British Journal of Nutrition (2014), **112**, 976–983
doi:10.1017/S0007114514001780

Dietary isoflavone intake is not statistically significantly associated with breast cancer risk in the Multiethnic Cohort

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(Submitted 8 January 2014 – Final revision received 6 June 2014 – Accepted 10 June 2014 – First published online 24 July 2014)

Abstract

Given the high intake levels of soya and low incidence rates of breast cancer in Asian countries, isoflavones, substances with an oestrogen-like structure occurring principally in soyabeans, are postulated to be cancer protective. In the present study, we examined the association of dietary isoflavone intake with breast cancer risk in 84 450 women (896 *in situ* and 3873 invasive cases) who were part of the Multiethnic Cohort (Japanese Americans, whites, Latinos, African Americans and Native Hawaiians) with a wide range of soya intake levels. The absolute levels of dietary isoflavone intake estimated from a baseline FFQ were categorised into quartiles, with the highest quartile being further subdivided to assess high dietary intake. The respective intake values for the quartiles (Q1, Q2, Q3, and lower and upper Q4) were 0–<3·2, 3·2–<6·7, 6·7–<12·9, 12·9–<20·3, and 20·3–178·7 mg/d. After a mean follow-up period of 13 years, hazard ratios (HR) and 95% CI were calculated using Cox regression models stratified by age and adjusted for known confounders. Linear trends were tested by modelling continuous variables of interest assigned the median value within the corresponding quartile. No statistically significant association was observed between dietary isoflavone intake and overall breast cancer risk (HR for upper Q4 *v.* Q1: 0·96 (95% CI 0·85, 1·08); *P* trend=0·40). While the test for interaction was not significant (*P*=0·14), stratified analyses suggested possible ethnic/racial differences in risk estimates, indicating that higher isoflavone intakes may be protective in Latina, African American and Japanese American women. These results are in agreement with those of previous meta-analyses showing no protection of isoflavones at low intake levels, but suggesting inverse associations in populations consuming high amounts of soya.

Key words: Isoflavones: Breast cancer: Ethnicity: Prospective cohorts

Breast cancer remains the second leading cause of cancer death among women in the USA[1]. Despite advances in prevention and control, the incidence of breast cancer varies considerably by race/ethnicity; compared with non-Hispanic white women, Asian women generally have lower age-adjusted incidence rates in the USA[2]. Some of these differences can be attributed to dietary factors, such as soya food consumption in Asian populations. Despite the fact that intake is measured in multiple ways across studies, several meta-analyses[3–6] have reported a modest protective effect of higher soya intake on breast cancer risk in Asian women. Soyabeans are a rich source of isoflavones, which are hypothesised to be natural oestrogen receptor (ER) modulators that possess both oestrogen-like and anti-oestrogenic properties[7]. Although the consumption of soya foods in the USA is on the rise, differences in intake, as well as in breast cancer incidence rates, remain between populations that traditionally consume high amounts of soya foods and women of other ethnicities[8]. Many epidemiological studies[7,9,10] on soya intake and breast cancer risk have involved Asian populations that consume traditional soya foods including tofu, fermented soyabean paste and sprouts. A small number of case–control studies[11–15] have examined the association of dietary isoflavone intake with breast cancer

Abbreviations: ER, oestrogen receptor; HR, hazard ratios; MEC, Multiethnic Cohort Study.

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risk in Asian women according to hormone receptor status, and some of these have suggested differential influences by ER status, pointing to possible limited protection in ER+ subtypes. The present study examined the association of dietary isoflavone intake with breast cancer risk in *in situ* and invasive cases, as well as by ER status, and in Japanese American, white, Latina, African American and Native Hawaiian women using population-based, observational data from the Multiethnic Cohort Study (MEC).

Materials and methods

Study population

The MEC was established to investigate the association of lifestyle and genetic factors with chronic disease. Details of study design, recruitment methods, response rates and baseline characteristics have been published elsewhere[16]. Briefly, the cohort consists of 215 251 men and women who were aged between 45 and 75 years at the time of recruitment and were selected from five racial/ethnic populations: Japanese Americans; whites; Latinos; African Americans; Native Hawaiians. Potential participants were identified through drivers' licence files from the Department of Motor Vehicles, voter registration lists and Health Care Financing Administration data files primarily from Los Angeles County, California and the state of Hawaii. The participants completed a mailed twenty-six-page questionnaire in 1993–6 that included questions on demographic and lifestyle factors such as physical activity, cigarette smoking status, diet, anthropometric measures, personal history of medical conditions and family history of cancer, as well as reproductive history and exogenous hormone use only among women[16,17]. Individuals who reported more than one ancestry were assigned to one of the categories according to the following priority ranking: African America; Native Hawaiian; Latino; Japanese American; white. The institutional review boards of the University of Southern California and the University of Hawaii approved the study protocol.

