Breast Care

Review Article · Übersichtsarbeit

Breast Care 2009;4:22–29
DOI: 10.1159/000200980

Published online: February 20, 2009

Isoflavones – Mechanism of Action and Impact on Breast Cancer Risk

Johannes Stubert Bernd Gerber

Department of Obstetrics and Gynecology, University of Rostock, Germany

Key Words
Phytoestrogens · Isoflavones · Breast cancer · Nutrition · Prevention · Risk factors

Summary
Isoflavones are plant-derived substances with weak estrogenic effects. Asian populations are high consumers of soy products which are rich in isoflavones. The lower breast cancer incidence in Asian women compared with Western women has been associated with the possibility of a preventive isoflavone effect on cancer risk. The aim of this review is to give an overview of current research data on the influence of isoflavones on the risk of primary breast cancer development as well as the risk of recurrence in breast cancer patients. Despite inconsistencies in the available data, an inverse correlation between isoflavone intake and risk of breast cancer is likely. However, a negative impact on breast cancer disease, especially on hormone receptor-positive tumors, cannot be excluded at present.

Schlüsselwörter
Phytoöstrogene · Isoflavone · Brustkrebs · Ernährung · Prävention · Risikofaktoren

Zusammenfassung
Isoflavone sind Substanzen pflanzlichen Ursprungs, welche schwach östrogene Wirkung aufweisen. Die Bevölkerung Asiens zeichnet sich durch einen hohen Konsum an isoflavonreicher Sojakost aus. Die im Vergleich zu westlichen Frauen niedrigere Brustkrebsinzidenz bei Asiatinnen wurde mit der Möglichkeit einer präventiven Isoflavonwirkung auf das Krebsrisiko in Verbindung gebracht. Die vorliegende Arbeit gibt einen Überblick über aktuelle Studien sowohl zum Einfluss von Isoflavonen auf das Risiko der Brustkrebsentstehung als auch auf das Rezidivrisiko nach Brustkrebserkrankung. Trotz inkonsistenter Studienlage scheint zwischen Isoflavonaufnahme und Brustkrebsrisiko ein inverser Effekt zu bestehen. Nach Brustkrebserkrankung kann ein negativer Effekt, insbesondere bei hormonsensitiven Tumoren, nicht ausgeschlossen werden.

Introduction

The annual breast cancer incidence rates in Western European countries and in the USA range from about 80 to 100 per 100,000 women. In contrast, the incidence rate in Japan, a country with a comparable level of industrialization, is with 33/100,000 only about a third of that in the USA [1]. Migration studies show a shift from low to higher incidence rates among Asian-American women within one generation after migration, and suggest environmental influences to be responsible for the observed difference in breast cancer risk. The consumption of soy food, which is rich in biologically active phytoestrogens, is traditionally high in Japan. Consequently, phytoestrogens have been connected with the low breast cancer incidence rates in the high-soy-consuming Asian populations. The most common class of phytoestrogens in soy products are isoflavones. Therefore, almost all studies elucidated the role of soy products and isoflavones, respectively. Of further interest, especially in Western populations, is the safety of isoflavone intake among breast cancer patients [2]. The alleged effects

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Dr. med. Johannes Stubert
Universitätsfrauenklinik und Poliklinik am Klinikum Südstadt
Südring 81, 18059 Rostock, Germany
Tel. +49 381440145-00, Fax -99
johannes.stubert@uni-rostock.de

of isoflavones on reducing menopausal vasomotor symptoms make them a commonly used alternative therapy option instead of hormone therapy with estrogens or estrogen/progestin combinations. A meta-analysis of 6 trials on the influence of soy isoflavones on the number of hot flashes per day revealed a weak beneficial effect for soy isoflavones compared with placebo (mean difference in the number of hot flashes per day –1.22, 95% confidence interval (CI) = –2.02 to –0.42) [3]. However, the findings of these trials were rather contradictory and the study assessment of poor quality. A further meta-analysis of another 6 trials compared isoflavone extracts from red clover with placebo and showed a weak difference of only –0.44, 95% CI = –0.47 to 0.58 [3]. This beneficial trend could be confirmed by 2 current randomized, double-blinded placebo-controlled trials (doses of dietary isoflavone intake 40–100 mg/day for 12 weeks to 12 months) [4, 5].

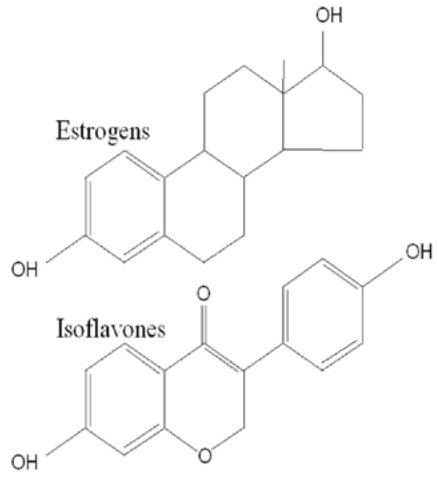

Fig. 1. Chemical structures of estrogens and isoflavones indicating the similarities between both substance classes.

Classification, Structure, and Metabolism of Isoflavones

Phytoestrogens are non-steroidal plant-derived substances that show remote structural and definite functional similarities to estradiol. Beside the lignans, the isoflavones represent the main class of phytoestrogens. In contrast to less investigated other groups of phytoestrogens, e.g. coumestans and stilbenes, which in a lower amount are contained in food, isoflavones are well studied. The most important representatives of this phytoestrogen class are genistein, daidzein, and glycitein. Their chemical structure consists of 2 phenolic rings that enable these substances to bind the estrogen receptor (ER) (fig. 1). The estrogenic potency of isoflavones is about 100-fold (coumestrol) to 1,000-fold (daidzein) weaker than that of 17β-estradiol [6, 7]. But compared with 17β-estradiol peak levels in premenopausal women, the serum levels of isoflavones after dietary intake can be higher in the same manner. In contrast to 17β-estradiol, isoflavones prefer to bind to estrogen receptor β (ERβ), which partially explains their different effects [8]. Depending on ambient estrogen concentrations, isoflavones may exert their effects as estrogen antagonists in a high estrogen environment, or they may act as estrogen agonists in a low estrogen environment [9–11]. Therefore, isoflavones are also referred to as selective ER modulators (SERMs) [12, 13]. Furthermore, isoflavones show several non-hormonal effects such as inhibition of aromatase activity [14, 15], downregulation of protein tyrosine kinases (PTK) [16], or promoter methylation with modulation of gene expression [17]. However, the relevance of these effects still remains unclear.

