Published in final edited form as:
Int J Cancer. 2011 March 15; 128(6): 1434–1441. doi:10.1002/ijc.25703.

Dietary Polyunsaturated Fatty Acids and Breast Cancer Risk in Chinese Women: A Prospective Cohort Study

Harvey J Murff, **Xiao-Ou Shu**, **Honglan Li**, **Gong Yang**, **Xiaoyan Wu**, **Hui Cai**, **Wanqing Wen**, **Yu-Tang Gao**, and **Wei Zheng**
Department of Medicine, Vanderbilt Epidemiology Center and Vanderbilt-Ingram Cancer Center, Vanderbilt University School of Medicine, Nashville, Tennessee; Tennessee Valley Healthcare System, Veterans Affairs Medical Center, Nashville, TN; Department of Epidemiology, Shanghai Cancer Institute, Shanghai, China; and Department of Biostatistics, Public Health College, Harbin Medical University, Harbin, P. R. China

Abstract

Breast cancer is the most common cancer in women. Controversy exists regarding the role of dietary fat in breast cancer etiology. We investigated the association of dietary polyunsaturated fatty acids (PUFA) and the ratio of n-6 PUFAs to marine-derived n-3 PUFAs with breast cancer risk in the Shanghai Women's Health Study, a prospective cohort study including 72,571 cancer-free participants at baseline. Dietary fatty acid intake was determined using food frequency questionnaires. We used Cox proportional hazards analysis to estimate the relative risks (RR) and 95% confidence intervals (CI) for the association of breast cancer risk with dietary fatty acids consumption. In 583,998 person-years of follow-up, we identified 712 breast cancer cases. We found no association of breast cancer risk to dietary intake of linoleic acid, arachidonic acid, α-linolenic acid, or marine-derived n-3 PUFA. We found a statistically significant interaction between n-6 PUFA intake, marine-derived n-3 PUFA intake and breast cancer risk ($p = 0.008$). Women with lower intake (the lowest tertile) of marine-derived n-3 PUFA and higher intake (the highest tertile) of n-6 PUFA had an increase risk for breast cancer (RR=2.06; 95% CI=1.27-3.34) compared to women with higher intake (the highest tertile) of marine-derived n-3 PUFAs and lower intake (the lowest tertile) of n-6 PUFAs after adjusting for potential confounders. The relative amounts of n-6 PUFA to marine-derived n-3 PUFAs may be more important for breast cancer risk than individual dietary amounts of these fatty acids.

Keywords

Breast Neoplasms; Fatty Acids, Unsaturated; Risk Factors; Cohort Studies

Introduction

The role of dietary fat in the etiology of breast cancer remains controversial.[1-3] Part of the uncertainty is due to the complex composition of dietary fat. Dietary fat represents a heterogenous group of lipids and fatty acids that can vary by carbon chain length, number of double bonds, position of double bonds, and spatial configuration. If individual fatty acids have conflicting effects on breast cancer risk, then analytic approaches incorporating composite dietary fat measurements (such as total fat or total polyunsaturated fat) might be misleading. This concern is plausible, as animal models have demonstrated that long chain

Correspondence and request for reprints: Harvey J. Murff, M.D., M.P.H., Vanderbilt Epidemiology Center, Institute for Medicine and Public Health, 2525 West End Avenue, Suite 600, Sixth Floor, Nashville, TN 37203-1738, Harvey.j.murff@vanderbilt.edu.

PUFAs have differential effects on mammary tumorigenesis based on double bond position.[4-7]

Meta-analyses of mouse mammary tumor models have suggested that n-6 PUFAs, such as linoleic acid (LA) and arachidonic acid (ARA) have a tumor promoting effect.[4, 6] A potential mechanisms behind the cancer promoting effects of n-6 PUFAs is through the production of proinflammatory eicosanoids such as prostaglandin E_2, which promotes angiogenesis and hinders apoptosis. Alternatively, marine-derived n-3 PUFAs, such as eicosapentanoic acid (EPA) and docosahexanoic acid (DHA) are the precursor molecules to eicosanoids that are less inflammatory when compared to ARA derived prostanoids.[8-9] As such the substitution of n-3 PUFA for n-6 PUFAs within the diet could have a chemopreventive effect.[10] Animal models have not consistently demonstrated that n-3 PUFAs reduce the risk of mammary carcinogenesis;[5, 7] however, background diet, specifically intake of n-6 PUFA may mitigate the protective effect on n-3 PUFAs.[11]

We used data collected as part of the Shanghai Women's Health Study, a prospective cohort study, to investigate the association of breast cancer risk to dietary polyunsaturated fatty acid intake. We also evaluated the impact of marine derived n-3 PUFAs on breast cancer risk in individuals reporting different dietary intakes of n-6 PUFAs.

