Effects of Breast Cancer Treatments on Plasma Nutrient Levels: Implications for Epidemiological Studies

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Abstract

The interpretation of case-control studies in which blood nutrient levels are examined as etiological factors in cancer is complicated by the possibility that either the disease or its treatment may alter these levels. Circulating levels of selected nutrients were examined prior to diagnostic biopsy and compared with levels 3 to 4 months after diagnosis among 71 women with breast cancer and 95 women with benign breast disease. Among women with benign breast disease or women with breast cancer who were not given postsurgical adjuvant drug therapy, levels of α-carotene, lycopene, α-tocopherol, cholesterol, and triglycerides did not change over time. In contrast, women who received chemotherapy had increased levels of cholesterol, retinol, and α- and γ-tocopherol, and women on antiestrogen therapy showed increased levels of triglycerides and α-tocopherol. Overall, the concentrations of carotenoids (lycopene, α-carotene, and β-carotene) did not change in breast cancer cases, although subgroup analyses showed increased levels of β-carotene among cases not receiving drug treatment and decreased levels among those receiving antiestrogens. In summary, blood levels of some nutrients did not appear to be affected by breast cancer or its treatments, but changes were noted for levels of plasma lipids, tocopherols, retinol, and β-carotene. Those investigating the etiological relationship between breast cancer and circulating nutrients need to consider these effects in designing and interpreting epidemiological studies.

Introduction

Much research has been devoted to studying the relationship between diet and cancer using case-control designs. However, due to limitations in dietary methodology (1, 2), particularly the potential for dietary interviews or questionnaires to misclassify individuals according to their nutrient intake, alternative indicators of nutritional status have been sought.

The use of circulating biochemical indicators of nutritional status is promising, but the interpretation of results in case-control studies is complicated by the possibility that either the disease or its treatment may alter these levels. Some studies have investigated breast cancer by enrolling cases after surgery, potentially during treatment or shortly thereafter (3, 4). Thus, questions can be raised regarding the etiological relevance of the findings. Even with study designs that collect blood samples months after therapy has been completed, questions can be raised regarding the length of time needed for blood levels to reflect "usual" status. Blood samples taken upon hospital admission (5, 6) also may not represent typical levels, since patients are likely to have modified behaviors prior to admission and/or since the tumor may affect blood nutrient levels. Thus, methodological studies analyzing blood samples at various time periods before and after breast cancer diagnosis can be informative.

Some studies have evaluated the effects of steroid therapy. Although the influence of antiestrogen therapy on plasma lipids and lipoprotein profiles have been documented (7–14), most studies were limited by small numbers and/or lack of assays prior to diagnosis and treatment. Little research has been devoted to the effects of antiestrogen therapies on plasma micronutrient levels or on the effects of cytotoxic treatments on plasma lipids and micronutrients.

To better understand the potential problems of interpreting circulating biochemical indicators of nutritional status in case-control studies, we examined the levels of selected nutrients before and after the diagnosis and treatment of breast cancer.

Materials and Methods

Between September 1985 and September 1986, study subjects were enrolled from the breast clinic at Roswell Park Memorial Institute and from the offices of two private surgeons in Buffalo, New York. All participants had lived in upstate New York or Pennsylvania for at least 1 year. Eligible patients were women 30 to 80 years of age who were being evaluated for a breast mass but who had no previous history of cancer. All consecutive subjects scheduled for diagnostic biopsies, as well as those not requiring surgical evaluation, were approached for participation in the study, and informed consent was obtained. Each subject completed a questionnaire, which provided information on dietary intake and breast cancer risk factors. Fasting blood samples were obtained both before the diagnostic breast biopsy and 3 to 4 months later at a follow-up visit or by appointment for those not scheduled for a biopsy or return visit.

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Two-hundred seventy-one patients were requested to participate in the overall study, of which 236 (87%) consented and completed the questionnaire and 221 (81%) donated at least the first blood sample. Of these participants, 198 (90%) also donated a second blood sample 3 to 4 months later. There were no significant differences in the participation rates or in the time interval between the first and second blood samples between cases and benign breast disease patients.

