Evaluation of Antioxidant Intakes in Relation to Inflammatory Markers Expression Within the Normal Breast Tissue of Breast Cancer Patients

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Abstract

Chronic inflammation may be a causative factor in breast cancer. One possible underlying mechanism is the generation of oxidative stress, which may favor tumorigenic processes. Antioxidant consumption may, therefore, help reduce tissue inflammation levels. However, few studies have explored this relation in breast tissue. We aimed to evaluate correlations between antioxidant (vitamin A/retinol, vitamin C, vitamin E, β-carotene, α-carotene, lycopene, lutein/zeaxanthin, β-cryptoxanthin, selenium, and zinc) intakes and protein expression levels of interleukin (IL)-6, tumor necrosis factor-α, C-reactive protein, cyclooxygenase-2, leptin, serum amyloid A1, signal transducer and activator of transcription 3, IL-8, IL-10, lactoferrin, and transforming growth factor-β measured in the normal breast tissue of 160 women diagnosed with breast cancer. Antioxidant intakes were collected using a self-administered food frequency questionnaire. Inflammation marker expression was assessed by immunohistochemistry. Correlations between antioxidant intakes and inflammatory marker expression were evaluated using Spearman's partial correlation coefficients (*r*) for all women and for premenopausal and postmenopausal women separately. After Bonferroni correction, negative correlations were observed between dietary β-tocopherol and IL-10 expression in all women combined (*r* = −0.26, *P* = .003) and among postmenopausal women (*r* = −0.39, *P* = .003). For all women, a negative correlation was found between total zinc intakes and IL-10 (*r* = −0.26, *P* = .002). Among postmenopausal women, dietary selenium intake was negatively correlated with the expression of lactoferrin (*r* = −0.39, *P* = .003). No associations were observed in premenopausal women. Our findings suggest that consumption of specific antioxidants, including β-tocopherol, zinc, and selenium, may act on the breast tissue through mechanisms affecting the expression of some inflammation markers, particularly among postmenopausal women.

Keywords

food, diet, antioxidants, inflammation mediators, local inflammation, chronic inflammation, carcinogenesis

Introduction

Chronic inflammation is being increasingly recognized as an etiologic factor for breast cancer.[1-4] Inflammation within the breast tissue may create a hostile local microenvironment favoring breast epithelial cell transformation, cancer cell proliferation and invasion, and tumor-related angiogenesis.[3] Possible mechanisms by which inflammation can contribute to tumorigenesis include disrupting the normal balance of pro- and anti-inflammatory mediators, which can facilitate tumor promotion and progression.[2] Alternatively, inflammation-induced oxidative stress may also drive malignant cell transformation by causing damage to important cellular

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components (eg, DNA, proteins, and lipids). In turn, these damages can cause inflammation within the tissue because numerous oxidative stress-sensitive transcription factors can mediate inflammatory responses.[5] Antioxidants may help reduce tissue-level inflammation through different mechanisms. Some antioxidants such as vitamins (A, E, and C), lycopene, lutein, and β-carotene act as reducing agents by restoring redox balance within tissue.[2]

Regarding inflammation and breast cancer, experiments have shown that antioxidants like vitamins A, E, and C as well as β-carotene regulate inflammatory signaling pathways in vitro and in vivo.[6,7] Retinoic acid and its receptors regulate several important signaling pathways, including inflammatory response pathways such as the nuclear factor NF-κB pathway.[6,8,9] Vitamin C has shown the ability to interfere in inflammatory paths by inhibiting the activation of NF-κB.[10,11] Furthermore, evidence from in vitro and in animal models of breast cancer[7,12] suggests that vitamin E can also affect these pathways by activating the peroxisome proliferator-activated receptor (PPAR)-γ pathway. PPAR-γ is able to interfere with NF-κB and to inhibit inflammatory markers such as cyclooxygenase-2 (COX-2).[13,14] These mechanisms could link vitamin E and inflammation and explain its benefits against breast cancer. Moreover, micronutrients, such as selenium and zinc, have also been shown to affect the expression of genes coding for many proteins involved in inflammation and immune responses.[15,16] More important, they are essential cofactors for antioxidant enzymes.[15]

Although data are somewhat inconsistent, there is some evidence suggesting associations between antioxidant intakes and breast cancer risk, which is consistent with the notion that inflammation, and resulting oxidative stresses, may be involved in the carcinogenic process. However, less is known about the link between antioxidants and inflammation among breast cancer patients. Among the few studies that examined the association between antioxidant intakes and circulating levels of inflammatory markers (3 observational[17-19] and 3 experimental[20-22]), intakes of retinol, carotenoids, lycopene, vitamin C or E tended to be negatively associated with C-reactive protein (CRP),[17,18,20] interleukin (IL)-1β,[19] or leptin[21] and positively associated with adiponectin.[21] However, since inflammatory marker concentrations were obtained from blood analysis, these may not adequately reflect levels found in the tumor microenvironment (breast tissue). Indeed, studies have shown that circulating levels of inflammatory markers such as leptin, adiponectin, and transforming growth factor (TGF)-β differ from concentrations measured in mammary tissue.[23,24] Therefore, inflammatory marker levels determined from blood samples may not give the same information as that obtained from in situ measurements in breast tissue. Up to now, a single case-control study has examined the relationship between antioxidants and inflammation in breast tissue. In that study, the concentrations of α-tocopherol, retinol, and β-carotene were assessed in the adipose breast tissue adjacent to the tumor from the case group consisting of 25 patients with noninvasive breast cancers.[25] Although a positive correlation between retinol concentration and IL-10 gene expression was observed, the small sample size limits generalization of results.

In this study, our working hypothesis was that antioxidant intakes influence the inflammatory profile of the normal breast tissue, which may contribute to breast cancer development. So, we took advantage of our tissue collection generated for the purposes of a study aiming to characterize breast density and expression of molecular markers[26] in order to evaluate the relation of general dietary intakes of vitamin A/retinol, vitamin C, vitamin E, β-carotene, α-carotene, lycopene, lutein/zeaxanthin, β-cryptoxanthin, selenium, and zinc with the protein expression of markers known as pro-inflammatory (IL-6, tumor necrosis factor-α [TNF-α], CRP, COX-2, leptin, and serum amyloid A1 [SAA1], signal transducer and activator of transcription 3 markers [STAT3], and IL-8) and anti-inflammatory (IL-10, lactoferrin, TGF-β) in the normal breast tissue of breast cancer patients.

