Use of dietary supplements containing soy isoflavones and breast cancer risk among women aged >50 y: a prospective study

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ABSTRACT

Background: Soy-based dietary supplements have been promoted as natural alternatives to menopausal hormone therapy, but their potential effect on breast cancer development is controversial.

Objectives: We examined the relation between the consumption of soy supplements and the risk of breast cancer, overall and by tumor hormone receptor status, among women aged >50 y.

Methods: In total, 76,442 women from the Etude Epidemiologique aupres de Femmes de la Mutuelle Generale de l'Education Nationale (E3N) cohort, born between 1925 and 1950, were followed from 2000 to 2011 (11.2 y on average, starting at a mean age of 59.5 y; 3608 incident breast cancers), with soy supplement use assessed every 2–3 y. HRs of breast cancer were estimated with the use of multivariable Cox models.

Results: Compared with never using soy supplements, the HRs associated with current use of soy supplements were 0.92 (95% CI: 0.76, 1.11) for all, 0.78 (95% CI: 0.60, 0.99) for estrogen receptor (ER)–positive, and 2.01 (95% CI: 1.41, 2.86) for ER-negative breast cancers. There was no association between past use of soy supplements and breast cancer. HRs for current use were 1.36 (95% CI: 0.95, 1.93) and 0.82 (95% CI: 0.65, 1.02) among women with and without a family history of breast cancer, respectively (P-interaction = 0.03) and 1.06 (95% CI: 0.87, 1.30) ≥5 y after menopause compared with 0.50 (95% CI: 0.31, 0.81) in premenopause or ≤5 y postmenopause (P-interaction = 0.04).

Conclusions: In this cohort of women aged >50 y, we report opposing associations of soy supplements with ER-positive and ER-negative breast cancer risk. Our results also caution against the use of these supplements in women with a family history of breast cancer. Whether the risk profile of soy supplements could be more favorable among premenopausal or recently postmenopausal women deserves further investigation. *Am J Clin Nutr* 2019;109:597–605.

Keywords: breast cancer, cohort, prospective study, women aged over 50 years, dietary supplements, soy, isoflavones, hormone receptors

Introduction

Dietary supplements containing phytoestrogens have been available since the 1990s and have been promoted mainly as natural alternatives to menopausal hormone therapy (MHT), the most effective treatment for symptoms due to estrogen deprivation such as hot flushes, night sweats, or vaginal

This research was carried out using data from the INSERM (French National Institutes for Health and Medical Research) E3N cohort, which was established and maintained with the support of the Mutuelle Générale de l'Education Nationale, Gustave Roussy, and the French League against Cancer (LNCC). This study was supported by a grant (IMD20131229259) from the Fondation pour la Recherche Médicale. The work reported in this article was partly performed during the stay of AF as a Visiting Scientist at the International Agency for Research on Cancer.

Supplemental Material is available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/ajcn/.

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Abbreviations used: ER, estrogen receptor; E3N, Etude Epidemiologique aupres de Femmes de la Mutuelle Generale de l'Education Nationale; GPER, G protein–coupled estrogen receptor; MHT, menopausal hormone therapy; PR, progesterone receptor; Q2000, questionnaire sent in June 2000; Q2002, questionnaire sent in July 2002; Q2005, questionnaire sent in July 2005; Q2008, questionnaire sent in June 2008.

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dryness. Most of these supplements contain soy extracts rich in phytoestrogens, called isoflavones, which can exert estrogen-like effects (1). Although there is no conclusive evidence that phytoestrogen supplements effectively reduce menopausal symptoms (2, 3), soy supplement sales likely benefited from the reporting in 2002–2003 that MHT increased cardiovascular and breast cancer risk (4, 5). In France, the soy supplement market expanded substantially from 1998, and in particular following MHT prescription decline in 2003 (6).

The potential effect of soy-based dietary supplements on breast cancer development is controversial. Due to their structural similarity to 17-β-estradiol, isoflavones can bind to estrogen receptors (ERs) and thus exert proliferative or antiproliferative effects on already transformed breast cancer cells, depending on factors such as endogenous estrogen concentrations (7–9). Isoflavones have been shown to exert potentially protective effects toward breast cancer tumorigenesis in experimental models, including at the initiation phase, through nonhormonal pathways (7–10). Although nutritional epidemiology studies suggest that the consumption, from childhood, of an Asian soy-rich diet could reduce the risk of breast cancer (11–14), it is difficult to anticipate the effect of the consumption of isoflavones at doses found in dietary supplements by peri- or postmenopausal Western women. Four observational epidemiologic studies have investigated that effect (15–18). Three were population-based case-control studies that reported suggestive inverse associations between ever using "soy medications" (16), ever using herbal products containing soy isoflavones or red clover for menopausal disorders (17), and ever using high-content ($\geq$0.676 mg/d) isoflavone supplements (18) and breast cancer risk. It should be noted that these case-control studies may have been affected by differential recall and participation biases, and only one (18) reported a statistically significant association. The only cohort study to evaluate the use of soy-based supplements and the risk for incident breast cancer showed no association (HR: 1.04; 95% CI: 0.74, 1.48, among past or current regular users of soy-based supplements compared with nonusers at baseline) (15). However, its statistical power was also limited, with only 36 incident cases of invasive breast cancer among exposed women.

