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Erythrocyte membrane fatty acids and breast cancer risk: a prospective analysis in the Nurses' Health Study II

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Abstract

The roles of specific fatty acids in breast cancer etiology are unclear, particularly among premenopausal women. We examined 34 individual fatty acids, measured in blood erythrocytes collected between 1996–1999, and breast cancer risk in a nested case-control study of primarily premenopausal women in the Nurses' Health Study II. Breast cancer cases diagnosed after blood collection and before June 2010 (n=794) were matched to controls and conditional logistic regression was used to estimate OR's (95% CI's) for associations of fatty acids with breast cancer; unconditional logistic regression was used for stratified analyses. Fatty acids were not significantly associated with breast cancer risk overall; however, heterogeneity by body mass index (BMI) was observed. Among overweight/obese women (BMI≥25), several odd-chain saturated (SFA, e.g. 17:0, OR_{Q4vsQ1}(95%CI) =1.85 (1.18–2.88), p_{trend}=0.006 p_{int}<0.001), *trans* (TFA, e.g. 18:1, OR_{Q4vsQ1}(95%CI) =2.33 (1.45–3.77), p_{trend}<0.001, p_{int}=0.007) and dairy-derived fatty acids (SFA 15:0 + 17:0 + TFA 16:1n-7t; OR_{Q4vsQ1}(95%CI) =1.83(1.16–2.89), p_{trend}=0.005, p_{int}<0.001) were positively associated, and n-3 polyunsaturated fatty acids (n-3 PUFA, e.g. alpha-linolenic acid; OR_{Q4vsQ1}(95%CI) =0.57 (0.36–0.89), p_{trend}=0.017, p_{int}=0.03) were inversely associated with breast cancer. Total SFA were inversely associated with breast cancer among women with BMI<25 (OR_{Q4vsQ1}(95%CI) =0.68 (0.46–0.98), p_{trend}=0.05, p_{int}=0.01). Thus, while specific fatty acids were not associated with breast cancer overall, our findings suggest positive associations of several SFA, TFA and dairy-derived fatty acids and inverse associations of n-3 PUFA with breast cancer

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among overweight/obese women. Given these fatty acids are influenced by diet, and therefore are potentially modifiable, further investigation of these associations among overweight/obese women is warranted.

Keywords

Breast cancer; fat; erythrocyte; fatty acids; diet

Introduction

Dietary fat intake has long been hypothesized to increase breast cancer risk, although no overall association has been observed in a large pooled analysis [1], a recent meta-analysis of 10 prospective studies [2], or in randomized trials [3, 4]. However, dietary fat is composed of many different fatty acids with distinct biological effects; and individual fatty acids may have differing associations with breast cancer risk. Findings from epidemiologic studies of dietary fatty acid intake and breast cancer risk have been inconsistent [5–9], and it is possible that biomarkers could provide better indications of intake of some fatty acids than assessments of diet. In addition, fatty acids that are synthesized and/or transformed *in vivo* may contribute to carcinogenesis, but are not captured by dietary intake [10]. Thus, circulating fatty acid concentrations that represent both dietary intake and internal transformation of fatty acids may provide a more direct measure of endogenous exposure.

Findings from prospective studies of plasma or serum fatty acids and breast cancer risk (N=58–363 cases), have been mixed [11–16]; with inverse associations observed with polyunsaturated fatty acids (PUFA)[11, 12, 15, 16] and positive associations with *trans* fatty acids (TFA) [12, 14, 15] and specific dairy-derived fatty acids [12, 14]. However, these studies included limited data on premenopausal women (≤91 cases), and relied on serum measures of fatty acids, which may only reflect dietary intake over several days to weeks. The fatty acid composition of the erythrocyte membrane is thought to represent an integrated measure of the interactions between dietary fatty acid intake, other dietary factors and patterns of fatty acid metabolism; and reflects dietary intake over several months [17]. To our knowledge, five prior studies of primarily postmenopausal women (N=46–322 cases) have examined erythrocyte membrane fatty acids and breast cancer risk, and results have been inconsistent [18–22]. For example, positive associations of erythrocyte saturated fatty acids (SFA) and breast cancer were observed among Asian women [21, 22], but not in other populations [18–20]. Although findings from studies of both erythrocyte and plasma or serum fatty acids and breast cancer have been mixed, studies of circulating fatty acids have largely been conducted among postmenopausal women. Given that dietary intake of animal fat [6, 7] and TFA [8] have been associated with breast cancer risk among pre- but not postmenopausal women, fatty acids may be particularly important in breast cancer etiology among premenopausal women. Notably, associations of several erythrocyte fatty acids and breast cancer risk varied according to menopausal status in the only prior study to report stratified associations [20]; although this case-control study was small (n=46 total breast cancer cases) and only considered five individual fatty acids.

Fatty acids may influence breast cancer etiology through inflammatory processes, as inflammation promotes tumor growth, angiogenesis, invasion and metastasis [23]. For example, TFA intake has been associated with increased circulating levels of inflammatory markers [24] and dairy-derived SFA and TFA, as well as TFA from partially hydrogenated oils (industrial *trans*), are associated with adverse metabolic effects [25]. In contrast, n-3 PUFAs may reduce breast cancer risk through their anti-inflammatory properties [26]. The saturation indices (SI) in blood cell membranes represent the ratios of the two most common SFA in tissues and the monounsaturated fatty acids (MUFA) that are direct metabolites of these SFA; palmitic/palmitoleic acid (SI_{n-7}) and stearic/oleic acid (SI_{n-9}). Lower SI ratios reflect higher activity of several enzymes involved in lipid metabolism, including fatty acid synthase and steroyl coenzyme-A desaturase (SCD1), which are often overexpressed in breast cancer [27]. Limited prospective data suggests inverse associations of SI ratios (lower SCD1 activity) and breast cancer risk [13, 14, 19]; although other studies have been inconclusive [12, 16, 18].

The Nurses' Health Study II (NHSII), with over 29,000 archived blood samples collected in 1996–1999, represents a unique opportunity to evaluate associations of specific fatty acids in erythrocyte membranes and subsequent risk of breast cancer among predominantly premenopausal women. Based on plausible biological mechanisms and prior evidence, we hypothesized that odd-chain SFA and TFA derived from animal fat (dairy-derived FA), TFA from processed foods (industrial *trans*), and the SCD1 activity, measured by the endogenous desaturation of SFA to MUFA (lower SI_{n-7} and SI_{n-9} ratios) would be associated with increased breast cancer risk; and that n-3 and n-6 PUFA would be inversely associated with breast cancer risk.

Materials and Methods

Study population

The NHSII cohort began in 1989 among 116,429 female registered nurses, aged 25–42 years from 14 states across the United States. Women continue to be followed via biennial questionnaire to assess lifestyle factors and disease diagnoses, with cumulative follow-up rates >90%. Between 1996 and 1999, 29,611 NHSII participants, ages 32–54 years, provided blood samples and answered a questionnaire assessing the date and time the sample was drawn, the number of hours since the last meal, current weight, and recent medication use. Further details of the blood collection procedure for NHSII have been described previously [28]. Briefly, participants had blood drawn and shipped the sample on ice to our laboratory via overnight courier. All samples were processed in our laboratory into plasma, white blood cell, and red blood cell components and have been stored at ≤130 degrees C in continuously monitored liquid nitrogen freezers.

Case and control selection

NHSII participants who were cancer-free at the time of blood collection and diagnosed before June 1, 2007 were included as cases in this nested case-control study. Breast cancer cases (n=794) were each matched to a single control on age at blood draw (+/−1 year), menopausal status at blood draw and diagnosis, self-reported race/ethnicity, fasting status

(<2, 2–4, 5–7, 8–11, ≥12 hours since last meal), and month (+/−1 month), and time of day of blood collection (+/−2 hours). Premenopausal women were also matched on luteal day, and postmenopausal women were also matched on menopausal hormone therapy use at blood draw (yes/no). Given that over 99% of reported breast cancer cases in the NHSII were confirmed upon medical record review [29], we included 27 breast cancer cases confirmed by the nurse when no medical records were available. This study was approved by the Committee on the Use of Human Subjects in Research at the Brigham and Women's Hospital (Boston, MA).

