ORIGINAL RESEARCH

Nutritional Epidemiology and Public Health

CURRENT DEVELOPMENTS IN NUTRITION

A Prospective Analysis of Plasma Phospholipid Fatty Acids and Breast Cancer Risk in 2 Provinces in Canada

Marnie Newell,[1] Sunita Ghosh,[2] Susan Goruk,[1] Mohammedreza Pakseresht,[1] Jennifer E Vena,[1,3] Trevor JB Dummer,[4,5] and Catherine J Field[1]

[1]Department of Agricultural, Food, and Nutritional Science, University of Alberta, Edmonton, Canada; [2]Department of Medical Oncology, University of Alberta, Edmonton, Canada; [3]Alberta's Tomorrow Project, CancerCare Alberta, Alberta Health Services, Calgary, Canada; [4]School of Population and Public Health, University of British Columbia, Vancouver, Canada; and [5]Cancer Control Research, BC Cancer, Vancouver, Canada

ABSTRACT

Background: Studies suggest that fatty acid status influences breast cancer etiology, yet the roles of individual fatty acids in breast cancer risk are unclear, specifically when central adiposity and menopausal status are considered.

Objectives: This study examined the associations of fatty acid status with breast cancer risk including location, menopausal status, and waist-to-hip ratio as key variables.

Methods: Prediagnostic plasma phospholipid fatty acids were measured in women with breast cancer ($n = 393$) and age-matched controls ($n = 786$) from a nested case-control prospective study within Alberta's Tomorrow Project (ATP) and British Columbia Generations Project (BCGP) cohorts. Binary logistic regression models were used to evaluate associations of fatty acids and breast cancer risk with subgroup analysis for menopausal status and waist-to-hip ratio.

Results: Women from BCGP had a higher n–3 (ω-3) fatty acid status compared with the ATP (6.4% ± 0.08% vs. 5.3% ± 0.06%; $P < 0.001$), so subsequent analysis was blocked by cohort. Overall, fatty acids had inconsistent associations with risk. In the ATP among premenopausal women, total long-chain n–3 fatty acids ($OR_{Q4vsQ1} = 1.78$; 95% CI: 0.58, 5.43; P-trend = 0.007, P-interaction = 0.07) were positively associated with breast cancer risk, whereas in BCGP, DHA ($OR_{Q4vsQ1} = 0.66$; 95% CI: 0.28, 1.53; P-trend = 0.03, P-interaction = 0.05) and total long-chain n–3 fatty acids ($OR_{Q4vsQ1} = 0.66$; 95% CI: 0.28, 1.54; P-trend = 0.03) were associated with decreased cancer risk when the waist-to-hip ratio was <0.85.

Conclusions: Our findings suggest that regional variations in fatty acid status influence breast cancer risk, resulting in positive associations of total long-chain n–3 fatty acids in premenopausal ATP women and negative associations of these fatty acids in BCGP women with a waist-to-hip ratio below guidelines. This study highlights the complexity and difficulty in using fatty acid status to predict breast cancer risk in diverse populations without the consideration of other risk factors. *Curr Dev Nutr* 2021;5:nzab022.

Keywords: ATP, BCGP, biomarkers, breast cancer, risk, fatty acids

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Supplemental Tables 1–11 are 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/cdn/.

Address correspondence to CJF (e-mail: catherine.field@ualberta.ca).

Abbreviations used: AA, arachidonic acid; ALA, α-linolenic acid; ATP, Alberta's Tomorrow Project; BCGP, British Columbia Generations Project; DI_{18}, desaturase index (ratio of product to substrate, 18:1n-9:18:0); ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; LC, long-chain; SCD, stearoyl-CoA desaturase.

Introduction

Globally, breast cancer is the most frequently diagnosed female cancer and the leading cause of cancer-related death in women (1). In Canada, preventable dietary and lifestyle factors, including excess body weight, are estimated to contribute to ∼8% of all cancers (2), yet globally, epidemiological evidence linking dietary fat intake to breast cancer risk is inconsistent (3–10) and does not address complete fatty acid status. Thus, measurement of plasma phospholipids could be a more reliable biomarker and indicator of fatty acid status than dietary assessment (11). As phospholipids are primarily synthesized within the body, they provide a quantitative measurement of the composition of fats that are bioavailable, which overcomes the potential limitations of food-frequency and dietary-assessment questionnaires. Plasma phospholipid analysis is a reasonable measure of long-chain (LC) PUFAs. They are reflective of the sum of processes including dietary intake and fatty acid synthesis and utilization. Dietary intake studies have suggested that LC n–3 PUFAs, including EPA and DHA, could be protective, although the

data are conflicting (3, 12). Fatty acids are integral components of cellular processes involved in many cancer hallmarks, including cellular proliferation, apoptosis, cell growth, and metastasis (13, 14). In in vitro and in vivo models, EPA and DHA have demonstrated an anticancer effect (15); therefore, it is plausible that they could have a role in prevention as well.

To date, several epidemiological studies have explored circulating fatty acids as biomarkers of breast cancer risk with conflicting findings (16–37), but most have not considered geographic variations in dietary intake, body fatness, or the influence of menopause on fatty acid composition. In this unique nested case-control study from a Canadian population, we studied 2 regionally distinct populations—the Alberta's Tomorrow Project (ATP) and British Columbia Generations Project (BCGP) cohorts—to examine the associations of fatty acid status with breast cancer risk, including location, menopausal status, and waist-to-hip ratio as key variables.

Methods

Study population

The ATP and BCGP are prospective cohort studies and part of the larger CanPath (Canadian Partnership for Tomorrow's Health, country-wide prospective cohort), created to investigate lifestyle, diet, environmental, and genetic influences on risk of chronic diseases including cancer (38). Detailed methodologies for the study design and recruitment for both cohorts have been previously published (39, 40). In brief, participants in both cohorts were between the ages of 35 and 69 y; provided a health and lifestyle questionnaire, physical measurements, and biosamples; and consented to data linkage (including cancer registry). A total of 31,072 participants in ATP and 29,796 participants in BCGP were recruited between the years 2001 and 2015 (ATP) and 2009 and 2016 (BCGP). Nonfasted blood samples were collected prediagnosis and therefore not subjected to any potential data collection biases following standard protocols, separated into blood components (RBCs, serum, plasma, and buffy coat) and stored either in liquid nitrogen or in a −80°C freezer (39, 41). Ethical approval for the ATP for recruitment and data collection was obtained by the former Alberta Cancer Board Research Ethics Committee and the University of Calgary Conjoint Health Research Ethics Board. Ethical approval for the BCGP for data collection and recruitment was obtained by the University of British Columbia–British Columbia Cancer Agency Research Ethics Board.

Nested cohort

Breast cancer cases in women that occurred from time of blood sample collection (respective study inclusion date) to 31 December, 2019 were identified through linkage to the Alberta Cancer Registry and the British Columbia Cancer Registry. From these 2 cohorts, 393 females with a breast cancer diagnosis and age-matched control women ($n = 786$, matched 2:1 with cancer cases) with no cancer (as of 31 December, 2019) were identified. The current analysis includes 614 women from the ATP (203 cases and 411 controls) and 514 women from the BCGP (174 cases and 340 controls). Fifty-one samples were excluded from the analysis due to insufficient sample for phospholipid analysis or sample degradation prior to arrival in the laboratory. **Figure 1** depicts the flow diagram of final sample selection for fatty acid analysis. Descriptive information on breast cancer subsets and hormone receptor status of the included population is provided in **Supplemental Tables 1** and **2**. Ethical approval for the current study was provided by the Health Research Ethics Board of Alberta Cancer Committee (HREBA.CC-17-0344).

Dietary intake

In ATP, dietary intake over 1 y was estimated using the validated Canadian Diet History Questionnaire, as described earlier (40). Briefly, this questionnaire was modified from the US National Cancer Institute's Diet History Questionnaire, which contains intake questions on 124 food items and portion sizes. Estimated nutrient intake was determined using the Canadian Nutrient File (42). Dietary intake and supplement use from this FFQ were available for 256 subjects from ATP (70 cases and 186 controls) and were used to determine if the plasma phospholipid n–3 status related to estimated dietary intake. Unfortunately, there were no dietary data collected for the BCGP.

Plasma phospholipid analysis

Cases and controls from both cohorts were processed within the same batch, and laboratory personnel were blinded to participant information. To determine fatty acid concentration, 10 μg C15 phosphatidylcholine internal standard (Nuchek Prep, Inc.) was added to 200 μL plasma and phospholipids were extracted using a Folch method as previously described (43, 44). Briefly, lipids were extracted from the plasma sample and total phospholipids were separated by spotting the samples on a heat-activated silica gel "G" TLC plate (Analtech) and developing plate in a chamber with solvent containing 80:20:1 petroleum ether:diethyl ether:acetic acid. Methyl ester bands were prepared by a mixture of BF_3(boron trifluoride) and hexane at 100°C. Total phospholipid fatty acids were separated by automated GLC 7890A (Agilent Technologies) on a CP-Sil 88 column (100 m × 0.25 mm; Agilent) (45). To control for variations between batches of samples, control measures were used in addition to the internal standard (concentration = 20 μg/mL), and individual GC peaks were identified and validated against phospholipid standards (GLC-502 and GLC-643) from NuChek Prep, Inc., which were run for each batch to verify retention time and quantification for each individual fatty acid.

Statistical analyses

Means and SDs are reported for the continuous variables and frequencies and proportions are used to describe the categorical variables. To assess characteristics of the study population between cases and controls, independent *t* tests for continuous variables and chi-square tests for categorical variables were used. Missing values were excluded from calculations. ORs and their 95% CIs were determined using binary logistic regression models to evaluate the association between the outcome variable (cases vs. controls) breast cancer risk and fatty acid status (both relative % and concentration). Cutoff points for quartile analysis of plasma fatty acids were calculated for individual cohorts based on distribution of plasma concentrations in control women. The quartile cutoff points for each cohort are provided in **Supplemental Table 3**.

