Dietary Modulation of Omega-3/Omega-6 Polyunsaturated Fatty Acid Ratios in Patients With Breast Cancer

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Background: **Polyunsaturated fatty acids of the omega-6 (ω-6) class, as found in corn and safflower oils, can act as precursors for intermediates involved in the growth of mammary tumors when fed to animals, whereas polyunsaturated fatty acids of the omega-3 (ω-3) class, as found in fish oil, can inhibit these effects. The effects of dietary intervention on the ratios of these fatty acids in breast and other adipose tissues have not previously been prospectively studied.** ***Purpose:*** **The present investigation was conducted to study the impact on the ratio of ω-3 and ω-6 polyunsaturated fatty acid in plasma and in adipose tissue of the breast and buttocks when women with breast cancer consume a low-fat diet and fish oil supplements.** ***Methods:*** **Twenty-five women with high-risk localized breast cancer were enrolled in a dietary intervention program that required them to eat a low-fat diet and take a daily fish oil supplement throughout a 3-month period. Breast and gluteal fat biopsy specimens were obtained from each woman before and after dietary intervention. The fatty acid compositions of specimens of plasma, breast fat, and gluteal fat were determined by gas-liquid chromatography. Statistical analysis involved use of a two-sided paired *t* test.** ***Results:*** **After dietary intervention, a reduction in the level of total ω-6 polyunsaturated fatty acids in the plasma was observed ($P<.0003$); moreover, total ω-3 polyunsaturated fatty acids increased approximately threefold ($P<.0001$) and the ω-3/ω-6 polyunsaturated fatty acids ratio increased approximately fourfold (i.e., mean values increased from 0.09 to 0.41; $P = .0001$). An increase in total ω-3 polyunsaturated fatty acids in breast adipose tissue was observed following dietary intervention ($P = .04$); the ω-3/ω-6 polyunsaturated fatty acid ratio increased from a mean value of 0.05 to 0.07 ($P = .0001$). An increase in total ω-3 polyunsaturated fatty acids was observed in gluteal adipose tissue following the intervention ($P = .05$); however, the ratio of ω-3 to ω-6 polyunsaturated fatty acids (mean ratio values of 0.036-0.045; $P = .06$) was unchanged.** ***Conclusion:*** **Short-term dietary intervention can lead to statistically significant increases in ω-3/ω-6 polyunsaturated fatty acid ratios in plasma and breast adipose tissue. Breast adipose tissue changed more rapidly than gluteal adipose tissue in response to the dietary modification tested in this study. Therefore, gluteal adipose tissue may not be a useful surrogate to study the effect of diet on breast adipose tissue. [J Natl Cancer Inst 1997;89:1123-31]**

The incidence of breast cancer differs markedly between countries in North America or Europe and Japan (*1*). These differences may be attributed to the contrasting patterns of dietary fat intake. Women in North America and Europe consume a high-fat diet containing omega-6 (ω-6) polyunsaturated fatty acids, primarily linoleic acid (18:2, ω-6; where chain length is indicated by a number separated by a colon from a second number designating the number of double bonds, ω-6 represents the numbering of the carbon atoms from the methyl end, and the location of the first double bond is indicated by a single number) present in corn and safflower oils, whereas the traditional Japanese diet is a low-fat diet containing omega-3 (ω-3) polyunsaturated fatty acid, primarily eicosapentaenoic acid (20:5, ω-3) and docosahexaenoic acid (22:6, ω-3) found in fish oil. One theory holds that the effect of diet on the incidence of breast cancer is mediated through the total fat content, although the results of

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See ''Notes'' following ''References.''

prospective studies have not supported this hypothesis [reviewed in (*2*)]. An intervention trial (*3*) of total fat reduction is under way. An alternative hypothesis, that the nutritional modulation of breast cancer risk is mediated via the balance of polyunsaturated fatty acids in the diet, has not been tested in prospective studies.

Several lines of evidence have indicated that the various polyunsaturated fatty acids differ in their ability to affect mammary tumor formation and growth. In murine models with either 7,12-dimethylbenz[*a*]anthracene-induced mammary tumors or tumors derived from human breast cancer cell lines growing in immunodeficient mice, diets containing large amounts of linoleic acid are linked with increased mammary tumor promotion and with more aggressive tumor characteristics (*4,5*). In these models, the addition of ω-3 polyunsaturated fatty acids to the diet can block the promoting effects of ω-6 polyunsaturated fatty acids (*6,7*). In addition, feeding of high-fat diets based on coconut or olive oils is less effective in promoting tumor growth (*8,9*). Although the exact mechanisms by which polyunsaturated fatty acids exert their effect on tumor cell growth have not been identified, it is thought that eicosanoids derived from arachidonic acid play an important role in these processes (*10*). Linoleic acid can be converted to arachidonic acid (20:4, ω-6) by desaturation and elongation reactions. Arachidonic acid is rapidly incorporated into membrane phospholipids by transacylases. Various exogenous stimuli, including many growth factors, result in the release of arachidonic acid from membrane phospholipids. Arachidonic acid is further metabolized by the cyclooxygenase systems to 2-series (prostaglandins of 2-series have two double bonds, and this is described by a subscript, e.g., PGE_2) prostaglandins or through the 5-lipoxygenase systems to leukotrienes and 5-hydroxy-eicosatetraenoic acid (*11*). Fish oils, containing high amounts of eicosapentaenoic acid and docosahexaenoic acid, can antagonize the overproduction of eicosanoids derived from ω-6 polyunsaturated fatty acids by competing as a substrate for both cyclooxygenase and lipoxygenase systems (*12*) and at the same time producing 3-series prostaglandins and 5-series leukotrienes (both of which are eicosanoids; those of 3- and 5-series have 3 and 5 double bonds, respectively, and are described by a subscript PGE_3 and LTB_5, respectively) (*13*). The cyclooxygenase pathway product endoperoxide is converted to 3-series prostaglandins whereas the lipoxygenase pathway product 5-hydroxy-eicosatetraenoic acid is metabolized to leukotrienes of the 5-series. Production of eicosanoids are tightly controlled in normal cells. Eicosanoids are rapidly inactivated by catabolic enzymes but may reach higher levels in tumor tissue (*14*). It has been shown that the cyclooxygenase product, PGE_2, and the lipoxygenase products, leukotrienes B_4 and C_4 (*15*), may be associated with breast tumor promotion through their effects on signal transduction. In addition, large quantities of prostaglandins have been found in both human and animal tumors (*15*). In contrast, the 3- and 5-series eicosanoids derived from eicosapentaenoic acid cause less tissue inflammation, and their net effect is to inhibit production of the active metabolites of arachidonic acid (*16*). Thus, the ratio of precursor fatty acids (eicosapentaenoic acid/arachidonic acid) may determine the spectrum of eicosanoids produced by the cell and thereby attenuate the biologic effects of growth factor stimuli.