Materials and Methods

The Shanghai Women's Health Study (SWHS) is a population-based prospective cohort study that enrolled 74942 women aged 40 to 70 years from seven urban communities in Shanghai from 1996 to 2000. A detailed study rationale and design has been published previously.[12] Briefly, study participants completed a baseline survey including information on dietary habits, reproductive history, hormone use, physical activity, personal medical history, smoking and alcohol history, occupational history and family cancer history. The overall study participation rate was 92.7%. In this study, we excluded participants with a prior history of cancer at baseline or with a missing date of cancer diagnosis (n = 1586). In addition, we excluded subjects with missing values for body mass index on the baseline survey (n = 59) and subjects reporting implausible total energy intakes (n = 726, caloric intake less than or greater than the 0.5 percentile for the entire cohort).

The cohort has been followed with a combination of biennial in-home interviews and annual record linkage with the Shanghai Cancer Registry and Shanghai Vital Statistics database. The in-home interviews collect interim health history details. The follow-up rates for the first, second and the third follow-up surveys were 99.8%, 98.7%, and 96.7% respectively. Follow-up rates have been optimized as almost the entire cohort (99.98%) has continued to live in Shanghai with 80% remaining in the same neighborhood over the course of the study.[12] All breast cancer cases identified through cancer registry matches are verified by medical charts from the diagnostic hospital. For this study we included all incident breast cancer cases (n = 712) diagnosed from baseline enrollment to December 2007.

At baseline (from 1996 to 2000), participants completed a comprehensive dietary assessment questionnaire. A second dietary assessment was completed during the first dietary follow-up survey from 2000 to 2004. Data were obtained regarding usual dietary intake over the past 12 months. Individual nutrient intakes for specific fatty acids and other nutrients were calculated by the product of the amount of each food consumed by the nutrient content of the specific food based on the Chinese Food Composition Table.[13] This food frequency questionnaire has been validated through comparisons of dietary intake derived from the FFQ to 24-hour dietary recalls in a dietary calibration study among 200 SWHS participants. [14] Although this study did not investigate specific PUFAs, it did

determine Pearson's correlation coefficients for intakes of fish (the major contributor for n-3 highly unsaturated fatty acids) and red meat (a major contributor towards ARA intake). For fish ($r = 0.50$) and red meat($r = 0.52$) the agreement of FFQ surveys and the average of multiple 24h dietary recalls was moderate to good.

To reduce error in assessing usual dietary intake, we calculate the mean reported dietary intake for specific nutrients based on the baseline and first follow-up dietary questionnaires. This mean value was used for all subsequent analyses. For participants who were diagnosed with any cancer type or diabetes mellitus during the period between the baseline FFQ and the second follow-up FFQ, only the baseline reported fatty acid intake was used for the data analysis given the concern that some women may have changed their dietary habits after the diagnosis of these diseases. We calculated dietary n-6 PUFA intake by combining LA and ARA. Dietary n-3 PUFA intake was calculated by combining α-linolenic acid (ALA), EPA, and DHA. Dietary marine-derived n-3 PUFA intake was calculated by combining EPA and DHA.

Baseline characteristics between breast cancer cases and non-cases were compared using the Chi-squared statistics for categorical variables and Students T-test for continuous variables. The levels of correlation between continuous baseline characteristics and dietary n-6 PUFA and marine-derived n-3 PUFA intake was determined using Spearman rank correlation as these variables were not normally distributed. We compared dietary median intake of n-6 and marine-derived n-3 PUFA by categorical baseline variables using Wilcoxon rank-order sum test.

Cox proportional hazards analysis was used to estimate the relative risks and 95% confidence intervals for the association of breast cancer risk with dietary fatty acids consumption. Entry time into the model was age at enrollment and exit time was age at censoring due to death or loss to follow-up. Age was used as the time scale in all models. Dietary intake of fatty acids were adjusted for energy intake using the residual method.[15] Dietary PUFAs were categorized into quintiles based on the overall distribution of the energy-adjusted nutrient intakes of the cohort. A priori covariates included for adjustment were age at cohort entry (continuous), body mass index (kg/m^2), family history of breast cancer (yes, no), highest obtained educational level (elementary school, middle school, high school, college or higher), smoking status (ever, never), alcohol use (ever, never), regular physical activity in past 5 years (yes, no), use of hormone replacement therapy (ever, never), personal diagnosis of diabetes mellitus (yes, no), total red meat consumption (grams/day), total fish consumption (grams/day), total vitamin E intake (IU/day), age at menopause (less than or equal to 50 years, greater than 50 years), age at first pregnancy (less than or equal to 20 years, greater than 20 years and less than or equal to 30 years, greater than 30 years), and parity (nulliparous, 1 to 2 pregnancies, greater than 2 pregnancies). Fish intake was included within the models as these food sources represent the major contributors to marine-derived n-3 PUFAs but might contain other potential confounding factors such as methylmercury or dioxins[16-17]. To determine whether the inclusion of fish intake might result in over adjustment of our models due to its strong correlation to marine-derived n-3 PUFAs we constructed models including fish intake as both a continuous variable and as a mean-centered variable. Our results were not appreciably changed and all data presented includes fish intake as a continuous variable. Because vitamin E intake may influence the effects of marine-derived fatty acids by modifying lipid peroxidation, we included this nutrient in all models as a potential confounder.[18-19] Menopausal status was included within the model as a time-dependant covariate. Models were stratified by birth cohort in five year intervals. Test for linear trend were conducted by including the median intake within each PUFA quintile within the model.

