Long-term dietary calcium intake and breast cancer risk in a prospective cohort of women

Susanna C Larsson, Leif Bergkvist, and Alicja Wolk

ABSTRACT

Background: Calcium may potentially influence the risk of breast cancer because of its role in regulating cell proliferation, differentiation, and apoptosis. However, prospective studies of calcium intake in relation to breast cancer incidence are sparse.

Objective: The objective of this study was to prospectively examine the association between calcium intake and the risk of breast cancer by estrogen receptor (ER) and progesterone receptor (PR) status of the breast.

Design: The Swedish Mammography Cohort is a population-based prospective cohort of 61,433 women who were cancer-free at enrollment in 1987–1989. Dietary calcium intake was assessed with a food-frequency questionnaire at baseline and again in 1997. Cox proportional hazards models were used to estimate rate ratios (RRs) and 95% CIs, adjusted for breast cancer risk factors.

Results: During an average of 17.4 years of follow-up, 2952 incident cases of breast cancer were ascertained. Dietary calcium intake was not associated with risk of overall breast cancer; the multivariate RR for the highest compared with the lowest quintile of calcium intake was 0.97 (95% CI: 0.87, 1.09; P for trend: 0.49). There was a statistically significant inverse trend for ER-negative/PR-negative (ER−/PR−) breast cancer (P for trend: 0.02); the multivariate RR for the comparison of extreme quintiles of calcium intake was 0.66 (95% CI: 0.44, 0.99). Calcium intake was not associated with ER-positive/PR-positive (ER+/PR+) or ER+/PR− tumors.

Conclusions: Our findings do not support an association between dietary calcium intake and overall breast cancer risk. The inverse relation between calcium intake and ER−/PR− breast cancer requires confirmation in other studies.


INTRODUCTION

Calcium may play a role in breast carcinogenesis because of its importance in regulating cell proliferation, differentiation, and apoptosis (1–3). In experimental studies, calcium reduces cell proliferation and induces differentiation of mammary cells (4, 5). Moreover, high calcium intake has been shown to decrease fat-induced epithelial hyperproliferation of the mammary gland and chemically induced mammary carcinogenesis in rodents (4, 6). Findings from cross-sectional studies indicate that dietary calcium intake is inversely related to mammographic breast density (7–9), which is strongly positively associated with a risk of breast cancer (10). Epidemiologic studies have also suggested that a high dietary calcium intake is associated with a reduced risk of benign proliferative epithelial disorders of the breast (11, 12), putative precursors of breast cancer.

The association between calcium intake and breast cancer risk was examined in 6 hospital-based and 4 population-based case-control studies, and most of these studies showed an inverse association (13, 14). However, most of these studies were small (≤310 cases) or failed to control for potential confounders (13, 14). Large prospective cohort studies of calcium intake in relation to incidence of breast cancer are limited (15–17) and only 2 studies have evaluated whether the association varies by hormone receptor status of the breast tumor (15, 17).

We analyzed data from a population-based prospective cohort of Swedish women to test the hypothesis that a high calcium intake is associated with a decreased risk of breast cancer. We investigated whether the association varies by the estrogen receptor (ER) and progesterone receptor (PR) status of the breast tumor.

SUBJECTS AND METHODS

Study cohort

The Swedish Mammography Cohort was established in 1987–1989 in Västmanland County and in 1988–1990 in Uppsala County in central Sweden. All women born between 1917 and 1948 in Västmanland County and between 1914 and 1948 in Uppsala County received a mailed invitation to be screened by mammography. Enclosed with this invitation was a 6-page questionnaire that elicited information on diet, body size, reproductive factors, family history of breast cancer, and other factors. A completed questionnaire was obtained from 66,651 women, representing 74% of the source population. In late autumn of 1997, all cohort members who were still alive and living in the study area received a new questionnaire that was expanded to...
include about 350 items concerning diet and other lifestyle factors (including physical activity); 39,227 women (70%) completed the second questionnaire.

