Commentary

Mammographic breast density as a biomarker of effects of isoflavones on the female breast

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Received: 9 July 2001
Revisions requested: 24 July 2001
Revisions received: 12 September 2001
Accepted: 18 September 2001
Published: 25 October 2001

Breast Cancer Res 2002, **4**:1-4

(Print ISSN 1465-5411; Online ISSN 1465-542X)

Abstract

Isoflavones possess both estrogenic and anti-estrogenic actions, and are hypothesized to protect against breast cancer. However, two intervention studies of markers of proliferation on breast tissue have raised concerns that soy isoflavones may have an estrogenic effect on breast tissue. Increased mammographic breast density is associated with an elevated risk of breast cancer, although the mechanism underlying this relationship has not been explained. Estrogens increase and anti-estrogens decrease breast density. Breast density may therefore serve as a biomarker of estrogenic or anti-estrogenic effects of a treatment on breast tissue. The effect of isoflavones on breast density is under investigation.

Keywords: breast cancer, diet, hormones, isoflavones, mammographic density

Isoflavones

Isoflavones are polyphenolic compounds naturally present in foods of plant origin that bear a striking structural similarity to the mammalian estrogens. Soybeans, and products made from soybeans, are by far the richest identified sources of isoflavones (Table 1), and it has been hypothesized that soy consumption in Asian populations may contribute to the lower incidence of breast cancer and other hormone-dependent conditions in Eastern versus Western populations [1]. Isoflavones are capable of binding to estrogen receptors α and β [2], and they are weak estrogens. In common with other weak estrogens, and similar to selective estrogen receptor modulators such as tamoxifen, isoflavones can act as both estrogen agonists and estrogen antagonists, the latter suggesting potential anti-proliferative effects of isoflavones on breast tissue. In Western diets, the lignans, another class of phytoestrogens, are quantitatively more important than isoflavones, but the evidence for lignans possessing estrogenic and/or anti-estrogenic activities is less well established, and fewer data are available on their potential beneficial health effects in humans.

Epidemiology

There are few prospective studies on the effects of isoflavones on breast cancer risk. In a recent large-scale, population-based case–control study among Chinese women, intake of soy foods during adolescence, a period when breast tissue is particularly sensitive to environmental stimuli, was associated with a significant reduction in breast cancer risk in later life [3]. In one of the largest studies to date, however, neither tofu nor miso consumption had a significant effect on risk of breast cancer among almost 35,000 women living in Hiroshima and Nagasaki, Japan [4]. No strong confirmation of an effect of soy on reducing risk of breast cancer is thus apparent from exist-

IGF = insulin-like growth factor; IGFBP = insulin-like growth factor binding protein.

Table 1

Isoflavone content of some common foods

	μg/kg wet weight (Da + Gen)
Peas, fresh, cooked	nd
Asparagus, cooked	nd
Carrots, old, cooked	nd
Cucumber, with skin	2.9
Potatoes, old, cooked	7.4
Potatoes, new, cooked	37.5
Red kidney beans, cooked	222.7
Chickpeas, dried, cooked	241.4
French beans, cooked	350.3
Beansprouts, mung, raw	5736
Soy cheese	34,000
Soymilk	44,000
Tofu yogurt	151,000
Soy hot dog, tempeh burger	188,000
Tofu	242,000
Soybeans, dried, cooked	469,000
Miso	1,265,000

Data from Liggins *et al.* [24], and Reinli and Block [25]. Da, Daidzein; Gen, Genistein; nd, not detected.

ing studies, but no studies to date have identified an increased risk of breast cancer associated with elevated intakes of isoflavones.

Clinical intervention trials

There are many trials in the literature designed to investigate the mechanisms whereby isoflavones may protect against breast cancer. However, two have raised concerns that isoflavones may exert estrogenic effects on breast tissue. Petrakis *et al*. [5] reported an increase in the presence of epithelial hyperplasia in nipple aspirate fluid from premenopausal and postmenopausal women following a 6-month soy protein intervention, and Hargreaves *et al*. [6] reported changes in estrogen-regulated proteins in nipple aspirate fluid that were indicative of an estrogenic response among premenopausal women following a 14-day soy protein intervention. Interestingly, Hargreaves *et al*. also reported significantly higher concentrations of both daidzein and genistein in nipple aspirate fluid than in paired serum samples, suggesting that isoflavones may accumulate within the breast ducts [6]. Thus, although isoflavones are thought to be protective over breast cancer, it is not clear what effect they may have on breast tissue, and a bioassay of their effects is needed.

Breast density

The mammographic appearance of the breast differs between individuals according to the relative proportions of radiologically dense connective and epithelial tissues, and of radiologically lucent fat. Increased radiological density has consistently been associated with an increased risk of breast cancer since the 1970s. Investigators have used both qualitative (e.g. Wolfe's classification system) and quantitative assessments of breast density (reviewed in [7]). Quantitative assessments, including computer-assisted techniques, have generally been associated with larger gradients in risk of breast cancer than qualitative assessments [7] and, to date, the majority of studies have expressed breast density as a percentage of the breast occupied by dense tissue. The absolute area of dense tissue is also associated with risk of breast cancer (see [7] and references cited therein). In a study among women from a multi-ethnic population, women with small total breast areas had higher percent densities but lower absolute densities than women with large total breast areas [8]. It is not yet clear, however, whether one method of expressing breast density is preferable over the other.

In a review of case–control studies using quantitative methods of determining density, odds ratios for women in the highest versus lowest extents of density (expressed as percent density) ranged from 2.1 to 6.0 [7]. These odds ratios are of a greater magnitude than relative risks incurred from established breast cancer risk factors such as early age at menarche and late age at menopause [9].

