ASSOCIATIONS BETWEEN BREAST CANCER
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Abstract

A case-control study investigating the association between plasma lipids and breast cancer was conducted in Buffalo, New York. Women aged 30-80 completed a questionnaire and donated a fasting blood sample prior to diagnostic breast biopsies. The 83 women found to have breast cancer (cases) had significantly higher plasma triglyceride values than did the 113 women found not to have breast cancer (controls). Lower beta-carotene values were associated with breast cancer, but only in those women with elevated triglyceride or cholesterol. Plasma cholesterol values were lower in those breast cancer cases presenting with a more advanced stage of disease suggesting that metabolic effects of clinical and preclinical breast cancer may lower cholesterol levels. The observation of a positive association with triglycerides and a negative association with beta-carotene is consistent with the hypothesis that women who develop breast cancer may consume diets higher in total and saturated fat, and lower in carotene-rich vegetables.

Running title: Breast cancer and plasma lipids

Key Words: Breast cancer, benign breast disease, plasma, diet, cholesterol, triglyceride, beta-carotene

Introduction

Much research has been devoted to the association between breast cancer and various indicators of fat consumption. A number of ecological studies have shown a direct association between breast cancer rates and per capita fat intake (1-6), total fat estimated from a food frequency questionnaire (7), and animal but not vegetable fat (8,9). Results from analytical studies of individuals, however, have been inconsistent. Investigators have generally reported no significant association between total dietary fat intake and breast cancer (10-17), although a few studies have shown weak direct associations (18,19). Most ecological studies (20-22) and analytical studies (23-28) using food items have suggested that diets more frequently including foods high in total and saturated fat are associated with breast cancer. One other investigation (29) reported no association between consumption of high fat food items and fatal breast cancer. Other research of individuals using estimated dietary intake of saturated fat have been equivocal (11,13,14,18). In summary, ecological studies generally indicate a direct association between breast cancer and total and saturated fat, while analytical studies have found either a weak association or none at all.

The relationship between plasma cholesterol levels and breast cancer is also unclear. Investigations using case-control designs report higher (30), lower (31) and similar plasma cholesterol levels (32) in breast cancer cases compared with controls. An inverse association between plasma cholesterol and overall cancers in women has been suggested in two cohort studies (33,34); however, the differences

between cases and controls were not statistically significant. Two prospective studies (35,36) reported significantly higher cholesterol values among women who later developed breast cancer, while two other prospective studies (37,38) found no association. However, further analyses of these data revealed a tendency for cholesterol values to be lower in women with breast cancer (39), or in women with overall cancers (36,40) when that cancer was diagnosed less than two years after the blood sample was drawn. These results support the concept that preclinical cancer can lower serum cholesterol.

The inconsistencies noted in the studies of dietary fat and breast cancer may be related, in part, to the oftentimes serious limitations of dietary methodology (41-43). In theory, one approach which may obviate this difficulty might be the use of metabolic indicators of the consumption of high fat diets. We, therefore, investigated the association between two plasma lipids and breast cancer. Although plasma triglycerides and cholesterol do not always reflect dietary intake, high levels of these lipids are consistent with high total and saturated fat dietary patterns (44-53). The inconsistencies in the plasma cholesterol studies and the suggestion of a preclinical effect warranted further investigation because the effect of disease on circulating levels of cholesterol would be of methodological interest to epidemiological studies obtaining blood samples on cancer patients. In addition, because of the interest in beta-carotene and cancer risk (54) and because beta-carotene may be an important anti-oxidant against lipid peroxidation (55), the relationships between plasma beta-carotene and plasma lipids and cancer risk were investigated.

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Methods

Between September 1985 and September 1986, women were enrolled from
the breast clinic at Roswell Park Memorial Institute, and from the
offices of two private surgeons in Buffalo, New York. Eligible patients
were 30 to 80 years of age, had no previous history of cancer and had
lived in upstate New York or Pennsylvania for at least one year. After
subjects were scheduled for a diagnostic biopsy, informed consent was
obtained and they were given a questionnaire and directions for the
blood collection procedure. The self-administered questionnaire
ascertained information regarding the subjects' medical and reproductive
history.

