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# REPORTS

# Phytoestrogens and Indicators of Breast Cancer Prognosis

**Tam C. Ha, Philippa M. Lyons-Wall, Douglas E. Moore, Bruce N. Tattam, John Boyages, Owen A. Ung, and Richard J. Taylor**

***Abstract:*** *Breast cancer incidence is lower and survival is longer in Asian women residing in Japan, China, or the Philippines than Caucasian women residing in the United States. Phytoestrogen intake has been examined as a possible reason for the disparity in breast cancer incidence and survival. This study examined the association between phytoestrogen intake prior to diagnosis of breast cancer and indicators of breast cancer prognosis (tumor size, estrogen and progesterone receptor status, histological grade, lymphovascular invasion, nodal spread, and stage) in 128 women, aged 40–79 yr, newly diagnosed with invasive breast cancer. After controlling for significant confounding factors, higher intakes of phytoestrogens were associated with favorable indicators of breast cancer. In women with higher intakes of phytoestrogens, there was a 32% reduction in the odds of being diagnosed with any stage of cancer other than stage 1 (95% confidence interval, CI = 0.49–0.93;* P = *0.02), a 38% reduction in odds of being diagnosed with positive lymphovascular invasion (95% CI = 0.40–0.95;* P = *0.03), and a 66% increase in the odds of being diagnosed with a positive progesterone receptor (95% CI = 1.06–2.58;* P = *0.03). We conclude that phytoestrogen intake prior to diagnosis may improve prognosis of breast cancer.*

## Introduction

Breast cancer is the most commonly diagnosed cancer in Caucasian women after non-melanoma skin cancer (1). The incidence, however, is lower in Asian women residing in Japan, China, or the Philippines than in Caucasian counterparts in the United States (1–3). On migration to the United States, the incidence of breast cancer in Asian women approaches that of women in the United States after two to three generations (4). This change in breast cancer incidence prompted investigation of environmental causes and, in particular, investigations of dietary phytoestrogens, which are consumed in higher quantities in Asian countries than in Western countries. Phytoestrogens are plant compounds that possess estrogenic and antiestrogenic properties. The two main groups of phytoestrogens found in the human diet are isoflavonoids and lignans, although the majority of epidemiological and experimental studies have focused on isoflavonoids. These compounds are found in uniquely high concentrations in soy foods such as bean curd, tofu, and tempeh. Breast cancer survival is also longer in Asian women compared with Caucasian women, although this observation is not yet entirely explained (3,5).

Epidemiological studies in Asian women provide some evidence that a higher consumption of soy products (6–9) or excretion of isoflavonoids (10) is associated with a lower risk of breast cancer (9,11), although not all findings are consistent (12,13). The inconsistencies between studies could be due to limitations in the measurement of soy foods or isoflavonoid intakes because a number of the case-control studies were not specifically designed to investigate the relationship between intakes of soy and/or isoflavonoids and breast cancer risk. In Western populations where intakes of isoflavonoids tend to be lower, associations have also been reported between breast risk and isoflavonoid excretion (14), although another study did not confirm these associations (15).

A proposed mechanism by which isoflavonoids could influence breast cancer prognosis is via their low affinity binding for estrogen receptors (ERs). In the co-presence of estradiol, isoflavonoids compete for binding to ERs (16) and can exert an antiestrogenic response by displacing the more biologically active estrogen. Isoflavonoids could also reduce

T. C. Ha and R. J. Taylor are affiliated with the School of Public Health, The University of Sydney, Australia. R. J. Taylor is also affiliated with the New South Wales Breast Cancer Institute, The University of Sydney Westmead, Australia. P. M. Lyons-Wall is affiliated with the School of Public Health, Queensland University of Technology, Australia. D. E. Moore and B. N. Tattam are affiliated with the Faculty of Pharmacy, The University of Sydney, Australia. J. Boyages and O. A. Ung are affiliated with the New South Wales Breast Cancer Institute, The University of Sydney Westmead, Australia.

the proliferation of breast cancer cells through effects independent of the ER, for example, via inhibition of topoisomerase II (17), and induction of $G_2$-M cell cycle arrest and apoptosis (18). Genistein can inhibit angiogenesis (19) and has also been shown to down-regulate matrix metalloproteinase-9 and up-regulate the tissue inhibitor of metalloproteinase-1, which are proteins associated with the regulation of invasion by tumor cells (20). Taken together, these in vitro studies provide several possible mechanisms for protective effects of isoflavonoids in breast cancer cell lines, and it is biologically plausible that they could contribute to some of the protective effects of soy and/or isoflavonoids on breast cancer observed in epidemiological studies.