Potentially, therefore, breast density is an intermediate risk marker for breast cancer. The mechanism underlying increased breast density is not fully understood, but a number of factors have been associated with breast density changes.

Hormones

There is much evidence that breast density is sensitive to changes in a woman's hormonal milieu. Breast density increases when a woman begins hormone replacement therapy and decreases when she discontinues (see [10] and references cited therein). Furthermore, fewer women have dense breasts after the menopause compared with before the menopause (reviewed in [7]). It has recently been shown that the anti-estrogen tamoxifen decreases breast density [11–13]. A hormonal contraceptive regimen designed to reduce exposure of the breast to estrogen and progesterone also reduced mammographic density [14]. The contrasting effects of estrogens and anti-estrogens on breast density, and the increased risk of breast cancer associated with dense breasts, suggest that it may be useful as a biomarker of estrogenic or anti-estrogenic effects of a treatment on breast tissue. High circulating levels of insulin-like growth factor (IGF)-I and low circulating levels of IGF binding protein (IGFBP)-3

have also been associated with increased mammographic density in premenopausal women [15]. Since IGFs increase cell proliferation and IGFBPs inhibit proliferation, it has been suggested that increased breast density might result from stimulation of cell proliferation by IGF-I and/or impaired inhibition of proliferation resulting from low levels of IGFBP-3 [15].

Genetic factors

A family history of breast cancer has been positively associated with breast density in some, although not all, studies. In a cohort study of families with a history of breast cancer, segregation analysis suggested that a major autosomal gene influences breast density (see [7] and references cited therein). It has been suggested that *BRCA1* carriers might have increased breast density due to mammary epithelial hyperplasia brought about by a defect in the tumor suppressor gene, but to date this has not been shown [16]. One study has examined polymorphisms in low penetrance genes controlling steroid hormone metabolism in relation to percent breast density in women selected on the basis of having Wolfe's P2 or DY breast patterns. Sample sizes within genotypes were small, however, and there were no clear associations between polymorphisms in CYP17 (T → C substitution at the 5′ promoter), CYP19 (G → T substitution on intron 6) or estrogen receptor (C → T substitution on intron 1) genes and percentage breast density [17].

Diet

Data from both observational and intervention studies on diet and mammographic densities have suggested that intakes of certain nutrients can influence breast density. A 2-year low-fat, high-carbohydrate intervention resulted in a significantly greater decrease in absolute area of breast density than occurred in controls consuming a self-selected diet, particularly among women who became postmenopausal during the study [18]. Further analysis of this subgroup showed that reduction in total or saturated fat intake or cholesterol intake was significantly associated with the decrease in dense area, and the most significant dietary variable associated with the reduction in percentage density was reduction in cholesterol intake [19]. In a large cohort study of breast cancer families, however, increased breast density was associated with decreased intakes of saturated fat in premenopausal women [20]. Positive associations between alcohol intakes and breast density have generally been found, although in the breast cancer family cohort white wine was positively associated and red wine negatively associated with density [20]. Positive associations between breast density and total protein and carbohydrate intakes [21] and with intakes of vitamins B_{12}, C, and E [20] have also been described. It remains to be determined, however, by what mechanism the diet influences breast density, but it has been suggested that effects may occur via diet-induced alterations in sex hormone levels [18].

Isoflavones and breast density

If breast density is a marker of breast cancer risk, and isoflavones protect against breast cancer, more lucent breasts might be expected to occur in Asian women. Two cross-sectional studies have examined this. In the first, premenopausal Japanese women had a significantly lower proportion of dense breast patterns and a higher proportion of lucent breast patterns than did British women. The authors did not measure diet, but suggested their findings might be attributable to environmental factors such as diet, since they were unable to find correlations between measurements of endocrine function and breast density [22]. A second cross-sectional study of a multi-ethnic population living in Hawaii, in which soy intakes and mammographic densities were investigated, was not able to confirm this suggestion. Chinese and Japanese women had higher percent densities than Caucasian and Native Hawaiian women (but had lower absolute densities), and there was a significant trend towards higher percent breast density with increasing quartiles of soy-food intake. This relationship was not apparent using absolute area of dense tissue [8]. In a randomized, placebo-controlled trial of an isoflavone supplement (40 mg clover-derived isoflavones per day for 1 year) in women selected on the basis of having Wolfe's P2 or DY breast patterns, a decrease in density was observed in the intervention and placebo groups, but the difference between treatments was not statistically significant ($P > 0.05$). When divided into tertiles according to age, women aged 56–65 years in the isoflavone group experienced a significantly greater decrease in density than women aged 56–65 years in the placebo group ($P < 0.05$). Sample sizes within tertiles of age were small, however, and a larger study is needed to confirm these findings [23]. In a subset of women who had been genotyped for polymorphisms in CYP17 (T → C substitution at the 5′ promoter), CYP19 (G → T substitution on intron 6), and estrogen receptor (C → T substitution on intron 1) genes, there was no effect of genotype on change in breast density [17].

Conclusions and future work

Despite the rapid increase in the extent of research into isoflavones in recent years, there remains a lack of data on which to base recommendations regarding intakes either for patients with breast cancer or healthy women wishing to reduce their risk of breast cancer. Mammographic breast density has the potential to act as a biomarker of estrogenic or anti-estrogenic effects of isoflavones on breast tissue. Further studies are needed to determine the effects of higher doses of isoflavones, effects of long-term isoflavone consumption, and effects of isoflavones consumed at various stages of life on breast tissue. Placebo-controlled intervention trials are the optimal method for determining the effects of a standardized dose of isoflavones. Such trials are potentially problematic, however, in terms of ethical issues associated with the

necessity to carry out repeat mammography. Observational prospective or cross-sectional study designs could provide information on the effects of dietary isoflavones without the need for additional mammography, and would enable large populations to be studied in relatively short periods of time.

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