Table 3. Odds ratios and 95% confidence intervals of breast cancer for combinations of dietary intake of isoflavones and polymorphisms in estrogen receptor genes among Japanese

| | All subjects | | | | Premenopausal women | | | | Postmenopausal women | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Isoflavone intake (mg/day), tertile category | | | *P* for trend | Isoflavone intake (mg/day), tertile category | | | *P* for trend | Isoflavone intake (mg/day), tertile category | | | *P* for trend |
| | 1 | 2 | 3 | | 1 | 2 | 3 | | 1 | 2 | 3 | |
| Estrogen receptor alpha gene (rs9340799) | | | | | | | | | | | | |
| AA | | | | | | | | | | | | |
| No.† | 109/83 | 76/90 | 88/83 | | 54/41 | 30/31 | 33/19 | | 55/42 | 46/59 | 55/64 | |
| OR‡ | 1 | 0.73 | 0.78 | 0.32 | 1 | 0.68 | 1.13 | 0.96 | 1 | 0.75 | 0.64 | 0.15 |
| (95% CI) | | (0.45–1.18) | (0.47–1.29) | | | (0.34–1.35) | (0.53–2.39) | | | (0.41–1.34) | (0.36–1.15) | |
| AG + GG | | | | | | | | | | | | |
| No.† | 42/46 | 42/39 | 31/47 | | 25/25 | 22/12 | 12/7 | | 17/21 | 20/27 | 19/40 | |
| OR‡ | **0.52** | 0.68 | 0.51 | 0.75 | 0.64 | 1.38 | 1.13 | 0.54 | 0.59 | 0.56 | **0.38** | 0.15 |
| (95% CI) | **(0.27–0.99)** | (0.37–1.24) | (0.26–1.01) | | (0.31–1.35) | (0.59–3.23) | (0.39–3.30) | | (0.26–1.32) | (0.26–1.20) | **(0.18–0.79)** | |
| | *P* for interaction = 0.39 | | | | *P* for interaction = 0.15 | | | | *P* for interaction = 0.87 | | | |
| Estrogen receptor alpha gene (rs1913474) | | | | | | | | | | | | |
| CC | | | | | | | | | | | | |
| No.† | 41/38 | 32/42 | 27/33 | | 20/16 | 16/12 | 13/4 | | 21/22 | 16/30 | 14/29 | |
| OR‡ | 1 | 0.68 | 0.76 | 0.62 | 1 | 1.15 | 2.39 | 0.08 | 1 | 0.60 | 0.47 | 0.09 |
| (95% CI) | | (0.34–1.36) | (0.37–1.59) | | | (0.40–3.26) | (0.61–9.30) | | | (0.24–1.47) | (0.18–1.21) | |
| CT + TT | | | | | | | | | | | | |
| No.† | 110/91 | 86/87 | 92/97 | | 59/50 | 36/31 | 32/22 | | 51/41 | 50/56 | 60/75 | |
| OR‡ | 0.97 | 0.97 | 0.84 | 0.33 | 0.91 | 0.86 | 0.97 | 0.99 | 1.20 | 1.08 | 0.80 | 0.14 |
| (95% CI) | (0.54–1.74) | (0.55–1.72) | (0.45–1.55) | | (0.41–2.02) | (0.37–2.04) | (0.39–2.44) | | (0.56–2.59) | (0.51–2.29) | (0.38–1.68) | |
| | *P* for interaction = 0.69 | | | | *P* for interaction = 0.58 | | | | *P* for interaction = 0.73 | | | |
| Estrogen receptor alpha gene (rs2234693) | | | | | | | | | | | | |
| TT | | | | | | | | | | | | |
| No.† | 58/36 | 38/41 | 48/38 | | 33/21 | 12/16 | 21/11 | | 25/15 | 26/25 | 27/27 | |
| OR‡ | 1 | 0.55 | 0.68 | 0.54 | 1 | 0.41 | 1.15 | 0.77 | 1 | 0.79 | 0.58 | 0.28 |
| (95% CI) | | (0.26–1.16) | (0.32–1.43) | | | (0.15–1.12) | (0.43–3.10) | | | (0.32–1.92) | (0.24–1.40) | |
| TC + CC | | | | | | | | | | | | |
| No.† | 93/93 | 80/88 | 71/92 | | 46/45 | 40/27 | 24/15 | | 47/48 | 40/61 | 47/77 | |
| OR‡ | **0.51** | **0.52** | **0.42** | 0.46 | 0.64 | 0.99 | 0.86 | 0.39 | 0.54 | **0.42** | **0.35** | 0.14 |
| (95% CI) | **(0.28–0.96)** | **(0.28–0.98)** | **(0.21–0.82)** | | (0.31–1.34) | (0.45–2.16) | (0.35–2.15) | | (0.24–1.21) | **(0.19–0.92)** | **(0.16–0.76)** | |
| | *P* for interaction = 0.37 | | | | *P* for interaction = 0.08 | | | | P for interaction = 0.97 | | | |
| Estrogen receptor beta gene (rs4986938) | | | | | | | | | | | | |
| GG | | | | | | | | | | | | |
| No.† | 115/86 | 88/96 | 86/99 | | 57/46 | 39/32 | 32/21 | | 58/40 | 49/64 | 54/78 | |
| OR‡ | 1 | 0.74 | 0.65 | 0.06 | 1 | 0.96 | 1.03 | 0.94 | 1 | 0.60 | **0.47** | **0.01** |
| (95% CI) | | (0.47–1.16) | (0.39–1.07) | | | (0.51–1.83) | (0.49–2.15) | | | (0.33–1.07) | **(0.27–0.84)** | |
| GA + AA | | | | | | | | | | | | |
| No.† | 36/43 | 30/33 | 33/31 | | 22/20 | 13/11 | 13/5 | | 14/23 | 17/22 | 20/26 | |
| OR‡ | 0.57 | 0.78 | 0.90 | 0.23 | 0.80 | 0.91 | 1.99 | 0.20 | 0.47 | 0.80 | 0.62 | 0.49 |
| (95% CI) | (0.31–1.08) | (0.40–1.50) | (0.45–1.82) | | (0.37–1.72) | (0.35–2.34) | (0.62–6.46) | | (0.21–1.06) | (0.36–1.75) | (0.28–1.35) | |
| | *P* for interaction = 0.17 | | | | *P* for interaction = 0.48 | | | | *P* for interaction = 0.11 | | | |
| Estrogen receptor beta gene (rs1256049) | | | | | | | | | | | | |
| GG | | | | | | | | | | | | |
| No.† | 85/62 | 59/62 | 59/58 | | 43/32 | 28/20 | 23/12 | | 42/30 | 31/42 | 36/46 | |
| OR‡ | 1 | 0.74 | 0.82 | 0.16 | 1 | 1.05 | 0.98 | 0.80 | 1 | 0.56 | 0.51 | 0.08 |
| (95% CI) | | (0.43–1.27) | (0.46–1.48) | | | (0.49–2.27) | (0.39–2.47) | | | (0.28–1.13) | (0.26–1.01) | |
| GA + AA | | | | | | | | | | | | |
| No.† | 66/67 | 59/67 | 60/72 | | 36/34 | 24/23 | 22/14 | | 30/33 | 35/44 | 38/58 | |
| OR‡ | 0.70 | 0.74 | 0.61 | 0.93 | 0.79 | 0.78 | 1.31 | 0.20 | 0.50 | 0.60 | **0.41** | 0.35 |
| (95% CI) | (0.41–1.19) | (0.43–1.27) | (0.35–1.07) | | (0.39–1.58) | (0.36–1.69) | (0.55–3.08) | | (0.24–1.03) | (0.31–1.18) | **(0.21–0.80)** | |
| | *P* for interaction = 0.63 | | | | *P* for interaction = 0.65 | | | | *P* for interaction = 0.31 | | | |

†No. of patients with cancer/No. of controls.
‡Conditional model adjusting for menopausal status (premenopausal, postmenopausal), number of births (0, 1, 2, 3, 4, 5+), family history of breast cancer (yes, no), smoking status (never, past, current smoker), moderate physical activity in the past 5 years (no, less than 3 days/month, 1–4 days/week, more than 5 days/week), and vitamin supplement use (yes, no). For stratified analyses according to menopausal status, an unconditional model adjusting for age, area, number of births (0, 1, 2, 3, 4, 5+), family history of breast cancer (yes, no), smoking status (never, past, current smoker), moderate physical activity in the past 5 years (no, less than 3 days/month, 1–4 days/week, more than 5 days/week), and vitamin supplement use (yes, no). ORs and 95% CIs with statistical significance are written in bold letter. CIs, confidence intervals; OR, odds ratio.

