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AUTHORITY

USAMRMC Ltr., 6 May 98

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY *(Leave blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	July 1996	Annual (1 Jul 95 - 30 Jun 96)

4. TITLE AND SUBTITLE Molecular Markers of Carotenoid and Polyunsaturated Fatty Acid Intake and Breast Cancer Risk in a Heterogeneous Population

5. FUNDING NUMBERS

DAMD17-94-J-4200

6. AUTHOR(S)

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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)

Commander
U.S. Army Medical Research and Materiel Command
Fort Detrick, Frederick, MD 21702-5012

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT

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13. ABSTRACT *(Maximum 200*

The primary objective of the ongoing project is to explore relationships between breast cancer and tissue levels of specific fatty acids and carotenoids. Research during the past year focused on four broad areas. First, adipose tissue- and plasma-based micronutrient measures were compared. Repeat measures data were employed to compensate for measurement error in these comparisons. Variability between measures was substantial and dependent upon other factors, particularly gender. Second, the relationship between the essential omega-3 and -6 fatty acids as well as monounsaturates fatty acids with breast cancer was examined. The ratio of omega-3 to omega-6 polyunsaturates showed a protective association with breast cancer. Monounsaturates (particularly oleic acid) appeared powerfully protective in one center, but this association was not consistent acrosss centers. Third, the relationship between adipose tissue carotenoids and breast cancer was investigated. Much weaker associations were observed for these micronutrients than those noted for the fatty acids above or for trans fatty acids in last year's analyses. This led to adoption of a strategy focusing on potential effects of micronutrient levels on the associations between fatty acids and breast cancer, rather than vice-versa, in our ongoing research.

14. SUBJECT TERMS	15. NUMBER OF PAGES
Breast Cancer, Fatty Acids, Adipose Tissue, Antioxidants, Carotenoids, Tocopherol, Epidemiology, Case-Control Study, Diet, Plasma	120
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Limited

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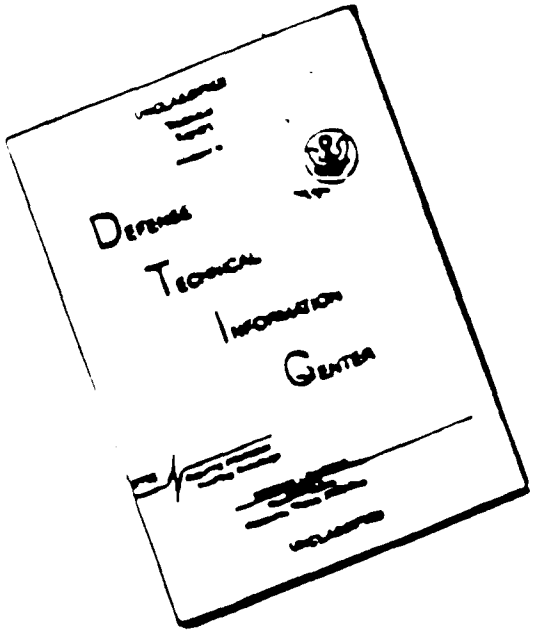

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GRANT NUMBER DAMD17-94-J-4200

TITLE: Molecular Markers of Carotenoid and Polyunsaturated Fatty Acid Intake and Breast Cancer Risk in a Heterogeneous Population

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REPORT DATE: July 1996

TYPE OF REPORT: Annual

PREPARED FOR: Commander
U.S. Army Medical Research and Materiel Command
Fort Detrick, Frederick, Maryland 21702-5012

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Terrence LeMieux 7-10-96

PI - Signature Date

1996 Annual Report

Molecular Markers of Carotenoid and Polyunsaturated Fatty Acid Intake and Breast Cancer Risk in a Heterogeneous Population (EURAMIC)

1996 Annual Report

Molecular Markers of Carotenoid and Polyunsaturated Fatty Acid Intake and Breast Cancer Risk in a Heterogeneous Population (EURAMIC)

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Introduction

1.1 The problem under investigation

It is widely believed that dietary intake plays an important role in the development of breast cancer. This hypothesis is supported by migrant studies as well as case-control and ecologic analyses. However, translation of this concept into adequate knowledge for the development of precise preventive measures has been hampered by our lack of understanding of the inconsistencies seen between studies. The bulk of attention to date has been focused on two nutrients, beta carotene and fatty acids. The potential preventive effects of beta carotene on breast cancer, and the apparent increase in risk associated with fatty acid consumption, are important relationships under investigation. However, epidemiologic cohort and case-control studies have not produced consistent evidence supporting these two relationships. Part of the explanation for this may be due to the composition of the fat. Different fatty acids have very different metabolic pathways and differ in their impact on prostaglandin production, their potential for oxidation and their utility for energy production. Another reason for the inconsistencies may be the inaccuracy and biases associated with the dietary assessment tools used in most studies.

The major purpose of this research is to examine the correlation between breast cancer risk and the stored concentrations of specific fatty acids, including the omega-6, omega-3, and trans fatty acids, in post menopausal women recruited in a consistent fashion from 5 centers spanning western Europe. The first year of the study addressed the validity of the fatty acid measurements, their collinearity, and the development of appropriate models for estimating their associations with breast cancer risk. After completing the analyses on trans fatty acids initiated in the first year of the project, the second year of the study focused on the relationships between essential fatty acids of the omega-6 and omega-3 families and breast cancer, and explored the relationship with monounsaturated fats. Evaluation of the associations between adipose tissue micronutrients (carotenoids and tocopherols) and breast cancer were also initiated. In addition, the agreement between adipose and plasma-based micronutrient biomarkers was assessed, and investigation of dietary data on micronutrient and fat intake available for a subset of the study population was begun.

1.2 Background and significance

Last year's Annual Report presented an extensive treatment of the evidence regarding the potential role of dietary fat and micronutrient intake in breast cancer as well as the methods available to assess such exposures. To avoid repetition only a brief synopsis is presented this year; interested readers are referred to last year's Report for further detail.

The possible involvement of dietary fat in many diseases, including breast cancer, continues to receive extensive study. These studies have largely met with inconsistent results. The conflicting results regarding fat intake may be due to the physiologic importance of individual fatty acids and the dietary assessment tools used to estimate levels of fat intake. Most studies have addressed the intake of only total fat or a few major fat classes. The uncertain accuracy of

estimates of fat intake obtained through traditional dietary assessment tools may have further contributed to the inconsistency of results. The use of adipose tissue as a biomarker of fatty acid intake presents an alternative exposure measure that avoids the problems of recall and nutrient database limitations typical of dietary interview or questionnaire-based assessment techniques. It also allows direct and reasonably accurate quantification of minor fatty acids such as the omega-3 polyunsaturates. Differences in physiologic activity of specific fatty acids makes the accurate assessment of minor yet physiologically active components like the omega-3 polyunsaturates particularly desirable.

1.3 Investigational strategy

The primary information for this study is derived from measurements of fatty acid, carotenoid, and tocopherol concentrations in fat collected by gluteal adipose tissue biopsy. Information is available from 392 newly diagnosed cases of breast cancer and 467 matched controls recruited across 5 centers in western Europe following a standardized protocol. The fatty acids and micronutrients were assayed in central laboratories under strict quality control procedures. Additional information on other important risk factors and effect modifiers was obtained for all of these women via an interview-administered questionnaire. In addition, analyses of plasma micronutrients are available for a substantial subset of the study population. Computerized diet histories were obtained from women enrolled at one center, contributing data for 158 individuals to this project. These will serve as a reference for comparisons of nutrient levels in adipose tissue and plasma with intake through the diet. This population will also be used to examine the role of glucosinolate intake in the development of breast cancer.

The advantage of this multi-centre study, carried out in different countries, is that the larger range of exposure to fatty acids and antioxidant vitamins resulting from major differences in food consumption potentially allows detection of associations more modest than those detectable in a more homogeneous population of the same size.

Body: Research Activity

2.1 Experimental methodology

In order to gain the additional power provided by the study of diverse individuals, a multi-country case-control study of breast cancer in Europe was designed and carried out.[1] In this study, 6 centers in 5 countries recruited incident cases of postmenopausal breast cancer. (See Appendix A for a list of collaborating centers and their key personnel.) Breast cancer cases were defined as female subjects aged 50 to 75 years, with first diagnosis of breast cancer (ICD-code 174), histologically classified as ductal carcinoma, with primary tumors less than 5 cm, axillary lymph nodes stage < N3, without any clinical indication of distant metastases at discharge. The methods of recruitment and data collection were described at length in sections 2.1.1-2.1.6 of the 1995 Annual Report, and will not be repeated here. One center recruited too few subjects for inclusion in the analyses. This left a total of five centers in the dataset used for the current project.

2.2 Analysis process

Adipose tissue sample analyses from five countries were pooled into common-format datasets. Extensive data verification included validation of transcription and pooling, and resolution of any questions via original records. Biomarker distributions and their correlations were employed in creating variables to address the study hypotheses. Optimal methods of adjusting for recognized risk factors were developed, based on considerations of risk magnitude, muticollinearity, and degree of impact on risk estimates for hypothesized exposures. The following sections detail the types and methods of analyses performed on the data.

2.2.1 Dataset creation and cleaning

Data from disparate files gathered in different parts of the EURAMIC study were pooled together into common-format datasets for manipulation and analysis by our preferred system for computerized statistical analysis, SAS. Appendix B contains summaries of the logs detailing the programming employed to create the datasets used in the analyses.

A list of variables and their definitions is located in Appendix C. This is complemented by brief guide to fatty acid nomenclature, presented in Appendix D. In addition to creating variables for individual fatty acids measured in the EURAMIC study, grouping schemes were created that combine individual fatty acids that share common actions or potential effects (e.g. total omega-3 polyunsaturated fatty acids, which may compete for the same metabolic pathways as the most common polyunsaturates of the omega-6 family). Also, new variables were created to address potential interrelationships between individual fatty acids and to facilitate differentiation between effects of different subtypes of fatty acids (e.g. trans*pufa, C18:1 trans/ C18:1 total). These created grouping schemes and variables are noted in the abovementioned variable dictionary.

After creation of the pooled dataset, values for key variables were verified by comparing the original separate data files with corresponding values on the pooled dataset. The distribution of values for individual fatty acids and micronutrients were examined for outliers, values that are physically impossible and values that are incongruent with other values. After locating any questionable data, the original data sources (e.g. chromatograms) were reviewed to verify that any apparently questionable values were transcribed and coded correctly, and that they were the result of valid laboratory assays. Transcription or coding errors were then corrected, and invalid assay results were excluded from the dataset.

2.2.2 Computing requests

Computing requests are the written forms of documentation and communication between the project staff and the statistical programmer. The requests provide specific details needed to complete the desired tasks, such as descriptive information about the analyses requested, the name of the data file to be used, any inclusion or exclusion criteria, and related requests. Other identifying information is supplied on the computing request form, such as the requester's name, the project code, the priority level, and the deadline, if any. An examples of the current format employed for computer requests is presented in Appendix E. Further details regarding computing requests are available in section 2.2.2 of the previous Annual Report.

2.2.3 Project meetings

Times for two project team meetings are set aside each week. Meetings take place at least once a week to discuss the results of the analyses and to evaluate progress toward completion of the stated tasks. The project team includes the primary investigator, the post-doctoral researcher, doctoral students working on the project, and the statistical programmer. Additional meetings are held on an as-needed basis, either with the project team alone or in combination with biostatisticians collaborating on the project.

2.2.4 Data storage and security

Redundant storage systems are employed to guard against accidental loss of data. All active datasets are stored both on the university's computer network and on floppy disks. Also, taped copies of all network files exist offsite to insure preservation of data in the event of system failure. A complete back-up of the network is performed on the last Friday of every month. In addition, everything that has changed on the network is saved once a week. Print-outs of completed analyses are stored in the study coordinator's office.

The programmer's computer on which data is stored is secured physically by a locked door, and can only be accessed by the programmer's private code. Also, the network is password protected so that only the programmer and her supervisors have access to the data. Passwords are changed monthly to further insure security of the datasets.

2.2.5 Statistical analyses

Descriptive statistics such as means and medians were obtained through the SAS procedures MEANS and UNIVARIATE. Variable distributions were assessed through the UNIVARIATE, PLOT, and QC procedures. UNIVARIATE provides information on skew and kurtosis as well as statistical tests of normality. PLOT provides graphical plots of individual data points. QC features a Box-Cox option that evaluates data transforms to determine those that yield the most normal distribution.

In exploring associations with disease, crude mean levels of fatty acids or micronutrients among cases and controls were compared using Student's t-tests and chi-square analyses. Potential confounders and/or effect modifiers were identified through both correlation analyses (CORR procedure) and stratified and multivariate logistic regression analysis (LOGISTIC procedure for unconditional regression, PHREG for conditional regression analyses). Models of relative risk were constructed taking into account factors with the potential to confound the relationship between exposure and disease outcome in the study population, using logistic regression analysis.

The fatty acids were analyzed individually, and grouped in classes according to chain length, the degree of saturation and the position of the double bonds, and the presence of trans-bonds. Multivariate analyses were conducted using multiple logistic regression on the outcome breast cancer with maximum likelihood estimation of the regression coefficients and their standard errors. Tests for trend were also performed by assigning the median value of the category to each subject and modeling this as a continuous variable.

In addition to the analyses of relationship between exposures and breast cancer analyses performed, original data from a measurement reproducibility study conducted by the same labs involved in the EURAMIC breast cancer study was analyzed to quantify sources of measurement error and analytical variability. This information was used to determine the overall variability of laboratory measures for each fatty acid and the relative contribution of specific sources of variability--variation between measurements on the same individual (within-person variability), and variation between measures on different individuals (between-person variability). The components of variance were obtained from the VAR COMP procedure. These components were used to correct for the role of measurement error in comparisons of agreement between adipose-based and plasma-based measures of micronutrients. They are also being used in new methods developed to assess the potential impact of measurement error on breast cancer risk estimates.

2.2.6 Other activities

Presentation at the ASCN 36th annual meeting

An abstract summarizing the findings regarding omega-6 polyunsaturated fatty acids and breast cancer was presented at the 36th annual meeting of the American Society for Clinical Nutrition. The meeting took place in Washington, DC, on April 14-17th, 1996. That abstract is enclosed as part of Appendix H in this report. The meeting included a debate on trans fatty acids and health, but as in the trans fatty acid symposium held at the

prior year's meeting, the focus was almost entirely on cardiovascular disease, with no epidemiologic data presented for other diseases. Discussions with participants confirmed the scarcity of specific information on trans exposure and cancer in human populations, and the need for progress in this area. Several abstracts dealt with the association between supplementation or naturally-occurring intake of one or more carotenoids with levels measured in blood or other tissue. None dealt specifically with plasma-adipose correlations and the factors affecting them, however, indicating that the research detailed in section 2.3.2 of this Report will provide a valuable addition to current scientific knowledge.

Presentation at the SER annual meeting

An abstract summarizing findings regarding the relationship of omega-3 fatty acids and the balance between omega-3 and omega-6 fatty acids with breast cancer was presented at the meeting of the Society for Epidemiologic Research in Boston, Massachusetts. The meeting took place June 12-15, 1996.

2.3 Progress on targeted tasks

Having examined the distributions of each of the fatty acids within and across centers to construct categories for statistical modeling and tested for collinearity between fatty acids during year 1, the stage was set for analyses to examine the relationships between fatty acids, carotenoids, and glucosinolates and breast cancer. The associations of individual fatty acids and the three carotenoids measured in adipose tissue (beta- and alpha-carotene, lycopene) with breast cancer were modeled using logistic regression. The impact of other factors, including recruitment center, smoking and drinking habits, and established breast cancer risk factors like age and reproductive history, on these associations was evaluated. The interrelationships between serum and adipose tissue micronutrients were determined. To facilitate these and other analyses, a quality control dataset was constructed and information from this dataset was used to address the potential impact of measurement error the relationship between adipose and serum-based micronutrient measurements. Diet history data available for a subset of the study population were recompiled to yield daily food intakes, which were in turn used to create intakes by particular food groups of potential interest in the study of diet and breast cancer (e.g., glucosinolate-rich cruciferous vegetables [cabbage], lycopene sources [tomatoes]). Preliminary analysis of the relationship between food intakes and breast cancer was begun.

The second year's activities have thus included elements originally targeted for year 3 (initiation of food intake and glucosinolate analyses). Testing the breast cancer-micronutrient associations for potential interactions between fatty acids and carotenoids, originally targeted for year 2, is not yet fully complete, thus balancing out the advanced progress on the food intake/glucosinolate objectives. The earlier start on the food intake and glucosinolate analyses has proven prudent, since acquisition of state-of-the-art nutrient information for the German population in English translation and the development of a database incorporating that information proceeded more slowly than anticipated.

Executive Summary of Progress

- Data cleaning and verification was undertaken for the plasma micronutrient dataset to insure accuracy of the data to be used in comparisons of adipose and plasma micronutrient biomarkers.
- The distributions of the major adipose tissue and plasma micronutrient variables were explored through univariate statistical analyses and graphical plots.
- Correlation analyses were carried out to assess the relationships between adipose and plasma measurements of corresponding micronutrients.
- Quality control data were employed to quantify specific sources of measurement error for adipose tissue and plasma micronutrients as well as adipose tissue trans fatty acid.
- Data on measurement error were used to adjust the adipose-plasma correlation analyses for the effects of that error.
- New techniques to evaluate the effect of measurement error on logistic regression models were developed. These techniques were tested by applying them to the model of the association between trans fatty acids and breast cancer detailed in the previous annual report. Application of these techniques to other models is proceeding.
- The relationship between beta-carotene and breast cancer was evaluated by EURAMIC project researchers. Very weak evidence of a protective effect for beta-carotene was noted.
- Logistic regression analyses were initiated to evaluate the relationship of the carotenoid antioxidants lycopene, beta-carotene, and alpha-carotene, singly and in concert, to breast cancer. Analyses to date have revealed little consistent association.
- The hypothesis that polyunsaturated fatty acids of the omega-3 family are protective against breast cancer was tested.
- While omega-3 fatty acids considered in isolation showed a weakly protective association overall, the ratio of omega-3 to omega-6 polyunsaturates evidenced a statistically significant protective association. As omega-6 polyunsaturate levels rose, the apparent effect per unit of omega-3 effect appeared to fall.
- The relationship between monounsaturated fatty acids and breast cancer was also explored. Monounsaturates in general and oleic acid in particular appear very strongly protective in one study center. This protective pattern is not in evidence in other centers, however. The direction of association for individual monounsaturated fatty acids is not uniform, even within centers.
- A new dataset incorporating information available on food consumption for a subset of the study population was created. Data were cleaned, verified, and then used to calculate consumption for glucosinolate-rich foods. Analyses are underway to determine whether estimated consumption of total glucosinolates or specific glucosinolate-rich foods appear protective.
- A manuscript was prepared on the polyunsaturated fatty acid findings; a manuscript on the adipose vs. plasma micronutrient findings is nearing completion.

2.3.1 Basic descriptive analyses

Descriptive analyses (including calculation of mean, standard deviation, minimum, and maximum) were presented for individual fatty acid variables in last year's report. Table 2.3.1 #1 focused on the fatty acids derived from exogenous sources--those fatty acids for which adipose tissue stores best serve as a measure of exposure. Table 2.3.1 #2 provided information on all individual fatty acids. Presentation and discussion of these results will not be repeated in this year's Report. However, an additional tabulation based on medians rather than means is presented in section 2.3.3, Table #1, of this year's report.

2.3.2 Specific analyses: Continuation of trans fatty acid-breast cancer analyses

As related in section 2.3.2 of the 1995 Annual Report for this project, adipose tissue trans fatty acids showed a statistically significant association with breast cancer in the study population. These initial analyses excluded subjects whose adipose tissue samples weighed less than 15mg, as a precaution against the possibility that smaller samples led to less accurate measurements and hence potentially distorted results. Following that time, analyses employing an alternative exclusion criterion were completed. This criterion was based on discordance between the amount of fat in the sample estimated from chromatographic assay results and the actual weight of the sample (See Appendix C under "EXCL"). All samples that met this criterion were included in the analyses, regardless of the sample's weight. We found no indication that samples weighing less than 15mg differed systematically from larger samples in their results for trans fatty acids. Tables 2.3.2 #1 and #2 present the results of analyses after omission of the 15mg exclusion. These can be compared with the results presented in Table 2.3.2. in the 1995 Annual Report. Models of the association between trans fatty acids and breast cancer yielded similar estimates of effect regardless of whether samples weighing under 15 mg were excluded. As the under-15 mg exclusion reduced the number of subjects available for analyses without any apparent affect on the validity of results, its use was discontinued.

Table 2.3.2 #1: **Logistic Regression Models of Breast Cancer by Trans Fatty Acid**

Independent Variable(s) in Model	N[1]	Odds Ratio for Trans (95% C. L.) [2]
Trans fatty acid	698	1.46 (1.19, 1.79)
Trans fatty acid, with covariates[3]	645	1.40 (1.02, 1.93)
Trans fatty acid with covariates, PUFA and interaction term[4]	645	5.87 (2.45, 14.05)

[1]N = Number of individuals in model.

[2]Odd ratios for the difference between 75th and 25th percentiles of adipose trans %; 75th and 25th percentiles are 1.60 and 0.68 , respectively, yielding a difference of 0.92.

[3]Covariates include BMI, study center, cigarettes/day currently smoked, ex-smoker status, age in years, SES as 3-level categorical variable, post-menopausal estrogen supplementation, and current alcohol consumption in grams/week.

[4]Interaction term represents the interaction of trans and polyunsaturated fatty acid (PUFA).

Table 2.3.2#2: Breast Cancer ORs for Trans Fatty Acid, by Tertile of PUFA

Independent Variable(s) in Model	N[1]	Trans Fatty Acid OR.-trans as % of total FA (OR for difference between 75th and 25th percentiles)[2]		
		Low PUFA[3]	Moderate PUFA[3]	High PUFA[3]
Trans fatty acid	231;237;230	3.12 (2.00, 4.86)	1.50 (0.97, 2.32)	0.89 (0.66, 1.19)
Trans fatty acid, with covariates[4]	216;214;215	2.60 (1.31, 5.16)	0.83 (0.40, 1.74)	Nonconvergent model
Trans fatty acid, with covariates[4] - centers	216;214;215	3.65 (2.17, 6.14)	1.88 (1.14, 3.09)	0.97 (0.66, 1.40)

[1] N = Number of individuals in each PUFA tertile.

[2] Percentiles based on total study population (698 subjects). For trans %, 75th and 25th percentiles are 15.9 and 6.8, respectively, yielding a difference of 9.10.

[3] Tertiles based on total study population. Starting points for the second and third tertiles were 12.14 and 15.09 for trans %.

[4] Covariates include BMI, study center dummy variables, cigarettes/day currently smoked, ex-smoker status, age in years, SES as 3-level categorical variable, post-menopausal estrogen supplementation, and current alcohol consumption in grams/week, unless otherwise noted.

2.3.3 Specific analyses: Omega-3 and omega-6 fatty acids

Basic descriptive statistics on the omega-3 and omega-6 fatty acids were presented in the 1995 Annual Report, section 2.3.3. Many of these variables proved to be highly skewed. Since the means presented in Table 2.3.3 #1 of the previous Report are susceptible to distortion by extreme values, a new tabulation based on median values instead of means was compiled. This is presented in the current Table 2.3.3 #1. Significant variation in both omega-3 and omega-6 fatty acid medians between centers is apparent. The patterns seen for medians is very similar to that previously reported for means. The omega-6 polyunsaturate linoleic acid (C18:2 ω6) is the predominant polyunsaturate in all centers. Polyunsaturates of the omega-3 family are much less common. Medians for both families of polyunsaturates vary substantially across centers. Malaga and Zurich exhibit the lowest median omega-3 level coupled with the highest level of omega-6 when cases and controls are considered together. In contrast, the highest median docosahexaenoic acid (DHA, C22:6 ω3)--the major long-chain omega-3 fatty acid--occurs in Malaga and Berlin, while Zurich clearly sports the lowest median DHA. Trans fatty acids are least predominant in Malaga and most predominant in Zeist and Coleraine. Oleic acid and total monounsaturates are highest in Malaga, consistent with the anticipated higher consumption of olive oil in Mediterranean Spain than in other parts of Europe. The differences in median fatty acid stores across participating centers, as well as the differences in means recounted in last year's Report, favor prospects for the detection of any exposure-disease relationship(s) that might exist.

Analyzing the relationship between polyunsaturated fatty acids and breast cancer is of central importance for two reasons. First and foremost, laboratory models of mammary carcinogenesis provide stronger support for a role of these fatty acids than for saturated or monounsaturated fats. Second, these fatty acids are dubbed "essential" because they must be obtained through the diet, since they cannot be endogenously synthesized from other families of fats. This makes adipose tissue, the biomarker used in this project, an excellent marker for their long-term intake. Extensive analyses of the associations between polyunsaturated fatty acids of the omega-3 and omega-6 families with breast cancer were carried out for this project during the past year. A manuscript relating the findings of these analyses has been prepared and is included in Appendix H. A condensed account of the analyses undertaken and their results is presented in the following paragraphs.

A simple comparison of median levels of omega-3 and omega-6 fatty acids in adipose tissue (Table 2.3.3#1) reveals that cases tended to have lower omega-3 and higher omega-6 fatty acid stores than controls. The difference in omega-6 fatty acids is almost entirely attributable to Malaga, where the median omega-6 percentage among cases was 17.9 compared to 11.7 for controls.

To control for other risk factors, logistic regression analyses were carried out. For these analyses, a conditional regression technique was employed. In this approach, cases were matched with controls from the same age bracket and recruitment center--the analyses were "conditioned" on age and center. These analyses confirmed a striking adverse association between omega-6 polyunsaturates and breast cancer (Table 2.3.3#2) in the combined study population. The sum of omega-3 fatty acids in adipose tissue yielded an adjusted odds ratio for breast cancer of 0.88 (95% C.L. 0.67--1.15), suggestive but statistically weak. The picture changes considerably when the balance between omega-3 and omega-6 fatty acid is addressed by modeling the ratio of omega-3 to

omega-6 fatty acids. The protective association of total omega-3 with disease is much stronger (OR=0.70; 0.52--0.92), and there is little difference between the ORs for alpha-linolenic acid (ALA) and docosahexaenoic acid (DHA).

Switching to center-specific analyses, confirmed that the deleterious association between omega-6 polyunsaturates and breast cancer was confined to the Spanish population (Table 2.3.3#3). These center-specific models also yielded no consistent association for total omega-3, alpha-linolenic, or docosahexaenoic acid. The ratio of omega-3 to omega-6 and of DHA to omega-6, on the other hand, yielded an odds ratio below one for the majority of centers.