The current analysis examined the relation between the consumption of dietary supplements containing soy isoflavones assessed at different times during follow-up and the risk of breast cancer, overall and by tumor characteristics, among women aged >50 y enrolled in the Etude Epidémiologique auprès de Femmes de la Mutuelle Générale de l'Education Nationale (E3N) cohort.

Methods

Cohort

The E3N cohort has been described previously (19). Briefly, it comprises 98,995 French women born between 1925 and 1950, who were registered with the health insurance plan for public education system employees, covering mainly teachers, and who gave written informed consent. Women completed self-administered questionnaires sent every 2–3 y since 1990 to obtain and update information on various characteristics and occurrence of selected diseases. The E3N study was approved by the French National Commission for Data Protection and Privacy. The STrengthening the Reporting of OBservational studies in Epidemiology (STROBE) guidelines were followed to perform and report the study.

Ascertainment of cases

Occurrence of cancer was identified from the self-administered questionnaires, which enquired about any cancer diagnosis (including date and site), next-of-kin spontaneous reports, and the national cause-of-death registry. Pathology reports were obtained for 95% of incident breast cancers. Information on ER and progesterone receptor (PR) status, invasive or in situ status, and histologic subtype was extracted from these reports. The present analyses included all breast cancer cases, including those for whom pathology reports had not been obtained, since the proportion of false-positive self-reports was very low (<5%).

Data relative to the use of soy supplements

The consumption of soy supplements was first documented in the questionnaire sent in June 2000 (Q2000), and the information was updated in the questionnaires sent in July 2002 (Q2002), July 2005 (Q2005), and June 2008 (Q2008).

In Q2000 and Q2002, participants were asked whether they were "currently, at least 3 times per week, consuming a soy-based dietary supplement." For women who declared such consumption, we further obtained the names and brands of the corresponding products through an additional questionnaire sent in February 2004 (response rate: 95%). In Q2005, participants were asked to indicate the name, brand, and date of last use of any phytoestrogen-containing dietary supplement that they had ever used. That information was updated in Q2008. We developed a database of 186 dietary supplements containing soy that were found on the French market between 1999 and 2008 (**Supplemental Material**), which allowed us to identify supplements containing soy isoflavones among participants' self-reports.

Endpoints and follow-up

The prespecified considered endpoint was the diagnosis of a primary breast cancer (as first cancer). Person-years of follow-up were calculated from the completion date of Q2000, Q2002, Q2005, or Q2008, whichever was completed first, and until the date of diagnosis of any cancer (other than basal cell skin carcinoma or in situ colorectal cancer), the end of the last follow-up cycle, or 7 December 2011 (date at which the last questionnaire considered for identifying cancer occurrence was sent to participants), whichever occurred first. A follow-up cycle started at the date of response to a questionnaire collecting information on exposure and ended either at the date of answer to the following questionnaire or, for nonresponders, at the date it was sent to participants.

Study population

A total of 76,442 women free of cancer at follow-up start were included in the analyses (**Figure 1** Supplementary data).