To test for a possible interaction between n-6 PUFA and marine-derived n-3 PUFA, we stratified energy-adjusted intake of both fatty acid types into tertiles. We used the likelihood ratio test to evaluate potential multiplicative interactions of the two variables by comparing the models with and without the cross product term of these variables. All statistical analyses were conducted using SAS version 9.1 (SAS Institute). All p values were two-sided and significant level was set at 0.05.

Results

Women who developed breast cancer were more likely to have a family history of breast cancer, have a higher educational level, a younger age at menarche, and an older age at menopause, be nulliparous, and older at first pregnancy compared to non-cases. (Table 1) There were no differences between cases and non-cases with regards to age, alcohol use, tobacco use, hormone replacement therapy use, body mass index, waist-to-hip ratio, or consumption of red meat, fish or vitamin E.

Consumption of n-6 PUFA was moderately correlated to red meat intake ($r = 0.47$, p-value <0.0001) and fish intake ($r = 0.43$, p-value $= 0.0001$). Intake of marine-derived n-3 PUFA was strongly correlated with fish intake ($r = 0.89$, p-value <0.0001) and weakly correlated red meat intake ($r = 0.25$, p-value <0.0001). (Table 2)

In multivariate models, we found no association between intake of dietary LA, ARA, ALA, and marine-derived n-3 PUFA and breast cancer risk. (Table 3) We found no association between ratios of n-6 to n-3 PUFA or n-6 to marine-derived n-3 PUFAs and breast cancer risk. There was a non-significant trend (p-value = 0.10) for an increased risk of breast cancer with increasing ratio of n-6 PUFA to marine-derived n-3 PUFA.

We evaluated the joint effect of n-6 PUFA consumption and marine-derived n-3 PUFA consumption on breast cancer risk. (Table 4) In general, the risk of breast cancer was increased with decreasing intake of n-3 PUFA and increasing intake of n-6 PUFA. Women who had the highest intake of n-6 PUFAs and the lowest intake of marine-derived n-3 PUFAs were at the highest risk of breast cancer (RR = 2.06, 95% CI = 1.27-3.34), compared to women in the lowest tertile of n-6 PUFA and highest tertile of marine-derived n-3 PUFA intake. For women with the highest intake of marine-derived n-3 PUFA, women in the highest tertile of n-6 PUFA had an increased risk of breast cancer compared to women in the lowest tertile of n-6 PUFA intake (RR = 1.63, 95% CI 1.06-2.51). There was a statistically significant interaction between total n-6 PUFA intake, marine-derived n-3 PUFA intake and breast cancer risk ($p = 0.008$).

Conclusions

In this large prospective study, we found no significant main effects for an association between dietary PUFAs intake and breast cancer risk however we did find a significant interaction between n-6 and marine-derived n-3 PUFAs. Women who reported consuming high levels on n-6 PUFAs concomitantly with low levels of marine derived n-3 PUFAs were at a 2-fold increased risk for breast cancer compared to women consuming high levels of marine-derived n-3 PUFA and lower levels of n-6 PUFA.

We found no main effects for any individual n-6 PUFA or marine derived n-3 PUFA, which is consistent with most prior prospective studies.[20-27] Nevertheless, some prospective cohort studies have described both increased [28] and decreased [29] risks of breast cancer associated with n-6 PUFA intake. Three prospective studies have found a reduced risk of breast cancer associated with dietary n-3 PUFA intake which appears predominately in post-menopausal

women. [30-32] We found no significant differences in individual PUFAs effect estimates between pre- and post-menopausal women (data not shown).

Because of the competition between n-6 PUFA and n-3 PUFAs as enzyme substrates and within membrane phospholipids, it has been suggested that an analytic approach focusing on a single fatty acid at a time may be insufficient.[33] Several studies have tried to address these limitations by investigated the ratio of n-6 PUFAs to n-3 PUFA. The findings have been mixed with some studies finding an increased ratio of n-6 PUFA to n-3 PUFA to be associated with a increased breast cancer risk [23, 30, 33-37] while other studies being largely null.[26, 28, 31, 38-39] Similar to Thiebaut et al. we found a significant interaction between n-6 and n-3 PUFAs.[23]