There are known associations between demographic and lifestyle factors with breast cancer and to account for this, multiple covariates, identified a priori, were adjusted for in the effort to reduce potential confounding. Variables included in the adjusted models were as follows:

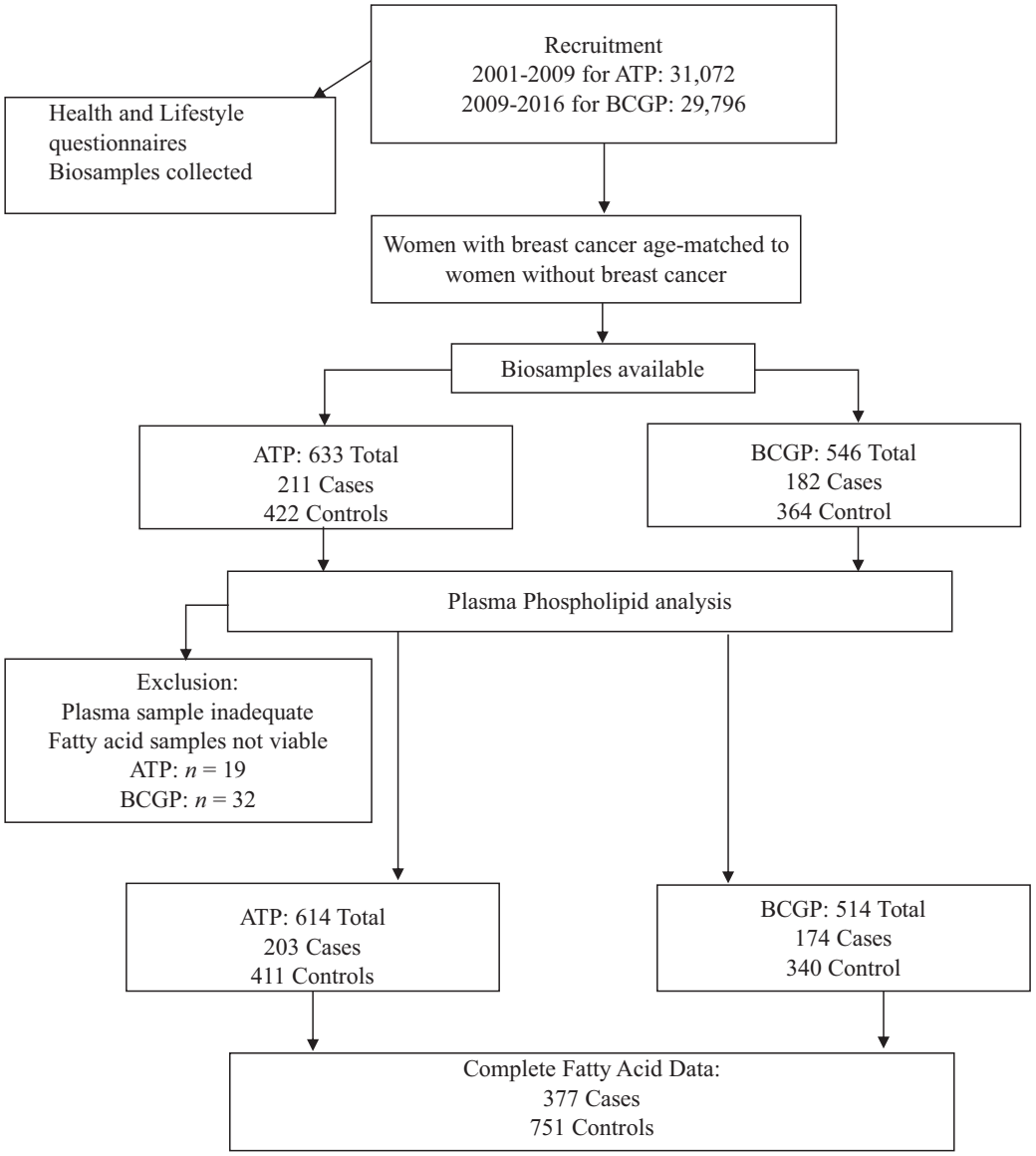

FIGURE 1 Flow diagram of final sample selection for fatty acid analysis from ATP and BCGP

age, BMI (kg/m^2; continuous variable), height (continuous variable), alcohol intake [never, infrequent (≤1–4/mo), and frequent (>2/wk)], combination of age at first birth and parity (nulliparous; first birth before 30 y with 1–2 children; first birth before 30 y with >3 children; first birth after 30 y), age at menopause, menopausal hormone use (never and ever), family history of breast cancer, education (≤high school, some postsecondary, undergraduate degree, or advanced degree), ethnicity (white or nonwhite), and age at menarche (<11 or >11 y). Means of fatty acids within each quartile were used to test for trends.

To determine if differences existed for fatty acid status in conjunction with other defined parameters, models were stratified according to BMI (healthy, overweight, obese) or menopausal status at baseline (pre or post), and subanalyses were conducted. Statistical tests were 2-sided. SPSS version 25 (released 2017, IBM SPSS Statistics for Windows, version 25.0; IBM Corporation) was used for all statistical analyses.

Results

Overall, the majority of anthropometric and demographic characteristics at time of plasma ascertainment were similar between cases and controls when both ATP and BCGP cohorts were considered together with the following exceptions: cases had higher waist-to-hip ratios, higher alcohol consumption, had longer oral contraceptive use, more first-degree relatives with breast cancer, and lower estimated mean total physical activity (**Supplemental Table 4**; $P < 0.04$). However, anthropometric and demographic characteristics were different between the ATP and BCGP cohorts, including living area, BMI distribution, waist-to-hip ratio, marital status, education, ethnicity, alcohol use, hysterectomy, gravidity, age at first pregnancy, oral contraceptive use, family history of cancer, and number of first-degree relatives with breast cancer (**Table 1**). The mean time between sample collection and breast cancer diagnoses

TABLE 1 Descriptive statistics for the ATP and BCGP participants[1]

		ATP, *n* (%)		BCGP, *n* (%)	
	n	Cases (*n* = 203)	Controls (*n* = 411)	Cases (*n* = 174)	Controls (*n* = 340)
Age, y	1128		(%)		(%)
35–49		34 (17)	67 (16)	29 (17)	73 (21)
50–59		69 (34)	131 (32)	54 (31)	94 (28)
60–80		100 (49)	213 (52)	91 (52)	173 (51)
Living area	976				
Rural		25 (12)	70 (17)	33 (24)	30 (13)
Urban		178 (88)	341 (83)	106 (76)	193 (87)
BMI (kg/m^2)	1075				
Underweight (<18.5)		0 (0)	3 (1)	1 (1)	3 (1)
Healthy weight (18.5 to <25)		74 (37)	141 (34)	76 (48)	150 (49)
Overweight (25 to <30)		61 (30)	130 (32)	44 (28)	87 (28)
Obese class 1 (30 to <35)		50 (25)	93 (23)	22 (14)	44 (14)
Obese class 2 (35 to <40)		9 (4)	25 (6)	9 (6)	14 (4)
Obese class 3 (≥40)		7 (3)	18 (4)	5 (3)	9 (3)
Waist-to-hip ratio[2]	1062				
Below guidelines		80 (41)	166 (42)	71 (46)	188 (60)
Above guidelines		117 (59)	230 (58)	85 (54)	125 (40)
Marital status	1124				
Married or cohabitating		139 (68)	305 (74)	122 (71)	205 (60)
Divorced, separated, widowed		48 (24)	77 (19)	35 (20)	84 (25)
Single, never married		16 (8)	29 (7)	14 (8)	50 (15)
Education	1124				
Elementary school		4 (2)	9 (2)	3 (2)	6 (2)
High school		53 (26)	90 (22)	34 (20)	60 (16)
Trade or vocational school		18 (9)	51 (12)	17 (10)	31 (8)
Diploma (community college, pre-university)		54 (26)	101 (24)	43 (25)	73 (20)
University certificate below Bachelor's level		7 (3)	31 (8)	7 (4)	20 (5)
Bachelor's degree		47 (23)	95 (23)	36 (21)	79 (21)
Graduate degree		20 (10)	34 (8)	31 (18)	70 (19)
Income level, $	1089				
<50,000		58 (29)	118 (29)	42 (27)	96 (30)
50,000–99,999		73 (36)	140 (34)	63 (40)	133 (41)
>100,000		70 (35)	150 (37)	52 (33)	92 (29)
Ethnicity–White	1114				
Yes		193 (96)	391 (96)	152 (89)	294 (87)
No		7 (4)	15 (4)	19 (11)	44 (13)
Smoking status	1121				
Never		103 (52)	219 (53)	96 (56)	169 (50)
Former		83 (41)	171 (42)	69 (40)	154 (46)
Current		14 (7)	21 (5)	6 (4)	13 (4)
Alcohol	1121				
Never		4 (2)	21 (5)	3 (2)	18 (5)
≥1/mo		73 (36)	149 (36)	46 (27)	96 (28)
2–4/mo		43 (21)	111 (27)	39 (23)	66 (20)
2–3/wk		50 (25)	66 (16)	30 (18)	64 (19)
>4/wk		33 (16)	63 (15)	53 (31)	93 (28)
Menopausal status	1119				
Premenopausal		59 (29)	97 (24)	41 (24)	96 (28)
Postmenopausal		142 (71)	313 (76)	130 (76)	241 (72)
Age at menopause, y	792				
≤44		39 (28)	76 (25)	21 (17)	50 (22)
45–49		26 (19)	51 (17)	31 (25)	49 (22)
≥50		74 (53)	174 (58)	73 (58)	128 (56)
Mammogram	1121				
Never		12 (6)	20 (5)	7 (4)	14 (4)
<6 mo		51 (25)	121 (30)	45 (27)	95 (28)
6 mo to <1 y		71 (35)	125 (30)	61 (36)	109 (32)

(*Continued*)

TABLE 1 (*Continued*)