To our knowledge, only one study has examined the role of soy food intake and breast cancer survival (21). Other studies examining dietary factors and breast cancer survival have investigated dietary fat, protein, carbohydrate, and vitamins A, C, and E, with a diversity of opinion remaining (22–24). Our cross-sectional analytical study examined the association of two main groups of phytoestrogens, isoflavonoids and lignans, with seven indicators of breast cancer prognosis: tumor size, estrogen and progesterone receptor status, lymphovascular invasion, tumor grade, axillary nodal involvement, and cancer stage. These indicators of breast cancer prognosis have already been shown to be good predictors of actual survival (25–27).

It is possible that an earlier stage of diagnosis could result from health-related behaviors associated with phytoestrogen intake, such as mammographic screening, rather than from higher consumption of phytoestrogens per se. Behaviors influencing time of diagnosis were measured via three modes of presentation categories, that is, methods by which patients initially became aware of their cancer. The three modes of presentation were mammographic screening; breast self-examination, irregular breast examination, doctor discovered, and imaging; and symptomatic. Possible confounding was investigated by examining the level of phytoestrogen intake in each mode of presentation group. Our aim was to examine the association between dietary phytoestrogen intake prior to diagnosis and indicators of breast cancer prognosis.



## Methods

### Subjects

Subjects were recruited from the NSW Breast Cancer Institute, Westmead Hospital, Sydney, Australia, between October 2000 and November 2001. Eligible patients were women who were ≥40 and <80 yr of age and newly diagnosed with histologically confirmed invasive breast cancer. Subjects were excluded if they had a previous history of other malignancies (excluding skin cancers other than melanoma), were deemed too frail to physically participate, or were not able to speak and read English sufficiently to participate (total excluded $n = 5$ for these categories). Institutional ethics approval and informed written consent from individual subjects were obtained. Of the 128 eligible patients, 104 participated in the study, 86% of whom were Caucasian (the remainder was comprised of Indians, Fijians, Asians, and Africans). Information obtained on potential confounders (see Table 1) included age at diagnosis, reproductive variables (age at menarche, age at first full-term delivery, gravida, parity, breastfeeding, and menopausal status), tobacco smoking, family history of breast cancer in a first-degree relative, oral contraceptive and hormone therapy usage, and anthropometric variables [body mass index (BMI), kg/m$^2$; four skinfold thicknesses to derive percent body fat (suprailiac, subscapular, biceps, and triceps) (28); and height, weight, and waist and hip circumference]. No significant differences in potential confounders were found between study subjects and nonsubjects. The majority of study subjects underwent surgery (97%) within 1–2 wk of being diagnosed with breast cancer, providing adequate pathological staging for the study population and data on indicators of breast cancer prognosis. Breast cancer characteristics that were collected included tumor size, histological grade, axillary nodal involvement, lymphovascular invasion, stage of cancer, and estrogen and progesterone receptor status. If a patient did not undergo surgery, tumor variables were based first on mammographic findings ($n = 2$) and then ultrasound results, respectively; if these were not available, clinical measurements ($n = 1$), as indicated by the treating medical team, were recorded.