Unfortunately, the results of many studies are inconsistent due to a lot of bias [18, 19]. So far, there is low evidence for any well-defined estrogen-like or anti-estrogen-like effect in humans [20, 21]. One possible reason for the apparently incoherent findings could be the quantitatively and qualitatively variable composition of phytoestrogens in food stuff and their

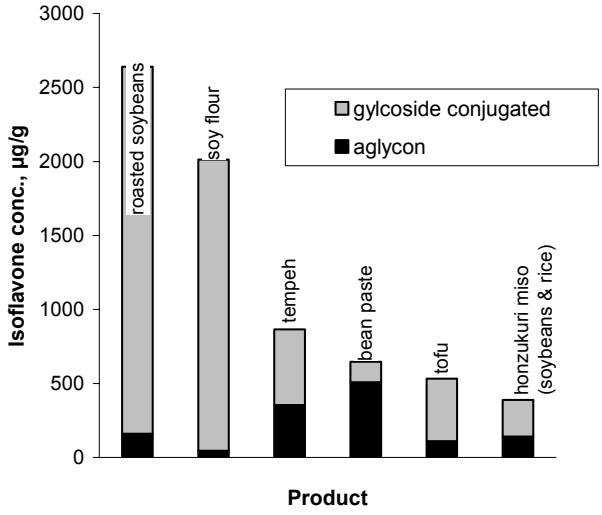

Fig. 2. Total isoflavone concentrations (µg/g) of fermented and unfermented soy products. Relatively higher content of aglycons in the fermented soy products tempeh, bean paste, and miso [81].

complex metabolism with the synthesis of miscellaneous derivates which partially show diverse biologic activities. Isoflavones are contained in plants as glycosidic conjugates. After consumption, they are hydrolyzed by intestinal bacteria forming aglycons. The aglycons are metabolized in the intestinal wall and in the liver, and become glucoronide conjugates. Further metabolism to substances with different estrogenic potency is possible (e.g. equol, O-desmethylangolensin, p-ethylphenol). Last but not least, there is a big variability regarding the inter- and intra-individual metabolism – depending on differences in gut microflora (use of antibiotics!), genetic polymorphisms, or intestinal transit time [22, 23]. For example, the daidzein metabolite equol has been found in urine samples of only 20–30% of Japanese women, and depends also on dairy product intake [24].

The main natural dietary source of isoflavones are soy products which in Asian countries are traditionally consumed in

high doses. With the exception of soy milk and soy infant formulas, isoflavone concentrations in soy products are comparatively high (0.5–3.0 mg/g). Soy products are followed by other legumes, particularly runner beans *(Phaseolus coccineus)* and mung beans *(Vigna radiata),* as well as flaxseed *(Linum usitatissimum).* But concentrations in these products are about 100-fold to 1,000-fold lower than in soy products [25]. However, the isoflavone levels of soy food differ considerably among the various preparations with high content in soy flour and powder and lower content in processed soy food like tofu or miso (fig. 2). Boiling can decrease the isoflavone content of soybeans by more than half, but aglycons, which are found in high concentrations in fermented soy products like miso, are very stable at high temperature [25, 26].

The daily phytoestrogen intake is about 20–50 mg per person in East and Southeast Asian countries and is less than 1 mg in European countries and in the USA [27–32]. Interestingly, doughnuts constitute approximately 20% of the average daily intake of genistein and 15% of daidzein due to the high content of added soy flour in the USA [33].

High Isoflavone Consumption and Breast Cancer Risk

Given the great difference in soy consumption among Western versus Asian populations, these 2 groups should be examined separately. The first report of a breast cancer-preventive effect of a high phytoestrogen intake was published in 1991 for a population of Chinese women [34]. The meta-analysis of Wu et al. [35] gives a comprehensive overview on recent study findings (table 1). It included 8 (1 cohort, 7 case-control) studies with almost complete assessment of the total soy intake in Asian or in Asian-American populations. A further 6 studies were excluded because of incomplete assessment of dietary soy intake. The analysis shows a statistically significant risk reduction of breast cancer by 29% for Asian women with continuous high soy intake (odds ratio (OR) = 0.71, 95% CI = 0.6–0.85). Cut off point for high intake was 20 mg soy isoflavones or more per day, lowest intake was 5 mg or less. Also, a moderate consumption of 10 mg per day showed a statistically significant risk reduction of 12% (OR = 0.88, 95% CI = 0.78–0.98). The described significant inverse correlation was observed in both pre- and postmenopausal women [35]. A large prospective cohort study including a total of 35,303 Singapore Chinese women confirmed the previous data. High soy intake (> 10.6 mg isoflavone/1,000 kcal) was associated with a significant breast cancer risk reduction compared with lower daily intake of soy products (risk ratio (RR) = 0.82, 95% CI = 0.70–0.97). The level of significance was reached only in a subgroup analysis of postmenopausal women, and amongst them the ones above the median body mass index (BMI > 24 kg/m^2) (RR = 0.67, 95% CI = 0.51–0.88) were even more significant [36].

In a recent Japanese study (nested case-control within a prospective cohort of 24,226 women), high serum levels of genistein were statistically significantly inversely correlated to breast cancer risk in comparison to low serum levels (OR = 0.34, 95% CI = 0.16–0.74, p < 0.01). However, the medium isoflavone intake was 32.5 mg/day in the patient group and 32.1 mg/day in the control group. Therefore, the study did not find a statistically significant difference between isoflavone intake and breast cancer risk (p = 0.36). Furthermore, the authors assumed a threshold level of isoflavone effect due to the lack of data on a dose-response relationship. Unlike genistein, the second main isoflavone, daidzein, did not show any beneficial effect on breast cancer risk reduction (OR = 0.71, 95% CI = 0.35–1.44, p = 0.34). None of the findings were substantially influenced by potential confounders such as menopausal status or BMI. Although the study was well arranged, attention should be paid to the short half-live of isoflavones in the blood (only 6–8 h). To minimize the attenuation of risk estimates derived from random measurement errors, the authors matched fasting time between cases and controls [37]. In addition, another recently published Chinese case-control study showed a significant breast cancer risk reduction in women with high plasma levels of genistein (OR 0.26, 95% CI 0.13–0.50) [38].

