Effects of High Fruit-Vegetable and/or Low-Fat Intervention on Breast Nipple Aspirate Fluid Micronutrient Levels

Zora Djuric,1 Gang Chen,2 Jianwei Ren,1 Raghu Venkatramanamoorthy,3 Chandice Y. Covington,4 Omer Kucuk,3 and Lance K. Heilbrun3

1Department of Family Medicine, University of Michigan, Ann Arbor, Michigan; 2Pennsylvania State University, College of Medicine, Hershey, Pennsylvania; 3Barbara Ann Karmanos Cancer Institute, Wayne State University, Detroit, Michigan; and 4School of Nursing, University of North Dakota, Grand Forks, North Dakota

Abstract

Background: A change in diet is known to affect micronutrient levels in blood but to what extent diet can affect micronutrient levels in the breast is not yet well established. Methods: Healthy, premenopausal women with a family history of breast cancer were randomized across four diet arms for 1 year in a 2 × 2 factorial design study: control, low-fat, high fruit-vegetable, and combination low-fat/high fruit-vegetable diets. Subjects were asked to collect breast nipple aspirate fluid (NAF) at 0, 6, and 12 months, and levels of micronutrients were measured in the fluid. Results: A total of 122 women were enrolled, 97 were retained for 12 months, and sufficient NAF for analysis was available from 59 women at baseline, 49 at 6 months, and 50 at 12 months. Repeated measures mixed-model ANOVA was used to model the data using cholesterol levels and lactation duration as covariates, where appropriate. The high fruit-vegetable intervention, regardless of fat intake, significantly increased total carotenoid levels in NAF. In the low-fat arm, levels of total carotenoids decreased over time relative to control. Levels of total tocopherols and retinol did not change significantly. Levels of 15-F2t-isoprostane, a marker of lipid peroxidation, also did not change significantly over time, although there was a decrease observed in the combination arm. Conclusions: These results indicate that total carotenoid levels in NAF can be significantly increased in the breast NAF with a high fruit-vegetable diet. A low-fat diet that was achieved with little increase in fruit and vegetable intake, however, decreased NAF carotenoid levels.

Introduction

Breast nipple aspirate fluids (NAF) can be obtained noninvasively from the breast in most women (1-7). NAF contains a large variety of substances, including hormones, growth factors, and mutagens, and is especially high in protein and lipids (1, 7-15). Intraindividual and interindividual variation in NAF composition is large (16-18), but the biological basis and the consequences of this variation are not well understood. NAF bathes the ductal epithelial cells and therefore might be expected to influence risk of ductal carcinoma. Differences have been found in breast fluid hormone levels between subjects with breast cancer, benign breast disease, and normal breasts (5, 19-21). Oxidized cholesterol metabolites were elevated in the breast fluid of women at increased risk for breast cancer (21). Increased levels of cholesterol β-epoxide have been associated with the presence of increasing atypia of the exfoliated cells (22, 23). Some of the components of NAF are influenced by diet (3, 24-27), and this may be one mechanism by which diet can affect breast cancer risk. One dietary change that seems important for breast cancer risk is increased consumption of fruits and vegetables. Although fruit-vegetable interventions determined by food frequency questionnaires have not indicated much association of diet and breast cancer risk in cohort studies (28), increased serum levels of β-carotene, which is a carotenoid found in many types of fruits and vegetables, were associated with strong protective effects in three large prospective studies (29-31). NAF micronutrient levels may more directly influence breast cancer risk and should be modified by diet. Carotenoids in breast milk are responsive to dietary supplementation (32, 33), and increases in serum predicted increases in breast milk (34). Carotenoids and tocopherols have antioxidant properties that can protect cells from oxidative damage, and they also function via many other pathways relevant to cancer prevention (35). Increased fruit and vegetable consumption and decreased fat intakes have been shown to decrease levels of 15-F2t-isoprostane, a marker of oxidative stress in urine (36, 37). We conducted a clinical trial that randomized healthy, premenopausal women with a family history of breast cancer to one of four diets for 12 months: nonintervention, low-fat, high fruit-vegetable, and combination low-fat/high fruit-vegetable. The purpose of the study was to examine the potential of these dietary factors to modulate biomarkers of oxidative stress in blood and NAF. The low-fat goal was 15% of energy from fat and the high fruit-vegetable goal was nine servings per day in specific categories to increase variety of intake (38). In our previous report of blood carotenoids in this same clinical trial, an increase in fruit and vegetable intake was associated with an increased blood carotenoid levels but a low-fat diet was associated with decreased γ-tocopherol levels (39). This seemed to be due to compromised intake of γ-tocopherol during low-fat intervention, regardless of whether fruit and vegetable consumption was concomitantly increased. Low-fat intake did not seem to adversely affect carotenoid levels in plasma, indicating that a diet that is 15% of energy from fat is sufficient for absorption of dietary carotenoids. Levels in NAF, however, might not be affected in the same way as blood levels. This present report examined levels of carotenoids, tocopherols, retinol, and 15-F2t-isoprostane in NAF obtained from women in the trial at 0, 6, and 12 months.