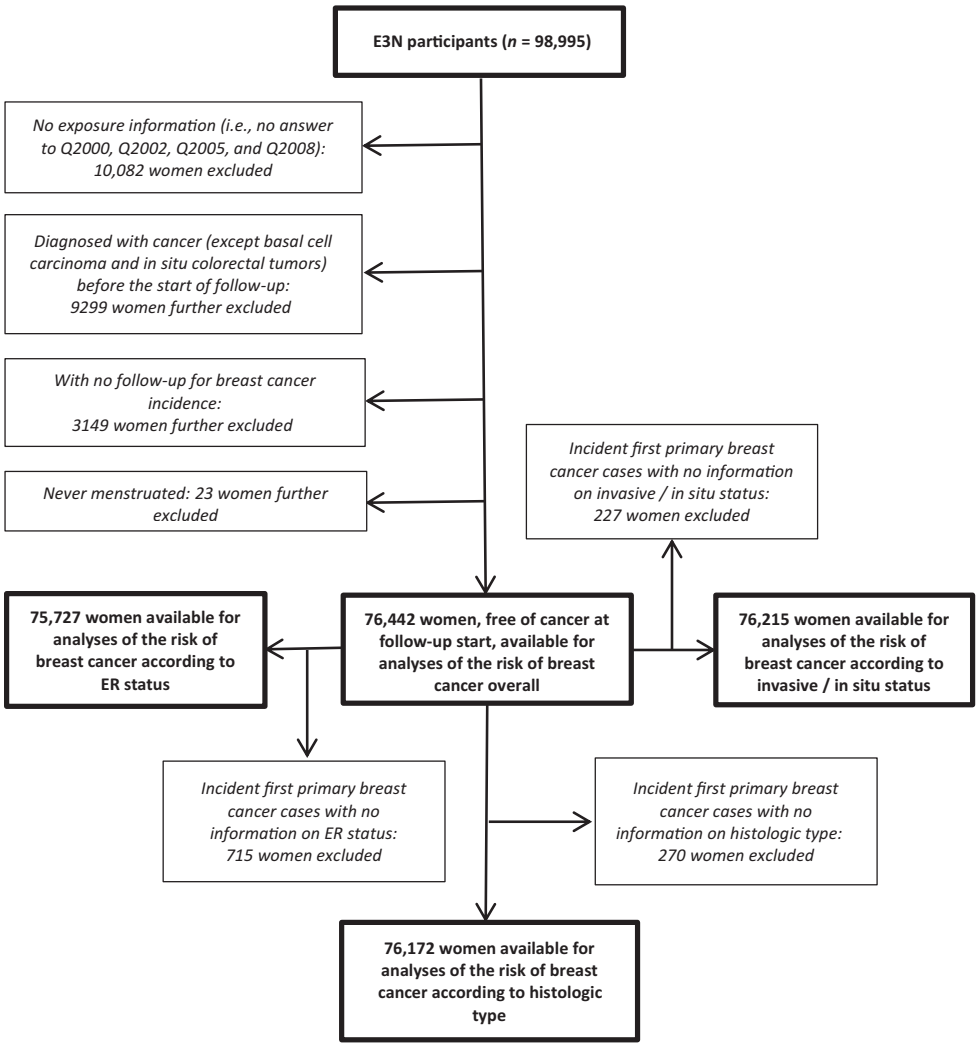

FIGURE 1 Flowchart for the analysis of the association between dietary supplements in soy isoflavones and the risk of breast cancer in the E3N cohort (2000–2011). ER, estrogen receptor; E3N, Etude Epidemiologique aupres de Femmes de la Mutuelle Generale de l'Education Nationale; Q2000, questionnaire sent in June 2000; Q2002, questionnaire sent in July 2002; Q2005, questionnaire sent in July 2005; Q2008, questionnaire sent in June 2008.

Statistical analysis

HRs and 95% CIs of breast cancer were estimated through the use of Cox proportional hazards regression models for left-truncated and right-censored data. Exposure to soy supplements was introduced into the Cox models by the use of time-varying variables, the information being updated at the start of each follow-up cycle. Exposure was prospectively categorized as current use, past use, and never use, as shown in **Figure 2**. For women who did not answer a questionnaire, the

	Q2000–Q2002	Q2002–Q2005	Q2005–Q2008	After Q2008
Current use =	Current use reported in Q2000	Current use reported in Q2002	Use within the past month reported in Q2005	Use within the past month reported in Q2008
Past use =	—	No current use in Q2002 but current use in Q2000	No use within the past month reported in Q2005, but current use in Q2000 or Q2002, or last use more than 1 mo ago reported in Q2005	No use within the past month reported in Q2008, but current use in Q2000 or Q2002, or ever use in Q2005, or last use >1 mo ago reported in Q2008
Never use =	No current use in Q2000[1]	Neither current nor past use	Neither current nor past use	Neither current nor past use

FIGURE 2 Classification of exposure to dietary supplements in soy isoflavones during follow-up (E3N cohort, 2000–2011). The arrow shows the follow-up cycles (start date–end date) during which the exposure definitions (below the arrow) apply. [1]Reasonable assumption since, in France, the soy supplement market expanded only from 1998 (6). E3N, Etude Epidemiologique aupres de Femmes de la Mutuelle Generale de l'Education Nationale;Q2000, questionnaire sent in June 2000; Q2002, questionnaire sent in July 2002; Q2005, questionnaire sent in July 2005; Q2008, questionnaire sent in June 2008.

TABLE 1 Selected self-reported baseline characteristics of participants by use of dietary supplements in soy isoflavones at the end of follow-up E3N cohort, 2000–2011[1]