Laboratory assays

Erythrocyte fatty acid concentrations were assayed using gas-liquid chromatography [30] in Dr. Hannia Campos' laboratory at the Harvard T.H. Chan School of Public Health, Department of Nutrition. The samples were labeled to mask case-control status, and matched case-control sets were handled identically and together, and assayed in the same analytical run. The order within each case-control set was determined at random. Masked replicates from pooled specimens (~10% of samples) were analyzed to monitor quality control. These aliquots were indistinguishable from the participant specimens, and were interspersed among them without the knowledge of the laboratory personnel. A total of 39 individual fatty acids were analyzed and expressed as a percentage of total fatty acids. However, concentrations were undetectable for all (or the majority of) participants for several SFAs (octanoic acid, decanoic acid and tridecanoic acid) and TFAs (myristelaidic acid, and eicosenoic acid); and these were therefore excluded from the study. Out of the 34 remaining fatty acids, 8 fatty acids with levels close to the detection limit had coefficients of variation (CVs) >20% and up to 95%, (lauric acid, mystristic acid, pentadecanoic acid, mysristoleic acid, docosadienoic acid, palmitelaidic acid, linolelaidic acid, and octadecadienoic acid). Because our *a priori* hypotheses included individual fatty acids, we include all 34 fatty acids in the analysis. However, our interpretations are cautious for fatty acids with higher CVs.

Fatty acids

We examined erythrocyte fatty acids individually, and in the following groups by type; SFA, monounsaturated fatty acids (MUFA), n-3 PUFA, n-6 PUFA, and TFA. When possible, we use established common names for fatty acids; but also include the isomer notations specifying the number of carbon bonds and double bonds separated by colon, with "n" indicating the distance of first double bond from the methyl end of the chain. The letters "c" and "t" indicate whether the double bonds are in a *cis* or *trans* configuration. The following fatty acids were included in the analysis:

SFA: lauric acid (12:0), mystristic acid (14:0), pentadecanoic acid (15:0), palmitic acid (16:0), margaric acid (17:0), stearic acid (18:0), nonadecanoic acid (19:0), arachidic acid (20:0), behenic acid (22:0), tricosanoic acid (23:0), and lignoceric acid (24:0).

MUFA: mysristoleic acid (14: 1n-5c), pentadecenoic acid (15:1n-5c), palmitoleic acid (16:1n-7c), oleic acid (18:1n-9c), octadecenoic (18:1n-7c), gondoic acid (20:1n-9c), nervonic acid (24:1n-9c).

n-3 PUFA: alpha-linolenic acid (ALA; 18:3n-3c), eicosapenaenoic acid (EPA; 20:5n-3c), docosapentaenoic acid (DPA; 22:5n-3c), and docosahexaenoic acid (DHA; 22:6n-3c).

n-6 PUFA: linoleic acid (18:2n-6cc), gamma-linoleic acid (18:3n-6c), eicosadienoic acid (20:2n-6c), dihomo-gamma linolenic acid (20:3n-6c), arachidonic acid (20:4n-6c), docosadienoic acid (22:2n-6c), and aolrenic acid (22:4n-6c).

TFA: palmitelaidic acid (16:1n-7t), linolelaidic acid (18:2n-6t), octadecadienoic acid (18:2n-7c), 18:1 *trans* (18:1n-12t + 18:1n-9t + 18:1n-7t) and 18:2 *trans* (18:2n-6ct + 18:2n-6tc).

In addition to the fatty acids listed above, we calculated the ratio of total n-6 PUFA to total n-3 PUFA, as this ratio has been associated with breast cancer risk [31]and is hypothesized to predict several chronic inflammatory diseases [32]. In secondary analyses, we also examined associations for total marine n-3 PUFA (EPA, DPA, & DHA), as these longer-chain fatty acids reflect a different food source than ALA. The saturation indices, SI_{n-7} (palmitic/palmitoleic acid) and SI_{n-9} (stearic/oleic acid), were considered as indicators of the steroyl coenzyme-A desaturase activity [33, 34]. We also examined SFA and TFA primarily from milk or meat from cattle or other ruminants (15:0 + 17:0 + 16:1n-7t), termed dairy-derived fatty acids, and TFA from partially hydrogenated oils (18:1 *trans* + 18:2 *trans*), termed industrial *trans* for analysis.

Statistical methods

We assessed Spearman correlations amongst individual and grouped fatty acids. The distribution of each fatty acid by case/control status was examined and quintiles were created based on the distribution among controls. We evaluated differences in fatty acid concentrations between cases and controls using the Wilcoxon signed-rank test, accounting for matched status. Relative risks (RR) and 95% confidence intervals (CI) of breast cancer in relation to individual and groups of fatty acids were estimated using conditional logistic regression models [35]. Information on potential covariates, including family history of breast cancer, history of biopsy-confirmed benign breast disease, age at menarche, age at first birth/parity, history of breastfeeding, non-steroidal anti-inflammatory drug (NSAID) and aspirin use, dietary quality and intake of macro- and micro-nutrients, alcohol consumption, smoking status, physical activity, and weight at blood draw was obtained from the questionnaire administered at the time of blood collection or the biennial questionnaire immediately prior to blood draw. Weight at age 18 years and adult height, used to calculate BMI at 18 years, was obtained from the 1989 questionnaire, and weight change since age 18 was calculated from weight at blood draw. The final model included age at menarche, age at first birth/parity, breastfeeding, family history of breast cancer, history of biopsy-confirmed benign breast disease, BMI at age 18, weight change between age 18 and blood collection, alcohol consumption and physical activity as categorized in the footnote of Table 3. For covariates with missing data (≤5.5%), we assigned to the missing data the mode for categorical variables, and the median for continuous variables. We modeled the medians of fatty acid quintiles as a continuous variable and used the Wald test to examine linear trend.

We conducted stratified analyses by menopausal status (at blood collection and diagnosis,), BMI at blood collection (<25 vs. ≥25 kg/m^2), waist circumference (dichotomized at the median), plasma total carotenoids (dichotomized at the median) and by tumor estrogen receptor status (ER +/−), tumor grade (1–3) and tumor size (</≥2 cm), using unconditional logistic regression, additionally controlling for matching factors. The likelihood ratio test was used to compare models with and without interaction terms between the stratification variables and the specific fatty acid concentrations (medians of the quintiles as a continuous variable). To assess heterogeneity by tumor characteristics, competing risk analysis with data duplication methods were used [36]. Although erythrocyte fatty acid levels were not significantly different between fasting and non-fasting samples [37], we conducted *a priori* sensitivity analysis restricted to those with fasting blood samples (n=532 pairs) to examine whether fasting status influenced associations. To preclude the influence of preclinical disease, we conducted additional analyses in which cases diagnosed within the first 2 years of follow-up were excluded (n=116 pairs). We also assessed models additionally adjusted for NSAID use and overall dietary quality, measured by the Alternative Healthy Eating Index (AHEI), in secondary analysis. We evaluated potential non-linearity between fatty acid concentrations and breast cancer risk non-parametrically using restricted cubic splines [38, 39]. In secondary analyses, we used previously published reproducibility data for the single fatty acid measures taken at two time points over 2 to 3 years [40], to correct for random within-person variation and laboratory error [41]. Given that statistical outliers may represent true values and that our analysis was based on quantiles, which are robust to extreme values, we did not exclude statistical outliers in our analysis. All statistical tests were two-sided and *p* values were considered statistically significant at <0.05; analyses were conducted in SAS v.9.3 (SAS Institute, Cary, NC).

Results

Breast cancer cases were similar to controls with regard to most demographic and reproductive characteristics (Table 1). However, compared with controls, breast cancer cases gained less weight since age 18, were less physically active, less likely to be parous, and more likely to have a history of benign breast disease and a family history of breast cancer (Table 1).

Median (10th–90th percentile) concentrations of erythrocyte fatty acids by case/control status are shown in Table 2. Among both cases and controls, the most abundant individual fatty acids were palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1n-9c), linoleic acid (18:2n-6cc), and arachidonic acid (20:4n-6c), collectively representing ~75% of total fatty acid concentrations. Relative concentrations of fatty acids did not differ substantially between cases and controls (all p-values>0.04); although the SI_{n-7} was slightly higher among cases. Some individual fatty acids were modestly correlated; median (10th–90th percentile) Spearman correlation coefficients of 0.21 (0.05–0.79) for positively correlated and −0.20 (−0.37–−0.04) for inversely correlated individual fatty acids. For fatty acid groups by type, Spearman correlations ranged from −0.75 (SFA and n-6 PUFA) to 0.20 (SFA and TFA).