0.16–0.85) (*P* for interaction = 0.11, 0.08 and 0.21, respectively) (Tables 3 and 4). Moreover, we found no remarkable difference in the association between isoflavone intake and breast cancer risk by the four other polymorphisms.

## Discussion

In these case-control studies of Japanese, Japanese Brazilians, and non-Japanese Brazilians, we found that a statistically significant



doi: 10.1111/j.1349-7006.2009.01118.x

Table 4. Odds ratios and 95% confidence intervals of breast cancer for combinations of dietary intake of isoflavones and polymorphisms in estrogen receptor genes among Japanese Brazilian and non-Japanese Brazilian subjects

| | Japanese Brazilians living in São Paulo, Brazil | | Non-Japanese Brazilians living in São Paulo, Brazil | |
|---|---|---|---|---|
| | Isoflavone intake (mg/day), median category | | Isoflavone intake (mg/day) | |
| | 1 | 2 | Non-consumers | Consumers |
| Estrogen receptor alpha gene (rs9340799) | | | | |
| AA | | | | |
| No.† | 31/21 | 23/29 | 145/157 | 16/25 |
| OR‡ | 1 | **0.36** | 1 | 0.68 |
| (95% CI) | | **(0.14–0.95)** | | (0.32–1.43) |
| AG + GG | | | | |
| No.† | 15/18 | 10/11 | 198/161 | 20/36 |
| OR‡ | 0.44 | 0.34 | 1.23 | 0.61 |
| (95% CI) | (0.14–1.45) | (0.09–1.32) | (0.89–1.68) | (0.33–1.13) |
| | *P* for interaction = 0.36 | | *P* for interaction = 0.52 | |
| Estrogen receptor alpha gene (rs1913474) | | | | |
| CC | | | | |
| No.† | 13/12 | 12/12 | 213/204 | 24/35 |
| OR‡ | 1 | 0.76 | 1 | 0.65 |
| (95% CI) | | (0.18–3.18) | | (0.36–1.19) |
| CT + TT | | | | |
| No.† | 33/27 | 21/28 | 129/114 | 12/26 |
| OR‡ | 1.25 | 0.55 | 1.13 | 0.49 |
| (95% CI) | (0.42–3.72) | (0.17–1.78) | (0.82–1.56) | (0.24–1.01) |
| | *P* for interaction = 0.52 | | *P* for interaction = 0.40 | |
| Estrogen receptor alpha gene (rs2234693) | | | | |
| TT | | | | |
| No.† | 17/12 | 8/10 | 97/106 | 10/16 |
| OR‡ | 1 | 0.41 | 1 | 0.57 |
| (95% CI) | | (0.10–1.65) | | (0.22–1.47) |
| TC + CC | | | | |
| No.† | 29/27 | 25/30 | 246/212 | 26/45 |
| OR‡ | 0.65 | 0.36 | 1.20 | 0.65 |
| (95% CI) | (0.23–1.84) | (0.12–1.08) | (0.84–1.71) | (0.37–1.15) |
| | *P* for interaction = 0.71 | | *P* for interaction = 0.94 | |
| Estrogen receptor beta gene (rs4986938) | | | | |
| GG | | | | |
| No.† | 38/30 | 21/30 | 156/148 | 13/28 |
| OR‡ | 1 | **0.31** | 1 | **0.37** |
| (95% CI) | | **(0.12–0.78)** | | **(0.16–0.85)** |
| GA + AA | | | | |
| No.† | 8/9 | 12/10 | 156/170 | 23/33 |
| OR‡ | 0.62 | 0.97 | 0.97 | 0.68 |
| (95% CI) | (0.16–2.35) | (0.31–3.01) | (0.70–1.35) | (0.37–1.24) |
| | *P* for interaction = 0.08 | | *P* for interaction = 0.21 | |
| Estrogen receptor beta gene (rs1256049) | | | | |
| GG | | | | |
| No.† | 27/23 | 20/25 | 308/286 | 34/59 |
| OR‡ | 1 | 0.49 | 1 | **0.55** |
| (95% CI) | | (0.21–1.17) | | **(0.35–0.90)** |
| GA + AA | | | | |
| No.† | 19/16 | 13/15 | 35/32 | 2/2 |
| OR‡ | 0.97 | 0.53 | 1.10 | 0.84 |
| (95% CI) | (0.36–2.58) | (0.18–1.58) | (0.64–1.87) | (0.10–6.97) |
| | *P* for interaction = 0.89 | | *P* for interaction = 0.78 | |

†No. of patients with cancer/No. of controls.
‡Conditional model adjusting for menopausal status (premenopausal, postmenopausal), number of births (0, 1, 2, 3, 4, 5+), family history of breast cancer (yes, no), smoking status (never, past, current smokers), moderate physical activity in the past 5 years (no, less than 3 days/month, 1–4 days/week, more than 5 days/week), and vitamin supplement use (yes, no). ORs and 95% CIs with statistical significance are written in bold letter. CIs, confidence intervals; OR, odds ratio.

inverse association between isoflavone intake and breast cancer risk appeared only among women with the GG genotype of the rs4986938 polymorphism in the *ESR2* gene, but the interaction was not statistically significant. Our findings support the hypothesis that polymorphisms in the *ESR2* gene may modify the association between isoflavone intake and breast cancer risk.

To date, many studies investigating the possible effect of SNPs in the *ESR2* gene on breast cancer risk have focused on the

rs4986938 and rs1256049 polymorphisms, although their functional importance has yet to be clarified. Here, we found no association between either SNP and the risk of breast cancer, which is in general agreement with most previous studies.[16,17] In contrast, we did see an inverse association between isoflavone intake and breast cancer risk with the rs4986938 polymorphism in three populations, but only among women with the GG genotype. We also saw a suggestive interaction in the case-control studies of Japanese and Japanese Brazilians but not in the case-control study of non-Japanese Brazilians. Although the reason for the inconsistency in interactions among populations remains unclear, it might reflect the amount of intake, on the basis that the findings were relatively consistent among the populations with a high intake (Japanese and Japanese Brazilians). Moreover, the prevalence of the GG genotype of the rs4986938 polymorphism among the control group was higher in Japanese (72.4%) and Japanese Brazilians (75.9%) than in non-Japanese Brazilians (46.4%). This might partly explain the previous inconsistencies in results for isoflavone exposure and breast cancer risk between Asian and Western populations.[4]

To our knowledge, only two studies have investigated interactions between phytoestrogen exposure and polymorphisms in the *ESR2* gene in the risk of hormone-related diseases.[21,22] Hedelin *et al.* reported a significant interaction between phytoestrogen intake and a promoter SNP in the *ESR2* gene (rs2987983) in the risk of prostate cancer in a population-based case-control study in Sweden.[22] Tsuchiya *et al.* reported a significant interaction between urinary genistein level and RsaI polymorphism in the *ESR2* gene in the risk of advanced endometriosis among infertile Japanese women.[21] These findings suggest that isoflavones may reduce the risk of hormone-related diseases via a mechanism that involves estrogen receptor beta. Considering that functional data are not presently available, our finding suggests that the rs4986938 polymorphism, or some other genetic variants in strong linkage disequilibrium with this SNP, modify the protective effect of isoflavones on breast cancer. In this regard, we provide further evidence for a role of isoflavones in the development of breast cancer.

