The interassay coefficients of variation at three (low, medium, and high) concentrations of E_1, E_2, and androstenedione were between 7% and 16%.

Statistical Analysis. For each subject, we computed the usual daily intake of various nutrients by combining information obtained from the interview with nutrient values from the Singapore Food Composition Tables (21). Nutrient density expressed as weight per 4200 kJ (1000 kcal) was used in all data analyses as a means for adjusting for energy intake. The distributions of plasma levels of E_1, E_2, and androstenedione in the study population were skewed and were corrected to a large extent by transformation to logarithmic values. Therefore, all statistical analyses were performed on logarithmically transformed values. We present the geometric means and their 95% CIs, which were obtained by taking the antilog of the 95% CIs of logarithmically transformed values.

Of the 144 subjects, 35% ($n = 47$) gave blood samples within 2 h after a meal (breakfast in almost all cases), whereas 9% ($n = 12$) offered fasting blood samples (9 or more hours after a meal). We investigated whether the time interval since the last meal significantly influenced serum levels of E_1, E_2, and androstenedione. Although results were not statistically significant, levels of E_1, E_2, and androstenedione tended to be higher among those with fasting samples. Thus, the time interval between last meal and blood draw was included as a covariate in all analyses. We used ANOVA and analysis of covariance methods to assess the influence of age, menstrual and reproductive history, and diet and other lifestyle factors on levels of E_1, E_2, and androstenedione. Because age and BMI

Table 1 Adjusted[a] geometric mean (95% CI) for serum E_1, E_2, and androstenedione levels (pg/ml) by age at specimen, years since menopause, dialect group and education

Variable	*N*	E_1	E_2	Androstenedione
All subjects	144	30.3 (28.2–32.6)	13.2 (12.4–13.9)	429.3 (383.0–481.3)
Age at specimen collection (yrs)				
50–54	35	26.7	12.5	454.5
55–64	70	30.9	13.3	431.3
65–74	39	32.9	13.6	404.5
2p (linear trend)		0.34	0.61	0.20
Years since menopause				
<5	43	28.9	13.6	445.2
5–9	35	29.5	12.0	420.4
10–14	26	32.1	13.3	497.9
15+	40	31.6	13.7	381.8
2p (linear trend)		0.31	0.74	0.45
Education				
No formal schooling	68	30.2	13.1	422.1
Primary school	49	29.5	13.2	417.1
Secondary school or higher	27	32.4	13.3	472.3
2p (linear trend)		0.52	0.89	0.49
Dialect group				
Hokkien	74	28.8	12.8	408.7
Cantonese	70	32.0	13.5	452.2
2p		0.16	0.37	0.39

[a] Adjusted for time interval between blood draw and last meal.

were associated with estrogen levels (see "Results," Table 2), age (continuous variable) and BMI (continuous variable) were also included as covariates in all analyses that compared hormone levels between different categories of menstrual and reproductive factors, as well as dietary and other lifestyle factors. All results were essentially identical with or without adjustment for the time interval between last meal and blood draw. In the report, only the results that included time interval as a covariate are shown. For variables with three or more levels, we present *P*s for linear trends using the actual values of the continuous variable of interest. All *P*s quoted are two-tailed. Calculations were performed using the SAS statistical software system (SAS Institute, Cary, NC).

Results

Analysis of E_1, E_2, and androstenedione was conducted on 144 women, ranging in age from 50 to 74 years (mean age, 60.0 years; SD, 7.0 years), who had been menopausal for at least 1 year. Table 1 shows mean levels of E_1, E_2, and androstenedione for all subjects and separately by age, years since menopause, years of education, and the two Chinese dialect groups (see "Patients and Methods"). The three hormones under study did not differ significantly by these demographic factors.