Dietary isoflavone intake assessment

The baseline survey included a FFQ with questions on participants' average use and serving size of specific foods during the previous year. The participants could select the following options for questions on the frequency of consumption: never or hardly ever; 1 time/month; 2–3 times/month; 1 time/week; 2–3 times/week; 4–6 times/week; 1 time/d; ≥2 times/d. For many items, photographs of three different plated portions were provided to aid with serving size estimation. A calibration study indicated acceptable correlations (Pearson's r 0·50 for total isoflavones) between FFQ and 24 h recall-based dietary data[17] and estimated the percentages of women classified into opposite quartiles of absolute intake of isoflavones as 9 % in the highest quartile (Q4) for the FFQ and the lowest quartile (Q1) for the 24 h recalls and 2 % in Q1 for the FFQ and Q4 for the 24 h recalls. The respective values for nutrient density were 8 and 1 %. Total soya product consumption included self-reported intake of tofu, miso and vegetarian meats, which constituted the majority of soya foods according to the 24 h recalls that were performed when the FFQ was developed. A few additional foods not included in the FFQ, such as soya milk, green soyabeans and soyabean sprouts, accounted for only 6 % of total isoflavone intake reported in the 24 h recalls. Daily isoflavone intake (mg/d) was calculated using a food composition table and based on the intake of tofu, miso and vegetarian meats, as well as mixed dishes containing ingredients derived from soyabeans. For example, a 'stir-fried beef or pork and vegetables or fajitas' category made up of a combination of recipes, some with soya, was assigned a default isoflavone value. As the concentrations of isoflavones are very low in other food sources, their contribution to total intake can be considered negligible. Measurement error-corrected isoflavone densities per 4184 kJ were also calculated, but the results were not substantially different and, therefore, are not reported.

Exclusion criteria

Women were excluded if they did not belong to one of the five major ethnic groups listed above (n 8050), if they had missing or invalid dietary data (n 4611) or if they were diagnosed with breast cancer before the date of the baseline questionnaire administration (n 4787). Women with missing menopausal status data but who were aged ≥55 years at the time of recruitment were coded as postmenopausal (n 7358 recoded as postmenopausal). Otherwise, women with missing menopausal status data (n 1694) were excluded. Women with missing covariate data (n 15 176), i.e. age at menarche, oral contraceptive use, menopausal hormone use, BMI, age at first live birth, parity and years of education, were also excluded. After exclusion of these cases, 84 550 women were available for analysis.

Follow-up and case identification

The follow-up of participants began at the completion of the baseline questionnaire (or at 45 years of age for the few individuals who were not yet 45 years old) and continued to the earliest of the following endpoints: (1) diagnosis of breast cancer, (2) death or (3) end of follow-up (31 December 2007). All incident cases of *in situ* or invasive breast cancer (International Classification of Diseases for Oncology (ICD-O-3) code C50) were identified through record linkage to the Hawaii Tumor Registry, the Cancer Surveillance Program for Los Angeles County and the California State Cancer Registry; all cancer registries are part of the Surveillance, Epidemiology and End Results (SEER) programme of the National Cancer Institute. Deaths within the cohort were determined through annual linkage to state death certificate files in California and Hawaii and periodically to the National Death Index. During a mean follow-up period of 12·5 years (median = 13·7 years), a total of 4769 women with incident *in situ* breast cancer (n 896) and invasive breast cancer (n 3873) were identified.

Statistical analyses

The absolute levels of dietary soya isoflavone intake (mg/d) were categorised into quartiles (Q1–Q4) for the present analysis. In addition, the highest-intake quartile category was divided into lower and upper Q4 to evaluate the small group of women who reported very high soya intake. The range of 28·0–160·8 mg/d in the upper Q4 category was similar to intake levels (26–54 mg/d) described for Japanese populations[8] and to median values of the highest-intake categories reported in previous studies of Asian populations (>20 mg/d)[4]. Because the distribution of soya intake levels varied substantially across ethnic groups, ethnicity-specific quartiles for each group were created for stratified analyses. The characteristics of women at cohort entry were compared across quartiles. As dietary sources of isoflavones included not just soya but also other legumes, Pearson's correlation coefficients were computed between soya food consumption and total isoflavone intake across ethnic groups to evaluate the differences in the proportion of dietary isoflavones provided by soya food intake. Hazard ratios (HR) and 95% CI were calculated using Cox proportional-hazards models. The lowest-intake quartile group served as the reference in all models. Age was the underlying time variable in the Cox regression model, starting with a participant's age at cohort entry to the earliest of the three endpoints mentioned above. Cox regression models included ethnicity (Japanese American, white, Latina, African American and Native Hawaiian) as a stratum variable and adjusted for age at cohort entry (continuous), BMI (<20, 20–<25, 25–<30, or ≥30 kg/m^2), age at menarche (≤12, 13–14, or ≥15 years), age at first live birth (no children, ≤20, 21–30, or ≥31 years), parity (nulliparous, 1, 2–3, or ≥4 children), menopausal status (pre- or postmenopausal or age ≥55 years at cohort entry), education (≤12 or >12 years), oral contraceptive use (never or ≥1 month), menopausal hormone use (no current use/premenopausal, past oestrogen use, current oestrogen use/no progesterone use, or current oestrogen use with current/past progesterone use), family history of breast cancer (no or yes), total energy intake (log-transformed continuous), alcohol consumption (0 or >0 g/d), smoking status (never, past or current), diabetes (no or yes) and hypertension (no or yes). Although protective in some studies, physical activity was not included as a covariate in the present analysis because its inclusion did not materially change the risk estimates. Linear trends were tested by modelling the median values of the corresponding quartile as continuous variables.