	Exposure to dietary supplements in soy isoflavones at the end of follow-up		
Characteristics at baseline	Never user ($n = 65,644$)	Current user ($n = 1459$)	Past user ($n = 9092$)
Age, y	60.0 ± 6.7	56.4 ± 5.1	56.7 ± 5.2
Birth cohort			
1925–1929	5183 (7.9)	24 (1.6)	142 (1.6)
1930–1934	8876 (13.5)	66 (4.5)	495 (5.4)
1935–1939	12,961 (19.7)	177 (12.1)	1259 (13.8)
1940–1944	15,930 (24.3)	367 (25.2)	2292 (25.2)
1945+	22,694 (34.6)	825 (56.6)	4904 (53.9)
Years of education			
<12	8667 (13.2)	142 (9.7)	802 (8.8)
12–≤14	34,472 (52.5)	784 (53.7)	4845 (53.3)
>14	22,505 (34.3)	533 (36.5)	3445 (37.9)
BMI, kg/m^2			
<18.5	1927 (2.9)	51 (3.5)	259 (2.8)
18.5–<25	43,025 (65.5)	1060 (72.7)	6746 (74.2)
≥25	20,692 (31.5)	348 (23.9)	2087 (23.0)
Height (quartiles), cm			
<158	15,897 (24.2)	335 (23.0)	1958 (21.5)
158–<161	14,518 (22.1)	325 (22.3)	1971 (21.7)
161–<165	15,154 (23.1)	334 (22.9)	2199 (24.2)
≥165	20,075 (30.6)	465 (31.9)	2964 (32.6)
Smoking status			
Never smoker	35,433 (54.0)	725 (49.7)	4658 (51.2)
Current smoker	6566 (10.0)	142 (9.7)	742 (8.2)
Past smoker	23,645 (36.0)	592 (40.6)	3692 (40.6)
Age at menarche, y			
<13	29,199 (44.5)	684 (46.9)	4192 (46.1)
≥13	36,445 (55.5)	775 (53.1)	4900 (53.9)
Parity and age at first birth			
Nulliparous	7514 (11.4)	178 (12.2)	1007 (11.1)
First child before age 30 y, 1 or 2 children	32,478 (49.5)	795 (54.5)	5018 (55.2)
First child before age 30 y, ≥3 children	18,700 (28.5)	343 (23.5)	2161 (23.8)
First child after age 30 y	6952 (10.6)	143 (9.8)	906 (10.0)
Menopausal status and time since menopause			
Premenopausal	6482 (9.9)	279 (19.1)	1646 (18.1)
<5 y postmenopause	15,285 (23.3)	532 (36.5)	3194 (35.1)
5–<10 y postmenopause	14,972 (22.8)	342 (23.4)	2146 (23.6)
10–<15 y postmenopause	13,316 (20.3)	191 (13.1)	1363 (15.0)
≥15 y postmenopause	15,589 (23.7)	115 (7.9)	743 (8.2)
Use of oral contraceptives			
Never	26,825 (40.9)	438 (30.0)	2567 (28.2)
Ever	38,819 (59.1)	1021 (70.0)	6525 (71.8)
Use of estrogen-alone MHT			
Never	56,002 (85.3)	1286 (88.1)	7877 (86.6)
Recent	4311 (6.6)	75 (5.1)	555 (6.1)
Past	5317 (8.1)	96 (6.6)	657 (7.2)
Ever, but unknown recency	14 (0.0)	2 (0.1)	3 (0.0)
Use of estrogen + progesterone/dydrogesterone MHT			
Never	45,172 (68.8)	1027 (70.4)	6210 (68.3)
Recent	14,288 (21.8)	300 (20.6)	1918 (21.1)
Past	6184 (9.4)	132 (9.0)	964 (10.6)
Ever, but unknown recency	0 (0.0)	0 (0.0)	0 (0.0)
Use of estrogen + other progestogen MHT			
Never	41,782 (63.6)	943 (64.6)	5756 (63.3)
Recent	15,394 (23.5)	325 (22.3)	2037 (22.4)
Past	8467 (12.9)	191 (13.1)	1299 (14.3)
Ever, but unknown recency	1 (0.0)	0 (0.0)	0 (0.0)
Use of other/unknown MHT type			
Never	61,690 (94.0)	1380 (94.6)	8612 (94.7)
Recent	648 (1.0)	29 (2.0)	113 (1.2)

(*Continued*)

TABLE 1 (*Continued*)

Characteristics at baseline	Exposure to dietary supplements in soy isoflavones at the end of follow-up		
	Never user ($n = 65,644$)	Current user ($n = 1459$)	Past user ($n = 9092$)
Past	3301 (5.0)	50 (3.4)	366 (4.0)
Ever, but unknown recency	5 (0.0)	0 (0.0)	1 (0.0)
Personal history of benign breast biopsy			
No	63,112 (96.1)	1393 (95.5)	8671 (95.4)
Yes	2532 (3.9)	66 (4.5)	421 (4.6)
Family history of breast cancer			
No	49,896 (76.0)	1070 (73.3)	6722 (73.9)
In ≥1 first-degree relative	7536 (11.5)	188 (12.9)	1105 (12.2)
Only among second-degree relatives	8212 (12.5)	201 (13.8)	1265 (13.9)
Mammogram performed during the previous follow-up cycle			
No	7909 (12.0)	121 (8.3)	529 (5.8)
Yes	57,735 (88.0)	1338 (91.7)	8563 (94.2)
Alcohol consumption,[2] g/d	11.6 ± 13.9	11.2 ± 13.8	11.1 ± 13.2
Dietary lignan intake,[2] μg/d	1200 ± 441	1247 ± 465	1232 ± 451
Total dietary energy intake,[2] kcal/d	2130 ± 543	2142 ± 537	2172 ± 542

[1] Values are as *n* (%) or means ± SDs; $n = 76,442$. At the end of follow-up, 247 women were in the "unknown exposure" category (not shown). E3N, Etude Epidemiologique aupres de Femmes de la Mutuelle Generale de l'Education Nationale; MHT, menopausal hormone therapy.