We found a decreased risk of breast cancer among Japanese women with at least one minor allele of the rs9340799 or rs2234693 polymorphism in comparison with those with the major allele homozygote. Although these are the most frequently studied SNPs, results have been inconsistent.[18–20] Most studies have shown no association between the rs2234693 polymorphism and breast cancer risk.[18–20] On the other hand, several but not all studies have reported that the G allele of the rs9340799 polymorphism was associated with a decreased risk of breast cancer,[18,20] which is consistent with our findings in Japanese women. Since we failed to observe an overall consistency of findings in our three populations, however, our findings in Japanese women might be merely due to chance.

Although interactions between phytoestrogen exposure and polymorphisms in the *ESR1* gene in the risk of breast cancer have not been investigated, we are aware of two studies examining interactions on circulating sex hormone levels.[23,24] In their study of 125 postmenopausal women in the European Prospective Investigation of Cancer and Nutrition–Norfolk cohort, Low *et al.* reported that urinary and serum isoflavones were negatively correlated with plasma estradiol among women with the CC genotype for PvuII polymorphism in the *ESR1* gene, but not those with other genotypes.[23] Moreover, they reported a significant interaction between urinary lignans and rs9340835 polymorphism in the *ESR1* gene, affecting plasma estrone levels in a cross-sectional study of 1988 healthy postmenopausal women from the same cohort.[24] Although these studies imply the presence of gene–nutrient interaction, we found no remarkable difference in the association between isoflavone intake and breast cancer risk by polymorphisms in the *ESR1* gene. Further studies based on a comprehensive evaluation of this gene would clarify this gene–nutrient interaction.

Our study has methodological advantages over studies conducted previously. First, and unique to this study, we assessed gene–nutrient interactions using three populations with substantially different isoflavone intakes and allele frequencies of SNPs. For example, isoflavone intake differed considerably among the three populations, with median levels (interquartile range) in the control group of (mg/day) 40.7 (25.8–61.4) among Japanese, 13.4 (7.9–31.1) among Japanese Brazilians, and 0 (0–0) among non-Japanese Brazilians. In addition, allele frequency also differed among the populations, such as that of the G allele of the rs4986938 polymorphism in the *ESR2* gene, at 0.86 for Japanese, 0.87 for Japanese Brazilians, and 0.67 for non-Japanese Brazilians. Second, the overall consistency of findings in the three populations could allow the results to be more generalized than those from a single population.

Several limitations of the study also warrant mention. First, dietary intake of isoflavones was assessed after the diagnosis of breast cancer, and therefore, is sensitive to recall bias. Second, although the substantially high participation rates among both eligible patients with cancer and controls minimized potential biases related to control selection, the use of controls from medical checkup examinees and cancer-free patients, whose dietary habits may differ from those of the general population due to health consciousness or disease, might have lead to selection bias. For example, isoflavone intake was higher among women aged 50–69 years in the control group of the Nagano study (median intake = 46.3 mg/day) than in participants aged 50–69 years living in Nagano in the 10-year follow-up survey of the Japan Public Health Center-based Prospective Study (median intake = 38.8 mg/day), which used a similar FFQ and had a high response rate. Third, the evaluation of gene–nutrient interactions was performed in a relatively small number of patients with cancer. The interpretability of our results might therefore be limited.

Allowing for these methodological issues, we found a suggestive interaction between isoflavone intake and the rs4986938 polymorphism of the *ESR2* gene in the risk of breast cancer in case-control studies of Japanese and Japanese Brazilians. Our findings support the hypothesis that polymorphisms in the *ESR2* gene may modify the association between isoflavone intake and breast cancer risk. Further, they provide additional evidence that the mechanisms by which isoflavones may reduce the risk of breast cancer might involve estrogen receptor beta.

## Acknowledgments

This study was supported by a Grant-in-Aid for Research on Risk of Chemical Substances from the Ministry of Health, Labour and Welfare of Japan, and Grants-in-Aid for Scientific Research on Priority Areas (17015049) and for Young Scientists (B) (17790378 and 19790415) from the Ministry of Education, Culture, Sports, Science, and Technology of Japan and the Japan Society for the Promotion of Science.

We are grateful to the members of the São Paulo-Japan Breast Cancer Study Group: S. Ikeda and C. Nishimoto (Research Center for Cancer Prevention and Screening, National Cancer Center, Tokyo); C.I. Yamaguchi, C.M. Kunieda, and S.S. Sugama (Nikkei Disease Prevention Center, São Paulo); C.K. Taniguchi and J.A. Marques (Departamento de Ginecologia, Hospital Pérola Byington, São Paulo); M.R. Eichhorn (Departamento de Nutrição, Hospital Pérola Byington, São Paulo); H. Iyeyasu, M.S. Maciel, S.M.T. Carvalho, J.B.D. Collins, and C.E.M. Fontes (Departamento de Mastologia, Hospital A.C. Camargo, São Paulo); L.P. Kowalski and J.M.F. Toyota (Departamento de Cirurgia de Cabeça e Pescoço e Otorrinolaringologia, A.C. Camargo Hospital, São Paulo); E.M. Barbosa (Departamento de Mastologia, Instituto Brazileiro de Controle ao Câncer, São Paulo); O. Ferraro (Departamento de Mastologia, Hospital do Servidor Público Estadual Francisco Morato de Oliveira, São Paulo); R. Anzai (Departamento de Mastologia, Hospital Santa Cruz); E.H. Hotta and D.A. Petti (Instituto de Ginecologia e Mastologia, Hospital Beneficencia Portuguesa); and S. Mendes (Instituto Brazileiro de Mastologia e Ginecologia, Hospital Beneficencia Portuguesa).



doi: 10.1111/j.1349-7006.2009.01118.x

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Breast Cancer Res Treat
DOI 10.1007/s10549-008-0168-1

EPIDEMIOLOGY

# Dietary isoflavone intake and breast cancer risk in case–control studies in Japanese, Japanese Brazilians, and non-Japanese Brazilians

**Motoki Iwasaki · Gerson Shigeaki Hamada · Ines Nobuko Nishimoto · Mario Mourão Netto · Juvenal Motola Jr. · Fábio Martins Laginha · Yoshio Kasuga · Shiro Yokoyama · Hiroshi Onuma · Hideki Nishimura · Ritsu Kusama · Minatsu Kobayashi · Junko Ishihara · Seiichiro Yamamoto · Tomoyuki Hanaoka · Shoichiro Tsugane**