In contrast, Table 2 shows that BMI, age at menarche, parity, and age at first live birth had significant influences on hormone levels. E_1 and E_2 levels were 41% ($P = 0.02$) and 17% higher ($P = 0.34$), respectively, among subjects in the highest BMI category (24+) compared with those in the lowest BMI category (<20). Women who reported menarche at age 17 years or older compared with those who reported menarche at ages 16 years or younger showed lower levels of serum E_2 (9%; $P = 0.25$), E_1 (22%; $P = 0.02$), and androstenedione (29%; $P = 0.05$). The inverse association between age at menarche and levels of androstenedione was no longer statistically significant after adjustment for E_1. Similarly, the inverse association between age at menarche and levels of E_1 was not statistically significant after adjustment for levels of androstenedione. Nulliparous women had higher E_1 and E_2 levels than parous women, although these differences were not statistically significant (data not shown). Nulliparous women ($n = 7$), in combination with those who had a late first live birth (*i.e.*, at age 31 years or older; $n = 12$), displayed higher E_2 (20%; $P = 0.03$) and E_1 (10%; $P = 0.39$) levels than women who had a first live birth at age 30 years or younger. We further investigated hormone levels by subjects' self-reported menarche age (<17 *versus* 17+ years old) and parity status (age at first birth < 31 years old *versus* nulliparous or age at first birth = 31+). E_1 and E_2 levels were lowest among participants who started menstruating late (*i.e.*, ages 17 years or later), intermediate among those who started menstruating earlier (*i.e.*, before age 17 years) but had an early first live birth (*i.e.*, before age 31 years), and highest among those who started menstruating earlier and were nulliparous or had a first live birth at age 31 years or later (Table 2). These differences in E_1 (P for trend = 0.02) and E_2 (P for trend = 0.02) were statistically significant. Although there were relatively few current smokers in this population, they displayed significantly higher E_2 levels (28%; $P = 0.04$) than women who were former smokers ($n = 1$) or had never smoked ($n = 135$) after adjustment for BMI and other covariates.

Table 3 shows significant associations between intake of soy protein and E_1 levels. Specifically, E_1 levels were 15% lower ($P = 0.09$) among individuals in the highest quartile of soy protein intake (27.6 pg/ml) compared with those in the lowest quartile of intake (32.4 pg/ml). Similar patterns of differences in E_1 levels emerged in analyses by intake of isoflavonoids, a main constituent in soy beans ($P = 0.10$; Table 3). However, E_1 levels did not decline in a linear manner with increasing soy intake; an apparent reduction was observed only among those in the highest quartile of soy intake. The differences were strengthened when we compared E_1 values for those reporting the highest quartile of soy protein intake (27.6 pg/ml) with those in the other three quartiles of intake (32.2 pg/ml; $P = 0.047$). Similarly, women with above median intake of total soy showed lower E_1 levels than those with below median intake (28.7 *versus* 33.2 pg/ml; $P = 0.048$). E_1 levels were also

Table 2 Adjusted[a] geometric mean levels of serum E_1, E_2, and androstenedione (pg/ml) by body size, select menstrual and reproductive variables, and cigarette smoking status

Variable	*N*	E_1	E_2	Androstenedione
BMI (kg/m^2)				
<20	17	24.6	11.5	379.1
20−<24	92	29.9	13.4	436.4
24+	35	34.6	13.5	436.9
2p (linear trend)		0.02	0.34	0.59
Height (cm)[b]				
<152	33	30.6	14.1	472.5
152−<160	86	30.0	13.5	395.8
160+	25	31.1	11.2	500.3
2p (linear trend)		0.67	0.01	0.61
Age at menarche (yrs)[b]				
≤12	20	32.1	12.3	472.5
13–14	49	31.4	14.0	454.3
15–16	57	31.1	13.2	435.5
17+	18	24.6	12.0	316.1
2p (17+ *vs.* ≤16)		0.02	0.25	0.05
Age first live birth (yrs)/parous[b]				
≤20	35	30.1	12.6	338.4
21–25	50	30.6	13.0	463.8
26–30	40	29.1	13.0	484.3
31+/nulliparous	19	33.0	15.4	421.5
2p (31+/nulliparous *vs.* ≤30)		0.39	0.03	0.90
Menarche/parity[b]				
Menarche <17/31+/nulliparous	17	35.3	16.0	418.7
Menarche <17/other	109	30.9	13.0	453.2
Menarche 17+	18	24.6	12.1	316.9
2p (linear trend)		0.02	0.02	0.24
Cigarette smoking[b]				
Never/ex-smoker	136	30.2	13.0	432.9
Current smoker	8	31.4	16.7	372.0
2p		0.81	0.04	0.56

[a] Adjusted for time interval between blood draw and last meal, and age.
[b] Further adjusted for BMI.

inversely related to intake of other (*i.e.*, nonsoy) legumes; women with above median intake showed E_1 levels of 28.7 pg/ml compared with 32.7 pg/ml among those with below median intake ($P = 0.07$). When intake of all legumes (soy and nonsoy sources) was considered, mean E_1 levels were statistically significantly different between those with above median intake (28.3 pg/ml) and those with below median intake (33.5 pg/ml; $P = 0.03$). There were, however, no differences in levels of E_2 and androstenedione by soy intake.