Associations were generally similar in age-adjusted and multivariable models; thus, we only present the multivariable results (Table 3). We did not observe any significant associations of fatty acid concentrations and overall risk of breast cancer. However, a suggested positive association was observed between total TFA and risk of breast cancer, OR_{Q5vsQ1}(95% CI)=1.30 (0.92–1.84), p_{trend}=0.08, largely driven by the association with 18:1 *trans*, OR_{Q5vsQ1}(95% CI)=1.32 (0.94–1.86), p_{trend}=0.07.

For several fatty acids, the associations with breast cancer risk varied significantly by BMI (Table 4). For example, total SFA were inversely associated with risk of breast cancer among women with BMI<25 kg/m^2 (OR_{Q4vsQ1}(95% CI)=0.68 (0.46–0.98), p_{trend}=0.05), and a positive association was suggested among overweight/obese (BMI ≥ 25 kg/m^2) women, (OR_{Q4vsQ1}(95%CI) 1.41 (0.90–2.21), p_{trend}=0.07, p_{int}=0.01) (Table 4). Statistical interaction was also evident for several individual SFA including pentadecanoic acid (p_{int}=0.002, CV=23%), margric acid (p_{int}<0.001), stearic acid (p_{int}=0.003), nonadecanoic acid (p_{int}=0.01), and arachidic acid (p_{int}=0.02), with suggested inverse associations among women with BMI <25 kg/m^2 and significant positive associations among overweight/obese women. Associations between several n-3 PUFA also varied by BMI, with significant inverse associations observed for ALA (p_{trend}=0.02, p_{int}=0.03), EPA (p_{trend}=0.02, p_{int}=0.04), and DPA (p_{trend}=0.05, p_{int}=0.54) only among overweight/obese women. N-6 PUFA were not consistently associated with breast cancer risk in BMI-stratified models, although docosadienoic acid was positively associated (p_{trend}=0.001, p_{int}=0.01, CV=20.7%) and gamma-linolenic acid was inversely associated (p_{trend}=0.01, p_{int}=0.05) with breast cancer risk among overweight/obese women. We also observed significant interaction of associations between several TFA and dairy-derived fatty acids and breast cancer risk according to BMI, with positive associations limited to overweight/obese women for total TFA, palmitelaidic acid (CV=40.8%), 18:1 *trans*, dairy-derived fatty acids, and industrial *trans* (e.g., total TFA, OR_{Q4vsQ1}(95% CI) =1.88(1.17–3.03), p_{trend}=0.002, p_{int}=0.02). Finally, a suggested positive association of SI $ratio_{n-9}$ and breast cancer risk (p_{trend}=0.08) was observed among overweight/obese women, while no association was observed among women with BMI < 25 kg/m^2 (p_{int}=0.03). Given differences in associations of BMI and breast cancer risk according to menopausal status, we additionally examined BMI-stratified associations restricted to premenopausal women. In this analysis, SFA, TFA, dairy-derived fatty acids and n-3 PUFA were more strongly associated with breast cancer risk among overweight/obese premenopausal women (e.g., dairy-derived fatty acids, OR_{Q4vsQ1}(95%CI) =2.13 (1.22–3.71), compared to 1.83 (1.16–2.89) among all overweight/obese women.

We did not observe clear patterns of interactions in analyses stratified by ER status, abdominal obesity, plasma carotenoids, tumor grade, or tumor size (data not shown); although several significant associations were observed within strata. For example, a significant positive trend between 18:1 *trans* and breast cancer was observed only among those with greater abdominal adiposity (waist circumference ≥ 29 inches, p_{trend}=0.004, p_{int}=0.08). In analyses stratified by median plasma total carotenoids, positive associations with breast cancer risk were observed among women with higher carotenoid concentrations for total TFA group (p_{trend}=0.01, p_{int}=0.19), 18:1 *trans* (p_{trend}=0.01 p_{int}=0.23) and industrial *trans* (p_{trend}=0.01 p_{int}=0.21). Positive associations of several TFAs and breast cancer risk were evident for those with low grade tumors; total TFA (p_{trend}=0.02, p_{int}=0.33), 18:1 *trans*

(p_{trend}=0.01, p_{int}=0.21) and industrial *trans* (p_{trend}=0.03, p_{int}=0.32). TFA were also positively associated with risk of small (< 2 cm) breast cancers: similar findings were seen for total TFA (p_{trend}=0.003, p_{int}=0.26), palmitelaidic acid (p_{trend}=0.01, p_{int}=0.71, CV=40.8%), 18:1 *trans* (p_{trend}<0.001, p_{int}=0.15) and industrial *trans* (p_{trend}=0.002, p_{int}=0.25). Inverse associations were observed between levels of palmitoleic acid (p_{trend}=0.001, p_{int}=0.02) and gamma-linolenic acid (p_{trend}=0.01, p_{int}=0.01) and risk of small breast cancers. N-3 PUFA were inversely (p_{trend}=0.04, p_{int}=0.08) and the PUFA6:3 ratio was positively (p_{trend}=0.01, p_{int}=0.03) associated with risk of larger breast tumors (≥2 cm).

Results were similar in analyses excluding cases diagnosed during the first two years of follow-up, and in models additionally adjusted for NSAIDs and AHEI. However, inverse associations for DPA (p_{trend}=0.03) and positive associations of nonadecanoic acid (p_{trend}=0.07), total TFA (p_{trend}=0.01), 18:1 *trans* (p_{trend}=0.005) and industrial *trans* (p_{trend}=0.04) were observed in fasting samples (data not shown), while associations were not significant overall. In secondary analyses, total marine n-3 PUFA were not significantly associated with breast cancer risk, OR_{Q5vsQ1}(95% CI), 1.02 (0.72–1.44), p_{trend}=0.83, or in analyses stratified by BMI. The relationships between the majority of the individual fatty acids and breast cancer were, if any, linear; although a non-linear relationship was detected for mystristic acid (p-value for curvature=0.04, CV=41%). Given modest associations and relatively high ICCs, correcting for measurement error did not substantially change the results, albeit corrected relative risks were stronger. For example, for 18:1 *trans* (ICC (95%CI) =0.72 (0.55–0.84)), the uncorrected vs. corrected RR (95%CI) comparing the median fatty acid level of women in the highest vs. lowest quintile were 1.45 (1.06–1.99) and 1.62 (1.07–2.43), respectively.

Discussion

In this large, nested case-control study of primarily premenopausal women, erythrocyte fatty acids concentrations were not significantly associated with overall breast cancer risk. However, our findings suggest inverse associations of n-3 PUFA and positive associations of SFA, TFA and dairy-derived fatty acids with breast cancer risk among overweight and obese women.

Our findings that erythrocyte fatty acids were not associated with overall breast cancer risk are consistent with results from a large nested case-control study in Sweden [18]. However, in other studies, positive associations of SFA [21, 22], MUFA [19, 21, 22], and n-6 PUFA [21]; and inverse associations of n-3 PUFA [19, 21, 22], n-6 PUFA [19–22] and the SI ratios [19, 21, 22] have been observed. Our study was unique in its focus on premenopausal women, and this may have contributed to differences in findings across studies. However, we have observed positive associations of premenopausal dietary intake of animal fats [6, 7] and TFA [8] with breast cancer risk, suggesting the relevance of premenopausal dietary intake. To our knowledge, ours was the first study to examine associations of a comprehensive panel of erythrocyte TFA and breast cancer, and we observed a suggested positive association of total TFA and breast cancer risk, driven largely by the association with 18:1 *trans*. Although a single erythrocyte TFA (18:1n-9t) was not associated with breast cancer risk in a large study of postmenopausal women [19], positive associations have been observed in studies of

dietary TFA [8, 9], and in some [14] but not all [13] studies using serum TFA measures. Relative concentrations of TFA were similar in our study and prior studies using erythrocyte [19] and serum [13, 14] TFA measures; thus, differences across studies are not likely to be due to varying concentrations of TFAs across study populations.