From the baseline cohort of 66,651 women, we excluded those with an erroneous or missing national registration number. We also excluded questionnaires that lacked a date, the date the subject moved out of the study area, or the date of death. After the additional exclusion of women with implausible values for total energy intake (ie, 3 SDs from the mean value for loge-transformed energy intake) and those with a cancer diagnosis (except non-melanoma skin cancer) before baseline, the analytic cohort consisted of 61,433 women. Of these, 26,403 were premenopausal, 2574 were perimenopausal, and 32,456 were postmenopausal.

For analyses using information from the second questionnaire, 36,664 women were eligible after exclusion of those with implausible energy intake on the second dietary questionnaire and those who had been diagnosed with cancer between baseline and 1 January 1998. The study was approved by the ethics committees at the Uppsala University Hospital (Uppsala, Sweden) and the Karolinska Institutet (Stockholm, Sweden).

Assessment of diet

To assess diet, a food-frequency questionnaire (FFQ) was used that included 67 and 96 food items at baseline and in 1997, respectively. In these questionnaires, women were asked to indicate how often, on average, they had consumed each food item during the past 6 mo (baseline FFQ) or the last year (1997 FFQ). For most food items, participants could choose from 8 predefined categories for frequency of consumption, ranging from “never/seldom” to “4 or more times per day” (baseline FFQ) or “3 or times per day” (1997 FFQ). For some commonly consumed foods, such as milk, cultured milk (sour milk and yogurt), and cheese, participants reported the exact number of servings per day. The major food sources of calcium were dairy foods. In the baseline FFQ, intake of the following calcium-rich dairy foods was assessed: low-fat milk, medium-fat milk, whole milk, low-fat cultured milk, cultured milk, cheese, and ice cream. The 1997 FFQ included the same dairy foods and also cottage/cream cheese, sour cream, and cream. Dietary calcium intake was calculated by multiplying the frequency of consumption of each food item by its calcium content per age-specific serving with the use of food composition values obtained from the Swedish National Food Administration Database (18). The age-specific serving sizes were based on mean values obtained from 213 randomly chosen women from the study area who weighed and recorded their food intake for a mean of 27.8 d. On the 1997 questionnaire, participants reported use of multivitamins and calcium supplements according to duration and number of tablets taken per week. Total calcium intake included contributions from diet, multivitamins containing minerals, and individual calcium supplements.

The validity of the baseline FFQ was assessed by comparing responses from four 1-wk dietary records (3–4 mo apart) with responses from the FFQ among 129 women randomly selected from the cohort (A Wolk, unpublished data, 1992). The Pearson correlation coefficient between the FFQ and the dietary records was 0.5 for dietary calcium. The validity of the 1997 FFQ was reported previously; the Spearman correlation coefficient between the FFQ and fourteen 24-h recalls over 1 y was 0.77 for both dietary and total calcium intake (19).

Case ascertainment

We ascertained histologically confirmed incident cases of invasive breast cancer by linking the study cohort with the national and regional Swedish Cancer registers. The completeness of cancer follow-up was estimated to be almost 100% (20). Information on the ER and PR status of breast tumors was obtained by reviewing pathology laboratory work logs stored at Uppsala University Hospital (from 1987 to 1994) and by linkage with the clinical database (the Quality Register) at the Regional Oncology Centre in Uppsala (from January 1992 through December 2007), which was based on the patients’ original medical records. ER and PR status was evaluated by means of an Abbott immunoassay until 1997 and an immunohistochemical method thereafter. Cases with ≥0.1 fmol/µg cytosol DNA were considered hormone receptor-positive when the Abbott immunoassay was used. By the immunohistochemical method, cases were considered as receptor-positive when the percentage of positive cells was ≥10%, and receptor-negative when the percentage of positive cells was <10%. The Department of Pathology and Cytology at Uppsala University Hospital and Västerås Central Hospital were involved in this evaluation. Information on the dates of death for deceased participants was obtained from the Swedish Death Registry.