All patients were classified into one of three groups based on their clinical examination or pathology reports from the breast biopsies: cases (n = 83); benign breast disease patients (n = 113); or excluded (n = 40). The focus of these analyses were changes in blood nutrient levels among benign breast disease patients at average risk for breast cancer compared with women who were diagnosed with breast cancer. Thus, 40 patients who had biopsy reports describing lesions thought to place them at increased risk of breast cancer (predominantly of an atypical hyperplastic nature) were excluded (15, 16). The 113 benign breast disease patients in this analysis were those 79 women who were found to have benign lesions not associated with increased risk and 34 women who were referred to the specialists because of a suspicious mammogram or lump but were determined not to have a mass requiring biopsy. Cases were women found to have breast cancer (International Classification of Diseases 9, no. 174) and were classified according to stage of disease using standard postsurgical staging forms (17).

The fasting blood samples were centrifuged, and plasma was preserved in sodium ascorbate (10 mg/ml plasma) and frozen at −80°C until the end of the study. Samples were thawed once for portioning of aliquots and once for laboratory analyses. Aliquots from all subjects and first and second blood draws were analyzed in a random fashion, and all analyses were conducted blind to case-control status. Retinol, tocopherols, and carotenoids were analyzed by high-pressure liquid chromatography; retinol and tocopherols by a modification (18) of a standard method (19) and carotenoids by a modification (18) of a new method (20). Triglycerides were analyzed using a colorimetric assay (21) and cholesterol by a kit method (Sigma Chemical Co., St. Louis, MO).

All analyses were limited to women with values for both blood samples. Statistical analyses were conducted using SYSTAT (Systat, 1985 and 1990). Differences in means between nutrient levels from bloods obtained prior to biopsy (blood no. 1) and 3 to 4 months after surgery (blood no. 2) were tested with the paired t test, whereas differences between cases and benign breast disease patients in nutrient levels in blood no. 1 or blood no. 2 were tested with the independent t test. Blood nutrients were log-transformed for t tests but are presented as untransformed means. Differences between treatments were tested using analysis of covariance controlling for other potential confounding factors including age, Quetelet's index, dietary fats, and plasma lipids. Covariates and nutrient differences were standardized for analysis of covariance of treatment effects, and only significant ($P < 0.05$) predictors of the outcome were included in the models. Analyses of the change in fat-soluble nutrients (retinol, carotenoids, and tocopherols) over time controlled for the change in plasma lipids during that time. The Quetelet's index and dietary fat were measured only at baseline.

Table 1 Paired analysis of two blood samples taken 3–4 months apart from women with benign breast disease

Blood marker (units)	Blood no. 1	Blood no. 2
	Mean (SD)	Mean (SD)
β-Carotene (μg/dl)	13.3 (7.5)	12.7 (7.0)
α-Carotene (μg/dl)	3.4 (2.0)	3.5 (2.1)
Lycopene (μg/dl)	26.1 (12.4)	25.1 (12.1)
α-Tocopherol (μg/dl)	1351 (578)	1367 (587)
γ-Tocopherol (μg/dl)	179 (112)	190 (104)
Retinol (μg/dl)	54.6 (12.0)	55.9 (12.8)
Cholesterol (mg/dl)[a]	235 (53)	237 (55)
Triglyceride (mg/dl)	98 (56)	102 (56)

[a] n = 95 for cholesterol and n = 91 for all other analytes.

Results

The paired analyses of the two blood samples in the benign breast disease group are shown in Table 1. Although no treatments were prescribed for these patients, some may have initiated dietary changes or vitamin supplement usage, factors not evaluated. There were no significant changes in the plasma levels of the three carotenoids (β-carotene, α-carotene, and lycopene), the two tocopherols (α- and γ-tocopherol), retinol, or the two lipids (cholesterol and triglycerides).