Analyses were conducted for all cases and stratified by *in situ* and invasive behaviour. Heterogeneity in the risk estimates across ethnic groups, as well as Japanese *v.* other ethnic groups combined, was tested using a global Wald test of the cross-product terms for ethnicity and the ethnicity-specific trend variable of total intake or density of dietary isoflavones (assigning the ethnicity-specific median values within the corresponding quartiles). Heterogeneity in the risk estimates by ER status was evaluated using a competing risk model that compared proportional-hazards models of ER+ and ER− breast cancer cases by score test[18]. ER− breast cancer cases were censored at their diagnosis age in the ER+ model and ER+ cases were similarly censored in the ER− model. Subgroup analyses were also conducted by BMI status (<25 *v.* ≥25 kg/m^2) and alcohol consumption (0 *v.* >0 g/d) and for each of the five racial/ethnic groups. In sensitivity analyses, events were restricted to invasive cases, censoring *in situ* cases at the time of death or at the end of follow-up, or to only postmenopausal women. Breast cancer cases diagnosed within 2 or 4 years of cohort entry were also excluded. All statistical analyses were carried out in SAS version 9.3 (SAS Institute) with a two-sided P value <0·05 being considered statistically significant.

Results

In the present study population, values for the quartiles (Q1, Q2, Q3, and lower and upper Q4) of dietary isoflavone intake at cohort entry were 0–<3·2 (median: 1·7), 3·2–<6·7 (4·8), 6·7–<12·9 (9·1), 12·9–<20·3 (16·0), and 20·3–178·7 (29·6) mg/d, respectively (Table 1). Women differed significantly in demographic and reproductive characteristics, as well as in medical history, across quartiles of dietary isoflavone intake. Women who were Japanese American, Native Hawaiian or Latina tended to have higher daily intakes of isoflavones, as did women who were underweight or of normal weight, had never used oral contraceptives, had ≤12 years of education, had never consumed alcohol or smoked cigarettes, or had been diagnosed with diabetes. On the other hand, women who were obese tended to have lower intakes of isoflavones, as did women who were nulliparous. Across ethnic groups, total soya intake from tofu, miso and vegetarian meats was highly correlated with total isoflavone intake in Japanese American women (r 0·83; P<0·0001), reflecting their isoflavone intake primarily from these soya foods, followed by that in Native Hawaiian (r 0·80; P<0·0001) and white (r 0·67; P<0·0001) women. Correlations were lower in African American (r 0·37; P<0·0001) and Latina (r 0·23; P<0·0001) women, indicating lower reporting of intakes of these individual soya foods and higher consumption of mixed dishes. Japanese American women reported much higher soya intakes from the three types of soya foods included in the baseline FFQ (27 g/d) than the other groups. They mostly ate tofu and miso comprising 99% of the self-reported soya food intake (g/d), whereas the respective values were 97, 84, 70 and 64% for Native Hawaiian, white, Latina and African American women, respectively.

In multivariable models adjusted for all covariates (Table 2), no association between quartiles of absolute dietary isoflavone intake and overall breast cancer risk was observed, with a HR of 0·96 being detected for the highest-intake quartile category (P trend=0·36). Similar results were obtained in sensitivity analyses restricted to postmenopausal women (P trend=0·56) or to the 3873 cases of invasive breast cancer (P trend=0·24). Exclusion of cases diagnosed within 2 or 4 years of cohort entry or stratification of women by BMI category or alcohol consumption did not materially alter the results.

No heterogeneity in risk estimates was detected by ethnicity (P interaction=0·14) or ER status (P interaction=0·90).