Statistical analysis

For each participant, follow-up time accrued from the date of enrollment to the date of breast cancer diagnosis, death from any cause, or 31 December 2007, whichever occurred first. In analyses of ER/PR status, for women in Västmanland County, person-time of follow-up was counted from January 1998 because routine evaluation of ER and PR status was implemented in Västmanland County in 1997. For analyses using information from the 1997 questionnaire, the follow-up of all women started in January 1998. Calcium intake was adjusted for total energy using the residual method (21) and categorized into quintiles based on the distribution in the entire cohort.

To account for changes in diet during follow-up and to better represent long-term dietary intake, we used a cumulative average approach (22). Specifically, breast cancer incidence from baseline through 1997 was related to dietary calcium intake at baseline, and breast cancer incidence from 1998 through December 2007 was related to the average calcium intake at baseline and in 1997. In sensitivity analyses, we related calcium intake at baseline to breast cancer incidence during the entire follow-up. Moreover, calcium intake in 1997 was related to breast cancer incidence from 1998 through December 2007.

We used Cox proportional hazards models (23) to estimate incidence rate ratios (RRs) with 95% CIs for the association between calcium intake and breast cancer risk. To control as finely as possible for age and calendar time, and possible 2-way interactions between these 2 time scales, we stratified the models by age in months at start of follow-up and the year of enrollment. In multivariate models, we additionally adjusted for education (primary school, high school, university) and potential risk factors for breast cancer, including body mass index (in kg/m²).
<18.5, 18.5–24.9, 25–29.9, ≥30), height (in cm; continuous), parity (nulliparous, 1–2, ≥3), age at first birth (nulliparous, <26, 26–30, ≥31 y), age at menarche (<12, 13, ≥14 y), age at menopause (<51, ≥51 y), use of oral contraceptives (ever or never), use of postmenopausal hormones (ever or never), family history of breast cancer (yes or no), and intake of alcohol (nondrinkers and <3.4, 3.4–9.9, ≥10.0 g/d), dietary fiber (in quintiles), and total energy (kcal/d; continuous). We tested the proportional hazard assumption using the likelihood ratio test and found no departure from the assumption.

Tests for trend were performed by assigning the median value to each quintile of calcium intake and treating this value as a continuous variable in the model. Because calcium intake has been shown to reduce fat-induced epithelial hyperproliferation (4, 6), we conducted analyses stratified by fat intake (below or above the median intake in the cohort, ie, <55 or ≥55 g/d) to examine whether the association between calcium intake and breast cancer risk was modified by fat intake. The likelihood ratio test was used to test the significance in interaction models. All statistical analyses were conducted by using SAS version 9.1 (SAS Institute Inc, Cary, NC). All reported P values were 2-tailed.

RESULTS

The baseline characteristics of the study cohort by quintiles of dietary calcium intake are shown in Table 1. Compared with women with a low calcium intake, those with higher intakes were slightly more likely to have a postsecondary education and had lower alcohol and dietary fiber intake. Other characteristics did not vary appreciably across quintiles of calcium intake.

During an average of 17.4 y of follow-up (1,071, 164 person-years of observation), a total of 2952 of the 61,433 women in the cohort were diagnosed with invasive breast cancer. Information on ER and PR status was available for 2062 cases (information on ER/PR status was available first in 1997 for women in Västmanland County). Of these cases, 1286 (62.4%) were ER+/PR+, 417 (20.2%) were ER+/PR−, 266 (12.9%) were ER−/PR−, and 93 (4.5%) were ER−/PR−.

Long-term dietary calcium intake was not associated with the risk of overall breast cancer (Table 2). The association did not vary by menopausal status; the multivariate RRs for the highest compared with the lowest quintile of calcium intake were 0.94 (95% CI: 0.79, 1.13) in premenopausal women (n = 1244 cases) and 0.92 (95% CI: 0.78, 1.09) in postmenopausal women (n = 1584 cases). Analyses according to ER and PR status showed a statistically significant inverse association between calcium intake and risk of ER-negative/PR-negative (ER−/PR−) tumors (P for trend = 0.02) but not ER-positive/PR-positive (ER+/PR+) or ER+/PR− tumors. Compared with women in the lowest quintile of calcium intake, those in the highest quintile had a 34% lower risk of ER−/PR− breast cancer (RR: 0.66; 95% CI: 0.44, 0.99). Further adjustment for dietary vitamin D and conjugated linoleic acid intakes slightly strengthened the association (RR: 0.59; 95% CI: 0.38, 0.91). Exclusion of cases diagnosed within the first 3 y of follow-up did not change the results appreciably (RR: 0.63; 95% CI: 0.39, 1.01). In the analysis using data from the baseline questionnaire only, without updating of diet, the multivariate RR comparing extreme quintiles of calcium intake was 0.67 (95% CI: 0.47, 0.96).