Low Isoflavone Consumption and Breast Cancer Risk

The meta-analysis of Wu et al. [35] conducted in low-soy-consuming Western populations included 11 studies (table 2). Four of them were cohort or nested case-control studies, and 7 were simple case-control studies. Combined (OR = 1.04, 95% CI = 0.97–1.11) as well as separate testing of cohort (OR 1.08, 95% CI = 0.95–1.24) and case-control studies (OR = 1.02, 95% CI = 0.95–1.11) revealed no significant differences between low (0.15 mg or less) and high (0.8 mg or more) daily isoflavone intake and breast cancer risk. There were no differences in results by menopausal status [35]. The results of the recently published studies are consistent with previous studies. Neither a large prospective US American cohort study (Iowa Women's Health Study, only postmenopausal women) nor 2 equally large prospective European cohort studies (pre- and postmenopausal women), all based on food frequency questionnaires (FFQ), presented any statistically significant effects on breast cancer risk by isoflavone intake [39–41]. In one case-control study, a significant increase of risk for breast cancer in relation to higher isoflavone intake was observed (OR = 1.08, 95% CI = 1.00–1.16, p = 0.055) [42]. In this study, isoflavone consumption was indirectly estimated by measurement of excreted substances in urine samples of pre- and postmenopausal women. The significance level was reached in the total isoflavone group, and further in a subgroup analysis of ER-positive breast cancers for equol – a rather strong estrogenic metabolite of daidzein (OR = 1.07, 95% CI = 1.01–1.12, p = 0.013). The increase in breast cancer risk was only marginal, and furthermore the values of urine samples are inconsistent with the values of the isoflavone

Table 1. Isoflavone intake and breast cancer risk in high-consuming populations

Authors [Ref.]	Study design	Subjects, n	Results (95% CI)	Comments
Wu et al. [35]	meta-analysis of 8 studies (7 case-control, 1 cohort)	3,493 cases, 5,421 controls; cohort of 21,852 women	OR 0.71 (0.60–0.85)	≥ 20 mg vs. ≤ 5 mg daily isoflavone intake
Wu et al. [36]	cohort study	629 cases; cohort of 35,303 women	RR 0.82 (0.70–0.97)	> 10.6 mg/1,000 kcal vs. ≤ 10.6 mg/1,000 kcal intake of daily isoflavones
Iwasaki et al. [37]	nested case-control study	144 cases, 288 controls out of 24,226 women	OR 0.34 (0.16–0.74)	highest vs. lowest quartile of serum levels of genistein; no difference for daidzein serum levels and no significant difference for dietary isoflavone intake
Lampe et al. [38]	case-control study	196 cases, 1,002 controls	OR 0.26 (0.13–0.50)	highest vs. lowest quartile of plasma levels of genistein

CI = Confidence interval; OR = odds ratio; RR = risk ratio.

Table 2. Isoflavone intake and breast cancer risk in low-consuming populations

Authors [Ref.]	Study design	Subjects, n	Results (95% CI)	Comments
Wu et al. [35]	meta-analysis of 11 studies (7 case-control, 4 cohort)	8,533 cases, 8596 controls; cohort of 170,693 women	OR 1.04 (0.97–1.11)	≥ 0.8 mg vs. 0.15 mg daily isoflavone intake
	only case-control studies	6807 cases, 8596 controls	OR 1.02 (0.95–1.11)	
	only cohort studies	1726 cases; cohort of 170,693 women	OR 1.08 (0.95–1.24)	
Cutler et al. [39]	cohort study	2,529 cases; cohort of 34,708 postmenopausal women	not available	≥ 0.52 mg vs. ≤ 0.13 mg daily isoflavone intake; no significant difference for isoflavone intake
Hedelin et al. [40]	cohort study	1,014 cases; cohort of 45,448 women	RR 0.98 (0.83–1.17)	highest vs. lowest quartile of isoflavone intake (< 0.1 mg/day)
Travis et al. [41]	cohort study	585 cases; cohort of 37,643 women	HR 1.17 (0.79–1.71)	≥ 20 mg vs. < 10 mg daily isoflavone intake; only 29 cases with intake ≥ 20 mg
Ward et al. [42]	nested case-control study	237 cases, 952 controls out of 14,032 women	OR 1.08 (1.00–1.06)	high vs. low levels of urine isoflavones, results are inconsistent with the non-significant difference of isoflavone plasma levels
Verheus et al. [43]	nested case-control study	383 cases, 383 controls	OR 0.68 (0.47–0.98)	highest vs. lowest tertile of plasma levels of genistein, effect is stronger in postmenopausal women

CI = Confidence interval; OR = odds ratio; RR = risk ratio; HR = hazard ratio.

plasma levels. Even the authors of this study did not dismiss the possibility of a false-positive finding as a result of the conducted multiple subgroup analyses. In contrast thereto are the results of a big Dutch nested case-control study of pre- and postmenopausal women and measurement of plasma levels of isoflavones [43]. Except genistein, all isoflavones showed a non-significant reduction in breast cancer risk. Comparing the highest versus the lowest tertile of plasma levels, genistein was associated with a significant decrease in overall breast cancer risk (OR = 0.68, 95% CI = 0.47–0.98, p = 0.07). A subgroup analysis revealed that the protective effect was much stronger in post- than in premenopausal women (OR = 0.76, 95% CI 0.45–1.09, p = 0.09 vs. OR = 0.67, 95% CI = 0.29–1.56, p = 0.30). Although women of European or US American descent show much lower circulating levels of isoflavones than Asian women, the endogenous levels of phytoetrogens are 50–1,000 times higher than endogenous estrogen levels in postmenopausal women. Given this, the authors concluded that biological effects of isoflavones may occur also in low-soy-consuming populations.