**Table 1.** Distribution of Demographic Characteristics of Study Participants Diagnosed With Breast Cancer

| Characteristic | *n* (%) |
|---|---|
| Average age (yr) | 57 |
| Menopausal status | |
| Premenopausal | 27 (26%) |
| Postmenopausal | 77 (74%) |
| Average age at onset of menarche (yr) | 13 |
| Average age at first full-term delivery (yr) | 25 |
| Parity | 2.6 |
| Gravida | 3 |
| Family history of breast cancer | |
| First-degree ± other degree relatives | 18 (17.3%) |
| Other degree relatives only | 19 (18.3%) |
| No relatives with breast cancer | 67 (64.4%) |
| Oral contraceptive usage | |
| 0 yr | 34 (34.3%) |
| >0 and <5 yr | 25 (25.3%) |
| ≥5 and <10 yr | 14 (14.1%) |
| ≥10 yr | 26 (26.3%) |
| Hormone therapy usage | |
| 0 yr | 61 (59.8%) |
| >0 and <5 yr | 16 (15.7%) |
| ≥5 yr | 25 (24.5%) |
| Smoking | |
| Nonsmoker | 78 (77.2%) |
| Ex-smoker | 10 (9.9%) |
| Current smoker | 13 (12.9%) |
| Mode of presentation | |
| Routine mammographic screening | 38 (36.9%) |
| Breast self-examination/non–breast self-examination/doctor discovered/imaging | 18 (17.5%) |
| Symptomatic | 47 (45.6%) |

Two dietary questionnaires were used. Dietary phytoestrogen intake (isoflavonoids and lignans), in a typical month over the past year, was obtained by a specially designed 106-item phytoestrogen food-frequency questionnaire of phytoestrogen-derived foods available in an Australian market. The main sources of isoflavonoids were soy foods and soy products including bean curd (or tofu); the main sources of lignans were linseed (or flaxseed), legumes, fruits, and vegetables. Intakes of potential confounding dietary variables, including carbohydrate; fat; protein; vitamins A, C, and E; alcohol; dietary fiber; niacin; phosphorous; zinc; and calcium, were obtained from a validated food-frequency questionnaire (general food-frequency questionnaire) (29). Values of isoflavonoids (genistein and daidzein) in individual food items were obtained from the United States Department of Agriculture (USDA) database, and values for mammalian lignans (enterolactone and enterodiol) were obtained from published values by Thompson et al. (30,31). Manufacturer values for isoflavonoids and lignans were also included for food items where published values were not available from the USDA database (32). Aggregate measurements of isoflavonoids (mg/day) and lignans (mg/day) were obtained from the sum of all individual food items in the phytoestrogen questionnaire.

The convergent validity of the phytoestrogen questionnaire had been tested previously in our laboratory. Intake obtained by the questionnaire was compared with that obtained by a 7-day weighed food record taken over the same time interval in a group of 36 healthy adults, aged 20–49 yr and mean + SE BMI of 20.7 + 0.4 kg/m$^2$; none were on any special diet, and 31 were omnivores and 5 were vegetarians. The weighed record was chosen as a standard because it provided a more detailed and accurate description of the total phytoestrogen intake, recorded as eaten over the same time interval. The results showed significant associations between intakes of isoflavonoids ($r = 0.82$; $P < 0.0001$) or lignans ($r = 0.68$; $P < 0.0001$) obtained from questionnaire and weighed records (Spearman rank correlations). Median (range) intakes of isoflavonoids from the questionnaire and weighed records were 0.84 (0.0–38.6) and 1.16 (0.0–43.1) mg/day, and intakes of lignans were 1.09 (0.12–11.9) and 1.48 (0.57–7.32) mg/day, respectively; there were no significant differences between intakes obtained by the two methods (paired *t*-tests) (33). Therefore, there was good convergent validity between the phytoestrogen questionnaire and 7-day weighed food record for intakes of both isoflavonoids and lignans.

To assess the criterion validity of the phytoestrogen questionnaire, a subsample of study subjects ($n = 25$) provided three 24-h urine samples (two weekdays and one weekend day over a week), which were used for analysis of isoflavonoid and lignan concentrations in urine using liquid chromatography/mass spectrometry/mass spectrometry (LC/MS/MS) (34). Patients requiring chemotherapy immediately were not asked to participate in the urine collection due to potential chemotherapy interference with the LC/MS/MS assay. The major dietary isoflavonoids and their metabolites and lignans were chosen for urine analysis. After hydrolysis, analysis was conducted on the biologically active aglycone forms, including eight isoflavonoids (genistein, biochanin A, daidzein, equol, *O*-desmethylangolensin, dihydrogenistein, dihydrodaidzein, and formononetin) and two lignans (enterodiol and enterolactone). Details of the urine analysis have been described elsewhere (34). Briefly, a significant linear relationship was observed between dietary isoflavonoid intake obtained from the phytoestrogen questionnaire and isoflavonoid concentrations in urine ($r = 0.50$; $P = 0.01$). No significant relationship was observed between dietary lignan intake and excretion ($r = 0.16$; $P = 0.46$). Phytoestrogens are distributed into three biological matrices: urine, plasma, and feces. It is not possible to completely recover dietary phytoestrogens from urine; however, at low to moderate levels of soy protein consumption, a linear relationship has been shown between dietary phytoestrogen intake and excretion in urine (35), which provides a rationale for using urine to test criterion validity. The results in this article are based on values of dietary isoflavonoid and lignan intake (mg/day) derived from the phytoestrogen questionnaire. No subjects reported using complementary medicines, hormonal supplements, or dietary supplements containing phytoestrogens.