The association between calcium intake and breast cancer risk may be stronger among women with a high fat intake (4, 6). In fact, we observed a statistically significant interaction between calcium and fat intake in relation to risk of ER−/PR− breast cancer (P for interaction: 0.04). The multivariate RRs of ER−/PR− breast cancer for the highest compared with the lowest quintile of calcium intake were 0.88 (95% CI: 0.50, 0.54; P for trend: 0.54) among women with a low fat intake (<55 g/d; n = 139 cases) and 0.50 (95% CI: 0.27, 0.92; P for trend: 0.01) among women with a high fat intake (≥55 g/d; n = 127 cases).

### Table 1

Age-standardized baseline characteristics of 61,433 women in the Swedish Mammography Cohort by quintiles of energy-adjusted dietary calcium intake, 1987–1990

| Quintile of calcium intake (mg/d) | <727 (621) | 727–862 (800) | 863–980 (922) | 981–1124 (1046) | ≥1125 (1242) | P for trend
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<td><strong>Characteristics</strong></td>
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<tr>
<td>Age (y)</td>
<td>54.1 ± 9.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>54.0 ± 9.8</td>
<td>53.8 ± 9.7</td>
<td>53.4 ± 9.6</td>
<td>53.3 ± 9.6</td>
<td>&lt;0.001</td>
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<td>BMI (kg/m&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>24.7 ± 4.0</td>
<td>24.5 ± 3.8</td>
<td>24.7 ± 3.8</td>
<td>24.8 ± 3.8</td>
<td>25.1 ± 3.9</td>
<td>&lt;0.001</td>
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<td>Postsecondary education (%)</td>
<td>11.5</td>
<td>12.6</td>
<td>12.9</td>
<td>13.0</td>
<td>13.4</td>
<td>&lt;0.001</td>
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<td>Family history of breast cancer (%)</td>
<td>7.2</td>
<td>7.1</td>
<td>7.5</td>
<td>6.7</td>
<td>7.4</td>
<td>0.62</td>
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<tr>
<td>Oral contraceptive use (%)</td>
<td>54.4</td>
<td>54.3</td>
<td>54.2</td>
<td>53.4</td>
<td>53.7</td>
<td>0.10</td>
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<tr>
<td>Postmenopausal hormone use (%)</td>
<td>42.4</td>
<td>44.7</td>
<td>45.2</td>
<td>45.1</td>
<td>44.7</td>
<td>&lt;0.001</td>
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<tr>
<td>Nulliparous (%)</td>
<td>11.9</td>
<td>10.9</td>
<td>10.5</td>
<td>10.4</td>
<td>10.7</td>
<td>&lt;0.001</td>
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<tr>
<td>Age at first birth (y)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>23.8 ± 4.6</td>
<td>24.2 ± 4.5</td>
<td>24.3 ± 4.6</td>
<td>24.2 ± 4.5</td>
<td>24.0 ± 4.5</td>
<td>0.001</td>
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<td>Age at menarche (y)</td>
<td>13.3 ± 1.3</td>
<td>13.2 ± 1.3</td>
<td>13.2 ± 1.3</td>
<td>13.2 ± 1.3</td>
<td>13.2 ± 1.3</td>
<td>0.005</td>
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<tr>
<td>Age at menopause (y)</td>
<td>50.7 ± 4.8</td>
<td>50.7 ± 4.8</td>
<td>50.7 ± 4.7</td>
<td>50.7 ± 4.7</td>
<td>50.6 ± 4.9</td>
<td>0.001</td>
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<tr>
<td>Total energy intake (kcal/d)</td>
<td>1567 ± 525</td>
<td>1632 ± 461</td>
<td>1625 ± 454</td>
<td>1592 ± 428</td>
<td>1495 ± 419</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Alcohol intake (g/d)</td>
<td>2.8 ± 4.2</td>
<td>2.7 ± 3.6</td>
<td>2.5 ± 3.3</td>
<td>2.4 ± 3.2</td>
<td>2.2 ± 2.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Dietary fiber intake (g/d)</td>
<td>25.8 ± 6.2</td>
<td>24.6 ± 5.4</td>
<td>24.2 ± 5.2</td>
<td>23.8 ± 5.1</td>
<td>22.7 ± 5.5</td>
<td>&lt;0.001</td>
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<sup>a</sup>Medians in parentheses.