Part of the reasons for the discrepant finding between studies with regard to the ratio of n-6 PUFAs to n-3 PUFAs likely results from the choice of fatty acids to include within this summary measure. In prior studies total n-3 PUFAs has been calculated as: (ALA + EPA + DPA + DHA)[25, 37], (EPA + DPA + DHA)[23, 33], (ALA + EPA + DHA)[36], and (EPA + DHA).[30] We did not include ALA within our summary measure as it is poorly converted to EPA in humans[40-41] and anti-neoplastic effects have mainly been described with marine-derived (EPA and DHA) fatty acids.[7] We did not include DPA within our summary n-3 PUFA measure as it can be derived from either arachidonic acid (all-cis-4,7,10,13,16-docosapentanoic acid) or EPA (all-cis-7,10,13,16-docosapentanoic acid.[42] In addition, most dietary DPA is in the n-6 PUFA form and found in red meat as opposed to the n-3 PUFA form that is found predominately in seal oil. [43-44]

Our study has several strengths. The study is a prospective cohort that has eliminated potential for recall biases. In addition, follow-up for the SWHS has been excellent. Our study has a large sample size with almost 600,000 person-years of follow-up and has the power to detect even a weak association. The most important limitation of our study is related to the calculation of marine fatty acid dietary content. We have not examined the correlation between fatty acid biomarkers of n-3 PUFAs and FFQ in this population however, in other studies these correlations have ranged from 0.20 to 31.[45-46] These poor correlations are partially the result of the lack of specificity regarding fish species consumed and other factors which might impact n-3 PUFA content of fish. Non-differential misclassification errors could have attenuated our results. To try to reduce dietary fatty acid intake reporting errors, we averaged the dietary reports over two questionnaires.

In conclusion, we found a trend towards increased breast cancer risk with increasing dietary ratio of total n-6 PUFA to marine-derived n-3 PUFAs. The relative amounts of n-6 PUFA to marine-derived n-3 PUFAs may influence breast cancer risk, with low intake of marine-derived n-3 PUFAs associated with an increased risk for breast cancer in the setting of high n-6 PUFA intake.

Acknowledgments

Sources of support: USPHS grant R37CA70867 (Zheng) and K07CA114029 (Murff)

Abbreviations used

PUFA	polyunsaturated fatty acid
LA	linoleic acid
ARA	arachidonic acid

ALA	α-linolenic acid
EPA	eicosapentanoic acid
DPA	docosapentaenoic acid
DHA	docosahexanoic acid

References

1. Boyd NF, Stone J, Vogt KN, Connelly BS, Martin LJ, Minkin S. Dietary fat and breast cancer risk revisited: a meta-analysis of the published literature. Br J Cancer. 2003; 89:1672–85. [PubMed: 14583769]
2. Terry PD, Rohan TE, Wolk A. Intakes of fish and marine fatty acids and the risks of cancers of the breast and prostate and of other hormone-related cancers: a review of the epidemiologic evidence. Am J Clin Nutr. 2003; 77:532–43. [PubMed: 12600840]
3. Hunter DJ, Spiegelman D, Adami HO, Beeson L, van den Brandt PA, Folsom AR, Fraser GE, Goldbohm RA, Graham S, Howe GR, et al. Cohort studies of fat intake and the risk of breast cancer--a pooled analysis. N Engl J Med. 1996; 334:356–61. [PubMed: 8538706]
4. Fay MP, Freedman LS, Clifford CK, Midthune DN. Effect of different types and amounts of fat on the development of mammary tumors in rodents: a review. Cancer Res. 1997; 57:3979–88. [PubMed: 9307282]
5. Ip C. Review of the effects of trans fatty acids, oleic acid, n-3 polyunsaturated fatty acids, and conjugated linoleic acid on mammary carcinogenesis in animals. Am J Clin Nutr. 1997; 66:1523S–29S. [PubMed: 9394710]
6. Rose DP. Effects of dietary fatty acids on breast and prostate cancers: evidence from in vitro experiments and animal studies. Am J Clin Nutr. 1997; 66:1513S–22S. [PubMed: 9394709]
7. Rose DP, Connolly JM. Omega-3 fatty acids as cancer chemopreventive agents. Pharmacol Ther. 1999; 83:217–44. [PubMed: 10576293]
8. Calder PC. Dietary modification of inflammation with lipids. Proc Nutr Soc. 2002; 61:345–58. [PubMed: 12296294]
9. Wada M, DeLong CJ, Hong YH, Rieke CJ, Song I, Sidhu RS, Yuan C, Warnock M, Schmaier AH, Yokoyama C, Smyth EM, Wilson SJ, et al. Enzymes and receptors of prostaglandin pathways with arachidonic acid-derived versus eicosapentaenoic acid-derived substrates and products. J Biol Chem. 2007; 282:22254–66. [PubMed: 17519235]
10. Larsson SC, Kumlin M, Ingelman-Sundberg M, Wolk A. Dietary long-chain n-3 fatty acids for the prevention of cancer: a review of potential mechanisms. Am J Clin Nutr. 2004; 79:935–45. [PubMed: 15159222]
11. Cohen LA, Chen-Backlund JY, Sepkovic DW, Sugie S. Effect of varying proportions of dietary menhaden and corn oil on experimental rat mammary tumor promotion. Lipids. 1993; 28:449–56. [PubMed: 8316054]
12. Zheng W, Chow WH, Yang G, Jin F, Rothman N, Blair A, Li HL, Wen W, Ji BT, Li Q, Shu XO, Gao YT. The Shanghai Women's Health Study: rationale, study design, and baseline characteristics. Am J Epidemiol. 2005; 162:1123–31. [PubMed: 16236996]
13. Chinese Academy of Medical Sciences. Food Composition Tablesed. People's Health Publishing House; Beijing: 1991.
14. Shu XO, Yang G, Jin F, Liu D, Kushi L, Wen W, Gao YT, Zheng W. Validity and reproducibility of the food frequency questionnaire used in the Shanghai Women's Health Study. Eur J Clin Nutr. 2004; 58:17–23. [PubMed: 14679362]
15. Willett W, Stampfer MJ. Total energy intake: implications for epidemiologic analyses. Am J Epidemiol. 1986; 124:17–27. [PubMed: 3521261]
16. Hou H, She Y, Ma Y, Hu C, Zheng M, Zhang S. Investigations on methyl mercury contamination of fishes in the Second Songhua River. Biomed Environ Sci. 1988; 1:79–82. [PubMed: 3268111]