Paired analyses among breast cancer cases also indicated no significant differences for the three carotenoids, but plasma levels of tocopherols, retinol, cholesterol, and triglyceride showed significant increases in the second blood samples drawn from cases (Table 2). Values from the first blood sample showed significantly higher triglyceride values among cases compared with benign breast disease patients (P = 0.002), whereas γ- and α-tocopherol, retinol, and cholesterol concentrations were similar between the two groups. Similar comparisons of the second blood sample showed triglycerides, γ-tocopherol, and retinol levels were significantly higher compared with levels among benign breast disease patients (P = 0.002, P = 0.001, and P = 0.06, respectively).

To test differences within treatments, breast cancer cases were then stratified according to mutually exclusive postsurgical treatment groups. Subjects received either no drug treatment, chemotherapy, or tamoxifen. Although eight patients were treated with postoperative radiation and three with ovarian surgery, all but two

Table 2 Paired analysis of two blood samples taken 3–4 months apart from women with breast cancer

Blood marker (units)	n	Blood no. 1	Blood no. 2
		Mean (SD)	Mean (SD)
β-Carotene (μg/dl)	70	11.6 (9.0)	12.6 (10.3)
α-Carotene (μg/dl)	70	3.2 (2.3)	3.2 (2.6)
Lycopene (μg/dl)	70	21.7 (10.0)	22.8 (11.3)
α-Tocopherol (μg/dl)	68	1288 (518)	1379 (639)[a]
γ-Tocopherol (μg/dl)	68	193 (115)	250 (123)[b]
Retinol (μg/dl)	68	55.1 (16.1)	60.7 (20.3)[b]
Cholesterol (mg/dl)	71	232 (47)	247 (55)[b]
Triglyceride (mg/dl)	68	118 (51)	137 (84)[c]

[a] P = 0.08.
[b] $P < 0.01$.
[c] P = 0.03.

Table 3 Paired analysis of plasma nutrient levels within postsurgery treatment groups in breast cancer patients

Group	*n*	Blood no. 1	Blood no. 2	*P*[a]
		Mean (SD)	Mean (SD)	
Cholesterol (mg/dl)				
No drug treatment	41	242 (50)	251 (46)	0.096
Tamoxifen	15	222 (38)	221 (57)	0.987
Chemotherapy	14	213 (42)	256 (69)	0.015
Triglyceride (mg/dl)				
No drug treatment	39	116 (56)	114 (63)	0.804
Tamoxifen	14	119 (42)	175 (103)	0.033
Chemotherapy	14	127 (49)	168 (101)	0.091
γ-Tocopherol (μg/dl)				
No drug treatment	39	192 (104)	225 (105)	0.006
Tamoxifen	15	206 (166)	220 (138)	0.242
Chemotherapy	13	179 (79)	362 (104)	<0.001
α-Tocopherol (μg/dl)				
No drug treatment	39	1321 (515)	1335 (517)	0.631
Tamoxifen	15	1339 (626)	1564 (1023)[b]	0.151
Chemotherapy	13	1156 (407)	1313 (394)	0.017
Retinol (μg/dl)				
No drug treatment	39	53.5 (14.2)	58.4 (16.3)	0.029
Tamoxifen	15	63.3 (17.4)	64.5 (18.2)	0.590
Chemotherapy	13	51.7 (17.8)	64.1 (31.7)[b]	0.029
β-Carotene (μg/dl)				
No drug treatment	40	13.6 (11)	16.0 (12)	0.003
Tamoxifen	15	9.4 (5.2)	6.7 (4.9)	0.062
Chemotherapy	14	8.4 (4.8)	9.3 (4.1)	0.362

[a] Significance of paired *t* test performed on log-transformed data.
[b] Outlier α-tocopherol = 4965 μg/dl, retinol = 161 μg/dl.

completed their treatment at least 2 months before the second blood sample was drawn and were grouped in the category of no drug treatment. In general, the no treatment group was composed of earlier stage disease (68% *in situ* and Stage I); the tamoxifen group had slightly more severe disease, with positive nodal status and estrogen receptor-positive tumors (87% Stage II); whereas the chemotherapy group has the most advanced disease, with positive nodal status and estrogen receptor-negative tumors (43% Stages III–IV).