Isoflavones and Markers of Breast Cancer Risk

Assuming that estrogenic effects on breast tissue may indirectly estimate the risk of breast cancer development, some studies analyzed the impact of isoflavones on surrogate markers like mammographic density, measurement of the proliferation marker Ki-67 in breast tissue, and others [44, 45]. Several trials did not show any significant increase in breast tissue proliferation after treatment with isoflavones compared to placebo in both premenopausal and postmenopausal women. Doses of daily isoflavone intake ranged from 36 mg to 120 mg and duration of exposition from 14 days to 12 weeks [46–48]. Also, in accordance with the results on cell proliferation, the trials did not reveal a significant increase of mammographic breast density depending on isoflavone intake. Dietary daily isoflavone intake differed between 40 mg and 99 mg and study duration between 6 months and 3 years [49–53].

Isoflavones and Risk of Breast Cancer Recurrence

Most concerns regarding phytoestrogen intake after diagnosis of breast cancer are based on cell culture and animal studies which showed that phytoestrogens have growth stimulatory effects on human breast cancer cells (e.g. MCF-7) and on breast cancer xenoplants in athymic ovariectomized mice [54]. The induction of tumor growth by genistein is stronger than by daidzein. Surprisingly, the estrogenic metabolite equol did not enhance proliferation of breast cancer cells in the mouse model [54–57]. This means that phytoestrogens have a preventive effect on breast cancer development but have also stimulatory effects on the growth of ER-positive tumors in animal models and in cell culture. What could be the explanation for such divergent results? It may be hypothesized that both the cell culture system and the mouse model lack an endogenous estrogen environment. As a consequence, the low estrogen effect of isoflavones may stimulate tumor development. A milieu of higher endogenous estrogen may cause competition between isoflavones and estradiol for receptor binding sites and so the weaker estrogenic potency of isoflavones as well as the different binding preferences to the ERs α and β might lead to an anti-estrogenic effect. In a higher estrogen milieu isoflavonoids act in vitro as anti-estrogens, while in a low estrogen environment they act as estrogens as is characteristic for a postmenopausal situation [10]. In agreement, a more physiological mouse model with postmenopausal estrogen levels demonstrated an additional proliferative effect of genistein [58]. However, the addition of 17β-estradiol in only low postmenopausal levels of 20 pmol/l induced antiproliferative effects on MCF-7 cells by isoflavonoids [59]. Furthermore, the injection of murine or human ER-negative breast carcinoma cells into soy-fed BALB/c mice resulted in reduced metastasis and tumor growth [60, 61].

The data currently available on the recurrence risk of breast cancer in women are sparse. Two population-based cohort studies examined the influence of soy intake and isoflavone intake, respectively. Boyapati et al. [62] analyzed the disease-free survival (DFS) of a group of 1,459 breast cancer patients taken from the Shanghai Breast Cancer Study cohort. The median time of follow-up was 5.2 years. The study did not observe any statistically significant association with overall survival between total isoflavone intake and DFS (third tertile vs. first tertile of isoflavone intake: hazard ratio (HR) = 1.06, 95% CI = 0.79–1.42, p = 0.64). Also, the subgroup analysis did not reveal any significant difference with respect to hormone receptor status, BMI, or menopausal status. Unfortunately, no data on tamoxifen use were taken. The second study was conducted on 1,210 breast cancer patients from the Long Island Breast Cancer Study Project. The patients were instructed to complete a FFQ when diagnosed with cancer. The follow-up time was 5 years. For the estimated adjusted HR for the highest isoflavone intake (> 7.48 mg/day) compared with the lowest one (≤ 0.29 mg/day), there was a trend towards a reduced risk but without statistical significance among both premenopausal and postmenopausal women for the all-cause mortality (HR = 0.52, 95% CI = 0.33–0.82, p = 0.37). Although the trend was more evident within the postmenopausal group, it was not significant either (HR = 0.44, 95% CI = 0.24–0.81, p = 0.34). The HRs for breast cancer-specific mortality did show a similar trend but with lower risk reduction. The subgroup analysis for hormone receptor status did not reveal any significant difference [63]. Neither study assessed possible postdiagnostic changes of isoflavone consumption.

Isoflavones and Antihormonal Treatment

The possible functional interaction of isoflavones with antihormonal therapy appears to be rather obscure. The estrogenic properties of isoflavones may possibly antagonize the anti-estrogenic action of tamoxifen. After xenotransplantion of a tamoxifen-sensitive human breast cancer cell line (MCF-7) in BALB/c nude mice, genistein reduced the growth inhibitory effect of tamoxifen [64]. In vitro cell cultures for daidzein proved the same. Only high and non-physiological concentrations of isoflavones inhibited tumor cell proliferation [65]. In contrast, Mai et al. [66] demonstrated synergistic effects of isoflavones (e.g. genistein) and tamoxifen on MCF-7 cells. Furthermore, genistein, in the presence of tamoxifen, exerted a synergistic influence on a hormone receptor positive, HER2-overexpressing human breast cancer cell line – a receptor combination with frequently encountered tamoxifen resistance [67]. Here, genistein decreased epithelial growth factor receptor (EGFR), HER2, and ERα expression. The authors assumed a potential therapeutic use in ER+/HER2+ breast cancer cases with tamoxifen resistance, where genistein could potentially reconstitute tamoxifen sensitivity. No

influence on serum levels of tamoxifen and its metabolism was observed in female tamoxifen users [68]. Also, the activity of aromatase inhibitors (AI) seems to be influenced by isoflavones. Genistein abolished in the MCF-7 implanted BALB/c mouse model the growth inhibitory effect of the AI letrozole [69].

Given this, the effects of isoflavones on breast cancer survival are presently not clear. Adverse effects, particularly on hormone receptor-dependent breast cancer and on antihormonal therapies, cannot definitely be excluded even though the 2 cohort studies with over 2,500 patients did not demonstrate any detrimental outcome. Considering the potentially negative effect on breast cancer outcome, the German breast cancer commission of the 'Arbeitsgemeinschaft Gynäkologische Onkologie, AGO' recommends avoiding high isoflavone intake in breast cancer patients independent of the hormone receptor status [70].