Received: 21 July 2008 / Accepted: 20 August 2008


**Abstract** Although epidemiologic studies have shown an inverse association between isoflavones and breast cancer risk, little evidence for a dose–response relation is available. We conducted hospital-based case–control studies of patients aged 20–74 years with primary, incident, histologically confirmed invasive breast cancer, and matched controls from medical checkup examinees in Nagano, Japan and from cancer-free patients in São Paulo, Brazil. A total of 850 pairs (390 Japanese, 81 Japanese Brazilians and 379 non-Japanese Brazilians) completed validated food frequency questionnaires. The odds ratio of breast cancer according to isoflavone intake was estimated using a conditional logistic regression model. We found a statistically significant inverse association between isoflavone intake and the risk of breast cancer for Japanese Brazilians and non-Japanese Brazilians. For Japanese, a non-significant inverse association was limited to postmenopausal women. In the three populations combined, breast cancer risk linearly decreased from 'no' to 'moderate' isoflavone intake and thereafter leveled off. Compared to non-consumers, adjusted odds ratios (95% confidence interval) for consumers in increasing quintile intake categories (median intake in each category: 8.7, 23.1, 33.8, 45.7, and 71.3 mg/day) were 0.69 (0.44–1.09), 0.54 (0.31–0.94), 0.45 (0.26–0.77), 0.34 (0.19–0.62), and 0.43 (0.24–0.76), respectively. Overall, we found an inverse association between dietary isoflavone intake and risk of breast cancer. Our finding suggests a risk-reducing rather than risk-enhancing effect of isoflavones on breast cancer within the range achievable from dietary intake alone. In addition, women may benefit

M. Iwasaki (✉) · M. Kobayashi · J. Ishihara · T. Hanaoka · S. Tsugane
Epidemiology and Prevention Division, Research Center for Cancer Prevention and Screening, National Cancer Center, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan
e-mail: moiwasak@ncc.go.jp

G. S. Hamada
Nikkei Disease Prevention Center, São Paulo, Brazil

I. N. Nishimoto
Statistical Section/Head and Neck Surgery and Otorhinolaryngology Department, Hospital A.C. Camargo, São Paulo, Brazil

M. M. Netto
Breast Surgery Department, Hospital A.C. Camargo, São Paulo, Brazil

J. Motola Jr. · F. M. Laginha
Department of Breast Surgery, Hospital Pérola Byington, São Paulo, Brazil

Y. Kasuga
Department of Surgery, Nagano Matsushiro General Hospital, Nagano, Japan

S. Yokoyama · H. Onuma
Department of Breast and Thyroid Surgery, Nagano Red Cross Hospital, Nagano, Japan

H. Nishimura
Department of Surgery, Nagano Municipal Hospital, Nagano, Japan

R. Kusama
Department of Surgery, Nagano Hokushin General Hospital, Nagano, Japan

S. Yamamoto
Cancer Information Services and Surveillance Division, Center for Cancer Control and Information Services, National Cancer Center, Tokyo, Japan

from risk reduction if they consume at least moderate amounts of isoflavones.

**Keywords** Breast cancer · Dietary isoflavones · Case–control study · Immigrants

**Abbreviations**

| | |
|---|---|
| CI | Confidence interval |
| ER | Estrogen receptor |
| FFQ | Food-frequency questionnaire |
| OR | Odds ratio |
| PR | Progesterone receptor |

## Introduction

Soy foods, which are rich in isoflavones, are habitually consumed by Asian populations in large amounts. Isoflavones, of which genistein and daidzein are major examples, are classified as phytoestrogens, which are plant-derived non-steroidal compounds with estrogen-like biological properties. A high intake of isoflavones has therefore been hypothesized to contribute to the lower incidence of breast cancer in Asia than Western countries [1]. This hypothesis is supported by not only in vitro studies at high genistein concentrations and the majority of animal studies [2, 3] but also epidemiological studies [4–10]. In particular, a recent meta-analysis showed a small decrease in risk of breast cancer with higher soy intake [11] while a more recent meta-analysis indicated that risk reduction was limited to Asian populations [12]. In apparent contradiction to potential protective effects, however, genistein exhibits estrogenic properties at low concentrations, which could theoretically enhance breast cancer risk [2, 3], and some animal studies have in fact reported that genistein stimulates tumor development and growth [13, 14].

Although research remains insufficient for any comprehensive determination of whether isoflavones are protective or harmful for breast cancer, interest in soy foods and isoflavones is nevertheless increasing. This increase may reflect an expectation of potential benefits in a wide variety of medical conditions, including cancer of the endometrium and prostate as well as breast, cardiovascular diseases, osteoporosis, and menopausal symptoms. In fact, consumption of soy foods in the United States has increased over the past ten years, against fairly constant intake in Japan over the past four decades [15]. Moreover, phytoestrogen supplements are commercially marketed for use by postmenopausal women as natural and safe alternatives to hormone replacement therapy. A dose–response pattern, in particular the effect of relatively high-dose isoflavones on breast cancer risk, is thus now of concern. Nevertheless, little evidence of any dose–response relationship is available—indeed, we do not know the answer to 'how much isoflavones is needed?' This is partly because few studies have estimated isoflavone intake using a validated food-frequency questionnaire (FFQ) [4–6, 16, 17], and also because most studies in Western countries have involved only a small variation in isoflavone intake [6, 7, 16–20].

Here, to evaluate the dose–response relationship between isoflavone intake and the risk of breast cancer, ranging from zero to the relatively high levels achievable from dietary intake only, we conducted hospital-based case–control studies in Nagano, Japan and São Paulo, Brazil, areas with a low and middle incidence of breast cancer, respectively (age-standardized rate per 100,000 world population, 32.7 and 46.0 in 2002, respectively) [21], using validated FFQs with relatively high validity in three populations: Japanese living in Japan, Japanese Brazilians living in São Paulo, and non-Japanese Brazilians living in São Paulo. The mortality of breast cancer among these three populations has increased over the last 20 years, with that in Japanese Brazilians intermediate between that in Japanese and Brazilians [22]. In addition, because amounts and variations in isoflavone intake are expected to be high and large for Japanese, intermediate and relatively large for Japanese Brazilians, and low and small for non-Japanese Brazilians, respectively, these populations serve as suitable venues for studies of the effect of dose–response relations.

## Materials and methods

### Study subjects

These multicenter, hospital-based case–control studies of breast cancer were designed to determine lifestyle factors and genetic susceptibility to the risk of breast cancer and to compare potential risk factors among Japanese living in Nagano, Japan, and Japanese Brazilians and non-Japanese Brazilians living in São Paulo, Brazil. Eligible cases were a consecutive series of female patients aged 20–74 years with newly diagnosed and histologically confirmed invasive breast cancer. Cases were recruited between 2001 and 2005 at four hospitals in Nagano, and between 2001 and 2006 at eight hospitals in São Paulo. A total of 405 cases (98%) participated in Nagano, and 83 Japanese Brazilians (91%) and 389 non-Japanese Brazilians (99%) in São Paulo. In the study in Nagano, eligible controls were selected from medical checkup examinees in two of the four hospitals and confirmed not to have cancer. One control was matched for each case by age (within 3 years) and residential area during the study period. Among potential controls, one examinee refused to participate and two refused to provide blood samples. Consequently, we

obtained written informed consent from 405 matched pairs. In the study in São Paulo, eligible controls were preferentially selected from cancer-free patients who visited the same hospital as the index cases. One control was matched for each case by age (within 5 years) and ethnicity during the study period. Among potential controls, 22 patients refused to participate (participation rate = 96%). Consequently, we obtained written informed consent from 472 matched pairs (83 for Japanese Brazilians and 389 for non-Japanese Brazilians). The study protocol was approved by CONEP (Comissão Nacional de Ética em Pesquisa), Brasília, Brazil and by the institutional review board of the National Cancer Center, Tokyo, Japan.

## Data collection

Participants in Nagano were asked to complete a self-administered questionnaire, while in-person interviews were conducted by trained interviewers using a structured questionnaire in São Paulo. The two questionnaires contained closely similar questions concerning demographic characteristics, medical history, family history of cancer, menstrual and reproductive history, anthropometric factors, physical activity, and smoking habits. For dietary habits, we used a semi-quantitative FFQ (136 items for the Japanese version and 118 items for the Brazilian version) which was developed and validated in each population [23, 24]. Information on estrogen receptor (ER) and progesterone receptor (PR) status was obtained from medial records. Hormone receptor status was determined by either enzyme-linked immunoassay or immunohistochemical assay. Hormone receptor positivity values were determined either as specified by the laboratory that performed the assay, or in accordance with the laboratory's written interpretation thereof, or both.