Given the dramatic differences in point estimates for total omega-6 and ALA in one center (Malaga) compared to the others, regression models were performed excluding this center. Absolute levels of DHA and total omega-3 appeared more protective ((respective ORs with 95% CLs 0.95 (0.85--1.11), 0.89 (072--1.12) and 0.82 (0.60--1.06)) after exclusion of Malaga. The loss of sample size did, however, contribute to a loss of statistical significance in the associations for the ratio of total omega-3 and DHA to omega-6 ((respective ORs 0.79 (0.59--1.02) and 0.83 (0.65--1.05)).

The relative importance of the omega-3 components alpha-linolenic and docosahexaenoic acid was explored through models including both fatty acids simultaneously (Table 3). ALA and DHA, whether as a percentage of total fatty acids or as ratios to omega-6, yield fairly similar ORs with all centers pooled. The exclusion of Malaga strengthens the results for DHA ((0.78 (0.63--0.97)) and its ratio to total omega-6 ((0.81 (0.65--1.02)) while eliminating the association for ALA : omega-6 ratio ((0.99 (0.67--1.44)).

Analyses stratified by tertile of adipose omega-6 show that the apparent protective association of omega-3 fatty acid is strongest at lower omega-6 polyunsaturated fatty acid levels(Table 2.3.3#4). This is consistent with competition of omega-3 and omega-6 fatty acids for metabolic pathways, resulting in a greater impact of a given amount of omega-3 when background levels of omega-6 are lower. The similarity of results for DHA and total omega-3 indicates that the relationship is driven by DHA.

Discussion

No statistically significant association was found between the simple percentage of total omega-3 fatty acids in adipose tissue and incident breast cancer, although pooled analyses provided weak support for a protective association. An inverse association was observed between disease and the ratios of both omega-3 and DHA to omega-6 in adipose tissue. These findings are consistent with hypothesized anticarcinogenic mechanisms for omega-3 fatty acids.

The best known mechanism through which omega-3 fatty acids exert physiologic effects and hence could affect carcinogenesis is a modulation of eicosanoid metabolism. The most common polyunsaturated fatty acids in the typical Western diet belong to the omega-6 family, and the predominant member of that family is linoleic acid. Linoleic acid can be converted to arachidonic acid, which in turn serves as the parent compound for eicosanoids that can powerfully affect cell function. Supplying long-chain omega-3 fatty acids (predominantly DHA in marine oils)

competitively inhibits the delta-5 and -6 desaturase pathways necessary for conversion of linoleate to arachidonate. Once processed through these pathways, omega-3 fatty acids also give rise to a family of eicosanoids with effects often different or opposite to those produced by their counterparts from the omega-6 family.[2] Omega-3 fatty acids could thus inhibit tumor development and/or growth insofar as the production of the omega-6 family eicosanoids is a part of "normal" tumor growth and metabolism. Further, direct cytotoxic effects through peroxidation products of these polyunsaturated fatty acids have been proposed.[3]

The strong relationship of total omega-6 polyunsaturate with breast cancer in one center may be due to an inverse association of monounsaturated with omega-6 (and hence total polyunsaturated) fat, driven by higher olive oil consumption in that center. A protective effect of olive oil consumption is supported by many dietary studies on southern European populations,[4-8] although not all.[9,10] The protective association with total omega-3 to omega-6 ratio is more consistent across centers than the deleterious association with omega-6 described above. Further, the specificity of effect for the primary long-chain omega-3 fatty acid is congruent with the results of dietary studies linking fish consumption to lower breast cancer rates.[4,11-16]

Table 2.3.3 #1: Descriptive Statistics for Adipose Tissue Fatty Acids in Cases and Controls, by Center

Medians followed by 25th and 75th percentile

Fatty Acid(s) [1]	All Centers		Berlin		Zeist		Coleraine		Zurich		Malaga	
	cases	controls	cases	controls	cases	controls	cases	controls	cases	controls	cases	controls
N [2]	291	407	16	103	70	63	95	99	54	74	56	68
Total ω3 polyunsaturate [as % total fatty acid]	0.77 0.62-.93	0.81 0.64-.97	1.01 0.93-1.22	0.95 0.81-1.11	0.89 0.74-0.98	0.92 0.78-.99	0.83 0.74-1.02	0.89 0.79-.98	0.57 0.53-.71	0.62 0.57-.72	0.66 0.57-.75	0.58 0.52-.65
α-linolenic acid (ALA) [as % total]	0.59 0.43-.71	0.59 0.44-.74	0.69 0.59-80	0.69 0.59-0.80	0.67 0.59-0.78	0.68 0.54-.76	0.67 0.59-.78	0.71 0.62-0.78	0.45 0.37-.50	0.48 0.41-.50	0.36 0.32-.42	0.35 0.32-.39
Docosahexaenoic acid (DHA) [as % total]	0.15 0.10-.21	0.16 0.12-.21	0.25 0.18-.33	0.18 0.15-0.25	0.14 0.09-0.18	0.18 0.11-.23	0.12 0.09-.17	0.14 0.10-.18	0.13 0.08-.16	0.12 0.10-.16	0.23 0.18-.31	0.20 0.14-.23
Total ω6 polyunsaturate [as % total fatty acid]	13.17 10.80-16.51	12.30 10.72-14.58	11.48 10.82-13.10	11.43 10.15-13.15	14.22 11.64-15.95	14.19 11.83-16.16	11.26 9.56-14.22	11.61 9.99-15.00	13.29 11.73-14.84	13.41 12.14-15.48	17.88 13.37-22.12	11.74 10.62-13.69
Linoleic acid (LA) [as % total fatty acid]	12.35 10.23-15.57	11.49 9.90-13.66	10.62 9.78-11.82	10.68 9.38-12.40	13.41 10.91-15.30	13.37 10.99-15.31	10.52 8.95-13.48	10.91 9.46-14.17	12.62 10.97-13.81	12.45 11.40-14.61	16.69 12.38-21.11	10.96 9.59-12.63
Total ω3: total ω6 [X 100]	5.69 4.34-7.64	6.40 4.95-8.32	9.18 8.45-11.08	8.45 7.34-9.60	6.44 5.32-7.32	6.42 5.39-7.21	7.44 6.03-9.05	7.44 5.90-9.02	4.61 3.57-5.39	4.56 4.12-5.40	3.67 3.06-4.72	5.04 4.09-5.69

Total DHA: total ω6 [X 100]	1.05 0.76-1.54	1.30 0.90-1.79	1.99 1.50-3.01	1.64 1.29-2.21	0.97 0.60-1.53	1.18 0.70-1.70	1.05 0.67-1.45	1.23 0.79-1.48	0.90 0.64-1.22	0.92 0.69-1.18	1.36 0.93-1.66	1.70 1.16-1.99
Total ALA : total ω6 [X 100]	2.23 1.51-3.00	2.34 1.71-3.11	3.03 2.57-3.44	3.00 2.64-3.45	2.49 1.98-2.90	2.31 2.02-2.80	3.00 2.47-3.54	2.94 2.44-3.76	1.66 1.37-1.95	1.70 1.39-1.98	1.05 0.78-1.45	1.48 1.27-1.69
Total monounsaturated [as % total fatty acid]	55.57 52.87-58.43	56.60 53.58-59.77	57.07 55.18-59.05	57.48 55.23-60.19	55.29 52.69-57.99	53.58 51.11-56.33	55.26 52.82-57.93	54.94 52.87-57.52	54.89 52.10-56.83	55.51 52.00-57.08	58.45 53.69-63.20	66.11 63.46-68.36

[1] Specific fatty acid as a percentage of all fatty acids present in tissue sample. ω3 = omega-3 = sum of a-linolenic, eicosapentaenoic, and docosahexaenoic acid; ω6 = omega-6 = sum of linoleic, γ-linolenic, eicosadienoic, dihomo γ-linolenic, and arachidonic acid. A brief key to fatty acid nomenclature is provided in Appendix C; information regarding coding of specific fatty acid variables used in the project dataset can be found in Appendix F.
[2] Number of subjects.

Table 2.3.3#2: Conditional logistic regression models of breast cancer by fatty acids: all centers pooled

Fatty Acid Variable(s)	Odds Ratio (95% C.L.s) [1]	
	Without covariates	With covariates[2]
As a % of all adipose tissue fatty acids...		
Total omega-3	0.88 (0.68--1.15)	0.88 (0.68--1.15)
ALA	0.86 (0.63--1.16)	0.86 (0.63--1.16)
ALA (in model also containing DHA)		0.87 (0.63--1.21)
DHA	0.99 (0.82--1.21)	1.00 (0.82--1.23)
DHA (in model also containing ALA)		0.92 (0.76--1.11)
Total omega-6	1.22 (0.99--1.52)	1.27 (1.01--1.58)
Ratio to total omega-6 fatty acid...		
Total omega-3	0.72 (0.55--0.94)	0.70 (0.52--0.92)
ALA	0.79 (0.56--1.10)	0.77 (0.55--1.09)
ALA (in model also containing DHA)		0.79 (0.55--1.14)
DHA	0.82 (0.66--1.01)	0.82 (0.66--1.02)
DHA (in model also containing ALA)		0.84 (0.69--1.04)

[1] Odds ratio for the difference between 75th and 25th percentiles in the control population. See Table 1, column 3 for actual percentiles. All models are conditioned on recruitment center and age. Total omega-3, DHA, omega-3 : omega-6 ratio, and DHA : omega-6 ratio models use log-transformed fatty acid values.

[2] For total omega-3 and DHA, covariates include BMI, nulliparity, age at birth of first child, and current alcohol consumption in grams/week; for omega-6 and fatty acid ratio models, covariates also include SES.

Table 2.3.3#3: Center-Specific Models of Breast Cancer by Adipose Tissue Fatty Acids[1]

Fatty acid	Berlin	Zeist	Coleraine	Zurich	Malaga
N[2]	118	122	193	125	124
Total omega-3 as % of all fatty acids	1.32 (0.50, 3.49)	0.71 (0.41, 1.22)	0.82 (0.50, 1.33)	0.51 (0.26, 0.99)	2.98 (1.40, 6.32)
DHA as % of all fatty acids	1.24 (0.53, 2.91)	0.73 (0.52, 1.01)	0.72 (0.51, 1.02)	0.86 (0.51, 1.44)	2.48 (1.35, 4.56)
ALA as % of all fatty acids	1.01 (0.45, 2.28)	0.95 (0.55, 1.63)	0.82 (0.49, 1.37)	0.46 (0.17, 1.29)	2.47 (0.40, 15.37)
Total omega-6 as % of all fatty acids	1.44 (0.50, 4.10)	0.87 (0.51, 1.48)	0.88 (0.62, 1.24)	0.81 (0.44, 1.47)	3.39 (2.06, 5.59)
Total omega-3 : omega-6 ratio	2.98 (1.40, 6.32)	0.79 (0.42, 1.47)	1.01 (0.61, 1.67)	0.63 (0.30, 1.32)	0.40 (0.22, 0.74)
DHA : omega-6 ratio	1.08 (0.47, 2.45)	0.75 (0.53, 1.06)	0.78 (0.55, 1.10)	0.94 (0.54, 1.64)	0.91 (0.56, 1.47)
ALA : omega-6 ratio	0.88 (0.30, 2.59)	1.06 (0.52, 2.17)	1.01 (0.62, 1.65)	0.60 (0.20, 1.79)	0.04 (0.01, 0.18)

[1] Center-specific fatty acid odds ratios for model conditioned on age, accompanied by 95% C.I. in parentheses. Odd ratios are based on difference between 75th and 25th percentiles in the control population. See Table 1, column 3 for listing of actual percentiles.

[2] N = number of observations for model (subjects with missing covariate information or no matching control in their age group excluded).

[3] Cov. = covariates including age in years, BMI, current alcohol consumption in grams/week, age at first birth, and nulliparity.

Table 2.3.3#4: Odds Ratios for Total Omega-3 and DHA after Stratification by Tertile of Total Omega-6 fatty acid

Independent Variable(s) in Model	N[1]	Fatty Acid O.R.-fatty acid as % of total FA[2]		
		Low omega-6[3]	Moderate omega-6[3]	High omega-6[3]
Total omega-3 fatty acid, with covariates[3, 4]	229, 225, 228	0.74 (0.41, 1.35)	0.84 (0.54, 1.29)	1.06 (0.67, 1.68)
DHA, with covariates[3, 4]	229, 225, 228	0.81 (0.54, 1.24)	0.79 (0.55, 1.13)	0.99 (0.72, 1.36)

1 N = Number of individuals in each of the ascending omega-6 fatty acid tertiles.
2 Odds ratio for the difference between 75th and 25th percentiles in the control population. See Table 1, column 3 for actual percentiles.
3 Tertiles based on total study population (698 subjects with valid fatty acid assays, before losses due to missing covariates). Starting points for the second and third tertiles were 12.14 and 15.09 for omega-6 %.
4 Conditioned on recruitment center only; age included in model as continuous covariate.
5 Covariates include age (years), BMI, current alcohol consumption in grams/week, and age at birth of first child.

2.3.4 Specific analyses: Adipose tissue versus plasma micronutrients

The relationship between adipose and blood-based measurements of the same micronutrients is of potentially great importance for studies employing biomarkers. Correlations between levels of micronutrients measured in plasma with those in adipose tissue reflect the equilibrium between current circulating levels and longer-term depot stores. Knowledge of the degree to which adipose tissue measurements predict plasma levels is important in comparing results of studies that include only one biomarker or the other. In addition, a very strong correlation between adipose and plasma measures would indicate that the relatively less invasive plasma-based approach yields essentially similar results to an approach using adipose tissue, which would have practical implications for future study designs.

Data from the EURAMIC study were employed to investigate the agreement between adipose tissue and plasma-based micronutrient measures. In addition to fat biopsies, blood samples were collected for a subset of the total EURAMIC population. These blood samples were analyzed to determine their plasma concentrations of vitamin A, three carotenoids (beta-carotene, lycopene, and lutein), and vitamin E. Most of these micronutrients were also assayed in adipose tissue as part of the main EURAMIC study. Table 2.3.4#1 compares the micronutrients measured in adipose tissue with those measured in plasma. Lutein has no measured plasma counterpart, and retinol has only a distant surrogate measure, total vitamin A.

Table 2.3.4 #1: Micronutrients measured in adipose tissue and plasma

Adipose tissue micronutrient	Corresponding plasma micronutrient
Carotenoids	
Beta-carotene	Beta-carotene
	Alpha-carotene
Lutein	None
Lycopene	Lycopene
Retinol	None directly; vitamin A closest approximation
Tocopherols	
Alpha-tocopherol	Vitamin E (includes both alpha and gamma tocopherol)
Gamma-tocopherol	Vitamin E (includes both alpha and gamma tocopherol)

Evaluation of the association between adipose and plasma micronutrient measures required the creation of a dataset incorporating the plasma micronutrient results. This dataset was then combined with relevant adipose tissue micronutrient results and data on potential confounders from the pooled breast cancer dataset used in the main analyses (PLBC0196) and the EURAMIC myocardial infarct dataset. The program used to create the final combined plasma-adipose tissue analytical dataset, dubbed PLSR1195, is presented in Appendix B.

Following creation of the analytical dataset, descriptive analyses were performed on all micronutrients to identify potential outliers and invalid values. After validation of all suspect values and exclusion of those values derived from faulty chromatograms, the distribution of each

micronutrient was assessed for normality. Most of the micronutrients had significantly non-normal distributions (Table 2.3.4#2). Since the assumption of normality is central for correlation and linear regression analyses, a transformation yielding nominal normality was determined for each micronutrient. Natural log transformation produced approximate normality in three more micronutrients, but the rest remained significantly non-normal. An empirical procedure, the Box-Cox regression algorithm available as part of SAS's QC module, was then used to determine the optimum normalizing transform(s) for the remainder of the micronutrients. Table 2.3.4#3 presents the transformations ultimately adopted for each micronutrient.

To avoid any possible influence of the disease process on results, investigation of the relationship between adipose tissue and plasma micronutrients was restricted to controls. These controls included men from the EURAMIC myocardial infarct study as well as women from the breast cancer study. Valid data on both adipose and plasma micronutrients were available for 208-211 subjects, depending on the micronutrient in question (some subjects had missing or invalid values for one or more, but not all, micronutrients). The breakdown of subjects by gender and other characteristics is presented in Table 2.3.4#4.

Relationships between micronutrients were assessed through a combination of correlation analyses, graphical plots, and regression analyses. Table 2.3.4#5 presents the correlation between major plasma micronutrients. The strongest correlation is that between plasma lycopene and lutein. Lutein and vitamin E also correlate strongly. Nearly all of the plasma show statistically significant intercorrelation, with the abovementioned relationships for lutein being clearly the strongest. Since lutein has no measured counterpart in plasma, the key point for subsequent analyses is that the correlations between plasma lycopene, beta-carotene, and vitamin E are strong, but not sufficiently so as to approach collinearity. These biomarkers do, then, appear to measure distinct things, rather than to be interchangeable indicators.

Scatter plots of adipose against plasma micronutrient values provided a means of graphically evaluating the relationship between these tissue biomarkers. In particular, separate plots for men and women were compared to assess the potential impact of gender on results. These comparisons revealed apparently substantial differences between men and women in the relationship between adipose and plasma b-carotene (see Figure 2.3.4#1) and for adipose retinol and plasma b-carotene. Thus it appeared that, at least for b-carotene and retinol, gender-specific analyses or control for gender would be advisable. Gender appeared less important in plots for lycopene, vitamin E, and a-tocopherol.

To determine what factors in addition to gender might influence the relationship between micronutrients, multivariate linear regression analyses were carried out. These analyses utilized forward selection to identify the factors with significant predictive power in modeling each of the micronutrients. Table 2.3.4#6 presents the results of these analyses. Age was a significant predictor for several plasma and adipose micronutrients (plasma vitamins A and E, adipose alpha- and gamma-tocopherol). Body mass index was a significant predictor only for adipose micronutrients (beta-carotene, lycopene, alpha-tocopherol, and retinol). Lack of vitamin C supplementation predicted lower levels of most plasma and adipose micronutrients. Presumably

vitamin C supplementers also tend to take multivitamin supplements (no information on such supplementation was available), which would be expected to increase tissue levels of many micronutrients. In addition, users may tend to consume greater amounts of fruits and vegetables containing the measured micronutrients. Diabetes predicted lower plasma beta-carotene, but showed no significant association with other micronutrients, so it is unclear whether the plasma beta-carotene finding reflects a physiologic effect or a statistical artifact. Smoking status was significantly associated with levels of several micronutrients (beta-carotene (in men), lutein, and vitamin E) in plasma, but not in adipose tissue, which could indicate a depletion of circulating micronutrients without an effect on body stores; drinking status, in contrast, showed significant predictive value for both plasma and adipose lycopene, but only among women. Total polyunsaturated fatty acid in adipose tissue was associated with significantly lower adipose tissue retinol in both men and women, but showed no significant association with other micronutrients. Total PUFA was examined due to the possibility lipid peroxidation associated with PUFA intake increases antioxidant demand. The absence of association with the major antioxidant micronutrients makes the significance of the observed retinol-PUFA association uncertain.

Based on the results of these regression analyses, gender, age, BMI, smoking, drinking, and diabetes were singled out for attention as potential confounders of the relationship between plasma and adipose micronutrients. Control for vitamin supplement use was deemed undesirable, as this would constitute controlling for the same exposure that that the biomarkers are used to measure. Since no plasma-adipose comparison of retinol could be made due to the absence of data on plasma retinol, adipose PUFA was not included in subsequent analyses.

Correlation analyses provide a quantitative measure of the association between adipose and plasma biomarkers. Table 2.3.4#7 presents the results of initial correlation analyses, with and without stratification by gender. Table 2.3.4#8 presents corresponding results after adjustment for age, body mass index, smoking and drinking through the use of partial correlations. (Analyses including diabetes were also conducted, but diabetes turned out to have no effect after stratification on smoking status, so it is not included here). In general, correlations tended to strengthen somewhat after control for the additional factors, although for the most part the impact was small. The greatest impact of adjustment on results with both genders combined was observed for beta-carotene ($R^2 = 0.29$ unadjusted vs. 0.40 adjusted). Adjustment had no effect on results after stratification by gender, however, indicating that the additional factors acted as a surrogate for gender (or vice versa). The greatest impact on gender-specific results was observed for lycopene among women ($R^2 = 0.40$ vs. 0.25). The only other impact of note on the correlations was on that for adipose beta-carotene with plasma vitamin A, which became significantly negative among women after adjustment. As a whole, factors other than gender appeared to have little bearing on the association between levels of micronutrients measured in adipose tissue and those measured in plasma, with the exception of smoking. Smokers had weaker adipose-plasma correlations both for beta-carotene and for alpha-tocopherol, but not for lycopene. This implies that with the possible exception of lycopene, the relationship between adipose and plasma has only limited sensitivity to factors other than gender and smoking.

Only two micronutrients were measured directly in both plasma and adipose tissue: beta-carotene and lycopene. Since alpha-tocopherol is typically the major component of vitamin E (which includes other tocopherols as well), adipose alpha-tocopherol and plasma vitamin E are also fairly directly comparable. All three of these biomarker pairs showed highly significant correlations in the pooled population, and the correlations remained significant after stratification by sex and adjustment for age, BMI, smoking, and drinking. Levels of the vitamin A precursors alpha and beta-carotene in adipose tissue showed a statistically significant negative correlation with plasma vitamin A. The nearly complete absence of this association in men coupled with the strikingly stronger correlation of plasma and adipose beta-carotene observed in women suggests that hormonal or other factors associated with gender may be particularly critical for beta-and alpha-carotene - but not necessarily for other carotenoids, such as lycopene.

Table 2.3.4#2: Summary of tests for normality of distribution--Micronutrients in plasma and adipose tissue: Controls only

Micronutrient	Untransformed		Natural log transformed	
	Nominally non-normal?	Test for non-normality: P <	Nominally non-normal?	Test for non-normality: P <
Plasma				
Beta carotene	Yes	0.0001	Yes	0.0003
Lutein	Yes	0.0001	Yes	0.0164
Lycopene	Yes	0.0001	Yes	0.0003
Vitamin E	Yes	0.0001	No	0.3181
Vitamin A	No	0.3334	No	0.4203
Adipose				
Beta carotene	Yes	0.0001	Yes	0.0001
Lycopene	Yes	0.0001	Yes	0.0001
Alpha tocopherol	Yes	0.0001	No	0.8110
Retinol	Yes	0.0001	No[1]	0.0926

Table 2.3.4#3: Transformations used to produce distribution without significant non-normality

Micronutrient	Transform
Plasma	
Beta carotene	0.40
Lutein	0.40
Lycopene	0.40
Vitamin E	Natural log
Vitamin A	None--transform unnecessary
Adipose	
Beta carotene	0.40
Lycopene	0.40
Alpha tocopherol	Natural log
Retinol	0.40

Table 2.3.4#5: Correlations among plasma micronutrients

Controls only, EXCL=0
Pearson Correlation Coefficients (P-values) after normalization

Micronutrient	Beta-carotene	Lycopene	Lutein	Vitamin A	Vitamin E
Beta-carotene	**1.000**	**0.323** (<0.001)	**0.205** (0.003)	**-0.045** (0.515)	**0.286** (<0.001)
Lycopene	**0.323** (<0.001)	**1.000**	**0.591** (<0.001)	**0.148** (0.031)	**0.352** (<0.001)
Lutein	**0.205** (0.003)	**0.591** (<0.001)	**1.000**	**0.187** (0.007)	**0.415** (<0.001)
Vitamin A	**-0.045** (0.515)	**0.148** (0.031)	**0.187** (0.007)	**1.000**	**0.295** (<0.001)
Vitamin E	**0.286** (<0.001)	**0.352** (<0.001)	**0.415** (<0.001)	**0.295** (<0.001)	**1.000**

N=208 for (correlations involving) b-carotene, 211 for all others.
Normalizing transformations employed: b-carotene, lycopene, and lutein = 0.40 ; vitamin A = none ; vitamin E = natural log

Table 2.3.4#6: Predictors of Normalized Plasma and Adipose Tissue micronutrient levels
Controls only, EXCL=0

Micronutrient	Predictor	Parameter estimate (S.E.)	Partial p-value	Regression coefficient (pooled p-value)
Plasma beta-carotene	**No Vitamin C**	-0.228 (0.105)	0.031	0.079
	Current Smoking	-0.023 (0.010)	0.018	(<0.001)
	Diabetic	-0.865 (0.418)	0.040	
Plasma lutein (women)	**No Vitamin C**	0.056 (0.090)	0.538	0.008
	Former Smoker	-0.061 (0.162)	0.707	(0.802)
Plasma lutein (men)	**No Vitamin C**	-0.724 (0.152)	<0.001	0.330
	Former Smoker	-0.156 (0.195)	0.425	(<0.001)
Plasma lycopene (women)	**No Vitamin C**	-0.213 (0.127)	0.097	0.124
	Current Drinker	0.574 (0.213)	0.008	(0.003)
Plasma lycopene (men)	**No Vitamin C**	-0.582 (0.180)	0.002	0.226
	Current Drinker	- 0.303 (0.425)	0.478	(<0.001)
Plasma vitamin E	**No Vitamin C**	-0.059 (0.025)	0.022	0.092
	Age	0.007 (0.002)	<0.001	(<0.001)
	Current Smoking	0.005 (0.002)	0.027	
Plasma vitamin A (women)	**Age**	0.747 (0.635)	0.242	0.031 (0.155)
Plasma vitamin A (men)	**Age**	0.681 (0.437)	0.123	0.040
			0.188	(0.169)
Adipose beta-carotene (women)	**No Vitamin C**	-0.079 (0.035)	0.024	0.045
	BMI	-0.003 (0.006)	0.568	(0.064)
Adipose beta-carotene (men)	**No Vitamin C**	-0.062 (0.034)	0.070	0.250
	BMI	-0.037 (0.007)	<0.001	(<0.001)
Adipose beta-carotene (women)	**No Vitamin C**	-0.079 (0.034)	0.023	0.042 (0.023)
Adipose beta-carotene (men)	**No Vitamin C**	-0.048 (0.037)	0.201	0.019 (0.201)

Adipose lycopene (women)	**Current Drinker**	0.080 (0.036)	0.027	0.126
	No Vitamin C	-0.046 (0.021)	0.033	(0.006)
	BMI	-0.003 (0.003)	-0.824	
Adipose lycopene (men)	**Current Drinker**	0.025 (0.059)	0.670	0.174
	No Vitamin C	0.003 (0.024)	0.902	(0.004)
	BMI	-0.020 (0.006)	<0.001	
Adipose lycopene (women)	**Current Drinker**	0.076 (0.035)	0.033	0.120
	No Vitamin C	-0.047 (0.021)	0.029	(0.003)
Adipose lycopene (men)	**Current Drinker**	0.024 (0.063)	0.702	0.037
	No Vitamin C	0.008 (0.025)	0.754	(0.380)
Adipose alpha-tocopherol (women)	**No Vitamin C**	-0.240 (0.052)	<0.001	0.195
	Age	0.015 (0.006)	0.023	(<0.001)
	BMI	0.002 (0.009)	0.820	
Adipose alpha-tocopherol (men)	**No Vitamin C**	-0.226 (0.081)	0.007	0.138
	Age	0.014 (0.006)	0.020	(0.006)
	BMI	-0.025 (0.018)	0.174	
Adipose gamma-tocopherol (women)	**No Vitamin C**	-0.240 (0.052)	<0.001	0.195
	Age	0.015 (0.006)	0.020	(<0.001)
Adipose gamma-tocopherol (men)	**No Vitamin C**	-0.228 (0.080)	0.006	0.128
	Age	0.012 (0.006)	0.034	(0.003)
Adipose retinol (women)	**Adipose PUFA**	-0.016 (0.004)	<0.001	0.158
	BMI	-0.008 (0.004)	0.023	(<0.001)
	No Vitamin C	-0.055 (0.019)	0.004	
Adipose retinol (men)	**Adipose PUFA**	-0.016 (0.004)	<0.001	0.137
	No Vitamin C	-0.057 (0.019)	0.003	(<0.001)

Results are stratified by gender whenever gender itself was a statistically significant predictor.
No vitamin C = Does not take supplements containing vitamin C

Table 2.3.4#7: Correlations between plasma and adipose micronutrients, unadjusted for other factors

	Correlation coefficient[1]		
	Pooled	**Women**	**Men**
Adipose b-carotene vs. plasma b-carotene	0.293 (<0.001)	0.313 (<0.001)	0.525 (<0.001)
Adipose lycopene vs. plasma lycopene	0.260 (<0.001)	0.397 (<0.001)	0.194 (0.072)
Adipose a-carotene vs. plasma vitamin A	-0.213 (0.002)	-0.209 (0.021)	0.013 (0.908)
Adipose b-carotene vs. plasma vitamin A	-0.212 (0.002)	-0.102 (0.272)	-0.030 (0.783)
Adipose retinol vs. plasma vitamin A	0.120 (0.084)	-0.003 (0.970)	0.280 (0.008)
Adipose a-tocopherol vs. plasma vitamin E	0.367 (<0.001)	0.432 (<0.001)	0.263 (0.012)
Adipose g-tocopherol vs. plasma vitamin E	-0.019 (0.789)	0.140 (0.127)	-0.260 (0.013)

Table 2.3.4#8: Correlations between plasma and adipose micronutrients after adjustment for age, BMI, smoking status, and current drinking status

	Correlation coefficient[1]		
	Pooled	**Women**	**Men**
Adipose b-carotene vs. plasma b-carotene	0.396 (<0.001)	0.311 (0.001)	0.528 (<0.001)
Adipose lycopene vs. plasma lycopene	0.197 (0.007)	0.252 (0.009)	0.224 (0.047)
Adipose a-carotene vs. plasma vitamin A	-0.242 (<0.001)	-0.242 (0.012)	0.018 (0.873)
Adipose b-carotene vs. plasma vitamin A	-0.287 (<0.001)	-0.228 (0.019)	-0.018 (0.873)
Adipose retinol vs. plasma vitamin A	0.136 (0.061)	0.041 (0.673)	0.343 (0.002)
Adipose a-tocopherol vs. plasma vitamin E	0.359 (<0.001)	0.389 (<0.001)	0.357 (0.001)
Adipose g-tocopherol vs. plasma vitamin E	-0.019 (0.795)	0.127 (0.196)	-0.205 (0.071)

Note:
1. Pearson correlation coefficients (P-values) after normalization.