### Data Analysis

Isoflavonoid and lignan dietary intake data (mg/day) were not normally distributed, and logarithmic transformations (natural logarithm) were required to normalize distributions. Dietary phytoestrogen intake was examined for isoflavonoids and lignans separately and for phytoestrogens as a combined isoflavonoid and lignan variable (see Table 2). The association between indicators of breast cancer prognosis (outcome factor) and phytoestrogen intake (study factor) was analyzed by controlling for age and other statistically significant confounders using logistic regression in a series of separate analyses. The strong a priori evidence of an association between age and breast cancer survival rendered adjustment for age necessary in all statistical models regardless of actual statistical significance to allow for any residual effects. Subsample analysis of phytoestrogen intake in the mammographic screening group alone was also conducted to assess potential confounding by health-related behaviors associated with phytoestrogen intake. Sample size was calculated for this study based on a geometric difference of 1.07 mg/day of dietary isoflavonoid intake between tumor size of ≤15 mm and >15 mm, power of 80% and two-sided 5% significance level, and it was estimated that at least 100 study subjects were required for the study. All statistical tests were two sided and conducted at the 95% level.

Statistical analysis was conducted using the SAS (for Windows, version 8, SAS Institute, Inc., Chicago, IL). Nutrient content was obtained using Foodworks (Xyris Software, version 2, Brisbane, Australia) and the NUTTAB database for Australian foods. Associations between phytoestrogens and indicators of breast cancer prognosis are presented as odds ratios (ORs) [95% confidence intervals (CIs)].

**Table 2.** Comparison of Age-Adjusted Mean Intake of Lignans, Isoflavonoids, and Combined Total Phytoestrogens Within Prognostic Breast Cancer Variable Groups (geometric mean mg/day)

| Prognostic Variable | *n* | Isoflavonoid Intake (mg/day) | *P* Value | Lignan Intake (mg/day) | *P* Value | Phytoestrogen Intake (mg/day) | *P* Value |
|---|---|---|---|---|---|---|---|
| Estrogen receptor | | | | | | | |
| Positive | 87 | 1.13 | 0.43 | 2.03 | 0.43 | 4.99 | 0.40 |
| Negative | 13 | 0.6 | | 1.7 | | 3.42 | |
| Progesterone receptor | | | | | | | |
| Positive | 76 | 1.40 | 0.05 | 2.16 | 0.04 | 5.76 | 0.02 |
| Negative | 24 | 0.42 | | 1.53 | | 2.61 | |
| Lymphovascular invasion | | | | | | | |
| Positive | 29 | 0.55 | 0.12 | 1.62 | 0.08 | 2.86 | 0.03 |
| Negative | 67 | 1.43 | | 2.15 | | 6.07 | |
| Grade | | | | | | | |
| 1 | 41 | 0.74 | No | 1.75 | 0.15[a] | 3.93 | No |
| 2 | 33 | 2.34 | linear | 2.16 | | 7.14 | linear |
| 3 | 26 | 0.83 | trend | 2.25 | | 4.51 | trend |
| Positive lymph nodes | | | | | | | |
| 0 | 63 | 1.26 | 0.16[a] | 2.04 | No | 5.39 | 0.14[a] |
| 1–3 | 21 | 0.89 | | 1.74 | linear | 4.08 | |
| 4+ | 10 | 0.34 | | 1.95 | trend | 2.58 | |
| Tumor size | | | | | | | |
| ≤15 mm | 54 | 1.7 | 0.06 | 2.26 | 0.03 | 7.06 | 0.004 |
| ≥15 mm | 46 | 0.63 | | 1.64 | | 2.98 | |
| Stage | | | | | | | |
| I | 58 | 1.73 | 0.03 | 2.17 | 0.14 | 6.53 | 0.02 |
| All other stages combined | 43 | 0.55 | | 1.74 | | 3.14 | |

*a:* *P* value is for linear trend.