Methods

Study Population

This study was reviewed and approved by the research ethics committee of the Centre de recherche du CHU de Quebec-Université Laval.

Selection of the study population was described elsewhere.[26] Briefly, the study subjects were women who underwent surgery for breast cancer at the Centre des maladies du sein Deschênes-Fabia between January 2011 and May 2012. Women were eligible if they were 69 years old or younger, were not currently pregnant, had a diagnosis of breast cancer and underwent partial or total mastectomy, received a digital mammogram within the 6 months prior to their surgery, never had a diagnosis of cancer at any site excluding the actual breast cancer, never had any breast surgery including breast reduction or implants, never taken selective estrogen receptor modulators such as tamoxifen or raloxifene, and had not received radiotherapy or chemotherapy prior to breast surgery. All study participants provided written informed consent.

Of the 226 women that met the eligibility criteria, 168 accepted to participate in the study. Of these, 8 were subsequently excluded from the analyses: 4 had a history of prior breast surgery and 4 did not return the food frequency questionnaire (FFQ). Finally, data for 160 women (81 premenopausal and 79 postmenopausal) were available for this study.

Data Collection

Anthropometric data were collected by a trained research nurse. Women were weighed (kg) wearing light clothing

without shoes and their height was assessed. Waist circumference was measured using a soft tape midway between the lowest rib margin and the iliac crest in a standing position, and hip circumference was measured over the widest area of the gluteal region. These anthropometric measures were used to estimate the body mass index (BMI; weight [kg]/height [m²]) and the waist-to-hip ratio (WHR; waist circumference [cm]/hip circumference [cm]).

A phone interview was conducted by a trained research assistant during the month following the breast surgery. Information collected included age at surgery (years), duration of oral contraceptive use (years), age at menarche (years), age at first pregnancy (years), parity (yes vs no), number of live births, breastfeeding duration (months), first-degree family history of breast cancer (yes vs no), current use of nonsteroidal anti-inflammatory drugs (yes vs no), duration of hormone replacement therapy (HRT) use (years), educational level (elementary-secondary, college or university degree completed), smoking status (former or current smoker vs never smoked), alcohol consumption (drinks/week), and some health problems associated with inflammation or metabolic syndrome (rheumatoid arthritis, osteoarthritis, Crohn's disease, asthma, chronic bronchitis, emphysema, high blood cholesterol, stroke, hypertension, angina, heart attack, or diabetes; yes vs no). Menopausal status (premenopausal vs postmenopausal) was determined as previously described.[26] The level of physical activity performed within the year preceding the diagnosis, expressed as metabolic equivalent (MET)-h/week,[27] was collected using the validated Past Year Total Physical Activity Questionnaire (PYTPAQ). The PYTPAQ has demonstrated a relatively high reliability and validity when compared to accelerometer data.[28]

Assessment of Dietary Intakes

Participants received a self-administered semiquantitative FFQ and were asked to return it on completion. Missing data were collected during the phone interview. This questionnaire is a modified version of the National Cancer Institute Diet History Questionnaire (DHQ-I), which has been validated in American populations in previous studies,[29-31] then adapted and validated for the Canadian population.[32] The DHQ-I is a 124-item food frequency instrument that collects information on diet and supplement use over the past year, including alcohol intake. A subset of questions ascertains seasonal food intake, food type, and the addition of fat. Data from the DHQ-I questionnaire were translated to nutrient intake at the Alberta Health Services—Cancer Care,[32] providing the daily total energy intake (kcal/day), intakes of vitamin A (international unit [IU]), retinol (μg retinol equivalents [RE] from vitamin A and from carotenes), α-carotene (μg), β-carotene (μg), vitamin C (mg), vitamin E (mg α-tocopherol equivalents [ATE]), α-tocopherol (mg),

β-tocopherol (mg), δ-tocopherol (mg), γ-tocopherol (mg), lutein/zeaxanthin (μg), lycopene (μg), and selenium (mg) from diet sources. The total daily intakes for vitamin A, retinol, vitamin C, vitamin E, and zinc were estimated by summing the contribution of all food items in the questionnaire and those of supplements used. To evaluate response consistency between the DHQ-I and the questionnaire filled during the phone interview, responses regarding alcohol intake were compared using Spearman rank correlation for reliability. The correlation coefficient was .89.

Inflammatory Marker Evaluation

Removed tissue from surgery was sent to the pathology department at Hôpital Saint-Sacrement du CHU de Québec-Université Laval where it was treated following standard operating procedures under strict quality control and quality assurance guidelines. Hematoxylin and eosin (H&E)–stained histological sections were prepared from mastectomy blocks. Two pathologists scrolled slides of each woman to identify tissue having histological characteristics of normal tissue located at more than 1 cm from the tumor.[33] H&E-stained slides were used as templates to target 6 cylindrical 1-mm-diameter epithelial tissue cores on the corresponding formalin-fixed paraffin-embedded block. We assumed that 6 cores/woman would be highly representative of the expression in the whole tissue section.[34] Cores were then extracted and randomly arrayed on recipient paraffin blocks using the semi-automated Tissue Puncher (Beecher Instruments Tissue Microarray Technology, Estigen, Sun Prairie, WI). On each tissue microarray (TMA) block, each participant was represented twice and 3 breast cancer cell lines (MCF-7, MDA-231, and SKBR-3) were placed in duplicate to serve as internal controls.