Received 9/11/06; revised 3/30/07; accepted 4/18/07.
Grant support: NIH grants U01CA77297 and CA22453.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
Requests for reprints: Zora Djuric, University of Michigan, 1500 East Hospital Drive, Room 2150 Cancer and Geriatrics Center, Ann Arbor, MI 48109-0930. Phone: 734-615-6210; Fax: 734-647-9617. E-mail: zoralong@umich.edu
Copyright © 2007 American Association for Cancer Research.

doi:10.1158/1055-9965.EPI-06-0766
Materials and Methods

Subjects. The Nutrition and Breast Health Study enrolled healthy, nonsmoking, premenopausal women, ages 21 to 50. Subjects had at least one first-degree relative with breast cancer but no personal history of cancer. The eligibility criteria included that they were not lactating at the time of enrollment. Details of the study methodology have been published (38). A total of 122 women was enrolled and the intervention lasted 12 months. The study was approved by the Institutional Review Board of Wayne State University.

Dietary Intervention. Women in the nonintervention arm received the Daily Food Guide Pyramid from the National Dairy Council as a guide for healthy eating and were asked to continue following their own usual diet. For the intervention arms, both individualized in-person counseling and monthly group meetings were implemented. The counseling was biweekly initially and then monthly once women became adept at meeting dietary goals. Women were given intake goals using modified American Dietetic Association exchange lists, and the two low-fat arms also received a daily fat-gram goal (38).

The goal in the low-fat arm was to reduce fat intake to 15% of total energy while keeping fruit and vegetable and total energy consumption constant. The percentage of energy from carbohydrates increased to ~70% of total energy, whereas protein content remained constant. The goal for the high fruit-vegetable arm was nine servings of fruits and vegetables per day in a specified variety: one serving of a dark green vegetable high in lutein, one serving of a dark orange vegetable high in α-carotene, one serving of a red product high in lycopene, two servings of other vegetables, two servings of vitamin C–rich fruits, and two servings of other fruits (one serving was defined as ~60 kcal for fruit and 25 kcal for most vegetables; ref. 38). Counseling for maintenance of baseline energy intakes included emphasis on substitution of other fruits and vegetables for other carbohydrates. For the combination arm, both grams of fat and servings of fruits and vegetables were enumerated to meet goals of 15% energy from fat and nine servings per day of fruits and vegetables. This diet resulted in energy from fat largely being replaced with energy from fruits and vegetables.

Four-day food records indicated excellent compliance to the diets with fat intakes reaching ~16% of energy in the low-fat and combination arms and fruit-vegetable intakes exceeding goals and reaching ~11 servings per day in the high fruit-vegetable and combination arms (38).

Nipple Aspiration and Samples. The basic approach has been described previously in more detail (40). The breast aspirator is similar to that used by Sartorius (41, 42), and it was an inexpensive, easy-to-use method for subjects to express their own breast fluids. This method differs somewhat from that used by other researchers (21, 42) in that manual chest wall pressure on the breast tissue during aspiration is not used nor recommended to prevent trauma to breast tissue. Subjects were taught the procedure at the study enrollment visit using a breast mannequin and printed guide, and they did the procedure by themselves at home (41). Participants were asked to provide breast nipple fluid samples at 0, 6, and 12 months, and they were paid $50 for each 2-week attempt at obtaining breast fluid. They brought the fluid with them to the study visits and at that time donated a fasting blood sample as well, for which an additional $25 was provided. Plasma was processed immediately and both plasma and NAF were stored at ~70°C.