17. Wu WZ, Schramm KW, Kettrup A. Bioaccumulation of polychlorinated dibenzo-p-dioxins and dibenzofurans in the foodweb of Ya-Er Lake area, China. Water Res. 2001; 35:1141–8. [PubMed: 11268834]
18. Cognault S, Jourdan ML, Germain E, Pitavy R, Morel E, Durand G, Bougnoux P, Lhuillery C. Effect of an alpha-linolenic acid-rich diet on rat mammary tumor growth depends on the dietary oxidative status. Nutr Cancer. 2000; 36:33–41. [PubMed: 10798214]
19. Gago-Dominguez M, Castelao JE, Pike MC, Sevanian A, Haile RW. Role of lipid peroxidation in the epidemiology and prevention of breast cancer. Cancer Epidemiol Biomarkers Prev. 2005; 14:2829–39. [PubMed: 16364997]
20. Holmes MD, Hunter DJ, Colditz GA, Stampfer MJ, Hankinson SE, Speizer FE, Rosner B, Willett WC. Association of dietary intake of fat and fatty acids with risk of breast cancer. JAMA. 1999; 281:914–20. [PubMed: 10078488]
21. Cho E, Spiegelman D, Hunter DJ, Chen WY, Stampfer MJ, Colditz GA, Willett WC. Premenopausal fat intake and risk of breast cancer. J Natl Cancer Inst. 2003; 95:1079–85. [PubMed: 12865454]
22. Saadatian-Elahi M, Toniolo P, Ferrari P, Goudable J, Akhmedkhanov A, Zeleniuch-Jacquotte A, Riboli E. Serum fatty acids and risk of breast cancer in a nested case-control study of the New York University Women's Health Study. Cancer Epidemiol Biomarkers Prev. 2002; 11:1353–60. [PubMed: 12433711]
23. Thiebaut AC, Chajes V, Gerber M, Boutron-Ruault MC, Joulin V, Lenoir G, Berrino F, Riboli E, Benichou J, Clavel-Chapelon F. Dietary intakes of omega-6 and omega-3 polyunsaturated fatty acids and the risk of breast cancer. Int J Cancer. 2009; 124:924–31. [PubMed: 19035453]
24. Wirfalt E, Vessby B, Mattisson I, Gullberg B, Olsson H, Berglund G. No relations between breast cancer risk and fatty acids of erythrocyte membranes in postmenopausal women of the Malmo Diet Cancer cohort (Sweden). Eur J Clin Nutr. 2004; 58:761–70. [PubMed: 15116079]
25. Takata Y, King IB, Neuhouser ML, Schaffer S, Barnett M, Thornquist M, Peters U, Goodman GE. Association of serum phospholipid fatty acids with breast cancer risk among postmenopausal cigarette smokers. Cancer Causes Control. 2009; 20:497–504. [PubMed: 19255861]
26. Chajes V, Hulten K, Van Kappel AL, Winkvist A, Kaaks R, Hallmans G, Lenner P, Riboli E. Fatty-acid composition in serum phospholipids and risk of breast cancer: an incident case-control study in Sweden. Int J Cancer. 1999; 83:585–90. [PubMed: 10521790]
27. Pala V, Krogh V, Muti P, Chajes V, Riboli E, Micheli A, Saadatian M, Sieri S, Berrino F. Erythrocyte membrane fatty acids and subsequent breast cancer: a prospective Italian study. J Natl Cancer Inst. 2001; 93:1088–95. [PubMed: 11459870]
28. Wirfalt E, Mattisson I, Gullberg B, Johansson U, Olsson H, Berglund G. Postmenopausal breast cancer is associated with high intakes of omega6 fatty acids (Sweden). Cancer Causes Control. 2002; 13:883–93. [PubMed: 12588084]
29. Rissanen H, Knekt P, Jarvinen R, Salminen I, Hakulinen T. Serum fatty acids and breast cancer incidence. Nutr Cancer. 2003; 45:168–75. [PubMed: 12881010]
30. Gago-Dominguez M, Yuan JM, Sun CL, Lee HP, Yu MC. Opposing effects of dietary n-3 and n-6 fatty acids on mammary carcinogenesis: The Singapore Chinese Health Study. Br J Cancer. 2003; 89:1686–92. [PubMed: 14583770]
31. Wakai K, Tamakoshi K, Date C, Fukui M, Suzuki S, Lin Y, Niwa Y, Nishio K, Yatsuya H, Kondo T, Tokudome S, Yamamoto A, et al. Dietary intakes of fat and fatty acids and risk of breast cancer: a prospective study in Japan. Cancer Sci. 2005; 96:590–9. [PubMed: 16128744]
32. Voorrips LE, Brants HA, Kardinaal AF, Hiddink GJ, van den Brandt PA, Goldbohm RA. Intake of conjugated linoleic acid, fat, and other fatty acids in relation to postmenopausal breast cancer: the Netherlands Cohort Study on Diet and Cancer. Am J Clin Nutr. 2002; 76:873–82. [PubMed: 12324303]
33. Bougnoux P, Giraudeau B, Couet C. Diet, cancer, and the lipidome. Cancer Epidemiol Biomarkers Prev. 2006; 15:416–21. [PubMed: 16537692]
34. Bagga D, Anders KH, Wang HJ, Glaspy JA. Long-chain n-3-to-n-6 polyunsaturated fatty acid ratios in breast adipose tissue from women with and without breast cancer. Nutr Cancer. 2002; 42:180–5. [PubMed: 12416257]