Table 1. Characteristics of women at cohort entry by quartiles (Q1–Q4) of isoflavone intake*

Characteristics	Q1	Q2	Q3	Lower Q4	Upper Q4	*P*
n	21 137	21 138	21 138	10 568	10 569	
Dietary isoflavone intake (mg/d)						
Median	1·7	4·8	9·1	16·0	29·6	
Range	0·0–<3·2	3·2–<6·7	6·7–<12·9	12·9–<20·3	20·3–178·7	
Ethnicity						<0·0001
Japanese American (*n* 23 890)	5·0	20·4	38·4	49·0	49·6	
White (*n* 21 758)	43·1	29·3	19·4	13·1	9·0	
Latina (*n* 16 725)	19·2	19·4	18·0	19·5	25·6	
African American (*n* 15 872)	29·5	23·6	14·6	8·8	6·2	
Native Hawaiian (*n* 6305)	3·3	7·3	9·6	9·7	9·5	
BMI (kg/m^2)						<0·0001
<20	6·8	7·2	8·4	10·2	10·3	
20–<25	36·6	37·9	39·8	41·2	40·8	
25–<30	32·1	31·7	31·0	30·3	29·6	
≥30	24·5	23·2	20·8	18·4	19·3	
Menopausal status						<0·0001
Premenopausal	14·1	16·4	17·4	15·0	13·8	
Postmenopausal†	85·9	83·6	82·6	85·0	86·2	
Age at menarche (years)						<0·0001
≤12	51·6	51·1	50·2	49·2	44·9	
13–14	37·6	38·1	38·4	38·2	39·2	
≥15	10·8	10·8	11·4	12·6	15·9	
Parity						<0·0001
No children	14·8	12·6	11·9	12·2	12·2	
1 child	12·6	12·0	10·8	10·3	10·1	
2–3 children	43·4	44·6	46·8	46·1	43·3	
≥ 4 children	29·3	30·9	30·6	31·4	34·4	
Age at first live birth (years)						<0·0001
No children	14·8	12·6	11·9	12·2	12·2	
≤20	32·4	31·5	27·1	24·1	24·7	
21–30	47·3	49·6	54·1	56·1	55·2	
≥31	5·5	6·4	6·9	7·7	8·0	
Oral contraceptive use						<0·0001
Never	53·0	53·2	56·6	61·1	65·6	
Ever (at least 1 month)	47·1	46·8	43·4	38·9	34·4	
Menopausal hormone therapy						<0·0001
No current use or premenopausal	52·1	53·6	54·0	53·2	55·5	
Past oestrogen use	17·1	16·3	15·1	16·4	15·2	
Current oestrogen use with no progesterone use	14·6	13·9	13·8	12·9	13·5	
Current oestrogen use with current/past progesterone use	16·2	16·2	17·1	17·5	15·9	
Family history of breast cancer						<0·0001
No	88·1	89·1	88·8	88·6	90·1	
Yes	11·9	10·9	11·2	11·4	9·9	
Education (years)						<0·0001
≤12	40·0	41·3	43·7	46·5	51·5	
>12	60·0	58·7	56·3	53·5	48·5	
Alcohol consumption (g/d)						<0·0001
0	54·9	57·7	62·7	66·8	69·7	
>0	45·1	42·3	37·4	33·2	30·3	
Smoking						<0·0001
Never	49·2	51·9	56·9	62·2	64·2	
Past	33·2	32·3	29·3	26·6	26·0	
Current	17·7	15·9	13·8	11·2	9·9	
Diabetes, yes	9·9	9·8	10·5	11·3	12·3	<0·0001
Hypertension, yes	38·0	37·4	37·25	37·2	37·3	0·52

* Data are presented as percentages within each quartile category or means and standard deviations, except for dietary isoflavone intake, which is reported as median and range. Percentages may not add to 100 due to rounding. Univariate comparisons across quartile categories were made using χ^2 test (categorical variables) or ANOVA (continuous variable).
† Postmenopausal or age ≥55 years at baseline.

In additional stratified analyses by ethnicity, no statistically significant associations were observed between dietary isoflavone intake and overall breast cancer risk (Table 3). Among Japanese American women who had higher median isoflavone intakes than other ethnic groups across quartiles (Table 3), an inverse association between high absolute isoflavone intake and overall breast cancer risk was close to statistical significance (HR for upper Q4 *v.* Q1: 0·86 (95 % CI 0·70, 1·05); *P* trend=0·06). However, no statistically significant heterogeneity (*P* interaction=0·90) was observed in the comparison of Japanese *v.* other ethnic groups combined. Similar non-significant inverse associations between the

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Table 2. Risk of breast cancer by quartiles (Q1–Q4) of dietary isoflavone intake*
(Hazard ratios (HR) and 95 % confidence intervals)