[2] Among women with available information on amount of alcohol consumption, dietary lignan, and total energy intakes at baseline ($n = 61,255$).

follow-up was censored during the period between the mailing date of the unanswered questionnaire and the date of the next answered questionnaire.

All models were adjusted for age as the underlying time metric and for the nondietary variables listed in **Table 1**, included either as constant values at baseline or as time-varying variables when updated in follow-up questionnaires (as detailed in Supplemental Material).

Other details about the statistical methods are given in the Supplemental methods. All analyses were performed with SAS software version 9.3 (SAS Institute, Inc.).

Results

Among the 76,442 women included in the analyses, 4.4% reported current use of a dietary supplement containing soy when answering Q2000, 5.3% when answering Q2002, 3.8% when answering Q2005, and 1.4% when answering Q2008. At the time they answered Q2002, Q2005, and Q2008, 2.6%, 10.6%, and 13.6% of responders were past users of a soy supplement, respectively. Among women who reported ever using soy supplements together with their brand names, 81% had used ≥1 of the 5 most-consumed soy supplements in the cohort, which contained between 3.75 and 37.5 mg soy isoflavones (present in their glucoside form in soy extract)/tablet, according to the manufacturers.

Table 1 presents selected self-reported baseline characteristics of participants, according to whether they were current, past, or never users of soy supplements at the end of follow-up. Overall, current or past users of soy supplements tended to be younger (and hence less likely to be postmenopausal), to have a higher educational level, to have fewer children, and were more likely to have a BMI (in kg/m^2) <25, to have used oral contraceptives, and to have recently underwent a mammogram compared with women who never used soy supplements.

Participants had a mean ± SD age of 59.5 ± 6.6 y (range: 49–83 y) at the start of follow-up and were followed for a median of 11.2 y (IQR = 8.0–11.4 y, total number of person-years = 718,757), during which time a total of 3608 cases of first primary breast cancer were diagnosed.

No association was observed between the use of soy supplements and the overall risk of breast cancer: current users had a multivariable HR of 0.92 (95% CI: 0.76, 1.11) and past users of 1.01 (95% CI: 0.88, 1.16) compared with never users (**Table 2**). Sensitivity analyses showed no indication of confounding by alcohol consumption or dietary lignan intake after adjusting for total energy intake (excluding energy from alcohol) among the population who answered a diet history questionnaire in 1993 or 2005 ($n = 61,445$) (20); no variation in the results when analyses were restricted to women who self-reported performing a mammogram in the previous follow-up cycle ($n = 73,732$); and no variation in results when using multiple imputation for missing covariate data (data not shown).

The HRs for the association of soy supplements with different subtypes of breast cancer are shown in Table 2. Current use of soy supplements was associated with a lower risk of ER-positive (ER+) breast cancer (HR: 0.78; 95% CI: 0.60, 0.99) and with a higher risk of ER-negative (ER–) breast cancer (HR: 2.01; 95% CI: 1.41, 2.86) compared with never using supplements (P-homogeneity < 0.0001). These associations were observed independent of PR status. The association between current use of soy supplements and breast cancer risk did not vary according to the invasive or in situ status or to histologic subtype. No association was observed for past use compared with never use of soy supplements, whatever the tumor subtype considered.

There was no interaction between soy supplement use (current, past, never) and age ($P = 0.65$), smoking status ($P = 0.77$), MHT use ($P = 0.18$), postmenopausal BMI ($P = 0.87$), personal history of benign breast disease ($P = 0.52$), level of alcohol consumption ($P = 0.67$), or dietary lignan intake ($P = 0.51$) (data not shown).