Fatty acids in the plasma are derived from endogenous and exogenous sources. These fatty acids are incorporated into the intracellular stores and membrane phospholipids. The fatty acid composition of cell membrane phospholipids can influence the biosynthesis of eicosanoids during a cellular response to an extracellular stimuli (*11*). It is possible that the ratio of ω-3/ω-6 fatty acids entering the cellular pool from dietary sources can alter the ratio of eicosanoid precursor fatty acids in membrane phospholipids. While most of the case–control studies (*17-20*) that have compared the fatty acid composition of breast and/or gluteal adipose tissue between women with breast cancer and those with benign breast disease have failed to show a difference in storage of ω-3 fatty acids, one study (*21*) has observed a lower percentage of docosahexaenoic acid in breast adipose tissue of postmenopausal patients with breast cancer. Moreover, the considerations outlined above suggest that it is the ratio of ω-3/ω-6 rather than the absolute amount of either family of polyunsaturated fatty acid that is relevant to the breast cancer problem. We can speculate that the ratios of fatty acids in various diets may explain the international differences in breast cancer risk, and it might be prudent to intervene to alter the ω-3/ω-6 ratios in the diet and the ratios of polyunsaturated fatty acids that can be maintained in tissue phospholipids. A recent study (*22*) investigated the correlation between the fatty acid content of adipose tissue of buttocks and breast cancer risk in postmenopausal women in five European countries. They found no correlation between the higher concentration of ω-6 polyunsaturated fatty acids in adipose tissue and breast cancer. The study suggested to us the possibility that the ratio of ω-3/ω-6 polyunsaturated fatty acids may provide a way to predict an increased risk of breast cancer.

The fatty acid composition of adipose tissue reflects long-term dietary intake of fatty acids (*23*). Dietary fatty acid composition is reflected in the composition of stored triacylglycerols (*24*) and therefore may be subject to alteration by diet. This modification of stored fatty acids in breast fat could have important implications for breast cancer in view of the importance of breast adipocytes for storage and release of fatty acids that are required for normal differentiation, proliferation, and morphogenesis of breast epithelial cells (*25-27*). The effects of dietary changes in ω-3/ω-6 polyunsaturated fatty acids on the composition of human breast adipose tissue have not been studied in prospective intervention studies.

On the basis of the hypothesis that ω-3 polyunsaturated fatty acids may counteract the influence of ω-6 polyunsaturated fatty acids on specific processes in tumor development, this investigation was conducted to study the impact of short-term intervention with a low-fat diet and fish oil supplementation on ω-3/ω-6 polyunsaturated fatty acids ratios in plasma and breast adipose tissue. Furthermore, it is not known whether breast adipose tissue fatty acid composition is identical to that of other storage sites, since differences have been observed among different sites (*28,29*). To compare the composition of breast adipose tissue with other adipose tissue storage sites, we also studied the fatty acid composition of breast adipose tissue and gluteal adipose tissue before and after intervention with a low-fat diet and fish oil supplements in patients with breast cancer.

Methods

Subjects and Study Design

Thirty-six women aged 29-62 years of age with histologically documented breast cancer were recruited and studied in the Bowyer Oncology Center at the University of California at Los Angeles. The study was conducted from November 1995 through July 1996. All patients had high-risk (stage II with >10 lymph nodes involved or stage III with any number of lymph nodes involved) localized breast cancer and had completed high-dose chemotherapy with peripheral blood progenitor cell transplantation or stage IV breast cancer that had failed to respond to conventional therapy. The median time of enrollment of patients after the transplant was 497 days (range, 48-1242 days). Patients were excluded if they were on prescribed anticoagulants, had undergone bilateral mastectomy, or were currently following a low-fat diet or taking fish oil supplements. Twenty-five of the 36 women completed the study, and only their results are included in this report. Five patients were removed from the study before dietary intervention was complete because they had disease recurrence, four were noncompliant with follow-up, and two provided insufficient tissue samples for analysis. Written informed consent was obtained from all women and all procedures were approved by the University of California, Los Angeles, Human Subject Protection Committee.

The duration of the dietary intervention for each patient was 3 months. Eligible patients underwent a baseline evaluation of physical characteristics, health, and diet prior to starting the diet intervention studies. Medical history and physical examination and review of pathology were done prior to enrollment of subjects in the study. Body weight was measured by use of a calibrated clinic scale, and height was measured by use of a wall-mounted stadiometer. The percent body fat and lean body mass were measured with the use of a bioelectrical impedance meter. The waist-to-hip ratio was determined by measuring the hip and waist circumference with a standard measuring tape.

Potential subjects were required to complete brief questionnaires on their medical and reproductive histories. Each potential subject met with the study nutritionist and was asked to complete a food-frequency questionnaire and a 4-day food record. These data were returned to the study nutritionist before entry in the diet intervention.

Breast and gluteal fat biopsies for determination of the levels of fatty acids were conducted before each subject started the diet intervention. Blood samples were collected from patients after they had fasted for 12 hours, and they were used for determination of the level of total fatty acids in the plasma.

After completion of the baseline measurements, subjects underwent an individualized program targeted to reduce dietary fat intake to 15% of total calories. All subjects were instructed to consume a very low-fat, high-fiber diet and fish oil for a duration of 3 months. Patients were required to meet with the nutritionist once a week for the first month and then every 2 weeks during the second and third months of intervention. Patients received both written and one-on-one instructions on how to consume a diet containing 15% fat. Fish oil is a commercially available nutritional supplement and was provided by the Pharmavite Corp. (Mission Hill, CA) in 1000-mg capsules containing 100% fish oil composed of 18% eicosapentaenoic acid and 12% docosahexaenoic acid. Patients were required to take 10 capsules daily to achieve a goal of 3 g ω-3-polyunsaturated fatty acids daily. Patients were also given and asked to consume 800 IU of vitamin E each day, since the requirement for vitamin E increases as the amount of polyunsaturated fatty acids in the diet increases. This diet provided 15% of calories from fat, 15% from protein, and 70% from carbohydrate. Patients were asked to maintain a food diary for the entire 3-month period of dietary intervention. The study nutritionist read each subject's food diary every week to assess compliance with the diet. In addition, 4-day food records were completed at the end of each month for computer-analyzed nutrient analysis.