The NAF samples were collected every other day during the 2-week collection period. Droplets of fluid were collected with a heparinized Natelson blood collection capillary tube (Fisher), and a rubber bulb was then used to transfer the fluid. The first and last collections were placed in cytologic preservative and the five other collections were combined into an amber Eppendorf tube. The Eppendorf tube was kept in a Dyna Chill portable ~15°C cooler (Research Products International). This container was prefrozen so that the fluid froze as it came in contact with the sides of the Eppendorf tube. The fluid was stored frozen in the home freezers using the Dyna Chill insulated container to protect against the freeze/thaw cycles during the collection period. Storage was at ~70°C after it was taken to the laboratory (within a few days of the last collection). The self-collection method for NAF can be less intimidating to women than being subjected to clinic-based procedures, but a limitation is that this can result in variability with regard to collection technique and storage.

Laboratory Analyses. The breast fluid was thawed and weighed aliquots were removed for the laboratory analyses. Approximately 1 mg NAF was required for 15-F2t-isoprostane analyses, 2 mg for cholesterol, and 1 mg for protein, and 1 to 90 mg of any remaining NAF were used for fat-soluble micronutrient analyses. All aliquots were diluted with dextrose solution [5% USP dextrose, 50 mmol/L mannitol, 10 mmol/L Tris (pH 7.4)] before analysis. All but the micronutrient measurements were made immediately after thawing the fluid; micronutrients were determined after freezing and thawing the samples once more.

Levels of total 15-F2t-isoprostane were determined using a kit from Cayman Chemical Co. using a modified Sep-Pak procedure we described previously (43). Approximately 1 mg fluid was diluted to 200 μL with the dextrose solution, and the same procedure recommended for total 15-F2t-isoprostanes in plasma was followed, assaying two dilutions, each in duplicate. Seven samples had high levels of 15-F2t-isoprostanes and resulted in readings that were above the calibration curve limits. These samples were deleted from the final analysis since repeat analyses.

The analyses of fat-soluble micronutrients in NAF were done by Craft Technologies using a C-30 high-performance liquid chromatography column. Samples that were analyzed but had levels below the limit of detection were assigned a value that was one half the limit of detection. Total carotenoid levels were calculated from the sum of lutein, zeaxanthin, β-cryptoxanthin, α-cryptoxanthin, trans-luteopene, cis-luteopene, α-carotene, trans-β-carotene, and cis-β-carotene. Total tocopherol levels were calculated from the sum of α-tocopherol, γ-tocopherol, and δ-tocopherol. Plasma micronutrient was analyzed in-house also using a C-30 column as reported previously (39). Both of the laboratories analyzing samples for this study calibrated their assays using standards from the National Institutes of Standards Technology.

Statistical Methods. The baseline levels of micronutrients, 15-F2t-isoprostane, and cholesterol in NAF and in plasma were summarized with simple descriptive statistics. For variables measured in both NAF and plasma, the strength of the NAF/plasma linear association was assessed via Kendall’s rank correlation coefficient (44). For micronutrients and for 15-F2t-isoprostane, the potential confounding due to NAF cholesterol and/or plasma cholesterol was adjusted for via Kendall’s partial rank correlation coefficient (44). The 11 Kendall’s rank correlation coefficients were each tested (versus a null value of 0), and adjustment for the resulting multiple comparisons problem was made via the false discovery rate method (45). Because the sampling distribution of Kendall’s partial rank correlation coefficient is still unknown, significance testing of adjusted (i.e., partial) rankings is not possible (44).