While our hypotheses were not confirmed in our main analysis; interestingly, in *a priori* BMI-stratified analyses, several SFA, TFA and dairy-derived fatty acids (15:0, 17:0, and 16:1n-7t) were positively associated, and n-3 PUFA were inversely associated with breast cancer risk among overweight and obese women. Associations of dietary n-3 and n-6 PUFA and breast cancer risk did not vary by BMI in a prospective cohort study among Japanese women [42]. However, dietary n-3 PUFA were also associated with a lower risk of breast cancer among obese women in a large population-based case-control study in Mexico [43].; although this retrospective study relied on dietary recall after breast cancer diagnosis, which entails potential consequences for differential misclassification. To our knowledge, no prior study has examined associations of erythrocyte or serum fatty acids and breast cancer risk according to BMI; and the consistent heterogeneity observed in our study suggests that fatty acids may be particularly important for breast cancer risk in a state of adiposity.

Observed associations of fatty acids and breast cancer risk among overweight/obese women in this study may reflect effects of dietary intake of these fatty acids. The sensitivity of fatty acid composition in erythrocyte membranes to dietary intake differs across individual fatty acid types; fatty acids which are not endogenously synthesized (odd-chain SFA, n-3 PUFA, n-6 PUFA, and TFA) more likely reflect dietary intake than fatty acids that can be produced through metabolic processes, such as even-chain SFA and MUFA [44, 45]. Odd-chain SFA are largely derived from dietary consumption of dairy products, although these are also found in beef fat and fish, and erythrocyte contents of 15:0 and 16:1n-7t are sometimes used as biomarkers of dairy fat intake [37]. Thus, our findings that odd-chain SFA, TFA and dairy-derived fatty acids are positively associated with breast cancer risk among overweight and obese women, suggest the potential role of dietary intake of these fatty acids, largely through consumption of milk and other dairy products [46]. Moreover, given that n-3 PUFA largely reflect intake, dietary consumption of n-3 PUFA, particularly ALA and EPA, may reduce breast cancer risk among overweight and obese women. Therefore, dietary intake of these fatty acids among overweight and obese women and breast cancer risk should be examined in more detail.

Obesity is characterized by chronic low-grade inflammation and SFA, TFA and dairy-derived fatty acids may promote breast carcinogenesis in an obesogenic environment by exacerbating the inflammatory mechanisms altered in obesity. In fact, both SFA [47] and TFA [24] have been linked to elevated plasma markers of inflammation. Given that fatty acids can be metabolized into multiple lipid mediators of inflammation [48], effects of obesity on lipid metabolism may also explain observed associations of SFA, TFA and dairy-derived fatty acids among overweight/obese women. However, the associations persisted even with adjustment for BMI and waist circumference among overweight/obese women, suggesting that the observed associations are not due entirely to altered lipid metabolism as a result of adiposity [49]. Further studies are warranted to replicate our findings and to

assess how BMI might modulate associations of primarily odd-chain SFA, TFA and dairy-derived fatty acids with breast cancer.

Among overweight and obese women in this study, we observed significant inverse associations of total n-3 PUFA, as well as the individual n-3 PUFAs, ALA, EPA and DPA with breast cancer, while no associations were observed among women with BMI<25 kg/m^2. N-3 PUFA may reduce breast cancer by inhibiting production of eicosanoids [50]. It is also possible that the anti-inflammatory effects of n-3 PUFAs [26] may be more influential in a state of chronic inflammation resulting from increased adiposity. For example, n-3 PUFA may improve adipokine levels, enhance insulin sensitivity, and minimize inflammatory processes that are altered in obesity [43]. In BMI-stratified analyses of n-6 PUFA and breast cancer, docosadienoic acid was positively associated and gamma-linolenic acid was inversely associated with breast cancer among overweight/obese women. In experimental studies, gamma-linolenic acid inhibits the overexpression and hyperactivity of the fatty acid synthase oncogene closely linked to malignant transformation of mammary cells [51], which might contribute to our inverse finding among overweight/obese women; however, prior epidemiologic studies have suggested both positive [21] and inverse [22] associations with breast cancer. No significant interactions with BMI were observed for other n-6 PUFAs, or in analyses stratified by waist circumference, carotenoids, menopausal status, and ER status. Thus, our findings do not suggest a strong role of erythrocyte n-6 PUFAs in breast cancer risk.

We did not observe consistent associations of endogenously synthesized fatty acids (even-chain SFA and MUFA) and the saturation index ratios with breast cancer risk in this study. The SI ratios represent the activity of the enzyme delta-9 desaturase that converts SFA to MUFA, their direct metabolites. Thus, a higher SI ratio represents lower activity of the enzyme, while a lower SI ratio suggests higher conversion of the SFA to MUFA. Lower desaturase enzyme activity is thought to reduce breast cancer risk by limiting cancer cell proliferation and invasiveness, and impairing tumor formation and growth [33]. Thus, while inverse associations with the SI ratios observed in prior studies [19, 21] may be biologically plausible, our results do not support that delta-9 desaturase activity, converting SFA to MUFA, is important in breast cancer risk among predominately premenopausal women.

To our knowledge, ours is the largest prospective study of circulating fatty acids and breast cancer risk among primarily premenopausal women to date. Fatty acid composition in erythrocyte membranes reflects dietary intake over several months [17], and is a significant predictor of other disease outcomes, including heart disease [37], and non-aggressive prostate cancer [52]. Additional strengths of this study include a comprehensive assessment of erythrocyte TFA, many of which had not been evaluated in regard to breast cancer risk previously. Further, this study incorporated information on BMI, menopausal status, carotenoids, and tumor characteristics, including ER status, tumor size and grade. We also conducted several sensitivity analyses to ensure that results were not affected by outliers, fasting status or by the presence of preclinical disease. With available reproducibility data, we were also able to assess the potential influence of measurement error and explicitly correct our estimates in secondary analyses. Finally, this study was nested in a large, well-

characterized, prospective cohort with extensive covariate information and an ongoing, high rate of follow-up.

Although we only had a single blood sample to reflect long-term fatty acid levels, erythrocyte fatty acids capture a longer period of exposure than serum, and a single measure was reproducible over time among postmenopausal women in NHS, with a median 3-year ICC of 0.58; range 0.00 (lauric acid) to 0.87 (arachidonic acid) [40]. We evaluated a number of variables as potential confounders and adjustment had minimal effect on our results; however, residual confounding is possible. We were unable to separate the individual 18:1 TFA (18:1n-12t, 18:1n-9t, 18:1n-7t). Thus, the industrial TFA group includes 18:1n-7t (vaccenic acid), which originates largely from ruminant sources. However, given the observed risk estimates, the inability to separate out the 18:1 trans isomers is unlikely to significantly alter the study findings. It is also possible that we were unable to detect specific associations among fatty acids with considerable non-differential measurement error (i.e., those with high CVs); and given the observed significant findings, true associations are likely to be even stronger. While, we assessed multiple associations in this study, the analyses were guided by strong *a priori* and biologically justifiable hypotheses. Nonetheless, if we statistically accounted for multiple comparisons using the Bonferroni correction, none of the observed associations would reach statistical significance. Thus, given the large number of associations tested, we interpret our results with caution.

In this large nested case-control study of primarily premenopausal women, erythrocyte fatty acid concentrations were not associated with breast cancer risk overall. However, our findings suggest that among overweight/obese women, SFA, TFA and dairy-derived fatty acids may increase and n-3 PUFA may reduce breast cancer risk. Given these fatty acids are potentially modifiable by diet, and that two-thirds of American women are overweight or obese, further investigation of these associations among overweight/obese women is warranted.

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Novelty

The roles of specific fatty acids in breast cancer etiology are unclear, particularly among premenopausal women. In this large nested case-control study of primarily premenopausal women, erythrocyte fatty acids were not associated with breast cancer risk overall. However, our findings suggest potential inverse associations of n-3 polyunsaturated fatty acids and positive associations of saturated, *trans*, and dairy-derived fatty acids with breast cancer risk among overweight and obese women.

Table 1

Characteristics of breast cancer cases and matched controls, Nurses' Health Study II

	Case (n=794)	Control (n=794)
Age at blood collection (yr)	44.7(4.5)	44.8(4.4)
Age at menarche (yr)	12.4(1.3)	12.5(1.4)
BMI at age 18 (kg/m2)	20.8(2.8)	21.1(3.0)
BMI at blood collection (kg/m^2)	25.1(5.0)	25.8(6.0)
Weight change since age 18 (kg)	11.7(11.9)	12.8(13.4)
Total physical activity (METs/week)	17.9 (15.5)	18.8 (16.4)
Number of children	2.2(0.9)	2.3(1.0)
Age at first birth (yr)[a]	26.6(4.7)	26.2(4.6)
Parity, %	78.6	81.9
Ever breast fed[b], %	79.5	79.4
Premenopausal, %	77.6	76.8
History of biopsy-confirmed benign breast disease, %	23.4	15.4
Family history of breast cancer, %	17.3	9.9
NSAIDs current regular use[c], %	13.3	13.8
Total fat (grams/day)	59.0(12.3)	58.4(12.5)
Total Saturated Fat (grams/day)	20.4(5.4)	20.2(5.5)
Total Monounsaturated Fat (grams/day)	23.0(5.4)	22.7(5.2)
Total Polyunsaturated Fat (grams/day)	9.8(2.3)	9.7(2.4)
Alcohol consumption (grams/day)	3.8(6.9)	3.3(5.8)

Values are means(SD) or percentages.