To evaluate inflammation in the breast tissue, 11 markers were selected based on (1) their known involvement in the inflammatory process; (2) their potential involvement in breast tissue inflammation or breast cancer risk, based on the literature; and (3) the commercial availability of validated antibodies. Serial 4-μm-thick sections were cut from each TMA block, and the first and last sections of each block were H&E stained for histological evaluation. Immunoperoxidase staining was performed following conventional immunohistochemistry protocols. Briefly, TMA cut sections were deparaffinized in toluene and rehydrated in serial ethanol solutions. For COX-2, SAA1, STAT3, IL-8, TGF-β, IL-10, and lactoferrin staining, heat-induced epitope retrieval was performed using prewarmed citrate buffer (pH 6.0) for 12 minutes. Slides were incubated in 3% hydrogen peroxide. Sections were then labeled with the following primary antibodies: mouse monoclonal antibody (mAb) raised against IL-6 (Santa Cruz Biotechnology, sc-130326), TNF-α (clone 52B83, Santa Cruz Biotechnology, sc-130326), CRP (clone Y284, Epitomics,

1568-1), COX-2 (clone COX229, Invitrogen, 358200), SAA1 (clone 3C11-2C1, Abgent, AT375a), STAT3 (clone EP2147Y, Epitomics, 2236-1), IL-8 (Proteintech Group, 60141-1-Ig), TGF-β (clone TB21, AbDserotec, MCA797), IL-10 (clone JES3-12G8, AbDserotec, MCA2250), lacto-ferrin (clone EPR4338, Epitomics, 3271-1), and rabbit polyclonal raised against leptin (Ob [A-20], Santa Cruz Biotechnology, sc-842). Immunoreactivity was detected by 3,3′-diaminobenzidine (DAB) solution. Slides were counterstained with hematoxylin. Finally, TMA stained slides were scanned using the Nanozoomer 2.0 RS (Hamamatsu Photonics, Japan) to generate high-resolution images. To ensure quality control, sections were obtained from specifically prepared control TMA blocks, which included normal (tonsils, breast, thymus, colon, and liver), inflammatory (colon), and malignant (breast, colon, liver, lung, ovary, prostate, and kidney) human tissues. Thus, for each staining run and each antibody, appropriate positive and negative control sections, in which primary antibody was substituted by antibody diluent, were included.

The expression level of each inflammatory marker was visually assessed by one reader blinded to all women's information using the semiquantitative quick score method validated for the estrogen receptor in breast carcinomas.[35] Briefly, tissue sections were classified as positive according to staining intensity (0 = none, 1 = mild, 2 = medium, 3 = high) and the percentage of labeled cells (0 = 0%, 1 = 1% to 9%, 2 = 10% to 50%, 3 = >50%). The quick score was obtained by multiplying the 2 scores. For some women, the quick score could not be determined for all inflammatory markers because the tissue was either damaged, absent, or impossible to interpret in all cores arrayed on the TMA blocks. Each marker was evaluated only in the epithelial component, except for COX-2, which was also evaluated in the stromal component.[36] The reproducibility of the analysis was assessed on 5 randomly selected TMA slides that were reevaluated by 2 readers. The intraobserver κ was 0.75 (95% confidence interval [CI] = 0.64-0.86), and the interobserver κ was 0.74 (95% CI = 0.63-0.84). Furthermore, concordance between the expression of inflammatory markers in TMA cores and a section of the corresponding whole tissue were assessed on 10 randomly selected women. We observed a concordance of 81.5% (70% to 100%) with a κ of 0.62 (95% CI = 0.45-0.78).

Statistical Analysis

Antioxidant intakes were natural log transformed and adjusted for total energy using the residual method.[37] Associations were studied in separate models for antioxidant intakes from food only or in combination with supplements (total). For the analysis of dietary antioxidants only, models were adjusted for any antioxidant (vitamin A, retinol, vitamin C, vitamin E, and/or zinc) supplement use (yes/no). Since similar results were obtained using antioxidant-specific supplement use (yes vs no) or any antioxidant supplement use (yes vs no), models were adjusted for any antioxidant supplement use.

Given the skewed distribution of quick scores, relationships between inflammatory marker expression and antioxidant intakes were explored using the Spearman rank-based correlation. Partial correlation coefficients were obtained after adjusting for covariates potentially associated with at least one inflammatory marker in univariate analyses ($P <$ .10). Given the strong correlations between waist circumference, BMI, and WHR, only waist circumference was included in the models since its correlations with quick scores were stronger in univariate analyses. Considering that menopausal status is a factor affecting the pattern of cytokines,[4,38-42] the correlations were tested in all women combined as well as in pre- and postmenopausal women separately.

All tests were 2-sided, and a P value <.05 was considered statistically significant. Since 12 markers (COX-2 was evaluated in both the epithelial and stromal components) were assessed, we further evaluated statistical significance using the conservative Bonferroni P value (.05/12 = .004) to control the family-wise error rate. All analyses were performed using SAS software (version 9.3; SAS Institute, Inc, Cary, NC).

Results

Characteristics of the 160 women combined and stratified by menopausal status are provided in Table 1. Briefly, the overall mean age was 52.5 years (standard deviation [SD] ±7.8). Mean ages for premenopausal and postmenopausal women were 46.9 years (SD ±5.7) and 58.3 years (SD ±4.9), respectively. Compared to postmenopausal women, premenopausal women had lower mean anthropometric measurements and were more physically active. In addition, they were more likely to have breastfed (39.5% vs 36.7%), have postsecondary education (67.9% vs 53.2%), and take supplemental antioxidants (33.3% vs 29.1%). Conversely, postmenopausal women reported greater mean daily dietary intakes of several antioxidants. They were also more likely to have used HRT (57.0% vs 9.9%), have a first-degree relative with breast cancer (25.3% vs 16.1%), be a former or current smoker (64.6% vs 51.9%), use nonsteroidal anti-inflammatory drugs (8.7% vs 3.7%), and have an inflammatory or metabolic health problem (73.4% vs 33.3%). Total energy intake for all women ranged between 641 and 3686 kcal/day.

Associations between antioxidant intakes and inflammatory markers in the whole cohort are shown in Table 2. Dietary intakes of β-tocopherol, as well as total zinc intakes from food and supplements, negatively correlated with IL-10 expression. These results remained significant after adjusting for multiple comparisons ($r = -0.26$, $P = .003$, and $r = -0.26$, $P = .002$, respectively). A

Table 1. Characteristics of the Study Population.