35. Maillard V, Bougnoux P, Ferrari P, Jourdan ML, Pinault M, Lavillonniere F, Body G, Le Floch O, Chajes V. N-3 and N-6 fatty acids in breast adipose tissue and relative risk of breast cancer in a case-control study in Tours, France. Int J Cancer. 2002; 98:78–83. [PubMed: 11857389]
36. Simonsen N, van't Veer P, Strain JJ, Martin-Moreno JM, Huttunen JK, Navajas JF, Martin BC, Thamm M, Kardinaal AF, Kok FJ, Kohlmeier L. Adipose tissue omega-3 and omega-6 fatty acid content and breast cancer in the EURAMIC study. European Community Multicenter Study on Antioxidants, Myocardial Infarction, and Breast Cancer. Am J Epidemiol. 1998; 147:342–52. [PubMed: 9508101]
37. Goodstine SL, Zheng T, Holford TR, Ward BA, Carter D, Owens PH, Mayne ST. Dietary (n-3)/(n-6) fatty acid ratio: possible relationship to premenopausal but not postmenopausal breast cancer risk in U.S. women. J Nutr. 2003; 133:1409–14. [PubMed: 12730430]
38. Vatten LJ, Bjerve KS, Andersen A, Jellum E. Polyunsaturated fatty acids in serum phospholipids and risk of breast cancer: a case-control study from the Janus serum bank in Norway. Eur J Cancer. 1993; 29A:532–8. [PubMed: 8435206]
39. Shannon J, King IB, Moshofsky R, Lampe JW, Gao DL, Ray RM, Thomas DB. Erythrocyte fatty acids and breast cancer risk: a case-control study in Shanghai, China. Am J Clin Nutr. 2007; 85:1090–7. [PubMed: 17413110]
40. Arterburn LM, Hall EB, Oken H. Distribution, interconversion, and dose response of n-3 fatty acids in humans. Am J Clin Nutr. 2006; 83:1467S–76S. [PubMed: 16841856]
41. Plourde M, Cunnane SC. Extremely limited synthesis of long chain polyunsaturates in adults: implications for their dietary essentiality and use as supplements. Appl Physiol Nutr Metab. 2007; 32:619–34. [PubMed: 17622276]
42. Chow, CK. Fatty acids in foods and their health implications. 2nd ed.. M. Dekker; New York: 2000.
43. Howe P, Meyer B, Record S, Baghurst K. Dietary intake of long-chain omega-3 polyunsaturated fatty acids: contribution of meat sources. Nutrition. 2006; 22:47–53. [PubMed: 16289978]
44. Meyer BJ, Lane AE, Mann NJ. Comparison of seal oil to tuna oil on plasma lipid levels and blood pressure in hypertriglyceridaemic subjects. Lipids. 2009; 44:827–35. [PubMed: 19727884]
45. Baylin A, Campos H. The use of fatty acid biomarkers to reflect dietary intake. Current opinion in lipidology. 2006; 17:22–7. [PubMed: 16407712]
46. Ma J, Folsom AR, Shahar E, Eckfeldt JH, The Atherosclerosis Risk in Communities (ARIC) Study Investigators. Plasma fatty acid composition as an indicator of habitual dietary fat intake in middle-aged adults. Am J Clin Nutr. 1995; 62:564–71. [PubMed: 7661118]

Table 1

Comparison of cases and non-cases on demographics and selected breast cancer risk factors