[a] Age at first birth among women with children indicated on 1995 questionnaire

[b] Percent of breastfeeding among women with children on 1995 questionnaire

[c] Non-steroidal anti-inflammatory medication, regular use defined as ≥2 times/week

Table 2

Concentrations of erythrocyte fatty acids (% of total fatty acids) among cases and controls, Nurses' Health Study II

	case		control		
	Median	**10–90th%**	**Median**	**10–90th%**	**p-value**
Total Saturated Fatty Acids	42.2	40.0–46.8	42.3	40.2–46.4	0.33
Lauric acid (12:0)	0.01	0.0–0.04	0.01	0.0–0.04	0.77
Mystristic acid (14:0)	0.4	0.2–0.7	0.4	0.2–0.7	0.69
Pentadecanoic acid (15:0)	0.1	0.1–0.2	0.1	0.1–0.2	0.92
Palmitic acid (16:0)	20.7	18.9–22.8	20.7	18.9–22.8	0.66
Margric acid (17:0)	0.4	0.3–0.5	0.4	0.3–0.5	0.59
Stearic acid (18:0)	14.9	13.4–18.7	15.0	13.5–18.4	0.89
Nonadecanoic acid(19:0)	0.1	0.1–0.2	0.1	0.1–0.2	0.71
Arachidic acid (20:0)	0.4	0.3–0.5	0.4	0.3–0.5	0.71
Behenic acid (22:0)	1.5	1.2–2.0	1.5	1.2–2.0	0.50
Tricosanoic acid (23:0)	0.3	0.2–0.4	0.3	0.2–0.3	0.95
Lignoceric acid (24:0)	3.1	2.3–4.1	3.1	2.3–4.2	0.16
Total Monounsaturated Fatty Acids	16.1	14.7–17.9	16.2	14.6–17.9	0.38
Mysristoleic acid (14: 1n-5c)	0.01	0.0–0.03	0.01	0.0–0.03	0.38
Pentadecenoic acid (15: 1n-5c)	0.04	0.03–0.05	0.04	0.03–0.05	0.61
Palmitoleic acid (16: 1n-7c)	0.5	0.3–0.9	0.5	0.3–0.9	0.09
Oleic acid (18: 1n-9c)	12.3	11.1–13.9	12.4	11.1–13.8	0.57
Octadecenoic acid (18: 1n-7c)	1.1	1.0–1.3	1.1	1.0–1.3	0.47
Gondoic acid (20: 1n-9c)	0.2	0.2–0.24	0.2	0.2–0.24	0.04
Nervonic acid (24: 1n-9c)	1.8	1.3–2.5	1.8	1.3–2.5	0.64
n-3 Polyunsaturated Fatty Acids	5.3	4.2–7.1	5.3	4.2–7.0	0.79
Alpha-linolenic acid (ALA, 18: 3n-3c)	0.2	0.1–0.3	0.2	0.1–0.3	0.99
Eicosapentaenoic acid (EPA,20:5n-3c)	0.4	0.3–0.7	0.4	0.3–0.7	0.80
Docosapentaenoic acid (DPA,22:5n-3c)	1.8	1.4–2.1	1.8	1.4–2.2	0.21
Docosahexaenoic acid (DHA, 22:6n-3c)	3.0	2.1–4.3	2.9	2.1–4.2	0.38

	case		control		
	Median	**10–90th%**	**Median**	**10–90th%**	**p-value**
n-6 Polyunsaturated Fatty Acids	32.3	28.1–35.0	32.1	28.1–34.9	0.64
Linoleic acid (18:2n-6cc)	13.6	11.3–16.2	13.4	11.1–15.9	0.11
Gamma-linolenic acid (18:3n-6c)	0.1	0.1–0.2	0.1	0.1–0.2	0.05
Eicosadienoic acid (20:2n-6c)	0.3	0.2–0.3	0.3	0.2–0.3	0.98
Dihomogammalinolenic acid (20:3n-6c)	1.5	1.2–2.0	1.5	1.2–2.0	0.24
Arachidonic acid (20:4n-6c)	13.4	11.3–15.0	13.4	11.5–15.3	0.21
Docosadienoic acid (22:2n-6c)	0.1	0.06–0.11	0.1	0.06–0.11	0.22
Aolrenic acid (22:4n-6c)	2.9	2.2–3.5	2.9	2.2–3.6	0.60
Total *Trans* Fatty Acids	2.2	1.5–2.9	2.1	1.5–2.9	0.41
Palmitelaidic acid (16:1n-7t)	0.2	0.1–0.2	0.2	0.1–0.2	0.17
Linolelaidic acid (18:2n-6t)	0.02	0.01–0.03	0.02	0.01–0.03	0.62
Octadecadienoic acid (18:2n-7c)	0.1	0.04–0.1	0.1	0.04–0.1	0.45
18:1 *trans*	1.6	1.1–2.3	1.6	1.1–2.2	0.26
18:2 *trans*	0.3	0.2–0.4	0.3	0.2–0.4	0.26
Dairy-derived Fatty Acids[a]	0.7	0.6–0.9	0.7	0.6–0.9	0.54
Industrial *trans*[b]	1.9	1.3–2.6	1.9	1.3–2.6	0.42
Total n-6/n-3 Ratio	6.0	4.3–7.7	6.0	4.3–7.7	0.98
SI ratio$_{n-7}$[c]	44.1	23.4–72.7	42.6	23.5–69.6	0.04
SI ratio$_{n-9}$[d]	1.2	1.0–1.6	1.2	1.0–1.5	0.77

[a] Dairy-derived Fatty Acids=15:0 + 17:0 + 16:1n-7t

[b] Industrial *trans*=18:1 *trans*+18:2 *trans*

[c] SI ratio$_{n-7}$ = Palmitic acid/Palmitoleic acid

[d] SI ratio$_{n-9}$=Stearic acid/Oleic acid

Table 3

Multivariable-adjusted* relative risk (95% CI) of breast cancer according to quintiles of erythrocyte fatty acid concentration, Nurses' Health Study II