	All (N = 160)	Premenopausal (n = 81)	Postmenopausal (n = 79)
	Mean ± SD	Mean ± SD	Mean ± SD
Age at surgery (years)	52.5 ± 7.8	46.9 ± 5.7	58.3 ± 4.9
Age at first pregnancy (years)[a]	25.8 ± 4.1	26.1 ± 4.4	25.6 ± 3.8
Age at menarche (years)	12.6 ± 1.5	12.4 ± 1.3	12.8 ± 1.7
Body mass index (kg/m^2)	27.1 ± 5.7	26.4 ± 5.8	27.7 ± 5.6
Waist circumference (cm)	86.9 ± 12.8	83.8 ± 12.3	90.1 ± 12.6
Waist-to-hip ratio	0.81 ± 0.06	0.80 ± 0.06	0.83 ± 0.05
Alcohol intake (drinks/week)	4.4 ± 4.6	4.6 ± 4.3	4.1 ± 5.0
Physical activity (METs-h/week)	117 ± 50	136 ± 47	98 ± 45
Total daily energy intake (kcal)	1696 ± 594	1701 ± 588	1691 ± 604
Vitamin daily intakes from food			
Vitamin A (IU)	12611 ± 9208	11492 ± 8108	13758 ± 10138
Total retinol (µg RE)	2648 ± 1860	2440 ± 1659	2861 ± 2034
Vitamin C (mg)	147 ± 92	137 ± 84	158 ± 98
Vitamin E (mg ATE)	9.0 ± 4.0	9.0 ± 4.1	9.0 ± 3.9
α-Tocopherol (mg)	8.4 ± 3.7	8.4 ± 3.9	8.3 ± 3.6
β-Tocopherol (mg)	0.26 ± 0.13	0.26 ± 0.14	0.27 ± 0.14
δ-Tocopherol (mg)	1.8 ± 1.0	1.7 ± 0.9	2.0 ± 1.1
γ-Tocopherol (mg)	11.3 ± 5.2	11.3 ± 5.4	11.2 ± 4.9
α-Carotene (µg)	1020 ± 901	895 ± 774	1148 ± 1003
β-Carotene (µg)	5606 ± 4782	5065 ± 4036	6161 ± 5411
β-Cryptoxanthin (µg)	196 ± 157	180 ± 140	212 ± 173
Lutein + zeaxanthin (µg)	4592 ± 5275	4474 ± 4254	4712 ± 6175
Lycopene (µg)	7485 ± 5835	7389 ± 5346	7583 ± 6330
Zinc (mg)	9.9 ± 4.8	9.8 ± 4.7	10.0 ± 4.9
Selenium (mg)	84 ± 34	85 ± 34	84 ± 33
	Median (Q1; Q4; n)	Median (Q1; Q4; n)	Median (Q1; Q4; n)
Pro-inflammatory mediator quickscore			
IL-6	2 (0; 6; 155)	3 (1; 6; 79)	2 (0; 3; 76)
TNF-α	2 (0; 6; 156)	3 (1; 6; 79)	1 (0; 3; 77)
CRP	2 (0; 4; 157)	3 (1; 6; 79)	1 (0; 2; 78)
COX-2e	6 (3; 6; 156)	6 (6; 9; 79)	6 (3; 6; 77)
COX-2s	1 (1; 2; 157)	2 (1; 3; 79)	1 (1; 2; 78)
Leptin	6 (2; 6; 155)	6 (4; 9; 79)	6 (1; 6; 76)
SAA1	3 (3; 3; 152)	3 (3; 3; 79)	3 (2; 3; 73)
STAT3	3 (3; 6; 156)	3 (3; 6; 79)	3 (3; 6; 77)
IL-8	3 (3; 3; 157)	3 (3; 3; 79)	3 (3; 3; 78)
Anti-inflammatory mediator quickscore			
IL-10	6 (3; 6; 149)	6 (3; 6; 77)	3 (2; 6; 72)
Lactoferrin	3 (2; 6; 153)	4 (2; 6; 79)	3 (2; 6; 74)
TGF-β	1 (0; 3; 157)	1 (0; 3; 79)	0 (0; 3; 78)
	n (%)	n (%)	n (%)
Parity	116 (72.5)	58 (71.6)	58 (73.4)
Breastfeeding among parous	61 (38.1)	32 (39.5)	29 (36.7)
Oral contraceptive ever used	153 (95.6)	78 (96.3)	75 (94.9)
Hormone replacement therapy ever used	53 (33.1)	8 (9.9)	45 (57.0)
First-degree family history of breast cancer	33 (20.6)	13 (16.1)	20 (25.3)
Former or current smoker	93 (58.1)	42 (51.9)	51 (64.6)
Education level			
Elementary-secondary school completed	63 (39.4)	26 (32.1)	37 (46.8)
College completed	53 (33.1)	32 (39.5)	21 (26.6)
University completed	44 (27.5)	23 (28.4)	21 (26.6)
Current use of NS anti-inflammatory drugs	10 (6.3)	3 (3.7)	7 (8.7)
Health problem[b]	85 (53.1)	27 (33.3)	58 (73.4)
Antioxidant supplement use	50 (31.3)	27 (33.3)	23 (29.1)

Abbreviations: ATE, α-tocopherol equivalent; CRP, C-reactive protein; COX-2e, cyclooxygenase-2 in epithelium; COX-2s, cyclooxygenase-2 in stroma; NS, nonsteroidal; IL-8, interleukin-8; IL-10, interleukin-10; IU, international unit; RE, retinol equivalent; SAA1, serum amyloid A1; STAT3, signal transducer and activator of transcription 3; TGF-β, transforming growth factor-β; TNF-α, tumor necrosis factor-α.

[a]Of 160 women, 116 had been pregnant. At the time of the study, 58 were premenopausal and 58 were postmenopausal.

[b]Affected by at least one of the following health problems associated with inflammation or metabolic syndrome: rheumatoid arthritis, osteoarthritis, Crohn's disease, asthma, chronic bronchitis, emphysema, high blood cholesterol, stroke, hypertension, angina, heart attack, or diabetes.

Table 2. Correlations Between Antioxidant Intakes and Expression of Inflammatory Markers in Normal Breast Tissue Among All the Study Population.