		ATP, *n* (%)		BCGP, *n* (%)	
	n	Cases (*n* = 203)	Controls (*n* = 411)	Cases (*n* = 174)	Controls (*n* = 340)
1–2 y ago		46 (23)	99 (24)	42 (25)	86 (25)
>2 to 3 y ago		12 (6)	29 (7)	4 (24)	20 (6)
>3 y ago		11 (5)	16 (4)	10 (6)	14 (4)
Hysterectomy	1124				
None		151 (74)	302 (73)	132 (78)	284 (84)
Simple		52 (26)	109 (27)	38 (22)	56 (16)
Total or bilateral oopherectomy	1115				
Yes		32 (16)	60 (15)	24 (14)	38 (11)
No		169 (84)	350 (85)	146 (86)	296 (89)
Age at menarche, y	1090				
<11		15 (8)	23 (6)	7 (4)	21 (6)
11		19 (10)	42 (10)	22 (13)	28 (9)
12		61 (31)	133 (33)	45 (27)	91 (28)
13		56 (28)	110 (27)	53 (31)	104 (32)
≥14		46 (23)	95 (24)	39 (23)	80 (25)
Gravidity	1121				
0		27 (13)	57 (14)	32 (19)	75 (22)
1		18 (9)	34 (8)	24 (14)	46 (14)
2–3		105 (52)	215 (52)	87 (51)	167 (49)
>3		53 (26)	103 (25)	28 (16)	50 (15)
Age at first pregnancy	1120				
Not applicable		27 (13)	57 (14)	32 (19)	75 (22)
<21 y		46 (23)	92 (22)	38 (22)	67 (20)
21–25 y		54 (27)	122 (30)	32 (19)	66 (20)
25-30 y		41 (20)	86 (21)	36 (21)	69 (20)
>30 y		35 (17)	53 (13)	33 (19)	59 (18)
Months of lactation	1072				
Not applicable		27 (13)	57 (14)	32 (19)	75 (25)
0		44 (22)	74 (18)	23 (14)	44 (15)
≤6 mo		38 (19)	93 (23)	31 (19)	57 (19)
7 to <12 mo		29 (14)	48 (12)	23 (14)	28 (9)
12-18 mo		16 (8)	53 (13)	22 (14)	30 (10)
>18 mo		49 (24)	85 (21)	31 (19)	63 (21)
Oral contraceptive use	787				
Never		23 (12)	49 (12)	14 (15)	48 (52)
1–4 y		48 (24)	123 (30)	19 (20)	17 (19)
5–9 y		50 (25)	104 (26)	25 (26)	12 (13)
≥10 y		76 (38)	129 (32)	36 (38)	14 (15)
Hormone fertility	1125				
Yes		15 (7)	19 (5)	7 (4)	19 (6)
Never		188 (93)	392 (95)	164 (96)	321 (94)
Hormone replacement	1119				
Yes		88 (43)	181 (44)	135 (40)	72 (43)
No		115 (57)	230 (56)	201 (60)	97 (57)
Years of HRT	1085				
Never		115 (58)	230 (57)	97 (60)	201 (63)
0–4 y		30 (15)	81 (20)	25 (15)	57 (18)
5–9 y		23 (12)	38 (9)	25 (15)	28 (9)
≥10 y		32 (16)	54 (13)	15 (10)	34 (11)
Self-reported family history of cancer	1113				
Yes		141 (70)	285 (70)	117 (69)	200 (60)
No		59 (30)	125 (30)	52 (31)	134 (40)
Number of first-degree relatives with BC	1124				
None		171 (84)	370 (90)	134 (78)	299 (88)
≥1		32 (16)	41 (10)	37 (22)	40 (12)
Physical activity	728				
Mean total physical activity, MET-min/wk		2904 ± 204	3622 ± 172	2876 ± 290	2556 ± 314

[1] *n* = 1128. ATP, Alberta's Tomorrow Project; BC, breast cancer; BCGP, British Columbia Generations Project; HRT, hormone replacement therapy; MET, metabolic equivalent task.
[2] Waist-to-hip ratio below guidelines: <0.85; above guidelines: ≥0.85.

for cases was 2.8 ± 0.10 y, and there was no difference between cohorts ($P = 0.42$). Eighty-five percent of the cases were estrogen receptor positive (ER+), 16% were human epidermal growth factor receptor 2 positive (HER2+), and 9% were triple negative [ER− PR− (progesterone receptor negative) HER2−] (Supplemental Tables 1 and 2). Two-sided Student's *t* tests were performed and no differences in fatty acid content based on receptor status of breast cancer cases were observed; therefore this was not used as a cofactor in analyses.

To confirm that plasma is reflective of dietary intake, we first assessed the Spearman's rank correlation coefficients of n–3 LC-PUFAs between reported dietary intake and plasma phospholipid fatty acid status in a subcohort of ATP (**Supplemental Table 5**). Weak correlations were observed between plasma relative percentage of LC n–3 fatty acids ($r = 0.21$, $P < 0.01$), DHA ($r = 0.25$, $P < 0.001$), the combination of EPA + DHA ($r = 0.24$, $P < 0.001$), and energy-adjusted n–3 fatty acid consumption (grams/1000 kJ) in this subcohort of the study. These correlations remained consistent when assessed between plasma relative percentage of fatty acid and daily fish consumption, or unadjusted fatty acid consumption (Supplemental Table 5).

The mean phospholipid fatty acid content (relative %: **Table 2**; concentration: **Supplemental Table 6**) varied between cohorts, with BCGP participants having 20% higher n–3 PUFAs, including EPA, docosapentaenoic acid (22:5n–3), and DHA, with lower SFAs and a lower n–6:n–3 ratio compared with the ATP participants. Regardless of cancer status, we determined 84% of the fatty acids or fatty acid combinations were different between the 2 cohorts. For this reason, all subsequent analysis was blocked by cohort. A few differences were observed between cases and controls for individual fatty acids (Table 2). In ATP, arachidonic acid (AA; 20:4n−6) was higher in cases versus controls ($P < 0.02$, relative % and concentration), and total concentrations of MUFAs, PUFAs, and total n–6 fatty acids were also higher in cases versus controls ($P < 0.04$). Cases in BCGP had lower vaccenic acid (18:1 c11; relative % and concentration) and lower oleic acid (18:1n–9; relative percentage) compared with controls.

We first assessed the data by running principal components analysis to determine if there were clusters of fatty acids that grouped together to predict risk. However, this analysis did not elucidate any clusters that characterized risk. Multivariable ORs for breast cancer by quartiles of plasma phospholipid fatty acid were then assessed, with quartile 1 being the lowest relative percentage (**Table 3**). Associations of fatty acids were similar in univariate and adjusted models; therefore, we have presented the adjusted results herein. In ATP, positive associations between fatty acids and overall breast cancer risk were observed for total LC n–6, AA, DHA, and the combination of EPA + DHA and a negative association was observed for total SFAs and breast cancer risk. In BCGP, a positive association between fatty acids and overall breast cancer risk was observed for the ratio of n–6:n–3, with an observed positive trend in the ratio of AA:DHA + EPA. Negative associations for vaccenic acid and total LC n–3 fatty acids were also observed.

Breast cancer etiology differs depending on hormonal changes and menopausal status and this could be impacted by nutritional factors including fatty acid composition (46); therefore, the data were stratified to examine differences in fatty acid status by menopausal status. Regardless of breast cancer status or cohort, fatty acid status was different between pre- and postmenopausal women (**Supplemental Table 7**). While menopausal status did not affect the overall amount (relative %) of SFAs, MUFAs, and PUFAs, the balance of n–6 and n–3 fatty acids within PUFAs was different: premenopausal women had lower total n–3 and more total n–6 (yet lower total LC n–6) than postmenopausal women.

Interestingly, only in ATP did the association with breast cancer risk and several fatty acids vary by menopausal status [**Table 4** and **Supplemental Tables 8** (ATP) and **9** (BCGP)]. Specifically, positive associations between fatty acids and breast cancer risk were observed in premenopausal women for the desaturation index [ratio of oleic acid:stearic acid (18:1n–9:18:0)] and total LC n–3, driven by a positive association of EPA + DHA. A negative association was observed for total SFAs, driven by the negative associations observed in (stearic acid) 18: and (lignoceric acid) 24:0. In several instances, the second quartile conferred the highest level of risk and could be responsible for the overall positive association. This was observed for total n–3, total LC n–3, DHA, and the combination of EPA + DHA. In postmenopausal women, positive associations were observed for DHA and total LC n–6 including AA, while a negative association was observed for (palmitic acid) 16:0 and (alpha linolenic acid) 18:3n–3. Statistical interactions were observed for oleic acid (P-interaction = 0.04) and total LC n–6 fatty acids (P-interaction = 0.05), suggesting positive associations in postmenopausal women versus premenopausal women. Conversely, statistical interactions observed for linoleic acid (P-interaction = 0.05), α-linolenic acid (ALA; 18:3n−3) (P-interaction = 0.03), total n–3 (P-interaction = 0.09), and total LC n–3 (P-interaction = 0.07) suggested inverse associations in postmenopausal women compared with premenopausal women. In the BCGP cohort no clear associations or trends were observed in fatty acids when stratified by menopausal status.

Stratification by BMI (in kg/m^2; 18 to <25, 25 to <30 and ≥30) did not elucidate any clear patterns of risk (data not shown); however, stratifying by waist-to-hip ratio produced associations between some fatty acids and breast cancer risk [**Table 5** and **Supplemental Tables 10** (ATP) and **11** (BCGP)]. In the ATP cohort, positive associations with breast cancer risk were observed for 16:0 and DHA and negative associations for ALA and the ratio of AA:DHA when the waist-to-hip ratio was <0.85. When the waist-to-hip ratio was ≥0.85, positive associations with breast cancer risk were observed for DHA and total LC n–6 (largely influenced by AA) and a negative association was observed for SFAs. In ATP, interactions were observed in 3 instances, suggesting decreased risk with increased 16:0 (P-interaction = 0.05), and increased risk with increased (octadecenoic acid) 18:1n–7 (P-interaction = 0.04), or the ratio of AA:DHA (P-interaction = 0.002) if waist-to-hip was ≥0.85. In the BCGP cohort, when stratified by waist-to-hip ratio, several fatty acids were associated with decreased risk of breast cancer when waist-to-hip ratio was <0.85, including vaccenic acid, DHA, EPA + DHA, total n–3, and total LC n–3. The ratios of n–6:n–3 and AA:DHA were associated with increased breast cancer risk when the waist-to-hip ratio was <0.85. EPA was associated with decreased risk when waist-to-hip ratio was ≥0.85. There was a statistical interaction for DHA (P-interaction = 0.05), suggesting a negative association among women with a waist-to-hip ratio of <0.85 compared with women with a waist-to-hip ≥0.85. In 2 other instances, statistical interactions were observed: EPA (P-interaction = 0.002) and the ratio of AA:DHA (P-interaction = 0.05); however, they are not linear interactions and suggest there may be an optimal range for reduced risk.