	Quintiles of erythrocyte fatty acid concentrations					
	Q1	**Q2**	**Q3**	**Q4**	**Q5**	**p_{trend}**
Total Saturated Fatty Acids	1.0 (ref)	0.75 (0.53–1.06)	0.89 (0.63–1.25)	0.61 (0.43–0.89)	0.85 (0.60–1.21)	0.47
Lauric acid (12:0)	1.0 (ref)	0.78 (0.54–1.14)	1.12 (0.77–1.63)	0.80 (0.54–1.18)	0.86 (0.58–1.29)	0.48
Mystristic acid (14:0)	1.0 (ref)	0.83 (0.60–1.16)	0.80 (0.57–1.14)	0.79 (0.56–1.12)	0.91 (0.63–1.31)	0.80
Pentadecanoic acid (15:0)	1.0 (ref)	1.21 (0.87–1.69)	0.91 (0.64–1.29)	1.07 (0.76–1.51)	1.08 (0.76–1.54)	0.95
Palmitic acid (16:0)	1.0 (ref)	0.98 (0.69–1.39)	1.02 (0.71–1.46)	0.79 (0.53–1.17)	0.79 (0.51–1.22)	0.19
Margric acid (17:0)	1.0 (ref)	1.23 (0.87–1.74)	1.09 (0.78–1.53)	1.13 (0.80–1.60)	1.13 (0.80–1.61)	0.75
Stearic acid (18:0)	1.0 (ref)	1.21 (0.85–1.71)	1.16 (0.82–1.64)	0.83 (0.57–1.19)	1.15 (0.80–1.64)	0.94
Nonadecanoic acid(19:0)	1.0 (ref)	1.16 (0.83–1.63)	1.23 (0.87–1.73)	1.05 (0.74–1.50)	1.18 (0.81–1.71)	0.57
Arachidic acid (20:0)	1.0 (ref)	1.18 (0.85–1.64)	0.98 (0.70–1.38)	1.07 (0.75–1.51)	1.08 (0.75–1.55)	0.89
Behenic acid (22:0)	1.0 (ref)	1.30 (0.92–1.84)	1.34 (0.93–1.93)	1.15 (0.78–1.69)	1.17 (0.77–1.77)	0.81
Tricosanoic acid (23:0)	1.0 (ref)	1.01 (0.72–1.43)	0.96 (0.66–1.42)	0.91 (0.61–1.35)	1.10 (0.71–1.70)	0.81
Lignoceric acid (24:0)	1.0 (ref)	1.00 (0.70–1.44)	1.05 (0.71–1.55)	0.88 (0.58–1.35)	0.83 (0.52–1.33)	0.34
Total Monounsaturated Fatty Acids	1.0 (ref)	0.87 (0.63–1.21)	0.99 (0.70–1.39)	0.82 (0.57–1.16)	0.91 (0.64–1.30)	0.56
Mysristoleic acid (14: 1n-5c)	1.0 (ref)	0.88 (0.63–1.24)	0.81 (0.58–1.15)	0.88 (0.62–1.26)	0.82 (0.57–1.18)	0.42
Pentadecenoic acid (15: 1n-5c)	1.0 (ref)	0.92 (0.66–1.29)	1.16 (0.83–1.62)	0.96 (0.68–1.35)	1.06 (0.74–1.51)	0.70
Palmitoleic acid (16: 1n-7c)	1.0 (ref)	0.91 (0.65–1.26)	0.70 (0.49–1.00)	0.85 (0.60–1.21)	0.72 (0.49–1.04)	0.13
Oleic acid (18: 1n-9c)	1.0 (ref)	1.05 (0.76–1.44)	0.93 (0.67–1.30)	0.78 (0.55–1.10)	0.93 (0.66–1.31)	0.37
Octadecenoic acid (18: 1n-7c)	1.0 (ref)	0.85 (0.61–1.19)	0.91 (0.65–1.27)	1.06 (0.76–1.47)	1.00 (0.71–1.40)	0.64
Gondoic acid (20: 1n-9c)	1.0 (ref)	1.10 (0.79–1.55)	1.16 (0.82–1.65)	1.19 (0.83–1.69)	1.36 (0.95–1.94)	0.09
Nervonic acid (24: 1n-9c)	1.0 (ref)	1.22 (0.88–1.68)	0.99 (0.69–1.42)	1.08 (0.75–1.56)	1.06 (0.70–1.61)	0.86
n-3 Polyunsaturated Fatty Acids	1.0 (ref)	1.07 (0.78–1.49)	0.86 (0.62–1.20)	0.82 (0.58–1.16)	1.02 (0.72–1.44)	0.75
Alpha-linolenic acid (ALA, 18: 3n-3c)	1.0 (ref)	0.70 (0.50–0.98)	0.84 (0.61–1.17)	0.76 (0.54–1.05)	0.82 (0.58–1.16)	0.37
Eicosapentaenoic acid (EPA,20:5n-3c)	1.0 (ref)	1.23 (0.88–1.71)	1.04 (0.74–1.46)	1.03 (0.73–1.46)	1.00 (0.71–1.43)	0.62
Docosapentaenoic acid (DPA,22:5n-3c)	1.0 (ref)	0.80 (0.58–1.10)	0.84 (0.60–1.17)	0.74 (0.52–1.05)	0.71 (0.49–1.03)	0.07
Docosahexaenoic acid (DHA, 22:6n-3c)	1.0 (ref)	1.04 (0.75–1.45)	0.88 (0.63–1.24)	1.00 (0.71–1.40)	1.10 (0.78–1.54)	0.62

	Quintiles of erythrocyte fatty acid concentrations					
	Q1	**Q2**	**Q3**	**Q4**	**Q5**	**p_{trend}**
n-6 Polyunsaturated Fatty Acids	1.0 (ref)	0.80 (0.57–1.13)	1.05 (0.76–1.46)	1.25 (0.91–1.72)	0.98 (0.69–1.40)	0.33
Linoleic acid (18:2n-6cc)	1.0 (ref)	1.12 (0.80–1.56)	1.05 (0.75–1.47)	1.24 (0.88–1.74)	1.24 (0.88–1.75)	0.19
Gamma-linolenic acid (18:3n-6c)	1.0 (ref)	1.04 (0.76–1.42)	0.75 (0.54–1.06)	0.85 (0.61–1.19)	0.76 (0.54–1.07)	0.08
Eicosadienoic acid (20:2n-6c)	1.0 (ref)	1.08 (0.77–1.52)	0.71 (0.50–1.03)	0.80 (0.56–1.14)	1.09 (0.75–1.58)	1.00
Dihomogammalinolenic acid (20:3n-6c)	1.0 (ref)	1.18 (0.85–1.63)	1.02 (0.73–1.42)	1.02 (0.72–1.43)	1.13 (0.80–1.60)	0.76
Arachidonic acid (20:4n-6c)	1.0 (ref)	0.91 (0.66–1.26)	0.86 (0.62–1.19)	1.13 (0.81–1.57)	0.81 (0.58–1.14)	0.51
Docosadienoic acid (22:2n-6c)	1.0 (ref)	1.07 (0.77–1.50)	1.03 (0.72–1.46)	1.21 (0.86–1.71)	1.20 (0.83–1.73)	0.25
Aolrenic acid (22:4n-6c)	1.0 (ref)	1.00 (0.71–1.41)	1.07 (0.77–1.49)	1.40 (0.99–1.96)	1.01 (0.69–1.47)	0.35
Total *Trans* Fatty Acids	1.0 (ref)	0.99 (0.72–1.38)	1.09 (0.78–1.53)	1.14 (0.81–1.60)	1.30 (0.92–1.84)	0.08
Palmitelaidic acid (16:1n-7t)	1.0 (ref)	0.92 (0.66–1.29)	1.20 (0.86–1.66)	1.07 (0.77–1.49)	1.12 (0.78–1.60)	0.40
Linolelaidic acid (18:2n-6t)	1.0 (ref)	1.05 (0.75–1.46)	0.99 (0.70–1.38)	1.07 (0.76–1.51)	1.02 (0.70–1.49)	0.89
Octadecadienoic acid (18:2n-7c)	1.0 (ref)	1.46 (1.02–2.09)	1.32 (0.88–1.98)	1.18 (0.78–1.78)	1.17 (0.75–1.83)	0.85
18:1 *trans*	1.0 (ref)	0.96 (0.69–1.34)	1.09 (0.78–1.53)	1.10 (0.78–1.55)	1.32 (0.94–1.86)	0.07
18:2 *trans*	1.0 (ref)	0.95 (0.68–1.31)	1.01 (0.73–1.40)	1.05 (0.76–1.47)	0.81 (0.57–1.15)	0.40
Dairy-derived Fatty Acids[a]	1.0 (ref)	1.13 (0.81–1.60)	1.12 (0.79–1.57)	1.12 (0.80–1.57)	1.21 (0.85–1.71)	0.37
Industrial *trans*[b]	1.0 (ref)	1.05 (0.75–1.45)	1.19 (0.85–1.65)	1.25 (0.89–1.77)	1.21 (0.85–1.72)	0.19
Total n-6/n-3 Ratio	1.0 (ref)	0.88 (0.63–1.24)	1.08 (0.78–1.52)	1.00 (0.71–1.42)	1.09 (0.78–1.55)	0.45
SI $ratio_{n-7}$[c]	1.0 (ref)	1.04 (0.74–1.46)	0.97 (0.68–1.38)	1.06 (0.74–1.52)	1.32 (0.91–1.92)	0.13
SI $ratio_{n-9}$[d]	1.0 (ref)	1.05 (0.75–1.47)	0.93 (0.66–1.31)	1.04 (0.73–1.47)	1.05 (0.75–1.49)	0.75

Adjusted for: age at menarche (<12, 13–14, 14+ years), age at first birth/parity (nulliparous, 1–2 children <25 years, 3+ children <25 years, 1–2 children ≥25 years, and 3+ children ≥25 years), lactation (yes/no), family history of breast cancer (yes/no), history of benign breast disease (yes/no),, alcohol consumption(</≥5 grams/day), BMI at age 18 (<21, 21–23 and ≥23 kg/m^2), weight change between age 18 and blood collection(continuous), physical activity (<3, 3 to <9, 9 to <18, 18 to <27 and 27+ MET-hours/week)