<sup>b</sup>Across categories of calcium intake. Generalized linear models were used for continuous variables, and chi-square tests were used for categorical variables.

<sup>c</sup>Mean ± SD (all such values).

<sup>d</sup>Parous women only.
The association between calcium intake and risk of overall breast cancer and ER+/PR+ or ER+/PR− tumors was not modified by fat intake.

To examine whether intake of total or supplemental calcium was associated with the risk of breast cancer, we used information from the 1997 questionnaire. During a mean follow-up of 9.4 y, from January 1998 through December 2007, we ascertained 1008 incident cases of invasive breast cancer, including 110 ER+/PR− tumors. Twenty-three percent of participants reported use of vitamin supplements. The mean intakes of dietary, total, and supplemental calcium were 1043, 1106, and 63 mg, respectively. We observed no association between dietary or total calcium intake and risk of breast cancer, but the associations were not statistically significant, possibly because of low statistical power; the multivariate RR comparing extreme quintiles of intake was 1.00 (95% CI: 0.82, 1.22) or overall breast cancer (multivariate RR comparing extreme quintiles of intake: 0.58 (95% CI: 0.50, 0.99)).

In this large prospective cohort study of Swedish women, we observed no association between dietary calcium intake and risk of overall breast cancer. However, dietary calcium intake was significantly inversely associated with the risk of developing ER−/PR− breast cancer. Women with the highest calcium intake had a 34% lower risk of ER−/PR− breast cancer compared with those with the lowest intake.

Five previous prospective studies examined the association between calcium intake and breast cancer risk. In the Nurses’ Health Study of 88,691 women (including 3482 cases), a high dietary calcium intake (>1000 mg/d compared with ≤500 mg/d) was associated with a statistically significant 33% lower risk of breast cancer among premenopausal women (16); no significant association was observed among postmenopausal women or for total calcium intake from diet and supplements combined or for nondairy calcium. Results from the Women’s Health Study, which included 10,578 premenopausal women (n = 276 cases) and 20,909 postmenopausal women (n = 743 cases), showed an inverse association between total calcium intake and premenopausal breast cancer risk, with an RR of 0.61 (95% CI: 0.40, 0.92) for women with the highest intake (≥1366 mg/d) compared with those with the lowest (<617 mg/d); no significant association was observed for calcium from diet intake alone (17). In the Cancer Prevention Study II Nutrition Cohort of 68,567 postmenopausal women (n = 2855 cases), those in the highest category of dietary calcium intake (>1250 mg/d) had a 20% lower risk of breast cancer compared with those in the lowest category (≤500 mg/d; RR: 0.80; 95% CI: 0.67, 0.95); no significant association was observed for total calcium intake (15). Two small cohort studies—one in Finland (4697 women, n = 88 cases) (24) and one in France (3627 women, n = 92 cases) (25)—also showed a statistically significant inverse relation between dietary calcium intake and risk of breast cancer. Although these studies are consistent in showing an inverse