Intake of soy (expressed as soy protein, isoflavones, tofu equivalents), nonsoy legumes, and total legumes was not significantly associated with level of education (no formal schooling, primary school, secondary school, or higher), dialect group (Cantonese or Hokkien), BMI (<20, 20 to <24, 24+), age at menarche (≤16 years or 17+ years), and parity/age at first live birth (age at first live birth ≤ 30 years or nulliparous/age at first live birth age = 31+). However, current cigarette smokers had a significantly lower intake of soy protein ($P = 0.006$) than non-cigarette smokers/ex-cigarette smokers, but their intake of nonsoy legumes was not significantly lower ($P = 0.75$).

Table 4 shows hormone levels by other, selective dietary factors including intake of alcohol, coffee, and tea. Hormone levels differed little by self-reported intake of alcohol, but very few women were regular alcohol users in this population. Daily coffee and daily tea drinkers did not differ from nondaily drinkers of these beverages in levels of E_1, E_2, and androstenedione. Table 4 also shows hormone levels by quartiles of intake (expressed in densities) of total fat and fiber, nutrients found to influence endogenous hormone levels in some studies. Concentrations of E_1, E_2, and androstenedione did not differ by intake levels of total fat in this population. This was true even when we compared hormone levels between those in the lowest decile of fat intake (<10% fat calories) and those with higher intakes. Hormone levels also did not differ by intake levels of saturated, monounsaturated, or polyunsaturated fat. There were no differences in E_1, E_2, and androstenedione levels when individuals in the highest quartile of fiber (>10.26 g/day) were compared with those consuming less fiber. There were no significant associations between intake of various micronutrients (including vitamins A, C, and E; calcium; total and specific carotenoids; and isothiocyanates) and serum hormone levels investigated (data not shown). Physical activity (*e.g.*, reported levels of strenuous activity per week and hours of sitting per day) did not significantly influence serum levels of E_1, E_2, and androstenedione (data not shown).

We conducted stepwise regression analysis to identify all significant and independent determinants of serum E_1 and E_2 in this population. Serum E_1 levels were significantly influenced by high BMI ($P = 0.006$), nulliparity/early age at menarche ($P = 0.01$), and soy protein intake ($P = 0.04$). Nulliparity/early age at menarche ($P = 0.03$) and current cigarette smoking ($P = 0.06$) were significantly associated with serum E_2 levels.

Table 3 Adjusted[a] geometric mean levels of serum E_1, E_2, and androstenedione (pg/ml) by daily soy intake

Food/nutrient density	E_1	E_2	Androstenedione
Soy protein (% kcal)			
≤0.9	32.4	13.1	462.8
>0.9 to ≤1.4	33.2	13.5	483.8
>1.4 to ≤2.1	30.9	12.8	392.7
>2.1	27.6	13.9	402.6
2p (>2.1 *vs.* ≤2.1)	0.047	0.40	0.44
Total isoflavonoids (mg/1000 kcal)			
≤6.3	30.9	12.9	439.0
>6.3 to ≤10.6	37.1	14.5	477.8
>10.6 to ≤16.5	29.3	13.0	410.3
>16.5	27.8	13.2	416.7
2p (>16.5 *vs.* ≤16.5)	0.07	0.74	0.68
Total soy products (g/1000 kcal)[b]			
≤40.1	31.9	12.4	487.2
>40.1 to ≤64.7	34.4	14.1	453.5
>64.7 to ≤98.1	29.9	13.5	396.0
>98.1	27.8	13.3	412.5
2p (above *vs.* below median)	0.048	0.92	0.26
Nonsoy legumes (g/1000 kcal)			
≤0.1	30.9	13.2	386.4
>0.1 to ≤1.2	34.6	13.2	541.9
>1.2 to <2.8	29.4	14.3	388.9
≥2.8	27.7	12.4	449.2
2p (above *vs.* below median)	0.07	0.74	0.38
Total legumes index[c]			
≤96.5	33.6	13.3	454.9
>96.5 to ≤145.0	33.4	13.4	465.1
>145.0 to ≤192.0	28.6	13.3	391.7
>192.0	27.9	13.5	424.8
2p (above *vs.* below median)	0.03	0.92	0.34