Patients were classified into one of three groups based on their
pathology reports from the diagnostic breast biopsies; cases (n=83),
controls (n=113) or excluded (n=40). Patients with biopsy reports that
described lesions thought to be at increased risk of breast cancer,
predominantly of an atypical hyperplastic nature, were excluded from the
control group because these lesions, though not malignant, are thought
to correlate with much higher risk of subsequent breast cancer (56-58).
Subjects with the remaining benign lesions, such as lipomas or fibromas,
were included in the control group, as well as 34 patients who completed
the diagnostic workup, but were determined not to have masses requiring
biopsy.

Fasting blood samples were drawn from subjects prior to their
biopsies, or on a designated day for the 34 patients without biopsies.
The samples were centrifuged, plasma was preserved in sodium ascorbate
(10 mg/ml plasma) and frozen at -80° until all samples had been

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collected. Samples were thawed once for portioning of aliquots and once for the laboratory analysis. Triglycerides were analyzed by a manual colorimetric method (59) and total cholesterol was assessed using a spectrophotometric kit method (Sigma Chemical Co., St. Louis, MO).

Statistical analyses were performed using SYSTAT (version 3.0, Systat, 1985) and the supplementary logit module on an AT&T 6300 Personal Computer. Differences in means were tested with the student's t-test. Nutrients were log-transformed and categorized into quartiles based on the distribution in the controls. Multivariate logit analyses were conducted with case status as the dependent variable; there were three possible outcome categories but only the case versus control results are presented here. In the multivariate analyses, the non-nutrient breast cancer risk factors used as covariates were age, age at menarche, age at menopause, age at first birth, parity, menopausal status, family history in a first degree relative, Quetelet's index, marital status and income. The logit models and variables were assessed using the log likelihood ratio test. Tests for trend were conducted using the continuous form of the nutrient variable in the logit analyses and in the ordinary least squares regression of stage of disease on the nutrient of interest. A general stage variable was used with 4 categories (In situ, Stage I, Stage II, and Stage III & IV), and a more specific stage variable was created with 20 ordinal categories based on the severity of disease designated from categories in the standard T-N-M staging form (60).

Results

Comparisons of the baseline characteristics for the cases and controls has indicated (61) that the cases were significantly older than the controls. Many of the known breast cancer risk factors (such as family history of breast cancer, higher weight, earlier age at menarche) were not more prevalent in cases than in controls, suggesting that these controls were at high-risk for breast cancer. Therefore, this study compared women with breast cancer to women at high-risk for breast cancer.

A comparison of the mean cholesterol levels (Table 1) indicated no differences between cases and controls, however there was a significant trend of decreasing cholesterol with increasing stage of breast cancer. Mean triglyceride levels for controls were lower than for cases (p=0.001). A slight trend of decreasing triglyceride levels is apparent with more advanced stage of disease, however this trend is not statistically significant (p=0.20).

The unadjusted logit model indicated no association between cholesterol and breast cancer (Table 2), but adjustment for the other risk factors and for other nutrients (triglyceride and beta-carotene) indicated a statistically significant association. Although not apparent from the odds ratios, a significant test for trend indicated an increasing risk of being a case associated with decreasing cholesterol values (test for trend p=0.03). Therefore, lower cholesterol values were associated with case status.

The unadjusted model for triglycerides indicated a significant trend of decreasing risk with decreasing triglyceride values (Table 3).

Adjustment for the other risk factors diminished this association, however the best-fit model included the other nutrients and predicted a 4-fold increased risk of breast cancer in the highest compared with the lowest triglyceride quartile.

These models were assessed by adding and testing variables and interactions hypothesized to be important in predicting case status. Previous analysis indicated that low beta-carotene levels were associated with increased risk of breast cancer (61). A test of the interaction between beta-carotene and triglyceride, and beta-carotene and cholesterol indicated that both interactions were significant predictors of case status ($p<0.005$ for the addition of both interactions) and that the interactions were related to one another (17). The results from the one and two interaction models were similar; for ease of interpretation, the results from the one interaction model are presented.