TABLE 2 Plasma phospholipid fatty acid composition in relative percentage (%) among breast cancer cases and controls[1]

	Entire cohort			ATP			BCGP			*P*, ATP vs. BCGP[2]
Fatty acids	**Cases (*n* = 377)**	**Control (*n* = 751)**	***P***	**Cases (*n* = 203)**	**Control (*n* = 411)**	***P***	**Cases (*n* = 174)**	**Control (*n* = 340)**	***P***	
Saturates										
Total SFAs[3]	46.6 ± 0.1	46.8 ± 0.1	0.18	47.3 ± 0.1	47.6 ± 0.1	0.04	45.8 ± 0.2	45.8 ± 0.1	0.83	<0.001
14:0 (Myristic acid)	0.3 ± 0.0	0.4 ± 0.1	0.57	0.3 ± 0.0	0.3 ± 0.0	0.12	0.4 ± 0.0	0.4 ± 0.0	0.36	<0.001
16:0 (Palmitic acid)	28.8 ± 0.1	28.9 ± 0.1	0.44	29.0 ± 0.1	29.3 ± 0.1	0.12	28.5 ± 0.1	28.4 ± 0.1	0.46	<0.001
17:0 (Margric acid)	0.43 ± 0.0	0.44 ± 0.0	0.32	0.44 ± 0.0	0.45 ± 0.0	0.24	0.42 ± 0.0	0.42 ± 0.0	0.94	<0.001
18:0 (Stearic acid)	15.4 ± 0.1	15.5 ± 0.0	0.62	15.9 ± 0.1	15.8 ± 0.1	0.67	14.9 ± 0.1	15.1 ± 0.1	0.22	<0.001
20:0 (Arachidic acid)	0.506 ± 0.0	0.510 ± 0.0	0.65	0.496 ± 0.0	0.518 ± 0.0	0.04	0.518 ± 0.0	0.499 ± 0.0	0.12	
24:0 (Lignoceric acid)	1.08 ± 0.0	1.11 ± 0.0	0.10	1.08 ± 0.0	1.17 ± 0.0	0.01	1.07 ± 0.0	1.04 ± 0.0	0.36	<0.001
Monounsaturates										
Total MUFAs[4]	13.8 ± 0.1	13.9 ± 0.0	0.23	13.9 ± 0.1	13.9 ± 0.1	0.86	13.6 ± 0.1	13.9 ± 0.1	0.05	
16:1n–7 (Palmitoleic acid)	0.7 ± 0.0	0.7 ± 0.0	0.70	0.7 ± 0.0	0.7 ± 0.0	0.88	0.8 ± 0.0	08 ± 0.0	0.51	<0.001
18:1 c11 (Vaccenic acid)	0.21 ± 0.0	0.24 ± 0.0	0.02	0.22 ± 0.0	0.24 ± 0.0	0.24	0.19 ± 0.0	0.23 ± 0.0	0.04	
18:1n–9 (Oleic acid)	9.4 ± 0.1	9.5 ± 0.0	0.33	9.5 ± 0.1	9.4 ± 0.1	0.38	9.3 ± 0.1	9.6 ± 0.1	0.01	
18:1n–7 (Octadecenoic acid)	1.4 ± 0.0	1.4 ± 0.0	0.90	1.4 ± 0.0	14 ± 0.0	0.47	1.3 ± 0.0	1.3 ± 0.0	0.36	<0.001
24:1n–9 (Nervonic acid)	2.1 ± 0.0	2.1 ± 0.0	0.94	2.1 ± 0.0	2.1 ± 0.0	0.25	2.0 ± 0.0	2.0 ± 0.0	0.15	<0.001
Polyunsaturates										
Total PUFAs[5]	39.0 ± 0.1	38.7 ± 0.1	0.05	38.3 ± 0.2	37.9 ± 0.1	0.05	39.9 ± 0.2	39.7 ± 0.1	0.45	<0.001
Total n–6	33.2 ± 0.1	32.9 ± 0.1	0.07	32.9 ± 0.2	32.6 ± 0.1	0.07	33.5 ± 0.2	33.3 ± 0.1	0.44	<0.001
Total long-chain n–6[6]	13.8 ± 0.1	13.5 ± 0.1	0.01	13.8 ± 0.1	13.5 ± 0.1	0.03	13.9 ± 0.2	13.6 ± 0.1	0.18	
18:2n–6 (Linoleic acid)	19.2 ± 0.1	19.3 ± 0.1	0.82	19.0 ± 0.2	19.0 ± 0.12	0.96	19.5 ± 0.2	19.5 ± 0.2	0.76	<0.001
18:3n–6 (γ-Linolenic acid)	0.10 ± 0.0	0.09 ± 0.0	0.14	0.09 ± 0.0	0.08 ± 0.0	0.21	0.11 ± 0.0	0.10 ± 0.0	0.40	
20:2n–6 (Eicosadienoic acid)	0.29 ± 0.0	0.28 ± 0.0	0.20	0.27 ± 0.0	0.27 ± 0.0	0.53	0.30 ± 0.0	0.29 ± 0.0	0.28	<0.001
20:3n–6 (Dihomo-γ-linolenic acid)	4.1 ± 0.0	4.1 ± 0.03	0.78	4.2 ± 0.0	4.1 ± 0.04	0.95	4.0 ± 0.1	4.0 ± 0.0	0.72	<0.01
20:4n–6 (AA)	9.0 ± 0.1	8.8 ± 0.1	0.01	9.0 ± 0.1	8.6 ± 0.1	0.02	9.1 ± 0.1	8.9 ± 0.1	0.18	<0.01
22:4n–6 (Adrenic acid)	0.25 ± 0.0	0.25 ± 0.0	0.96	0.24 ± 0.0	0.23 ± 0.0	0.03	0.26 ± 0.0	0.27 ± 0.0	0.26	<0.001
22:5n–6 (Osbond acid)	0.17 ± 0.0	0.17 ± 0.0	0.93	0.17 ± 0.0	0.18 ± 0.0	0.16	0.17 ± 0.0	0.16 ± 0.0	0.14	<0.001
Total n–3	5.9 ± 0.1	5.8 ± 0.1	0.89	5.4 ± 0.1	5.3 ± 0.1	0.80	6.4 ± 0.1	6.4 ± 0.1	0.88	<0.001
Total long-chain n–3[7]	5.4 ± 0.1	5.4 ± 0.1	0.97	5.0 ± 0.1	5.0 ± 0.1	0.80	6.0 ± 0.1	6.0 ± 0.1	0.72	<0.001
18:3n–3 (α-Linolenic acid)	0.41 ± 0.0	0.39 ± 0.0	0.07	0.36 ± 0.0	0.36 ± 0.0	0.99	0.46 ± 0.0	0.42 ± 0.0	0.03	<0.01
20:4n–3 (Eicosatetraenoic acid)	0.64 ± 0.0	0.63 ± 0.0	0.50	0.62 ± 0.0	0.64 ± 0.0	0.31	0.66 ± 0.0	0.62 ± 0.0	0.07	
20:5n–3 (EPA)	1.2 ± 0.0	1.2 ± 0.0	0.47	1.0 ± 0.0	1.1 ± 0.0	0.35	1.3 ± 0.1	1.4 ± 0.0	0.79	<0.001
22:5n–3 (DPA)	0.77 ± 0.0	0.78 ± 0.0	0.57	0.76 ± 0.0	0.74 ± 0.0	0.24	0.80 ± 0.0	0.81 ± 0.0	0.64	<0.001
22:6n–3 (DHA)	2.9 ± 0.0	2.8 ± 0.0	0.78	2.6 ± 0.0	2.5 ± 0.0	0.26	3.2 ± 0.1	3.2 ± 0.0	0.46	<0.001
Ratios										
Total n–6:total n–3	6.1 ± 0.1	6.1 ± 0.1	0.74	6.5 ± 0.1	6.5 ± 0.1	0.63	5.6 ± 0.1	5.6 ± 1.7	0.89	<0.001
AA:DHA	3.5 ± 0.1	3.4 ± 0.0	0.79	3.7 ± 0.1	3.8 ± 0.1	0.64	3.1 ± 0.1	3.0 ± 0.1	0.25	<0.001
AA:EPA + DHA	2.5 ± 0.0	2.4 ± 0.0	0.66	2.7 ± 0.1	2.7 ± 0.0	0.79	2.3 ± 0.1	2.2 ± 0.0	0.04	<0.001
DI_{16}	0.02 ± 0.0	0.02 ± 0.0	0.63	0.02 ± 0.0	0.02 ± 0.0	0.83	0.03 ± 0.0	0.03 ± 0.0	0.70	<0.001
DI_{18}	0.62 ± 0.0	0.62 ± 0.0	0.81	0.61 ± 0.0	0.60 ± 0.0	0.56	0.63 ± 0.0	0.64 ± 0.0	0.28	<0.001

[1] AA, arachidonic acid; ATP, Alberta's Tomorrow Project; BCGP, British Columbia Generations Project; DI_{16}, desaturation index of 16:1:16:0; DI_{18}, desaturation index of 18:1:18:0; DPA, docosapentaenoic acid.
[2] ATP vs. BCGP (overall differences between pooled cases and controls from each cohort) as assessed by independent *t* tests.
[3] SFAs = 14:0, 16:0, 17:0, 18:0, 20:0, 24:0.
[4] MUFAs = 16:1n–7, 18:1 c11, 18:1n–9, 18:1n–7, 24:1n–9.
[5] PUFAs = 18:2n–6, 18:3n–6, 20:2n–6, 20:3n–6, 20:4n–6, 22:4n–6, 22:5n–6, 18:3n–3, 20:4n–3, 20:5n–3, 22:5n–3, 22:6n–3.
[6] Total long-chain n–6 = 20:2n–6, 20:3n–6, 20:4n–6, 22:4n–6, 22:5n–6.
[7] Total long-chain n–3 = 20:4n–3, 20:5n–3, 22:5n–3, 22:6n–3.