Interaction of Isoflavone Intake and Mammary Development

Three studies evaluated the role of isoflavone intake and time of exposure. In all of the 3 studies, intake of isoflavones during adolescence was associated with a reduction of breast cancer risk. Shu et al. [71] found a statistically significant risk reduction between lowest and highest quintile of soy intake in Chinese women of OR = 0.51, 95% CI = 0.40–0.65, $p < 0.001$ (case-control study with 1,459 breast cancer cases, FFQ for the time between the ages of 13 and 15 years). A case-control study conducted by Wu et al. [72] on 501 Asian-American women with breast cancer revealed similar results. The comparison between the group with a generally high intake of isoflavones during adolescence (between ages 12 and 18) and adult life and the generally low intake group showed a significant risk reduction of OR = 0.51, 95% CI = 0.36–0.78, $p \leq 0.001$. High intake in adults who had a low intake during adolescence did not reveal a significant risk reduction (OR = 0.93, 95% CI = 0.58–1.48). High isoflavone intake only during adolescence seems to reduce the breast cancer risk to a lower degree (OR = 0.77, 95% CI = 0.51–1.16). The third study on 3,024 Canadian breast cancer patients (case-control) showed that higher phytoestrogen intake during adolescence was associated with a reduced breast cancer risk (OR = 0.71, 95% CI = 0.62–0.82, $p < 0.001$) [73]. Also, intake of soy-based formula in the first year of live could have a preventive effect on breast cancer development in later life. A small Canadian case-control study on 728 women with 372 cases of breast cancer revealed a risk reduction for the soy formula-fed group of OR = 0.42 (only soy milk vs. only breast/cow's milk in the first 4 months). The effect is not significant (95% CI = 0.13–1.40) not least due to the small study population [74]. The protective impact of isoflavones, especially of genistein, on breast tissue during the prepubertal and pubertal period was confirmed by several animal studies on rats [75]. The underlying mechanisms of action are not yet clear, but prepubertal soy feeding of female rats generates a reduction in the number of terminal end buds (TEBs) and an increase in lobular differentiation. The human equivalent to the TEB is the terminal ductal lobular unit (TDLU) which is the morphologic origin of most breast cancer cases. The structural changes are accompanied by a functional maturation of the gland with reduction of the cancer-sensitive stem cell pool and molecular switch to a less cancer-sensitive phenotype, respectively. Effects are determined by genetic and epigenetic phenomena and result amongst others in an upregulation of the tumor suppressor genes BRCA1 and PTEN. Warri et al. [75] proposed that early soy exposure might have a similar protective effect on the breast as early pregnancy. The findings are in agreement with the recently postulated importance of the sensitive window of pubertal breast development on cancer genesis [76–78].

Discussion and Open Questions

Due to the inconsistency of the data, an interpretation of the various study findings is hardly possible. The findings did however repeatedly reveal the following 2 main differences: Firstly, there is a discrepancy in breast cancer prevention between Western and Asian populations, that cannot be explained only by different amounts of daily isoflavone intake. Secondly, there is an obvious discrepancy between the beneficial trend of using isoflavone for purposes of breast cancer prevention in human studies and the detrimental effects on breast cancer growth in cell culture or in mouse models. Besides methodical problems, the age period of isoflavone exposure may be relevant for the incoherence of the data, but at present many questions about isoflavone effects and particularly its safety have not yet been answered: What is the epidemiologic evidence on isoflavones and breast cancer risk? Is there a dose-response relationship or a threshold level? What about high-risk situations (especially BRCA mutations)?

The positive effects of high isoflavone intake in Asian women have been demonstrated by a sufficient number of powerful studies. In populations with low isoflavone intake, data have not shown a clear preventive effect on breast cancer development, but also lack evidence of any adverse influence on breast cancer risk. In conclusion, isoflavones can presently be regarded as relatively safe, but the effect of high 'Asian-like' isoflavone intake on Western populations remains uncertain. Therefore, formulas with high doses of phytoestrogens should be avoided. Further studies should evaluate the role of phytoestrogens on breast cancer survival.

Our current knowledge about the safety of isoflavones in cases of breast cancer is really rudimentary. Patients often ask for alternative treatment options like phytoestrogens, but presently we have only few human studies dealing with

this problem and so we are not able to give any satisfactory answer. Further investigations have to consider not only the hormone receptor status, but also the HER2 expression and histological properties. Also, does the endogenous estrogen environment influence the effects of the isoflavones? What is the impact of other phytoestrogens such as lignans and flavones? – An interesting question because of its common presence in Western diet products like tomatoes, berries, citrus fruits and many other fruits, vegetables, and herbs. Recent studies reported a modest decrease in risk of breast cancer in relation to intake of some flavones (apigenin, luteolin) [79, 80]. – What is the impact of different isoflavone metabolites and differences in metabolism? Which influence do non-estrogenic effects have (e.g. inhibition of tyrosine kinases, influence on enzymatic estrogen metabolism, epigenetic modulation)?