TABLE 2 HRs for different subtypes of breast cancer associated with use of dietary supplements in soy isoflavones: E3N cohort, 2000–2011[1]

	Exposure to dietary supplements in soy isoflavones						
	Never use		Current use		Past use		
Breast cancer characteristics (no. of women included)	No. of cases	HR[2] (95% CI)	No. of cases	HR[2] (95% CI)	No. of cases	HR[2] (95% CI)	*P*-homogeneity[3]
All breast cancers ($n = 76,442$)	3241	1 (reference)	114	0.92 (0.76, 1.11)	245	1.01 (0.88, 1.16)	0.42
According to ER status ($n = 75,727$)[4]							
ER+	2190	1 (reference)	63	0.78 (0.60, 0.99)	169	1.03 (0.88, 1.22)	0.054
ER–	406	1 (reference)	35	2.01 (1.41, 2.86)	24	0.81 (0.53, 1.23)	0.0007
P-homogeneity[5]	—	—	—	<0.0001	—	0.28	—
According to combined ER and PR status ($n = 75,587$)[6]							
ER+ PR+	1534	1 (reference)	44	0.79 (0.59, 1.07)	117	0.99 (0.82, 1.21)	0.21
ER+ PR–	529	1 (reference)	17	0.81 (0.49, 1.31)	47	1.17 (0.86, 1.60)	0.19
ER– PR–	350	1 (reference)	29	1.84 (1.25, 2.71)	22	0.80 (0.52, 1.25)	0.004
ER– PR+	50	1 (reference)	6	3.80 (1.60, 9.02)	2	0.83 (0.20, 3.56)	0.07
According to invasive/in situ status ($n = 76,215$)[7]							
Invasive	2657	1 (reference)	102	1.01 (0.83, 1.24)	187	0.95 (0.82, 1.11)	0.62
In situ	384	1 (reference)	10	0.63 (0.33, 1.18)	34	1.13 (0.78, 1.62)	0.11
P-homogeneity[5]	—	—	—	0.15	—	0.41	—
According to histologic subtype ($n = 76,172$)[8]							
Ductal	2246	1 (reference)	74	0.84 (0.67, 1.06)	163	0.94 (0.80, 1.11)	0.44
Lobular	467	1 (reference)	24	1.43 (0.95, 2.17)	38	1.19 (0.84, 1.68)	0.48
Other	288	1 (reference)	12	1.15 (0.64, 2.06)	19	1.07 (0.66, 1.73)	0.84
P-homogeneity[5]	—	—	—	0.08	—	0.46	—

[1] $n = 76,442$. Eight breast cancer cases occurred in the "unknown exposure" category (6 with information available on ER status, 6 with information available on combined ER/PR status, 7 with information available on invasive/in situ status, and 7 with information available on histologic subtype; not shown). ER, estrogen receptor; E3N, Etude Epidemiologique aupres de Femmes de la Mutuelle Generale de l'Education Nationale; PR, progesterone receptor; +, positive, ; -, negative.

[2] From a Cox proportional hazards model with age as the time scale, stratified by birth cohort, and adjusted for years of education, height, age at menarche, family history of breast cancer, use of oral contraceptives, parity and age at first full-term birth, menopausal status and time since menopause, BMI cross-classified by menopausal status, use of menopausal hormone therapy, personal history of benign breast biopsy, smoking status, and mammogram performed during the previous follow-up cycle. Categories used are those displayed in Table 1.

[3] *P* value for assessing homogeneity in HRs associated with current use and past use of dietary supplements in soy isoflavones.

[4] Of the 76,442 women in the analytic cohort, 715 incident first primary breast cancer cases were excluded because of missing information on ER status.

[5] *P* value for assessing homogeneity in HRs of the different subtypes of breast cancer.

[6] Of the 76,442 women in the analytic cohort, 855 incident first primary breast cancer cases were excluded because of missing information on combined ER/PR status.

[7] Of the 76,442 women in the analytic cohort, 227 incident first primary breast cancer cases were excluded because of missing information on invasive/in situ status.

[8] Of the 76,442 women in the analytic cohort, 270 incident first primary breast cancer cases were excluded because of missing information on histologic subtype.

However, there was effect modification by family history of breast cancer (*P*-interaction = 0.03) and menopausal status combined with time since menopause (*P*-interaction = 0.04), as shown in **Table 3**. HRs for current soy supplement use were 1.36 (95% CI: 0.95, 1.93) and 0.82 (95% CI: 0.65, 1.02) among women with and without family history of breast cancer, respectively. Following stratification by menopausal status, the association of soy supplement use and breast cancer risk was 1.06 (95% CI: 0.87, 1.30) ≥5 y after menopause onset and 0.50 (95% CI: 0.31, 0.81) in premenopause or <5 y postmenopause. The inverse association of current use of soy supplements with ER+ breast cancer risk was reduced, whereas the positive association with ER– breast cancer risk was stronger among women with history of breast cancer in first-degree relatives or ≥5 y after menopause onset compared with women with no family history of breast cancer or in premenopause or <5 y postmenopause (Table 3).

Discussion

In this prospective study in French women aged >50 y, there was no overall association between current or past use of dietary supplements containing soy isoflavones and breast cancer risk. However, when investigated by ER status, we observed opposing associations such that there was a lower risk of ER+ and a higher risk of ER– breast cancers in current users. The higher risk of ER– breast cancer was particularly strong among women with a history of breast cancer in first-degree relatives, whereas the lower risk of ER+ breast cancer was statistically significant only in premenopausal or recently postmenopausal women and in women with no family history of breast cancer.