## Dietary assessment

In the FFQ, participants were questioned on how often they consumed the individual food items (frequency of consumption), as well as relative sizes compared to standard portions. Response choices for frequency were never or less than once/month, 1–3 times/month, 1–2 times/week, 3–4 times/week, 5–6 times/week, once/day, 2–3 times/day, 4–6 times/day, and 7 times/day or more, and relative sizes to a standard portion were small (50% smaller than standard), medium (same as standard), and large (50% larger). For the Japanese version, white rice intake was determined in terms of the relative size of the rice bowl used and the frequency of intake, with the nine choices of less than 1–10 bowls per day. Frequency for miso soup intake was given in the six choices of almost never, 1–3 times/month, 1–2 times/week, 3–4 times/week, 5–6 times/week, or daily, while amount was given in nine categories ranging from less than 1–10 bowls per day, without reference to the relative size of the bowl used. Daily food intake was calculated by multiplying frequency by standard portion and relative size for each food item in the FFQ. Daily intakes of genistein and daidzein were calculated using a food composition table of isoflavones developed previously [25, 26]. Isoflavone intake was defined for this study as the sum of genistein and daidzein intake. Other nutrients were calculated using the Japanese Standard Tables of Food Composition, 5th ed. for the Japanese version [27] and the United States Department of Agriculture (USDA) food composition tables for the Brazilian version [28]. For some Japanese-specific foods in the Brazilian version, the Japanese Standard Tables of Food Composition, 5th ed. was used.

The validity of isoflavone intake estimated from the Japanese version of the FFQ was evaluated in a subsample of the Japan Public Health Center-based Prospective Study, which includes Nagano as one of the study areas. The estimated intake according to the FFQ was compared to that in four consecutive 7-day dietary records, one conducted in each the four seasons. Spearman's correlation coefficients between energy-adjusted genistein and daidzein intake estimated from the FFQ and from dietary records were 0.59 for genistein and 0.60 for daidzein [24]. For the Brazilian version, the validity of isoflavone intake estimated from the FFQ was evaluated in a subsample of the control group in this case–control study by comparing the estimated intake according to the FFQ to that in two consecutive 4-day dietary records, one each in two seasons. Spearman's correlation coefficients between energy-adjusted genistein and daidzein intake estimated from the FFQ and from dietary records were 0.76 for genistein and 0.76 for daidzein (unpublished data).

## Statistical analysis

We excluded subjects who reported extremely low or high total energy intake ($<500$ or $\geq 4000$ Kcal), leaving 390 pairs of Japanese, 81 pairs of Japanese Brazilians and 379 pairs of non-Japanese Brazilians for use in the present analyses. Comparison of baseline characteristics between cases and controls was evaluated by the Mantel-Haenszel test using matched-pair strata in each population. Dietary intake of isoflavones was adjusted for total energy intake by the residual method and divided into median or tertile categories based on control distribution for Japanese and Japanese Brazilians, respectively. Because of the small proportion of consumers, non-Japanese Brazilians were categorized into non-consumers and consumers of isoflavones. Using a conditional logistic regression model, we calculated odds ratios (ORs) and 95% confidence intervals

(CIs) of breast cancer for isoflavone intake. An unconditional logistic regression model was used for stratified analyses according to menopausal status. Associations between isoflavone intake and hormone receptor-defined breast cancer were assessed by an unconditional polytomous logistic regression model. Linear trends for ORs were tested in the logistic regression model using the exposure categories as ordinal variables. The following variables, which were mainly selected based on comparison of baseline characteristics between cases and controls, were adjusted for as potential confounders: menopausal status, number of births, family history of breast cancer, smoking status, moderate physical activity in the past 5 years, and vitamin supplement use. We did not include a history of benign breast disease as a covariate since we regarded it as an intermediate variable in the causal pathway between isoflavone intake and breast cancer. All p values reported are two-sided, and significance level was set at $P < 0.05$. All statistical analyses were performed with SAS software version 9.1 (SAS Institute, Inc., Cary, NC).

## Results

### Characteristics of cases and controls and isoflavone intake (Table 1)

For Japanese, the proportion of premenopausal women, current smokers, and vitamin supplement users was higher in cases than in controls, and cases tended to have a family history of breast cancer and history of benign breast disease. Cases were less likely than controls to breast-feed, be physically active, and eat vegetables. For Japanese Brazilians, cases were less likely than controls to give birth and be physically active and more likely to eat vegetables and fruits. For non-Japanese Brazilians, the proportion of premenopausal women and current smokers was higher in cases than controls while the proportion of physically active women and vitamin supplement users was lower. Isoflavone intake substantially varied among populations, with mean intakes (mg/day) in control subjects of 46.1 for Japanese, 24.9 for Japanese Brazilians, and 4.4 for non-Japanese Brazilians. Because genistein and daidzein intakes were highly correlated, with a Spearman's correlation coefficient for the three populations of 0.99, only isoflavone intake was used for the following analyses.

### ORs in the three populations (Table 2)

We found a statistically significant inverse association between isoflavone intake and the risk of breast cancer for Japanese Brazilians and non-Japanese Brazilians but not for Japanese. Adjusted OR for the highest versus lowest tertile of isoflavone intake was 0.25 (95% CI 0.09–0.68; $P$ for trend <0.01) for Japanese Brazilians. For non-Japanese Brazilians, adjusted OR for consumers versus non-consumers of isoflavones was 0.56 (95% CI 0.35–0.90). No substantial change was seen after further adjustment for other potential confounders, such as age at menarche, age at menopause, age at first birth, history of breast feeding, body mass index, alcohol drinking, or vegetable and fruit intake.

A stratified analysis according to menopausal status revealed that an inverse association was limited to postmenopausal women in Japan although it was not statistically significant. Adjusted OR for the highest versus lowest tertile of isoflavone intake was 0.62 (95% CI 0.38–1.01; $P$ for trend = 0.06) for postmenopausal women, but 1.35 (95% CI 0.72–2.54; $P$ for trend = 0.41) for premenopausal women. The inverse association was stronger in premenopausal than postmenopausal women for Japanese Brazilians but no remarkable difference between the two strata was seen for non-Japanese Brazilians.

### ORs of hormone receptor-defined breast cancer (Table 3)

Information on the combined ER and PR status of the breast tumor was available for 387 (99%) Japanese, 61 (75%) Japanese Brazilians, and 264 (70%) non-Japanese Brazilians cases. The following subtypes were used for modeling in an unconditional polytomous logistic regression model: positive for both receptors (ER+/PR+), ER-positive and PR-negative (ER+/PR−), and negative for both receptors (ER−/PR−) for Japanese, and ER+/PR+, ER+/PR−, ER−/PR−, and unknown for Japanese Brazilians and non-Japanese Brazilians. Overall, we found no remarkable difference in risk by hormone receptor-defined subtype.