References

1 Parkin DM, Bray F, Ferlay J, Pisani P: Global cancer statistics, 2002. CA Cancer J Clin 2005;55:74–108.
2 Hanf V, Gonder U: Nutrition and primary prevention of breast cancer: foods, nutrients and breast cancer risk. Eur J Obstet Gynecol Reprod Biol 2005; 123:139–149.
3 Nelson HD, Vesco KK, Haney E, Fu R, Nedrow A, Miller J, Nicolaidis C, Walker M, Humphrey L: Nonhormonal therapies for menopausal hot flashes: systematic review and meta-analysis. JAMA 2006; 295:2057–2071.
4 Khaodhiar L, Ricciotti HA, Li L, Pan W, Schickel M, Zhou J, Blackburn GL: Daidzein-rich isoflavone aglycones are potentially effective in reducing hot flashes in menopausal women. Menopause 2008;15: 125–132.
5 Nahas EA, Nahas-Neto J, Orsatti FL, Carvalho EP, Oliveira ML, Dias R: Efficacy and safety of a soy isoflavone extract in postmenopausal women: a randomized, double-blind, and placebo-controlled study. Maturitas 2007;58:249–258.
6 Korner W, Spengler P, Bolz U, Schuller W, Hanf V, Metzger JW: Substances with estrogenic activity in effluents of sewage treatment plants in southwestern Germany. 2. Biological analysis. Environ Toxicol Chem 2001;20:2142–2151.
7 Korner W, Hanf V, Schuller W, Bartsch H, Zwirner M, Hagenmaier H: Validation and application of a rapid in vitro assay for assessing the estrogenic potency of halogenated phenolic chemicals. Chemosphere 1998;37:2395–2407.
8 Morito K, Hirose T, Kinjo J, Hirakawa T, Okawa M, Nohara T, Ogawa S, Inoue S, Muramatsu M, Masamune Y: Interaction of phytoestrogens with estrogen receptors alpha and beta. Biol Pharm Bull 2001; 24:351–356.
9 Morito K, Aomori T, Hirose T, Kinjo J, Hasegawa J, Ogawa S, Inoue S, Muramatsu M, Masamune Y: Interaction of phytoestrogens with estrogen receptors alpha and beta (II). Biol Pharm Bull 2002;25: 48–52.
10 Hwang CS, Kwak HS, Lim HJ, Lee SH, Kang YS, Choe TB, Hur HG, Han KO: Isoflavone metabolites and their in vitro dual functions: they can act as an estrogenic agonist or antagonist depending on the estrogen concentration. J Steroid Biochem Mol Biol 2006;101:246–253.
11 De Lemos ML: Effects of soy phytoestrogens genistein and daidzein on breast cancer growth. Ann Pharmacother 2001;35:1118–1121.
12 Brzezinski A, Debi A: Phytoestrogens: the 'natural' selective estrogen receptor modulators? Eur J Obstet Gynecol Reprod Biol 1999;85:47–51.
13 Chang EC, Charn TH, Park SH, Helferich WG, Komm B, Katzenellenbogen JA, Katzenellenbogen BS: Estrogen Receptors alpha and beta as determinants of gene expression: influence of ligand, dose, and chromatin binding. Mol Endocrinol 2008;22: 1032–1043.
14 Wang Y, Man GW, Chan FL, Chen S, Leung LK: The red clover (Trifolium pratense) isoflavone biochanin A inhibits aromatase activity and expression. Br J Nutr 2008;99:303–310.
15 Rice S, Whitehead SA: Phytoestrogens and breast cancer – promoters or protectors? Endocr Relat Cancer 2006;13:995–1015.
16 Akiyama T, Ishida J, Nakagawa S, Ogawara H, Watanabe S, Itoh N, Shibuya M, Fukami Y: Genistein, a specific inhibitor of tyrosine-specific protein kinases. J Biol Chem 1987;262:5592–5595.
17 King-Batoon A, Leszczynska JM, Klein CB: Modulation of gene methylation by genistein or lycopene in breast cancer cells. Environ Mol Mutagen 2008;49: 36–45.
18 Gerber B, Muller H, Reimer T, Krause A, Friese K: Nutrition and lifestyle factors on the risk of developing breast cancer. Breast Cancer Res Treat 2003;79: 265–276.
19 Gerber B, Scholz C, Reimer T, Briese V, Janni W: Complementary and alternative therapeutic approaches in patients with early breast cancer: a systematic review. Breast Cancer Res Treat 2006;95: 199–209.
20 Edmunds KM, Holloway AC, Crankshaw DJ, Agarwal SK, Foster WG: The effects of dietary phytoestrogens on aromatase activity in human endometrial stromal cells. Reprod Nutr Dev 2005;45: 709–720.
21 Sammartino A, Di Carlo C, Mandato VD, Bifulco G, Di Stefano M, Nappi C: Effects of genistein on the endometrium: ultrasonographic evaluation. Gynecol Endocrinol 2003;17:45–49.
22 Yu ZT, Yao W, Zhu WY: Isolation and identification of equol-producing bacterial strains from cultures of pig faeces. FEMS Microbiol Lett 2008;282: 73–80.
23 Halm BM, Franke AA, Ashburn LA, Hebshi SM, Wilkens LR: Oral antibiotics decrease urinary isoflavonoid excretion in children after soy consumption. Nutr Cancer 2008;60:14–22.
24 Nagata C, Ueno T, Uchiyama S, Nagao Y, Yamamoto S, Shibuya C, Kashiki Y, Shimizu H: Dietary and lifestyle correlates of urinary excretion status of equol in Japanese women. Nutr Cancer 2008;60: 49–54.
25 Liggins J, Bluck LJ, Runswick S, Atkinson C, Coward WA, Bingham SA: Daidzein and genistein contents of vegetables. Br J Nutr 2000;84:717–725.
26 Setchell KD: Phytoestrogens: the biochemistry, physiology, and implications for human health of soy isoflavones. Am J Clin Nutr 1998;68:1333S–1346S.
27 Chen Z, Zheng W, Custer LJ, Dai Q, Shu XO, Jin F, Franke AA: Usual dietary consumption of soy foods and its correlation with the excretion rate of isoflavonoids in overnight urine samples among Chinese women in Shanghai. Nutr Cancer 1999;33: 82–87.
28 Wakai K, Egami I, Kato K, Kawamura T, Tamakoshi A, Lin Y, Nakayama T, Wada M, Ohno Y: Dietary intake and sources of isoflavones among Japanese. Nutr Cancer 1999;33:139–145.
29 Surh J, Kim MJ, Koh E, Kim YK, Kwon H: Estimated intakes of isoflavones and coumestrol in Korean population. Int J Food Sci Nutr 2006;57:325–344.
30 Mulligan AA, Welch AA, McTaggart AA, Bhaniani A, Bingham SA: Intakes and sources of soya foods and isoflavones in a UK population cohort study (EPIC-Norfolk). Eur J Clin Nutr 2007;61:248–254.
31 Boker LK, van der Schouw YT, De Kleijn MJ, Jacques PF, Grobbee DE, Peeters PH: Intake of dietary phytoestrogens by Dutch women. J Nutr 2002; 132:1319–1328.
32 De Kleijn MJ, van der Schouw YT, Wilson PW, Adlercreutz H, Mazur W, Grobbee DE, Jacques PF: Intake of dietary phytoestrogens is low in postmenopausal women in the United States: the Framingham study(1–4). J Nutr 2001;131:1826–1832.
33 Horn-Ross PL, Lee M, John EM, Koo J: Sources of phytoestrogen exposure among non-Asian women in California, USA. Cancer Causes Control 2000;11: 299–302.
34 Lee HP, Gourley L, Duffy SW, Esteve J, Lee J, Day NE: Dietary effects on breast-cancer risk in Singapore. Lancet 1991;337:1197–1200.
35 Wu AH, Yu MC, Tseng CC, Pike MC: Epidemiology of soy exposures and breast cancer risk. Br J Cancer 2008;98:9–14.
36 Wu AH, Koh WP, Wang R, Lee HP, Yu MC: Soy intake and breast cancer risk in Singapore Chinese Health Study. Br J Cancer 2008;99:196–200.
37 Iwasaki M, Inoue M, Otani T, Sasazuki S, Kurahashi N, Miura T, Yamamoto S, Tsugane S: Plasma isoflavone level and subsequent risk of breast cancer among Japanese women: a nested case-control study from the Japan Public Health Center-based prospective study group. J Clin Oncol 2008;26: 1677–1683.
38 Lampe JW, Nishino Y, Ray RM, Wu C, Li W, Lin MG, Gao DL, Hu Y, Shannon J, Stalsberg H, Porter PL, Frankenfeld CL, Wahala K, Thomas DB: Plasma isoflavones and fibrocystic breast conditions and breast cancer among women in Shanghai, China. Cancer Epidemiol Biomarkers Prev 2007; 16:2579–2586.
39 Cutler GJ, Nettleton JA, Ross JA, Harnack LJ, Jacobs DR, Jr., Scrafford CG, Barraj LM, Mink PJ, Robien K: Dietary flavonoid intake and risk of cancer in postmenopausal women: the Iowa Women's Health Study. Int J Cancer 2008;123:664–671.
40 Hedelin M, Lof M, Olsson M, Adlercreutz H, Sandin S, Weiderpass E: Dietary phytoestrogens are not associated with risk of overall breast cancer but diets rich in coumestrol are inversely associated with risk of estrogen receptor and progesterone receptor negative breast tumors in Swedish women. J Nutr 2008;138:938–945.