[a] Dairy-derived fatty acids=15:0 + 17:0 + 16:1n-7t

[b] Industrial *trans*=18:1 *trans*+18:2 *trans*

[c] SI ratio$_{n\text{-}7}$= Palmitic acid/Palmitoleic acid

[d] SI ratio$_{n\text{-}9}$=Stearic acid/Oleic acid

Table 4

Multivariable-adjusted* relative risk (95% CI) of breast cancer according to quartiles of erythrocyte fatty acid concentration, stratified by BMI at blood collection, Nurses' Health Study II

	BMI < 25 kg/m^2(max 468 cases/428 controls)					BMI ≥ 25 kg/m^2 (max 325 cases/365 controls)					
	Quartiles of erythrocyte fatty acid concentrations					Quartiles of erythrocyte fatty acid concentrations					
	Q1	Q2	Q3	Q4	p_{trend}	Q1	Q2	Q3	Q4	p_{trend}	p_{int}
Total Saturated Fatty Acids	1.0 (ref)	0.76 (0.52–1.12)	0.57 (0.39–0.84)	0.68 (0.46–0.98)	0.05	1.0 (ref)	0.91 (0.58–1.44)	1.11 (0.70–1.76)	1.41 (0.90–2.21)	0.07	0.01
Lauric acid (12:0)	1.0 (ref)	0.94 (0.65–1.38)	0.97 (0.66–1.42)	0.87 (0.59–1.28)	0.50	1.0 (ref)	0.85 (0.54–1.34)	0.95 (0.61–1.49)	0.92 (0.58–1.46)	0.92	0.69
Mystristic acid (14:0)	1.0 (ref)	0.76 (0.52–1.10)	0.75 (0.51–1.11)	0.87 (0.60–1.27)	0.65	1.0 (ref)	0.85 (0.53–1.38)	0.97 (0.62–1.53)	1.08 (0.68–1.70)	0.52	0.44
Pentadecanoic acid (15:0)	1.0 (ref)	1.13 (0.76–1.67)	0.76 (0.51–1.14)	0.81 (0.54–1.19)	0.11	1.0 (ref)	1.09 (0.70–1.71)	1.31 (0.84–2.05)	1.68 (1.07–2.64)	0.02	0.002
Palmitic acid (16:0)	1.0 (ref)	1.30 (0.90–1.89)	1.00 (0.68–1.46)	0.91 (0.61–1.37)	0.46	1.0 (ref)	0.78 (0.50–1.23)	0.96 (0.60–1.54)	0.86 (0.54–1.36)	0.71	0.80
Margric acid (17:0)	1.0 (ref)	1.12 (0.73–1.72)	0.74 (0.48–1.13)	0.78 (0.51–1.19)	0.08	1.0 (ref)	1.15 (0.75–1.76)	1.28 (0.80–2.04)	1.85 (1.18–2.88)	0.01	0.000
Stearic acid (18:0)	1.0 (ref)	0.96 (0.65–1.42)	0.78 (0.52–1.15)	0.72 (0.49–1.06)	0.06	1.0 (ref)	0.96 (0.60–1.52)	1.01 (0.65–1.58)	1.58 (1.01–2.47)	0.02	0.003
Nonadecanoic acid (19:0)	1.0 (ref)	1.25 (0.82–1.89)	0.91 (0.60–1.38)	0.78 (0.51–1.19)	0.08	1.0 (ref)	1.28 (0.84–1.97)	1.38 (0.88–2.17)	1.71 (1.05–2.78)	0.03	0.01
Arachidic acid (20:0)	1.0 (ref)	1.26 (0.84–1.91)	0.74 (0.49–1.12)	0.87 (0.58–1.32)	0.18	1.0 (ref)	0.99 (0.65–1.50)	1.23 (0.77–1.96)	1.53 (0.97–2.41)	0.05	0.02
Behenic acid (22:0)	1.0 (ref)	1.52 (1.03–2.25)	1.39 (0.93–2.08)	1.20 (0.81–1.79)	0.68	1.0 (ref)	0.90 (0.58–1.40)	0.80 (0.51–1.26)	1.05 (0.66–1.68)	0.88	0.87
Tricosanoic acid (23:0)	1.0 (ref)	1.14 (0.77–1.68)	1.02 (0.69–1.51)	1.24 (0.84–1.85)	0.37	1.0 (ref)	0.99 (0.63–1.56)	0.99 (0.64–1.56)	0.99 (0.64–1.55)	0.98	0.56
Lignoceric acid (24:0)	1.0 (ref)	1.20 (0.82–1.75)	1.16 (0.79–1.69)	0.91 (0.61–1.35)	0.60	1.0 (ref)	0.62 (0.39–0.98)	0.74 (0.47–1.16)	0.82 (0.52–1.27)	0.61	0.83
Total Monounsaturated Fatty Acids	1.0 (ref)	1.18 (0.82–1.70)	0.85 (0.58–1.24)	1.02 (0.67–1.54)	0.69	1.0 (ref)	0.83 (0.52–1.33)	0.80 (0.49–1.29)	0.68 (0.42–1.09)	0.12	0.25
Mysristoleic acid (14:1n-5c)	1.0 (ref)	0.78 (0.54–1.13)	0.70 (0.47–1.02)	0.77 (0.53–1.13)	0.20	1.0 (ref)	0.69 (0.43–1.12)	0.84 (0.52–1.34)	0.96 (0.60–1.51)	0.57	0.20
Pentadecenoic acid (15:1n-5c)	1.0 (ref)	1.58 (1.04–2.41)	1.18 (0.79–1.77)	1.10 (0.74–1.63)	0.88	1.0 (ref)	0.69 (0.45–1.06)	1.00 (0.64–1.56)	1.15 (0.71–1.85)	0.41	0.32
Palmitoleic acid (16: 1n-7c)	1.0 (ref)	0.74 (0.52–1.05)	0.70 (0.48–1.02)	0.83 (0.52–1.30)	0.38	1.0 (ref)	1.17 (0.70–1.96)	1.10 (0.67–1.81)	0.89 (0.54–1.47)	0.34	0.82
Oleic acid (18: 1n-9c)	1.0 (ref)	0.82 (0.57–1.19)	0.85 (0.58–1.25)	0.89 (0.60–1.32)	0.54	1.0 (ref)	0.69 (0.43–1.10)	0.63 (0.40–1.00)	0.67 (0.42–1.05)	0.08	0.30
Octadecenoic acid (18:1n-7c)	1.0 (ref)	0.87 (0.58–1.29)	1.29 (0.87–1.89)	0.94 (0.63–1.40)	0.89	1.0 (ref)	0.64 (0.41–1.02)	0.84 (0.55–1.31)	1.01 (0.65–1.58)	0.68	0.96
Gondoic acid (20: 1n-9c)	1.0 (ref)	0.97 (0.64–1.46)	1.01 (0.68–1.52)	1.14 (0.77–1.68)	0.44	1.0 (ref)	1.22 (0.79–1.90)	1.19 (0.76–1.87)	1.46 (0.92–2.31)	0.12	0.71
Nervonic acid (24: 1n-9c)	1.0 (ref)	0.79 (0.54–1.17)	0.95 (0.65–1.40)	0.98 (0.67–1.43)	0.87	1.0 (ref)	1.10 (0.70–1.73)	0.92 (0.59–1.45)	1.05 (0.66–1.65)	1.00	0.77
n-3 Polyunsaturated Fatty Acids	1.0 (ref)	1.32 (0.88–1.97)	1.03 (0.70–1.51)	1.11 (0.76–1.64)	0.87	1.0 (ref)	0.74 (0.48–1.13)	0.68 (0.43–1.07)	0.68 (0.42–1.08)	0.11	0.23
Alpha-linolenic acid (ALA, 18: 3n-3c)	1.0 (ref)	0.81 (0.55–1.19)	0.93 (0.63–1.37)	1.07 (0.72–1.59)	0.47	1.0 (ref)	0.76 (0.48–1.20)	0.76 (0.49–1.17)	0.57 (0.36–0.89)	0.02	0.03