### Dose–response pattern (Table 4; Fig. 1)

To evaluate dose–response relations using a wide range of isoflavone intake, we combined individual study data from three populations and categorized the subjects into six groups, namely non-consumers and quintiles among isoflavone consumers based on the combined control distribution. Compared to non-consumers, adjusted ORs (95% CI) for consumers in increasing quintile categories (median intake in each category: 8.7, 23.1, 33.8, 45.7, and 71.3 mg/day) based on a conditional logistic regression model were 0.69 (0.44–1.09), 0.54 (0.31–0.94), 0.45 (0.26–0.77), 0.34 (0.19–0.62), and 0.43 (0.24–0.76), respectively. A stratified analysis according to menopausal status based on an unconditional logistic regression model revealed that this inverse association was more prominent in postmenopausal

**Table 1** Characteristics of case and matched control subjects

| | Japanese living in Nagano, Japan | | | Japanese Brazilians living in São Paulo, Brazil | | | Non-Japanese Brazilians living in São Paulo, Brazil | | |
|---|---|---|---|---|---|---|---|---|---|
| | Case ($n = 390$) | Control ($n = 390$) | $P^a$ | Case ($n = 81$) | Control ($n = 81$) | $P^a$ | Case ($n = 379$) | Control ($n = 379$) | $P^a$ |
| Age (years), mean | 53.8 | 54.0 | – | 56.6 | 56.5 | – | 52.4 | 52.5 | – |
| Premenopausal women, % | 46 | 35 | <0.01 | 31 | 30 | 0.80 | 42 | 38 | 0.04 |
| Age at menopause (years), mean[b] | 49.0 | 49.4 | 0.15 | 49.9 | 50.6 | 0.73 | 49.1 | 48.4 | 0.13 |
| Age at menarche (years), mean[b] | 13.4 | 13.2 | 0.42 | 12.9 | 12.9 | 0.20 | 13.2 | 13.1 | 0.96 |
| Nulliparous women, % | 13 | 14 | 0.66 | 23 | 16 | 0.24 | 11 | 10 | 0.91 |
| Number of births (≥4 births), % | 2 | 3 | 0.16 | 7 | 20 | 0.02 | 29 | 35 | 0.10 |
| Age at first birth (years), mean[b, c] | 26.9 | 26.4 | 0.42 | 28.6 | 27.5 | 0.25 | 23.2 | 22.5 | 0.24 |
| Breast feeding (yes), %[c] | 91 | 96 | 0.03 | 92 | 91 | 0.56 | 88 | 91 | 0.67 |
| Oral contraceptives user, % | 3 | 3 | 1.00 | 29 | 36 | 0.30 | 63 | 65 | 0.62 |
| Family history of breast cancer, % | 11 | 6 | 0.02 | 15 | 12 | 0.65 | 6 | 6 | 0.88 |
| History of benign breast disease, % | 12 | 7 | 0.03 | 12 | 6 | 0.17 | 7 | 7 | 1.00 |
| Height (cm), mean[b] | 155.3 | 155.5 | 0.50 | 154.0 | 153.9 | 0.91 | 158.2 | 158.4 | 0.96 |
| Body mass index (kg/m$^2$), mean[b] | 22.7 | 23.0 | 0.07 | 24.3 | 24.5 | 0.43 | 26.6 | 26.1 | 0.11 |
| Smoking (current smoker), % | 8 | 5 | <0.01 | 11 | 2 | 0.07 | 17 | 11 | 0.04 |
| Alcohol drinking (regular drinker), % | 26 | 29 | 0.25 | 2 | 6 | 0.26 | 6 | 6 | 0.65 |
| Moderate physical activity past 5 years (yes), % | 32 | 40 | 0.02 | 19 | 32 | 0.03 | 9 | 14 | 0.03 |
| Vitamin supplement user, % | 18 | 12 | 0.03 | 19 | 26 | 0.27 | 3 | 9 | <0.01 |
| Total energy intake (kcal/day), mean[b] | 1881.6 | 1949.3 | 0.27 | 1662.0 | 1587.7 | 0.44 | 1847.0 | 1752.8 | 0.09 |
| Fish and shellfish intake (g/day), mean[b] | 87.6 | 94.4 | 0.11 | 27.4 | 30.5 | 0.56 | 13.7 | 16.6 | 0.24 |
| Meat or red meat intake (g/day), mean[b, d] | 58.1 | 57.6 | 0.36 | 54.3 | 53.3 | 0.44 | 72.1 | 64.2 | 0.14 |
| Vegetable intake (g/day), mean[b] | 257.6 | 310.5 | <0.01 | 146.7 | 93.0 | <0.01 | 77.7 | 86.4 | 0.96 |
| Fruit intake (g/day), mean[b] | 288.6 | 287.7 | 0.69 | 364.0 | 311.0 | 0.02 | 260.2 | 250.9 | 0.35 |
| Isoflavone intake (mg/day), mean[b] | 43.5 | 46.1 | <0.01 | 16.5 | 24.9 | 0.15 | 1.1 | 4.4 | 0.01 |
| Genistein intake (mg/day), mean[b] | 27.0 | 28.6 | <0.01 | 10.2 | 15.8 | 0.15 | 0.73 | 3.1 | 0.01 |
| Daidzein intake (mg/day), mean[b] | 16.5 | 17.5 | <0.01 | 6.3 | 9.1 | 0.15 | 0.33 | 1.4 | 0.01 |

[a] $P$ for Mantel-Haenszel test with matched-pair strata

[b] Adjusted for age

[c] Among parous women

[d] Meat intake for Japanese and red meat intake for Japanese Brazilians and non-Japanese Brazilians

than premenopausal women. To clarify the effect of high isoflavone intake in detail, subjects were further categorized into 11 groups, namely non-consumers and deciles of isoflavone consumers. We found a linear decrease in breast cancer risk from zero to moderate intake (20–30 mg/day) and a leveling-off thereafter based on a conditional logistic regression model (Fig. 1). No increasing trend was found for relatively high intake.

## Discussion

In these case–control studies of Japanese, Japanese Brazilians, and non-Japanese Brazilians, overall, we found an inverse association between dietary isoflavone intake and the risk of breast cancer. Our finding is in general agreement with those of a recent meta-analysis [11] and in five of the ten previous studies examining the association between isoflavone intake as estimated by FFQ and breast cancer risk [4–8]. It is noteworthy that, although several experimental studies have suggested adverse effects from soy constituents [2, 3, 13, 14], no epidemiological study estimating isoflavone intake by FFQ has reported an increased risk of breast cancer. Our study also suggests a risk-reducing rather than risk-enhancing effect of isoflavones on breast cancer within the range achievable from dietary intake alone. It remains unclear, however, whether isoflavone exposure other than dietary intake is associated with the risk of breast cancer.

We found a linear decrease in breast cancer risk from zero to moderate intake (20–30 mg/day) and thereafter a leveling-off. This dose–responses pattern might imply the

**Table 2** Odds ratios (ORs) and 95% confidence intervals (CIs) of breast cancer according to dietary isoflavone intakes