At the end of 3 months of the diet intervention, all measurements that were conducted at baseline were repeated. As described above, they included the following: a physical examination by the physician—measurement of height, weight, percent body fat, and waist-to-hip ratio measurements; fasting blood samples for plasma total fatty acids; and a 4-day food record. The nutrient analysis of all the food records was done by use of the Minnesota-based Nutrient Data System at the University of California, Los Angeles. Patients underwent repeat biopsies of breast and gluteal fat to evaluate the effect of the dietary intervention.

Plasma samples from fasting patients were obtained by standard venipuncture technique and collected into vacutainer tubes, for a total of 15 mL for measurement of total fatty acids. These measurements were done at baseline and at the end of 3 months of diet intervention.

A punch biopsy of breast fat (1 g) was conducted to measure tissue fatty acids. This was accomplished with a 3-mm disposable punch biopsy instrument. Separate gluteal fat samples were obtained. Fat samples were obtained at baseline and at the end of 3 months of diet intervention.

Analysis of Fatty Acid Composition

The total fatty acids in plasma and breast and gluteal adipose tissue biopsy specimens were extracted and converted to methyl esters by the direct one-step transesterification method of Lepage and Roy (*30*). Fatty acid methyl esters were separated and quantified by use of a Hewlett-Packard 5890 A series II gas–liquid chromatograph fitted with a model 7673 automatic split-injection system and a flame ionization detector. The integrator was calibrated with a calibration mixture containing all fatty acids found in plasma or tissue (NuChek Preparation Inc., Elysian, MN). The standard curve for each individual fatty acid was formed by running different concentrations of the mixture. Quantification was based on the known quantity of the internal standard added to the unknown samples and the standard mixture. Calculations of the quantitative amounts of fatty acids in the biologic sample were based on the response ratio and the ratio of the unknown amounts of fatty acid in the sample to the known quantity of each fatty acid from the calibration curve. Results were expressed as absolute (μmol/L) of total fatty acids and relative percent of total fatty acids. The fatty acid methyl esters identified include the following: C6:0, C8:0, C10:0, C12:0, C14:0, C14:1, C16:0, C16:1, C18:0, C18:1 (ω-9) cis, C18:2 (ω-6) cis, C18:3 (ω-3), C20:0, C20:1 (ω-9), C20:2 (ω-9), C20:3 (ω-6), C20:4 (ω-6), C20:5 (ω-3), C22:0, C22:1 (ω-9), C24:0, C24:1 (ω-9), and C22:6 (ω-3).

Statistical Analysis

Means of individual fatty acids were calculated, and the total sum of fatty acids of each family analyzed (saturated, monounsaturated, ω-6, ω-3, and polyunsaturated fatty acids) was determined. Statistical analysis was conducted by use of a two-sided paired *t* test to assess the effects of intervention with a low-fat diet and fish oil supplementation for 3 months, by comparing results before and after the intervention. The relation between plasma, breast, and gluteal fatty acid changes in ratios of eicosapentaenoic acid and arachidonic acid was investigated by use of Pearson's correlation coefficients. All *P* values reported are from use of two-sided statistical tests. Statistical analyses were performed by use of the Statview computer program (Abacus Concepts, Inc., Berkeley, CA).

Results

The median age of patients who completed the study was 45.5 years (range, 29-62 years). No adverse events were noted as a consequence of short-term intervention with a low-fat diet and fish oil supplementation. In addition, breast and gluteal fat biopsy procedures were tolerated well by all subjects.

Intervention with a low-fat, fish oil-supplemented (LF/FOS) diet resulted in a decrease in energy consumption, from a mean of 1336.4 to 1137 kcal/day ($P<.05$) as measured by the 4-day food-record analysis. The mean percentage of calories from protein increased significantly, from 17.1% to 19.0%, and, from carbohydrate, from 58.6% to 68.1% ($P<.05$). The mean percentage of fat decreased significantly from 25.6% of total calories to 14.8% ($P<.05$). This represented a decrease in the mean amount of fat consumed, from 37.2 to 17.9 g/day, and the amounts of carbohydrate and protein in the diet remained relatively stable.

The effects of the diet on the subjects' body composition were determined at baseline and at the end of the 3-month intervention. There was a small but statistically significant decrease in mean weight (62.86 ± 1.86 kg before intervention and 61.73 ± 1.81 kg after intervention; $P = .02$) and body mass index (kg/m^2) (23.3 ± 0.56 before intervention and 22.9 ± 0.53 after intervention; $P = .02$). However, there were no significant changes in the mean percent body fat ($P = .58$) or mean waist-

Table 1. Effects of consumption of a low-fat, fish oil-supplemented (LF/FOS) diet for 3 months on plasma fatty acids

Fatty acid*	Baseline	LF/FOS diet	P‡
	Fatty acid, μmol/L†		
14:0	96.9 ± 10.7	75.0 ± 8.6	.02
14:1	4.5 ± 2.3	5.3 ± 2.5	.60
16:0	2886.9 ± 201.0	2536.1 ± 155.4	.02
16:1	351.8 ± 32.5	303.9 ± 29.4	.06
18:0	696.5 ± 52.2	612.7 ± 42.6	.06
18:1 (ω-9)	2528.5 ± 181.0	1772.4 ± 128.8	<.0001
18:2 (ω-6) (LA)	3555.1 ± 292.9	2545.6 ± 174.9	<.0007
18:3 (ω-3)	30.1 ± 8.3	18.4 ± 6.1	.08
20:0	1.3 ± 1.3	2.3 ± 1.6	.33
20:3 (ω-6)	98.8 ± 14.4	14.5 ± 5.1	<.0001
20:4 (ω-6) (AA)	777.8 ± 61.1	581.5 ± 38.1	<.0001
20:5 (ω-3) (EPA)	33.8 ± 16.6	686.1 ± 82.8	<.0001
22:0	30.1 ± 5.5	10.8 ± 3.0	.005
22:6 (ω-3) (DHA)	330.8 ± 58.3	529.2 ± 61.8	.001
24:0	42.9 ± 7.2	21.3 ± 4.5	.003
24:1 (ω-9)	69.9 ± 7.2	83.3 ± 6.5	.01
Total ω-6	4432.8 ± 345.2	3141.7 ± 204.4	.0003
Total ω-3	394.7 ± 69.6	1233.6 ± 133.9	<.0001
ω-3/ω-6 ratio	0.09	0.41	<.0001
EPA/AA ratio	0.052	1.226	<.0001

*LA = linoleic acid; AA = arachidonic acid; EPA = eicosapentaenoic acid; and DHA = docosahexaenoic acid.