	Total isoflavone intake (mg/d)		All (*in situ* and invasive) cases (*n* 4769)			Postmenopausal women only (*n* 4112)			Invasive breast cancer cases only (*n* 3873)			ER+ cases only (*n* 2393)†			ER− cases only (*n* 625)†		
	Median	Range	Cases (*n*)	HR	95 % CI	Cases (*n*)	HR	95 % CI	Cases (*n*)	HR	95 % CI	Cases (*n*)	HR	95 % CI	Cases (*n*)	HR	95 % CI
Q1	1·7	0·0–<3·2	1162	1·00	–	1009	1·00	–	975	1·00	–	609	1·00	–	155	1·00	–
Q2	4·8	3·2–<6·7	1174	0·99	0·91, 1·08	1014	1·01	0·92, 1·11	958	0·97	0·88, 1·06	607	0·99	0·88, 1·11	162	1·07	0·85, 1·34
Q3	9·1	6·7–<12·9	1242	1·00	0·92, 1·10	1048	1·01	0·92, 1·12	984	0·96	0·87, 1·07	596	0·95	0·83, 1·07	155	1·07	0·84, 1·37
Lower Q4	16·0	12·9–<20·3	605	0·96	0·86, 1·07	520	0·96	0·85, 1·08	488	0·92	0·81, 1·05	291	0·90	0·77, 1·06	81	1·16	0·85, 1·57
Upper Q4	29·6	20·3–178·7	586	0·96	0·85, 1·08	521	0·98	0·86, 1·12	468	0·92	0·81, 1·05	290	0·94	0·80, 1·12	72	1·06	0·76, 1·47
P trend*				0·40			0·56			0·24			0·44			0·77	

ER, oestrogen receptor
*HR and 95 % CI adjusted for ethnicity (Japanese American, White, Latina, African American and Native Hawaiian included as a strata variable), age at cohort entry (continuous), BMI (<20, 20–<25, 25–<30, or ≥30 kg/m^2), age at menarche (≤12, 13–14, or ≥15 years), age at first live birth (no children, ≤20, 21–30, or ≥31 years), parity (nulliparous, 1, 2–3, or ≥4 children), menopausal status (premenopausal or postmenopausal or age ≥55 years at cohort entry), years of education (≤12 or >12 years), oral contraceptive use (never or ≥1 month), menopausal hormone use (no current use or premenopausal, past oestrogen use, current oestrogen use with no progesterone use, or current oestrogen use with current/past progesterone use), family history of breast cancer (no or yes), total energy intake (log-transformed continuous), alcohol consumption (0 or >0 g/d), smoking status (never, past or current), diabetes (no or yes) and hypertension (no or yes). Linear trends were tested by modelling continuous variables of interest assigned the median value within the corresponding quartile. Linear trends were tested by modelling continuous variables assigned the median value within the corresponding quartile.
†ER status + or −.

highest-intake quartile category and overall breast cancer risk were also observed in Latina women (HR for upper Q4 *v.* Q1: 0·89 (95 % CI 0·65, 1·21); *P* trend=0·41) and African American women (HR for upper Q4 *v.* Q1: 0·87 (95 % CI 0·68, 1·12); *P* trend=0·22). Interestingly, Native Hawaiian women in the highest-total isoflavone intake group had a non-significant increase in breast cancer risk (HR for the upper Q4 *v.* Q1: 1·45 (95 % CI 1·02, 2·07); *P* trend=0·10). Moreover, there was a significant positive dose–response relationship between daily isoflavone intake and breast cancer risk in the forty-four ER− breast cancer cases among Native Hawaiian women (HR for upper Q4 *v.* Q1: 3·87 (95 % CI 1·30, 11·54); *P* trend <0·01). This relationship was attenuated when modelling nutrient density for isoflavone intake (*P* trend=0·04; data not shown), despite showing similar risk estimates across quartile categories (HR for upper Q4 *v.* Q1: 3·06 (95 % CI 1·15, 8·13)). In Latina women, while total isoflavone intake and overall breast cancer risk were not significantly associated, a lower risk of ER+, but not of ER−, breast cancer was observed with a higher density of dietary isoflavones (*P* trend=0·02 and 0·68, respectively). No associations with ER+/ER− cancers were observed in Japanese American women or in any of the other ethnic groups.

Discussion

In this prospective cohort study conducted in a multiethnic population with a broad range of dietary intake levels, we found no statistically significant association between isoflavone intake and overall breast cancer risk. Despite the oestrogen-like structure of isoflavones, these null findings persisted irrespective of ER status or when restricted to invasive breast cancer cases. Although the analyses did not demonstrate a statistically significant interaction with ethnicity, stratified analyses indicated possible ethnic differences in risk estimates, in particular, a weak protective association of higher isoflavone intakes in some ethnic groups. These results are in agreement with those of previous meta-analyses showing no association in white populations with low soya intakes but protective effects in Asian populations consuming high amounts of soya[4,5].