TABLE 3 Multivariate ORs (95% CIs) of breast cancer according to quartiles of plasma phospholipid fatty acid relative percentage in the ATP and BCGP[1]

	Cohort							
	ATP				BCGP			
Fatty acids	Q2 vs. Q1[2]	Q3 vs. Q1	Q4 vs. Q1	*P*-trend	Q2 vs. Q1	Q3 vs. Q1	Q4 vs. Q1	*P*-trend
Saturates								
Total SFAs[3]	0.71 (0.43, 1.17)	1.00 (0.61, 1.64)	0.54 (0.32, 0.91)*	0.05*	1.40 (0.78, 2.48)	0.63 (0.34, 1.18)	1.09 (0.60, 1.96)	0.08
14:0 (Myristic acid)	0.71 (0.43, 1.18)	0.71 (0.43, 1.18)	0.79 (0.48, 1.30)	0.49	1.09 (0.60, 1.97)	1.55 (0.84, 2.84)	1.38 (0.75, 2.54)	0.44
16:0 (Palmitic acid)	1.26 (0.76, 2.06)	0.86 (0.52, 1.43)	0.75 (0.44, 1.25)	0.23	1.24 (0.69, 2.22)	0.87 (0.47, 1.61)	1.37 (0.67, 1.90)	0.44
18:0 (Stearic acid)	0.79 (0.47, 1.33)	0.72 (0.43, 1.21)	0.88 (0.52, 1.50)	0.62	0.82 (0.47, 1.46)	0.51 (0.28, 0.94)	0.74 (0.41, 1.34)	0.19
24:0 (Lignoceric acid)	1.11 (0.68, 1.80)	0.65 (0.39, 1.08)	0.63 (0.38, 1.05)	0.06	0.80 (0.44, 1.45)	1.45 (0.82, 2.59)	0.81 (0.45, 1.48)	0.15
Monounsaturates								
Total MUFAs[4]	0.98 (0.60, 1.62)	0.78 (0.47, 1.29)	0.86 (0.52, 1.42)	0.74	1.05 (0.59, 1.85)	0.81 (0.46, 1.43)	0.70 (0.38, 1.30)	0.54
16:1n–7 (Palmitoleic acid)	1.08 (0.66, 1.78)	0.90 (0.54, 1.51)	0.91 (0.54, 1.51)	0.88	0.68 (0.38, 1.22)	1.02 (0.56, 1.84)	1.28 (0.71, 2.30)	0.68
18:1 c11 (Vaccenic acid)	0.96 (0.58, 1.57)	0.54 (0.32, 0.92)*	0.86 (0.52, 1.41)	0.09	0.66 (0.40, 1.10)	0.99 (0.56, 1.76)	0.45 (0.24, 0.84)*	0.04*
18:1n–9 (Oleic acid)	0.83 (0.50, 1.38)	0.97 (0.59, 1.61)	0.98 (0.59, 1.63)	0.88	1.40 (0.76, 2.58)	1.48 (0.80, 2.74)	1.11 (0.60, 2.05)	0.53
18:1n–7 (Octadecenoic acid)	0.94 (0.57, 1.55)	0.95 (0.57, 1.57)	1.23 (0.74, 2.04)	0.69	1.40 (0.79, 2.49)	1.11 (0.62, 1.99)	0.88 (0.48, 1.61)	0.46
24:1n–9 (Nervonic acid)	0.90 (0.55, 1.48)	0.76 (0.46, 1.26)	0.73 (0.44, 1.21)	0.59	1.00 (0.56, 1.83)	1.27 (0.70, 2.29)	1.23 (0.68, 2.25)	0.78
Polyunsaturates								
Total PUFAs[5]	0.99 (0.58, 1.70)	1.90 (1.15, 3.13)*	1.15 (0.68, 1.94)	0.03*	1.50 (0.82, 2.76)	1.29 (0.70, 2.40)	1.60 (0.88, 2.91)	0.43
Total n–6	1.11 (0.66, 1.87)	1.38 (0.82, 2.31)	1.46 (0.88, 2.42)	0.42	0.74 (0.40, 1.36)	1.24 (0.69, 2.20)	1.08 (0.60, 1.93)	0.40
Total long-chain n–6[6]	0.66 (0.39, 1.14)	1.17 (0.70, 1.96)	1.60 (0.95, 2.67)	0.01*	1.34 (0.74, 2.43)	0.92 (0.49, 1.70)	1.46 (0.79, 2.70)	0.35
18:2n–6 (Linoleic acid)	0.75 (0.45, 1.24)	0.59 (0.35, 0.99)*	0.87 (0.52, 1.43)	0.22	1.25 (0.69, 2.26)	0.91 (0.50, 1.66)	1.42 (0.77, 2.61)	0.44
18:3n–6 (γ-Linolenic acid)	1.49 (0.90, 2.48)	0.86 (0.51, 1.47)	1.33 (0.79, 2.24)	0.14	1.12 (0.61, 2.05)	1.81 (1.01, 3.26)*	1.44 (0.78, 2.63)	0.19
20:2n–6 (Eicosadienoic acid)	1.32 (0.79, 2.12)	1.00 (0.60, 1.69)	1.47 (0.88, 2.45)	0.33	1.12 (0.61, 2.04)	1.16 (0.64, 2.10)	1.07 (0.58, 1.95)	0.96
20:3n–6 (Dihomo-γ-linolenic acid)	0.88 (0.53, 1.47)	0.83 (0.49, 1.41)	0.98 (0.58, 1.68)	0.87	0.83 (0.45, 1.51)	0.84 (0.46, 1.54)	1.04 (0.56, 1.94)	0.83
20:4n–6 (AA)	0.93 (0.55, 1.59)	1.35 (0.80, 2.26)	1.67 (0.99, 2.81)*	0.09	0.98 (0.54, 1.79)	1.32 (0.62, 2.06)	1.24 (0.67, 2.27)	0.86
Total n–3	1.94 (1.15, 3.26)*	1.51 (0.89, 2.57)	1.50 (0.88, 2.56)	0.10	1.32 (0.74, 2.34)	0.52 (0.28, 0.99)	0.94 (0.51, 1.75)	0.03*
Total long-chain n–3[7]	1.92 (1.14, 3.23)*	1.67 (0.99, 2.81)*	1.56 (0.92, 2.67)	0.09	1.22 (0.69, 2.16)	0.50 (0.27, 0.95)*	0.86 (0.46, 1.59)	0.03*
18:3n–3 (α-Linolenic acid)	0.52 (0.31, 0.87)*	0.97 (0.59, 1.59)	0.86 (0.52, 1.43)	0.06	1.63 (0.91, 2.93)	1.33 (0.72, 2.45)	1.57 (0.87, 2.86)	0.36
20:4n–3 (Eicosatetraenoic acid)	0.82 (0.50, 1.35)	0.76 (0.46, 1.26)	0.80 (0.48, 1.32)	0.72	1.08 (0.59, 1.96)	1.11 (0.61, 2.04)	1.33 (0.74, 2.40)	0.80
20:5n–3 (EPA)	1.09 (0.66, 1.82)	1.14 (0.69, 1.90)	1.05 (0.63, 1.76)	0.96	0.87 (0.48, 1.58)	0.89 (0.50, 1.61)	0.81 (0.44, 1.49)	0.92
22:5n–3 (DPA)	1.21 (0.72, 2.02)	1.04 (0.62, 1.74)	1.47 (0.88, 2.44)	0.44	0.83 (0.46, 1.47)	0.72 (0.40, 1.30)	0.66 (0.36, 1.21)	0.56
22:6 n-3 (DHA)	2.70 (1.59, 4.58)*	1.97 (1.15, 3.37)*	1.87 (1.08, 3.23)*	0.003*	1.39 (0.78, 2.48)	0.94 (0.52, 1.70)	0.76 (0.41, 1.42)	0.26
EPA + DHA	2.35 (1.39, 3.98)*	1.90 (1.12, 3.23)*	1.85 (1.08, 3.18)*	0.01*	1.30 (0.73, 2.31)	0.69 (0.37, 1.27)	0.88 (0.47, 1.62)	0.20
Ratios								
Total n–6:total n–3	0.94 (0.57, 1.55)	0.92 (0.55, 1.52)	0.72 (0.43, 1.22)	0.64	0.62 (0.33, 1.15)	1.45 (0.81, 2.59)	1.26 (0.68, 2.35)	0.04*
AA:DHA	1.25 (0.75, 2.08)	1.53 (0.92, 2.54)	0.82 (0.48, 1.39)	0.09	0.92 (0.50, 1.68)	1.13 (0.62, 2.08)	1.55 (0.85, 2.83)	0.33
AA:EPA + DHA	1.04 (0.62, 1.75)	1.72 (1.04, 2.85)*	0.73 (0.43, 1.24)	0.009*	1.18 (0.65, 2.17)	1.25 (0.68, 2.30)	1.99 (1.08, 3.69)*	0.14
DI_{16}	0.97 (0.58, 1.61)	0.94 (0.57, 1.56)	0.93 (0.57, 1.52)	0.99	1.20 (0.66, 2.20)	1.10 (0.61, 1.96)	1.06 (0.58, 1.93)	0.94
DI_{18}	1.02 (0.62, 1.70)	0.92 (0.55, 1.55)	1.21 (0.73, 2.02)	0.75	0.96 (0.54, 1.71)	0.59 (0.33, 1.06)	0.93 (0.52, 1.67)	0.28

[1]Adjusted for smoking ever, alcohol consumption, hysterectomy ever, BMI, physical activity, age at first pregnancy, age at menopause, age at menarche. AA, arachidonic acid; ATP, Alberta's Tomorrow Project; BCGP, British Columbia Generations Project; DI_{16}, desaturation index of 16:1:16:0; DI_{18}, desaturation index of 18:1:18:0; DPA, docosapentaenoic acid; Q, quartile.
[2]Quartile 1: lowest fatty acid content; reference quartile. *Significant compared with quartile 1, $P < 0.05$.
[3]SFAs = 14:0, 16:0, 17:0, 18:0, 20:0, 24:0.
[4]MUFAs = 16:1n–7, 18:1 c11, 18:1n–9, 18:1n–7, 24:1n–9.
[5]PUFAs = 18:2n–6, 18:3n–6, 20:2n–6, 20:3n–6, 20:4n–6, 22:4n–6, 22:5n–6, 18:3n–3, 20:4n–3, 20:5n–3, 22:5n–3, 22:6n–3.
[6]Total long-chain n–6 = 20:2n–6, 20:3n–6, 20:4n–6, 22:4n–6, 22:5n–6.
[7]Total long-chain n–3 = 20:4n–3, 20:5n–3, 22:5n–3, 22:6n–3.