## Results

Mean age at breast cancer diagnosis was 57 yr (range 40–79 yr). The response rate was 80% for the phytoestrogen questionnaire and 81% for the general food-frequency questionnaire.

### Association of Phytoestrogen Intake With Indicators of Breast Cancer Prognosis

The associations between isoflavonoid intake and indicators of breast cancer prognosis, adjusted for confounders, are displayed in Table 3. For every unit increase in isoflavonoid consumption (mg/day), the odds of being diagnosed with a positive progesterone receptor were significantly associated with a 22% increase in risk (OR = 1.22; 95% CI = 1.00–1.49; $P = 0.05$), whereas the odds of being diagnosed with a higher stage of breast cancer (all stages combined except for stage I) were significantly associated with a 17% reduction in risk (OR = 0.83; 95% CI = 0.70–0.98; $P = 0.03$).

The associations between lignan intake and indicators of breast cancer prognosis, adjusted for confounders, are displayed in Table 4. For every unit increase in lignan consumption (mg/day), the odds of being diagnosed with a positive progesterone receptor were significantly associated with a 142% increase in risk (OR = 2.42; 95% CI = 1.03–5.68; $P = 0.04$), and the odds of being diagnosed with a positive lymphovascular invasion were significantly associated with a 68% reduction in risk (OR = 0.32; 95% CI = 0.12–0.85; $P = 0.02$). The odds of being diagnosed with a tumor size of >15 mm were significantly associated with a 50% reduction in risk (OR = 0.50; 95% CI = 0.26–0.94; $P = 0.03$).

The associations between phytoestrogen intake (isoflavonoids and lignans combined) (mg/day) and indicators of breast cancer prognosis, adjusted for confounders, are displayed in Table 5. For every unit increase in phytoestrogen consumption (mg/day), the odds of being diagnosed with a positive progesterone receptor were significantly associated with a 66% increase in risk (OR = 1.66; 95% CI = 1.06–2.56; $P = 0.04$), and the odds of being diagnosed with a positive lymphovascular invasion were significantly associated with a 38% reduction in risk (OR = 0.62; 95% CI = 0.40–0.95; $P = 0.03$). The odds of being diagnosed with a tumor size of >15 mm were significantly associated with a 37% reduction in risk (OR = 0.63; 95% CI = 0.45–0.87; $P = 0.006$), and the odds of being diagnosed with a higher stage of breast cancer (all stages combined except for stage I) were significantly associated with a 32% reduction in risk (OR = 0.68; 95% CI = 0.49–0.93; $P = 0.02$).

### Assessment of Other Confounding Factors Resulting in an Earlier Diagnosis of Breast Cancer

There was a trend toward a higher intake of isoflavonoids among patients who attended mammographic screening compared with those diagnosed via other mode of presentation groups; however, the association was not significant ($P = 0.07$). Therefore, mode of presentation as an indicator of behaviors resulting in an earlier diagnosis

**Table 3.** Overall Association of Isoflavonoid Intake (mg/day) and Indicators of Breast Cancer Prognosis[a]

| Outcome Factor (*y*) (coding 0, 1) | *n* | Confounders | Odds Ratio (95% CI) | *P* Value |
|---|---|---|---|---|
| Estrogen receptor status (0 = negative, 1 = positive) | 100 | Age | 1.11 (0.87-1.43) | 0.40 |
| Progesterone receptor status (0 = negative, 1 = positive) | 100 | Age | 1.22[b] (1.00-1.49) | 0.05 |
| Both estrogen and progesterone receptor positive versus other combinations (0 = all other combinations, 1 = both estrogen and progesterone positive) | 100 | Age | 1.23[b] (1.02-1.48) | 0.03 |
| Lymphovascular invasion (0 = negative invasion, 1 = positive invasion) | 96 | Age, age at first full-term delivery | 0.86 (0.70-1.05) | 0.13 |
| Grade (0 = grade 1 + 2, 1 = grade 3) | 100 | Age, alcohol consumption, hormone therapy usage, % energy from other dietary sources[c] | 1.02 (0.82-1.27) | 0.85 |
| Nodal status (0 = negative, 1 = positive) | 94 | Age | 0.90 (0.75-1.07) | 0.24 |
| Size group (0 = ≤15 mm, 1 = >15 mm) | 100 | Age, breastfeeding | 0.86 (0.72-1.02) | 0.08 |
| Stage group (0 = stage I, 1 = all other stages combined) | 101 | Age | 0.83[b] (0.70-0.98) | 0.03 |