	Breast cancer cases (n = 712)	Non-cases (n = 71,859)	*P* value[3]
Age at baseline (yrs)	52.7±8.84[1]	52.4±9.05	0.47
Family history of breast cancer (%)	3.5[2]	1.8	0.001
Education (%)			
Elementary school	13.2	21.3	<0.0001
Middle school	32.6	37.3	
High school	35.0	27.9	
≥ College	19.1	13.5	
Ever use of alcohol (%)	2.1	2.2	0.83
Ever smoker (%)	2.0	2.7	0.21
Age at menarche (yrs)	14.7±1.76	14.9±1.74	0.006
Postmenopausal (%)	48.2	48.9	0.72
Age at menopause (yrs)	49.2±4.53	48.6±4.34	0.006
Use of hormone replacement therapy (%)	2.7	2.1	0.26
Nulliparity (%)	3.8	2.7	0.0003
Age at first pregnancy (yrs)	26.3±4.04	25.5±4.08	<0.0001
Body mass index (kg/m^2)	24.2±3.36	24.0±3.42	0.11
Waist-to-hip ratio	0.81±0.05	0.81±0.05	0.18
Total energy intake (kcals/day)	1651.2±330.6	1647.2±339.6	0.76
Vitamin E intake (IU/d)	13.6±5.18	13.6±5.26	0.83
Red meat intake (g/d)	46.1±28.0	48.1±29.9	0.08
Fish intake (g/d)	51.1±37.5	50.0±38.3	0.44

[1] Mean ± S.D. (all such values)

[2] Frequency

[3] Chi-square test for frequencies and Student's *T*-test for means

Table 2

Spearman correlations and median values for baseline characteristics and total n-6 PUFA and marine-derived n-3 PUFA intake

	Total n-6 PUFA (g/d)			Marine-derived n-3 PUFA (g/d)		
	r_s[1]	Median (IQR)	P value[2]	r_s	Median (IQR)	P value
Marine-derived n-3 PUFA (g/d)	0.37		<0.0001	-		-
Age (yrs)	−0.06		<0.0001	−0.27		<0.0001
Body mass index (kg/m^2)	0.002		0.52	−0.07		<0.0001
Waist-to-hip ratio	−0.05		<0.0001	−0.10		<0.0001
Total energy intake (kcals/day)	0.67		<0.0001	0.33		<0.0001
Vitamin E intake (IU/d)	0.85		<0.0001	0.42		<0.0001
Red meat intake (g/d)	0.47		<0.0001	0.25		<0.0001
Fish intake (g/d)	0.43		<0.0001	0.89		<0.0001
Age at menarche (yrs)	−0.07		<0.0001	−0.09		<0.0001
Age at menopause (yrs)	0.02		0.0001	−0.01		0.06
Age at first birth (yrs)	0.05		<0.0001	0.12		<0.0001
Parity (number of children)	−0.07		<0.0001	−0.17		<0.0001
Family history of breast cancer						
Yes		6.47 (3.18)	<0.0001		0.073 (0.086)	0.01
No		6.19 (3.22)			0.068 (0.087)	
Alcohol use						
Ever		6.55 (3.46)	<0.0001		0.078 (0.097)	<0.0001
Never		6.19 (3.21)			0.068 (0.086)	
Tobacco use						
Ever		5.76 (3.53)	<0.0001		0.049 (0.078)	<0.0001
Never		6.20 (3.21)			0.069 (0.087)	
Use of hormone replacement therapy						
Ever		6.60 (3.28)	<0.0001		0.075 (0.082)	<0.0001
Never		6.19 (3.21)			0.068 (0.087)	
Menopausal status						
Pre- or peri-menopausal		6.32 (3.15)	<0.0001		0.081 (0.093)	<0.0001

	Total n-6 PUFA (g/d)			Marine-derived n-3 PUFA (g/d)		
	r_s[1]	Median (IQR)	P value[2]	r_s	Median (IQR)	P value
Postmenopausal		6.06 (3.27)			0.054 (0.075)	
Education						
Elementary school		5.45 (3.13)			0.038 (0.057)	
Middle school		6.25 (3.19)	<0.0001		0.073 (0.088)	<0.0001
High school		6.47 (3.18)			0.081 (0.094)	
≥ College		6.56 (3.15)			0.078 (0.086)	

[1] r_S = Spearman's rank order correlation coefficient

[2] Spearman's rho and Wilcoxon rank sums test for median values

Table 3

Breast cancer risks associated with dietary intake of polyunsaturated fatty acids