Table 2.3.4#9: Correlations between plasma and adipose micronutrients by current smoking status

	Correlation coefficient[1]			Parial correlation coefficient[2]	
	Pooled	Non-smoker	Smoker	Non-smoker	Smoker
Adipose b-carotene vs. plasma b-carotene	0.293 (<0.001)	0.408 (<0.001)	0.324 (0.039)	0.432 (<0.001)	0.195 (0.241)
Adipose b-carotene vs. plasma lycopene		0.045 (0.561)	-0.029 (0.857)	0.039 (0.616)	-0.079 (0.639)
Adipose b-carotene vs. plasma vitamin A	-0.212 (0.002)	-0.217 (0.005)	-0.350 (0.025)	-0.267 (<0.001)	-0.301 (0.066)
Adipose lycopene vs. plasma lycopene	0.260 (<0.001)	0.231 (0.003)	0.338 (0.031)	0.229 (0.003)	0.338 (0.038)
Adipose lycopene vs. plasma b-carotene		0.337 (<0.001)	0.268 (0.090)	0.349 (<0.001)	0.237 (0.152)
Adipose lycopene vs. plasma lutein		0.011 (0.891)	0.214 (0.179)	0.026 (0.742)	0.189 (0.255)
Adipose lycopene vs. plasma vitamin A		-0.153 (0.047)	-0.279 (0.077)	-0.152 (0.052)	-0.252 (0.126)
Adipose a-carotene vs. plasma b-carotene		0.374 (<0.001)	0.106 (0.520)	0.385 (<0.001)	0.032 (0.848)
Adipose a-carotene vs. plasma lutein		0.157 (0.041)	0.233 (0.153)	0.172 (0.028)	0.189 (0.256)
Adipose a-carotene vs. plasma lycopene		0.094 (0.222)	-0.026 (0.876)	0.100 (0.205)	-0.057 (0.733)
Adipose a-carotene vs. plasma vitamin A	-0.213 (0.002)	-0.216 (0.005)	-0.202 (0.217)	-0.229 (0.003)	-0.183 (0.271)
Adipose retinol vs. plasma b-carotene		0.282 (<0.001)	-0.020 (0.902)	0.281 (<0.001)	0.004 (0.979)
Adipose retinol vs. plasma lutein		0.015 (0.852)	-0.189 (0.231)	0.020 (0.800)	-0.171 (0.303)

Adipose retinol vs. plasma lycopene		0.127 (0.101)	-0.009 (0.957)	0.126 (0.108)	-0.246 (0.137)
Adipose retinol vs. plasma vitamin A	0.120 (0.084)	0.141 (0.069)	-0.043 (0.786)	0.151 (0.053)	-0.021 (0.899)
Adipose a-tocopherol vs. plasma vitamin E	0.367 (<0.001)	0.424 (<0.001)	0.178 (0.260)	0.397 (<0.001)	0.124 (0.460)
Adipose g-tocopherol vs. plasma vitamin E	-0.019 (0.789)	0.031 (0.692)	-0.234 (0.136)	0.021 (0.795)	-0.016 (0.924)

Note:
1. Pearson correlation coefficients (P-values) after normalization.
2. Partial Pearson correlation coefficients on age (P-values) after normalization.

Conclusions:

Measurements of counterpart micronutrients (Beta-carotene, lycopene, and alpha-tocopherol/vitamin E) in adipose tissue and plasma show significant correlation. Correlations for different micronutrients are not consistent, however--some tend to covary directly, others inversely between the two tissues (e.g., adipose alpha or beta-carotene and plasma vitamin A). Even for directly comparable micronutrient measures, while the correlations are strong there is considerable discontinuity between the two measures even after taking measurement error into account. Thus, the measures are not interchangeable. Since adipose tissue is a longer-term marker, less subject to rapid change in response to diet than blood-based measures, much of the observed difference could reflect transient fluctuations in plasma micronutrient levels--particularly as only a single reading, rather than the average of several readings over time, was available for plasma micronutrient. Physiological differences could also contribute to the differences. Levels of an important micronutrient in plasma may well be more tightly regulated than levels in a storage depot, for example. This would tend to make the storage depot more important as a marker of long-term intake, but not necessarily more important etiologically if the level of micronutrient in the blood bathing the tissues is a key factor. Ideally, information on micronutrients from both tissues would be useful in studies of potential associations with disease. Differences between the markers may provide insights into possible etiologic mechanisms--and congruent results with different markers would provide stronger evidence of association than results based on a single marker in isolation.

Where micronutrient measures based on either adipose tissue or serum are employed, important potential predictors appear to include gender, age, body mass index, smoking, drinking, and vitamin supplement use. The importance of controlling for supplement

use is debatable, as it constitutes both an alternative marker and, for many micronutrients, a direct source of exposure. The other factors appear to possess varying importance depending on the marker in question. Body mass index, for example, is associated with the level of many micronutrients in adipose tissue, but not in plasma. The key factors affecting the correlation between adipose tissue and plasma micronutrients were clearly gender and, less universally, smoking. The effect of gender may reflect differences in metabolism, differences in range of intakes, or both. The observation of a lower correlation between measures of beta-carotene and alpha-tocopherol, but not of lycopene, among smokers is consistent with results seen for plasma and skin samples in a recent study (Peng et al. 1995). Control for other factors tends to strengthen correlations somewhat. Comparisons of adipose and plasma biomarkers should therefore pay particular attention to gender and (at least for beta-carotene and alpha-tocopherol) smoking, while drinking habits, body mass index, and age should also be addressed if data (and subject numbers) permit.

2.3.5 Specific analyses: Monounsaturated fatty acids and breast cancer

Previous analyses of the EURAMIC study's data on adipose tissue fatty acids and breast cancer focused on the essential and trans fatty acids (See 2.3.2 and 2.3.3). The essential fatty acids include the polyunsaturates of the omega-3 and omega-6 families. Necessary for normal growth and development, these fatty acids cannot be synthesized from other types (i.e., monounsaturates of saturates) and thus their direct supply in the diet is essential. Although not essential fatty acids, most trans fatty acid in the tissues also come from the diet. Due to these characteristics, adipose tissue stores are at their best as indicators of dietary intake for essential and trans fats.

Correlations between diet and tissue levels are generally not as strong for other fatty acid classes (monounsaturated and saturated fatty acids), but they are nevertheless associated. The monounsaturate oleic acid is the major fatty acid component of adipose tissue. Although recent studies in American populations have found fairly poor correlations ($r < 0.26$) between adipose oleate and estimated dietary intake (London 1991[17], Hunter 1992[18]), comparisons including European populations with a broad range of oleic acid intakes produced much better correlations (r up to 0.46) (Beynen 1980[19], Fordyce 1983[20]).

The current analyses explore the relationships between monounsaturated fatty acids and disease. Since these fatty acids can be synthesized endogenously as well as being acquired through the diet directly, definitive distinction between intake and internal synthesis as the source of observed tissue levels is not possible. In addition, tissue levels of fatty acids, whether of dietary or of other origin, may be more relevant to potential associations of fatty acids with disease than simple intake, which may not reflect effective dose due to interindividual differences in absorption or metabolism. Thus, while the interpretation of relationships between tissue stores and breast cancer is not as straightforward for monounsaturated fatty acids as it is for polyunsaturates, such relationships could still provide valuable insight into the potential role of these fatty acids in disease.

Dietary sources of monounsaturated fatty acids are varied. Most dietary monounsaturates are either 16, 18, or 22 carbons in length. Palmitoleic acid, the 16 carbon monounsaturate of the omega-7 family (C16:1n7), is a general minor component of animal and vegetable fat, and a more major component of some nuts and fish oils. Cis-vaccenic acid, an 18 carbon monounsaturated fatty acid of the omega-7 family (C18:1n7), is a minor component of most seed oils and a larger component in seafoods. Oleic acid, the major monounsaturated fatty acid, belongs to the omega-9 family (C18:1n9); while olive oil is its richest dietary source, it is also the major component of most animal fats and makes up a sizable fraction of most vegetable oils. Erucic acid, the 22 carbon monounsaturate of the omega-9 family (C22:1n9), is a very minor dietary component, associated primarily with rapeseed oil. Myristoleic acid is another minor dietary monounsaturate, 14 carbons in length (C14:1n7), and is associated primarily with butter.

A quick overview of the levels of individual monounsaturated fatty acids in adipose tissue reveals that the 14, 20, and 22-carbon monounsaturates are very minor constituents of adipose fat, averaging less than 1% of the total fatty acids present (Figure 2.3.5 #1). Cis-vaccenic acid is more abundant, averaging around 2%. Palmitoleic is next in abundance, averaging over 6% in most centers, but oleic acid is by far the most common monounsaturate (or fat in general) in adipose tissue, averaging over

40%.

A simple comparison of mean fatty acid percentages in cases and controls for each of the study centers reveals the protective association between oleic acid and breast cancer in Malaga previously described in section 2.3.3. Outside of Malaga, however, cases and controls have similar oleic acid levels. Cis-vaccenic acid is modestly higher among cases in 4 out of 5 centers, while palmitoleic acid is lower in 3 out of 5. Of the minor monounsaturates, the 14-carbon isomer shows a tendency toward lower levels in cases, and the 20 and 22-carbon isomers show little consistent case-control difference. Erucic acid is, however, far more common in the Berlin population than in the others, and within that population it is more common among cases.

To assess the statistical strength of the associations between the monounsaturates and breast cancer, and to control for the potential effects of other risk factors, unconditional logistic regression analyses were carried out for each center (Table 2.3.5 #1). All risk factors showing a significant correlation with any of the individual monounsaturated fatty acids within any center were included in the adjusted models. These risk factors included age, body mass index, parity, age at birth of first child, and family history of breast cancer. Odds ratios were computed based on the difference between the 75th and 25th percentiles of the fatty acid in the control population for all centers combined.

Oleic acid had a statistically significant protective association with breast cancer in Malaga (OR= 0.40, 95% CL 0.28, 0.58). In contrast, most of the other centers yielded ORs above one, with the OR for Zeist reaching nominal statistical significance after adjustment for other risk factors (2.36; 1.01, 5.50). Adjusted ORs for palmitoleic and cis-vaccenic acids paralleled the findings from the comparison of means. The OR reached statistical significance for palmitoleic acid in Coleraine (OR 0.50, 0.28--0.88) and for cis-vaccenic acid in Malaga (4.43, 1.68--11.68). None of the center-specific ORs for the minor monounsaturates reached statistical significance with the exception of myristoleic acid in Berlin (OR 0.16, 0.04--0.66), and subject numbers in Berlin were too small to allow adjustment for other risk factors. Erucic acid was below the limit of detection in most samples, and below 0.01% for most samples in which it was detected. Consequently, it was modeled as a 3-level variable rather than using the original continuous data. Assigning a value of 1, 2, or 3 for below detection, moderate, and high adipose tissue levels, erucic acid appears protective in 3 out of 5 centers, reaching nominal significance in Zurich and Zeist (Table 2.3.5 #3). Odds ratios for the moderate and high categories using the below-detection group as a referent, however, reveal only weak evidence of a dose-response pattern in Zurich and Zeist, and indicate that most of the relationship is due to the influence of the high category. Subject numbers in Berlin do not permit stratification into moderate and high, at least not using cutpoints from the pooled EURAMIC population. Malaga displays statistically weak indications of association between high C22:1 and breast cancer, while there is little evidence of any association in Coleraine.

The statistically strong but directionally opposite associations of oleic and cis-vaccenic acid with breast cancer in Malaga raise the possibility that confounding or effect modification could be involved in the associations between those fatty acids and breast cancer. To investigate this possibility, the study population was divided into tertiles for each fatty acid. Due to the significantly higher oleic acid levels in Malaga, populations in and outside of Malaga were analyzed separately. Odds ratios for medium and high vs. low oleic acid were computed for each tertile of cis-vaccenic acid, and vice-versa.

Table 2.3.5 #3 presents the results of these analyses.

Computing results for centers outside Malaga and for Malaga separately, and dividing each of these center groupings into tertiles based on the total population for that particular grouping (thereby producing a more even distribution of subjects per tertile than analyses using control-based cutpoints, which yielded particularly uneven distributions by tertile in Malaga), leads to the following conclusions. 1) Cis-vaccenic acid shows an adverse association with breast cancer at both low and medium tertiles of oleic acid in Malaga, with indications of dose-response but no indication of interaction. However, there aren't enough cases at high oleic to model, and numbers are insufficient to support adjustment for other covariates at any tertile of oleic acid. 2) Outside Malaga, cis-vaccenic appears mildly protective in medium and high oleic tertiles, with hints of dose-response, but is mildly associated with cancer in the low oleic acid tertile. None of the tertile-specific results approach statistical significance. 3) The association of cis-vaccenic with breast cancer in Malaga does not appear attributable to residual confounding due to oleic acid, since oleic and cis-vaccenic acid show virtually no correlation in Malaga (Pearson and Spearman correlation coefficients -0.04 and -0.07, respectively). Outside Malaga, the reversal of the apparent protective association for cis-vaccenic in the low oleic acid tertile renders the association difficult to interpret; cis-vaccenic is a relatively minor component of total monounsaturated fat anyway, so one would expect that if it did have potential effects on risk, these effects would be most evident at low oleic acid levels. Instead, there are indications of dose-respondent protection at medium and high oleic, but the opposite (association with risk, with a reverse dose-response) at low. 4) Within Malaga, oleic acid appears powerfully protective for both tertiles of cis-vaccenic acid (medium and high) that contain adequate case numbers to model. 5) Outside Malaga, oleic acid is modestly associated with breast cancer at all tertiles of cis-vaccenic acid after control for other risk factors, with some indication of dose-response. None of the individual associations reach statistical significance, however. No interaction between cis-vaccenic and oleic acid is apparent.

Regression models on the combined population from all centers provide a summary measure of association for the total study population (Table 2.3.5 #4). Models are conditioned on center and age, with the inclusion of other risk factors as covariates. Palmitoleic and oleic acid both appear significantly protective when all centers are combined. Associations for the other monounsaturates fail to achieve statistical significance, although the ORs for myristoleic and cis-vaccenic acid approach nominal significance.

Monounsaturated fatty acids may be interconverted in the body. The ratio between monounsaturates of differing chain length was explored as a potential indicator of the degree of desaturation and elongation occurring in the tissues. The greater the degree of such activity occurring, the higher the ratio of longer to shorter chain fatty acids that would be expected. Ratios of 18 to 16, 20 to 18, and 20+ to 18 carbon-length monounsaturates were compared (the ratio of 22 to lesser lengths was not compared directly since erucic acid was not detected in most subjects, and appeared almost exclusively in Berlin when it was detected). For each comparison of longer vs. shorter-chain monounsaturates, increased ratios were associated with breast cancer. These results did not approach statistical significance, however. In addition, it should be recalled that above-detection-limit levels of the longest-chain monounsaturate, erucic acid, appeared protective, so the association did not strengthen consistently as higher chain lengths were compared.

Discussion

Oleic acid showed a stronger relationship with breast cancer than any other monounsaturate. Within the Spanish study center population it appeared powerfully protective. This is consistent with evidence from several recent studies in Mediterranean populations suggesting that olive oil consumption may protect against breast cancer. Outside the Spanish center, however, oleic acid showed only a modest association with breast cancer--and not in a protective direction. That does not necessarily contradict the olive oil hypothesis. Animal fats are rich sources of oleic acid, olive oil consumption is presumably much lower in the other European study populations than in Spain, and hence these populations' adipose oleic acid levels may reflect intake of animal fat much more than that of olive oil. Stratification by levels of cis-vaccenic acid, a monounsaturate found in other seed oils, had little impact on the results inside or outside of Malaga. The balance between oleic acid and monounsaturates from seed oil sources thus does not appear important.

Evidence of an association for other monounsaturates with breast cancer is strongest for cis-vaccenic acid and palmitoleic acid. The association for cis-vaccenic acid is in the same direction for 4 out of 5 centers yet lacks statistical significance when results for all centers are combined. In addition, outside Malaga cis-vaccenic shows a mildly protective association at moderate and high oleic acid tertiles, but this reverses when oleic is low. The weak statistical strength of the cis-vacennic results coupled with their inconsistency across oleic acid tertiles cautions against giving them much weight. Palmitoleic acid shows less consistency across centers, with a protective association in 3 and an opposite association in 2. Despite this, with all results combined the protective relationship reaches statistical significance. The fact that palmitoleic acid is found in fish oils raised the intriguing possibility that its protective association reflects the effects of concomitant omega-3 fatty acid consumption. This hypothesis is dispelled by the correlations observed with the major omega-3 fatty acid in fish oil, docosahexaenoic acid, in those centers showing a protective association for palmitoleic (-0.27, +0.11, and -0.12 for Berlin, Coleraine, and Zeist, respectively). Another possible interpretation of a protective association for palmitoleic acid is that the level of this fatty acid reflects a reduced need for synthesis of other medium-chain fatty acids. When dietary sources are in short supply, including situations of essential fatty acid deficiency, exogenous synthesis is increased. While the ratios of 18 to 16-carbon monounsaturates (i.e., palmitoleic acid) and ratios of even longer-chain monounsaturates were in fact associated with breast cancer, the association was much weaker than that observed for palmitoleic acid itself. The balance between monounsaturates of differing chain lengths, in fact, appears to offer less explanatory power than either palmitoleic, cis-vaccenic, or oleic acid considered in isolation.

The epidemiologic literature provides little support for a protective role of palmitoleic acid in breast cancer. Since the finding resulted from exploratory analyses including five additional monounsaturates, rather than the testing of an a priori hypothesis based on evidence of protective potential for palmitoleic acid, these results must be considered speculative pending confirmatory findings in another study population.

None of the other, minor monounsaturates yield statistically significant results for all centers overall. Myristoleic acid appears protective in 3 out of 5 centers, significantly so in Berlin. A strong correlation

with palmitoleic acid (center-specific Pearson's correlation coefficients 0.55-0.76) indicates that the appearance of protection is secondary to the association with that fatty acid. And while erucic acid also shows signs of a protective association with breast cancer, it is such a minor constituent of fat that it is near or below the limit of detection in most samples. The referent category used in the erucic acid analyses actually represents samples in which no C22:1 was identified. This may have come about because there was too little to measure or because the erucic acid peak was small enough that it did not integrate separately from an adjacent peak. The results for the high and the medium vs. the "low" category make it clear that if only the nonzero values are considered, there would be little evidence of an association between erucic acid and breast cancer, protective or otherwise. Further, the very low levels of C22:1 seen even in most of the "medium" category indicate that the difference between low and medium may not be very meaningful, leaving the whole relationship hinging upon the handful of samples in the "high" category. This limitation greatly compromises the evidence for an association.

Table 2.3.5 #1: Results of initial unconditional logistic regression models for individual monounsaturated fatty acids by center
ORs for fatty acid followed by 95% CLs in parentheses

Variable(s) in model	Zurich (N=128; 126)	Malaga (N=124; 124)	Coleraine (N=194; 193)	Berlin (N= 62)	Zeist (N=133; 121)
C14_1cis	0.68 (0.35, 1.33)	1.98 (0.52, 7.56)	0.79 (0.50, 1.25)	0.16 (0.04, 0.66)	0.95 (0.61, 1.48)
C14_1cis + covariates	0.64 (0.31, 1.31)	2.02 (0.46, 8.83)	0.74 (0.45, 1.22)	*	0.99 (0.60, 1.63)
C16_1n_7	0.57 (0.31, 1.05)	1.04 (0.36, 3.00)	0.61 (0.36, 1.03)	0.23 (0.06, 0.83)	1.22 (0.70, 2.13)
C16_1n_7 + covariates	0.52 (0.26, 1.03)	1.13 (0.36, 3.55)	0.50 (0.28, 0.88)	*	1.54 (0.779, 2.98)
C18_1n_7	1.41 (0.95, 2.10)	3.49 (1.49, 8.22)	1.03 (0.71, 1.48)	1.65 (0.70, 3.88)	1.36 (0.96, 1.93)
C18_1n_7 + covariates	1.31 (0.84, 2.03)	4.43 (1.68, 11.68)	0.84 (0.56, 1.26)	*	1.47 (0.92, 2.34)
C18_1n_9	1.00 (0.53, 1.89)	0.43 (0.30, 0.60)	1.52 (0.85, 2.73)	1.09 (0.30, 4.03)	2.01 (0.96, 4.22)
C18_1n_9 + covariates	0.83 (0.42, 1.64)	0.40 (0.28, 0.58)	1.23 (0.66, 2.27)	*	2.36 (1.01, 5.50)
C20_1n_9	2.11 (0.88, 5.07)	0.73 (0.41, 1.32)	1.28 (0.79, 2.07)	1.20 (0.60, 2.39)	0.79 (0.47, 1.33)
C20_1n_9 + covariates	2.22 (0.86, 5.73)	0.60 (0.32, 1.13)	1.59 (0.94, 2.68)	*	0.80 (0.45, 1.40)

N = number of observations in crude and in with-covariate model, respectively. For Zeist, N is one less for C18_1n_7 due to missing value for one subject
*Unresolved model. Quasicomplete separation in the sample points.
Odds ratios based on difference between 75th and 25th percentiles from control population of pooled dataset. Specifically: C14_1cis = 0.33; C16_1n_7 = 3.22; C18_1n_7 = 0.49; C18_1n_9 = 4.86; C20_1n_9 = 0.17.
Covariates = Age BMI AGE_BR0 AGE_BR2 AGE_BR3 BCFAMILY

Table 2.3.5 #2: Results of logistic regression models for erucic acid treated as a 3-level variable, by center

Variable	Zurich	Malaga	Coleraine	Berlin	Zeist
	3-level variable (low=0, medium=1, high=2): OR (95% CLs)				
C22:1cat, referent is <u>low</u>	0.64 (0.42--0.98)	1.37 (0.81--2.30)	0.99 (0.73--1.35)	0.58 (0.30--1.11)	0.38 (0.23--0.63)
+ covariates	0.64 (0.41--1.00)	1.31 (0.76--2.28)	1.04 (0.75--1.43)	Incomplete separation	0.38 (0.21--0.69)
	3-level categorical variable (low, medium, high): O.R. for high and for medium vs.low (95% CLs)				
C221cat, referent is <u>low</u>	H: 0.41 (0.17--0.97)	H: 2.44 (0.74--8.09)	H: 0.98 (0.53--1.81)	Incomplete	H: 0.15 (0.05--0.43)
	M: 0.62 (0.20--1.86)	M: 1.03 (0.48--2.23)	M: 1.04 (0.42--2.60)	separation	M: 0.34 (0.09--1.23)
+ covariates	H: 0.42 (0.17--1.04)	H: 2.29 (0.65--8.11)	H: 1.11 (0.58--2.12)	Incomplete	H: 0.16 (0.05--0.54)
	M: 0.46 (0.13--1.62)	M: 0.97 (0.44--2.18)	M: 0.73 (0.27--1.96)	separation	M: 0.28 (0.06--1.23)

Covariates = Age, body mass index, nulliparity, age at birth of first child, and history of breast cancer in the immediate family.