[a] Adjusted for BMI, time interval between blood draw and last meal, and age.
[b] Weights of all soy products were converted to grams of plain tofu-equivalents before summing (see "Patients and Methods" for details).
[c] This is a unit-free index. Quartiles are selected based on the sum of ranks of intake of soy legumes and nonsoy legumes (see "Patients and Methods" for details).

Table 4 Adjusted[a] geometric mean levels of serum E_1, E_2, and androstenedione (pg/ml) by other dietary factors

Variable	E_1	E_2	Androstenedione
Alcohol use			
No	30.3	13.1	426.8
Monthly or more	29.9	13.9	473.5
2p	0.93	0.66	0.69
Coffee			
<Daily	30.2	13.0	459.5
Daily	30.3	13.2	415.8
2p	0.96	0.78	0.44
Tea			
Occasionally	30.2	13.4	418.0
Weekly	29.7	12.9	414.5
Daily	31.6	12.7	490.0
2p (linear trend)	0.65	0.51	0.32
Total fat (% kcal)			
≤21.9	30.3	13.2	432.9
>21.9 to ≤25.6	32.0	14.6	450.7
>25.6 to ≤29.4	31.0	12.7	444.3
>29.4	29.4	13.1	403.9
2p (linear trend)	0.49	0.57	0.64
Saturated fat (% kcal)			
≤7.2	32.7	13.3	461.4
>7.2 to ≤8.9	28.5	13.2	395.7
>8.9 to ≤10.7	32.4	14.7	440.2
>10.7	28.8	12.7	435.5
2p (linear trend)	0.38	0.60	0.64
Monounsaturated fat (% kcal)			
≤7.2	29.5	12.9	421.6
>7.2 to ≤8.6	31.9	14.1	449.5
>8.6 to <10.0	31.7	14.2	414.8
>10.0	30.1	12.6	451.7
2p (linear trend)	0.97	0.70	0.76
Polyunsaturated fat (% kcal)			
≤4.0	33.3	13.9	453.9
>4.0 to ≤4.9	29.9	13.2	490.6
>4.9 to ≤6.3	31.8	13.9	436.3
>6.3	28.8	12.8	383.3
2p (linear trend)	0.46	0.44	0.39
Total fiber (g/1000 kcal)			
≤6.8	34.2	14.4	434.3
>6.8 to ≤8.4	28.0	12.5	452.2
>8.4 to ≤10.3	30.3	13.0	430.3
>10.3	30.3	13.6	422.4
2p (linear trend)	0.48	0.85	0.91

[a] Adjusted for BMI, time interval between blood draw and last meal, and age.

Discussion

Historically, there existed about a 6-fold difference in breast cancer rates between high-risk whites in the West and low-risk Asians in Japan and China. However, this large variation in risk is not due to underlying genetic differences because breast cancer rates in Asian Americans shift substantially toward those of American whites (8). Moreover, between the 1970s and 1990s, breast cancer incidence more than doubled in Singapore and Japan (20). Reasons for the recent increase in these traditionally low-risk Asian groups are not known. The identification of determinants of plasma estrogens and androstenedione levels in this cross-sectional study of Singaporean Chinese women provided some insights regarding the underlying causes of this increasing trend in breast cancer.

There has been tremendous interest to determine the role of soy in the etiology of breast cancer since Lee *et al.* (29, 30) first reported a reduced risk of breast cancer in association with high soy intake among Chinese women in Singapore. However, results from epidemiological studies (31) and dietary intervention studies (19, 32) are not all consistent. Results from this cross-sectional study support the hypothesis that high soy intake may reduce the risk of breast cancer by lowering endogenous estrogen levels, particularly E_1 levels, that cannot be explained by other determinants of E_1 (*i.e.*, BMI, age at menarche, and parity) in this population. Although soy intake may be a marker of other aspects of a traditional Asian lifestyle that is associated with lower endogenous E_1 levels, few other compelling candidates (dietary or nondietary factors) exist. In a cross-sectional study of premenopausal women (postmenopausal women were not included) in Japan, intake of soy products was significantly inversely correlated with serum E_2 levels after controlling for age, BMI, cycle length, and total energy (E_1 was not measured; Ref. 18). However, in a cross-sectional study of pre- and postmenopausal British women, soy milk intake was not associated with circulating E_2 levels, but E_1 levels were not measured in this study (19).