	TNF-α (n = 156), r (P)[a]	CRP (n = 157), r (P)[a]	COX-2e (n = 156), r (P)[a]	COX-2s (n = 157), r (P)[a]	SAA1 (n = 152), r (P)[a]	STAT3 (n = 156), r (P)[a]	IL-10 (n = 149), r (P)[a]	Lactoferrin (n = 153), r (P)[a]	TGF-β (n = 157), r (P)[a]
Dietary vitamin A[b,c]	−0.06 (.45)	−0.05 (.54)	0.01 (.94)	0.17 (.04)	0.01 (.88)	−0.06 (.46)	−0.08 (.35)	−0.07 (.43)	−0.10 (.23)
Total[d] vitamin A[c]	−0.06 (.46)	−0.02 (.83)	0.004 (.96)	0.18 (.04)	0.01 (.92)	−0.07 (.40)	−0.14 (.10)	−0.08 (.33)	−0.10 (.22)
Dietary retinol[b,e]	−0.07 (.43)	−0.05 (.53)	0.005 (.95)	0.18 (.03)	−0.005 (.96)	−0.06 (.46)	−0.08 (.35)	−0.069 (.46)	−0.09 (.29)
Total[d] retinol[e]	−0.07 (.44)	−0.01 (.87)	0.002 (.98)	0.17 (.04)	−0.01 (.94)	−0.07 (.40)	−0.17 (.05)	−0.07 (.39)	−0.09 (.27)
Dietary lycopene[b]	0.05 (.55)	0.0003 (1.00)	0.08 (.36)	0.12 (.17)	0.03 (.69)	−0.05 (.54)	−0.01 (.92)	0.02 (.83)	0.08 (.38)
Dietary vitamin C[b]	0.09 (.30)	−0.02 (.84)	0.09 (.31)	0.09 (.29)	0.02 (.80)	0.03 (.71)	−0.003 (.98)	−0.02 (.78)	0.02 (.82)
Total[d] vitamin C	0.06 (.47)	0.04 (.65)	0.15 (.08)	0.03 (.68)	0.03 (.69)	0.06 (.47)	−0.03 (.77)	0.11 (.21)	0.10 (.23)
Total[d] vitamin E[f]	−0.08 (.34)	0.05 (.52)	−0.03 (.71)	−0.05 (.56)	−0.01 (.93)	−0.002 (.98)	−0.23 (.01)	−0.004 (.96)	−0.01 (.90)
Dietary β-tocopherol[b]	0.02 (.80)	−0.10 (.22)	0.05 (.53)	0.01 (.88)	0.10 (.27)	0.06 (.47)	−0.26 (.003)	0.03 (.69)	−0.12 (.15)
Dietary δ-tocopherol[b]	−0.06 (.46)	−0.09 (.29)	−0.11 (.18)	−0.18 (.03)	−0.01 (.87)	−0.04 (.64)	−0.03 (.74)	0.01 (.87)	−0.07 (.39)
Dietary zinc[b]	0.05 (.57)	−0.03 (.71)	0.19 (.02)	−0.06 (.50)	−0.06 (.46)	0.01 (.89)	−0.10 (.26)	0.005 (.95)	−0.07 (.40)
Total[d] zinc	0.03 (.75)	0.05 (.53)	0.08 (.33)	−0.10 (.24)	−0.06 (.50)	−0.09 (.27)	−0.26 (.002)	−0.11 (.21)	0.11 (.19)
Dietary selenium[b]	−0.01 (.93)	−0.12 (.16)	0.08 (.34)	−0.01 (.95)	−0.05 (.59)	−0.11 (.19)	−0.001 (.99)	−0.12 (.18)	−0.11 (.21)

Abbreviations: *r*, Spearman's coefficient; TNF-α, tumor necrosis factor-α; CRP, C-reactive protein; COX-2e, cyclooxygenase-2 in epithelium; COX-2s, cyclooxygenase-2 in stroma; SAA1, serum amyloid A1; STAT3, signal transducer and activator of transcription 3; IL-10, interleukin-10; TGF-β, transforming growth factor-β.
[a]Correlations adjusted for total daily energy intake (kcal), age (years), menopausal status (premenopausal/postmenopausal), waist circumference (cm), duration of oral contraceptive use (years), duration of breastfeeding (months), duration of hormone replacement therapy use (years), smoking status (former or current/never), health problem associated with inflammation or metabolic syndrome (yes/no), alcohol intake (drinks/week), physical activity (metabolic equivalents of task per week), age at menarche (years), parity, age at first pregnancy (years).
[b]Correlation was further adjusted for antioxidant supplement use (yes/no).
[c]International unit of vitamin A.
[d]From food plus supplements.
[e]Retinol equivalent from vitamin A and β-carotene.
[f]α-Tocopherol equivalent.

few borderline correlations were found with a .05 significance level. Positive correlations were observed between intakes of vitamin A or retinol derived from food or from food plus supplement sources, and the stromal expression of COX-2 (all *r* ≥ 0.17, *P* < .05). Dietary δ-tocopherol intakes negatively correlated with the stromal expression of COX-2 (*r* = −0.18, *P* = .03), while dietary zinc intakes positively correlated with the epithelial expression of COX-2 (*r* = 0.19, *P* = .02). Last, total vitamin E (food and supplements) negatively correlated with IL-10 expression (*r* = −0.23, *P* = .01).

Table 3 shows the associations between antioxidant intakes and inflammatory markers among premenopausal women. Some significant correlations (*P* < .05) were found but none remained significant after Bonferroni correction (*P* < .004). Notably, total intakes of retinol inversely correlated with IL-10 expression (*r* = −0.26, *P* = .04). Also, higher intakes of dietary lycopene correlated with higher expression of TGF-β (*r* = 0.27, *P* = .03). Vitamin C from food and total vitamin C from food plus supplements, and dietary zinc positively correlated with TNF-α expression (*P* = .04, for all). Zinc intakes from food only were positively correlated with CRP (*r* = 0.25, *P* = .05), while total zinc positively correlated with CRP (*r* = 0.29, *P* = .02) and negatively correlated with STAT3 (*r* = −0.29, *P* = .02) and IL-10 (*r* = −0.33, *P* = .01). Finally, we observed that higher

amounts of dietary selenium were correlated with lower expression of SAA1 (*r* = −0.27, *P* = .03).

Associations between antioxidant intakes and inflammatory markers among postmenopausal women are shown in Table 4. Compared to premenopausal women, 2 correlations were significant at the Bonferroni significance level (*P* < .004) among postmenopausal women. Dietary intakes of β-tocopherol negatively correlated with IL-10 expression (*r* = −0.39, *P* = .003) and higher amounts of dietary selenium correlated with lower expression of lactoferrin (*r* = −0.39, *P* = .003). Positive correlations with a .05 significance level included total vitamin A, retinol from food, or total retinol intakes and COX-2 in stroma, lycopene intake and TNF-α, and total vitamin C and lactoferrin. Negative correlations significant at *P* < .05 were also observed for total retinol and TNF-α, dietary zinc and CRP, δ-tocopherol and COX-2 in stroma, and β-tocopherol intakes and TGF-β.