†Mean ± standard error (n = 25).

‡*P* value calculated by use of a two-sided paired *t* test.

to-hip ratio ($P = .46$) after 3 months of intervention with a LF/FOS diet.

Effect of a LF/FOS Diet on Plasma Fatty Acids

The changes in total fatty acids in plasma and adipose tissues of the subjects studied in this investigation are reported in both absolute amounts and relative percentages of fatty acids. Short-term intervention with a LF/FOS diet resulted in profound changes in plasma fatty acids (Table 1). There was a significant reduction in total ω-6 polyunsaturated fatty acids ($P = .0003$) and the predominant ω-6 fatty acids, linoleic acid ($P<.0007$) and arachidonic acid ($P<.0001$). There was a threefold increase in circulating total ω-3 fatty acids ($P<.0001$). Furthermore, the levels of the predominant fatty acids in fish oils, namely, eicosapentaenoic acid ($P<.0001$) and docosahexaenoic acid ($P = .001$), increased significantly and were the main contributors to the total ω-3 fatty acid pool in the circulation. Linolenic acid (18:3, ω-3) levels did not change. The mean ω-3/ω-6 polyunsaturated fatty acid ratio increased more than fourfold (from 0.09 to 0.41 [$P = .0001$]) and the eicosapentaenoic acid/arachidonic acid ratio increased more than 20-fold (from 0.05 to 1.23 [$P = .0001$]) in the plasma.

Effect of a LF/FOS Diet on Breast and Gluteal Adipose Tissue Fatty Acids

The results of analysis of fatty acids in the breast and gluteal fat are shown in Tables 2 and 3 and in Figs. 1-4. A 3-month intervention with a LF/FOS diet had no effect on relative percentages or concentration (μmol/g) of polyunsaturated fatty acids stores in either breast or gluteal fat tissue. However, when polyunsaturated fatty acids concentration was differentiated into ω-6 and ω-3 families, the total ω-3 fatty acid deposits increased statistically significantly in both breast and gluteal fat, while total ω-6 polyunsaturated fatty acids remained unchanged. The effects of fish oil supplementation were reflected in significant increases in eicosapentaenoic acid and docosahexaenoic acid deposits in both sites. The incorporation of ω-3 fatty acid led to statistically significant increases in ω-3/ω-6 and eicosapentaenoic acid/arachidonic acid ratios in breast and gluteal fat. Similar trends were observed when the concentrations and relative percentages of the fatty acids in the tissues were studied.

Table 2. Effects of consumption of a low-fat, fish oil-supplemented (LF/FOS) diet for 3 months on breast fat fatty acids

Fatty acid*	Relative, %†			μmol/g†		
	Baseline	LF/FOS	P‡	Baseline	LF/FOS	P‡
12:0	0.40 ± 0.03	0.36 ± 0.03	.03	10.13 ± 1.40	8.91 ± 1.18	.46
14:0	2.61 ± 0.11	2.46 ± 0.09	.01	59.25 ± 5.67	55.87 ± 5.21	.65
14:1	0.27 ± 0.02	0.25 ± 0.02	.12	6.71 ± 0.75	6.13 ± 0.71	.52
16:0	21.4 ± 0.37	20.91 ± 0.38	.15	413.00 ± 35.02	413.42 ± 36.22	.99
16:1	4.43 ± 0.24	4.49 ± 0.29	.74	88.03 ± 8.65	89.93 ± 10.11	.86
18:0	5.12 ± 0.16	5.20 ± 0.23	.64	84.82 ± 8.17	86.47 ± 8.44	.89
18:1 (ω-9)	45.87 ± 0.45	45.75 ± 0.52	.61	795.27 ± 75.07	786.94 ± 65.57	.93
18:2 (ω-6) (LA)	16.98 ± 0.52	17.12 ± 0.53	.56	294.96 ± 29.05	305.80 ± 28.99	.79
18:3 (ω-3)	0.80 ± 0.04	0.79 ± 0.04	.99	14.58 ± 1.77	14.70 ± 1.59	.96
20:1 (ω-9)	0.85 ± 0.04	0.96 ± 0.02	.01	13.81 ± 1.44	15.17 ± 1.40	.51
20:2 (ω-6)	0.40 ± 0.02	0.41 ± 0.02	.78	6.83 ± 0.74	7.06 ± 0.69	.82
20:3 (ω-6)	0.15 ± 0.02	0.18 ± 0.02	.27	2.04 ± 0.48	2.68 ± 0.38	.23
20:4 (ω-6) (AA)	0.34 ± 0.03	0.41 ± 0.03	.03	5.34 ± 0.61	6.26 ± 0.60	.19
20:5 (ω-3) (EPA)	0	0.16 ± 0.03	.0001	0	3.40 ± 0.67	.0001
22:6 (ω-3) (DHA)	0.13 ± 0.04	0.38 ± 0.06	.0001	1.47 ± 0.41	5.05 ± 0.74	.0001
Total saturated	29.63 ± 0.48	29.08 ± 0.47	.06	569.58 ± 49.26	567.43 ± 49.47	.97
Total monounsaturated	51.42 ± 0.53	51.44 ± 0.57	.90	903.83 ± 84.66	898.23 ± 75.49	.96
Total polyunsaturated	18.80 ± 0.54	19.45 ± 0.55	.03	325.23 ± 32.12	344.94 ± 32.86	.67
Total ω-6	17.87 ± 0.51	18.12 ± 0.54	.54	309.17 ± 30.43	321.79 ± 30.36	.77
Total ω-3	0.92 ± 0.06	1.33 ± 0.09	.0001	16.06 ± 1.87	23.15 ± 2.78	.04
ω-3/ω-6 ratio	0.052 ± 0.003	0.075 ± 0.005	.0001	0.05 ± 0.003	0.07 ± 0.005	.0001
EPA/AA ratio	0	0.44 ± 0.08	.0001	0	0.49 ± 0.12	.0005

*LA = linoleic acid; AA = arachidonic acid; EPA = eicosapentaenoic acid; and DHA = docosahexaenoic acid.