Although individual studies have reported conflicting results, four meta-analyses[3–6] of mostly case–control and prospective studies across countries and regions generally have suggested a protective effect of soya consumption on breast cancer risk in both pre- and postmenopausal women, especially among Asian women consuming high amounts of soya; however, one earlier meta-analysis[3], when restricted to Asian women, did not support this conclusion. In the present study among mostly (>80 %) postmenopausal women with varying soya intake levels, no statistically significant association was detected after 13 years of follow-up as well as no heterogeneity in risk estimates across racial/ethnic groups was detected. Whereas we failed to observe a statistically significant association using FFQ-based dietary intake data, in a case–control study[19] nested within the subset of MEC participants with urine samples, a lower breast cancer risk was observed in postmenopausal women with higher urinary prediagnostic isoflavone excretion levels. In a comparison of 251 breast cancer cases and

Table 3. Risk of breast cancer (*in situ* and invasive) by ethnicity-specific quartiles (Q1–Q4) of dietary isoflavone intake*
(Hazard ratios (HR) and 95 % confidence intervals)

	Japanese American (*n* 23 890)					White (*n* 21 758)					Latina (*n* 16 725)				
	Median	Range	Cases (*n*)	HR	95 % CI	Median	Range	Cases (*n*)	HR	95 % CI	Median	Range	Cases (*n*)	HR	95 % CI
Total isoflavone intake (mg/d)															
Q1	5·0	0·1–<7·1	359	1·00	–	0·9	0·0–1·8	312	1·00	–	1·7	0·0–<3·2	194	1·00	–
Q2	9·0	7·1–<11·4	427	1·16	1·00, 1·33	2·8	1·8–<3·9	315	1·05	0·90, 1·23	4·8	3·2–<7·0	175	0·92	0·75, 1·14
Q3	14·5	11·4–<18·9	386	1·02	0·88, 1·19	5·4	3·9–<7·7	324	1·10	0·94, 1·29	9·9	7·0–<14·7	161	0·88	0·70, 1·10
Lower Q4	22·4	18·9–<28·0	212	1·12	0·93, 1·34	9·2	7·7–<11·8	146	1·02	0·83, 1·25	18·6	14·7–<24·0	71	0·80	0·60, 1·07
Upper Q4	36·8	28·0–160·8	163	0·86	0·70, 1·05	17·3	11·8–114·1	148	1·06	0·86, 1·30	34·8	24·0–178·7	74	0·89	0·65, 1·21
P trend*			0·06					0·78					0·41		
P heterogeneity†											0·14				

	African American (*n* 15 872)					Native Hawaiian (*n* 6305)				
	Median	Range	Cases (*n*)	HR	95 % CI	Median	Range	Cases (*n*)	HR	95 % CI
Total isoflavone intake (mg/d)										
Q1	1·1	0·0–<2·0	238	1·00	–	3·4	0·1–<5·2	96	1·00	–
Q2	3·0	2·0–<4·2	207	0·86	0·71, 1·03	7·0	5·2–<9·2	109	1·14	0·86, 1·50
Q3	5·5	4·2–<7·5	225	0·92	0·76, 1·11	11·6	9·2–<15·4	124	1·26	0·95, 1·67
Lower Q4	8·9	7·5–<11·3	80	0·65	0·50, 0·84	18·4	15·4–<23·7	47	0·95	0·66, 1·38
Upper Q4	17·4	11·3–112·3	107	0·87	0·68, 1·12	33·5	23·7–165·8	69	1·45	1·02, 2·07
P trend*			0·22					0·10		
P heterogeneity†										

* HR and 95 % CI adjusted for ethnicity (Japanese American, white, Latina, African American and Native Hawaiian included as a strata variable), age at cohort entry (continuous), BMI (<20, 20–<25, 25–<30, or ≥30 kg/m^2), age at menarche (≤12, 13–14, or ≥15 years), age at first live birth (no children, ≤20, 21–30, or ≥31 years), parity (nulliparous, 1, 2–3, or ≥4 children), menopausal status (premenopausal or postmenopausal or age ≥55 years at cohort entry), years of education (≤12 or >12 years), oral contraceptive use (never or ≥1 month), menopausal hormone use (no current use or premenopausal, past oestrogen use, current oestrogen use with no progesterone use, or current oestrogen use with current/past progesterone use), family history of breast cancer (no or yes), total energy intake (log-transformed continuous), alcohol consumption (0 or >0 g/d), smoking (never, past or current), diabetes (no or yes) and hypertension (no or yes). Linear trends were tested by modelling continuous variables of interest assigned the median value within the corresponding quartile. Linear trends were tested by modelling continuous variables assigned the median value within the corresponding quartile.

† Heterogeneity in the risk estimates across ethnic groups was tested by a global Wald test of the cross-product terms for ethnicity and the ethnicity-specific trend variable of total intake or density of dietary isoflavones (assigning the ethnicity-specific median values within the corresponding quartiles).

https://doi.org/10.1017/S0007114514001780 Published online by Cambridge University Press

462 matched controls, a significant linear trend test showed a lower risk in all women with higher levels of daidzein+genistein+equol excretion (OR for Q4 *v.* Q1: 0·69 (95% CI 0·43, 1·10); *P* trend=0·04), especially among Japanese American women (OR for Q4 *v.* Q1: 0·53 (95% CI 0·24, 1·16); *P* trend=0·003). However, other prospective studies that examined circulating or urinary isoflavone levels did not consistently observe protective effects[5].