	BMI < 25 kg/m^2(max 468 cases/428 controls)					BMI ≥ 25 kg/m^2 (max 325 cases/365 controls)					
	Quartiles of erythrocyte fatty acid concentrations					Quartiles of erythrocyte fatty acid concentrations					
	Q1	Q2	Q3	Q4	p_{trend}	Q1	Q2	Q3	Q4	p_{trend}	p_{int}
Eicosapentaenoic acid (EPA,20:5n-3c)	1.0 (ref)	1.11 (0.76–1.63)	1.12 (0.76–1.64)	1.18 (0.79–1.75)	0.46	1.0 (ref)	0.82 (0.51–1.30)	0.84 (0.53–1.32)	0.56 (0.34–0.89)	0.02	0.04
Docosapentaenoic acid (DPA,22:5n-3c)	1.0 (ref)	0.89 (0.60–1.32)	0.72 (0.48–1.08)	0.85 (0.57–1.27)	0.34	1.0 (ref)	0.81 (0.53–1.24)	0.82 (0.53–1.28)	0.62 (0.39–0.98)	0.05	0.54
Docosahexaenoic acid (DHA, 22:6n-3c)	1.0 (ref)	1.25 (0.84–1.87)	1.16 (0.78–1.73)	1.31 (0.88–1.94)	0.26	1.0 (ref)	0.68 (0.44–1.06)	0.67 (0.43–1.05)	0.77 (0.49–1.23)	0.34	0.20
n-6 Polyunsaturated Fatty Acids	1.0 (ref)	0.84 (0.56–1.25)	1.18 (0.80–1.73)	1.29 (0.88–1.90)	0.10	1.0 (ref)	1.03 (0.66–1.61)	1.10 (0.71–1.70)	0.74 (0.46–1.18)	0.37	0.09
Linoleic acid (18:2n-6cc)	1.0 (ref)	1.24 (0.81–1.88)	1.36 (0.91–2.04)	1.65 (1.11–2.46)	0.01	1.0 (ref)	0.73 (0.48–1.13)	0.91 (0.58–1.42)	0.79 (0.51–1.24)	0.43	0.04
Gamma-linolenic acid (18:3n-6c)	1.0 (ref)	0.94 (0.66–1.34)	1.00 (0.69–1.46)	1.08 (0.71–1.63)	0.68	1.0 (ref)	1.17 (0.71–1.92)	0.88 (0.55–1.43)	0.62 (0.38–1.01)	0.01	0.05
Eicosadienoic acid (20:2n-6c)	1.0 (ref)	1.08 (0.73–1.60)	0.90 (0.61–1.35)	1.25 (0.85–1.84)	0.31	1.0 (ref)	0.78 (0.50–1.20)	0.77 (0.50–1.19)	0.80 (0.51–1.24)	0.32	0.15
Dihomogammalinolenic acid (20:3n-6c)	1.0 (ref)	1.01 (0.70–1.45)	1.10 (0.76–1.59)	0.94 (0.62–1.43)	0.92	1.0 (ref)	0.95 (0.58–1.55)	0.76 (0.46–1.24)	0.94 (0.59–1.50)	0.79	0.80
Arachidonic acid (20:4n-6c)	1.0 (ref)	0.99 (0.67–1.46)	1.15 (0.79–1.68)	0.98 (0.66–1.44)	0.89	1.0 (ref)	0.57 (0.36–0.90)	0.88 (0.57–1.36)	0.78 (0.50–1.21)	0.50	0.52
Docosadienoic acid (22:2n-6c)	1.0 (ref)	0.91 (0.61–1.36)	0.92 (0.61–1.39)	0.84 (0.57–1.25)	0.41	1.0 (ref)	1.44 (0.92–2.27)	1.44 (0.92–2.25)	2.23 (1.39–3.58)	0.00	0.01
Aolrenic acid (22:4n-6c)	1.0 (ref)	1.02 (0.70–1.50)	0.86 (0.58–1.27)	1.04 (0.70–1.55)	0.96	1.0 (ref)	0.98 (0.62–1.55)	1.32 (0.84–2.06)	1.16 (0.73–1.85)	0.35	0.56
Total *Trans* Fatty Acids	1.0 (ref)	0.83 (0.56–1.25)	1.06 (0.71–1.57)	0.91 (0.61–1.36)	0.89	1.0 (ref)	0.95 (0.59–1.51)	1.49 (0.94–2.36)	1.88 (1.17–3.03)	0.002	0.02
Palmitelaidic acid (16:1n-7t)	1.0 (ref)	1.08 (0.71–1.63)	1.24 (0.83–1.86)	0.87 (0.58–1.30)	0.48	1.0 (ref)	1.12 (0.71–1.77)	1.34 (0.85–2.12)	2.41 (1.50–3.89)	0.000	0.000
Linolelaidic acid (18:2n-6t)	1.0 (ref)	0.85 (0.58–1.26)	0.88 (0.61–1.29)	0.90 (0.61–1.33)	0.65	1.0 (ref)	0.99 (0.63–1.57)	1.41 (0.88–2.24)	1.18 (0.75–1.86)	0.33	0.24
Octadecadienoic acid (18:2n-7c)	1.0 (ref)	1.20 (0.82–1.74)	1.07 (0.73–1.56)	0.83 (0.55–1.24)	0.29	1.0 (ref)	1.30 (0.80–2.13)	1.28 (0.79–2.08)	1.49 (0.92–2.42)	0.14	0.10
18:1 *trans*	1.0 (ref)	0.73 (0.49–1.10)	1.00 (0.67–1.49)	0.91 (0.61–1.35)	0.98	1.0 (ref)	1.18 (0.75–1.87)	1.46 (0.92–2.33)	2.33 (1.45–3.77)	0.000	0.01
18:2 *trans*	1.0 (ref)	0.83 (0.57–1.21)	1.05 (0.71–1.53)	0.78 (0.52–1.15)	0.38	1.0 (ref)	0.78 (0.49–1.24)	0.75 (0.47–1.19)	0.81 (0.51–1.29)	0.42	0.95
Dairy-derived Fatty Acids[a]	1.0 (ref)	0.91 (0.60–1.39)	0.88 (0.59–1.33)	0.72 (0.48–1.08)	0.09	1.0 (ref)	1.00 (0.64–1.55)	1.21 (0.77–1.89)	1.83 (1.16–2.89)	0.005	0.000
Industrial *trans*[b]	1.0 (ref)	0.76 (0.50–1.13)	1.07 (0.71–1.59)	0.90 (0.61–1.34)	0.98	1.0 (ref)	0.84 (0.52–1.33)	1.31 (0.84–2.06)	1.86 (1.16–2.97)	0.003	0.02
Total n-6/n-3 Ratio	1.0 (ref)	0.92 (0.62–1.36)	1.15 (0.78–1.69)	1.09 (0.74–1.62)	0.49	1.0 (ref)	0.87 (0.55–1.39)	1.10 (0.69–1.74)	1.19 (0.74–1.91)	0.32	0.90
SI ratio$_{n-7}$[c]	1.0 (ref)	0.77 (0.48–1.21)	0.88 (0.56–1.39)	1.09 (0.70–1.71)	0.23	1.0 (ref)	1.30 (0.86–1.98)	1.36 (0.87–2.14)	1.17 (0.70–1.94)	0.46	0.99
SI ratio$_{n-9}$[d]	1.0 (ref)	0.91 (0.61–1.36)	0.74 (0.50–1.11)	0.80 (0.54–1.19)	0.23	1.0 (ref)	0.96 (0.62–1.48)	1.08 (0.68–1.72)	1.45 (0.93–2.27)	0.08	0.03

Adjusted for: age at menarche (<12, 13–14, 14+ years), age at first birth/parity (nulliparous, 1–2 children <25 years, 3+ children <25 years, 1–2 children >25 years, and 3+ children >25 years), lactation (yes/no), family history of breast cancer (yes/no), history of benign breast disease (yes/no), alcohol consumption(</>5 grams/day), BMI at age 18 (<21, 21–23 and >23 kg/m2), weight change between age 18 and blood collection(continuous), physical activity (<3, 3 to <9, 9 to <18, 18 to <27 and 27+ MET-hours/week)

[a] Dairy-derived fatty acids=15:0 + 17:0 + 16:1n-7t

[b] Industrial *trans*=18:1 *trans*+18:2 *trans*

[c] SI ratio$_{n-7}$= Palmitic acid/Palmitoleic acid

[d] SI ratio$_{n-9}$=Stearic acid/Oleic acid