Table 2.3.5 #3: Odds Ratios for High and Medium tertiles of oleic acid vs. Low tertile, stratified by tertiles of cis-vaccenic acid, and vice-versa:

Malaga only and All Centers but Malaga

OR for Medium or High Fatty acid tertile vs. Low followed by 95% CLs

Malaga

Variable(s) in model	Low oleic		Medium oleic		High oleic	
	Med. cis-vaccenic OR	High cis-vaccenic OR	Med. cis-vaccenic OR	High cis-vaccenic OR	Med. cis-vaccenic OR	High cis-vaccenic OR
Cis-vaccenic acid	2.50 (0.39, 16.05)	3.00 (0.48, 18.93)	2.25 (0.35, 14.61)	5.06 (0.83, 30.75)	Nonconvergent	Nonconvergent
Cis-vaccenic acid + cov.	Nonconvergent	Nonconvergent	Nonconvergent	Nonconvergent	Nonconvergent	Nonconvergent
Variable(s) in model	Low cis-vaccenic		Medium cis-vaccenic		High cis-vaccenic	
	Med. oleic OR	High oleic OR	Med. oleic OR	High oleic OR	Med. oleic OR	High oleic OR
Oleic acid	Nonconvergent	Nonconvergent	0.10 (0.02, 0.64)	0.11 (0.02, 0.67)	0.19 (0.03, 1.11)	0.06 (0.01, 0.50)
Oleic acid + cov.	Nonconvergent	Nonconvergent	0.04 (0.00, 0.53)	0.04 (0.00, 0.46)	Nonconvergent	Nonconvergent

Centers Other Than Malaga

Variable(s) in model	Low oleic		Medium oleic		High oleic	
	Med. cis-vaccenic OR	High cis-vaccenic OR	Med. cis-vaccenic OR	High cis-vaccenic OR	Med. cis-vaccenic OR	High cis-vaccenic OR
Cis-vaccenic acid	1.43 (0.73, 2.78)	1.51 (0.63, 3.62)	1.07 (0.51, 2.22)	1.32 (0.62, 2.83)	1.01 (0.42, 2.39)	0.83 (0.39, 1.78)
Cis-vaccenic acid + cov.	1.31 (0.62, 2.78)	1.12 (0.39, 3.20)	0.92 (0.40, 2.11)	0.80 (0.31, 2.10)	1.00 (0.38, 2.65)	0.71 (0.27, 1.89)
Variable(s) in model	Low cis-vaccenic		Medium cis-vaccenic		High cis-vaccenic	
	Med. oleic OR	High oleic OR	Med. oleic OR	High oleic OR	Med. oleic OR	High oleic OR
Oleic acid	1.28 (0.63, 2.60)	1.54 (0.72, 3.32)	0.95 (0.48, 1.90)	1.09 (0.50, 2.37)	1.12 (0.45, 2.79)	0.85 (0.36, 2.03)
Oleic acid + cov.	1.67 (0.75, 3.74)	2.13 (0.86, 5,25)	1.17 (0.52, 2.63)	1.54 (0.62, 3.87)	1.87 (0.62, 5.70)	1.83 (0.61, 5.51)

Table 2.3.5 #4: Results of conditional logistic regression models of breast cancer by individual monounsaturated fatty acids: all centers pooled

Fatty Acid Variable(s)	Odds Ratio (95% C.L.s) [1]	
	Without covariates	With covariates[2]
As a % of all adipose tissue fatty acids...		
C14:1 ω5 (myristoleic)	0.77 (0.57--1.04)	0.75 (0.56--1.03)
C16:1 ω7 (palmitoleic acid)	0.74 (0.54--1.01)	0.68 (0.49--0.93)
C18:1 ω7 (cis-vaccenic acid)	1.38 (1.10--1.74)	1.26 (0.98--1.62)
C18:1 ω9 (oleic acid)	0.70 (0.57--0.86)	0.66 (0.53--0.82)
Total C18 cis monounsaturate	0.78 (0.58--0.89)	0.66 (0.53--0.83)
C20:1 ω9	1.08 (0.82--1.42)	1.11 (0.84--1.47)
Ratio measures		
18 cis to 16 cis monounsaturates	1.07 (0.90--1.27)	1.08 (0.90--1.30)
20 cis to 18 cis monounsaturates	1.09 (0.87--1.35)	1.16 (0.93--1.46)
20+22 to 18 cis monounsaturates	1.02 (0.84--1.23)	1.08 (0.89--1.32)

[1] Odds ratio for the difference between 75th and 25th percentiles in the control population. See Table 1, column 3 for actual percentiles. All models are conditioned on recruitment center and age.

[2] Covariates include BMI, nulliparity, age at birth of first child, and history of breast cancer in the immediate family.

Results exclude a potential low outlying value of C16:1 ω7 and "zero" values for C18:1w-7 and C20:1w-9 (a total of 10 for the former and 5 for the later fatty acid). Results with these values included were very similar, but may be less valid.

2.3.6 Specific analyses: Adipose tissue carotenoids and breast cancer

Analyses of the relationship between adipose tissue beta carotene and breast cancer conducted by members of the EURAMIC group gave little indication of an association.[21] The crude means for cases and controls were very similar: 0.91 vs. 0.94 ug/g. Models adjusting for other risk factors yielded similarly weak results: Table 2.3.6 #1 presents the basic findings. While the OR for the highest tertile of beta carotene dropped below 1.0, this difference did not approach statistical significance and there was no indication of dose-response.

Table 2.3.6 #1 ORs (with 95% C.L.s) for breast cancer by tertile of beta-carotene

Result	Beta-carotene tertile		
	Low	Medium	High
Age & center-adjusted	1.00	1.06	0.84
Multivariate adjusted[1]	1.00	1.03 (0.65, 1.62)	0.74 (0.45, 1.23)

[1] Adjusted for age, BMI, recruitment center, reproductive factors, smoking, and alcohol consumption.

To explore the potential importance of other carotenoids and possible interactions between them, we conducted analyses addressing the relationship of alpha-carotene and lycopene as well as beta-carotene to breast cancer. Center-specific models of breast cancer by these carotenoids revealed that results varied greatly by center (Table 2.3.6 #2). Beta-carotene appeared protective in two centers and detrimental in three, yet none of these results reached statistical significance. Alpha-carotene (not shown) largely paralleled beta-carotene, but with even weaker results due to greater measurement error. Lycopene, in contrast, appeared protective in all centers where beta-carotene appeared detrimental, and vice-versa. The association reached nominal statistical significance in Berlin. The very limited number of cases in this center, however, means that this result carries little weight overall.

Pooling all five centers produced the results summarized in Table 2.3.6 #3. Neither lycopene nor beta-carotene show much association with breast cancer, regardless of the mix of other risk factors included in the model. Inclusion of both carotenoids in the model simultaneously was found to result in a slightly more protective odds ratio for lycopene and a mildly adverse odds ratio for beta-carotene, both associations lacking statistical strength. The most dramatic change occurred with the inclusion of alpha-carotene (see Table 2.3.6 #3), which substantially altered the point estimate (OR) for beta carotene. Since it also greatly increased the confidence limits around this estimate, however, these results were not statistically meaningful. Simultaneous

inclusion of all three carotenoids proved impossible due to collinearity problems.

Alternative analyses were conducted to explore the effects of log transformation and the employment of an alternative exposure measure, total bodyfat. Models employing log-transformed carotenoid values (not shown) produced similar results to models using untransformed values. Models based on the estimated total amount of carotenoid carried in the bodyfat (Table 2.3.6 #4) produced results little different from those based on fat composition.

Taken altogether, the results to date indicate little association between breast cancer and adipose tissue carotenoid content. Of the carotenoids, lycopene shows more evidence of a relationship with breast cancer than does beta-carotene based on center-specific analyses, but the relationships for both micronutrients appear inconsistent and lacking in statistical significance.

Table 2.3.6 #2: **Center-Specific Models of Breast Cancer by Carotenoids** [1]

	Berlin	Zeist	Coleraine	Zurich	Malaga
N [2]	62	121	193	126	123
Lycopene, with covariates[3]	0.06 (0.00, 0.84)	0.81 (0.29, 2.22)	0.64 (0.25, 1.65)	1.24 (0.60, 2.57)	6.76 (1.31, 34.93)
b-carotene, with covariates[3]	4.41 (0.32, 60.38)	1.53 (0.46, 5.06)	1.92 (0.64, 5.75)	0.63 (0.28, 1.45)	0.22 (0.01, 4.57)

[1] Center-specific carotenoids odds ratios for model containing variables listed in first column, accompanied by 95% C.L. in parentheses.
[2] N = number of observations for model containing only trans fatty acid as independent variable followed (in parentheses) by number for model containing full covariate package as well.
[3] Covariates include BMI, study center, age in years, family history of BC, age at first child birth, and age at second and third children birth.

Table 2.3.6 #3: **Logistic Regression Models of Breast Cancer by Carotenoids**

Independent Variable(s) in Model	N[1]	Odds Ratio (95% C. L.)
Lycopene, with covariates[2]	625	0.97 (0.63, 1.48)
Beta-carotene, with covariates[2]		0.98 (0.60, 1.61)
Lycopene, with covariates[3]	603	0.93 (0.60, 1.45)
Beta-carotene, with covariates[3]		1.00 (0.97, 1.03)
Lycopene, with covariates[2] and alpha-carotene	625	0.91 (0.59, 1.42)
Beta-carotene, with covariates[2] and alpha-carotene		0.56 (0.25, 1.23)
Lycopene, with covariates[3] and alpha-carotene	603	0.88 (0.56, 1.38)
Beta-carotene, with covariates[2] and alpha-carotene		0.63 (0.29, 1.37)

[1] N = Number of individuals in model.
[2] Covariates include BMI, study center, age in years, family history of BC, age at first child birth, and age at second and third children birth.
[3] Covariates include BMI, study center, age in years, SES as 3-level categorical variable, post-menopausal estrogen supplementation, family history of BC, age at first child birth, age at second and third children birth, and current alcohol consumption in grams/week.

Table 2.3.6 #4: **Logistic Regression Models of Breast Cancer by Carotenoids (Total Bodyfat Burden)**

Independent Variable(s) in Model	N[1]	Odds Ratio (95% C. L.)
Lycopene, with covariates[2]	625	1.03 (0.67, 1.59)
beta-carotene, with covariates[2]		0.87 (0.56, 1.36)
Lycopene, with covariates[3]	603	0.98 (0.63, 1.53)
beta-carotene, with covariates[3]		0.88 (0.56, 1.36)
Lycopene, with covariates[2] and alpha-carotene	625	0.93 (0.58, 1.48)
beta-carotene, with covariates[2] and alpha-carotene		0.55 (0.25, 1.20)
Lycopene, with covariates[3] and alpha-carotene	603	0.89 (0.55, 1.44)
beta-carotene, with covariates[2] and alpha-carotene		0.61 (0.29, 1.30)

[1] N = Number of individuals in model.
[2] Covariates include BMI, study center, age in years, family history of BC, age at first child birth, and age at second and third children birth.
[3] Covariates include BMI, study center, age in years, SES as 3-level categorical variable, post-menopausal estrogen supplementation, family history of BC, age at first child birth, age at second and third children birth, and current alcohol consumption in grams/week.

2.3.7 Adjustment of logistic regression estimates for measurement error: Method development and preliminary application

The data used in this study are based on laboratory assays of adipose tissue samples. Use of this biomarker avoids many of the problems associated with estimating intake from dietary recall or record-based approaches. Nevertheless, laboratory-based methods are not error-free. The assay process is subject to measurement error: repeated readings on the same sample will differ in proportion to the variability introduced by basic instrumental error and changes in laboratory conditions.

Measurement error can be quantified through the use of quality control samples. Taking the major trans fatty acid variable (C18:1 trans 6 or 9) as an example, quality control information is available from multiple readings taken on replicate samples. The mean for all samples in the study was 2.24. The coefficient of variance calculated from the replicate samples was 0.073, indicating that the variance introduced by measurement variability is much less than the mean value of that fatty acid. The standard deviation of the mean was only 0.163, however, indicating that measurement variability was nearly half as large as the standard deviation about the mean.

Measurement error will tend to make an actual underlying difference more difficult to detect--the more uncertain one is regarding the measurement, the more uncertain one can be that the measured difference reflects the true difference. Methods to account for the effect of measurement error are well established in correlation analyses and have been developed for linear regression as well. These methods are not directly applicable to logistic regression, however--the modeling technique of choice for assessment of potential relationships between adipose tissue fatty acids and breast cancer in the current project. Two new techniques were therefore developed to estimate the impact of measurement error on logistic regression estimates.

Liang and Liu have published techniques for the adjustment of linear regression results for measurement error.[22] Dr. Larry Kupper of the Biostatistics Department at UNC-Chapel Hill developed a method by which these techniques could be adapted for logistic regression.

Two basic assumptions of this approach are as follows: 1) The coefficients of variation obtained from the repeatability study are to be treated as point estimates, or exactly known quantities. This avoids the considerable complication inherent in attempting to factor in the concept that the error estimates are themselves subject to error, which would require at minimum some kind of additional sensitivity analysis and possibly a major revision of the adjustment procedure to address. 2) The distribution of the variable to be adjusted is fairly normal, since the technique is developed out of normality-based approaches. Thus it may be necessary to normalize variables through transformation that were not normalized in the actual regression models run to date. The normality assumption is actually not critical to computation of adjusted point estimates (i.e. odds ratios), but is critical for valid hypothesis testing (i.e., statistical significance parameters such as 95% CLs or p-values).

The key information needed to estimate the potential effect of measurement error are the within and between subject components of the total variance of the measure. Variance within measurements on the same subject reflects variability associated with the assay itself, while between-subject variance reflects differences between individuals. The larger within-subject variance is relative to between-subject variance, the greater the potential for measurement error to obscure an actual association with exposure.

The most extensive relevant information on measurement error available came from a repeatability study conducted on healthy European volunteers. While these were not actual subjects in the EURAMIC study, their adipose tissue fatty acid measurements employed the same laboratory, methodology, and equipment as the EURAMIC study, during a proximal time-frame. Data came from assays on adipose tissue samples taken from a group of 56 individuals on two separate occasions, five months apart. From these data, within and between-subject components of variance were obtained through the SAS procedure VAR COMP. (The caveat here is the assumption that within-person differences are the result of typical analytical imprecision rather than a temporal effect, such as major dietary change or laboratory drift).

Some of the covariates included along with the fatty acid variable(s) in our original trans fatty acid models are probably measured with some error. Rather than assume that the error is too small to matter, or that error in the major exposure of interest is simply all that we are going to address, categorical variables that CAN be assumed to have near-zero measurement error were substituted for the covariates in question. Thus, for example, the number of cigarettes currently being smoked per day was replaced by a dichotomous "current smoker" variable. This approach strengthens the contention that all substantial measurement error in our model is addressed, since the fatty acid variable represents the only major source of such error.

For a test application of these newly developed techniques, the association between trans fatty acids and breast cancer was selected. The distribution of trans fatty acid in the breast cancer study population is significantly non-normal, even after log-transformation. To better meet the assumption of normality, the Box-Cox method in SAS's QC procedure was used to determine that the optimum power transform for this variable: 0.4. The between-person variance for trans fatty acid after application of this power transform proved far lower for the repeated measure study population than for the EURAMIC BC study population. Several factors may contribute to this: 1) the repeated measures study contains only healthy individuals, not a mixture of cases and controls; 2) the repeated measures study population was drawn from a single catchment area, not 5 centers with significantly different background trans fatty acid levels; and 3) the "optimal" normalizing transform for the pooled EURAMIC BC study population is actually a poor fit for the repeated measures study population's distribution--this may actually suppress between-person variability estimates for the repeated measures study population vs. the BC study population. Meeting the normality assumption of Liang-Liu based method thus necessitates an unconventional transform of the data from the main study, yet this transform does not fit the data from which the variability components are drawn. As many variables of potential interest are non-normally distributed, and their distributions cannot be normalized without resort to unusual transformations--which may limit generalizability of results and complicates their interpretation--

this is a major drawback.

As an alternative to the Liang/Liu based approach, development of a new method based on modified score equations was undertaken. Working with Dr. Kupper, Yue Wang, a doctoral student in the Biostatistics department at UNC-Chapel Hill, formulated an adjustment technique that does not rely on the assumption of normality. A computer program written in SAS code was developed that incorporated this new technique.

The following strategies were adopted to address adjustment for measurement error:
1) Forego transformation of the fatty acid variable and use the Wang-Kupper technique as the primary method of adjustment.
2) The most valid application of the repeated measures dataset is to estimate the within-person variance component, which would presumably not depend as greatly on between-center differences as does the between-person variance.
3) The rest of the adjustment procedures will employ the actual breast cancer study population. Populations with compatible center-specific means for trans fatty acid will be pooled for the measurement error correction.
4) The between-person variance component for purposes of measurement error correction will be calculated from the breast cancer study population, not the repeated measures population. The breast cancer study population is clearly a more appropriate source for the estimation of its own between-person variance than some external population.
5) Dichotomous current smoking and current alcohol consumption variables will be substituted for their continuous counterparts. This will minimize the potential impact of including additional variables (beyond trans fatty acid) measured with error in the covariate mix. The assumption is that smoker/nonsmoker and drinker/nonsmoker status are (more) virtually error-free measures relative to the number of cigarettes smoked per day or grams of alcohol consumed per week.
6) Odds ratios before and after adjustment for measurement will be computed using the intra-indivdual variance estimate from the repeated measure study and between-person variance estimated from the particular group of centers (see 2) above) under consideration.

Application and results of the adjustment strategy.

Variance component analysis yielded a within-subject variance for total trans fatty acid of 0.215 from the repeated measures population. For the breast cancer study population, ANOVA revealed that Coleraine and Zeist were the only centers whose trans fatty acid mean did not statistically significantly differ, based on Scheffe's test. Subsequent analyses were therefore performed using only the pooled population from Coleraine and Zeist. This insured that the within-subject variance obtained from the external population represented about the same proportion relative to the mean for each center included in the analyses. [Application of a single within-subject variance estimate to multiple populations implies that these populations share the same variance. Populations with significantly different means might also have different within-subject variances, however, so to maximize the validity of our analyses only those populations with nonsignificantly different means were included.]

The results of the measurement error adjustment techniques are compared with the unadjusted results for trans fatty acids in Table 2.3.6#1 The striking impact of adjustment underscores the

fact that, based on relevant knowledge regarding the amount of error in our biomarker-based measurements of adipose tissue trans fatty acids, the actual upper bound of the estimated effect (i.e., the exposure OR) for trans fatty acids is probably greatly underestimated by techniques that do not take such error into account.

The techniques involved in these analyses are still undergoing final testing. A method for calculating measurement-error corrected upper and lower confidence limits directly, rather than simply computing a new point estimate based on the potential effect of measurement error, is now being finalized. Several caveats also apply to interpretation of these results. First, trans fatty acids are fairly minor components of total adipose tissue fatty acid, and therefore are measured with greater error than many other components, such as total polyunsaturates or essential fatty acids of the omega-6 family. Second, the repeatability study actually assayed adipose samples taken at different times. The within-subject variance calculated from that study potentially includes variance due to actual changes in the composition of the subject's tissue between sampling times rather than strictly variance due to the assay itself, and therefore may overestimate the actual error. Third, the preliminary calculations were based only on the most-comparable pair of study centers rather than the entire study population, and thus the adjusted estimates are less stable than those derived from the entire study population would be.

As the adjustment techniques are finalized, including provision of direct computation of confidence limits, they will be reapplied to the project's fatty acid results. Of particular interest will be the computation of adjusted results for all centers pooled, since only by pooling all centers do the analyses acquire sufficiently large numbers of subjects to produce statistically significant results for the trans or the essential fatty acids. A center-by-center adjustment followed by weighted averaging of the adjusted results will be compared with a simplified approach wherein all centers are pooled and the results are adjusted as if all subjects came from a single population with shared variances.

Table 2.3.7: Comparison of Results for Models of Breast Cancer by Trans Fatty Acids According to Method of Adjustment for Measurement Error

Parameter Estimate for...		
Unadjusted Model	Liang-Liu Method	Wang-Kupper Method
0.43 ($p < 0.051$)	1.06	1.74

Models include Age, BMI, Hormone use, Current smoking status, Former smoking status, Current alcohol consumption, Socioeconomic status, and Center as covariates (all coded as dichotomous [yes vs. no] variables, except three levels [1 vs. 2 vs. 3] for low/medium/high SES).

2.3.8 Specific analyses: Dietary data

Dietary histories were obtained for most of the German women enrolled in the EURAMIC breast cancer study. Records of intake of individual food items by these women were stored in a separate dataset. In order to make the information useable for purposes of this project, a SAS dataset containing these data was created.

The resultant dataset contained 15,215 observations on a total of five 5 variables, including subject identification, food code, frequency of consumption for that food, and amount typically consumed when that food is eaten. This dataset was then checked for errors (e.g., inconsistent values, missing identifiers). Since the data consisted of intake estimates for a large number of individual food items, variables summarizing total estimated intake of key foods (e.g., allium vegetables, which includes both onion and garlic) were formulated. Food intake data are available for a total of 159 subjects, 51 of whom are cases.

A simple comparison of mean consumptions by cases and controls for selected food groups is presented in Table 2.3.8 #1. The richest dietary sources of glucosinolates are cabbages, broccoli, brusssel sprouts, and cauliflower, members of the Crucifera family. Average consumptions of five out of seven quantified glucosinolate sources (red, white, and cabbage, sauerkraut, and cauliflower) are lower in the German women with breast cancer than in their corresponding controls. The etiologic implications of these comparisons must be viewed with caution, as other risk factors have not as yet been controlled for. Their primary utility is the demonstration that a broad range of exposures exists and these exposures vary by disease status--characteristics encouraging more extensive analysis.

Currently underway is the combination of the food record data with a newly updated nutrient database obtained via the Koch Institute in Germany. Once this has been completed and checked, the food record dataset will be merged with the extensive data on adipose and plasma micronutrients and other risk factors available for these women. The relationship between reported dietary intake of carotenoids and glucosinolates with breast cancer will then be rigorously addressed, controlling for other risk factors and assessing the potential impact of differences in fatty acid intake. The relationships between nutrient measures obtained from adipose and plasma with those obtained from dietary interviews will also be compared.

Table 2.3.8: Estimated Mean Intake of Selected Glucosinolate-rich Foods for Cases and for Controls

Food	Mean intake (g/day)	
	Cases	Controls
Broccoli	21	17
Brussel Sprouts	59	45
Cabbage, red	49	59
Cabbage, savoy	16	18
Cabbage, white	27	35
Cauliflower	106	111
Sauerkraut	49	58

Primary Conclusions

The omega-3 fatty acids considered on their own appeared only mildly protective in the pooled study population. The ratio of omega-3 to omega-6 fatty acids, on the other hand, showed a statistically significant protective association with breast cancer. Further, more consistent center-specific results were seen for the ratio of docosahexaenoic acid, the major long-chain omega-3, than for the ratio of the medium-chain alpha-linolenic acid to omega-6. Taken together, this implies that 1) the balance between omega-3 and omega-6 fatty acids is more important than amounts of one or the other considered in isolation, and 2) the long-chain omega-3 s associated with fish appear more important than the omega-3s derived from land-based plant sources. As the results were only marginally statistically significant with all centers pooled and showed great variation between centers they are not conclusive. However, they lend support to the idea that altering the balance of dietary polyunsaturates in favor of omega-3 fats (perhaps through fish consumption or marine oil supplements) may provide a useful intervention to reduce the risk of breast cancer. Further investigation of this hypothesis is in order.

The powerful protective association of oleic acid with breast cancer in Malaga provides support for the hypothesis that olive oil consumption may be protective against breast cancer. The absence of this association in centers outside of Spain could also be construed to support this hypothesis insofar as that absence simply reflects the predominance of other sources of oleic acid (such as animal fats) over olive oil in the other centers. It should be borne in mind, however, that the question of whether olive oil itself or some other characteristic(s) of the traditional Mediterranean diet for which olive oil acts as a surrogate is at the heart of any observed association remains open. There was little evidence that the balance between longer and shorter chain-length monounsaturates, a potential index of endogenous synthetic activity, was related to breast cancer. Among the other monounsaturates, palmitoleic acid showed a nominally significant protective association with breast cancer with all centers combined, although the association was not consistent across centers. Pending a clear biological rationale and confirmation in another study, this finding must be classified as intriguing but speculative.
In contrast to the results for fatty acids, beta-carotene, alpha-carotene, and lycopene showed little evidence of a meaningful relationship with breast cancer. While this could be attributed to limitations of the biomarker, resulting in excessive measurement error, the available quality control data does not support this postulate. Another possibility is that the effects of these micronutrients are too small to be readily verified in a study of this size. One alternative that can-and will-be addressed by this project in subsequent analyses is the potential interaction between one or more of these carotenoids and fatty acids. Barring significant findings in these analyses, this project's results do not support an important role for these three carotenoids, singly or in concert, in breast cancer.

Plasma and adipose-tissue markers of micronutrient intake showed significant correlations when the same nutrients (e.g., beta-carotene) were measured in each tissue, particularly after adjustment for measurement error. At the same time, results for the two markers were not interchangeable. The use of both markers simultaneously could provide stronger verification of an exposure-disease relationship or yield additional insight into potential mechanisms involved

in such a relationship. Where only one marker can be employed, adipose tissue would be the best choice for long-term exposure, whereas plasma could be more useful for very recent exposure. Key factors to consider when using adipose tissue or plasma-based biomarkers include gender, age, body mass index, smoking, and drinking habits. The importance of each factor varies with the micronutrient and marker being employed. When both adipose and blood-based biomarkers are employed and compared, gender appeared to be the critical factor to take into account.