While isoflavone intake at the highest level for Japanese American women in the present study was comparable to mean amounts (26–54 mg/d) consumed in Japan[8], overall consumption was much lower, which possibly explains the lack of association. The low isoflavone intake in white women in the present study was also similar to levels reported for women in no-soya-consuming countries[20]. Moreover, it is possible that FFQ-related measurement errors attenuated the association towards the null, largely because the FFQ did not capture the intake of soyabeans, soya milk and other food items that constituted a small proportion (6%) of all isoflavones reported in 24 h recalls during the calibration study. An added problem is the broad use of soya as a component in a variety of food products such as canned tuna and white bread[21,22], which were not included in the food composition table used in the present study. The intake of isoflavones from dietary supplements and modern soya foods, such as soya bars, was also not captured, but these products were not common until recent years. Given the difficulty in capturing the intake of all soya-containing foods and supplements using FFQ, as well as imprecise estimates of isoflavone intake resulting from mixed dishes with default isoflavone values, the use of biomarkers, such as urinary isoflavone excretion levels, may provide a more accurate measure of total soya consumption. The differences in reproductive and other important characteristics related to breast cancer risk across quartiles of dietary isoflavone intake (Table 1) also indicate that these women probably differed in other unmeasured factors, which may have confounded the potential true relationship. We do not have a plausible explanation for the non-significant increase in breast cancer risk associated with the highest isoflavone intake in Native Hawaiian women, who also had high isoflavone intakes largely from soya foods, other than a possible chance finding. Finally, it has become apparent that timing of soya consumption is very important; animal and case–control studies have shown that soya intake during adolescence may be more important than that during adulthood[20,23]. Therefore, women in Hawaii and California may not have been sufficiently exposed to soya early in life to experience benefits at an older age.

Stratified analyses by ER status in the present study showed possible effect modification by ethnicity. These preliminary findings add to conflicting evidence from case–control studies suggesting differential effects of soya intake by hormone receptor status[11,12,15], possibly due to the oestrogen-like structure of isoflavones. Our findings, however, should be interpreted with caution, given the small sample sizes within ethnic strata and possible chance findings due to multiple comparisons.

The present study has several strengths. The multiethnic population allowed comparisons across ethnic groups with a broad range of dietary isoflavone intake levels. The large sample size and population-based cohort design strengthened the generalisability of the observed overall findings. The prospective study design minimised differential bias in self-reported dietary data. The mean follow-up period was longer than that in previous prospective studies (13 *v.* 4–11 years) in Asian countries[5]. There were also important limitations. Our estimation of soya intake was not complete. As has been discussed above, we estimated dietary isoflavone intake principally from the intake of soya foods, such as tofu, miso soup and vegetarian meat products. However, not all soya foods (e.g. green soyabeans, soya milk, health bars and cereals) were included in the baseline FFQ. The Japanese American population included in the study, consisting largely of the second and third generations of immigrants, probably had dietary and other lifestyle characteristics different from those of Japanese women in Japan. The smaller sample sizes in stratified analyses by ethnicity or ER status did not allow for robust risk estimates. We also did not have additional subtype information on breast cancer cases, such as progesterone receptor or HER-2 status, to conduct stratified analyses by these characteristics.

In our prospective cohort of mostly postmenopausal women, we observed no statistically significant association between estimated dietary isoflavone intake and overall breast cancer risk across racial/ethnic groups. These results are in agreement with the equivocal findings of previous FFQ-based studies evaluating the protective effects of soya consumption on breast cancer risk[3–6]. Given the measurement errors associated with dietary intake data, use of biomarker measures may clarify the literature by enhancing the assessment of isoflavone exposure in future investigations.

Acknowledgements

The authors thank all participants of the MEC.

The MEC was supported by National Cancer Institute grants R37 CA 54281 (principal investigator: L. N. Kolonel) and U01 CA 63464 (principal investigator: B. E. H.). R. E. and C. L. were supported by postdoctoral fellowships on grant R25 CA 90956. The tumour registries in Hawaii and Los Angeles are supported by NCI contracts N01 PC 35137 and N01 PC 35139.

The authors' contributions are as follows: G. M. and L. R. W. designed the research project; Y. M., S.-Y. P., R. K. M., A. D. S. and L. R. W. managed the data and carried out the statistical analyses; G. M., Y. M., R. E., R. K. M. and L. R. W. wrote the article; S.-Y. P., C. L., A. D. S., B. E. H., L. N. K. and L. L. M. critically revised the article; G. M. and L. R. W. had primary responsibility for the final content.