Table 4 summarizes the cholesterol by beta-carotene interaction. Within the highest cholesterol level, low beta-carotene was associated with an increased risk of breast cancer, and risk decreased as beta-carotene increased. A slightly elevated odds ratio was apparent within the median cholesterol level, in combination with low beta-carotene, however this confidence interval included unity. In contrast to the results from the main effect analysis, these results from the interaction analysis suggest an increased risk of breast cancer associated with the combination of higher cholesterol and lower beta-carotene values.

The evaluation of the triglyceride by beta-carotene interaction is shown in Table 5. There were no increased risks observed within the low triglyceride level, but a markedly increased risk associated with high triglyceride and low beta-carotene. There was also a suggestion of increased risk associated with low beta-carotene and the median level of triglyceride.

Discussion

Plasma Triglycerides

High triglyceride values in fasting blood samples were associated with breast cancer. Previous studies have found both elevated (30,62) and normal plasma triglyceride values (31,32) in breast cancer patients compared with controls. It is unclear whether there is a metabolic alteration due to the malignancy, dietary differences, or other factors resulting in higher triglyceride values among breast cancer cases.

One explanation for the high triglyceride values in our study is the possible presence of cachectin or "tumor necrosis factor" in some cases. Cachectin has been shown to inhibit lipoprotein lipase and thus inhibit clearance of triglycerides (63). Although there is some indication that cachectin may be present in small quantities early in the disease process, it is more prominent when wasting is observed in the later stages of disease (64). Wasting was not evident in the newly diagnosed cancers in this study, and in fact, the cases had relatively high Quetelet's index (mean ± SD = 25.8 ± 5.2). There was no trend of decreasing Quetelet's index with stage of disease, which provides indirect evidence that the disease or the malignancy was not mediating

the higher triglyceride levels. This fact, together with the higher mean triglyceride values in the earlier stages of disease, and the lack of a significant test for trend in triglyceride levels with stage of disease in this and another study (62) argue against an effect of cachectin.

These higher triglyceride values may be attributed to dietary differences among the cases. Studies have shown that higher triglyceride values are associated with diets higher in total fat (45), saturated fat (45,46,48,50,51,65) and simple carbohydrates (45,66. Williams et al (67) showed that diets low in polyunsaturated fat and plant-protein, and high in animal-protein were associated with high triglyceride levels. In the present study, the observed association of higher plasma cholesterol and lower plasma beta-carotene values with breast cancer supports the concept of cases consuming diets higher in saturated fat, animal protein and lower in polyunsaturated fat and in carotene-containing vegetables compared to controls. The dietary analysis in this study (17) demonstrated a lower intake of polyunsaturated fat among cases, although no association was evident between saturated fat and case status.

Higher triglyceride values also may be related to the hormonal environment. Elevated triglyceride values have been associated with oral contraceptive use (68), estrogen treatment (69,70) and the ovulatory phase of the menstrual cycle (71). A post hoc test controlling for exogenous estrogen use did not alter the results of higher triglyceride levels in cases compared with controls. However,

the elevated triglyceride levels may be an indicator of an environment higher in circulating estrogens.

Plasma Cholesterol

This study revealed that both high and low cholesterol levels were associated with breast cancer but most likely in very different ways. The apparent disparity can be reconciled by the flow diagram of hypothesized events shown in Figure 1. The intake of diets low in carotene-containing vegetables and high in saturated fats results in plasma that is low in beta-carotene and high in cholesterol and triglyceride levels. The combination of low beta-carotene and high cholesterol is associated with breast cancer, which is consistent with results from the evaluation of this interaction. After development and progression of the breast cancer, the disease process causes the cholesterol levels to be lowered. This is consistent with the observed lower cholesterol levels among breast cancer patients with more advanced disease.

Three investigations support these findings (30,31,72). Compared with the control groups, women with early stage breast cancers were shown to have higher cholesterol values (30,72), whereas women with metastatic breast cancer were shown to have lower cholesterol values (31). No association between plasma cholesterol and breast cancer was reported in a fourth investigation (32); this study was limited by small sample size (17 cases and 16 controls, power<50%), however.