Five soy intervention studies that included postmenopausal women offered some information on effects of short-term soy intake on circulating hormone levels. These studies compared circulating estrogen levels before and after soy supplementation (33) or between subjects randomized to a soy diet and those randomized to a control diet without soy (34–37). In three studies, circulating E_2 levels did not change in association with soy supplementation, but E_1 levels were not measured

(33–35). In a fourth study, levels of total E_2 were unchanged, whereas serum levels of E_1 and unbound E_2 (based on the ratio of E_2 to levels of sex hormone-binding globulin levels) were reduced in association with soy supplementation; the latter result was statistically significant (37). In a randomized crossover design study, plasma levels of E_1, E_1-sulfate, and E_2 decreased between 6% and 12% in association with supplementation of a "high" soy diet (132 mg isoflavones/day) compared with a "control" diet (7 mg isoflavones/day). The reduction in E_1-sulfate levels was statistically significant (36). Furthermore, in association with "high" soy intake (38), the same study participants showed a significant 18% reduction in urinary excretion of 4-hydroxylated E_1, a proposed genotoxic estrogen metabolite (39). In the same study, subjects consuming a "low" soy diet (65 mg isoflavones/day) also showed significantly lower (16%) levels of hydroxylated E_1 compared with individuals consuming the control diet (38), but blood estrogen levels were not significantly changed (36).

As in studies of hormonal responses to soy supplementation in premenopausal women (32), interpretation of results from this cross-sectional study of postmenopausal women is limited by our lack of understanding of the relative importance of the source of and amount of soy isoflavones. In addition, whereas E_2 is the most biologically active estrogen, E_1 has about 25% of the estrogenic activity of E_2 and is the predominant estrogen in postmenopausal women (40). Thus, measurements of both estrogens are preferred to provide a more complete assessment of the hormonal effects of soy in postmenopausal women. Our findings of a reduction in E_1 levels in association with soy intake may represent a reduction in the production and/or an increase in the elimination of E_1. There are supportive data from *in vitro* studies in which physiological concentrations of genistein showed inhibitory effects on 17β-hydroysteroid oxidoreductase type 1, an enzyme that is essential in the interconversion of E_1 to E_2 in adipose and other tissues (41). The reduction in E_1 levels may also reflect an inhibition in the aromatase enzyme, responsible for the conversion of androstenedione to E_1 in peripheral tissues. *In vitro* studies have shown an inhibitory effect of isoflavones on the aromatase enzyme (42), which may explain the lower E_1 levels in association with high soy intake. The results of Xu *et al.* (38) suggest that soy also may exert protection against breast cancer by modulating estrogen metabolism via reduced production of potentially more carcinogenic estrogen metabolites.

Fiber intake, *per se*, was not associated with serum E_1, E_2, and androstenedione levels (Table 4), but high intake of legumes, from soy and nonsoy foods, was associated with significant reductions in E_1 levels in this population (Table 3). High intake of legumes may be a marker of high intake of fiber or of a diet rich in plant foods (43). Although high intake of legumes and/or fiber may have a role in breast cancer, there is currently little direct support for this possibility from analytic epidemiological studies of breast cancer (43).

In our study, intake of dietary fat was not significantly associated with serum E_1, E_2, and androstenedione levels, consistent with most of the cross-sectional studies on dietary fat and endogenous estrogen levels conducted in Western populations. These previous studies have found either no association (10, 12) or even an inverse association (17) between estrogen levels and fat intake. The dietary fat intake levels in our population are substantially lower than those reported in Western populations and are more similar to those in Japan. However, our finding contrasts with results from a study of premenopausal women in Japan, in which E_2 levels increased significantly with increasing fat intake (Ref. 16; postmenopausal women were not included in that study). Results from this cross-sectional study are also not supportive of pooled results from dietary fat intervention studies. In a meta-analysis of 13 dietary intervention studies, E_2 levels were significantly reduced by 10% after approximately 2 months of participation in the low-fat intervention diet (range, 10–25% fat calories) compared with preintervention fat intake (range, 29–46% fat calories; Ref. 44).