41 Travis RC, Allen NE, Appleby PN, Spencer EA, Roddam AW, Key TJ: A prospective study of vegetarianism and isoflavone intake in relation to breast cancer risk in British women. Int J Cancer 2008;122: 705–710.
42 Ward H, Chapelais G, Kuhnle GG, Luben R, Khaw KT, Bingham S: Breast cancer risk in relation to urinary and serum biomarkers of phytoestrogen exposure in the European Prospective into Cancer-Norfolk cohort study. Breast Cancer Res 2008;10:R32.
43 Verheus M, van Gils CH, Keinan-Boker L, Grace PB, Bingham SA, Peeters PH: Plasma phytoestrogens and subsequent breast cancer risk. J Clin Oncol 2007;25:648–655.
44 Yager JD, Davidson NE: Estrogen carcinogenesis in breast cancer. N Engl J Med 2006;354:270–282.
45 Messina MJ, Wood CE: Soy isoflavones, estrogen therapy, and breast cancer risk: analysis and commentary. Nutr J 2008;7:17.
46 Cheng G, Wilczek B, Warner M, Gustafsson JA, Landgren BM: Isoflavone treatment for acute menopausal symptoms. Menopause 2007;14:468–473.
47 Sartippour MR, Rao JY, Apple S, Wu D, Henning S, Wang H, Elashoff R, Rubio R, Heber D, Brooks MN: A pilot clinical study of short-term isoflavone supplements in breast cancer patients. Nutr Cancer 2004;49:59–65.
48 Hargreaves DF, Potten CS, Harding C, Shaw LE, Morton MS, Roberts SA, Howell A, Bundred NJ: Two-week dietary soy supplementation has an estrogenic effect on normal premenopausal breast. J Clin Endocrinol Metab 1999;84:4017–4024.
49 Powles TJ, Howell A, Evans DG, McCloskey EV, Ashley S, Greenhalgh R, Affen J, Flook LA, Tidy A: Red clover isoflavones are safe and well tolerated in women with a family history of breast cancer. Menopause Int 2008;14:6–12.
50 Atkinson C, Warren RM, Sala E, Dowsett M, Dunning AM, Healey CS, Runswick S, Day NE, Bingham SA: Red-clover-derived isoflavones and mammographic breast density: a double-blind, randomized, placebo-controlled trial [ISRCTN42940165]. Breast Cancer Res 2004;6:R170–R179.
51 Maskarinec G, Williams AE, Carlin L: Mammographic densities in a one-year isoflavone intervention. Eur J Cancer Prev 2003;12:165–169.
52 Verheus M, van Gils CH, Kreijkamp-Kaspers S, Kok L, Peeters PH, Grobbee DE, van der Schouw YT: Soy protein containing isoflavones and mammographic density in a randomized controlled trial in postmenopausal women. Cancer Epidemiol Biomarkers Prev 2008;17:2632–2638.
53 Kataoka M, Atkinson C, Warren R, Sala E, Day NE, Highnam R, Warsi I, Bingham SA: Mammographic density using two computer-based methods in an isoflavone trial. Maturitas 2008;59:350–357.
54 Hsieh CY, Santell RC, Haslam SZ, Helferich WG: Estrogenic effects of genistein on the growth of estrogen receptor-positive human breast cancer (MCF-7) cells in vitro and in vivo. Cancer Res 1998;58:3833–3838.
55 Allred CD, Allred KF, Ju YH, Virant SM, Helferich WG: Soy diets containing varying amounts of genistein stimulate growth of estrogen-dependent (MCF-7) tumors in a dose-dependent manner. Cancer Res 2001;61:5045–5050.
56 Ju YH, Allred CD, Allred KF, Karko KL, Doerge DR, Helferich WG: Physiological concentrations of dietary genistein dose-dependently stimulate growth of estrogen-dependent human breast cancer (MCF-7) tumors implanted in athymic nude mice. J Nutr 2001;131:2957–2962.
57 Ju YH, Fultz J, Allred KF, Doerge DR, Helferich WG: Effects of dietary daidzein and its metabolite, equol, at physiological concentrations on the growth of estrogen-dependent human breast cancer (MCF-7) tumors implanted in ovariectomized athymic mice. Carcinogenesis 2006;27:856–863.
58 Ju YH, Allred KF, Allred CD, Helferich WG: Genistein stimulates growth of human breast cancer cells in a novel, postmenopausal animal model, with low plasma estradiol concentrations. Carcinogenesis 2006;27:1292–1299.
59 Imhof M, Molzer S, Imhof M: Effects of soy isoflavones on 17beta-estradiol-induced proliferation of MCF-7 breast cancer cells. Toxicol In Vitro 2008;22: 1452–1460.
60 Yan L, Li D, Yee JA: Dietary supplementation with isolated soy protein reduces metastasis of mammary carcinoma cells in mice. Clin Exp Metastasis 2002;19:535–540.