To our knowledge, only 4 epidemiologic studies have investigated the association of breast cancer risk with soy or isoflavone dietary supplements. These studies had either limited statistical power or retrospective designs, or both, and yielded inconsistent results (15–18) (see Introduction).

Soy isoflavones (mainly daidzein and genistein) are structurally similar to 17-β-estradiol. As such, they can bind to ERα (the form of ER measured clinically in the treatment of breast cancer patients), the activation of which increases cell proliferation. Thus, in the presence of high concentrations of estradiol, isoflavones may act as antiestrogens by competitively binding to ERα. Conversely, at low estrogen concentrations,

TABLE 3 HRs for breast cancer associated with use of dietary supplements in soy isoflavones in strata of significant effect modifiers: E3N cohort, 2000–2011[1]

	Exposure to dietary supplements in soy isoflavones						
	Never use		Current use		Past use		
	No. of cases	HR[2] (95% CI)	No. of cases	HR[2] (95% CI)	No. of cases	HR[2] (95% CI)	*P*-interaction[3]
All breast cancers							
No history of breast cancer in first-degree relatives	2660	1 (reference)	80	0.82 (0.65, 1.02)	191	0.97 (0.84, 1.13)	0.03
History of breast cancer in first-degree relatives	581	1 (reference)	34	1.36 (0.95, 1.93)	54	1.19 (0.89, 1.59)	
Premenopausal or <5 y postmenopause	448	1 (reference)	18	0.50 (0.31, 0.81)	20	0.98 (0.62, 1.56)	0.04
≥5 y postmenopause	2793	1 (reference)	96	1.06 (0.87, 1.30)	225	1.03 (0.89, 1.18)	
ER+ breast cancers							
No history of breast cancer in first-degree relatives	1800	1 (reference)	48	0.75 (0.56, 0.99)	131	1.00 (0.83, 1.20)	0.47
History of breast cancer in first-degree relatives	390	1 (reference)	15	0.91 (0.54, 1.53)	38	1.22 (0.86, 1.72)	
Premenopausal or <5 y postmenopause	291	1 (reference)	9	0.39 (0.20, 0.76)	15	1.13 (0.66, 1.94)	0.11
≥5 y postmenopause	1899	1 (reference)	54	0.90 (0.69, 1.18)	154	1.04 (0.88, 1.23)	
ER– breast cancers							
No history of breast cancer in first-degree relatives	347	1 (reference)	23	1.58 (1.03, 2.42)	20	0.77 (0.49, 1.23)	0.03
History of breast cancer in first-degree relatives	59	1 (reference)	12	4.23 (2.21, 8.07)	4	1.00 (0.35, 2.83)	
Premenopausal or <5 y postmenopause	73	1 (reference)	7	1.22 (0.55, 2.67)	3	0.78 (0.24, 2.55)	0.36
≥5 y postmenopause	333	1 (reference)	28	2.36 (1.59, 3.49)	21	0.81 (0.52, 1.27)	

[1] $n = 76{,}442$. ER, estrogen receptor; E3N, Etude Epidemiologique aupres de Femmes de la Mutuelle Generale de l'Education Nationale; +, positive; -, negative.

[2] From a Cox proportional hazards model with age as the time scale, stratified by birth cohort, and adjusted for years of education, height, age at menarche, family history of breast cancer, use of oral contraceptives, parity and age at first full-term birth, menopausal status and time since menopause, BMI cross-classified by menopausal status, use of menopausal hormone therapy, personal history of benign breast biopsy, smoking status, and mammogram performed during the previous follow-up cycle. Categories used are those displayed in Table 1.

[3] *P* value for assessing homogeneity in HRs associated with current use of soy supplements across the 2 categories considered (e.g., women with no history of breast cancer in first-degree relatives and women with such an history), as well as homogeneity in HRs associated with past use across these 2 categories.

as is the case in late menopause, isoflavones may exert weak estrogenic effects (21). However, isoflavones have a 10-fold higher affinity for ERβ, the activation of which has proapoptotic and prodifferentiation effects and has been shown to counteract the ERα-mediated stimulation of cell proliferation (22). Hence, isoflavone ERβ-mediated antiproliferative effects overcome ERα-mediated proliferative effects in ER+ cells [i.e., when ERα and ERβ are coexpressed (8)]. Isoflavones may also exert hormone-independent activity, such as proapoptotic, antioxidative, anti-inflammatory, and antiangiogenic effects, and these effects have been observed in vitro with high doses of isoflavones (7, 9, 23).

In vitro, isoflavones show either proliferative or antiproliferative effects on breast cells, depending on the evaluated dose, the estrogenic environment, and the cell ER availability (8, 24). The majority of studies on ovariectomized animals reported no effect of isoflavones on breast cell proliferation or mammary gland histopathologic features (23). However, a stimulating effect of genistein was observed in 2 mouse studies (25, 26). Because of the apparent complexity of the relations between isoflavones and breast cancer in laboratory studies, data from human studies are particularly important.