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Appendices

APPENDIX A: EURAMIC centers collaborating in the Breast Cancer Study

Finland
National Public Health Institute, Helsinki (A. Aro; J.K. Huttunen)

Germany
Institut für Sozialmedizin und Epidemiologie, Bundesgesundheitsamt, Berlin (M. Thamm; P.E. Kahl; L. Kohlmeier)

Northern Ireland
Dept. Biological & Biomedical Sciences, University of Ulster, Coleraine (C. Stewart; J.J. Strain)

Spain
Dept. of Preventive Medicine, University of Granada (M. Delgado, R. Galvez, J.M. Martin-Moreno (currently at the National School of Public Health, Madrid))

Dept.of Preventive Medicine, University of Malaga (J. Gòmez Aracena, J. Fernandez-Crehuet)

Switzerland
Institute of Social and Preventive Medicine, University of Zürich (B.C. Martin, F. Gutzwiller)

The Netherlands
TNO Toxicology and Nutrition Institute, Zeist (A. Kardinaal)
Wageningen Agricultural University (P. van 't Veer, F.J. Kok)

Appendix B: Dataset Creation Programming Logs

Annotated Creation log for PLBC0196 (the main adipose tissue composition and risk factor dataset used in Year 2 analyses) *

Selected programming code added	Comments
** A. TRANSFORMATIONS FOR SPECIFIC CENTERS* ** recoding of German data on "ever smoking";* *IF (CENTER EQ 8 AND SMOKE EQ 1) THEN EVERSMOK = 8;* ** recoding of SES values;*	Correcting coding of responses to question regarding ever-smoking in Berlin for consistency with coding from other centers: for all current smokers, this question was to be coded "inapplicable".
** for Zeist; if center=09 then do;* *IF (SES EQ 1 OR SES EQ 2) THEN SES = 1; else* *IF (SES EQ 3 OR SES EQ 4) THEN SES = 2; else* *IF (SES EQ 5) THEN SES = 3;* *end;* ** for remaining centers other than Berlin;* *else if (center < 8 | center > 8) then do;* *IF (SES EQ 1 OR SES EQ 2) THEN SES = 1; else* *IF (SES EQ 3) THEN SES = 2; else* *IF (SES EQ 4 OR SES EQ 5) THEN SES = 3; else* *end;*	Recode SES from 5 level to 3 level variable for all centers except Berlin (since SES already coded as 3 levels in Berlin in original dataset). A different recoding algorithm is used for Zeist, reflecting differences in the original SES coding criteria used in that center.
** B. Definition of variables to be used in analyses;* ** Recoding DISEASE code for CONTROLS from 2 to 0;* *IF (DISEASE EQ 2) THEN DISEASE = 0;*	Changing coding of disease status to yield a control = 0, case=1 format, for ease of analysis using SAS
** smoking;* ** correction of person with nr_cig=0 but smoke & eversmok missing (9/5/94);* *if (smoke=9 & eversmok=9 & nr_cig=0) then nr_cig=.;* *if id=7 then smoke=2; ** correction 6/12/95;*	Correction of apparently inconsistent value
** comparison never, ex- , pipe/cigar, and current cigarette smokers;* *TYP_SMOK = .;* *IF (SMOKE EQ 2 AND EVERSMOK EQ 2) THEN TYP_SMOK = 1;* *else* *IF (SMOKE EQ 2 AND EVERSMOK EQ 1) THEN TYP_SMOK = 2;* *else* *IF (SMOKE EQ 2 AND (CIGAR EQ 3 OR CIGAR EQ 4 OR PIPE EQ 3 OR PIPE EQ 4))* *THEN TYP_SMOK = 3;* *else* *IF (SMOKE EQ 1 OR SMOKE EQ 3) THEN TYP_SMOK = 4;*	Altering coding for TYP_SMOK so that current or former cigarette smokers who also currently smoke a pipe and/or cigars are not reclassified into the pipe and cigar smoker category

```
* categories for cigarette smoking;
        IF (NR_CIG EQ 88) THEN NR_CIG = .;
        CAT_CIG = .;
        IF (0 ge NR_CIG LE 5) THEN CAT_CIG = 1;
        IF (NR_CIG GT 5 AND NR_CIG LE 10) THEN CAT_CIG = 2;
else
        IF (NR_CIG GT 10 AND NR_CIG LE 20) THEN CAT_CIG = 3;
else
        IF (NR_CIG GT 20 AND NR_CIG NE 88) THEN CAT_CIG = 4;
      NEWSMOKE = TYP_SMOK;
      IF (TYP_SMOK EQ 4) THEN NEWSMOKE = CAT_CIG + 3;
```

Streamlining coding to save computational time

```
* no diabetes compared with diabetes present > 6 months;
            IF (DIAB_DUR EQ 1 OR DIAB_DUR EQ 2) THEN DIAB = 0;
            IF (DIAB_DUR EQ 3 OR DIAB_DUR EQ 4 OR
              DIAB_DUR EQ 5 OR DIAB_DUR EQ 6) THEN DIAB = 1;
```

To avoid differential misclassification, diabetes diagnosed < 6 months ago is recoded as no diabetes

```
*** Corrections to Zurich height, weight, and waist **** 6/22/95 ***;

if center=20 then do;
        if height=86 then do; height=160; weight=86; end;
else    if height=70 then do; height=163; weight=70; end;

        if waist=190 then waist=.;

end;
```

Corrections drawn from draft of 'Antioxidants & BC' manuscript

```
*** Corrections to Zeist alcohol variables **** 6/22/95 ****;
if center=9 then do;
        if id=9163 then alcohol=110; else
        if id=9562 then alcohol=30;  else
        if id=9577 then alcohol=20;  else
        if id=9581 then alcohol=55;

        if alcohol in (898,988,998,999)  then everdrnk = 1;

end;
```

Corrections supplied by Zeist in response to inquiry

```
* definition of alcohol drinkers and non-drinkers;
            DRINKNOW = .;
            IF (EVERDRNK EQ 1 OR EVERDRNK EQ 2) THEN
DRINKNOW = 0;
            IF (EVERDRNK EQ 8) THEN DRINKNOW = 1;
* exclude non-drinkers from alcohol-intake variable, to obtain variable to
* be used for DRINKNOWs only;
        if (DRINKNOW=0 & alcohol>0) then alcohol=.; *9/5/94 correction
                                    of coding;
```

New variables created for current or potential use

```
* Create variable for exogenous hormone use;
        EXOGHORM = 0;
        IF USE_OC = 1 OR HORM = 1 THEN EXOGHORM = 1;

* Revising the coding for horm to make non-use=0;
        if horm = 2 then horm=0;
```

New variables created for analysis

```
*** Correction of Benign Breast Disease for Berlin *** 6/22/95;
        if center=8 then do;
                if bbd=9 then bbd=5;
        else    if bbd=8 then bbd=4;
        end;
```

Corrections drawn from draft of 'Antioxidants & BC' manuscript

```
** Adjusting the Scaling **;
   biop_wgt=(biop_wgt/10);
   fa_tot_1=(fa_tot_1/10);
   fa_tot_i=(fa_tot_i/10);
   fa_biowt=(fa_biowt/10);
   selenium=(selenium/1000);

array recde(6) retinol gammatoc alphatoc lycopene alphacar betacar;
   do I=1 to 6;
     recde(i)=(recde(i)/100);
   end;
```

Returning data to specified units

```
** Assigning Missing and Zero ** 6/22/95 - adding .u for undetectable ***;
   if retinol = 9.88 then retinol=.u;
        else if retinol=9.99 then retinol=.;
   if gammatoc=988.88 then gammatoc=.u;
        else if gammatoc= 999.99 then gammatoc=.;
   if alphatoc=9888.88 then alphatoc=.u;
        else if alphatoc = 9999.99 then alphatoc=.;
   if lycopene =9.88 then lycopene=.u;
        else if lycopene=9.99 then lycopene=.;
   if biop_wgt=99.9 then biop_wgt=.;
   if alphacar=.98 then alphacar=.u;
        else if alphacar=.99 then alphacar=.;
   if betacar=9.88 then betacar=.u;
        else if betacar=9.99 then betacar=.;
   if selenium=9.888 then selenium=.u;
        else if selenium=9.999 then selenium=.;
   if fa_tot_1 in(9888.8,9999.9) then fa_tot_1=.;
   if fa_tot_i in(9888.8,9999.9) then fa_tot_i=.;
   if fa_biowt in(9888.8,9999.9) then fa_biowt=.;
```

Translating numerical 'missing' codes for micronutrients to missing values, adding a 'u' notation to values that are missing due to below limit of detection assay results rather than due to no sample being obtained or analyzed

Replacing codings of 'not applicable' for miscellaneous variables with "0"s where not applicable meant that the subject did not possess the characteristic in question

Recoding numerical "don't know" or "missing" codings to missing values.

```
array ZERO   disease biopsy yr_biop nails ses meth_wt
             meth_ht smoke eversmok why_stop cigar pipe caf_dcaf
             typ_cof everdrnk stop_alc startdrn
             dis_tx ch_diet diab_tx qualbiop dis_lab
             irryr;
    do over zero;
      if zero=9 then zero=.; else
           if zero=8 then zero=0;

    end;

    array zeroa sisters nr_brth; ** 6/22/95 **;
          do over zeroa;
                  if zeroa=9 then zeroa=.; ** exclusion, 9 only **;
          end;

    if startdrn=7 then startdrn=.; ** exclusion, 7 don't know **;

    array one    study tum_size nodes mother sishlth daughter
             dauhlth relhlth use_oc horm bbd ova_hist
             diab_dur special vit_c ;
    do over one;
      if one in(8,9) then one=.;
    end;

    array two    age days_sur mn_biop nr_cig cig_past age1
             max_ever age2 yr_stop coffee age_brth first_oc
             last_oc dur_oc menarche menopaus subsc
             vit_btch vit_numb FA_IS_NR irrday
             irrmnth;
    do over two;
       if two in(88,98,99) then two=.;
    end;

    array three  weight height waist hip alcohol FA_runnr humid
             sel_err;
    do over three;
       if three in (898,988,998,999) then three=.;
    end;

    array four   c14_1tr9 c18t11c6
             c182t912 c18_3n_6 c20_2n_6 c20_3n_6 c20_5n_3
             c22_0 c22_1 supc22_5 c12_0 c14_0 c14_1cis c15_0
             c16_0 c16_unkn c16_1n_7 c18_0 c18trans c18_1n_9
             c18_1n_7 c18_2n_6 c18_3n_3 c20_0 c20_1n_9
             iso_unkn c20_4n_6 c22_6n_3;
    do over four;
       if four =9888 then four=.u; *** 6/22/95 ***;
                    else if four=9999 then four=.;
       if four > .z then four=four/100;
    end;

    array five  FA_IS_wt nail_wt det_lim ;
    do over five;
       if five in(9888,9999) then five=.;
    end;

    if iricode='9999' then iricode=' ';
    if dur_horm in(99) then dur_horm=.;
```

Replacing codings of 'not applicable' for miscellaneous variables with "0"s where not applicable meant that the subject did not possess the characteristic in question Recoding numerical "don't know" or "missing" codings to missing values.

Translating numerical 'missing' codes for fatty acids to missing values, adding a 'u' notation to values that are missing due to below limit of detection assay results rather than due to no sample being obtained or analyzed, then returning data to specified units

Replacing codings of 'not applicable' for miscellaneous variables with "0"s where not applicable meant that the subject did not possess the characteristic in question Recoding numerical "don't know" or "missing" codings to missing values.

```
** Define a Keep variable **;
if biop_wgt < 15 then KEEP=0; else KEEP=1;
```

Creating a variable to identify samples with fairly low biopsy weight

```
        ********************************************
        ******   FAT Vars DEFINITION       **************
        ********************************************
* calculation of BMI and waist/hip ratio;
      IF (WAIST > .z AND HIP > .z) THEN WH_RATIO = WAIST/HIP;
                else  WH_RATIO = .;

IF (WEIGHT > .z AND HEIGHT > .z) THEN do;
      BMI = WEIGHT/((HEIGHT/100)**2);

** Creation of total body fat and lean mass variables (Formula for Women)**;

        BODY_FAT = WEIGHT -(.184 * WEIGHT + 34.5 * HEIGHT * .01 -
35.270) / .72;

        LEAN_WGT = WEIGHT - BODY_FAT;

end;
```

Creating variables for waist:hip ratio, BMI, estimated total body fat, and lean mass

```
IF BIOP_WGT > 0 THEN PERC_FAT = 100 * FA_TOT_1 / BIOP_WGT;
```

Creating variable for estimated percentage of fat in biopsy sample; variable is set to missing when biopsy weight is missing

```
************************************************************
************************************************************
**************  Hard Coding Corrections  *******************
************** to FA's and MICRONUTRIENTS  ******************
************************************************************
************************************************************

** Correction for GAMMATOC miscoded in original data BC002.DAT **;
if id = '7026' then gammatoc=100.88;

** Hard Coding FA Corrections ***;

if id='8256' then do;
     c18t11c6=0;
     c18_1n_9=47.40;
     c18_1n_7=2.16;
end;

if id='9411' then do;
     c18t11c6=0;
     c18_1n_9=43.24;
     c18_1n_7=1.67;
end;

** 6/22/95 Changes **;

if id in ('0077','0108','0146','0147','8257','8265') then do;
          c18_1n_9=c18_1n_7;
          c18_1n_7=0.00;
end;

if id = '9573' then c18_2n_6=6.53;

if id in ('8292', '1032') then do;
          biop_wgt=.;
          keep=0;
          perc_fat=.;
          do over four;
                    four=.;
          end;
end;
```

Correction of miscoded values and values based on apparently erroneous chromatographic interpretation

Setting BIOP_WGT and PERC_FAT to missing for two subjects (ID's 1032 and 8292) with invalid chromatgraphs, thus effectively excluding them from fatty acid and micronutrient analyses

```
if fa_tot_1 >= 10 & biop_wgt > 0 then do;
        if retinol =.u then retinol =0.05;
        if alphacar=.u then alphacar=0.035;
        if betacar =.u then betacar =0.0035;
        if lycopene=.u then lycopene=0.035;
        if alphatoc=.u then alphatoc=0.35;
        if gammatoc=.u then gammatoc=3.5;
end;
** end 1/12/96 **;
```

Setting micronutrient results that are missing due to 'below limit of detection' readings and were based on samples containing at least 10 mg of [estimated] fatty acid to the instrumental limit of detection for that micronutrient in a 10 mg sample

```
** modifications 1/12/96 **;
*Set zero values to limit of detection*;

IF .z < C18_3n_3 <= 0.0001 then C18_3n_3 = 0.2;
If .z < C22_6n_3 <= 0.0001  then C22_6n_3 = 0.025;
IF .z < C20_5n_3 <= 0.0001 then C20_5n_3 = 0.005;
```

Below limit of detection results for the omega-3 fatty acids were set to a value slightly below the lowest measured detectable value.

```
** end 6/22/95 **;

************************************************************
************************************************************
********** End Hardcoding for Micronutrients & FAs **********
************************************************************
************************************************************

        ***********************************************
        ****** Creating of Fatty Acid composite variables******
        ***********************************************

if biop_wgt > 0 then do;

 transfa= round(sum(of C14_1tr9, c18trans, C18t11c6, C182t912),0.01);
 satfa  = round(sum(of C12_0, C14_0, C15_0, C16_0, C18_0, C20_0,
                    C22_0),0.01);
 mufa   = round(sum(of C14_1cis, C16_1n_7, C18_1n_9, C18_1n_7,
                    C20_1n_9, C22_1),0.01);
 pufa   = round(sum(of C18_2n_6, C18_3n_6, C18_3n_3, C20_2n_6,
                    C20_3n_6, C20_4n_6, C20_5n_3, C22_6n_3),0.01);

N_3FA  = round(sum(of c18_3n_3, C20_5N_3, supc22_5, C22_6n_3),0.01);
N_6FA  = pufa - n_3fa;
```

Creation of new variables for our analyses

```
* Correction vitamins - part b: calculation fat-corrected values;
if perc_fat > 0 then do;

    ATOC_ADJ = 100 * ALPHATOC/PERC_FAT;
    BCAR_ADJ = 100 * BETACAR/PERC_FAT;
    RETI_ADJ = 100 * RETINOL /perc_fat;
    GTOC_ADJ = 100 * GAMMATOC/perc_fat;
    LYCO_ADJ = 100 * LYCOPENE/perc_fat;
    ACAR_ADJ = 100 * ALPHACAR/perc_fat;
```

Creation of micronutrient variables that express concentration in adipose tissue rather than concentration based on total tissue in the biopsy

```
if body_fat > .z then do;
    ATOC_ADF = ATOC_ADJ * BODY_FAT;
    BCAR_ADF = BCAR_ADJ * BODY_FAT;
    RETI_ADF = RETI_ADJ * BODY_fat;
    GTOC_ADF = GTOC_ADJ * BODY_fat;
    LYCO_ADF = LYCO_ADJ * BODY_fat;
    ACAR_ADF = ACAR_ADJ * BODY_fat;
```

Creation of micronutrient variables for total estimated mass of micronutrient carried in the body fat

```
ATOC_ADL = ATOC_ADF / LEAN_WGT;
    BCAR_ADL = BCAR_ADF / LEAN_WGT;
    RETI_ADL = RETI_ADF / LEAN_WGT;
    GTOC_ADL = GTOC_ADF / LEAN_WGT;
    LYCO_ADL = LYCO_ADF / LEAN_WGT;
    ACAR_ADL = ACAR_ADF / LEAN_WGT;

end;
end;
```

Creation of micronutrient variables for estimated mass of micronutrient as a fraction of total non-fat body mass

```
if body_fat > .z then do;

    TRFA_ADF = TRANSFA * BODY_FAT;
    PUFA_ADF = PUFA    * BODY_FAT;
    MUFA_ADF = MUFA    * BODY_fat;
    STFA_ADF = SATFA   * BODY_fat;
    N3FA_ADF = N_3fa   * BODY_fat;
    N6FA_ADF = N_6fa   * BODY_fat;

end;
```

Creation of fatty acid variables for total estimated mass of fatty acid carried in the body fat

```
if alphatoc > 0 then gamm_alp = gammatoc/alphatoc;
```

Creation of variable for gamma-tocopherol:alpha-tocopherol ratio

```
* Correction vitamins - part c: transformatiof of variables for analyses;
        LN_ATOC = LOG(ATOC_ADJ+1);
        LN_BCAR = LOG(BCAR_ADJ+1);
        LN_LYCO = LOG(LYCO_ADJ+1);
        LN_GTOC = LOG(GTOC_ADJ+1);
        LN_ACAR = LOG(ACAR_ADJ+1);

end;
```

Creation of natural log transformed micronutrient variables, with 1 added to values before transformation to shift values away from 0

```
* Create an EXCLUSION VARIABLE (EXCL) for ;
* regression equation of perc_fat on biop_wgt ;
      Y = 84.280 - 0.54413*BIOP_WGT; * Note: residual SD among
regression line equals 20.806;
            EXCL = 0;
            IF (PERC_FAT >= (Y + 2.5*20.806) OR PERC_FAT <= (Y -
2.5*20.806))
            THEN EXCL = 1;
* This means in practice: exclusion of fat-percentage of biopsy <30% or
>130%;
    USE =  (ALPHATOC NE . & BETACAR NE . & BELOWDET EQ 0 &
EXCL EQ 0);
```

Create variable to identify results with extreme percentages of fat based on comparison of measured sample mass with amount of fat estimated from chromatographic results.

```
        yr_born=92-age;
```

Creation of variable for year of birth

```
* Hard coding pack years to 0 if pack years is missing and # cig = 0 ***;

if packyc=. & nr_cig = 0 then packyc=0;

if typ_smok=2 then packyt  = packy; ** Ex-smokers **;
else               packyt  = packyc;

if packyt > .z then pacy_cat= input(packyt,cigyf.); else pacy_cat=.;

** Smoking variables ***;

if nr_cig=0 then cig_cat=0;
if nr_cig >0 then
cig_cat=1*(1<=nr_cig<=5)+2*(6<=nr_cig<=10)+3*(11<=nr_cig<=20)+
          4*(21<=nr_cig<=40)+5*(41<=nr_cig<=80)+6*(80<nr_cig);

*** Smoking Variables based on discussion Kohlmeier, Qaqish, Margolin,
Croghan,
*** and Simonsen;
CUR_SMOK = 0;
    IF (SMOKE EQ 1 OR (SMOKE EQ 3 and nr_cig >0)) THEN CUR_SMOK
= 1;

    if typ_smok=. then ex_smok=.; else
    if typ_smok=2 then ex_smok=1; else
                ex_smok=0;

    if cur_smok=. & ex_smok =. then ev_smok=.; else
    if ex_smok =1 | cur_smok=1 then ev_smok=1;
                    else ev_smok=0;

          if packyt > .z then do;
                    cur_pack=cur_smok*packyt;
                    ex_pack = ex_smok*packyt;
          end;

** Alcohol Variables **;

if alcohol > .z then alc_cat = input(alcohol, alcf.); else alc_cat=.;

if drinknow=1 then ev_drink=1; else
if everdrnk=2 then ev_drink=1; else
if everdrnk=. then ev_drink=.; else
if everdrnk=1 then ev_drink=0;

if drinknow=0 then cur_alc=0; else cur_alc=alcohol;

** Center variables **;

cencod1 = (center=20);
cencod2 = (center=08);
cencod3 = (center=13);
cencod4 = (center=17);
cencod5 = (center=09);
cencod6 = (center=21);
cencod7 = (center=13 | center=21);
```

Resetting 'missing' pack-year values for reported current smokers to 0 where subjects have indicated that they smoke less than 1 cigarette per day

Altering treatment of TYP_SMOK to prevent ex-smokers from being classified as piper or cigar smokers

Creating categorical variable for pack-years (current and ex-smokers combined)

Creating categorical variable for current smoking intensity

Creating 'rationalized' binary variables for current smoker and former smoker status exclusively and a binary ever-smoker variable

Creating separate pack-year variables for current smokers and for former smokers

Setting categorical alcohol consumption variable value to missing where quantitative alcohol consumption value is missing

Creating a binary variable identifying ever-drinkers (in place of separate variables for current drinkers and for not-current-but-former drinkers)

Creating quantitative current alcohol consumption variable wherein non-drinkers are assigned a value of 0 rather than set to missing

Creation of dummy variables for study center

```
*** Age_cat ****;
if age > .z then
age_cat=1*(age<50)+2*(50<=age<55)+3*(55<=age<60)+
        4*(60<=age<65)+5*(65<=age);
```

Creation of categorical age variable employing 5-year age bands up to age 65

```
*** VIT_USE variable *** 6/22/95;
if vit_c = 1 | vit_c=2 then vitc_use=1; else
if vit_c = 3          then vitc_use=0; else
if vit_c <= .z        then vitc_use=.;
```

Corrections drawn from draft of 'Antioxidants & BC' manuscript (adapted)

```
*** BBD_HIST variable *** 6/22/95;
if 1<= bbd <=4 then bbd_hist=1; else
if bbd = 5    then bbd_hist=0; else
if bbd <= .z  then bbd_hist=.;
```

Corrections drawn from draft of 'Antioxidants & BC' manuscript (adapted)

```
*** Month of Assay Variable *** 6/22/95 ***;
if 0 < vit_btch <= 7 then mn_run= 1; else
if 7 < vit_btch <=16 then mn_run= 3; else
if 16 < vit_btch <=18 then mn_run= 4; else
if     vit_btch  =19 then mn_run= 5; else
if 19 < vit_btch <=21 then mn_run= 6; else
if     vit_btch  =22 then mn_run= 7; else
if 22 < vit_btch <=29 then mn_run= 8; else
if 29 < vit_btch <=34 then mn_run= 9; else
if 34 < vit_btch <=37 then mn_run=10; else
if 37 < vit_btch <=44 then mn_run=11; else
if 44 < vit_btch <=51 then mn_run=12; else
                  mn_run=.;
```

Corrections drawn from draft of 'Antioxidants & BC' manuscript

```
*** Storage time Variable ***

6/22/95 ***;
if n(mn_run, mn_biop)<2 then storage=.; else
if mn_run >= mn_biop then storage=(mn_run-mn_biop); else
if mn_run <  mn_biop then storage=(12 + mn_run) - mn_biop;
```

Corrections drawn from draft of 'Antioxidants & BC' manuscript

```
************************
** AGE BRTH Variables **
************************;

if nr_brth=. then do;
          age_br0=.;
          age_br1=.;
          age_br2=.;
          age_br3=.;
end;

else do;

if nr_brth=0 & age_brth=. then age_br0=1; else age_br0=0;
if   0 < age_brth <= 24  then age_br1=1; else age_br1=0;
if  24 < age_brth <= 34  then age_br2=1; else age_br2=0;
if  34 < age_brth        then age_br3=1; else age_br3=0;

end;
```

Creation of dummy variables for age when first child was born, including variable for nulliparity (no child borne); dummies AGE-BR0, ..1, ..2, ..3 indicate nulliparity, age under 25, age under 35, age 35 or greater, respectively

```
** New variables Jan 12, 1996 **;

*define the strata variable to do conditional logisitic regression ;
if age > 40 then do;
if center in (8, 21) then
strat=center*100+ 3*(40<=age <55) + 5*(55<=age <65) + 7*(65<=age);
else strat=center*100+ 1*(40<=age <45) + 2*(45<=age<50) +
3*(50<=age<55) + 4*(55<=age<60)
+ 5*(60<=age<65) + 6*(65<=age<70) + 7*(70<=age);
end;
```

Creating groups of age and center-matched individuals for use in conditional logistic regression analyses.

```
******** Excess controls from Berlin marked for exclusion **;
if center not= 8 then xc_flag=0; else
if numid in
(8292, 8288, 8291, 8293, 8339, 8299, 8268, 8321, 8318, 8330, 8297, 8348,
8326, 8334, 8324, 8353, 8343,
 8270, 8331, 8335, 8349, 8317, 8358, 8290, 8357, 8257, 8284, 8269, 8355,
8253, 8356, 8400, 8282, 8336,
 8272, 8275, 8305, 8399, 8302, 8298, 8278, 8303, 8276, 8274, 8254, 8312,
8256, 8301, 8264, 8354, 8309,
 8311, 8310, 8283, 8333, 8329, 8258, 8314, 8347, 8325) then xc_flag=1;
else xc_flag=0;

** end changes Jan 12, 1996 *;
```

Creating indicator variable to facilitate exclusion of randomly selected subset of Berlin controls. The EURAMIC executive committee decided to reduce the original number of controls in Berlin to reduce the case-control imbalance in that center; the indicator variable allows easy comparison of results with and without these "extra" controls included.