| | Median isoflavone intake[a] | All subjects | | | | | | Premenopausal women | | | | Postmenopausal women | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | No. | | OR[b] | 95% CI | OR[c] | 95% CI | No. | | OR[d] | 95% CI | No. | | OR[d] | 95% CI |
| | | Case | Control | | | | | Case | Control | | | Case | Control | | |
| Japanese living in Nagano, Japan | | | | | | | | | | | | | | | |
| Tertile 1 | 22.1 | 152 | 129 | 1.00 | | 1.00 | | 80 | 67 | 1.00 | | 72 | 62 | 1.00 | |
| Tertile 2 | 39.6 | 118 | 131 | 0.75 | (0.53–1.07) | 0.86 | (0.59–1.27) | 52 | 44 | 0.99 | (0.58–1.71) | 66 | 87 | 0.79 | (0.48–1.29) |
| Tertile 3 | 69.1 | 120 | 130 | 0.75 | (0.52–1.10) | 0.83 | (0.54–1.28) | 46 | 26 | 1.35 | (0.72–2.54) | 74 | 104 | 0.62 | (0.38–1.01) |
| *P* for trend | | | | 0.12 | | 0.39 | | | | 0.41 | | | | 0.06 | |
| Japanese Brazilians living in São Paulo, Brazil | | | | | | | | | | | | | | | |
| Tertile 1 | 4.7 | 41 | 26 | 1.00 | | 1.00 | | | | | | | | | |
| Tertile 2 | 13.4 | 25 | 28 | 0.51 | (0.23–1.15) | 0.48 | (0.20–1.16) | | | | | | | | |
| Tertile 3 | 42.8 | 15 | 27 | **0.35** | **(0.15–0.80)** | **0.25** | **(0.09–0.68)** | | | | | | | | |
| *P* for trend | | | | **0.01** | | **<0.01** | | | | | | | | | |
| Median 1 | 8.0 | 48 | 40 | 1.00 | | 1.00 | | 16 | 10 | 1.00 | | 32 | 30 | 1.00 | |
| Median 2 | 35.0 | 33 | 41 | 0.68 | (0.37–1.26) | 0.52 | (0.26–1.06) | 9 | 14 | **0.17** | **(0.03–0.84)** | 24 | 27 | 0.84 | (0.37–1.92) |
| Non-Japanese Brazilians living in São Paulo, Brazil | | | | | | | | | | | | | | | |
| Non-consumers | 0 | 343 | 318 | 1.00 | | 1.00 | | 147 | 124 | 1.00 | | 196 | 194 | 1.00 | |
| Consumers | 15.0 | 36 | 61 | **0.54** | **(0.34–0.84)** | **0.56** | **(0.35–0.90)** | 14 | 21 | 0.54 | (0.26–1.13) | 22 | 40 | 0.58 | (0.33–1.03) |

[a] Crude intake (mg/day)

[b] Crude OR

[c] Conditional model adjusting for menopausal status (premenopausal women, postmenopausal women), number of births (0, 1, 2, 3, 4, 5+), family history of breast cancer (yes, no), smoking status (never, past, current smokers), moderate physical activity in the past 5 years (no, less than 3 days/month, 1–4 days/week, more than 5 days/week), and vitamin supplement use (yes, no)

[d] Unconditional model adjusting for matching factors (age and area for Japanese; age and hospital for Japanese Brazilians; age and ethnicity for non-Japanese Brazilians), number of births (0, 1, 2, 3, 4, 5+), family history of breast cancer (yes, no), smoking status (never, past, current smokers), moderate physical activity in the past 5 years (no, less than 3 days/month, 1–4 days/week, more than 5 days/week), and vitamin supplement use (yes, no)

Bold characters indicates statistically significant values

**Table 3** Odds ratios (ORs) and 95% confidence intervals (CIs) of hormone receptor-defined breast cancer according to dietary isoflavone intakes

| | No. of controls | ER+/PR+ | | | ER+/PR− | | | ER−/PR− | | | Unknown | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | No. of cases | OR[a] | 95% CI | No. of cases | OR[a] | 95% CI | No. of cases | OR[a] | 95% CI | No. of cases | OR[a] | 95% CI |
| Japanese living in Nagano, Japan, all subjects | | | | | | | | | | | | | |
| Tertile 1 | 129 | 82 | 1.00 | | 23 | 1.00 | | 38 | 1.00 | | | | |
| Tertile 2 | 131 | 70 | 0.98 | (0.64–1.51) | 24 | 1.10 | (0.58–2.08) | 21 | 0.58 | (0.32–1.07) | | | |
| Tertile 3 | 130 | 67 | 0.97 | (0.62–1.51) | 22 | 0.71 | (0.36–1.43) | 28 | 0.71 | (0.40–1.28) | | | |
| *P* for trend | | | 0.89 | | | 0.35 | | | 0.23 | | | | |
| Japanese living in Nagano, Japan, premenopausal women | | | | | | | | | | | | | |
| Tertile 1 | 67 | 46 | 1.00 | | 8 | 1.00 | | 18 | 1.00 | | | | |
| Tertile 2 | 44 | 40 | 1.35 | (0.74–2.46) | 4 | 0.80 | (0.22–2.89) | 6 | 0.47 | (0.17–1.32) | | | |
| Tertile 3 | 26 | 27 | 1.51 | (0.74–3.07) | 7 | 1.64 | (0.48–5.58) | 10 | 0.94 | (0.34–2.56) | | | |
| *P* for trend | | | 0.22 | | | 0.52 | | | 0.65 | | | | |
| Japanese living in Nagano, Japan, postmenopausal women | | | | | | | | | | | | | |
| Tertile 1 | 62 | 36 | 1.00 | | 15 | 1.00 | | 20 | 1.00 | | | | |
| Tertile 2 | 87 | 30 | 0.68 | (0.37–1.25) | 20 | 1.25 | (0.57–2.73) | 15 | 0.65 | (0.30–1.44) | | | |
| Tertile 3 | 104 | 40 | 0.68 | (0.38–1.22) | 15 | 0.53 | (0.22–1.26) | 18 | 0.57 | (0.27–1.22) | | | |
| *P* for trend | | | 0.21 | | | 0.14 | | | 0.15 | | | | |
| Japanese Brazilians living in São Paulo, Brazil, all subjects | | | | | | | | | | | | | |
| Median 1 | 40 | 24 | 1.00 | | 7 | 1.00 | | 7 | 1.00 | | 9 | 1.00 | |
| Median 2 | 41 | 16 | 0.63 | (0.27–1.45) | 2 | 0.22 | (0.04–1.36) | 4 | 0.34 | (0.08–1.49) | 11 | 1.24 | (0.38–4.03) |
| Non-Japanese Brazilians living in São Paulo, Brazil, all subjects | | | | | | | | | | | | | |
| Non-consumers | 318 | 97 | 1.00 | | 41 | 1.00 | | 76 | 1.00 | | 108 | 1.00 | |
| Consumers | 61 | 8 | 0.46 | (0.21–1.004) | 9 | 1.10 | (0.50–2.41) | 10 | 0.67 | (0.33–1.40) | 7 | **0.35** | **(0.16–0.80)** |

[a] Unconditional model adjusting for matching factors (age and area for Japanese; age and hospital for Japanese Brazilians; age and ethnicity for non-Japanese Brazilians), menopausal status (premenopausal women, postmenopausal women), number of births (0, 1, 2, 3, 4, 5+), family history of breast cancer (yes, no), smoking status (never, past, current smokers), moderate physical activity in past 5 years (no, less than 3 days/month, 1–4 days/week, more than 5 days/week), and vitamin supplement use (yes, no)

Bold characters indicates statistically significant values

**Table 4** Odds ratios (ORs) and 95% confidence intervals (CIs) of breast cancer according to dietary isoflavone intake based on combined individual study data from three populations