Our result of no association between current or past use of soy supplements and overall breast cancer risk is consistent with surrogate endpoint studies. Indeed, 8 randomized controlled trials that measured mammographic density and 2 that investigated histopathologic changes (proliferation) did not suggest deleterious effects of exposure to isoflavone supplements on the mammary gland in healthy postmenopausal women (as reviewed in reference 23).

However, the potential proliferative effects of soy isoflavones on already transformed breast cancer cells have raised concerns about soy supplement consumption in populations at high risk of breast cancer (6, 27, 28). We consistently observed a higher increase in risk of ER– breast cancer associated with current use of soy supplements in women with a history of breast cancer in first-degree relatives, which is a strong risk factor for breast cancer, than in women with no such history (HR: 4.23 compared with 1.58, respectively).

We found a lower risk of ER+ breast cancer associated with current use of soy supplements, which was restricted to premenopausal and early postmenopausal women. That finding is in line with the hypothesis that isoflavones would exert antiestrogenic or weak estrogenic effects depending on the estrogen milieu (as discussed above).

Our results showed a doubling in the risk of ER– breast cancer (i.e., not the most common type but with a less favorable prognosis) associated with current use of soy supplements. This suggests a biological action of soy isoflavones involving other cellular regulatory pathways than those modulated by canonical ERs.

Breast cancer cells may also exhibit other membrane receptors such as G protein–coupled estrogen receptor (GPER), for which genistein and daidzein have a high affinity (29, 30). In several cancer cell lines, GPER induces mobilization of intracellular calcium stores and activation of mitogen-activated protein kinase and PI3K signaling pathways (31). It has been shown to decrease the proapoptotic BCL2 like 11 (*BCL-2-L11*) gene, favoring cell proliferation and survival (32). Another candidate is ERα-36, the truncated form of ER, which is expressed by triple-negative breast cancer cells (33). The activation of these agonist pathways by soy isoflavones might be counterbalanced by the above-mentioned antiproliferative effects, which may be particularly relevant to ER+ breast cancer cells and in the presence of premenopausal concentrations of estradiol.

The current study has several strengths, in particular the absence of differential recall bias in exposure data between cases and noncases, that derive from its prospective design. The number of exposed cases was relatively high ($n = 359$) compared with those in the only other published cohort analysis [i.e., 36 breast cancer cases among soy supplement users) (15)]. This allowed us to study the potential interactions with breast cancer risk factors, as well as associations according to tumor characteristics. Exposure data were updated at 2- to 3-y intervals over follow-up, which limits misclassification bias and allowed us to distinguish between current and past use. The analyses were adjusted for many potential confounders, although residual confounding may still have occurred. As screening bias may interfere with the findings if soy supplement users had mammograms more frequently than nonusers, we adjusted our analyses for recent mammogram ["mammogram performed in the preceding follow-up period (yes/no)"], and we verified that our conclusions remained unaltered after restricting the analyses to women reporting a recent mammogram. Finally, the evaluation of the use of soy supplements in the E3N cohort started in the year 2000, when the market of these products had just started to develop in France (5).

We also acknowledge certain limitations. First, some women may have been misclassified as never using soy supplements if they consumed soy supplements but had stopped before the Q2000 or Q2002 questionnaires, which enquired only about current use, or if they forgot some episodes of use when answering Q2005 or Q2008. However, such misclassification of exposure would likely not differentially affect cases and noncases and would therefore only bias the associations towards the null. Second, the 11-y follow-up is likely too short to show an effect of soy supplements on tumor initiation. Third, data for ER status were not available for 715 of the 3608 incident breast cancers, but we verified that soy supplement use was not significantly associated with ER status availability among cases (data not shown). Finally, information on the consumption of soy-based foods was not available in the study population. However, soy foods were probably consumed in small quantities: the mean intake of isoflavones in the general European and French population was estimated to be <1 mg/d, and the consumption of soy and soy-based products, the main contributors to isoflavone intake, was not frequent in France in 2006–2007 (23). This study focused on soy isoflavone intake in the form of dietary supplements used relatively late in life. Thus, the associations identified cannot be extrapolated to other circumstances, especially to the consumption of isoflavone-rich foods throughout life.

In conclusion, this large prospective study in French women aged >50 y found a lower risk of ER+ breast cancer but also a higher risk of ER– breast cancer during the consumption of soy supplements. In accordance with previous recommendations, based on the precautionary principle that breast cancer survivors and women at high risk of breast cancer should avoid consuming soy supplements (6), our results prompt special caution among women with a family history of breast cancer. Due to relatively low numbers of exposed cases in some strata (premenopausal or recently postmenopausal women, women with a family history of breast cancer, ER– breast cancers), our results need further investigation in other settings.

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