Among all women combined or women stratified by menopausal status, no association was observed between dietary α-carotene, dietary β-carotene, dietary vitamin E, dietary α-tocopherol, dietary γ-tocopherol, dietary β-cryptoxanthin, dietary lutein/zeaxanthin intakes, and any of the assessed inflammatory markers. Similarly, no antioxidants correlated with expression of IL-6, IL-8, or leptin within the breast tissue (data not shown).

Table 3. Correlations Between Antioxidant Intakes and Expression of Inflammatory Markers in Normal Breast Tissue Among Premenopausal Women.

	TNF-α (n = 79), r (P)[a]	CRP (n = 79), r (P)[a]	COX-2e (n = 79), r (P)[a]	COX-2s (n = 79), r (P)[a]	SAA1 (n = 79), r (P)[a]	STAT3 (n = 79), r (P)[a]	IL-10 (n = 77), r (P)[a]	Lactoferrin (n = 79), r (P)[a]	TGF-β (n = 79), r (P)[a]
Dietary vitamin A[b,c]	0.18 (.14)	0.12 (.34)	−0.04 (.75)	0.11 (.37)	0.05 (.70)	0.03 (.83)	−0.17 (.19)	−0.08 (.51)	−0.20 (.11)
Total[d] vitamin A[c]	0.20 (.10)	0.14 (.26)	−0.03 (.79)	0.12 (.34)	0.04 (.73)	0.03 (.79)	−0.23 (.07)	−0.09 (.48)	−0.24 (.06)
Dietary retinol[b,e]	0.17 (.17)	0.13 (.32)	−0.04 (.77)	0.11 (.38)	0.02 (.85)	0.02 (.85)	−0.17 (.17)	−0.08 (.52)	−0.19 (.14)
Total[d] retinol[e]	0.20 (.10)	0.15 (.22)	−0.04 (.73)	0.12 (.34)	0.02 (.87)	0.02 (.89)	−0.26 (.04)	−0.09 (.48)	−0.22 (.07)
Dietary lycopene[b]	−0.05 (.70)	−0.14 (.26)	0.11 (.36)	0.22 (.08)	−0.01 (.92)	0.02 (.90)	−0.05 (.69)	−0.20 (.12)	0.27 (.03)
Dietary vitamin C[b]	0.26 (.04)	0.06 (.66)	0.16 (.21)	−0.03 (.83)	−0.01 (.91)	−0.02 (.87)	−0.04 (.74)	−0.19 (.13)	−0.05 (.70)
Total[d] vitamin C	0.25 (.04)	0.15 (.22)	0.14 (.27)	0.14 (.27)	−0.03 (.79)	−0.05 (.67)	−0.09 (.46)	−0.20 (.12)	0.08 (.52)
Total[d] vitamin E[f]	−0.05 (.71)	0.12 (.34)	−0.08 (.52)	−0.13 (.31)	−0.04 (.78)	0.11 (.40)	−0.20 (.11)	−0.10 (.42)	0.07 (.59)
Dietary β-tocopherol[b]	0.10 (.43)	−0.17 (.16)	0.07 (.56)	0.07 (.60)	0.05 (.67)	0.10 (.43)	−0.16 (.21)	−0.03 (.80)	−0.05 (.69)
Dietary δ-tocopherol[b]	−0.02 (.90)	−0.03 (.79)	−0.17 (.18)	−0.09 (.50)	−0.14 (.28)	0.14 (.27)	0.08 (.55)	0.0002 (1.00)	−0.05 (.69)
Dietary zinc[b]	0.25 (.04)	0.25 (.05)	0.16 (.21)	0.14 (.27)	−0.18 (.14)	−0.13 (.31)	−0.07 (.58)	0.02 (.88)	−0.09 (.45)
Total[d] zinc	0.19 (.13)	0.29 (.02)	−0.06 (.61)	0.06 (.65)	−0.07 (.56)	−0.29 (.02)	−0.33 (.01)	−0.18 (.15)	0.09 (.47)
Dietary selenium[b]	0.07 (.57)	0.03 (.84)	0.02 (.86)	0.14 (.25)	−0.27 (.03)	−0.16 (.20)	0.03 (.81)	−0.14 (.26)	−0.18 (.14)

Abbreviations: r, Spearman's coefficient; TNF-α, tumor necrosis factor-α; CRP, C-reactive protein; COX-2e, cyclooxygenase-2 in epithelium; COX-2s, cyclooxygenase-2 in stroma; SAA1, serum amyloid A1; STAT3, signal transducer and activator of transcription 3; IL-8, interleukin-8, IL-10, interleukin-10; TGF-β, transforming growth factor-β.
[a]Correlations adjusted for total daily energy intake (kcal), age (years), waist circumference (cm), duration of oral contraceptive use (years), duration of breastfeeding (months), smoking status (former or current), health problem associated with inflammation or metabolic syndrome (yes/no), alcohol consumption (drinks/week), physical activity (metabolic equivalents of task per week), age at menarche (years), parity, age at first pregnancy (years, among parous women).
[b]Correlation was further adjusted for antioxidant supplement use (yes/no).
[c]International unit of vitamin A.
[d]From food plus supplements.
[e]Retinol equivalent from vitamin A and β-carotene.
[f]α-Tocopherol equivalent.

Discussion

In this study, we found that dietary β-tocopherol intakes negatively correlated with the expression of IL-10 in the whole study population and among postmenopausal women. Furthermore, total zinc derived from food and supplements also negatively correlated with this marker in the whole cohort. These results remained significant even after applying a much more conservative significance level to account for multiple comparisons. Our findings support the idea that some antioxidant intakes whether derived from the diet alone or in combination with supplements are associated with the expression of certain inflammatory markers. Furthermore, results suggest that these relationships may be limited to postmenopausal women. To our knowledge, this is the first study to evaluate the intake of various antioxidants in relation to the protein expression of numerous inflammatory markers within the normal breast tissue of women with breast cancer.