Appendix C (Continued)
Creation of PLSR0895 (Serum and Adipose Tissue micronutrient and fatty acid dataset)

```
*****************************************************************
* REQUEST:        E0052                                         *
* TITLE:          Update Serum-Adipose Dataset -- Adding Vars   *
* DESCRIPTION:    Update the serum-adipose dataset PLSR0895 to  *
*                 include additional variables.                 *
* MANUSCRIPT:     SAPDASx20                                     *
* SUBSTUDY:       serum                                         *
*                                                               *
* REQUESTOR:      Simonsen                                      *
* PROGRAMMER:     Carry W. Croghan                              *
* DATE:           Oct., 1995                                    *
*---------------------------------------------------------------*
* JOBNAME:        E0052C00                                      *
* LANGUAGE:       SAS 6.10                                      *
* DESCRIPTION:    Updating Pooled Serum-Adipose dataset         *
* RELATED:        e0029, e0033, e0048                           *
* HISTORY:        E0048C02 -- CWC                               *
*                 E0052C00 -- modified                          *
*---------------------------------------------------------------*
* INPUT:          c:\euramic\pooled\sr\sasdata\LKPS002.SSD      *
*                 c:\euramic\pooled\bc\sasdata\LKPB038.SSD      *
*                 c:\epi\sasdata\(PLMI0795, PLBC0695)           *
* OUTPUT:         c:\epi\sasdata\PLSR1095                       *
*****************************************************************;
*---------------------------------------------------------------*
|  Macro Variables                                              |
*---------------------------------------------------------------*;
%let rtBC=0695;
%let rtMI=0795;
%let rt=1095;
%let infile1= sr.lkps002;
%let outfile= EP.plsr&rt;
*---------------------------------------------------------------*
|  getting correct SES from LKPB038 for Mal.                    |
*---------------------------------------------------------------*;
** This will not be needed once PLBC SES is corrected **;
data plbc&rtbc(drop = oldses);
      merge       bc.lkpb038(keep=id ses rename=(ses=oldses id=numid))
            ep.plbc&rtbc(in=k);
      by numid;
if k;
if center=21 then ses=oldses;
run;

*---------------------------------------------------------------*
|  Create Datasets                                              |
*---------------------------------------------------------------*;

proc sort data=&infile1;
      by study id;
run;

data temp(keep=id study age bcmother bcsister age_br0 age_br1 age_br2 age_br3
            cfather cmother hbp_hist ses
            c22_6n_3 c18_3n_3 c20_5n_3 mufa pufa satfa n_3fa n_6fa
            biop_wgt center perc_fat qualbiop vit_btch excl
            bmi body_fat hip waist wh_ratio lean_wgt disease
```

```
            ch_diet cur_smok ex_smok nr_cig diab
            drinknow alcohol horm use_oc vit_c cur_alc
            ALPHACAR ALPHATOC BCAR BETACAR GAMMATOC retinol
            LUT LYC  LYCOPENE VIT_A  VIT_E atoc_adj--gamm_alp

      rename=(vit_a=bvita bcar=bbcar lut=blute lyc=blyco vit_e=bvite
            retinol=reti alphacar=acar betacar=bcar lycopene=lyco
            alphatoc=atoc gammatoc=gtoc c18_3n_3=c1833 c20_5n_3=c2053
            c22_6n_3=c2263 n_3fa=n3fa n_6fa=n6fa satfa=stfa
            n6fa_adf=n6faaf n3fa_adf=n3faaf
            ATOC_ADJ=ATOCAJ BCAR_ADJ=BCARAJ RETI_ADJ=RETIAJ
            GTOC_ADJ=GTOCAJ LYCO_ADJ=LYCOAJ ACAR_ADJ=ACARAJ
            ATOC_ADF=ATOCAF BCAR_ADF=BCARAF RETI_ADF=RETIAF
            GTOC_ADF=GTOCAF LYCO_ADF=LYCOAF ACAR_ADF=ACARAF
            ATOC_ADL=ATOCAL BCAR_ADL=BCARAL RETI_ADL=RETIAL
            GTOC_ADL=GTOCAL LYCO_ADL=LYCOAL ACAR_ADL=ACARAL
            TRFA_ADF=TRFAAF PUFA_ADF=PUFAAF MUFA_ADF=MUFAAF
            STFA_ADF=STFAAF ));
length id $ 10;

      merge       plbc&rtBC(drop=id in=keep1)
            &infile1(rename=(id=numid)in=keep0)
            EP.plmi&rtMI(drop=id in=keep2);
      by study numid;

if keep0 & (keep1 or keep2);

if study = 1 then id='MI'||trim(left(put(put(numid,z4.),$4.))); else
if study = 2 then id='BC'||trim(left(put(put(numid,z4.),$4.)));

array bcvar bcmother bcsister age_br0 age_br1 age_br2 age_br3 use_oc horm;
array mivar cfather cmother hbp_hist ;

if study=1 then do;
      n3fa_adf=n_3fa*body_fat;
      n6fa_adf=n_6fa*body_fat;
      do over bcvar;          ** Setting the BC vars to 0 for MI Subjects;
            bcvar=0;
      end;
end;

else if study=2 then do;      ** Setting the MI vars to 0 for BC Subjects;
      do over mivar;
            mivar=0;
      end;
end;

run;
```

Appendix C (Continued)

Creation of QCA & QCB (Quality control datasets for evaluation of sources of variance)

```
*****************************************************************
* REQUEST:        E0038                                         *
* TITLE:          Create dataset for Variablity of Adipose &    *
*                 Serum Measures                                *
*                                                               *
* DESCRIPTION:    Create a dataset containing adipose and serum *
*                 quality control data supplied by TNO.         *
*                                                               *
* MANUSCRIPT:     QCPASx10                                      *
* SUBSTUDY:       QC                                            *
*                                                               *
* REQUESTOR:      Simonsen & Steck                              *
*                                                               *
* PROGRAMMER:     Carry W. Croghan                              *
* DATE:           May., 1995                                    *
*                                                               *
*---------------------------------------------------------------*
* JOBNAME:        E0038C00                                      *
* LANGUAGE:       SAS 6.10                                      *
*                                                               *
* DESCRIPTION:    Reading in ASCII files PKRT05.DAT and         *
*                 PKRT06.DAT.  Creating a V604 SASdataset       *
*                                                               *
* RELATED:        none                                          *
* HISTORY:        none                                          *
*---------------------------------------------------------------*
* INPUT:          PKRT05.DAT, PKRT06.DAT                        *
* OUTPUT:         E0038C00.sas                                  *
*****************************************************************;

*---------------------------------------------------------------*
|       Creating Temp05 from PKRT05.DAT                         |
*---------------------------------------------------------------*;

data temp05;
      infile in05;
      input       id $ 8-11 bcarsupl 8 avbatch 24-25 avpostn 26-27 vbiowt 28-30
            avretin 31-33 avgtoc 34-38 avatoc 39-44 avlyco 45-47
            avacar 48-49 avbcar 50-52 qualbio 53;

time=1;

***Recoding BCARSUPL ***;

      bcarsupl=bcarsupl-1;

***Recoding Missing values ***;
            if qualbio = 9 then qualbio = .;

array n2 avbatch avpostn avretin avacar;
      do over n2;
            if n2=99 then n2=.;
      else  if n2=98 then n2=.;
      end;
```

```
array n3 vbiowt avretin avlyco avbcar;
      do over n3;
            if n3=999 then n3=.;
      else  if n3=988 then n3=.;
      end;

            if avgtoc = 99999  then avgtoc = .; else
            if avgtoc = 98888  then avgtoc = .;

            if avatoc = 999999 then avatoc = .; else
            if avatoc = 988888 then avatoc = .;

*-------------------------------------------------------------*
|       Creating Temp06 from PKRT06.DAT                       |
*-------------------------------------------------------------*;

data temp06;
      infile in06;
      input       id $ 8-11 bcarsupl 8 avbatch 24-25 avpostn 26-27 vbiowt 28-30
            avretin 31-33 avgtoc 34-38 avatoc 39-44 avlyco 45-47
            avacar 48-49 avbcar 50-52 qualbio 53;

time=2;
***Recoding BCARSUPL ***;

      bcarsupl=bcarsupl-1;

***Recoding Missing values ***;

            if qualbio = 9 then qualbio = .;

array n2 avbatch avpostn avretin avacar;
      do over n2;
            if n2=99 then n2=.;
      end;

array n3 vbiowt avretin avlyco avbcar;
      do over n3;
            if n3=999 then n3=.;
      end;

            if avgtoc = 99999  then avgtoc = .;
            if avatoc = 999999 then avatoc = .;

**  JOB E0038C00 IS DONE.  **;
```

Appendix C: Variable Dictionary for the EURAMIC BC Dataset

[Unless noted as "CREATED," variables were already present in the original pooled dataset BC002.DAT.]

Center and Subject ID coding

Variable	Definition
ID	Recoded subject ID. 4 digits, first digit coding for center, other three for individual subject. (E.g., 1234 stands for center #1-Malaga, subject #234.)
BERL	Dummy code for Berlin center. 1=Berlin. CREATED
CENTER	2-digit code for center. 08=Berlin, 09=Zeist, 13=Granada, 17=Coleraine, 20=Zurich, and 21=Malaga.
CENCOD1-7	Binary code for group originating from one particular center. For example, CENCOD1=1 if Zurich, 0 if otherwise. Specific center denoted by other CENCODs: 2 = Berlin, 3 =Granada, 4=Coleraine, 5=Zeist, 6=Malaga, 7=Spain-Granada OR Malaga. Note that CENCOD7 combines CENCODs 3 and 6, so 3 and 6 should not be included in models simultaneously with 7. CREATED.
COLE	Indicator code for Coleraine center. 1=Coleraine. CREATED
DISEASE	Disease status provided by center. Recoded to 0=control, 1=case in BCMaster. Originally 1=case, 2=control.
GRAN	Indicator code for Granada center. 1=Granada. CREATED
MALA	Indicator code for Malaga center. 1=Malaga. CREATED
XC_FLAG	1 = Extra Berlin control, 0 = otherwise. CREATED
ZEIS	Indicator code for Zeist center. 1=Zeist. CREATED
ZURI	Indicator code for Zurich center. 1=Zurich. CREATED

Demographic

Variable	Definition
AGE	Age in years
AGE_CAT	5 categories, coded 1-5. 1= <50, 2=50-<55,...5=5 >=65. CREATED
SES	Socioeconomic status. 3 categories created from the original 5 category variable in BC002.DAT. 1=low, 2=medium, 3=high.
YR_BORN	Year born (92 - age). CREATED

Dimensions & bodymass

Variable	Definition
BMI	Body mass index. =WEIGHT/ [(HEIGHT/100)**2]. CREATED using Trans003.
BODY_FAT	estimated total fat burden. For men, =WEIGHT - [(0.297*WEIGHT + 19.5*HEIGHT*0.01-14.013) / 0.72]. For women, =WEIGHT - [(0.184*WEIGHT + 34.5*HEIGHT*0.01-35.270) / 0.72]. CREATED
HEIGHT	Height in cm. "888","999" values recoded as missing in BCMaster.
HIP	Hip circumference in cm
LEAN_WGT	Estimated total lean body mass. =WEIGHT - BODY_FAT. CREATED
METH_HT	Method of height ascertainment. 1=interview, 2=measurement.
METH_WT	Method of weight ascertainment. 1=interview, 2=measurement.
PERC_FAT	Percentage of fat in biopsy sample. =FA_TOT_1 / BIOP_WGT. CREATED
SUBSC	Subscapula, in mm.
WAIST	Waist circumference in cm.
WEIGHT	Weight in kg.
WH_RATIO	Waist:Hip ratio =WAIST/HIP. CREATED using Trans003.
Y	Predicted percent fat. CREATED

Personal History and Health Habits

Smoking

Variable	Definition
AGE1	Age began smoking for CURRENT SMOKERS ONLY.
AGE2	Age began smoking for EX-SMOKERS ONLY.
AGESTSM	Age stopped smoking = YR_STOP - YB. Set to 0 if EVERSMOK=2. CREATED
CIGAR	Smoke cigars? 1=no, 2=used to, 3=yes occasionally, 4=yes regularly.
CIG_PAST	Number of cigarettes smoking per day one year ago. Set to 0 if SMOKE=2 in BCMaster. CREATED
CSMOKE	1 = not current smoker, 2 = current regular smoker CREATED
CUR_PACK	Pack-years for CURRENT cigarette smokers. CUR_SMOK * PACKYT. CREATED

CUR_SMOK	Current cigarette smoker. 0=not current, 1=current. In addition to current smokers smoking "regularly" (SMOKE=1), current smokers who reported smoking only "occasionally" and at least one cigarette a day are also assigned a value of 1. CREATED using Trans003.
EV_SMOK	Ever smoker of cigarettes. 0=never smoker, 1=ever smoker. CREATED from CUR_SMOK and EX_SMOK.
EVERSMOK	Did you ever smoke? 1=yes (i.e., ex-smoker), 2=no, 8="not applicable"(i.e., current smoker).
EX_PACK	pack-years for EX-cigarette smokers. EX_SMOK * PACKYT. CREATED
EX_SMOK	Ex-smoker of cigarettes. 0=not ex-smoker, 1=ex-smoker. CREATED from TYP_SMOK.
MAXEVER	Maximum number of cigarettes per day ever smoked for EX-smokers (only). In original BC dataset a few current smokers also had values, although this data was not supposed to be obtained from them. Set to 0 if EVERSMOK=2 in BCMaster
NR_CIG	# of cigarettes CURRENTLY smoking per day. "88" recoded to missing in BCMaster. Set to 0 if SMOKE=2 in BCMaster, and set to missing for one individual with NR_CIG reported as 0 but SMOKE and EVERSMOK missing
PACKPD	Packs per day for EX-smokers = MAX_EVER/20. Set to 0 if EVERSMOK=2 CREATED
PACKPDC	Packs per day for CURRENT smokers = nr_cig/20. Set to 0 if SMOKE=2. CREATED.
PACKY	Pack-years for EX-smokers = packpd*ysm. CREATED
PACKYC	Pack-years for CURRENT smokers = packpdc*ysmc. Set to 0 if SMOKE=2. Also set to 0 if PACKYC missing in original dataset but NR_CIG = 0. Created in BCMaster.
PACY_CAT	Categorical pack-year variable. 0=0, 1=1-10, 2=11-20, 3=21-40, 4=>40. Created in BCMaster.
PACKYT	Pack-years for ALL cigarette smokers. Encompasses PACKY and PACKYC, as available and appropriate. CREATED.
PIPE	Smoke a pipe? 1=no, 2=used to, 3=yes occasionally, 4=yes regularly.
SMOKE	Smoke cigarettes now? 1=yes,regularly, 2=no, 3=occasionally.

TYP_SMOK	Smoker type. 1=never smoker, 2=ex-smoker, 3=current pipe &/or cigar, 4=current cigarette smoker ("regularly" OR "occasionally"). CREATED from SMOKE and EVERSMOK using Trans003 transforms.
WHY_STOP	Why did you stop smoking? 1=health, 2=other, 8=not applicable. CREATED using Trans003.
YEXSM	Years being an ex-smoker =age-agestsm. Set to 0 if EVERSMOK=2. CREATED
YR_STOP	Year stopped smoking (for EX-SMOKERS).
YSM	Years smoked for EX-smokers =agestsm-age2 Set to 0 if EVERSMOK=2. CREATED
YSMC	Years smoked for CURRENT smokers =age-age1. Set to 0 if SMOKE=2. CREATED

Alcohol consumption

Variable	Definition
ALCOHOL	Current alcohol intake in grams/wk. "0" recoded to missing using Trans003. Also, intakes above 0 for reported current non-drinkers (DRINKNOW=0), or were recoded to missing [we assume that former drinking was being reported in these individuals]. Consumption for reported non-drinkers set to 0 rather than missing in BC dataset. CREATED
ALC_CAT	4 category current alcohol consumption variable, gms/week. 0=0, 1=1-50, 2=51-150, 2=>150. CREATED
CUR_ALC	Current alcohol consumption (g/wk) CREATED from original ALCOHOL and DRINKNOW.
DRINKNOW	Current alcohol drinker. 0=not current drinker, 1=current drinker. (DRINKNOW = 0 if EVERDRNK=1 or 2, = 1 if EVERDRNK=8). CREATED
EVERDRNK	Ex-alcohol drinker. 1="never drinking", 2="stopped drinking", 8="not applicable" from original questionnaire (note that current drinkers were coded "8"). Variable was used to create EV_DRINK in BCMaster.
EV_DRINK	Ever an alcohol drinker. 0=never drinker, 1=ever drinker. (EV_DRINK = 1 if DRINKNOW=1 or EVERDRNK=2, = 0 if EVERDRNK=1, = . if VERDRNK=.). CREATED

STARTDRN	Ordinal categorical variable for how many years ago current alcohol drinkers began drinking. From BC002, but values of 7 ("don't know") recoded to missing.
STOP_ALC	Reason quit drinking. 1=health, 2=other reasons, 8=not applicable (i.e., still drinking or never drank alcohol). CREATED using Trans003.

Other dietary factors

Variable	Definition
CAF_DCAF	Is coffee usually consumed caffeinated? 1=caffeinated, 2=decaffeinated, 3=caf, decaf equally, 4=don't know, 8=not applicable.
CH_DIET	Changed dietary habits (within past year) 1=no, 2=yes, definitely, 3=yes, moderately, 4=don't know, 8=not applicable.
COFFEE	Number of cups of coffee currently consumed per day
TYP_COF	Type of coffee usually consumed. 1=boiled, 2=filtered, 3=instant, 4=don't know, 8=not applicable.
VIT_C	Take vitamin C tablets 1=yes, regularly, 2=yes, occasionally, 3=no, 8=don't know.
VITC_USE	Vitamin C supplement usage: 1=yes, 0=no. CREATED from VIT_C: if VIT_C = 1 or 2, then VITC_USE = 1; if VIT_C = 3, then VITC_USE = 0; if VIT_C = 8 or missing, then VITC_USE = missing.

Hormonal status & parity

Variable	Definition
AGE_BR0	1 = No children, 0 = Children. CREATED
AGE_BR1	1 = First child before age 25, 0 = not. CREATED
AGE_BR2	1 = First child between age 25-34, 0 = not. CREATED
AGE_BR3	1 = First child after age 34, 0 = not. CREATED
AGE_BRTH	Age at first birth.
DAUGHTER	Number of daughters.
DUR_HORM	Duration of estrogen therapy (months).
DUR_OC	Duration of oral contraceptive use (yrs).
EXOGHORM	Receiving exogenous hormones through estrogen replacement therapy or oral contraception. 0=no, 1=yes. Based on HORM and USE_OC. CREATED.

FIRST_OC	Age at first use of oral contraceptive.
HORM	Estrogen replacement.
LAST_OC	Age at last use of oral contraceptive..
MENARCHE	Age at menarche.
MENOPAUS	Age at menopause.
NR_BRTH	Number of births.
USE_OC	Oral contraceptive use.

Health history and current status

Variable	Definition
BBD	Previous benign breast disease.
BBD_HIST	History of benign breast disease: 0=No, 1=Yes. CREATED from BBD: if BBD = 1-4, then BBD_HIST = 1; if BBD = 5, then BBD_HIST = 0; if BBD = 8 or missing, then BBD_HIST = missing.
BCDAUGHT	History of breast cancer in subject's daughter(s). 0=No, 1=Yes. CREATED from original DAUHLTH variable in BC002.DAT. DAUHLTH values of 8 or 9 ("don't know" or "missing") set to missing in BCDAUGHT.
BCFAMILY	History of breast cancer in immediate family [mother, sister(s), daughter(s)]. CREATED from BCMOTHER, BCSISTER, and BCDAUGHT
BCMOTHER	History of breast cancer in subject's mother. 0=No, 1=Yes. CREATED from MOTHER, Ans' recode of original MOTHHLTH variable in BC002.DAT. MOTHER values of 8 or 9 ("don't know" or "missing") set to missing in BCMOTHER.
BCSISTER	History of breast cancer in subject's sister(s). 0=No, 1=Yes. CREATED from original SISHLTH variable in BC002.DAT. SISHLTH values of 8 or 9 ("don't know" or "missing") set to missing in BCSISTER.
DAUHLTH	Number of daughters with breast cancer.
DAYS_SUR	Days after surgery.
DIAB	Current diabetes, diagnosed at least 6 months ago. 0=no, 1=yes. CREATED from DIAB_DUR and DIAB_TX based on Trans003 suggestions.
DIAB_DUR	Duration of diabetes. 1=no diabetes, 2=dx< mo ago, 3=dx6-12 mo ago, 4=dx 1-2 yrs ago, 5=dx 2-5 yrs ago, 6=dx>5 yrs ago, 8=don't know.
DIAB_TX	Diabetes treatment?. 1=yes, only diet, 2=yes, diet and tablets, 3=yes, diet and insulin, 8=not applicable, no diabetes.
DISEASE	Disease status provided by center. Recoded to 0=control, 1=case in BCMaster. Originally 1=case, 2=control.
DIS_TX	Disease treated (BY SPECIALIST) 1=CVD, 2=GI, 3=lung, 4=kidney, 5=other, 7=don't know, 8=not applicable.
MOTHER	Mother's health.

NODES	Regional lymph node involvement. Regional lymph node involvement. 0=N0 (none involved), 1=N1 (involvement of at least one movable ipsilateral axillary node) , 2=N2 (involvement of ipsilateral node that is fixed to one another or to other structures). Controls originally coded as "8" for "not applicable"; recoded to 0 rather than missing in our PLBC dataset to prevent loss of subjects when controlling for node involvement and to preclude inclusion of cases with missing nodal information (as were all from Zeist) with controls. BC only.
OVA_HIST	Previous surgery.
RELHLTH	Other relatives with breast cancer.
SISHLTH	Number of sisters with breast cancer.
SISTERS	Number of sisters.
SPECIAL	Visit a specialist? (for DIS_TX?) 1=yes, 2=no.
TUM_SIZE	Tumor size. 0=in situ, 1=T1 (<2cm), 2=T2 (2-5cm), 3=T3 (>5cm), 4=T4 (Tumor of any size with direct extension to the chest wall or skin). All controls recoded from "8" (for "not applicable") to missing for this variable--unlike treatment of nodes, since TIS is not equivalent to absence of tumor. BC only.

Specimen and sampling characteristics

General

Variable	Definition
BELOWDET	A-toc, B-car, or Se sample assay reading below the limit of detection. 0=not below detection, 1=a-toc and/or B-car below detection, 2=selenium below detection CREATED.
BIOPSY	Adipose tissue biopsy available? 1=yes, 2=informed consent not granted, 3=unsuccessful biopsy attempt, 4=no biopsy, but other data available.
BIOP_WGT	Adipose tissue biopsy weight, in mg*10. 3 digit code
DIS_LAB	Disease status as coded in lab. 0=control, 1=case.

EXCL	Exclude extreme PERC_FAT values. For men, 1= PERC_FAT > (Y+2.5*20.208) or < (Y-2.5*20.208), where Y=78.301-(0.38522*BIOP_WGT), otherwise EXCL=0. For women, 1= PERC_FAT > (Y+2.5*20.806) or < (Y-2.5*20.806), where Y=84.280-(0.54423*BIOP_WGT), otherwise EXCL=0. CREATED based on BCREAD02.ME.
FA_BIOWT	Biopsy weight (mg) by TNO.
FA_IS_NR	Standard batch number.
FA_IS_WT	Amount of IS (gm/biopsy).
FA_RUNNR	Analysis batch.
FA_TOT_1	Sum of all individual fatty acids measured in sample, expressed as mg*100. Based on area under chromatograph peaks with assumption that all fatty acids have the same response factor (i.e., 1). Used in calculation of PERC_FAT, which is in turn used to calculate antioxidant values adjusted for % of fat in samples (e.g., ATOC_ADJ).
FA_TOT_I	Fatty acid biopsy ("true" response factor).
KEEP	Binary code for subjects with at least 15mg sample. 1=FA_biowt >= 15. CREATED.
MN_BIOP	Month of biopsy.
QUALBIOP	Quality of biopsy. 1=clean, 2=a little blood, 3=much blood, 4=other contamination
USE	Variable for exclusion of individuals with missing or below detection a-toc and/or B-car values OR extreme PERC_FAT values according to EXCL algorithm. 1= Neither ALPHATOC nor BETACAR missing, BELOWDET = 0, and EXCL = 0. CREATED
VIT_BTCH	Vitamin assay batch. 2 digit code.
VIT_NUMB	Vitamin assay serial number.
YR_BIOP	Year of biopsy.

Center

Variable	Definition
CENTER	Center--original coding for centers 1-6.
CENCOD1-7	Binary code for group originating from one particular center. See Center and Subject ID coding.

Fatty acids

Individual

Variable	Definition
C12_0	C12:0
C14_0	C14:0
C14_1CIS	C14:1cis
C14_1TR9	C14:1 trans 9
C15_0	C15:0
C16_0	C16:0
C16_1N_7	C:16:1 w-7
C16_UNKN	C16, type unknown
C182T912	C18:2 trans 9 & 12
C18T11C6	C18:1 trans 11 & cis 6
C18TRANS	C18:1 trans 6 & 9
C18_0	C18:0
C18_1N_7	C18:1 n-7
C18_1N_9	C18:1 n-9
C18_2N_6	C18:2 n-6
C18_3N_3	C18:3 n-3
C18_3N_6	C18:3 n-6
C20_0	C20:0
C20_1N_9	C20:1 cis 11 (n-9)
C20_2N_6	C20:2 n-6
C20_3N_6	C20:3 n-6
C20_4N_6	C20:4 n-6
C20_5N_3	Eicosapentaenoic acid, C20:5 n-3
C22_0	C22:0
C22_1	C22:1
C22_6N_3	Decosahexaenoic acid, C22:6 n-3

Trans Fatty Acids

Variable	Definition
TRANSFA	Total trans FA as a percentage of total FA in sample. For BC, sum of C14_1TR9, C182T912, C18T11C6, and C18TRANS. CREATED
TRFA_ADF	Trans fatty acid, total burden bodyfat. CREATED

Monounsaturated

Variable	Definition
MUFA	Total monounsaturated FA as a percentage of total FA in sample.
MUFA_ADF	Monounsaturated FA, total burden bodyfat. CREATED

Polyunsaturated

Variable	Definition
N_3FA	Omega-3 fatty acid as a percentage of total FA in sample. For BC=Sum of C18_3N_3, C20_5N_3, supC22_5, and C22_6N_3. For MI=Sum of C18_3N_3, C20_5N_3, C22_5N_3, and C22_6N_3. (Note supC22_5 vs. C22_5N_3 for BC vs. MI.) CREATED
N3FA_ADF	Omega-3 FA, total burden bodyfat. CREATED
N_6FA	Omega-6 fatty acid as a percentage of total FA in sample. =PUFA - N_3FA. CREATED
N6FA_ADF	Omega-6 FA, total burden bodyfat. CREATED
PUFA	Total polyunsaturated FA as a percentage of total FA in sample. CREATED
PUFA_ADF	Polyunsaturated FA, total burden bodyfat. CREATED

Saturated

Variable	Definition
SATFA	Total saturated FA as a percentage of total FA in sample. CREATED
STFA_ADF	Saturated FA, total burden bodyfat. CREATED.