There was a marginally significant increase in cholesterol level in the no drug treatment group (Table 3) and a markedly increased cholesterol level in the chemotherapy group. In addition, covariance analysis indicated that the change in cholesterol levels over time was different between cases not on drug therapy and the chemotherapy group, after controlling for age and Quetelet's index ($P = 0.08$). This difference over time was diminished by controlling for baseline dietary fat intakes, since the chemotherapy group had significantly higher dietary fat intakes at baseline than did the no treatment group. Both the chemotherapy and the no treatment groups had significantly greater changes in cholesterol over time than did the benign breast disease patients after adjusting for age and Quetelet's index ($P < 0.01$ and $P = 0.02$, respectively).

Triglyceride levels were significantly increased in the tamoxifen group (Table 3) and marginally significantly increased in the chemotherapy group. Both of these changes over time were also significantly different from the change in the no treatment group ($P = 0.006$ and $P = 0.042$, respectively) and remained significantly different after controlling for age, Quetelet's index, and baseline dietary fat intakes. The lack of change in triglyceride values in the no treatment cases was similar to that of the benign breast disease patients, while the changes evident in the tamoxifen and chemotherapy groups were significantly different from values for the benign breast disease group ($P = 0.001$ and $P = 0.025$, respectively) after adjustment for age and Quetelet's index.

The significant increases over time in plasma retinol and α- and γ-tocopherol in the breast cancer patients (Table 2) but not in the benign breast disease patients (Table 1) suggested further investigation by treatment groups. Levels of γ-tocopherol rose in all case groups and were significantly increased in the no treatment and chemotherapy groups (Table 3). Compared with no drug treatment, the women with chemotherapy had significantly greater increases in γ-tocopherol over time ($P < 0.001$). For α-tocopherol, the increase in the chemotherapy group was not significantly greater than in the no treatment group, while the increase in the tamoxifen group was significant after controlling for cholesterol differences ($P = 0.002$). Within the chemotherapy group, however, the change was significant because of the relatively low initial levels. Retinol concentrations within treatment groups increased in the no treatment and chemotherapy groups but not in the tamoxifen group. The larger change in the chemotherapy group was significantly different from the change in the no treatment group ($P = 0.006$). Similar analyses by treatment group indicated no differences in α-carotene or lycopene concentrations over time. β-Carotene, however, decreased in the tamoxifen group compared with a slight increase in the no treatment group ($P = 0.002$). These changes over time could not be explained by differences in plasma lipids, tocopherols, age, or Quetelet's index. Interestingly, both treatment groups had lower mean levels in blood 1 compared with the mean concentration in the no treatment group. Outliers were noted for several of these micronutrients; analyses after the removal of outliers showed results similar to those presented.

Discussion

This study demonstrated significant changes in plasma nutrients over a 3–4-month period of time after breast cancer diagnosis and surgical treatment, whereas benign breast disease patients did not show changes in nutrient status over a similar time period. Adjuvant therapy with tamoxifen was associated with decreased levels of β-carotene and increased levels of triglycerides and α-tocopherol, whereas chemotherapy was associated with elevation in levels of cholesterol, γ-tocopherol, and retinol. Within the group of cases not receiving drug treatment, there were increases in cholesterol, γ-tocopherol, and retinol concentrations after the diagnosis. These results suggest that in case-control studies, nutrient values in blood samples drawn from cases after surgery and/or treatment may not be accurate indicators of levels before diagnosis.

Women in the no drug treatment group by definition had earlier-stage disease than the women in the treatment groups. Therefore, comparison of the difference in values over time between no drug treatment and treatment also may be due to the effect of disease progression

during that time period. It is unlikely, however, that disease progression within such a short period of time would result in such marked changes in nutrient levels. Whether due to disease or to treatment, the observed changes in nutrient levels after treatment are of methodological importance. In contrast, it is unknown why some nutrient levels changed in the no drug treatment group, although we can speculate that there may have been life-style changes after diagnosis. It remains unclear whether blood samples taken at the time of diagnosis are atypically low (related to disease or behavior) or whether the later blood sample reflects changed behaviors. Further evaluations with sequential blood sampling and evaluation of life-style changes in women with breast cancer would be worthwhile.