†Mean ± SE (n = 25).

‡*P* value calculated by use of a two-sided paired *t* test.

Table 3. Effects of consumption of a low-fat, fish oil-supplemented (LF/FOS) diet for 3 months on gluteal fat fatty acids

	Relative, %†			μmol/g†		
Fatty acid*	Baseline	LF/FOS	*P*‡	Baseline	LF/FOS	*P*‡
12:0	0.18 ± 0.02	0.18 ± 0.02	.82	2.84 ± 0.57	3.23 ± 0.60	.59
14:0	1.84 ± 0.07	1.83 ± 0.08	.93	24.54 ± 4.03	29.87 ± 4.21	.34
14:1	0.28 ± 0.02	0.28 ± 0.03	.95	4.43 ± 0.95	5.62 ± 1.16	.41
16:0	18.13 ± 0.37	18.13 ± 0.38	.99	207.72 ± 31.98	254.75 ± 35.74	.31
16:1	8.37 ± 0.44	7.90 ± 0.51	.20	101.16 ± 17.32	122.83 ± 21.64	.38
18:0	2.45 ± 0.16	2.88 ± 0.35	.18	22.20 ± 4.10	28.64 ± 4.18	.27
18:1 (ω-9)	49.36 ± 0.36	48.58 ± 0.52	.11	526.31 ± 87.06	636.44 ± 96.36	.33
18:2 (ω-6) (LA)	17.23 ± 0.54	17.28 ± 0.52	.91	190.06 ± 33.09	231.76 ± 38.00	.34
18:3 (ω-3)	0.61 ± 0.06	0.66 ± 0.06	.44	7.49 ± 1.44	10.45 ± 2.16	.16
20:1 (ω-9)	0.70 ± 0.05	0.85 ± 0.06	.05	7.96 ± 1.46	11.15 ± 1.87	.12
20:2 (ω-6)	0.32 ± 0.04	0.37 ± 0.03	.28	3.81 ± 0.80	5.18 ± 0.86	.17
20:3 (ω-6)	0.09 ± 0.02	0.13 ± 0.02	.19	0.95 ± 0.30	1.76 ± 0.52	.12
20:4 (ω-6) (AA)	0.35 ± 0.04	0.60 ± 0.19	.23	3.26 ± 0.63	4.87 ± 0.92	.08
20:5 (ω-3) (EPA)	0	0.07 ± 0.02	.003	0	1.27 ± 0.50	.02
22:6 (ω-3) (DHA)	0.06 ± 0.02	0.18 ± 0.04	.0006	0.75 ± 0.29	2.56 ± 0.89	.01
Total saturated	22.63 ± 0.51	23.07 ± 0.87	.10	257.92 ± 40.45	317.28 ± 44.07	.31
Total monounsaturated	58.70 ± 0.55	57.61 ± 0.87	.10	639.85 ± 105.81	776.08 ± 119.68	.33
Total polyunsaturated	18.66 ± 0.60	19.28 ± 0.59	.18	206.33 ± 36.18	257.83 ± 43.40	.29
Total ω-6	17.99 ± 0.56	18.38 ± 0.55	.35	198.08 ± 34.67	243.56 ± 40.13	.32
Total ω-3	0.67 ± 0.07	0.90 ± 0.10	.02	8.24 ± 1.65	14.27 ± 3.43	.05
ω-3/ω-6 ratio	0.036 ± 0.003	0.048 ± 0.005	.03	0.036 ± 0.004	0.045 ± 0.005	.11
EPA/AA ratio	0	0.19 ± 0.07	.03	0	0.17 ± 0.06	.02

*LA = linoleic acid; AA = arachidonic acid; EPA = eicosapentaenoic acid; and DHA = docosahexaenoic acid.
†Mean ± SE (n = 25).
‡*P* value calculated by use of a two-sided paired *t* test.

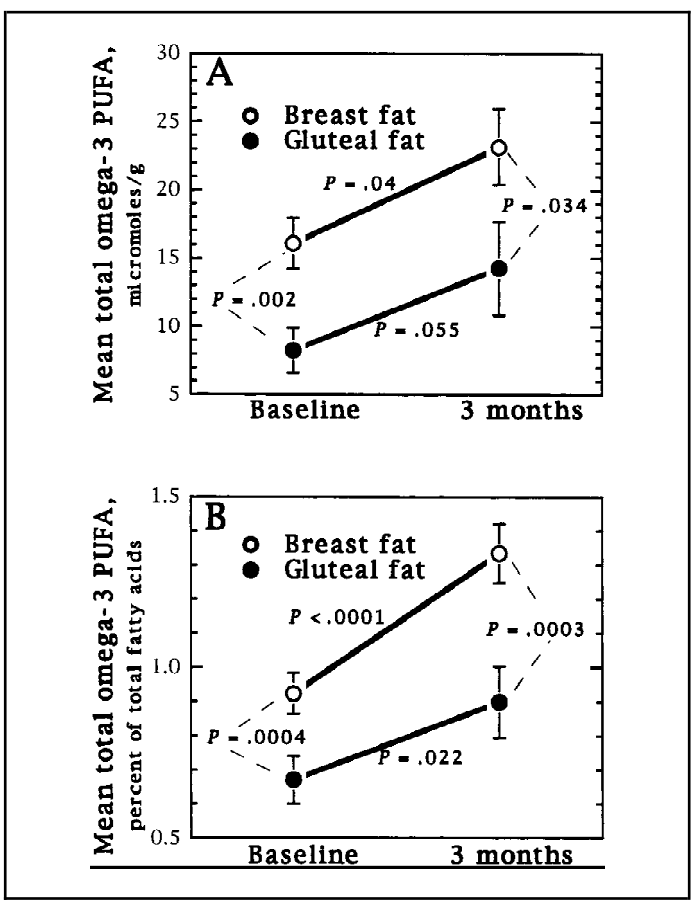

Fig. 1. Changes in mean total omega-3 polyunsaturated fatty acids content of breast and gluteal fat with low-fat, fish oil-supplemented diet intervention for 3 months (*P* = results of two-sided paired *t* tests; n = 25). The bars represent standard errors.