			Age-adjusted	Multivariate adjusted[1]
	Cases	Person-Years	RR (95% CI)	RR (95% CI)
n-6 PUFA				
Linoleic Acid (g/d)				
Q1 (4.53)[2]	149	116,974	1.00	1.00
Q2 (5.12)	137	117,244	0.92 (0.76-1.20)	0.92 (0.72-1.18)
Q3 (5.90)	135	117,169	0.90 (0.71-1.14)	0.92 (0.71-1.20)
Q4 (6.92)	141	116,877	0.95 (0.76-1.20)	0.99 (0.75-1.30)
Q5 (9.27)	150	115,732	1.03 (0.82-1.29)	1.13 (0.82-1.54)
P_{trend}			0.50	0.20
Arachidonic Acid (g/d)				
Q1 (0.03)	124	115,681	1.00	1.00
Q2 (0.04)	163	116,548	1.32 (1.04-1.66)	1.25 (0.98-1.60)
Q3 (0.05)	143	117,290	1.15 (0.90-1.47)	1.08 (0.83-1.39)
Q4 (0.06)	138	117,311	1.11 (0.87-1.42)	1.00 (0.76-1.32)
Q5 (0.08)	144	117,166	1.19 (0.93-1.52)	1.06 (0.78-1.45)
P_{trend}			0.63	0.65
n-3 PUFA				
α-linolenic acid (g/d)				
Q1 (0.63)	153	117,108	1.00	1.00
Q2 (0.75)	127	117,524	0.82 (0.65-1.04)	0.81 (0.63-1.04)
Q3 (0.88)	149	116,991	0.98 (0.78-1.23)	1.00 (0.77-1.29)
Q4 (1.04)	139	116,923	0.93 (0.74-1.17)	0.96 (0.72-1.27)
Q5 (1.39)	144	115,450	0.98 (0.78-1.22)	1.07 (0.76-1.50)
P_{trend}			0.74	0.28
Marine-derived n-3 PUFA (g/d)				
Q1 (0.02)	150	118,094	1.00	1.00
Q2 (0.04)	147	116,243	1.00 (0.80-1.26)	0.94 (0.73-1.19)
Q3 (0.07)	123	116,949	0.84 (0.66-1.07)	0.76 (0.59-1.00)
Q4 (0.11)	158	116,688	1.10 (0.88-1.38)	0.95 (0.72-1.25)
Q5 (0.20)	134	116,021	0.95 (0.75-1.20)	0.74 (0.52-1.06)
P_{trend}			0.95	0.21
n-6/ n-3 PUFA				
Q1 (5.18)	147	116,280	1.00	1.00
Q2 (5.83)	137	116,854	0.91 (0.72-1.15)	0.93 (0.73-1.19)
Q3 (6.29)	146	117,215	0.95 (0.75-1.20)	0.98 (0.76-1.26)
Q4 (6.78)	131	117,065	0.86 (0.68-1.09)	0.90 (0.69-1.18)
Q5 (7.64)	151	116,580	0.98 (0.77-1.23)	1.02 (0.77-1.34)
P_{trend}			0.79	0.88

			Age-adjusted	Multivariate adjusted[1]
	Cases	Person-Years	RR (95% CI)	RR (95% CI)
n-6/marine-derived n-3 PUFA				
Q1 (42.0)	139	118,229	1.00	1.00
Q2 (64.3)	147	117,028	1.06 (0.84-1.34)	1.11 (0.87-1.41)
Q3 (91.0)	133	116,888	0.95 (0.74-1.20)	1.03 (0.79-1.34)
Q4 (140.2)	147	116,942	1.05 (0.83-1.33)	1.18 (0.89-1.57)
Q5 (314.5)	146	114,909	1.05 (0.83-1.33)	1.29 (0.95-1.75)
P_{trend}			0.67	0.10

[1] Adjusted for age, body mass index, total energy, family history of breast cancer, alcohol use, tobacco use, education, use of hormone replacement therapy, personal history of diabetes, menopausal status, age at menopause, age at menarche, parity, age at first pregnancy, level of physical activity, red meat intake, fish intake and vitamin E intake.

[2] Median value

Table 4

Breast cancer risk[1] stratified by intake of n-6 polyunsaturated fatty acids and marine-derived n-3 polyunsaturated fatty acids

Marine-derived n-3 PUFA[3] (g/d)	n-6 PUFA[2] (g/day)					
	≤5.24		>5.24-≤7.28		>7.28	
	Cases	HR (95% CI)	Cases	HR (95% CI)	Cases	HR (95% CI)
>0.10	36	1.00 (ref)	65	1.07 (0.70-1.63)	132	1.63 (1.06-2.51)
0.046-0.10	91	1.64 (1.10-2.46)	81	1.24 (0.82-1.88)	64	1.22 (0.77-1.94)
≤0.045	122	1.42 (0.94-2.13)	59	1.14 (0.73-1.80)	62	2.06 (1.27-3.34)
					$P_{interaction}$	0.008

[1] Adjusted for age, body mass index, total energy, family history of breast cancer, alcohol use, tobacco use, education, use of hormone replacement therapy, personal history of diabetes, menopausal status, age at menopause, age at menarche, parity, age at first pregnancy, level of physical activity, red meat intake, fish intake and vitamin E intake.

[2] n-6 PUFA = linoleic acid + arachidonic acid

[3] Marine-derived n-3 PUFA = eicosapentanoic acid + docosahexanoic acid