The observed trend of decreasing cholesterol with increasing severity of disease in the present study is consistent with a metabolic

alteration of cholesterol values caused by the disease. Although the trend of lower cholesterol values with more advanced disease has been observed in colon cancer (73), no evidence was found for this phenomenon in breast cancer (74). Several cohort studies, however, suggested a preclincial effect (36,39,40). Only one study (39) suggested a preclinical cholesterol effect in breast cancer, but these results were not significant. In the Iowa cohort (36), the mean cholesterol values were 12 mg/dl lower in female cases diagnosed within 2 years of the blood drawing compared with cases diagnosed later but these authors did not determine the statistical significance of this difference. There was also a suggestion of a preclinical effect in overall cancers in women in the NHANES data (40).

Although the mechanism remains to be elucidated, cholesterol may be metabolized more quickly during the disease process resulting in lowered plasma levels. Hill et al (75) and Gorbach (76) have postulated that breast cancer patients may have increased conversion of biliary steroids into estrogens that could become reabsorbed. MacMahon and coworkers (77) reported that urinary estrogen excretion was higher among cases compared to controls, suggesting higher plasma estrogen levels. Plasma studies have been inconsistent, but evaluation of populations at low and high-risk of breast cancer support the concept of higher plasma estrogen levels in high-risk populations (78). Another explanation for the lower cholesterol in the cases could be an aberration in the enterohepatic circulation. There may be increased loss of cholesterol via fecal bile acids and neutral steroids as has been noted in breast cancer (79) and colon cancer patients (80).

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Alternatively, the lower cholesterol levels may be unrelated to metabolic disturbances, perhaps being of genetic and/or dietary origin. The trend in stage could be explained by a more rapid progression of the disease, with less opportunity for early detection and a later stage of cancer at diagnosis. This hypothesis could best be tested using prospective data with periodic blood samples and consistent screening.

Commentary

The main effect of cholesterol was only marginally significant and indicated that lower cholesterol was associated with case status. The interaction with beta-carotene was highly significant, however, and described the relationship between these nutrients and breast cancer more precisely. These results demonstrate the importance of investigating nutrient-nutrient interactions in relation to cancer risk. Although there are difficulties in interpreting statistical interactions (81-83), nutrients are known to interact with one another biologically, making it prudent to investigate interactions and describe the conditional nature of the effect more fully.

The increased risk associated with low beta-carotene is consistent with cases consuming diets lower in carotene-containing vegetables because plasma beta-carotene has been shown to reflect dietary intake of carotenes (84-86). Furthermore, this risk is higher in combination with the higher plasma lipid levels suggesting an interrelationship between these nutrients and carcinogenesis. These findings are intriguing and deserve further attention.

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Table 1
Effect of diagnosis and stage on mean values of
plasma cholesterol (mg/dl) and triglycerides (mg/dl)

Group	Cholesterol			Triglycerides		
	N	Mean	(SD)	N	Mean	(SD)
General Diagnoses						
Controls	101	237	(53)	100	99	(55)**
Cases	81	233	(48)	81	123	(64)
Cancer Stages						
In Situ	6	253	(71)	6	141	(55)
Stage I	26	245	(53)	25	122	(51)
Stage II	37	225	(36)	37	128	(78)
Stage III+IV	12	221	(54)*	12	105	(39)

* Significant (p=0.02) test for trend with "specific" stages
** Controls significantly (p=0.001) different from cases

Table 2
Changes in the case-control odds ratios for
cholesterol quartiles with adjustments for other variables

| Adjustment | Cholesterol Quartiles | | | | Trend[b] |
	High Q1	Q2	Q3	Low Q4[a]	p-value
Unadjusted	0.85[c] (0.38,1.87)[d]	0.53 (0.23,1.27)	0.70 (0.31,1.57)	1.00	0.60
Other risk Factors[e]	0.41 (0.14,1.14)	0.24 (0.08,0.73)	0.45 (0.16,1.28)	1.00	0.03
Other risk Factors Triglyceride Beta-carotene	0.38 (0.12,1.16)	0.26 (0.08,0.85)	0.52 (0.18,1.56)	1.00	0.03

a Reference group
b Test for trend across quartiles
c Odds ratio of quartile compared with the fourth quartile
d 95% confidence limits
e Adjusted for age, age at first birth, family history, menarche,
  Quetelet index, parity, age at menopause, income and marital
  status.