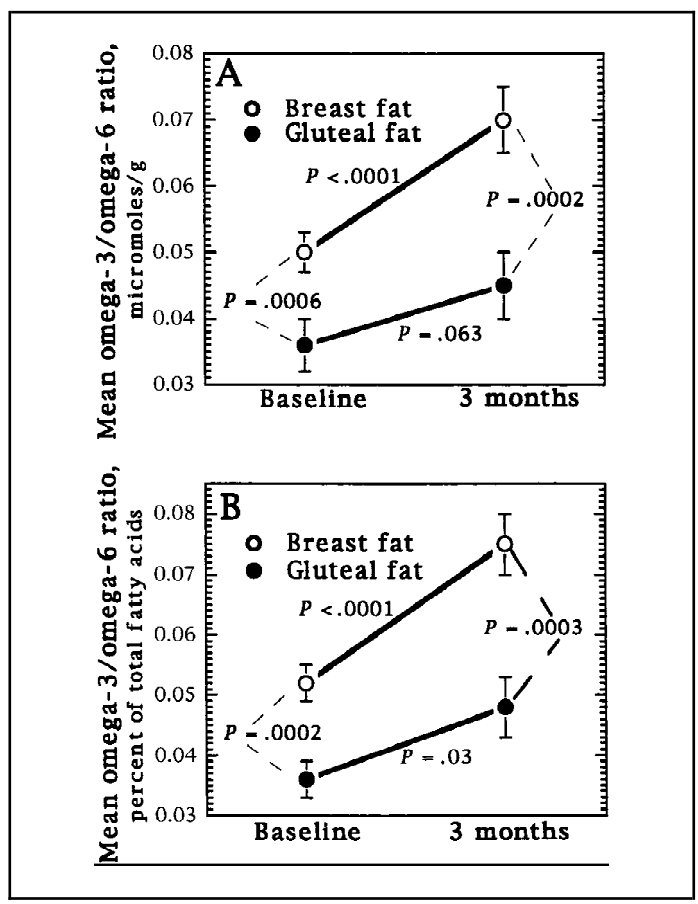

Fig. 2. Changes in mean omega-3/omega-6 ratio polyunsaturated fatty acids ratio of breast and gluteal fat with low-fat, fish oil-supplemented diet intervention for 3 months (*P* = results of two-sided paired *t* tests; n = 25). The bars represent standard errors.

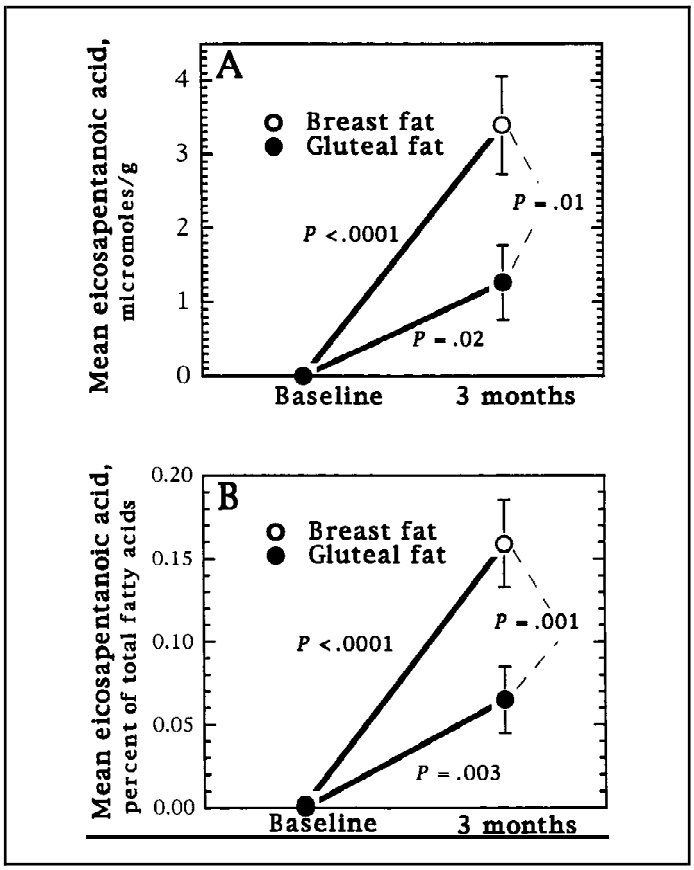

Fig. 3. Changes in mean eicosapentaenoic acid content of breast and gluteal fat with low-fat, fish oil-supplemented diet intervention for 3 months (P = results of two-sided paired t tests; n = 25). The bars represent standard errors.

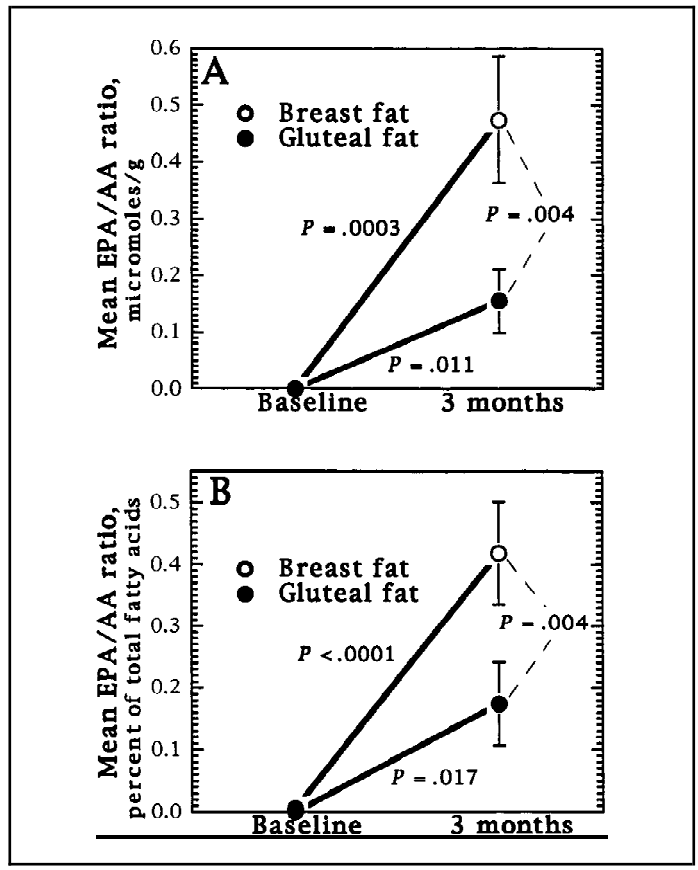

Fig. 4. Changes in mean eicosapentaenoic acid/arachidonic acid ratio of breast and gluteal fat with low-fat, fish oil-supplemented diet intervention for 3 months (P = results of two-sided paired t tests; n = 25). The bars represent standard errors.