*a:* Logistic regression is used; outcome factors (*y*) are indicators of breast cancer prognosis (left-hand column), and the study factor (*x*) is isoflavonoid intake [ln (isoflavonoids)(mg/day)]. The odds ratio is the change in odds for each natural logarithmic unit increase in mean isoflavonoid intake and being diagnosed with the breast cancer indicator coded as 1 compared with breast cancer indicator coded as 0. CI, confidence interval.

*b:* Statistically significant at 95% level ($P < 0.05$).

*c:* Percentage of energy from food components other than fat, carbohydrates, and alcohol, for example, sugar alcohols.

**Table 4.** Overall Association of Lignan Intake (mg/day) and Indicators of Breast Cancer Prognosis[a]

| Outcome Factor (*y*) (coding 0, 1) | *n* | Confounders | Odds Ratio (95% CI) | *P* Value |
|---|---|---|---|---|
| Estrogen receptor status (0 = negative, 1 = positive) | 100 | Age | 1.47 (0.57-3.76) | 0.42 |
| Progesterone receptor status (0 = negative, 1 = positive) | 100 | Age | 2.42[b] (1.03-5.68) | 0.04 |
| Both estrogen and progesterone receptor positive versus other combinations (0 = all other combinations, 1 = both estrogen and progesterone positive) | 100 | Age | 2.15[b] (1.01-4.61) | 0.05 |
| Lymphovascular invasion (0 = negative, 1 = positive) | 96 | Age, vitamin C | 0.32[b] (0.12-0.85) | 0.02 |
| Grade (0 = grade 1 + 2, 1 = grade 3) | 100 | Age, hormone therapy usage, alcohol consumption, % energy from other sources[c] | 1.48 (0.74-2.98) | 0.27 |
| Nodal status (0 = negative, 1 = positive) | 94 | Age | 0.78 (0.40-1.49) | 0.44 |
| Size group (0 = ≤15 mm, 1 = >15 mm) | 100 | Age<br>Waist-to-hip ratio | 0.50[b] (0.26-0.94) | 0.03 |
| Stage group (0 = stage I, 1 = all other stages combined) | 101 | Age | 0.63 (0.34-1.16) | 0.14 |

*a:* Logistic regression is used; outcome factors (*y*) are indicators of breast cancer prognosis (left-hand column), and the study factor (*x*) is lignan intake (mg/day). The odds ratio is the change in odds for each natural logarithmic unit increase in mean lignan intake and being diagnosed with the breast cancer indicator coded as 1. CI, confidence interval.

*b:* Statistically significant at 95% level ($P < 0.05$).

cannot be considered to unduly influence the results of isoflavonoid intake in relation to indicators of breast cancer prognosis.

This potential source of confounding was investigated further by examining the relationship between isoflavonoid intake and progesterone receptor status, which is not influenced by the mode of presentation, in the subsample analysis of women who presented via mammographic screening. ORs for the association between isoflavonoid intake and progesterone receptor status were comparable in both the whole group analysis (OR = 1.22; 95% CI = 1.00–1.49; $P = 0.05$) and in the subsample analysis (OR = 1.21; 95% CI = 0.84–1.75; $P = 0.30$). The lack of statistical significance in the subsample analysis is likely due to the smaller numbers