TABLE 4 Multivariate ORs (95% CIs) of breast cancer according to quartiles of plasma phospholipid fatty acid relative percentage by menopause status in the Alberta's Tomorrow Project and British Columbia Generations Project[1]

	Premenopausal				Postmenopausal				
Fatty acids	**Q2 vs. Q1[2]**	**Q3 vs. Q1**	**Q4 vs. Q1**	***P*-trend**	**Q2 vs. Q1[2]**	**Q3 vs. Q1**	**Q4 vs. Q1**	***P*-trend**	***P*-interaction**
Alberta's Tomorrow Project									
Total SFAs[3]	0.27 (0.09, 0.80)*	0.74 (0.28, 1.94)	0.22 (0.08, 0.65)*	0.02*	0.92 (0.51, 1.67)	1.15 (0.64, 2.06)	0.72 (0.39, 1.33)	0.49	0.54
Total MUFAs[4]	0.74 (0.24, 2.21)	0.58 (0.18, 1.84)	1.53 (0.50, 4.65)	0.24	1.12 (0.63, 1.98)	0.87 (0.49, 1.55)	0.63 (0.34, 1.14)	0.31	0.07
Total PUFAs[5]	1.07 (0.37, 3.11)	2.05 (0.77, 5.45)	1.41 (0.48, 4.15)	0.44	0.99 (0.53, 1.86)	1.98 (1.09, 3.62)*	1.15 (0.63, 2.11)	0.07	0.99
Total long-chain n–6[6]	0.38 (0.15, 1.01)*	0.41 (0.16, 1.05)*	1.76 (0.51, 6.11)	0.03*	0.91 (0.46, 1.80)	1.95 (1.03, 3.71)*	2.03 (1.10, 3.74)*	0.01*	0.05*
18:2n–6 (Linoleic acid)	2.24 (0.73, 6.86)	0.74 (0.22, 2.51)	1.70 (0.53, 5.45)	0.15	0.50 (0.27, 0.91)*	0.60 (0.34, 1.06)	0.76 (0.43, 1.36)	0.11	0.05*
20:4n–6 (AA)	1.22 (0.48, 3.10)	0.82 (0.31, 2.22)	3.07 (0.89, 10.60)	0.19	0.78 (0.40, 1.52)	1.70 (0.92, 3.16)	1.59 (0.88, 2.89)	0.04*	0.09
Total n–3	5.39 (2.05, 14.17)*	2.09 (0.77, 5.68)	1.40 (0.47, 4.16)	0.007*	1.25 (0.66, 2.37)	1.27 (0.67, 2.39)	1.36 (0.73, 2.53)	0.81	0.09*
Total long-chain n–3[7]	5.42 (2.0, 14.16)*	1.82 (0.66, 5.02)	1.78 (0.58, 5.43)	0.007*	1.20 (0.63, 2.28)	1.52 (0.82, 2.84)	1.39 (0.74, 2.58)	0.58	0.07*
18:3n–3 (α-Linolenic acid)	0.79 (0.24, 2.55)	1.38 (0.48, 3.91)	3.05 (1.01, 9.26)*	0.07	0.47 (0.26, 0.84)*	0.93 (0.52, 1.66)	0.56 (0.30, 1.01)*	0.03*	0.03*
22:6n–3 (DHA)	3.42 (1.26, 9.27)*	2.76 (1.05, 7.28)*	2.44 (0.81, 7.29)	0.07	2.58 (1.36, 4.89)*	1.75 (0.90, 3.38)	1.75 (0.91, 3.35)	0.04*	0.90
EPA + DHA	5.72 (2.09, 15.70)*	2.60 (0.95, 7.13)	2.06 (0.65, 6.55)	0.009*	1.74 (0.92, 3.3)	1.68 (0.89, 3.17)	1.70 (0.91, 3.18)	0.28	0.37
AA:DHA	2.38 (0.85, 6.38)	1.17 (0.40, 3.44)	0.68 (0.24, 1.93)	0.10	1.01 (0.57, 1.79)	1.72 (0.99, 2.97)*	0.88 (0.49, 1.58)	0.13	0.14
AA:EPA + DHA	1.12 (0.39, 3.23)	1.66 (0.61, 4.53)	0.57 (0.20, 1.58)	0.18	1.04 (0.57, 1.88)	1.77 (0.98, 3.18)*	0.82 (0.44, 1.55)	0.07	0.86
DI_{16}	1.86 (0.71, 4.89)	1.27 (0.47, 3.41)	1.16 (0.44, 3.10)	0.64	0.98 (0.53, 1.79)	1.66 (0.93, 2.99)	0.89 (0.47, 1.67)	0.82	0.50
DI_{18}	0.63 (0.18, 2.22)	1.04 (0.33, 3.23)	2.93 (0.98, 8.81)*	0.02	1.20 (0.69, 2.10)	0.95 (0.52, 1.72)	0.79 (0.43, 1.46)	0.61	0.01*
British Columbia Generations Project									
Total SFAs[3]	1.72 (0.54, 5.46)	0.42 (0.10, 1.69)	1.56 (0.50, 4.88)	0.20	1.22 (0.61, 2.42)	0.65 (0.31, 1.34)	0.88 (0.43, 1.79)	0.36	0.48
Total MUFAs[4]	0.69 (0.21, 2.29)	0.76 (0.24, 2.37)	0.68 (0.20, 2.41)	0.92	1.23 (0.62, 2.41)	0.83 (0.42, 1.65)	0.80 (0.39, 1.65)	0.61	0.86
Total PUFAs[5]	0.49 (0.14, 1.78)	0.58 (0.16, 2.07)	1.24 (0.42, 3.63)	0.40	2.13 (1.04, 4.39)	1.58 (0.76, 3.27)	1.74 (0.83, 3.62)	0.22	0.18
Total long-chain n–6[6]	1.16 (0.36, 3.73)	2.01 (0.61, 6.68)	1.43 (0.35, 5.94)	0.67	1.33(0.65, 2.72)	0.68 (0.32, 1.44)	1.48 (0.73, 3.00)	0.15	0.24
18:2n–6 (Linoleic acid)	0.95 (0.23, 3.85)	0.71 (0.20, 2.57)	0.81 (0.24, 2.69)	0.95	1.32 (0.66, 2.63)	0.98 (0.49, 1.96)	1.69 (0.82, 3.51)	0.40	0.87
20:4n–6 (AA)	1.96 (0.60, 6.37)	3.72 (1.19, 11.61)*	0.80 (0.17, 3.69)	0.08	0.73 (0.35, 1.52)	0.73 (0.35–1.53)	1.26 (0.62, 2.58)	0.31	0.02*
Total n–3	0.73 (0.25, 2.13)	0.54 (0.15, 2.01)	0.75 (0.21, 2.68)	0.82	1.87 (0.90, 3.89)	0.63 (0.29, 1.35)	1.12 (0.53, 2.38)	0.02*	0.36
Total long-chain n–3[7]	0.81 (0.28, 2.31)	0.34(0.08, 1.40)	0.70 (0.19, 2.52)	0.52	1.52 (0.74, 3.14)	0.65 (0.30, 1.38)	0.98 (0.46, 2.07)	0.11	0.55
18:3n–3 (α-Linolenic acid)	2.35 (0.68, 8.19)	1.47 (0.38, 5.71)	3.46 (0.95, 12.58)	0.25	1.48 (0.74, 2.95)	1.50 (0.72, 3.10)	1.25 (0.62, 2.51)	0.66	0.25
22:6n–3 (DHA)	1.86 (0.62, 5.54)	0.48 (0.12, 1.91)	1.15 (0.34, 3.90)	0.27	1.35 (0.66, 2.76)	1.10 (0.55, 2.21)	0.65 (0.31, 1.39)	0.25	0.41
EPA + DHA	1.11 (0.39, 3.15)	0.48 (0.12, 1.90)	1.06 (0.30, 3.72)	0.67	1.55 (0.75, 3.17)	0.81 (0.39, 1.67)	0.90 (0.43, 1.89)	0.25	0.76
AA:DHA	1.03 (0.28, 3.76)	1.41 (0.42, 4.78)	1.16 (0.31, 4.42)	0.94	0.98 (0.49, 1.97)	1.00 (0.48, 2.10)	1.88 (0.93, 3.80)	0.18	0.82
AA:EPA + DHA	2.17 (0.55, 8.54)	1.76 (0.45, 6.94)	1.68 (0.42, 6.68)	0.74	1.07 (0.53, 2.17)	1.20 (0.59, 2.44)	2.40 (1.16, 4.96)*	0.07	0.61
DI_{16}	1.87 (0.56, 6.29)	1.66 (0.51, 5.39)	1.49 (0.45, 5.03)	0.75	1.05 (0.50, 2.19)	1.07 (0.53, 2.17)	1.06 (0.52, 2.15)	1.00	0.98
DI_{18}	0.59 (016, 2.19)	0.32 (0.09, 1.13)	1.03 (0.29, 3.60)	0.20	1.56 (0.54, 2.04)	0.72 (0.36, 1.44)	0.84 (0.42, 1.67)	0.72	0.55

[1] Adjusted for smoking ever, alcohol consumption, hysterectomy ever, BMI, physical activity, age at first pregnancy, age at menopause, age at menarche. AA, arachidonic acid; DI_{16}, desaturation index of 16:1:16:0; DI_{18}, desaturation index of 18:1:18:0; Q, quartile.

[2] Quartile 1: lowest fatty acid content; reference quartile. *Significant compared with quartile 1, $P < 0.05$.

[3] SFAs = 14:0, 16:0, 17:0, 18:0, 20:0, 24:0.

[4] MUFAs = 16:1n–7, 18:1 c11, 18:1n–9, 18:1n–7, 24:1n–9.

[5] PUFAs = 18:2n–6, 18:3n–6, 20:2n–6, 20:3n–6, 20:4n–6, 22:4n–6, 22:5n–6, 18:3n–3, 20:4n–3, 20:5n–3, 22:5n–3, 22:6n–3.

[6] Total long-chain n–6 = 20:2n–6, 20:3n–6, 20:4n–6, 22:4n–6, 22:5n–6.

[7] Total long-chain n–3 = 20:4n–3, 20:5n–3, 22:5n–3, 22:6n–3.