**DISCUSSION**

In this large prospective cohort study of Swedish women, we observed no association between dietary calcium intake and risk of overall breast cancer. However, dietary calcium intake was significantly inversely associated with the risk of developing ER−/PR− breast cancer. Women with the highest calcium intake had a 34% lower risk of ER−/PR− breast cancer compared with those with the lowest intake.
association between calcium intake and risk of overall breast cancer, findings are mixed with regard to menopausal status and calcium source (dietary or total calcium). In our study, the lack of association between dietary calcium intake and overall breast cancer was consistent among premenopausal and postmenopausal women. We observed, however, a suggestive inverse association between calcium supplement use and risk of breast cancer. The absence of association between calcium intake and overall breast cancer risk in the study presented here may be due to the relatively high calcium intake in this study population. Only 4.7% of the population had an estimated calcium intake below 500 mg/d. Therefore, we had limited statistical power to examine whether very low calcium intake is associated with breast cancer risk. Previous studies included women with calcium intakes of ≤500 mg/d (15, 16) or < 617 mg/d (17) as the reference group.

Only 2 previous studies have reported results on calcium intake and breast cancer risk by hormone-receptor status (15, 17). In the Cancer Prevention Study II Nutrition Cohort (15), the inverse association with dietary calcium intake was somewhat stronger for ER+ tumors (n = 1283; RR for the highest compared with the lowest category of intake: 0.67; 95% CI: 0.51, 0.88) than for ER− tumors (n = 227; RR: 0.77; 95% CI: 0.40, 1.47) in postmenopausal women. In the Women’s Health Study, total calcium intake was nonsignificantly inversely associated with both ER+ tumors (n = 206; RR for the highest compared with the lowest quintile of intake: 0.64; 95% CI: 0.40, 1.03) and ER− tumors (n = 58; RR: 0.68; 95% CI: 0.26, 1.77) in premenopausal women (17).

In this cohort study, a high calcium intake was associated with a lower risk of ER−/PR− breast cancer, especially among women with a high fat intake. A potential explanation for the difference by hormonal receptor status may be related to the effect of calcium on fat-induced epithelial hyperproliferation. Studies in animals have suggested that a high calcium intake decreases fat-induced epithelial hyperproliferation of the mammary gland and chemically induced mammary carcinogenesis (4, 6). In addition, results from the Women’s Intervention Nutrition Study, a randomized clinical trial of 2437 women aged 48–79 y, showed that a low-fat dietary intervention significantly improved relapse-free survival of breast cancer patients with ER−/PR− breast cancer (26). The low-fat dietary intervention had no significant effect on relapse-free survival in women with ER+ or PR+ breast cancer (26).

This study has several strengths, including the prospective and population-based design, a large sample size, detailed information on diet, and information on hormone receptor status. The prospective design precluded recall bias, and the practically complete follow-up of the study population through linkage with computerized population-based registers of cancer and deaths minimizes the concern that our results were affected by differential loss to follow-up. Another strength is that exposure data were collected from participants at 2 time points. For our main analyses we used repeated assessments of diet, which provide a better measure of long-term intake than does the baseline diet, although the relation between calcium intake and breast cancer risk was not different for the baseline and updated diet, or for the most recent diet.

Our study is limited by the fact that dietary intake was assessed with a self-administered FFQ, which will inevitably lead to some error in the measurement of dietary calcium intake and attenuated risk estimates. Hence, we cannot exclude the possibility that we have overlooked a weak association between calcium intake and overall breast cancer risk. Another limitation is the lack of information about calcium supplement use at baseline. Finally, although we adjusted for potential confounders, we cannot rule out the possibility that our findings are due to confounding from other factors correlated with calcium intake.

In summary, the findings from this prospective study do not support an association between dietary calcium intake and risk of overall breast cancer. A high calcium intake was associated with a decreased risk of developing ER−/PR− breast cancer, especially among women with a high fat intake. This finding may be due to chance and needs to be confirmed in other large prospective studies.

The authors’ responsibilities were as follows—SCL and AW: study concept and design; AW: data collection; SCL: statistical analyses and writing of manuscript; SCL, LB, and AW: interpretation of results; and SCL, LB, and AW: critical revision of manuscript. None of the authors had any personal or financial conflicts of interest.

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