| | Non-consumers and quintile category among consumers | | | | | | *P* for trend |
|---|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 | 5 | |
| Median isoflavone intake (mg/day)[a] | 0 | 8.7 | 23.1 | 33.8 | 45.7 | 71.3 | |
| Japanese living in Nagano, Japan | | | | | | | |
| No. of cases/No. of controls | 0/0 | 49/31 | 93/90 | 89/85 | 72/96 | 87/88 | |
| Japanese Brazilians living in São Paulo, Brazil | | | | | | | |
| No. of cases/No. of controls | 9/5 | 46/41 | 16/12 | 3/8 | 1/6 | 6/9 | |
| Non-Japanese Brazilians living in São Paulo, Brazil | | | | | | | |
| No. of cases/No. of controls | 343/318 | 27/33 | 5/3 | 2/13 | 2/3 | 0/9 | |
| All subjects in three populations | | | | | | | |
| No. of cases/No. of controls | 352/323 | 122/105 | 114/105 | 94/106 | 75/105 | 93/106 | |
| OR | 1.00 | 0.69 | **0.54** | **0.45** | **0.34** | **0.43** | **<0.01** |
| (95% CI)[b] | | (0.44–1.09) | **(0.31–0.94)** | **(0.26–0.77)** | **(0.19–0.62)** | **(0.24–0.76)** | |
| Premenopausal women in three populations | | | | | | | |
| No. of cases/No. of controls | 150/127 | 48/37 | 58/52 | 49/37 | 23/30 | 36/23 | |
| OR | 1.00 | 0.68 | 0.44 | 0.54 | **0.27** | 0.62 | 0.27 |
| (95% CI)[c] | | (0.33–1.39) | (0.19–1.01) | (0.24–1.24) | **(0.10–0.69)** | (0.25–1.54) | |
| Postmenopausal women in three populations | | | | | | | |
| No. of cases/No. of controls | 202/196 | 74/68 | 56/53 | 45/69 | 52/75 | 57/83 | |
| OR | 1.00 | 0.70 | 0.52 | **0.31** | **0.34** | **0.33** | **<0.01** |
| (95% CI)[c] | | (0.40–1.24) | (0.26–1.04) | **(0.15–0.64)** | **(0.17–0.71)** | **(0.16–0.66)** | |

[a] Energy adjusted by residual method

[b] Conditional model adjusting for menopausal status (premenopausal women, postmenopausal women), number of births (0, 1, 2, 3, 4, 5+), family history of breast cancer (yes, no), smoking status (never, past, current smokers), moderate physical activity in the past 5 years (no, less than 3 days/month, 1–4 days/week, more than 5 days/week), and vitamin supplement use (yes, no)

[c] Unconditional model adjusting for age (continuous), study population (Japanese living in Nagano, Japan; Japanese Brazilians living in São Paulo, Brazil; non-Japanese Brazilians living in São Paulo, Brazil), number of births (0, 1, 2, 3, 4, 5+), family history of breast cancer (yes, no), smoking status (never, past, current smokers), moderate physical activity in the past 5 years (no, less than 3 days/month, 1–4 days/week, more than 5 days/week), and vitamin supplement use (yes, no)

Bold characters indicates statistically significant values

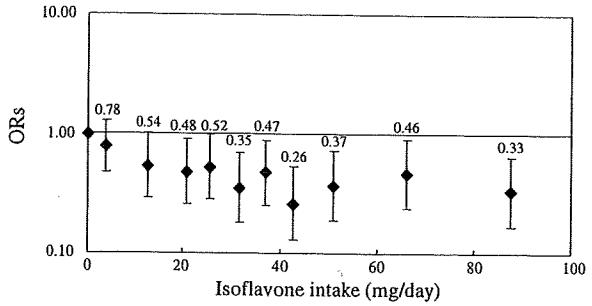


**Fig. 1** Odds ratios (ORs) and 95% confidence intervals of breast cancer according to dietary isoflavone intake based on combined individual data from three populations. Subjects were categorized into 11 groups: non-consumers and deciles of isoflavone consumers based on the control distribution. ORs were estimated using matching pairs with adjustment for menopausal status (premenopausal women, postmenopausal women), number of births (0, 1, 2, 3, 4, 5+), family history of breast cancer (yes, no), smoking status (never, past, current smokers), moderate physical activity in the past 5 years (no, less than 3 days/month, 1–4 days/week, more than 5 days/week), and vitamin supplement use (yes, no)

presence of a ceiling effect and suggests that women may benefit from risk reduction if they consume at least a moderate amount of isoflavones. Alternatively, it might merely reflect differences in measurement errors due to the use of different FFQs, selection bias, and residual confounding among the three populations, notwithstanding that it clearly reflected the results of separate analyses. Specifically, consumers had lower risk than non-consumers in non-Japanese Brazilians, whose average intake of isoflavone was 4.4 mg/day among the control group; the risk of breast cancer decreased with increasing intake of isoflavone in Japanese Brazilians, whose average intake of isoflavone was 24.9 mg/day among the control group; while higher intake of isoflavone was not associated with further risk reduction in Japanese, whose average intake of isoflavone was 46.1 mg/day among the control group. Confirmation of this pattern would require further prospective cohort studies using blood or urine samples as an exposure assessment, because these could minimize the measurement errors and selection bias mentioned above.

Our stratified analysis by menopausal status using data from the three populations combined showed that an inverse association was more prominent among postmenopausal than premenopausal women. In addition, our separate analyses showed somewhat different patterns in the three populations: the inverse association was limited to postmenopausal women in Japanese; it was stronger in premenopausal than postmenopausal women in Japanese Brazilians; and no remarkable difference was found in non-Japanese Brazilians. These findings are inconsistent with a recent meta-analysis showing an inverse association regardless of menopausal status [11]. Moreover, findings to date on the association of isoflavone intake and the risk of breast cancer stratified by menopausal status have been inconsistent, with one prospective cohort study in Japan [4] and one case–control study in the United States [8] reporting that an inverse association was limited to postmenopausal women; one case–control study in Japan [5] showing it was limited to premenopausal women; and one prospective cohort study in the United States [16] and three case–control studies [6, 17, 18] finding no difference between the two strata.

Several mechanisms by which isoflavones may reduce the risk of breast cancer have been proposed [2, 3]. The most prominent and thoroughly investigated mechanisms are mediated via estrogen receptors, arising due to the similar chemical structure of isoflavones to the human estrogen hormone and their binding affinity to estrogen receptors [3, 29]. Given that the action of estrogen on breast cell proliferation appears to be mediated by estrogen receptors, therefore, any association between isoflavone intake and breast cancer risk might differ by hormone receptor-defined subtype. The present study did not support this hypothesis, however, showing no apparent difference in risk by subtype. Moreover, results for the few studies to date have been inconsistent [7, 16, 18, 19]. Although our findings might merely be explained by a lack of statistical power, they suggest that the anti-cancer effects of isoflavones might be evoked not only by mechanisms mediated by estrogen receptors but also by other mechanisms, such as the modulation of endogenous hormones via inhibition of the key enzyme involved in estrogen biosynthesis and metabolism; the arrest of cell cycle progression; induction of apoptosis; inhibition of tyrosine kinase activity, topoisomerase II activity, and angiogenesis; and antioxidant activity [2, 3].

Our study has several methodological advantages over previous studies of isoflavones and the risk of breast cancer. First, isoflavone intake differed considerably among the three populations, with median levels (interquartile rage) in the control group (mg/day) of 40.6 (25.9–61.2) among Japanese, 13.4 (8.1–35.0) among Japanese Brazilians, and 0 (0–0) among non-Japanese Brazilians. This range allowed the detailed evaluation of dose–response relations, ranging from zero to a relatively high level achievable from dietary intake only, and is unique to the present study. Second, the overall consistency of findings in the three populations allowed for the greater generalizability of results as compared to those from a single population.

Several limitations of this study warrant mention. First, dietary intake of isoflavone was assessed after the diagnosis of breast cancer and is therefore sensitive to recall bias. Second, although the substantially high participation rates among both eligible cases and controls minimized potential biases related to control selection, the use of controls from

medical checkup examinees and cancer-free patients, whose dietary habits may differ from the general population due to health consciousness or disease, might have lead to selection bias. Third, stratified analyses were performed based on a relatively small number of cases. The interpretability of our results might therefore be limited.

Allowing for these methodological issues, we found an inverse association between dietary isoflavone intake and the risk of breast cancer in case–control studies of Japanese, Japanese Brazilians, and non-Japanese Brazilians. Our findings suggest a risk-reducing rather than risk-enhancing effect of isoflavones on breast cancer within the range achievable from dietary intake alone. In addition, women may benefit from risk reduction if they consume at least moderate amounts of isoflavones.

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

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