TABLE 5 Multivariate ORs (95% CIs) of breast cancer according to quartiles of plasma phospholipid fatty acid relative percentage by waist-to-hip ratio in Alberta's Tomorrow Project and British Columbia Generations Project[1]

	Waist-to-hip ratio <0.85				Waist-to-hip ratio ≥0.85				
Fatty acids	**Q2 vs. Q1[2]**	**Q3 vs. Q1**	**Q4 vs. Q1**	***P*-trend**	**Q2 vs. Q1[2]**	**Q3 vs. Q1**	**Q4 vs. Q1**	***P*-trend**	***P*-interaction**
Alberta's Tomorrow Project									
Total SFAs[3]	0.48 (0.22, 1.05)	0.58 (0.27, 1.26)	0.71 (0.32, 1.59)	0.28	1.08 (0.54, 2.20)	1.40 (0.71, 2.76)	0.52 (0.26, 1.07)	0.04*	0.06
Total MUFAs[4]	0.77 (0.34, 1.76)	0.69 (0.30, 1.57)	0.61 (0.27, 1.40)	0.69	1.03 (0.53, 2.00)	0.86 (0.44, 1.69)	0.99 (0.51, 1.93)	0.96	0.84
Total PUFAs[5]	1.30 (0.54, 3.13)	1.57 (0.68, 3.62)	1.38 (0.60, 3.20)	0.76	0.72 (0.35, 1.47)	2.11 (1.10, 4.07)*	0.95 (0.47, 1.90)	0.01*	0.48
Total long-chain n–6[6]	1.27 (0.56, 2.87)	1.33 (0.58, 3.05)	1.41 (0.62, 3.18)	0.85	0.47 (0.21, 1.02)	1.27 (0.63, 2.56)	1.81 (0.88, 3.72)	0.003*	0.32
18:2n–6 (Linoleic acid)	0.66 (0.29, 1.53)	0.42 (0.18, 1.00)*	0.74 (0.34, 1.62)	0.27	0.81 (0.42, 1.55)	0.73 (0.38, 1.42)	0.88 (0.44, 1.76)	0.82	0.74
20:4n–6 (AA)	0.67 (0.28, 1.59)	1.58 (0.69, 3.59)	1.21 (0.54, 2.69)	0.26	1.43 (0.69, 2.96)	1.46 (0.70, 3.04)	2.54 (1.22, 5.32)*	0.08	0.18
Total n–3	1.76 (0.76, 4.10)	1.68 (0.71, 3.94)	1.87 (0.78, 4.46)	0.48	2.02 (1.02, 4.02)*	1.44 (0.71, 2.90)	1.32 (0.65, 2.68)	0.24	0.95
Total long-chain n–3[7]	2.02 (0.85, 4.77)	2.22 (0.94, 5.20)	2.17 (0.90, 5.24)	0.24	1.80 (0.91, 2.57)	1.48 (0.74, 2.96)	1.30 (0.64, 2.64)	0.09	0.93
18:3n–3 (α-Linolenic acid)	0.29 (0.12, 0.69)*	0.66 (0.29, 1.49)	0.60 (0.27, 1.30)	0.05*	0.67 (0.34, 1.32)	1.03 (0.53, 1.99)	1.00 (0.50, 2.01)	0.56	0.47
22:6n–3 (DHA)	2.65 (1.06, 6.60)*	2.95 (1.20, 7.28)*	3.40 (1.37, 8.40)*	0.05*	2.92 (1.46, 5.82)*	1.52 (0.75, 3.11)	1.32 (0.63, 2.77)	0.02*	0.38
EPA + DHA	2.34 (1.00, 5.53)*	2.15 (0.90, 5.14)	2.79 (1.16, 6.72)*	0.12	2.51 (1.25, 5.02)*	1.86 (0.92, 3.77)	1.46 (0.71, 3.01)	0.06	0.71
AA:DHA	1.87 (0.85, 4.10)	0.84 (0.38, 1.87)	0.42 (0.17, 1.02)*	0.01	0.80 (0.39, 1.66)	2.31 (1.15, 4.65)*	1.12 (0.55, 2.30)	0.01*	0.002*
AA:EPA + DHA	1.40 (0.64, 3.07)	1.14 (0.51, 2.56)	0.48 (0.20, 1.15)	0.09	0.72 (0.35, 1.49)	2.14 (1.09, 4.18)*	0.96 (0.47, 1.95)	0.01*	0.07*
DI_{16}	0.97 (0.44, 2.17)	1.41 (0.64, 3.08)	1.04 (0.46, 2.35)	0.79	0.88 (0.44, 1.73)	0.64 (0.32, 1.27)	0.79 (0.42, 1.48)	0.63	0.34
DI_{18}	0.59 (0.25, 1.38)	0.44 (0.17, 1.11)	0.85 (0.38, 1.91)	0.26	1.48 (0.77, 2.85)	1.44 (0.75, 2.76)	1.38 (0.68, 2.79)	0.62	0.11
British Columbia Generations Project									
Total SFAs[3]	1.22 (0.58, 2.55)	0.68 (0.30, 1.56)	0.72 (0.31, 1.69)	0.50	1.54 (0.57, 4.16)	0.61 (0.22, 1.65)	1.44 (0.55, 3.72)	0.13	0.64
Total MUFAs[4]	0.81 (0.36, 1.82)	0.78 (0.34, 1.79)	0.77 (0.34, 1.78)	0.92	1.39 (0.58, 3.30)	0.74 (0.33, 1.66)	0.57 (0.22, 1.44)	0.28	0.51
Total PUFAs[5]	2.04 (0.78, 5.35)	1.50 (0.57, 3.97)	2.16 (0.85, 5.49)	0.36	1.06 (0.46, 2.48)	1.30 (0.56, 3.06)	1.32 (0.57, 3.05)	0.89	0.71
Total long-chain n–6[6]	1.44 (0.66, 3.14)	0.88 (0.37, 2.13)	1.91 (0.84, 4.36)	0.28	1.33 (0.51, 3.51)	0.93 (0.36, 2.38)	1.22 (0.47, 3.20)	0.83	0.89
18:2n–6 (Linoleic acid)	1.16 (0.46, 2.88)	0.83 (0.33, 2.05)	1.55 (0.66, 3.64)	0.44	1.33 (0.59, 2.99)	0.94 (0.42, 2.13)	1.26 (0.50, 3.19)	0.83	0.90
20:4n–6 (AA)	1.53 (0.68, 3.46)	1.41 (0.60, 3.32)	1.60 (0.69, 3.70)	0.68	0.56 (0.22, 1.43)	0.86 (0.35, 2.09)	0.98 (0.38, 2.53)	0.55	0.52
Total n–3	1.14 (0.50, 2.59)	0.30 (0.12, 0.77)*	0.76 (0.32, 1.77)	0.03*	1.66 (0.72, 3.85)	0.94 (0.38, 2.34)	1.21 (0.47, 3.08)	0.53	0.58
Total long-chain n–3[7]	1.05 (0.46, 2.36)	0.29 (0.12, 075)*	0.66 (0.28, 1.54)	0.03*	1.51 (0.66, 3.48)	0.87 (0.35, 2.16)	1.14 (0.44, 2.91)	0.60	0.62
18:3n–3 (α-Linolenic acid)	2.56 (1.09, 6.02)*	1.87 (0.76, 4.62)	1.84 (0.75, 4.50)	0.20	1.06 (0.46, 2.48)	0.97 (0.39, 2.40)	1.33 (0.56, 3.12)	0.89	0.47
22:6n–3 (DHA)	1.33 (0.58, 3.02)	0.38 (0.14, 0.97)*	0.66 (0.28, 1.53)	0.03*	1.31 (0.57, 3.05)	2.09 (0.91, 4.84)	0.86 (0.33, 2.26)	0.21	0.05*
EPA + DHA	1.02 (0.46, 2.26)	0.27 (0.10, 0.71)*	0.66 (0.29, 1.50)	0.03*	1.75 (0.75, 4.07)	1.57 (0.65, 3.78)	1.20 (0.46, 3.17)	0.57	0.13
AA:DHA	0.44 (0.18, 1.04)*	1.13 (0.48, 2.68)	1.88 (0.84, 4.17)	0.02*	2.02 (0.77, 5.26)	1.33 (0.53, 3.31)	1.44 (0.56, 3.70)	0.54	0.05*
AA:EPA + DHA	0.64 (0.28, 1.50)	1.26 (0.54, 2.95)	2.05 (0.92, 4.58)	0.06	2.34 (0.90, 6.08)	1.47 (0.58, 3.69)	2.28 (0.85, 6.13)	0.25	0.27
DI_{16}	2.18 (0.92, 5.15)	1.34 (0.56, 3.20)	1.38 (0.56, 3.40)	0.32	0.65 (0.26, 1.66)	0.97 (0.42, 2.25)	0.93 (0.40, 2.16)	0.80	0.31
DI_{18}	0.70 (0.29, 1.65)	0.59 (0.26, 1.35)	1.09 (0.48, 2.48)	0.41	1.23 (0.54, 2.80)	0.49 (0.20, 1.20)	0.71 (0.30, 1.70)	0.22	0.59

[1] Waist-to-hip ratio below guidelines <0.85 and above guidelines ≥0.85 (for women). Adjusted for smoking ever, alcohol consumption, hysterectomy ever, BMI, physical activity, age at first pregnancy, age at menopause, and age at menarche. AA, arachidonic acid; DI_{16}, desaturation index of 16:1:16:0; DI_{18}, desaturation index of 18:1:18:0; Q, quartile.
[2] Quartile 1: lowest fatty acid content; reference quartile. *Significant compared with quartile 1, $P < 0.05$.
[3] SFAs = 14:0, 16:0, 17:0, 18:0, 20:0, 24:0.
[4] MUFAs = 16:1n–7, 18:1 c11, 18:1n–9, 18:1n–7, 24:1n–9.
[5] PUFAs = 18:2n–6, 18:3n–6, 20:2n–6, 20:3n–6, 20:4n–6, 22:4n–6, 22:5n–6, 18:3n–3, 20:4n–3, 20:5n–3, 22:5n–3, 22:6n–3.
[6] Total long-chain n–6 = 20:2n–6, 20:3n–6, 20:4n–6, 22:4n–6, 22:5n–6.
[7] Total long-chain n–3 = 20:4n–3, 20:5n–3, 22:5n–3, 22:6n–3.

Discussion

In this large, prospective study of 2 geographically distinct Canadian cohorts, we found regional heterogenicity in fatty acid status, wherein women in the BCGP cohort had higher concentrations of plasma n–3 PUFAs, specifically EPA and DHA, compared with women in the ATP cohort. Considered in isolation, ATP and BCGP had inconsistent associations of plasma phospholipid fatty acid status with breast cancer risk. When adjusted for possible confounders, in ATP, SFAs were associated with reduced risk while AA, DHA, the combination of EPA + DHA, and LC n–6 fatty acids were associated with increased breast cancer risk, whereas in the BCGP, vaccenic acid and LC n–3 were inversely associated and the ratio of total n–6:total n–3 was positively associated with breast cancer risk. A priori stratification revealed that these associations were driven by waist-to-hip ratio in BCGP and both waist-to-hip ratio and menopausal status in ATP. Our study is unique as it has been conducted for the first time in a Canadian population, highlighting provincial variations in fatty acid status. To our knowledge, this is the first study to assess associations between breast cancer risk and fatty acids stratified by waist-to-hip ratio (a more accurate measurement of central adiposity compared with BMI).