Contributions by R. K. M. were made before her position at Purdue Pharma, L.P. The authors have no conflicts of interest to declare.

References

1. Howlader N, Noone AM, Krapcho M, *et al.* (2013) *SEER Cancer Statistics Review, 1975–2010.* Bethesda, MD: National Cancer Institute.

2. Gomez SL, Noone AM, Lichtensztajn DY, *et al.* (2013) Cancer incidence trends among Asian American populations in the United States, 1990–2008. *J Natl Cancer Inst* **105**, 1096–1110.
3. Trock BJ, Hilakivi-Clarke L & Clarke R (2006) Meta-analysis of soy intake and breast cancer risk. *J Natl Cancer Inst* **98**, 459–471.
4. Wu AH, Yu MC, Tseng CC, *et al.* (2008) Epidemiology of soy exposures and breast cancer risk. *Br J Cancer* **98**, 9–14.
5. Dong JY & Qin LQ (2011) Soy isoflavones consumption and risk of breast cancer incidence or recurrence: a meta-analysis of prospective studies. *Breast Cancer Res Treat* **125**, 315–323.
6. Qin LQ, Xu JY, Wang PY, *et al.* (2006) Soyfood intake in the prevention of breast cancer risk in women: a meta-analysis of observational epidemiological studies. *J Nutr Sci Vitaminol (Tokyo)* **52**, 428–436.
7. Shu XO, Zheng Y, Cai H, *et al.* (2009) Soy food intake and breast cancer survival. *JAMA* **302**, 2437–2443.
8. Messina M, Nagata C & Wu AH (2006) Estimated Asian adult soy protein and isoflavone intakes. *Nutr Cancer* **55**, 1–12.
9. Zhang M, Yang H & Holman CD (2009) Dietary intake of isoflavones and breast cancer risk by estrogen and progesterone receptor status. *Breast Cancer Res Treat* **118**, 553–563.
10. Kim MK, Kim JH, Nam SJ, *et al.* (2008) Dietary intake of soy protein and tofu in association with breast cancer risk based on a case–control study. *Nutr Cancer* **60**, 568–576.
11. Cho YA, Kim J, Park KS, *et al.* (2010) Effect of dietary soy intake on breast cancer risk according to menopause and hormone receptor status. *Eur J Clin Nutr* **64**, 924–932.
12. Suzuki T, Matsuo K, Tsunoda N, *et al.* (2008) Effect of soybean on breast cancer according to receptor status: a case–control study in Japan. *Int J Cancer* **123**, 1674–1680.
13. Dai Q, Shu XO, Jin F, *et al.* (2001) Population-based case–control study of soyfood intake and breast cancer risk in Shanghai. *Br J Cancer* **85**, 372–378.
14. Zaineddin AK, Buck K, Vrieling A, *et al.* (2012) The association between dietary lignans, phytoestrogen-rich foods, and fiber intake and postmenopausal breast cancer risk: a German case–control study. *Nutr Cancer* **64**, 652–665.
15. Zhang C, Ho SC, Lin F, *et al.* (2010) Soy product and isoflavone intake and breast cancer risk defined by hormone receptor status. *Cancer Sci* **101**, 501–507.
16. Kolonel LN, Henderson BE, Hankin JH, *et al.* (2000) A multiethnic cohort in Hawaii and Los Angeles: baseline characteristics. *Am J Epidemiol* **151**, 346–357.
17. Stram DO, Hankin JH, Wilkens LR, *et al.* (2000) Calibration of the dietary questionnaire for a multiethnic cohort in Hawaii and Los Angeles. *Am J Epidemiol* **151**, 358–370.
18. Therneau T & Grambsch P (2000) *Modeling Survival Data: Extending the Cox Model*. New York: Springer-Verlag.
19. Goodman MT, Shvetsov YB, Wilkens LR, *et al.* (2009) Urinary phytoestrogen excretion and postmenopausal breast cancer risk: the multiethnic cohort study. *Cancer Prev Res (Phila)* **2**, 887–894.
20. Messina M & Hilakivi-Clarke L (2009) Early intake appears to be the key to the proposed protective effects of soy intake against breast cancer. *Nutr Cancer* **61**, 792–798.
21. Horn-Ross PL, Lee M, John EM, *et al.* (2000) Sources of phytoestrogen exposure among non-Asian women in California, USA. *Cancer Causes Control* **11**, 299–302.
22. United States Department of Agriculture (2002) *Iowa State University Database on the Isoflavone Content of Foods, Release 1.3, 2002*. Beltsville, MD: USDA.
23. Nagata C (2010) Factors to consider in the association between soy isoflavone intake and breast cancer risk. *J Epidemiol* **20**, 83–89.

British Journal of Nutrition