Comparison of Breast and Gluteal Adipose Tissue Fatty Acids

A comparison of concentrations of breast and gluteal tissue fatty acids before the intervention showed significantly higher levels of total ω-6 fatty acids (P = .02), total ω-3 fatty acids (P = .002), and ω-3/ω-6 ratio (P = .0006) in breast fat versus gluteal fat. At the end of dietary intervention, breast fat maintained higher levels of total ω-3 fatty acids and a higher ω-3/ω-6 ratio than gluteal fat. A comparison of the relative percentages of fatty acids in breast and gluteal fat also showed significantly higher total ω-3 fatty acids (P = .0004) and a higher ω-3/ω-6 ratio (P = .0002) in breast fat versus gluteal fat before and after intervention (Figs. 1 and 2). Furthermore, both the concentration of eicosapentaenoic acid and the eicosapentaenoic acid/arachidonic acid ratio were significantly higher in breast fat versus gluteal fat at the end of 3 months of intervention with an LF/FOS diet (Figs. 3 and 4).

The changes in stored fatty acids in breast and gluteal fat after intervention with an LF/FOS diet for 3 months were also studied. Site-specific differences in amounts of incorporation of individual ω-3 polyunsaturated fatty acids were observed. The changes were significantly greater in breast fat compared with gluteal fat for eicosapentaenoic acid (mean value ± standard deviations: 3.40 ± 0.67 μmol/g versus 1.27 ± 0.50 μmol/g; P = .09) and docosahexaenoic acid (3.57 ± 0.69 μmol/g versus 1.80 ± 0.66 μmol/g; P = .05). Furthermore, breast fat compared with gluteal fat had a higher change in ω-3/ω-6 ratio (0.02 ± 0.004 μmol/g versus 0.009 ± .005 μmol/g; P = .07) and a significantly higher change in eicosapentaenoic acid/arachidonic acid ratio (0.48 ± 0.12 μmol/g versus 0.17 ± 0.07 μmol/g; P = .007) compared with gluteal fat with short-term supplementation with fish oils. Similar results were obtained when the concentrations were expressed as absolute amounts or relative percentages.

Before dietary intervention, there was no correlation between the concentration (μmol/g) of total ω-6 polyunsaturated fatty acids, total ω-3 polyunsaturated fatty acids, or the eicosapentaenoic acid/arachidonic acid and ω-3/ω-6 ratios from the two sites of adipose tissue. At the end of the LF/FOS diet intervention, there were statistically significant positive correlations between the change in the eicosapentaenoic acid/arachidonic acid ratio in plasma, breast fat, and gluteal fat (Table 4). The correlation was similar for both concentrations and relative percent change in the eicosapentaenoic acid/arachidonic acid ratio. The correlation for the change was higher between breast and gluteal fat compared with plasma and tissue. However, there was no correlation between plasma and the two sites of adipose tissue in terms of changes in total ω-6, total ω-3 polyunsaturated fatty acids, or ω-3/ω-6 ratios.

Discussion

We report the results of a study of the effects of short-term LF/FOS dietary intervention on the composition of breast and gluteal adipose tissue. A study (*31*) suggests that there is an

Table 4. Pearson correlation coefficients of change in EPA/AA ratio between plasma, breast fat, and gluteal fat in patients with breast cancer on low-fat, fish oil-supplemented diet for 3 months*

	Pearson correlation coefficient	*P*
	Mean change (relative, %)	
EPA/AA ratio		
Breast and gluteal fat	.82	.0001
Plasma and breast fat	.71	.0002
Plasma and gluteal fat	.66	.002
	Mean change (μmol/g)	
EPA/AA ratio		
Breast and gluteal fat	.81	.0001
Plasma and breast fat	.71	.0002
Plasma and gluteal fat	.66	.001

*EPA = eicosapentaenoic acid and AA = arachidonic acid.

inverse relationship between the incidence of breast cancer and the level of fish consumption, suggesting a protective role for ω-3 polyunsaturated fatty acids toward human breast cancer. Japan, which has a very high fish consumption, a low fat intake, and a very low mortality from breast cancer, was included in that analysis. However, for dietary fish oils to have an impact on the incidence of breast cancer, they should demonstrably alter the breast microenvironment. We have studied the effect of short-term consumption by patients with breast cancer of a LF/FOS diet on the fatty acid composition of breast adipose tissue.

The first purpose of this study was to determine if feeding the study subjects a LF/FOS diet could alter the ω-3/ω-6 fatty acid ratios in their plasma and adipose tissue. We found that short-term intervention with a LF/FOS diet altered the ω-3/ω-6 fatty acid ratio in both plasma and adipose tissue. The fourfold increase in the ratio in the plasma and the 1.4-fold increase in the ratio in breast and gluteal adipose tissues could be attributed to the consumption of a low-fat diet and ω-3 fatty acids in the form of fish oils by patients with breast cancer.

The main polyunsaturated fatty acids in high-fat Western diets is linoleic acid (18:2; ω-6), with lesser proportions of other fatty acids, such as linolenic acid (18:3; ω-3), arachidonic acid (20:4; ω-6), eicosapentaenoic acid, and docosahexaenoic acid. The intake of linoleic acid is on the order of 10-20 g/day, and this constitutes more than 85% of the total polyunsaturated fatty acids intake (*32*,*33*). The high level of ω-6 polyunsaturated fatty acids in tissues is a result of the predominance in the current food supply of linoleic acid, the precursor of arachidonic acid, which is further metabolized to 2-series eicosanoids. The LF/FOS diet used in our study supplied 15% of calories as fat, and the fatty acid composition of the fat was mainly ω-3 polyunsaturated fatty acids from fish oils rather than ω-6 polyunsaturated fatty acids as in the Western diet. This dietary modification led to a significant decrease in linoleic acid and arachidonic acid with a concomitant increase in the ω-3 fatty acids (eicosapentaenoic acid and docosahexaenoic acid) in plasma.