61 Kim HA, Jeong KS, Kim YK: Soy extract is more potent than genistein on tumor growth inhibition. Anticancer Res 2008;28:2837–2841.
62 Boyapati SM, Shu XO, Ruan ZX, Dai Q, Cai Q, Gao YT, Zheng W: Soyfood intake and breast cancer survival: a followup of the Shanghai Breast Cancer Study. Breast Cancer Res Treat 2005;92:11–17.
63 Fink BN, Steck SE, Wolff MS, Britton JA, Kabat GC, Gaudet MM, Abrahamson PE, Bell P, Schroeder JC, Teitelbaum SL, Neugut AI, Gammon MD: Dietary flavonoid intake and breast cancer survival among women on Long Island. Cancer Epidemiol Biomarkers Prev 2007;16:2285–2292.
64 Ju YH, Doerge DR, Allred KF, Allred CD, Helferich WG: Dietary genistein negates the inhibitory effect of tamoxifen on growth of estrogen-dependent human breast cancer (MCF-7) cells implanted in athymic mice. Cancer Res 2002;62:2474–2477.
65 Limer JL, Parkes AT, Speirs V: Differential response to phytoestrogens in endocrine sensitive and resistant breast cancer cells in vitro. Int J Cancer 2006;119:515–521.
66 Mai Z, Blackburn GL, Zhou JR: Soy phytochemicals synergistically enhance the preventive effect of tamoxifen on the growth of estrogen-dependent human breast carcinoma in mice. Carcinogenesis 2007;28:1217–1223.
67 Mai Z, Blackburn GL, Zhou JR: Genistein sensitizes inhibitory effect of tamoxifen on the growth of estrogen receptor-positive and HER2-overexpressing human breast cancer cells. Mol Carcinog 2007; 46:534–542.
68 Wu AH, Pike MC, Williams LD, Spicer D, Tseng CC, Churchwell MI, Doerge DR: Tamoxifen, soy, and lifestyle factors in Asian American women with breast cancer. J Clin Oncol 2007;25:3024–3030.
69 Ju YH, Doerge DR, Woodling KA, Hartman JA, Kwak J, Helferich WG: Dietary genistein negates the inhibitory effect of letrozole on the growth of aromatase-expressing estrogen-dependent human breast cancer cells (MCF-7Ca) in vivo. Carcinogenesis 2008;29:2162–2168.
70 Hanf V: Diagnosis and Treatment of Patients with Primary and Metastatic Breast Cancer: Complementary Therapy and Hormonal Treatment in Breast Cancer Survivors and (Plant) Alternatives and Survivorship. *www.ago-online.de/download/g_mamma_08_1_1_c_25_complementary_therapy_hrt_survivorship.pdf.* Version 2008; Arbeitsgemeinschaft Gynäkologische Onkologie, Kommission Mamma.
71 Shu XO, Jin F, Dai Q, Wen W, Potter JD, Kushi LH, Ruan Z, Gao YT, Zheng W: Soyfood intake during adolescence and subsequent risk of breast cancer among Chinese women. Cancer Epidemiol Biomarkers Prev 2001;10:483–488.
72 Wu AH, Wan P, Hankin J, Tseng CC, Yu MC, Pike MC: Adolescent and adult soy intake and risk of breast cancer in Asian-Americans. Carcinogenesis 2002;23:1491–1496.
73 Thanos J, Cotterchio M, Boucher BA, Kreiger N, Thompson LU: Adolescent dietary phytoestrogen intake and breast cancer risk (Canada). Cancer Causes Control 2006;17:1253–1261.
74 Boucher BA, Cotterchio M, Kreiger N, Thompson LU: Soy formula and breast cancer risk. Epidemiology 2008;19:165–166.
75 Warri A, Saarinen NM, Makela S, Hilakivi-Clarke L: The role of early life genistein exposures in modifying breast cancer risk. Br J Cancer 2008;98: 1485–1493.
76 Russo J, Mailo D, Hu YF, Balogh G, Sheriff F, Russo IH: Breast differentiation and its implication in cancer prevention. Clin Cancer Res 2005;11: 931s-936s.
77 Sivaraman L, Stephens LC, Markaverich BM, Clark JA, Krnacik S, Conneely OM, O'Malley BW, Medina D: Hormone-induced refractoriness to mammary carcinogenesis in Wistar-Furth rats. Carcinogenesis 1998;19:1573–1581.
78 Hilakivi-Clarke L: Nutritional modulation of terminal end buds: its relevance to breast cancer prevention. Curr Cancer Drug Targets 2007;7:465–474.
79 Gammon MD, Fink BN, Steck SE, Wolff MS: Soy intake and breast cancer: elucidation of an unanswered question. Br J Cancer 2008;98:2–3.
80 Bosetti C, Spertini L, Parpinel M, Gnagnarella P, Lagiou P, Negri E, Franceschi S, Montella M, Peterson J, Dwyer J, Giacosa A, La Vecchia C: Flavonoids and breast cancer risk in Italy. Cancer Epidemiol Biomarkers Prev 2005;14:805–808.
81 Wang H, Murphy PA: Isoflavone Content in Commercial Soybean Foods. J Agric Food Chem 1994; 42:1666–1673.