**Table 5.** Overall Association of Phytoestrogen Intake (mg/day) and Indicators of Breast Cancer Prognosis[a]

| Outcome Factor (*y*) (coding 0,1) | *n* | Confounders | Odds Ratio (95% CI) | *P* Value |
|---|---|---|---|---|
| Estrogen receptor status (0 = negative, 1 = positive) | 100 | Age | 1.24 (0.76–2.0) | 0.38 |
| Progesterone receptor status (0 = negative, 1 = positive) | 100 | Age | 1.66[b] (1.06–2.58) | 0.03 |
| Both estrogen and progesterone receptor positive versus other combinations (0 = all other combinations, 1 = both estrogen and progesterone positive) | 100 | Age | 1.53[b] (1.05–2.34) | 0.03 |
| Lymphovascular invasion (0 = negative, 1 = positive) | 96 | Age, vitamin C | 0.62[b] (0.40–0.95) | 0.03 |
| Grade (0 = grade 1 + 2, 1 = grade 3) | 100 | Age, hormone therapy usage, alcohol consumption, % energy from other dietary sources[c] | 1.08 (0.74–1.58) | 0.70 |
| Nodal status (0 = negative, 1 = positive) | 94 | Age | 0.80 (0.58–1.12) | 0.19 |
| Size group (0 = ≤15mm, 1 = >15 mm) | 100 | Age | 0.63[b] (0.45–0.87) | 0.006 |
| Stage group (0 = stage I, 1 = all other stages combined) | 101 | Age | 0.68[b] (0.49–0.93) | 0.02 |

*a:* Logistic regression is used; outcome factors (*y*) are indicators of breast cancer prognosis (left-hand column), and the study factor (*x*) is phytoestrogen intake (mg/day). The odds ratio is the change in odds for each natural logarithmic unit increase in mean phytoestrogen intake and being diagnosed with the breast cancer indicator coded as 1. CI, confidence interval.

*b:* Statistically significant at 95% level ($P < 0.05$).

*c:* Percentage of energy from food components other than fat, carbohydrates, and alcohol, for example, sugar alcohols.



($n = 36$). Therefore, potential confounding by behaviors resulting in an earlier diagnosis was not an issue in this study.

## Discussion

Our study found that a higher intake of dietary phytoestrogens at diagnosis was associated with favorable indicators of breast cancer prognosis, although not all relationships reached statistical significance. The only exception was the observation that a higher intake of lignans was associated with histological grade 3 tumors when compared with grade 1 and 2 combined; however, this association did not reach statistical significance. For all three measures of phytoestrogen intake, either individual isoflavonoids, lignans, or combined phytoestrogens, a higher intake was significantly associated with a positive progesterone receptor status. In terms of the relative importance of the indicators of breast cancer prognosis immediately after diagnosis, first tumor size and then number of positive axillary nodes for cancer are the two main independent predictors of disease-free survival and recurrence from breast cancer (27,36,37). There is currently no consensus on the ordering of prognostic significance for hormone receptor status, lymphovascular invasion, or histological grade, although these factors have been shown to be important predictors of breast cancer survival (25–27,38). Given that the findings are observed in a number of indicators, which all relate to a better prognosis, in particular, tumor size and nodal status where statistically significant associations were found, it is possible that these observations are mutually confirming and that a higher consumption of phytoestrogens is associated with a favorable breast cancer prognosis. It is biologically plausible that phytoestrogens may modify indicators of breast cancer prognosis, as supported by in vitro cell culture studies and in vivo animal studies with genistein, where anticarcinogenic properties including antioxidative, inhibition of topoisomerase II, antiestrogenic properties, and antiangiogenic characteristics have been identified (39–41). Hsieh et al. conducted in vitro experiments using the ER-positive MCF-7 cell line and showed that genistein was able to inhibit cellular proliferation at concentrations of 10 nM (39). Constantinou et al., using the estrogen-negative cell line MDA-MD-468, also showed that genistein at concentrations of 30 μM caused cancer cells to cease dividing (40). It is possible that the antiangiogenic properties of genistein, demonstrated by in vitro cell studies, may also be responsible for the retardation in tumor growth of chemically induced breast cancer in mice models (41). Collectively, these anticarcinogenic properties of genistein may result in a smaller tumor size at diagnosis in human populations and thus could explain the results obtained in our study whereby a higher isoflavonoid, lignan, and phytoestrogen intake was associated with a significantly smaller tumor size at diagnosis. From current knowledge, we are unable to explain the significant associations found between higher isoflavonoid, lignan, and combined phytoestrogen intakes and a positive progesterone receptor status.