Selected ratio variables

RAT36	(N_3FA / N_6FA) x 100 CREATED
RATALA6	(C18_3N_3 / N_6FA) x 50 CREATED
RATDHA6	(C22_6N_3 / N_6FA) x 100 CREATED
RATLO3	(LONGN_3 / N_6FA) x 100 CREATED

Antioxidants

Carotenoids

Variable	Definition

ALPHACAR	Alpha-carotene, ug/g*100 in total tissue sample. Note that this measure does not address differences in fat content of tissue samples. (See _ADJ version of variable.)
ACAR_ADF	Alpha-carotene in total adipose pool =ACAR_ADJ*BODY_FAT. CREATED
ACAR_ADJ	A-carotene, ug/g*100 in adipose tissue=ALPHACAR / PERC_FAT. CREATED
BETACAR	Beta-carotene, ug/g*100 in total tissue sample. Note that this measure does not address differences in fat content of tissue samples. (See _ADJ version of variable.)
BCAR_ADF	B carotene, total adipose pool =BCAR_ADJ*BODY_FAT. CREATED
BCAR_ADJ	B-carotene, ug/g*100 in adipose tissue=BETACAR / PERC_FAT. CREATED
LN_ACAR	Natural log ACAR_ADJ. CREATED
LN_BCAR	Natural log BCAR_ADJ. CREATED
LN_LYCO	Natural log transform of LYCO_ADJ. CREATED
LYCOPENE	Lycopene, ug/g*100 in total tissue sample. Note that this measure does not address differences in fat content of tissue samples. (See _ADJ version of variable.)
LYCO_ADF	Lycopene, total adipose pool =LYCO_ADJ*BODY_FAT. CREATED
LYCO_ADJ	Lycopene, ug/g*100 in adipose tissue =LYCOPENE / PERC_FAT. CREATED

Tocopherols

Variable	Definition
ALPHATOC	A-tocopherol, ug/g*100 in total tissue sample. Note that this measure does not address differences in fat content of tissue samples. (See _ADJ version of variable, ATOC_ADJ.)
ATOC_ADF	A-tocopherol, total adipose pool =ATOC_ADJ*BODY_FAT. CREATED
ATOC_ADJ	A-tocopherol, ug/g*100 in adipose tissue=ALPHATOC / PERC_FAT. CREATED
GAMMATOC	G-tocopherol, ug/g*100 in total tissue sample. Note that this measure does not address differences in fat content of tissue samples. (See _ADJ version of variable, GTOC_ADJ.)
GTOC_ADF	G-tocopherol, total adipose pool =GTOC_ADJ*BODY_FAT. CREATED

GTOC_ADJ	G-tocopherol, ug/g*100 in adipose tissue =GAMMATOC / PERC_FAT. CREATED
GAMM_ALP	Ratio of g- to a-tocopherol = GAMMATOC / ALPHATOC. CREATED
LN_ATOC	Natural log transform of ATOC_ADJ. CREATED
LN_GTOC	Natural log transform of GTOC_ADJ. CREATED

Others

Variable	Definition
RETINOL	Retinol, ug/g*100 in total tissue sample. Note that this measure does not address differences in fat content of tissue samples. (See _ADJ version of variable.)
RETI_ADF	Retinol, total adipose pool =LYCO_ADJ*BODY_FAT. CREATED
RETI_ADJ	Retinol, ug/g*100 in adipose tissue=RETINOL / PERC_FAT. CREATED

Abbreviations

BCMaster BC master dataset PLBC0695

CREATED Signifies that the variable is a new variable based on original data from BC002.DAT but created specifically for the current project.

E0016C00 Indicates computing request #16 from which the BCMaster file was created

FA Fatty acid

Trans003 TRANS003.TXT: a file presenting the EURAMIC coordinating center's recommended transformations of data.

Examples of Standard Naming Conventions Used for Variables Created for BC Analyses Only (not in permanent dataset):

Variable	Definition
MUFA25...MUFA75	25th, 50th & 75th percentiles of MUFA. CREATED
PUFA25...PUFA75	25th, 50th & 75th percentiles of PUFA. CREATED
QMUFA1...QMUFA4	1st...4th quartiles of monounsaturated FA. CREATED
QPUFA1...QPUFA4	1st...4th quartiles of polyunsaturated FA. CREATED
SMUFA	Scaled MUFA--MUFA unmodified. CREATED from E0016C00.
SPUFA	Scaled PUFA--PUFA unmodified. CREATED from E0016C00.
NN_3FA	Log-normalized N_3FA CREATED

PERN_3	Percentage of PUFA in omega-3 form. = 100*N_3FA/PUFA. CREATED

Appendix D: FATTY ACID NOMENCLATURE

The fatty acids addressed in this project are generally identified by means of a shorthand representation of their chemical structure, such as C18:2 ω-9 or C14:1tr9. The "C" prefix refers to the carbon chain around which fatty acids are built. The first number following the "C" identifies the length of the fatty acid in terms of the total number of carbons in its chain (e.g., C18). The fatty acid's degree of unsaturation is supplied by the number following the colon: a 0 for saturated fatty acids and a 1 or 2 for monounsaturated and polyunsaturated fatty acids, respectively (e.g., :2). In addition, some unsaturated fatty acids are separated into groups, or "families", based upon the distance (in carbons) from the terminal end of the fatty acid to the nearest unsaturated bond (e.g., ω-9). This number is also commonly referred to as the "omega" number, hence the designations omega-3 and omega-6 are often used in reference to these fatty acid families. Finally, a few unsaturated fatty acids are classified based on the positioning of side groups, which is represented by the notation "tr" (for trans) or "cis" accompanied by the location of the group on the carbon chain (e.g., tr9).

Occasionally names are used in place of structural nomenclature. These names and their corresponding structures are as follows:
Arachidonic acid = C20:4 ω-6
Docosahexaenoic acid = DHA = C22:6 ω-3
Eicosapentaenoic acid = EPA = C20:5 ω-3
Linoleic acid = LA = C18:2 ω-6
Oleic acid = C18:1 ω-9

Finally, some families or groups of fatty acids are also represented by summary terms. Summary terms employed in this project are:
MUFA = Monounsaturated fatty acid = sum of all ":1" fatty acids listed in Table 2.3.1 #1
PUFA = Polyunsaturated fatty acid = sum of all fatty acids with ":2" or greater in Table 2.3.1 #1
Total Trans = Trans fatty acids= sum of all fatty acids with the "tr" identifier in Table 2.3.1 #1
Total Saturated = Saturated fatty acid = sum of all ":0" fatty acids in Table 2.3.1 #1
ω-3 = Total omega-3 = Sum of all "ω-3" fatty acids in Table 2.3.1 #1
ω-6 = Total omega-6 = Sum of all "ω-6" fatty acids in Table 2.3.1 #1

Appendix E: Sample computing request

Request number E | | | | |

ADMINISTRATIVE USE ONLY

Computing Request

Request #: 68

Title: Lycopene and breast cancer

Priority: 2

Date: 3/14-15/96

Deadline: None

Requester: Simonsen/Kohlmeier

Basic Description: Explore the association between lycopene and breast cancer in the EURAMIC dataset, including the potential impact of other carotenoids as well as addressing other potential confounders.

Project Code: BCPDAxx5DR

Related Requests: 41, 56, 63, 65

ADMINISTRATIVE USE ONLY

Programmer:

Date Assigned:

Date Completed:

Specific Description / Instructions:

Dataset: PLBC0196

Inclusions/Exclusions: Exclude Granada, exclude ages <40, exclude subjects with EXCL=1, exclude "extra" Berlin controls (i.e., subjects with XC_FLAG =1)

Specifications:

1) Perform center-specific unconditional logistic regressions for the following models:

a) LYCO_ADJ
b) LYCO_ADJ + covariates
c) LYCO_ADJ ACAR_ADJ BCAR_ADJ
d) LYCO_ADJ ACAR_ADJ BCAR_ADJ + covariates

"Covariates" = AGE BMI SES HORM NR_CIG EX_SMOK CUR_ALC BCFAMILY *except* for Malaga, where EX_SMOK should not be included due to inadequate numbers.

2) For these analyses, combine AGE_BR2 & 3 into a single dummy variable (to prevent inadequate numbers in category). Then run model as in 1b) by center except adding AGE_BR dummy variables.

Variables: Same as req 65, i.e.,

All basic fatty acid variables (i.e., variables beginning with C.._...)

plus NN_3FA N_6FA N_DHA NLONG_N3 {see Request 56 for creation of these N... variables}

C141tCAT C221CAT {see Request 63 for creation of ...CAT variables}

Antioxidant variables: ALPHACAR BETACAR LYCOPENE RETINOL ALPHATOC GAMMATOC TCAR_ADJ ACAR_ADJ BCAR_ADJ LYCO_ADJ RETI_ADJ ATOC_ADJ GTOC_ADJ ACAR_ADF BCAR_ADF LYCO_ADF RETI_ADF ATOC_ADF GTOC_ADF

Other variables: AGE BMI BCFAMILY AGE_BR0,1,2,3 NR_CIG EX_SMOK WAIST HIP HEIGHT SES HORM DIAB CUR_ALC VIT_C USE_OC MENARCHE MENOPAUSE TUM_SIZE NODES BELOWDET QUALBIOP VIT_BTCH BIOP_WGHT BODY_FAT PERC_FAT EXCL FA_TOT_1 CENTER DISEASE

Analyses: Unconditional logistic regression

Appendix F: Summary of computing requests and associated programs

Listing of Computer Requests by Area of Inquiry - Breast Cancer
--including number of programs per request--
For period (Start - June 1995)

Request Number	Request Date	Requestor	Request Title	Priority	Date Assigned	Date Completed	COM1	Number of Programs
E0001	17MAY94	KOHLMEIER	BC DATA MERGING CORRECTIONS	2	17MAY94	15JUN94		8
E0002	17JUN94	HASTINGS	TRANSFA, MUFA, PUFA, & BC	2	09JUN94	01OCT94		27
E0003	17JUN94	HASTINGS	RERUN TRANSFA AND MUFA WITH QUINTILES	2	06JUL94	10JUL94		2
E0004	27JUN94	HASTINGS	ANOTHER RERUN OF TRANS FATTY ACID WITH Q	2	07JUL94	30AUG94		4
E0005	27JUN94	HASTINGS	CREATE 4 NEW VARS. AND REDO CONT. & QUIN	2	08JUL94	10JUL94		2
E0016	29SEP94	SIMONSEN	LOG REG W&W/O COVARIATES FOR TRANSFA & B	2	30SEP94	04JAN95		57
E0019	01NOV94	SIMONSEN	DIST OF CASES AND CONTROL BY CENTER, AGE	2	08DEC94	26JAN95		1
E0020	01NOV94	SIMONSEN	INDIVIDUAL TRANS LOG. REG.	2	04NOV94	11JAN95		18
E0023	15DEC94	SIMONSEN	LOG. REG. MODELS ADF FA QUINTILE & CONT.	2	08DEC94	26JAN95		6
E0027	11JAN95	CROGHAN/SIMONSEN	RECREATION OF BASIC BC DATASET WITH CORR	2	05JAN95	15FEB95		8
E0028	20JAN95	CROGHAN/SIMONSEN	GENERATION OF RESULTS AND TABLES FOR TRA	1	16FEB95	26MAY95		58
E0032	23FEB95	SIMONSEN	RESULTS FOR TRANS FAS & BC RISK PAPER	1	03MAR95	26MAY95		18
E0034	04APR95	SIMONSEN	14 AND 18 C SAT AND MONOUNSAT FA MODELS	2	04APR95	26JUL95		5
E0035	12APR95	SIMONSEN	OMEGA-3 FA AND BC: DESCRIPTIVE STATS	2	12APR95	26JUL95		1
E0036	12APR95	SIMONSEN	UPDATE PLBC DATASET FA CORRECTIONS	2	26APR95	28APR95		1
E0037	25APR95	SIMONSEN	CONDITIONAL REG: TRANS BC	2	26APR95	26JUL95		5
E0039	01MAY95	CROGHAN	FINAL DATA VERIFICATION TRANS & BC	1	01MAY95	03MAY95		0
E0043	31MAY95	SIMONSEN	MODELS OF BC BY OMEGA 3 AND 6 FA	2	31MAY95	19JUL95		2
E0044	08JUN95	SIMONSEN	BC POOLED CORRECTIONS JUNE 95	2	08JUN95	04AUG95		8
E0045	13JUN95	SIMONSEN	MORE TRANS-BC FUN:REANALYSES	1	16JUN95	17AUG95		16
E0047	23JUN95	SIMONSEN	TRANS-BC REANALYSES:EXCL & UPDATED DATA	2	12JUL95	17AUG95		0

PROJECT								247

N = 21

Request Number	Request Date	Requestor	Request Title	Priority	Date Assigned	Date Completed	COM1	Number of Programs
E0038	28APR95	SIMONSEN/STECK	DATASET CREATION FOR VARIABILITY OF ADIP	1	28APR95	26JUL95		4
E0040	05MAY95	STECK	ADIPOSE & SERUM VIT PLOTS OF DIFF VS MEA	1	05MAY95	26JUL95		11
PROJECT								15

N = 2

Listing of Computer Requests by Area of Inquiry - Serum Data
--including number of programs per request--
For period (Start - June 1995)

Request Number	Request Date	Requestor	Request Title	Priority	Date Assigned	Date Completed	COM1	Number of Programs
E0029	25JAN95	SIMONSEN	SERUM ANTIOXIDANT DATASET: DSCPT STAT &	2	12JAN95	26MAY95		9
E0033	31MAR95	SIMONSEN	SERUM V. ADIPOSE ANTIX ANALYSES (CLEANED	2	02APR95	26JUL95		0
PROJECT								9
Fiscal Year								271

N = 2

Listing of Computer Requests by Area of Inquiry - Breast Cancer
--including number of programs per request--
For period (July 1995 - July 1996)

Request Number	Request Date	Requestor	Request Title	Priority	Date Assigned	Date Completed	COM1	Number of Programs
E0046	06JUL95	STECK	BC & FA DESCRIPTIVE STATS	2	12JUL95	17AUG95		3
E0049	14JUL95	SIMONSEN	OMEGA-3 VS BC WITH UPDATED DATASET	2	19JUL95	22NOV95		6
E0053	10OCT95	SIMONSEN	MUFA AS MODIFIERS OF BC RISK	2	10OCT95	01MAY96		.
E0056	17NOV95	SIMONSEN	OMEGA ANAYSES II: NEW & IMPROVED!!	1	22NOV95	17JAN96		16
E0060	11JAN96	CROGHAN	JAN 96 UPDATE OF POOLED BC DATASET	2	11JAN96	24JAN96		3
E0061	18JAN96	SIMONSEN	OMEGA-3 BC MANUSCRIPT ANALYSES	1	19JAN96	29MAR96		18
E0063	31JAN96	SIMONSEN	MONOUNSATURATES: RELATIONSHIPS W/ OTHER	2	01FEB96	On going		.
E0065	08MAR96	SIMONSEN	CAROTENOID-FATTY ACID INTERACTIONS	2	08MAR96	03APR96		5
E0067	14MAR96	CROGHAN/KOHLMEIER	DISTRIBUTION OF ACLOHOL VARIABLES IN BC	1	14MAR96	03APR96		1
E0068	15MAR96	SIMONSEN/KOHLMEIER	LYCOPENE AND BREAST CANCER	1	15MAR96	On going		.

E0070	02APR96	SIMONSEN	DATA VERIFICATION FOR OMEGA-3 BC MANUSCR	1	03APR96	On going		.
E0071	04APR96	SIMONSEN	MEASUREMENT ERROR CORRECTION IN LOG REG:	2	12APR96	On going		.
E0073	06JUN96	SIMONSEN	POTENTIAL FATTY ACID-MICRONUTRIENT INTER	1	06JUN96	On going		.
E0074	03MAY96	SIMONSEN	OMEGA 3 UPDATED ANALYSIS	2	31MAY96	On going	NEED HARD COPY	.
PROJECT								52

N = 14

Listing of Computer Requests by Area of Inquiry - Dietary Data
--including number of programs per request--
For period (July 1995 - July 1996)

Request Number	Request Date	Requestor	Request Title	Priority	Date Assigned	Date Completed	COM1	Number of Programs
E0072	25APR96	SIMONSEN	CREATION OF DIETARY DATASETS FOR BERLIN	2	01MAY96	On going		.

N = 1

Listing of Computer Requests by Area of Inquiry - Quality Control
--including number of programs per request--
For period (July 1995 - July 1996)

Number

Request Number	Request Date	Requestor	Request Title	Priority	Date Assigned	Date Completed	COM1	of Programs
E0054	02NOV95	CROGHAN	REPEATABLITY DATA UPDATE W/ NEW DATA	2	02NOV95	17JAN96		6
E0055	02NOV95	SIMONSEN	SOURCES OF VARIABILITY: FATTY ACIDS	2	02NOV95	01MAY96		.
E0058	01DEC95	SU	SERUM & ADIPOSE MICRONUT RELATIONSHIPS	1	08DEC95	22MAR96		18
PROJECT								24

N = 3

Listing of Computer Requests by Area of Inquiry - Serum Data
--including number of programs per request--
For period (July 1995 - July 1996)

Number Request Number	Request Date	Requestor	Request Title	Priority	Date Assigned	Date Completed	COM1	of Programs
E0048	06JUL95	STECK	SERUM/ADIPOSE CORRECTIONS	2	12JUL95	17AUG95		30
E0050	16AUG95	HASTINGS	SERUM DATASET FOR EPI 269	2	17AUG95	17AUG95		1
E0051	17AUG95	SIMONSEN	SERUM V. ADIPOSE MICRONUTRIENTS	2	17AUG95	14MAR96	RE-OPENED	28
E0052	28SEP95	SIMONSEN	UPDATE SERUM-ADIPOSE DATASET	1	29SEP95	17JAN96		2
E0059	14DEC95	SU	SERUM ADIPOSE LOGISTIC REGRESSION	2	15DEC95	10APR96		7
E0062	24JAN96	SU	SER-ADI MICRONUT. DATA VERIFICATION	2	31JAN96	21FEB96		0
E0069	20MAR96	SU	SERUM-ADIPOSE MICRONUT CORR MANUSCRIPT	2	20MAR96	On going		.
E0075	17JUN96	SU	DATA VERIFICATION ON CRUCIFERAE META	2	03JUL96	On going		.
PROJECT								68
Fiscal Year								144

N = 8
Total N = 51

24JUL96

Appendix G: Abstracts Presented

The association between omega-6 fatty acids and breast cancer in a population of European women. Simonsen, N.; Strain, J.; van 't Veer, P., Kardinaal, A.; Fern' andez-Crehuet, J.; Huttunen, J.; Martin-Moreno, J.; Martin, B.; Thamm, M.; Kok, F; Kohlmeier, L. UNC-CH Sch. Publ. Health, Chapel Hill, NC, 27599, USA; Coleraine, N. Ireland; Zeist, Netherlands; Malaga, Spain; Helsinki, Finland; Madrid, Spain; Zurich, Switzerland; Berlin, Germany; Wageningen, Netherlands; UNC-CH, USA.

Laboratory reports that increasing amounts of polyunsaturated fatty acids (PUFA) of the omega-6 family (ω6) in culture or feed enhance mammary tumor activity have few parallels in studies of human populations. We found an unfavorable association of adipose ω6 PUFA with breast cancer in a population of postmenopausal European women recruited from 5 centers. After adjustment for other risk factors, the odds ratio for the 75th vs. 25th percentile was 1.27 (95% confidence limits of 1.01--1.58). Center-specific analyses, however, reveal weakly protective or absent associations for all centers except Malaga, cautioning against generalization of the positive association beyond that center.
{analyses supported by European Community funding and by U.S. Army grant DAMD17-94-J-4200, but do not necessarily reflect U.S. government policy or position}

Abstract presented at the American Society for Clinical Nutrition / Federation of American Societies for Experimental Biology annual meeting in Washington, DC, April 14-17, 1996.

Adipose tissue omega-3 fatty acids and breast cancer in a population of European women. N. Simonsen* (University of North Carolina, Chapel Hill NC 27599), J. J. Strain (University of Ulster at Coleraine, N. Ireland), P. van 't Veer (Agricultural University, Wageningen Netherlands), A. Kardinaal (TNO Nutrition and Food Research Institute, Zeist Netherlands), J. Fern'andez-Crehuet (University Hospital, Malaga Spain), J. Huttunen (National Public Health Institute, Helsinki Finland), J. Martin-Moreno (School of Public Health, Madrid Spain); B. Martin (Zurich University, Zurich Switzerland); M. Thamm (Robert Koch Institute, Berlin Germany); F. Kok (Agricultural University, Wageningen Netherlands); and L. Kohlmeier (University of North Carolina, Chapel Hill NC).

Laboratory evidence suggests that fatty acids of the omega-3 family compete with omega-6 fatty acids for metabolic pathways and may inhibit mammary tumors. Studies of fish intake in human populations generally support this idea. The present analyses explore the omega-3 hypothesis using adipose tissue fatty acid content determined by GLC in postmenopausal European women recruited for the EURAMIC study. Centers in five countries enrolled newly diagnosed primary breast cancer cases and controls.

The percentage of omega-3 fatty acids in adipose tissue did not differ significantly between the 291 cases (median 0.77) and the 407 controls (median 0.81). The ratio of omega-3 to omega-6 polyunsaturates was, however, inversely associated with breast cancer. The adjusted odds ratio for the 75th vs. 25th percentiles via conditional logistic regression controlling for age, recruitment center and other risk factors was 0.70 (95% C.L. 0.52--0.92). This was due to contributions from both major components of omega-3, α-linolenic and docosahexaenoic acid, in a protective direction. The relationship for α-linolenic acid was less consistent across centers. These results support the hypothesis that an increase in the proportion of omega-3 relative to omega-6 fatty acid, but not the absolute amount of omega-3, is protective against breast cancer.
{analyses supported by European Community funding and by U.S. Army grant DAMD17-94-J-4200, but do not necessarily reflect government policy or position.}

Abstract presented at the Society for Epidemiologic Research annual meeting in Boston, MA, June 12-15, 1996.

Appendix H: Manuscript Submitted

Adipose Tissue Omega-3 Fatty Acids and Breast Cancer in the EURAMIC Study

RUNNING TITLE: Adipose Omega-3 and Breast Cancer

Neal Simonsen, PhD, Pieter van 't Veer, PhD, John J. Strain, PhD, José M. Martin-Moreno, MD, DrPH, Jussi K. Huttunen, MD, Joaquin Fern'andez-Crehuet Navajas, MD, Blaise C. Martin, MD, DSc, Michael Thamm, Alwine F. M. Kardinaal, PhD, Frans J. Kok, PhD, Lenore Kohlmeier, PhD

Institutional affiliations: University of North Carolina, Chapel Hill, USA **(Kohlmeier, Simonsen);** Agricultural University, Wageningen, Netherlands (**Kok, van't Veer**); Human Nutrition Research Group, University of Ulster, Coleraine, Northern Ireland **(Strain);** Department of Epidemiology and Biostatistics, Centro Nacional de Epidemiologia, Madrid, Spain, **(Martin-Moreno);** National Public Health Institute, Helsinki, Finland **(Huttunen);** Department of Preventive medicine, Hospital Universitario, Facultad de Medicina, Malaga, Spain **(Fern'andez-Crehuet Navajas);** Institute for Social and Preventive Medicine of Zurich University, Zurich, Switzerland **(Martin);** Robert Koch Institute, Berlin, Germany **(Kohlmeier, Thamm);** TNO Nutrition and Food Research Institute, Zeist, Netherlands **(Kardinaal).**

The EURAMIC[1] Study was supported as a Concerted Action by the Commission of European Communities (DG-XII and DG-V). The national studies were financed by the Ulster Cancer Foundation and Milk Intervention Board (Co-responsibility Levy Disbursement, Reg(EEC) 1001/90 Contract 77.2), Dutch Ministry of Health, Spanish FIS and Ministry of Science and Education, German Federal Health Office, Cancer Research Switzerland (AKT76), and Swiss NSF (32-9257-87). These analyses were sponsored in part by the U.S. Department of the Army (grant DAMD17-94-J-4200). The content of this information does not necessarily reflect the policy or position of the U.S. government and no official endorsement should be inferred.

Please direct correspondence and reprint requests to: Neal R Simonsen, Department of Epidemiology, CB 7400, UNC-Chapel Hill, Chapel Hill, NC 27599

[1]EURAMIC = European community multicenter study on antioxidants, myocardial infarction, and breast cancer.

Other abbreviations: ALA = alpha-linolenic acid; BMI = body mass index; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; GLC = gas-liquid chromatography; OR = odds ratio.

Abstract

The fatty acid content of adipose tissue in postmenopausal European women was used to explore the hypothesis that fatty acids of the omega-3 family inhibit breast cancer. The percentage of omega-3 in adipose fat measured by GLC did not significantly differ between the 291 cases and the 407 controls. The ratio of omega-3 to omega-6 polyunsaturates was, however, protectively associated with breast cancer. For the difference between 25th and 75th percentiles of this measure, the odds ratio adjusted for other risk factors via logistic regression was 0.70 (95% confidence limits 0.52, 0.92). The contribution of docosahexaenoic acid appeared more consistent and more important to the total omega-3 relationship than that of alpha-linolenic acid. Total omega-6 displayed a significant association with disease, but this was confined to a single study center. These results support the hypothesis that an increased proportion, rather than the absolute amount, of omega-3 relative to omega-6 fatty acid is protective against breast cancer.

Key words: Breast cancer, omega-3 fatty acids, omega-6 fatty acids, adipose tissue.

Introduction

The variation in breast cancer rates across Western industrialized nations is marked. For every Japanese woman stricken with breast cancer each year, nearly five women in the United States are afflicted. Even within Europe sharp contrasts exist: in the neighboring countries of Spain and France, for example, annual female breast cancer incidences are 86 and 129 per 100,000, respectively.[1] Despite clear associations of hormonal, reproductive, and genetic factors with breast cancer risk, the majority of cases do not appear attributable to established risk factors.[2,3] Of the many dietary and environmental factors explored as possible sources of variation in breast cancer incidence, fat consumption has received the most focus, yet cohort studies provide little evidence that total fat consumption increases risk.[4,5,6] With growing awareness of the physiologic importance of individual fatty acids research has now shifted to the potential role of particular types of fat in the development of breast cancer.

Laboratory studies point to an involvement of essential fatty acids in tumor growth and metastasis.[7,8] Corn and other common seed oils provide rich sources of linoleic acid, the major fatty acid of the omega-6 family. Fish oils provide the predominant source of long-chain fatty acids (docosahexaenoic and eicosapentaenoic acid) of the omega-3 family. Substitution of fish for corn oil in the diet inhibits both spontaneous mammary tumor incidence and growth of transplanted tumors in rodent models.[9,10,11] Purified long-chain omega-3 fatty acids produce the same tumor growth inhibition seen with fish oil.[12]

Most of the relevant evidence regarding possible anticarcinogenic effects of omega-3 fatty acids in human populations comes from studies addressing fish consumption. Coldwater marine fish are the richest dietary source of long-chain omega-3 fatty acids. The preponderance of ecologic studies support an inverse relationship of fish consumption with breast cancer.[13-15] During the past five years, at least 7 case-control or cohort studies on populations from 6 different countries have contributed evidence regarding fish intake and breast cancer. Most report a statistically significant negative association between estimated fish or seafood consumption and disease, with the relationship often weaker or absent in premenopausal disease.[16-20] One reports no statistically significant association, although observing lower intakes in cases.[21] Another reports "no apparent association" between fish consumption and breast cancer, without presenting quantitative results.[22]

Three studies to date employed measurements of adipose tissue omega-3 content as an indicator of long-term exposure with mixed results.[20,23,24] One study using samples from breast tissue, proximal to and potentially affected by the tumor, found evidence of a protective association with breast cancer.[20] The other two, employing controls with conditions that omega-3 fatty acids might protect against, found no association.[23,24] To further investigate the relationship between adipose tissue as a biomarker of

omega-3 fatty acid exposure and breast cancer, a study using population-based controls and adipose tissue from a site remote from the tumor was therefore undertaken.

Materials and Methods

Subject Recruitment:

The EURAMIC breast cancer study was conducted in 5 European countries between 1990 and 1992.[25] Designed to test the relationship between long-term exposures to antioxidants and occurrence of breast cancer, adipose tissue antioxidant levels provided the primary measure of exposure in this case-control study. Fatty acid content of the adipose tissue samples was also determined. Fatty acid data from these samples are employed in this paper as a biomarker to investigate the relationship between exposure to exogenously derived omega-3 fatty acids and postmenopausal breast cancer in European populations differing greatly in their dietary fat intakes as well as breast cancer risks.[1,25,26] The primary hypothesis under exploration is that stores of exogenous essential fatty acids of the omega-3 family (reflecting exposure through diet) are related to breast cancer risk in post menopausal women, and that this association is modulated by stores of omega-6 fatty acids.