No study has reported negative correlations with intakes of β-tocopherol or total zinc and IL-10 expression in blood or tissues. Here, we observed that higher intakes of β-tocopherol and total zinc were associated with lower IL-10 expression levels in the breast tissue. This observation is quite surprising because IL-10 is known primarily as an anti-inflammatory cytokine that inhibits pro-inflammatory cytokine production.[43] Like several other cytokines, IL-10 can exert both dual proliferative and inhibitory effects

on breast tumor cells.[43] These paradoxical effects suggest a much more intricate role for IL-10 in breast cancer initiation and progression.[43] The dual role proposed here, however, is specific to cancer tissue, which is not evaluated in this study. Regarding normal breast tissue, a case-control study showed that expression of cytokine IL-10, in the adipose tissue adjacent to the breast tumor, was higher in breast cancer cases compared to controls.[25] Therefore, the low IL-10 expression that we observed in the normal mammary tissue of women with high intakes of β-tocopherol and zinc is compatible with this lower breast cancer risk. In our cohort, we observed that globally, the expression level of IL-10 in breast tissue was higher in postmenopausal than in premenopausal women, which agrees with studies reporting an increase in IL-10 levels in the blood after menopause indicating that hormonal status could influence IL-10 expression.[44,45] The biology underpinning a possible association between higher intakes of the specific β-tocopherol isoform and lower IL-10 expression in histologically normal breast tissue is unclear and controlled experiments must be conducted to confirm a causal relationship. Regarding zinc, ex vivo studies have shown that zinc treatment decreases the generation of IL-10 in mononuclear cells.[46,47] Further studies are required to confirm a similar effect in mammary epithelial cells.

We also observed that higher amounts of selenium were correlated with lower expression of lactoferrin but only

Table 4. Correlations Between Antioxidant Intakes and Expression of Inflammatory Markers in Normal Breast Tissue Among Postmenopausal Women.

	TNF-α (n = 77), r (P)[a]	CRP (n = 78), r (P)[a]	COX-2e (n = 77), r (P)[a]	COX-2s (n = 78), r (P)[a]	SAA1 (n = 73), r (P)[a]	STAT3 (n = 77), r (P)[a]	IL-10 (n = 72), r (P)[a]	Lactoferrin (n = 74), r (P)[a]	TGF-β (n = 78), r (P)[a]
Dietary vitamin A[b,c]	−0.23 (.07)	−0.14 (.26)	0.01 (.96)	0.23 (.07)	−0.004 (.97)	−0.10 (.42)	−0.10 (.48)	−0.09 (.52)	0.05 (.69)
Total[d] vitamin A[c]	−0.24 (.06)	−0.11 (.39)	−0.001 (.99)	0.27 (.03)	0.02 (.90)	−0.11 (.40)	−0.12 (.39)	−0.11 (.39)	0.04 (.78)
Dietary retinol[b,e]	−0.23 (.07)	−0.15 (.25)	−0.001(1.00)	0.25 (.05)	−0.01 (.92)	−0.10 (.44)	−0.09 (.49)	−0.07 (.58)	0.06 (.62)
Total[d] retinol[e]	−0.26 (.04)	−0.11 (.38)	−0.01 (.95)	0.28 (.02)	0.01 (.95)	−0.10 (.45)	−0.14 (.30)	−0.09 (.48)	0.04 (.74)
Dietary lycopene[b]	0.26 (.04)	0.18 (.17)	0.08 (.55)	0.05 (.68)	0.06 (.66)	−0.12 (.37)	0.05 (.72)	0.22 (.09)	−0.06 (.63)
Dietary vitamin C[b]	−0.04 (.75)	−0.02 (.89)	0.05 (.68)	0.13 (.31)	−0.04 (.77)	−0.0005(1.00)	0.06 (.63)	0.10 (.47)	0.17 (.19)
Total[d] vitamin C	−0.12 (.34)	−0.04 (.77)	0.15 (.25)	0.10 (.45)	0.01 (.91)	0.10 (.43)	−0.01 (.93)	0.29 (.03)	0.15 (.24)
Total[d] vitamin E[f]	−0.11 (.40)	0.03 (.82)	0.03 (.79)	0.06 (.65)	−0.03 (.84)	−0.10 (.42)	−0.24 (.07)	0.01 (.97)	−0.09 (.50)
Dietary β-tocopherol[b]	−0.04 (.76)	−0.10 (.43)	0.05 (.68)	−0.002 (.99)	0.12 (.39)	0.01 (.94)	−0.39 (.003)	−0.10 (.47)	−0.27 (.04)
Dietary δ-tocopherol[b]	−0.04 (.76)	−0.13 (.32)	0.07 (.57)	−0.26 (.04)	0.09 (.52)	−0.17 (.18)	−0.19 (.16)	−0.08 (.57)	−0.13 (.32)
Dietary zinc[b]	−0.04 (.75)	−0.30 (.02)	0.24 (.07)	−0.12 (.37)	0.03 (.84)	0.14 (.28)	−0.15 (.28)	−0.21 (.11)	0.02 (.91)
Total[d] zinc	−0.15 (.26)	−0.22 (.08)	0.19 (.14)	−0.18 (.16)	−0.09 (.52)	0.04 (.77)	−0.19 (.15)	−0.18 (.17)	0.16 (.22)
Dietary selenium[b]	0.02 (.85)	−0.23 (.07)	0.20 (.13)	−0.18 (.17)	0.12 (.36)	−0.12 (.37)	−0.10 (.48)	−0.39 (.003)	−0.06 (.66)

Abbreviations: r, Spearman's coefficient; TNF-α, tumor necrosis factor-α; CRP, C-reactive protein; COX-2e, cyclooxygenase-2 in epithelium; COX-2s, cyclooxygenase-2 in stroma; SAA1, serum amyloid A1; STAT3, signal transducer and activator of transcription 3; IL-8, interleukin-8, IL-10, interleukin-10; TGF-β, transforming growth factor-β.
[a]Correlations adjusted for total daily energy intake (kcal), age (years), waist circumference (cm), duration of oral contraceptive use (years), duration of breastfeeding (months), duration of hormone replacement therapy, smoking status (former or current), health problem associated with inflammation or metabolic syndrome (yes/no), alcohol consumption (drinks/week), physical activity (metabolic equivalents of task per week), age at menarche (years), parity, age at first pregnancy (years, among parous women).
[b]Correlation was further adjusted for antioxidant supplement use (yes/no).
[c]International unit of vitamin A.
[d]From food plus supplements.
[e]Retinol equivalent from vitamin A and β-carotene.
[f]α-Tocopherol equivalent.

among postmenopausal women. Selenium is commonly referred to as an antioxidant because it is required for the activity of selenoproteins, such as glutathione peroxidase, that play an important role in biological processes such as adaptive and innate immune responses, and antioxidant defense.[48,49] Lactoferrin is a natural forming iron-binding glycoprotein with antibacterial, antioxidant, and anticarcinogenic effects.[50-53] It is possible that if oxidative stress is well controlled by fully active selenoproteins, the contribution of lactoferrin in the regulation of oxidative stress is not required. In line with this idea, it is known that measures of iron stores increase from premenopause to postmenopause,[54] and it has been reported that high level of iron may have negative effects on selenium level in breastfed infants.[55] Therefore, high intakes of selenium in postmenopausal women could help maintain high levels of glutathione peroxidase activity, which in turn could help keep a balance between the oxidant/antioxidant systems within the breast tissue. However, this hypothesis must be further explored. For example, the correlation between selenium intakes and the level of oxidative stress in the breast tissue could be measured or carried out in a controlled experiment in animals.