Our study has several strengths, which include a high response rate to both dietary questionnaires, which reduced the potential for selection bias. No significant differences were found in demographic, medical, and anthropometric characteristics between study subjects and nonresponders, indicating that our sample was representative of the cross section of

women attending the NSW Breast Cancer Institute at Westmead Hospital, a major metropolitan hospital located in Sydney. By recruiting patients with newly diagnosed breast cancer, we attempted to collect dietary data that reflected dietary practices prior to diagnosis. A common difficulty with all studies of diet and breast cancer is the limited ability to measure retrospective dietary exposure accurately; however, phytoestrogens (isoflavonoids at least) are present uniquely in high quantities in specific foods that are easily recognizable, thereby increasing the accuracy of measuring these compounds.

One possible limitation to our study is that products containing soy ingredients were not included as items in the questionnaire if their isoflavonoid content was expected to be low, for example, commercial breads and bread mixes where soy flour is used as an improver to whiten the grain and processed luncheon meats, sausages, and hamburgers where soy flour acts as a binder. These sources could have made an important contribution to total isoflavonoid intake in study subjects with a low consumption of soy foods. However, we found a significant association between intake and excretion of isoflavonoids in a subsample of the study group, suggesting that our questionnaire did capture the predominant sources of isoflavonoids in our group of women.

Unlike our study, few past studies have used dietary instruments that had been formally tested for criterion validity within the study group and designed specifically for the measurement of dietary phytoestrogen intake in an Australian population (6). Criterion validation of dietary data, tested by LC/MS/MS urine analysis, showed that a linear relationship did exist for isoflavonoids but not for lignans, indicating that for isoflavonoids the range of intakes also reflected the level of urinary excretion, lending credibility to the dietary associations obtained with breast cancer prognosis. Our study utilized a food-frequency questionnaire designed specially for an Australian population, thereby capturing phytoestrogen-derived foods applicable to the study population. The collection of prior dietary information is of particular importance when investigating possible associations with malignancies as the development of cancer precedes the clinical diagnosis. No study subjects reported any major changes in their diets over the past year, and up to the last 5 yr, and, in particular, no changes in their consumption of soy foods.

Studies investigating phytoestrogen and breast cancer have mainly focused on breast cancer incidence and risk factors. Of the 10 published human studies involving phytoestrogens and breast cancer incidence, 7 have shown that a higher intake of soy was associated with a lower incidence of breast cancer, although not all findings reached statistical significance (7,9–11,14,42,43). No studies have shown that higher consumption of phytoestrogens was associated with higher risk of breast cancer. These studies suggest that the consumption of phytoestrogens plays a beneficial role in the etiology of breast cancer. It is perhaps not surprising that our study investigating phytoestrogens and indicators of breast cancer prognosis also found a beneficial role of phytoestrogens in the prognosis of breast cancer.

One study has been published examining the role of soy food intake and breast cancer survival in a Shanghai population (21). In this study, Boyapati et al. conclude that no overall association was observed between total soy protein intake or total isoflavonoid intake and disease-free breast cancer survival (21), possibly because there was no evaluation of the effect of tamoxifen usage on disease-free survival, a drug that is known to increase disease-free survival in ER-positive breast cancer (44). Although intake of soy foods was examined at a median time of 66 days after diagnosis, biochemical indicators of phytoestrogen status were not utilized to confirm intake. A strength of our study was that dietary exposure was confirmed by measurement of urinary phytoestrogen excretion, with a significant association observed between intake and excretion of isoflavonoids.

Boyapati et al. found that soy protein intake and disease-free survival did not vary by estrogen and progesterone receptor status, whereas our study found that a higher intake of phytoestrogens increased the odds of being diagnosed with both estrogen- and progesterone-positive receptors, although findings did not reach statistical significance for ER status. However, our results agree with the study by Touillaud et al. in which ER-positive tumors were associated with a higher intake of isoflavonoids (45).

Our findings have public health implications as breast cancer continues to be a condition where, at present, there have been no large improvements in survival in Australia (25). There is evidence from the literature that phytoestrogens may lower the incidence of breast cancer and implied evidence that Asian women have better breast cancer survival than Caucasian women. From our study, a higher consumption of phytoestrogens is associated with breast cancers of less severity, and it is possible that consumption of phytoestrogens before breast cancer diagnosis may lower the risk of breast cancer recurrence and improve survival.

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