The EURAMIC breast cancer study design has been extensively treated elsewhere.[25] Centers recruited incident cases of breast cancer from the gynecologic and surgical units of participating hospitals. Recruitment involved incident primary breast cancers (ICD-code 174) among post menopausal women 50-74 years of age at diagnosis. Eligible cases had tumors histologically classified as ductal carcinoma, with primary tumors less than 5 cm, axillary lymph nodes stage < N3, and with no clinical indication of distant metastases at discharge.

Centers drew controls from women without a history of breast cancer among the hospital catchment area population.[25] The sampling of controls employed frequency matching for age (5-year intervals). Potential cases or controls reporting within the past year a physician-prescribed change in diet other than sodium or total calorie reduction, altered use of dietary supplements containing α-tocopherol, β-carotene, or selenium, or weight loss exceeding 5 kg were excluded. Other grounds for exclusion included 1) a history of treatment for alcohol or other chemical abuse, 2) institutionalization, and 3) major psychiatric disorders that might compromise ability to give informed consent. The requirements of all relevant local committees on human experimentation were met, and informed consent was obtained from all subjects.

Adipose tissue was aspirated by needle from the subcutaneous buttock directly into vacutainer adapters.[27] To assist in acquiring the appropriate skills for sampling, and assure standardized procedures, a videotape showing the technique was distributed to all participating centers. After collection and during transit, samples were kept on dry ice or in liquid nitrogen prior to storage at -70°C. Quality control samples were included in the shipments. After saponification and acidification the free fatty acids were extracted with hexanol and methylated. Gas-liquid chromatography (Carlo Erba, HRGC 5300 Mega Series), with split injection, was conducted in Zeist using a 30 m long DB-23 column, I.D. 0.253 mm, phase layer 0.25 μ and helium as carrier gas, in a temperature programmed run.[28,29]

Data analyses:

Analyses employed the pooled dataset from the breast cancer component of the EURAMIC study, plus 54 additional frequency-matched controls from the Berlin center for whom fatty acid analyses are available. A standard EURAMIC algorithm identified unreliable assays by comparing chromatographically estimated fat weight with recorded weight of tissue samples.[25] In addition, chromatographic results were combed for samples showing inconsistent fatty acid values. Subjects with assays deemed unreliable or evident chromatographic problems were excluded from all analyses.

Measured omega-3 fatty acids included α-linolenic, eicosapentaenoic, and docosahexaenoic acid. Total omega-6 fatty acid reflected the sum of linoleic, γ-linolenic, eicosadienoic, dihomo γ-linolenic, and arachidonic acid, although the contribution of linoleic acid dwarfed that of all other components.

Initial analyses employed simple descriptive statistics to compare fatty acid distributions across study centers. Most additional analyses involved logistic regression modeling. All statistical analyses employed SAS version 6.10 software.

Logistic regression was used to assess the importance of potential confounders. Factors considered as potential confounders included age, body mass index (BMI), current and past smoking, current drinking, oral contraceptive use, supplemental hormone use, family history of breast cancer, parity, age

at first childbirth, age at menarche, socioeconomic status, and recruitment center. A forward selection process followed by backward elimination served to winnow the most important risk factors from the initial mix of demographic and health history variables.[30] Besides the omega-3 and omega-6 fatty acids, all variables meeting a statistical significance criterion of p=0.10 were selected for inclusion. In addition, center-specific analyses were undertaken to identify any variables with a potentially significant impact on point estimates for a fatty acid variable in one or more centers.

Reported risk estimates are the product of conditional logistic regression models unless otherwise noted. Except in center-specific analyses, models are conditioned on age and recruitment center. Other factors selected for inclusion in all fatty acid models (unless otherwise noted) included BMI, nulliparity (yes/no), and age at first childbirth. Age at first childbirth was modeled by dummy variables for age < 25 years, 25-34 years, and over 34 years, with age < 25 serving as the referent.

The natural log is employed as a normalizing transform for total omega-3 fatty acid and docosahexaenoic acid in all regression analyses; no normalizing transform was needed for α-linolenic or total omega-6 fatty acid. Where α-linolenic or docosahexaenoic acid levels fell below the limit of detection in a sample, values for those respective fatty acids were set to 0.2 or 0.025, a level slightly below the lowest value actually detected. With the exception of quintile comparisons, all odds ratios represent estimates of the difference in risk between the 75th and 25th percentiles for each fatty acid, with the percentiles based on that fatty acid's distribution in the control population.

Results

Valid adipose tissue fatty acid data were obtained for 698 women meeting the study inclusion criteria. While the refusal rate for adipose biopsy was higher for controls (nearly 59%) than for cases (14%), sufficient controls were recruited to match anticipated numbers of cases at each recruitment center. Actual case occurrence was lower than expected. In addition, the proportion of unusable biopsies among enrolled cases exceeded that among controls. This led to fewer cases (291) than controls (407) in the current analyses.

As expected, linoleic acid--a member of the omega-6 family--makes up the vast bulk of the polyunsaturates in all centers (Table 1). Less than a tenth of the polyunsaturates for any center belong to the omega-3 family, within which α-linolenic acid predominates. Medians for both families of polyunsaturates vary substantially across centers. Malaga and Zurich exhibit the lowest median omega-3, while Berlin and Coleraine show the lowest median omega-6 fatty acid. In contrast, the highest docosahexaenoic acid (DHA)--the major long-chain omega-3 fatty acid--occurs in Malaga and Berlin, while Zurich and Coleraine sport the lowest median DHA.

An examination of median adipose omega-3 fatty acid percentages in all centers combined reveals slightly lower levels in cases than controls, consistent with a protective association, but the difference is far less than the interquartile range. Center-specific comparisons for the major individual omega-3 fatty acids α-linolenic acid (ALA) and DHA similarly show little consistent difference with disease status. Omega-6 fatty acids actually show a more marked association for all centers combined, but most of the case-control difference occurs in a single center.

Center-specific logistic regression models of breast cancer reveal no consistent association between total omega-3, ALA, DHA, or total omega-6 and disease (Table 2). The ratio of omega-3 to omega-6 and of DHA to omega-6, on the other hand, yielded an odds ratio below one for the majority of centers. Total omega-6 and the ratio of ALA to omega-6 show statistically significant and directionally opposite associations with breast cancer in Malaga, yet inconsistent results for the other centers.

Table 3 presents pooled results for all centers. The sum of omega-3 fatty acids in adipose tissue yields an adjusted odds ratio for breast cancer of 0.88 (95% C.L. 0.67--1.15), results mirrored by α-linolenic acid (OR=0.8; 0.63--1.16). Docosahexaenoic acid--the major component of total omega-3 most associated with seafood--shows no association with disease after adjustment for other risk factors(OR=1.00; 0.82--1.23).

The picture changes considerably when the balance between omega-3 and omega-6 fatty acid is addressed by modeling the ratio of omega-3 to omega-6 fatty acids. The protective association of total omega-3 with disease is much stronger (OR=0.70; 0.52--0.92), and there is little difference between the ORs for ALA and DHA.

The point estimates for total omega-6 and ALA in one center (Malaga) differed dramatically from those for other centers. In regression models performed excluding this center, absolute levels of DHA and total omega-3 appear more protective ((respective ORs with 95% CLs 0.95 (0.85--1.11), 0.89 (072--1.12) and 0.82 (0.60--1.06)). The ratio of total omega-3 and DHA to omega-6 continue to show protective associations with little change in their point estimates, although the loss of sample size drains away the estimates' statistical significance ((respective ORs 0.79 (0.59--1.02) and 0.83 (0.65--1.05)).

The relative importance of the omega-3 components ALA and DHA was explored through models including both fatty acids simultaneously (Table 3). ALA and DHA, whether as a percentage of total fatty acids or as ratios to omega-6, yield fairly similar ORs with all centers pooled. The exclusion of Malaga strengthens the results for DHA ((0.78 (0.63--0.97)) and its ratio to total omega-6 ((0.81 (0.65--1.02)) while eliminating the association for ALA : omega-6 ratio ((0.99 (0.67--1.44)).

Analyses stratified by tertile of adipose omega-6 show that the apparent protective association of omega-3 fatty acid is strongest at lower omega-6 polyunsaturated fatty acid levels(Table 4). This is consistent with competition of omega-3 and omega-6 fatty acids for metabolic pathways, resulting in a greater impact of a given amount of omega-3 when background levels of omega-6 are lower. The similarity of results for DHA and total omega-3 indicates that the relationship is driven by DHA.

Discussion

This study did not find a statistically significant association between the simple percentage of total omega-3 fatty acids in adipose tissue and incident breast cancer, although pooled analyses provided weak support for the protective association suggested by other studies cited earlier. We did observe an inverse association between disease and the ratios of both omega-3 and DHA to omega-6 in adipose tissue. These findings are consistent with hypothesized anticarcinogenic mechanisms for omega-3 fatty acids.

The best known mechanism through which omega-3 fatty acids exert physiologic effects and hence could affect carcinogenesis is a modulation of eicosanoid metabolism. The most common polyunsaturated fatty acids in the typical Western diet belong to the omega-6 family, and the predominant member of that family is linoleic acid. Linoleic acid can be converted to arachidonic acid, which in turn serves as the parent compound for eicosanoids that can powerfully affect cell function. Supplying long-chain omega-3 fatty acids (predominantly DHA in marine oils) competitively inhibits the delta-5 and -6 desaturase pathways necessary for conversion of linoleate to arachidonate. Once processed through these pathways, omega-3 fatty acids also give rise to a family of eicosanoids with effects often different or opposite to those produced by their counterparts from the omega-6 family.[31] Omega-3 fatty acids could thus inhibit tumor development and/or growth insofar as the production of the omega-6 family eicosanoids is a part of "normal" tumor growth and metabolism.

Another possible anticarcinogenic mechanism is through suppression of hepatic fatty acid desaturase and fatty acid synthase activity seen with fish oil administration.[32,33] This suppression could potentially reduce the supply of eicosanoid or energy-producing fatty acids to tumor cells. The modification of hepatic phase I and phase II detoxification systems also noted with high dietary fish oil intake could potentially affect carcinogen detoxification and/or activation.[34] Further, direct cytotoxic effects through peroxidation products of these polyunsaturated fatty acids have been proposed.[7]

The hypothesized mechanism of anticarcinogenicity through competitive inhibition of omega-6 fatty acid metabolism implies that the absolute amount of omega-3 fatty acid taken in by an individual is less important than the balance between omega-3 and omega-6 intake. If intake of omega-6 is high, a larger amount of omega-3 is needed to offset that intake. This is consistent with our finding that the ratio of omega-3 to omega-6 fatty acids in adipose tissue shows more association with disease than does the simple percentage of omega-3 in that tissue. It would also explain the decreased point estimates for adipose omega-3 in the high omega-6 group (Table 4).

Interpretation of the current study's findings depends upon the viability of adipose tissue as a measure of exposure. Adipose tissue is thought to best reflect dietary exposures for the essential fatty acids, which cannot be endogenously synthesized (the polyunsaturates linoleic, α-linolenic, and the long-chain omega-3 fatty acids being of prime importance). For these fatty acids--the focus of the current analyses--adipose tissue represents a stable, long term reservoir which integrates exposure levels over time. Studies indicate a typical two year half life for polyunsaturated fatty acids in adipose tissue.[35-37]

The correlation coefficient for intake estimated from diet records and adipose tissue polyunsaturated fatty was around 0.35 in one major study.[38] This figure is not corrected for the inherent measurement error which can attenuate observed associations. In a comparison of estimates based on fat biopsies and 14-day dietary records among a Danish population, Pearson correlation coefficients adjusted for measurement error reached 0.57 for linoleic acid and 0.80 for DHA.[39] A Dutch study using multiple 24-hour recalls over the course of a year reported an adjusted coefficient of 0.77 for linoleic acid from adipose vs. recall, but did not examine correlations for omega-3 fatty acids.[40] Direct measurement of concentrations in storage tissue avoids potential inaccuracy or bias associated with dietary recall techniques, and is particularly important for substances such as the omega-3 fatty acids, whose concentrations in specific foods vary greatly by locality and time.[41]

To avoid bias due to rate of fat accretion or recent weight loss, we excluded women with recent weight loss exceeding 5 kg, and corrected for body mass index differences.

Three hospital-based case-control studies have examined tissue fatty acid composition in breast cancer. In the U.S., London et al.[23] reported nearly identical median percentages of long-chain omega-3s (primarily DHA, with a small EPA component) in gluteal adipose tissue from postmenopausal cases

and controls with nonproliferative breast disease. As in our study, no consistent trend across quintiles of total omega-3 or DHA were observed; relative to the lowest quintile, all quintiles of EPA yielded ORs of below one, but the downward trend was neither consistent nor statistically significant. Petrek et al.[24] examined abdominal and mammary adipose tissue in New York women with breast cancer and controls undergoing breast biopsy. Quantitative results were presented only for mammary tisuue, but reportedly neither tissue displayed any significant case-control differences in regard to omega-3 fatty acids. Although controls deemed to be at high risk for breast cancer were excluded, most major breast cancer risk factors were not controlled for in the analyses. In both of the U.S. studies, the use of women with breast disease as controls could be problematic if omega-3 fatty acids protect against nonproliferative breast disease, although such activity is not established. In Finland, Zhu et al.[20] found lower levels of the long-chain marine-origin fatty acid EPA in triglycerides of mammary adipose tissue samples taken from postmenopausal cases than in tissue taken from women with benign breast disease yet no difference in DHA, the major fatty acid in fish oil. Cases' mammary phospholipids, in contrast, had significantly lower DHA and nonsignificantly lower EPA. As previously noted, the use of breast adipose tissue in the Petrek and Zhu studies raises questions regarding the potential impact of localized tumor effects on fatty acid stores.

The lower response rate for controls presents a potential source of bias. If control selection favored individuals with 'healthier' habits also associated with higher omega-3 to omega-6 ratios, the observed results might have arisen through selection bias. However, adding adjustment for variables likely to be associated with healthy lifestyle--smoking and drinking habits, BMI, and SES--to the regression model, as well as family history of breast cancer, age at menarche, and age at menopause, did not weaken the observed results. (For example, the omega-3 / omega-6 OR was 0.69 (0.51, 0.93)).

Exploratory regressions including age as a continuous covariate produced very similar results to those conditioned on age, evidence against the existence of residual confounding due to the use of age bands.

The strong relationship of total omega-6 polyunsaturate with breast cancer in one center may be due to an inverse association of monounsaturated with omega-6 (and hence total polyunsaturated) fat, driven by higher olive oil consumption in that center. A protective effect of olive oil consumption is supported by many dietary studies on southern European populations,[16,21,42-44] although not all.[45,46]

Laboratory evidence supports an association between linoleic acid exposure and mammary malignancy.[47] Increasing the dietary balance of omega-3 vs. omega-6 appears protective. However, a switch to omega-6 polyunsaturates at the expense of saturated fat often appears favorable as well, which is at odds with the apparent deleterious association for omega-6 independent of omega-3. In addition, the heterogeneity of the total omega-6 association across centers argues against its generalizability.

In contrast, the protective association with total omega-3 to omega-6 ratio is relatively consistent across centers. Further, the specificity of effect for the primary long-chain omega-3 fatty acid is congruent with the results of dietary studies linking fish consumption to lower breast cancer rates.

In summary, although the percentage of omega-3 in adipose fat did not significantly differ between cases and controls in this study, the ratio of omega-3 to omega-6 polyunsaturates was inversely related to breast cancer. Both major components of omega-3, α-linolenic and docosahexaenoic acid, demonstrated associations in a protective direction when results from all centers were pooled. The relationship for docosahexaenoic acid appeared more consistent and more important to the total omega-3 relationship in most centers. The current study's results support the hypothesis that an increased proportion, rather than the absolute amount, of omega-3 relative to omega-6 fatty acid is protective against breast cancer.

Acknowledgements

A hearty thanks for the programming skills of Carry Croghan, which helped these analyses through to a successful conclusion, and for the efforts of the EURAMIC study's many collaborating researchers, without which these analyses would not have been possible.

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Table 1: Adipose Tissue Fatty Acids in Cases and Controls, by Center

Medians followed by 25th and 75th percentile

Fatty Acid(s)[1]	All Centers		Berlin		Zeist		Coleraine		Zurich		Malaga	
	cases	controls	cases	controls	cases	controls	cases	controls	cases	controls	cases	controls
N[2]	291	407	16	103	70	63	95	99	54	74	56	68
Total ω3 polyunsaturate [as % total fatty acid]	0.77 0.62-.93	0.81 0.64-.97	1.01 0.93-1.22	0.95 0.81-1.11	0.89 0.74-0.98	0.92 0.78-.99	0.83 0.74-1.02	0.89 0.79-.98	0.57 0.53-.71	0.62 0.57-.72	0.66 0.57-.75	0.58 0.52-.65
α-linolenic acid (ALA) [as % total]	0.59 0.43-.71	0.59 0.44-.74	0.69 0.59-80	0.69 0.59-0.80	0.67 0.59-0.78	0.68 0.54-.76	0.67 0.59-.78	0.71 0.62-0.78	0.45 0.37-.50	0.48 0.41-.50	0.36 0.32-.42	0.35 0.32-.39
Docosahexaenoic acid (DHA) [as % total]	0.15 0.10-.21	0.16 0.12-.21	0.25 0.18-.33	0.18 0.15-0.25	0.14 0.09-0.18	0.18 0.11-.23	0.12 0.09-.17	0.14 0.10-.18	0.13 0.08-.16	0.12 0.10-.16	0.23 0.18-.31	0.20 0.14-.23
Total ω6 polyunsaturate [as % total fatty acid]	13.17 10.80-16.51	12.30 10.72-14.58	11.48 10.82-13.10	11.43 10.15-13.15	14.22 11.64-15.95	14.19 11.83-16.16	11.26 9.56-14.22	11.61 9.99-15.00	13.29 11.73-14.84	13.41 12.14-15.48	17.88 13.37-22.12	11.74 10.62-13.69
Linoleic acid (LA) [as % total fatty acid]	12.35 10.23-15.57	11.49 9.90-13.66	10.62 9.78-11.82	10.68 9.38-12.40	13.41 10.91-15.30	13.37 10.99-15.31	10.52 8.95-13.48	10.91 9.46-14.17	12.62 10.97-13.81	12.45 11.40-14.61	16.69 12.38-21.11	10.96 9.59-12.63
Total ω3: total ω6 [X 100]	5.69 4.34-7.64	6.40 4.95-8.32	9.18 8.45-11.08	8.45 7.34-9.60	6.44 5.32-7.32	6.42 5.39-7.21	7.44 6.03-9.05	7.44 5.90-9.02	4.61 3.57-5.39	4.56 4.12-5.40	3.67 3.06-4.72	5.04 4.09-5.69

Total DHA: total ω6 [X 100]	1.05 0.76-1.54	1.30 0.90-1.79	1.99 1.50-3.01	1.64 1.29-2.21	0.97 0.60-1.53	1.18 0.70-1.70	1.05 0.67-1.45	1.23 0.79-1.48	0.90 0.64-1.22	0.92 0.69-1.18	1.36 0.93-1.66	1.70 1.16-1.99
Total ALA : total ω6 [X 50]	2.23 1.51-3.00	2.34 1.71-3.11	3.03 2.57-3.44	3.00 2.64-3.45	2.49 1.98-2.90	2.31 2.02-2.80	3.00 2.47-3.54	2.94 2.44-3.76	1.66 1.37-1.95	1.70 1.39-1.98	1.05 0.78-1.45	1.48 1.27-1.69
Total monounsaturated [as % total fatty acid]	55.57 52.87-58.43	56.60 53.58-59.77	57.07 55.18-59.05	57.48 55.23-60.19	55.29 52.69-57.99	53.58 51.11-56.33	55.26 52.82-57.93	54.94 52.87-57.52	54.89 52.10-56.83	55.51 52.00-57.08	58.45 53.69-63.20	66.11 63.46-68.36

[1] ω3 = omega-3 = sum of a-linolenic, eicosapentaenoic, and docosahexaenoic acid; ω6 = omega-6 = sum of linoleic, γ-linolenic, eicosadienoic, dihomo γ-linolenic, and arachidonic acid.
[2] Number of subjects.

Table 2: Center-Specific Models of Breast Cancer by Adipose Tissue Fatty Acids[1]

Fatty acid	Berlin	Zeist	Coleraine	Zurich	Malaga
N[2]	118	122	193	125	124
Total omega-3 as % of all fatty acids	1.32 (0.50, 3.49)	0.71 (0.41, 1.22)	0.82 (0.50, 1.33)	0.51 (0.26, 0.99)	2.98 (1.40, 6.32)
DHA as % of all fatty acids	1.24 (0.53, 2.91)	0.73 (0.52, 1.01)	0.72 (0.51, 1.02)	0.86 (0.51, 1.44)	2.48 (1.35, 4.56)
ALA as % of all fatty acids	1.01 (0.45, 2.28)	0.95 (0.55, 1.63)	0.82 (0.49, 1.37)	0.46 (0.17, 1.29)	2.47 (0.40, 15.37)
Total omega-6 as % of all fatty acids	1.44 (0.50, 4.10)	0.87 (0.51, 1.48)	0.88 (0.62, 1.24)	0.81 (0.44, 1.47)	3.39 (2.06, 5.59)
Total omega-3 : omega-6 ratio	2.98 (1.40, 6.32)	0.79 (0.42, 1.47)	1.01 (0.61, 1.67)	0.63 (0.30, 1.32)	0.40 (0.22, 0.74)
DHA : omega-6 ratio	1.08 (0.47, 2.45)	0.75 (0.53, 1.06)	0.78 (0.55, 1.10)	0.94 (0.54, 1.64)	0.91 (0.56, 1.47)
ALA : omega-6 ratio	0.88 (0.30, 2.59)	1.06 (0.52, 2.17)	1.01 (0.62, 1.65)	0.60 (0.20, 1.79)	0.04 (0.01, 0.18)

[1] Center-specific fatty acid odds ratios for model conditioned on age, accompanied by 95% C.I. in parentheses. Odd ratios are based on difference between 75th and 25th percentiles in the control population. See Table 1, column 3 for listing of actual percentiles.

[2] N = number of observations for model (subjects with missing covariate information or no matching control in their age group excluded).

[3] Cov. = covariates including age in years, BMI, current alcohol consumption in grams/week, age at first birth, and nulliparity.

Table 3: Conditional logistic regression models of breast cancer by fatty acids: all centers pooled

Fatty Acid Variable(s)	Odds Ratio (95% C.L.s) [1]	
	Without covariates	With covariates[2]
As a % of all adipose tissue fatty acids...		
Total omega-3	0.88 (0.68--1.15)	0.88 (0.68--1.15)
ALA	0.86 (0.63--1.16)	0.86 (0.63--1.16)
ALA (in model also containing DHA)		0.87 (0.63--1.21)
DHA	0.99 (0.82--1.21)	1.00 (0.82--1.23)
DHA (in model also containing ALA)		0.92 (0.76--1.11)
Total omega-6	1.22 (0.99--1.52)	1.27 (1.01--1.58)
Ratio to total omega-6 fatty acid...		
Total omega-3	0.72 (0.55--0.94)	0.70 (0.52--0.92)
ALA	0.79 (0.56--1.10)	0.77 (0.55--1.09)
ALA (in model also containing DHA)		0.79 (0.55--1.14)
DHA	0.82 (0.66--1.01)	0.82 (0.66--1.02)
DHA (in model also containing ALA)		0.84 (0.69--1.04)

[1] Odds ratio for the difference between 75th and 25th percentiles in the control population. See Table 1, column 3 for actual percentiles. All models are conditioned on recruitment center and and age. Total omega-3, DHA, omega-3 : omega-6 ratio, and DHA : omega-6 ratio models use log-transformed fatty acid values.

[2] For total omega-3 and DHA, covariates include BMI, nulliparity, age at birth of first child, and current alcohol consumption in grams/week; for omega-6 and fatty acid ratio models, covariates also include SES.

Table 4: Odds Ratios for Total Omega-3 and DHA after Stratification by Tertile of Total Omega-6 fatty acid

Independent Variable(s) in Model	N[1]	Fatty Acid O.R.-fatty acid as % of total FA[2]		
		Low omega-6[3]	Moderate omega-6[3]	High omega-6[3]
Total omega-3 fatty acid, with covariates[3, 4]	229, 225, 228	0.74 (0.41, 1.35)	0.84 (0.54, 1.29)	1.06 (0.67, 1.68)
DHA, with covariates[3, 4]	229, 225, 228	0.81 (0.54, 1.24)	0.79 (0.55, 1.13)	0.99 (0.72, 1.36)

1 N = Number of individuals in each of the ascending omega-6 fatty acid tertiles.
2 Odds ratio for the difference between 75th and 25th percentiles in the control population. See Table 1, column 3 for actual percentiles.
3 Tertiles based on total study population (698 subjects with valid fatty acid assays, before losses due to missing covariates). Starting points for the second and third tertiles were 12.14 and 15.09 for omega-6 %.
4 Conditioned on recruitment center only; age included in model as continuous covariate.
5 Covariates include age (years), BMI, current alcohol consumption in grams/week, and age at birth of first child.

DEPARTMENT OF THE ARMY
US ARMY MEDICAL RESEARCH AND MATERIEL COMMAND
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REPLY TO
ATTENTION OF:

MCMR-RMI-S (70-1y) 6 May 98

MEMORANDUM FOR Administrator, Defense Technical Information Center, ATTN: DTIC-OCP, Fort Belvoir, VA 22060-6218

SUBJECT: Request Change in Distribution Statement

1. The U.S. Army Medical Research and Materiel Command has reexamined the need for the limitation assigned to technical reports written for the following contracts. Request the limited distribution statement for these contracts be changed to "Approved for public release; distribution unlimited." These reports should be released to the National Technical Information Service.

Contract Number	Accession Document Number
DAMD17-94-J-4030	ADB215484
DAMD17-94-J-4138	ADB215863
DAMD17-94-J-4158	ADB215553
DAMD17-94-J-4278	ADB215864
DAMD17-94-J-4267	ADB216187
DAMD17-94-J-4200	ADB216054
DAMD17-94-J-4185	ADB219284
DAMD17-94-J-4172	ADB224562
DAMD17-94-J-4156	ADB216186
DAMD17-94-J-4082	ADB215979
DAMD17-94-J-4053	ADB216052
DAMD17-94-J-4028	ADB218953

2. Point of contact for this request is Ms. Betty Nelson at DSN 343-7328 or email: betty_nelson@ftdetrck-ccmail.army.mil.

FOR THE COMMANDER:

PHYLLIS M. RINEHART
Deputy Chief of Staff for
Information Management