The fact that higher vitamin A and retinol intakes tended to correlate with COX-2 expression in mammary stromal cells in postmenopausal women is interesting. Indeed, *cis*-retinoid acid was found to activate transcription of the

COX-2 gene in a cell culture model,[56] suggesting that retinoids could exert their effects in breast tissue through the activation of retinoic acid receptors. However, other regulatory mechanisms likely interact to modulate the expression of COX-2 in mammary stromal cells and could explain the different correlations observed for premenopausal and postmenopausal women.

Our study has several strengths. The measurement of vitamin A, vitamin C, vitamin E, zinc, and selenium mean daily intakes from dietary sources were consistent with the global daily mean intake calculated by Shivappa and colleagues from a world composite database.[57] Furthermore, studying the protein expression of several inflammatory markers in situ has the advantage of indicating local mechanisms by which the antioxidant is more likely to act on in the breast tissue. Unlike previous studies that have measured these markers in the blood,[17-22] our assessments may better reflect the local breast microenvironment since measures are directly obtained from the breast source. However, with these types of analyses, the risk of statistical multiplicity increases, which increases the likelihood of rare events. Given that our key findings remained statistically significant following Bonferroni correction suggests that our results are likely not due to chance.

This study has some limitations that must be considered when interpreting the results. The cross-sectional design used in the study and the use of correlation coefficients precludes

making causal inferences. However, reverse causation is unlikely because inflammation in the breast tissue should not influence dietary habits. Seventy-four percent of the approached women accepted to participate in this study. This is a good participation rate considering that patients were recently diagnosed. We believe that selection bias is unlikely because there is no a priori knowledge explaining that refusal to participate could be linked to dietary habits or breast tissue inflammation. Nonetheless, the prevalence of breast cancer risk factors in the study cohort may have been higher than the general population, thus limiting generalization of results. However, many characteristics of our sample, such as age, smoking status, BMI, total daily energy intake, were similar to those reported in a previous study on a group of healthy women of the Quebec region,[58] which reduces concerns about external validity.

Furthermore, although the analyses were performed on morphologically normal breast tissue, as determined by two experienced pathologists, the fact that normal tissue specimens were located near a breast cancer can raise concerns about field effects occurring in the tumor microenvironment. However, histological characteristics, gene expression profile, and expression of estrogen and progesterone receptors in biopsies taken near a breast tumor have been found to be similar to normal breast tissue (eg, tissue from breast reduction).[33,59] Moreover, to address this issue, we assessed the expression of 4 inflammatory markers (CRP, COX-2, leptin, and SAA1) across both breasts of 7 women having had a bilateral mastectomy for breast cancer in one breast and prophylactic mastectomy or benign breast lesion in the other breast. We observed an excellent concordance of 82% (71% to 100%) for the bilateral expression of inflammatory markers. Immunohistochemical analysis performed on tissue microarrays could also potentially introduce information bias since the expression levels of inflammatory markers are not always homogeneously expressed within a tissue. However, since a high concordance was observed between quick scores obtained from TMA cores and those of the corresponding whole tissue section, the risk of misclassification is minimal.

Misclassification may also have occurred with respect to the accuracy of determining antioxidant intakes from supplement sources. To ascertain antioxidant intakes from multivitamins, the diet history questionnaire (DHQ) was translated based on the formulation of most common multivitamin brands, such as regular Centrum and One-A-Day, but it does not discriminate between different formulations of multivitamins that target most specific populations. Notably, no selenium supplement intake was measured despite the fact that selenium is included in many formulations of multivitamins. This information bias may have mitigated our correlations between total antioxidant intakes and expression of inflammatory markers in the breast tissue. Another important limitation inherent to the DHQ is the fact that it assesses dietary habits over the past year. This type of instrument is subject to recall bias because it appeals to the memory. However, in our sample, all women who completed the DHQ were in a comparable situation and recovering from breast surgery for a recently diagnosed breast cancer. Therefore, their concerns about their diet should have been similar. Filling out a food frequency questionnaire also has the disadvantage of requiring the ability to properly conceptualize food. The accuracy of the measure depends on the list of items included in the questionnaire and some answers can be influenced by social desirability. Although the DHQ used in this study was validated for the Canadian population,[32] all these aforementioned factors could have potentially contributed to reducing the precision of antioxidant measurements and may have reduced the strength of the correlations found.

It is important to note that our sample size was small, which restricted the number of variables included in the models to those potentially associated with at least one inflammatory marker in univariate analyses when the *P* value was below .10. Although several variables associated with breast cancer risk and inflammation were included in the final models, residual confounding may still have occurred. Moreover, if variables were related to others not included in the analyses observed correlations could be misleading. Study size may also have contributed to insignificant results. For instance, we observed that correlations between antioxidant intakes and inflammatory marker expression in breast tissue differed according to menopausal status. However, stratified analyses may have been underpowered to detect significant associations.

Conclusions

In conclusion, this study presents an overall view of possible relations that could bind general antioxidant intake habits and the expression of various inflammatory markers within the breast tissue. Thus, potential links could exist between β-tocopherol or zinc intakes and expression of IL-10 and between selenium intakes and lactoferrin expression, particularly among postmenopausal women. These results suggest that antioxidant compounds may exert biological effects on breast tissue by mechanisms affecting the expression of inflammatory markers. The development of experimental studies where antioxidant intakes will be controlled is proposed to further elucidate the mechanisms involved in the relation between antioxidants and their impacts on breast tissue inflammation.

Declaration of Conflicting Interests

Funding

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