Substantial effects of breast cancer and/or its treatment were noted on levels of triglycerides and cholesterol. The effect of tamoxifen on plasma triglycerides has been noted previously (7–14), but the moderate effect of chemotherapy on plasma triglyceride levels has not been previously reported. Cholesterol increased substantially among women receiving chemotherapy. This was especially evident in women with more advanced disease who had lower prediagnostic cholesterol levels (data not shown). It was not possible to completely separate the effect of removal of a more advanced tumor from that of chemotherapy and/or from any subsequent dietary changes that might have occurred in that group. A possible explanation for a direct effect of chemotherapy on cholesterol levels, however, involves increased low-density lipoprotein receptor activity in tumor cells. It has been shown that patients with acute leukemia have hypocholesterolemia and increased low-density lipoprotein receptor activity in tumor tissue at diagnosis and that plasma cholesterol levels increase concurrently with the loss of tumor cells during chemotherapy (22, 23). There is also suggestive evidence for this mechanism in breast tissue (23). We have previously reported lowered cholesterol levels with stage of disease in this study group (24), which is congruent with the findings of hypocholesterolemia at diagnosis observed in case studies (22, 23). Although the present study is limited in sample size, especially among those with more advanced disease, our data suggest that both the disease and chemotherapy affect cholesterol levels.

Although tamoxifen has been reported to be associated with an approximate 10% decrease in total cholesterol (13, 14) we did not observe such a decrease in this study. Adjuvant therapy was not initiated in the present study until 4–6 weeks after surgery, which would have resulted in a minimum duration of treatment of 6–8 weeks at the time of the second blood collection. Love and coworkers (11) noted a decrease from baseline after 3 months of treatment. Thus, there may have been insufficient time for the cholesterol-lowering effect of tamoxifen to have become evident in our study.

The increased levels of α-tocopherol (25–28) and retinol (27, 28) between the first and second blood samples among cases, and especially among women who received chemotherapy, could have resulted from dietary changes, initiation of vitamin supplementation, or relatively low initial values. Likewise, slight changes in β-carotene in the no drug treatment and tamoxifen groups suggest that blood samples drawn after diagnosis may not be representative of a subject's usual status. These data suggest that it may be preferable to obtain 2 samples/individual to better estimate status (29). For example, in this study the mean β-carotene values of the two blood samples within the tamoxifen and chemotherapy groups were lower (8.8 and 8.1 μg/dl, respectively) than the means for the no drug treatment (14.8 μg/dl) or benign breast disease group (13.0 μg/dl). The mean of two blood samples may generate a more accurate estimate of usual status, but further work in this area is needed.

The lack of statistical differences in all nutrients tested at two time points in the benign breast disease patients, and in triglycerides, α-tocopherol, α-carotene, lycopene in cases receiving no drug treatment suggests that these markers are stable within individuals not on drug therapy over a limited period of time. Therefore, epidemiologists may be confident in testing untreated cases for some nutrients at 4 or more months after diagnosis in case-control studies. However, very few cases are likely to be found at the present time without adjuvant drug therapies, in contrast to the present study, which was conducted prior to the 1988 National Cancer Institute Clinical Alert recommending widespread usage of tamoxifen adjuvant therapy based on data from the Third International Conference on Adjuvant Therapy of Primary Breast Cancer (30).

Since many of the nutrients investigated were affected by breast cancer treatments, the interpretation of case-control studies of breast cancer should consider the timing of the blood collection. Although this study had a limited number of subjects in each treatment group, potentially important associations were observed. These associations should be examined in larger studies. Further investigation with earlier prediagnostic and later postdiagnostic or posttreatment samples would assist in determining opportune periods for specimen collections.

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