Table 3
Changes in the case-control odds ratios for triglyceride quartiles with adjustments for other variables

Adjustment	Triglyceride Quartiles			Low Q4[a]	Trend[b] p-value
	High Q1	Q2	Q3		
Unadjusted	3.90[c] (1.60,9.49)[d]	1.70 (0.65,4.43)	1.50 (0.57,3.97)	1.00	<0.01
Other Risk Factors[e]	2.84 (0.82,9.87)	1.08 (0.31,3.85)	1.39 (0.41,4.68)	1.00	0.10
Other Risk Factors Cholesterol Beta-carotene	4.19 (0.99,17.6)	1.57 (0.40,6.10)	1.64 (0.45,5.94)	1.00	0.07

a Reference quartile
b Test for trend of across quartiles
c Odds ratio
d 95% confidence limits
e Adjusted for age, age at first birth, family history, parity, age at menarche, Quetelet's index, age at menopause, income and marital status.

Table 4
Case-control adjusted[a] odds ratios with 3 reference groups
for the beta-carotene by cholesterol interaction

Cholesterol Level (percentile)[b]	Odds Ratios (CI)			
	Beta-Carotene Quartiles			
	Low Q1	Q2	Q3	High Q4[c]
Low[d] (25)[b]	0.33 (0.04,2.97) [6/7][e]	0.18 (0.02,1.79) [6/6]	0.12 (0.01,1.16) [6/5]	1.00 [1/2]
Median (50)	1.53 (0.35,6.61) [12/15]	0.66 (0.15,2.83) [6/12]	0.44 (0.11,1.86) [5/16]	1.00 [6/12]
High (75)	5.99 (1.10,32.6) [6/3]	2.04 (0.48,8.73) [5/7]	1.40 (0.33,5.93) [7/4]	1.00 [4/11]

a Adjusted for age, age at first birth, family history, age at
  menarche, Quetelet index, parity, age at menopause, income,
  marital status, beta-carotene, triglyceride, plus the beta-
  carotene by triglyceride and beta-carotene by cholesterol
  interactions; triglyceride held constant at its mean.
b Percentile based on ranking of all study subjects
c Reference group
d Low = 196 mg/dl, Median = 228 mg/dl, High = 262 mg/dl
e Number cases/controls

Table 5
Case-control adjusted[a] odds ratios with one reference group for the beta-carotene by triglyceride interaction

Triglyceride Level (percentile)[b]	Odds Ratios (CI) Beta-Carotene Quartiles			
	Low Q1	Q2	Q3	High Q4
Low[c] (25)[b]	0.83 (0.16,4.40) [3/9][e]	1.04 (0.20,5.38) [4/7]	1.14 (0.27,4.86) [3/7]	1.00[d] [2/7]
Median (50)	2.69 (0.71,10.3) [15/12]	1.52 (0.42,5.52) [7/13]	0.96 (0.27,3.43) [12/15]	1.00 [6/13]
High (75)	8.86 (1.81,43.3) [17/4]	2.23 (0.50,9.90) [6/5]	0.82 (0.17,4.01) [3/3]	1.00 [3/5]