The use of plasma for phospholipid fatty acid analysis provides an easily accessible, minimally invasive sample that has longer fatty acid stability compared with RBCs (47). Extraction of plasma phospholipids versus total plasma lipids avoids the pool of postprandial triacylglycerols and is believed to be a reliable estimation of a person's usual fatty acid status (47). Furthermore, the EPA and DHA plasma phospholipid contents observed in this study are similar to a previous study in pre- and postmenopausal Canadian women (48). While both ATP and BCGP are large, robust longitudinal studies, dietary intake data were not collected for BCGP; therefore, it is not possible to comment specifically on the status of EPA and DHA relative to dietary intake in this cohort. However, using the limited dietary intake available for ATP, we observed moderate but consistent correlations between recalled intake (diet and supplements) and plasma phospholipid DHA and EPA composition. In addition, a 2016 study that assessed dietary patterns in women at high risk for breast cancer reported plasma phospholipid ranges similar to the current study in women consuming a "modern diet" versus a "traditional diet." Dietary information for the dietary pattern study was obtained from a cohort of Canadian women in Ontario and British Columbia (49, 50). Taken together, this information infers the reliability of the plasma phospholipid data we obtained in the absence of dietary intake data.

The regional differences observed in our study are consistent with the discrepant findings previously reported in the literature (16–37). Current evidence, from studies using varied tissue sources of fatty acids—breast adipose tissue, erythrocytes, serum, and plasma—suggests a lower risk of breast cancer with higher fatty acid content of linoleic acid (16, 24, 26), stearic acid (18), ALA (19, 23), DHA (23), EPA (26, 28), total n–6 (16, 24), total n–3 (17), desaturase index (ratio of product to substrate, 18:1n-9:18:0) desaturase index (DI_{18}) (18), or PUFAs (24), as well as elevated risk of breast cancer with higher fatty acid content of palmitic acid (20, 26, 34), palmitoleic acid (26, 27, 36), oleic acid (20), MUFAs (20), SFAs (22, 34), and total n–3 (17). The difficulty in forming a strong conclusion from these studies could be due in part to a variety of factors: *1*) differences in dietary intake or dietary patterns based on geographic location (51–53); *2*) heterogeneity in tissue sources (47); *3*) controls in 2 instances were women with breast benign disease (19, 23); *4*) several studies only assessed postmenopausal women (17, 20, 25, 28, 29), whereas others had groups primarily containing premenopausal women (19, 26, 37); and *5*) failure of some studies to address (consider or list) in their analysis known risk factors such as BMI or age. Many studies that have used fatty acids as a biomarker are based on European or Asian populations (18, 20, 25–27), regions that have higher concentrations of EPA + DHA in blood fractions compared with North American populations (54). Two studies from the United States had DHA content similar to ATP (29, 37) and an Australian study had DHA content similar to our BCGP cohort (34), yet no clear associations between DHA and breast cancer risk were established in these studies. The regional variations in fatty acid content, including differences in concentrations of LC-PUFAs, underscore the complexity of using plasma phospholipid fatty acid status as a biomarker for breast cancer risk. The availability of 2 cohorts with distinctly different fatty acid profiles provided a means of assessing the impact of n–3 LC-PUFAs on breast cancer risk and offers evidence that suggests demographic or geographic influences on dietary intake and resultant fatty acid content impact future breast cancer risk. Indeed, the quartiles for relative percentage of DHA in BCGP were 25–30% higher than those in ATP and the results suggest that this variance in fatty acid content could influence risk when the data are stratified by menopause status or body composition.

In isolation, both menopausal status and body composition are both known to influence breast cancer risk (46). Breast cancer is a hormone-related, heterogeneous disease, whose etiology differs based on menopausal status (55, 56). In premenopausal women, the risk of breast cancer is inversely associated with higher body fatness, while the opposite is true for postmenopausal women (positively associated) (46). This is believed to be related to the fact that the major source of estrogen postmenopause comes from adipose tissue, resulting in obese women having higher serum concentrations of estradiol and a resultant increased risk (57). It is therefore plausible that in order to establish clear associations between fatty acid content and breast cancer risk, one must not only consider geographic location and the dietary influences on fatty acid status but also the menopausal status and measures of body-fat distribution of the women. The a priori stratification used in this study allowed us to further delineate the observations observed in the original multivariate analysis and determine that risk is dependent on the multifactorial combination of fatty acid status, menopause status, and body fatness. While no associations were observed in BCGP when stratified by menopausal status, in premenopausal women in ATP, SFAs (driven by stearic acid and 24:0) were associated with decreased risk, which is contrary to previously reported findings suggesting that higher concentrations of SFAs were associated with an increased risk (22, 34, 37). Interestingly, samples in these studies had lower amounts of total SFAs (40–42%), compared with the ATP cohort (~47%). How a higher content could be protective or how the balance or mixture of SFAs could influence risk should be explored further. From a mechanistic perspective, stearic acid has been suggested to induce apoptosis in breast cancer cells (58) and availability to tumors could therefore be protective against tumoral development. Dietary components including carbohydrates influence the status of fatty acids and could affect the fat-to-carbohydrate ratio as excess carbohydrates are converted to medium-

chain fatty acids and MUFAs through de novo lipogenesis (59). Therefore, we examined key fatty acids that could be reflective of a higher carbohydrate intake and at the desaturation ratios (ratio of product to substrate, 18:1n–9:18:0). The desaturation index can be used as a surrogate marker of stearoyl-CoA desaturase (SCD; Δ9 desaturase) activity as it is a possible indirect marker of de novo lipid synthesis. Increased SCD activity has been implicated in increased tumoral growth (60–62) and while it varies with breast cancer subtype, it is believed to influence breast cancer survival (63). It is important to use caution when interpreting associations derived from the calculated ratio as it is a surrogate marker of SCD because it does not take into consideration other factors that could influence SCD activity, nor is it solely representative of endogenous synthesis; however, DI_{18} was associated with increased risk in premenopausal ATP women. This combination of decreased OR with stearic acid and increased OR with DI_{18} has been previously observed in 2 European studies (18, 36) but was not observed in the BCGP cohort.

Essential fatty acids, including linoleic and LC n–3 fatty acids, must be obtained from the diet as they are not endogenously synthesized. The relation between the status of these fatty acids and breast cancer risk continues to be unclear (64). Statistical interactions for linoleic acid, ALA, and total LC n–3 suggested inverse associations in postmenopausal women compared with premenopausal women in ATP. This is in accordance with 2 prior studies that have suggested inverse associations with breast cancer for linoleic acid (16, 24). Contrary to positive epidemiological associations of EPA and DHA with risk reduction, in ATP, LC n–3 fatty acids were associated with increased risk of breast cancer in premenopausal women. This association of LC n–3 fatty acids with risk has been previously observed in 2 prospective studies of prostate cancer (65, 66) and these cohorts had a similar DHA status in phospholipids compared with our ATP cohort. However, it is important to note that, in ATP, women in the second quartile were associated with the highest risk and, while risk increased in the fourth quartile, it was trending downwards. This could suggest an optimal range for reduced risk. Furthermore, in concordance with a meta-analysis (12), we observed a trend towards risk reduction in postmenopausal women based on n–3 status, driven by a decrease in risk due associated with ALA. It has been suggested that the beneficial effects of these LC n–3 PUFAs occurs after long-term exposure. This is an interesting hypothesis and merits further investigation.

There have been several studies that have established an association between body fatness (primarily using BMI) and breast cancer risk, particularly in North American populations (46). In Alberta, it is estimated that 8% of breast cancer cases are attributable to being overweight/obese, as measured by BMI (2). Weight distribution and central adiposity, determined by the waist-to-hip ratio, are thought to be a better modality to assess regional adiposity compared with BMI alone as they have been shown to better predict morbidity and are considered a stronger predictor of all cancer risk (67). To our knowledge, this is the first nested case-control study using a prospective longitudinal cohort to examine associations between fatty acid status and waist-to-hip ratio. While there is a strong relation between obesity and dietary intake, associations of fatty acid status and breast cancer risk among healthy, overweight or obese women have not been thoroughly explored. The differences between the 2 cohorts, ATP and BCGP, provide a striking contrast and suggest the influence of specific fatty acids obtained from the diet on cancer risk. In the ATP cohort, in women with a waist-to-hip ratio <0.85, DHA was positively associated and the ratio of AA:DHA negatively associated with breast cancer. Yet, in the BCGP cohort, women with a waist-to-hip ratio <0.85 had a decreased risk of breast cancer with increased DHA, EPA + DHA, total n–3, and total LC n–3 and an increased risk of breast cancer with higher total n–6:total n–3 or AA:DHA ratios. These associations were attenuated in women with waist-to-hip ratios that were above guidelines, suggesting that the protective effect of these fatty acids on breast cancer risk may be attenuated in overweight or obese women.

Strengths of our study include the following: *1*) availability of cases and controls nested within 2 robust longitudinal population-based cohorts, with a large number of breast cancer incidences and extensive harmonized epidemiological data (68); *2*) biosamples that were obtained prediagnosis, therefore not subjected to any potential biases in collection of data; *3*) all biosamples were processed at the same facility and time frame to avoid any discrepancies in sample processing; and *4*) biolinkage to provincial cancer registries in Alberta and British Columbia to confirm cancer cases.

Limitations of the study include that both the ATP and BCGP cohorts do not encompass the same sociodemographic diversity observed in the Canadian population and could limit generalizability. For example, the 2 cohorts combined identified predominantly as white (92.4%) and there was a slightly higher proportion of women who were overweight or obese compared with the overall Canadian population (58.3% vs. 53.4% according to the Canadian Community Health Survey cycle 2.2) (39, 40). In addition, although the biological values obtained are reflective of net metabolic processes and are of scientific merit, dietary intake data were only available for a subcohort from the ATP and therefore we cannot confirm to what degree fatty acid status from a single nonfasting plasma sample represents dietary intake for the combined cohorts. Future longitudinal studies would benefit from including this metric in their data collection. Furthermore, it is possible that changes in dietary intake after sample collection could influence future cancer incidence. Finally, multiple associations were assessed in this study based on biologically justified a priori hypotheses. However, accounting for these comparisons with Bonferroni and Holm-Bonferroni corrections for statistical significance would reduce the number of associations observed to reach statistical significance and we interpret these results with caution. The available number of breast cancer cases in each cohort is a limitation of this study and it is possible that a larger cohort is needed to identify small differences in fatty acids associated with breast cancer risk.

In conclusion, our study is the first to demonstrate regional variations in fatty acid status and subsequent breast cancer risk in a Canadian population. Dietary intake affects LC n–3 fatty acid status and researchers need to consider that an optimal range and balance or mixture of other fatty acids could influence the protective effect of these LC-PUFAs. Furthermore, that these associations were observed with a priori stratification by menopausal status and central adiposity suggests the importance of modifiable dietary intake in addition to other metabolic or endocrine factors that potentially mediate fatty acid status. It highlights the complexity and difficulty in using a single biomarker to predict breast cancer risk. Further investigation into these associations could identify strategies for breast cancer prevention.

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