Although there was no decrease in the ω-6 fatty acids in the breast or gluteal adipose tissues of the subjects following their consumption of the LF/FOS diet for 3 months, their consumption of fish oils resulted in a statistically significant increase in total ω-3 fatty acids (specifically, eicosapentaenoic acid and docosahexaenoic acid) in those tissues. These short-term changes could not decrease the long-term accumulation of ω-6 fatty acids in the storage sites. Attempts to change the content of linoleic acid (18:2, ω-6) stored in adipose tissue of humans over the years have required nearly 3 years to reach a lower steady-state level (*34*). In contrast, the consumption of fish or fish oil supplements is an effective means of increasing ω-3 eicosanoid precursors in many organs of the body. In the present study, consumption of ω-3 fatty acids was associated with a statistically significant increase in the ω-3/ω-6 ratios and eicosapentaenoic acid/arachidonic acid ratios in breast and gluteal adipose tissues.

The second purpose of the study was to address the issue of differences in fatty acid profiles in fat obtained from different body sites. Adipose tissue is a reservoir of both dietary and endogenously synthesized fatty acids in the body. Several studies (*35*,*36*) have reported site-specific differences between visceral and subcutaneous fatty acid composition. Differences in fatty acid composition have been reported between various subcutaneous sites. Two studies (*28*,*37*) have reported reduced saturated and increased monounsaturated fatty acids in subcutaneous adipose triacylglycerols from gluteal versus abdominal sites whereas, another study (*38*) has reported increases in polyunsaturated fatty acids in addition to changes in saturated and monounsaturated fatty acids between the abdominal and inner or outer thigh. These studies (*28*,*35*-*38*) did not investigate the differences between long-chain ω-3 fatty acid and ω-3/ω-6 ratios between various adipose tissue sites.

Because gluteal fat has been used as a surrogate for breast fat in case–control studies of the relationship between polyunsaturated fatty acids in the diet and/or body stores and breast cancer risk, we studied the extent to which breast adipose tissue is representative of the body fat composition. There were significant differences in ω-3 fatty acids concentration and ω-3/ω-6 ratio between breast and gluteal adipose tissue both before and after intervention with the LF/FOS diet. In patients with breast cancer, breast fat was found to have maintained higher total ω-3 fatty acids stores and ω-3/ω-6 ratio compared with gluteal fat. Since the changes produced in the breast fat were statistically significantly greater compared with gluteal fat, it may be concluded that breast fat changed faster with dietary intervention. Such differences have not been previously reported and suggest that gluteal fat is an imperfect surrogate for breast fat, especially for intervention studies.

There are few data on the impact of consumption of fish oils rich in ω-3 polyunsaturated fatty acids on the concentration of fatty acids in different adipose tissue sites. In some earlier studies (*39*,*40*), a strong correlation was reported between the intake of ω-3 polyunsaturated fatty acids and related fatty acids concentration in gluteal adipose tissues. The effects of the LF/FOS diet intervention on breast and gluteal total fatty acids composition in patients with breast cancer were also examined in this study. The changes produced in total ω-3 and total ω-6 fatty acid and ω-3/ω-6 ratio were comparable between the two adipose tissue sites. However, there were statistically significant differences in the ω-3 fatty acids between the two sites. Following the dietary intervention, the concentrations of eicosapentaenoic acid and docosahexaenoic acid and also the ratio of eicosapentaenoic acid to arachidonic acid were higher in breast adipose tissue than

in gluteal adipose tissue. Furthermore, changes produced in the eicosapentaenoic acid/arachidonic acid ratio correlated between the two sites.

The mechanisms that might underlie the differences in fatty acid composition that we observed between the two sites in this study are presently unknown. Whether these differences reflect differences in metabolic activities, such as the rate of deposition of fat, the method of mobilization and rate of endogenous synthesis of fatty acid between the two sites are unknown. Fat depots in different regions of the body have separate functions that depend on the needs of the region that in turn may dictate the composition of the depot fat (*37*). The lack of correlation of fatty acids between two sites of adipose tissue before the intervention suggests that composition of gluteal adipose tissue may not serve as a good surrogate for breast cancer risk assessment. Although the extent of changes produced between the two sites was statistically correlated for eicosapentaenoic acid/arachidonic acid ratio, the significantly higher ratios maintained in the breast fat both before and after intervention may reflect a distinct microenvironment within the breast. It is not known whether the fatty acid composition in patients with breast cancer at either site is similar to that of disease-free patients. The possibility that the disease influences adipose tissue fatty acid composition has to be considered, since an altered metabolism of the host adipose tissue fatty acids has been reported in patients with colon cancer (*41*).

It has been shown that fatty acid composition of breast tissue fatty acids, including tumors, depends in part on fatty acid availability, which is influenced by dietary fatty acids (*42*). Thus, dietary intervention could provide an effective means to alter the fatty acid availability in tumor tissue and thereby possibly affect tumor growth. In addition, on the basis of the results of this study, it is possible to alter the ω-3/ω-6 polyunsaturated fatty acid ratio with short-term dietary intervention in patients with breast cancer. The higher ω-3/ω-6 ratio and the higher eicosapentaenoic acid/arachidonic acid ratio achieved in breast fat compared with gluteal fat following short-term intervention with an LF/FOS diet could have important implications for chemoprevention of breast cancer. Furthermore, there is a need to conduct long-term studies in patients with breast cancer to see if such ratios can be increased further and maintained locally within the breast to have a clinically significant impact on breast cancer. In future studies, we plan to study the relationship of ω-3/ω-6 ratios and prostaglandin production with breast cancer risk. The differential effect of dietary intervention on adipose tissue of the two sites and the lack of correlation of the changes achieved between the two sites indicate that it may be important to study the local adipose tissue within the breast to study the interaction of dietary polyunsaturated fatty acids and breast cancer risk.

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Notes

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