a Adjusted for age, age at first birth, family history, age at menarche, Quetelet index, parity, age at menopause, income, marital status, cholesterol, beta-carotene, plus the beta-carotene by triglyceride and beta-carotene by cholesterol interactions; cholesterol held constant at its mean.
b Percentile based on ranking of all study subjects
c Low = 67 mg/dl, Median = 95 mg/dl, High = 136 mg/dl
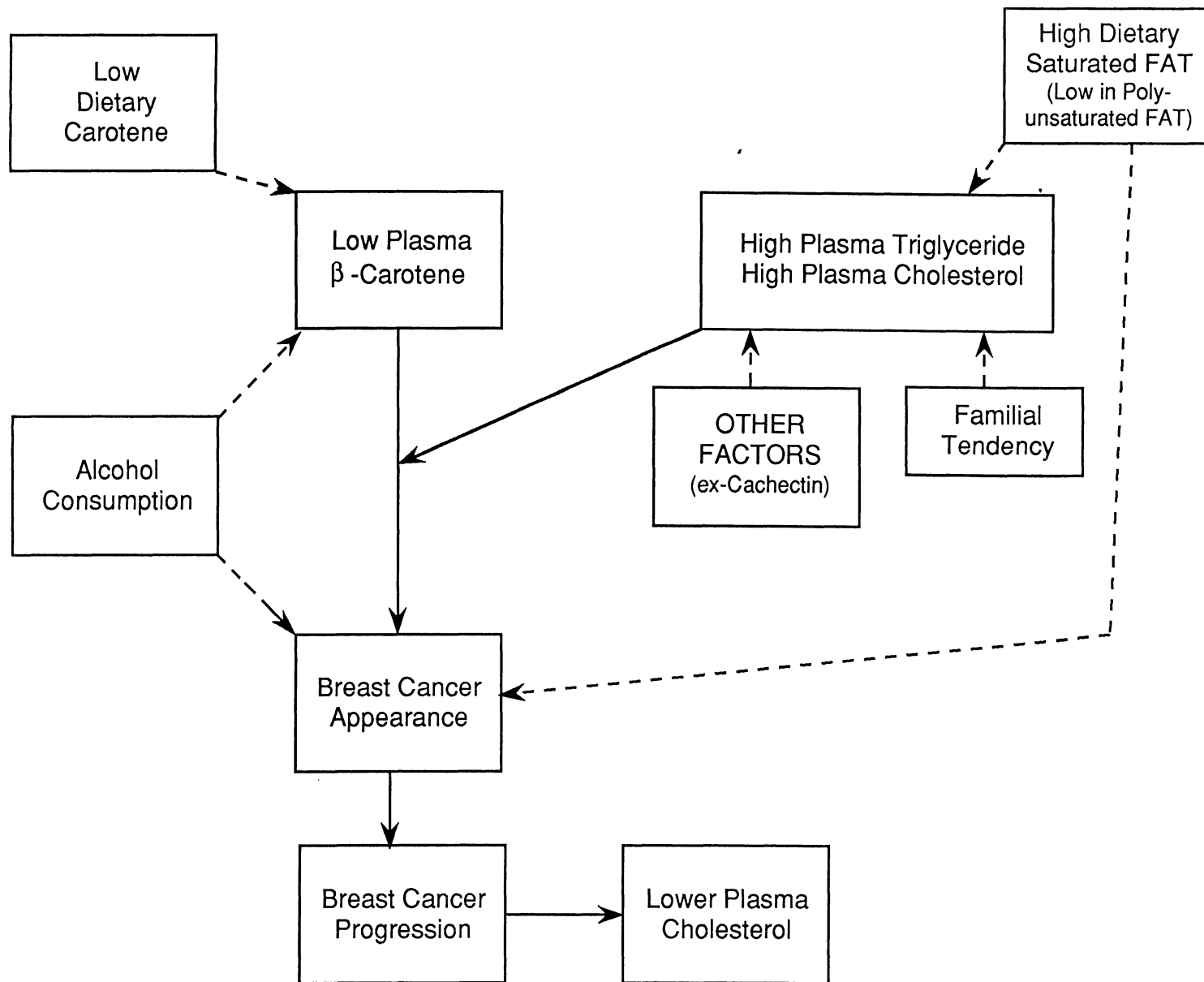d Reference group
e Number cases/controls

Figure 1. Flow diagram of the hypothesized association between three plasma nutrients and breast cancer. Direct arrows indicate direct associations, arrow on arrow indicates effect modification, dotted arrows indicate risk factors not evaluated in this investigation.