There is now accumulating evidence that high intake of alcohol may be associated with increasing levels of circulating estrogens (9, 13, 45). This question could not be addressed adequately in this population because of the low prevalence of alcohol use (Table 4). Intake of coffee and tea (primarily black tea) is prevalent in Singapore, but we observed no association between hormone levels and intake pattern of either beverage. Caffeine intake was not associated with serum E_1 levels in two other studies conducted among peri- and postmenopausal women (10, 12). However, in the study conducted by London *et al.* (10), levels of the percentage of free E_2 (but not total E_2) were significantly inversely associated with intake of caffeine. Among premenopausal Japanese women, serum E_2 levels were significantly inversely associated with green tea intake, whereas the inverse association with total caffeine intake was not statistically significant (46). Intake of green tea is uncommon among Chinese in Singapore and was not evaluated in this analysis.

Serum E_2 levels increased significantly among current smokers in this population, even after adjustment for BMI, menarche age, parity, and education. Many studies in diverse populations have investigated the relationship between cigarette smoking and risk of breast cancer (47, 49–54) and the relationship between cigarette smoking and endogenous estrogens (9, 48). Results have been inconsistent, in fact, even the direction of the relationships is uncertain. Since active smoking is uncommon ($<$6%) among women in our target population, those who smoke may have more westernized lifestyles. In other words, the observed association between active smoking and E_2 levels in the present study is likely an indirect one.

BMI had a substantial and significant influence on endogenous E_1 and E_2 levels among Singaporean Chinese, consistent with previous studies conducted in Caucasian populations (9, 11, 13, 55, 56). In this and previous studies, increases in estrogen levels were observed among women with high BMI (*i.e.*, $>$25). Interestingly, in this population, higher levels of E_1 and E_2 were also found among subjects with intermediate BMI (*i.e.*, 21–24) compared with those with lower BMI. Although E_1 was not significantly associated with BMI among postmenopausal women in Japan (14), height, as a marker of childhood/adolescent nutrient, was positively associated with E_1 levels in that study. High body size has been consistently associated with elevated breast cancer risk in studies conducted in China (57–59), Taiwan (53), Singapore (30, 52), and Japan (49–51, 60, 61) and among Asian Americans (62, 63). In addition, there is a suggestion that weight change (particularly weight increases) has a profound influence on breast cancer rates in Asian-American women (63).

Early age at menarche and nulliparity are well-established risk factors for breast cancer (1). Compatible with this observation, we found lower E_1 levels among individuals who started menstruation late (age 17 years or older) compared with those who started earlier. Nulliparous women and those with late age at first birth (31+ years) showed higher levels of E_1 and E_2 compared with parous women with an earlier age at first birth. When menarche and parity were considered together, women with late menarche (17+ years) showed the lowest E_1 and E_2

levels, whereas women with earlier menarche (16 years or earlier) and who were nulliparous or had a late first birth (after age 30 years) showed the highest E_1 and E_2 levels. These differences were highly significant. Although other studies have not consistently found age at menarche and parity to influence circulating estrogen levels (reviewed in Ref. 9), there are some supportive data. For example, in a cross-sectional study of American white adult women, there was a significant trend of decreasing estrogen levels with increasing age at menarche (11). In a follow-up study of Finnish girls, those who started menstruating at age 12 years or younger showed significantly higher levels of circulating E_2 levels in their 20s and 30s compared with girls who started menstruating at age 13 years or later. These results suggest that earlier onset of menstrual cycles is related to more intensive exposure to estrogen and a persistent effect of age at menarche on estrogen concentrations (64). Higher E_2 levels in nulliparous women compared with their parous sisters have been reported (65). Although menarche at age 17 years or later is infrequent even among native Asians today (66), this was a common phenomenon only 30 years ago (67). The dramatic global secular decline in age at menarche (66, 68), delay in childbearing and an increasing prevalence of nulliparity among Asian women (66, 68), and changes in diet (*i.e.*, reduction in soy intake) are likely contributors to the increasing trend in breast cancer in these regions.

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