Graphical displays were used to summarize the NAF micronutrient and 15-F2t-isoprostane levels by diet intervention arm and by time on study (Figs. 1-2). To deal with occasional missing data at any of the three time points, incomplete mixed-model repeated measures ANOVA was
used to model the mean levels of each NAF micronutrient. This allowed analysis of all available data, consistent with the intention to treat principle. Before any modeling, each NAF analyte required natural log (ln) transformation to achieve normality. The modeling of each NAF analyte was conducted using the MIXED procedure in Statistical Analysis System version 8.2 (44, 45).

Consistent with the 2 × 2 factorial study design, the two dietary intervention effect variables were the low-fat effect (LFE; yes/no) and the high fruit-vegetable effect (HFVE; yes/no). In cases where there was a significant interaction effect, the influence of each intervention effect variable was analyzed after stratifying on the other one, which is equivalent to doing selected contrasts of individual diet arms.

The linear effect of time (i.e., slope) was modeled, along with interaction effects of time with the LFE and with the HFVE (and their interaction). We also included NAF cholesterol (as a time-dependent covariate) and prior lactation duration (LD) as covariates in all models. Previous work has indicated that lactation can affect micronutrient levels in NAF (13, 40), making LD an important covariate, and blood cholesterol is a carrier for fat-soluble micronutrients. LD was a zero-inflated continuous covariate, with potentially different effects for LD ≤6 months versus LD >6 months (40). Therefore, we used two indicator variables to represent the three possible LD categories (0, 0 < LD ≤ 6, and LD > 6), with LD = 0 as the

Figure 1. Levels of retinol, total tocopherols, and 15-F_{2z}-isoprostane in NAF with time for the four diet groups. Statistics shown are the mean and SE of the natural log (ln)-transformed data.

Figure 2. Levels of total carotenoids in NAF with time on study for the four diet groups individually and for subjects with versus without high fruit-vegetable (FV) intervention. Statistics shown are the mean and SE of the natural log (ln)-transformed data. As a frame of reference in interpreting the transformed values, women who received a high fruit-vegetable intervention had a mean total carotenoid level of 607 at baseline and 1,164 ng/g fluid at 12 months.
Table 1. Micronutrient, 15-F2t-isoprostane, and cholesterol levels in NAFs and plasma at baseline in 59 women (given as ng per gram fluid, except for cholesterol given as mg/g fluid and mg/mL plasma)

<table>
<thead>
<tr>
<th>Micronutrient</th>
<th>Nipple aspirate (ng/g)</th>
<th>Plasma (ng/mL)</th>
<th>Kendall’s rank correlation*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median (SD)</td>
<td>Median (SD)</td>
<td>Unadjusted</td>
</tr>
<tr>
<td>Retinol</td>
<td>258 (1,166)</td>
<td>ND</td>
<td>—</td>
</tr>
<tr>
<td>Lutein</td>
<td>136 (233)</td>
<td>109 (103)</td>
<td>0.03</td>
</tr>
<tr>
<td>Zeaxanthin</td>
<td>91 (133)</td>
<td>23 (18)</td>
<td>0.08</td>
</tr>
<tr>
<td>α-Cryptoxanthin</td>
<td>30 (48)</td>
<td>ND</td>
<td>—</td>
</tr>
<tr>
<td>β-Cryptoxanthin</td>
<td>103 (230)</td>
<td>96 (53)</td>
<td>0.19</td>
</tr>
<tr>
<td>trans-Lycopene</td>
<td>25 (61)</td>
<td>496 (330)</td>
<td>0.18</td>
</tr>
<tr>
<td>cis-Lycopene</td>
<td>100 (200)</td>
<td>ND</td>
<td>—</td>
</tr>
<tr>
<td>α-Carotene</td>
<td>23 (46)</td>
<td>76 (67)</td>
<td>0.29</td>
</tr>
<tr>
<td>trans-β-Carotene</td>
<td>59 (136)</td>
<td>277 (202)</td>
<td>0.25</td>
</tr>
<tr>
<td>cis-β-Carotene</td>
<td>28 (53)</td>
<td>ND</td>
<td>—</td>
</tr>
<tr>
<td>Total carotenoids</td>
<td>575 (1,105)</td>
<td>1,075 (543)</td>
<td>0.19</td>
</tr>
<tr>
<td>δ-Tocopherol</td>
<td>447 (1,777)</td>
<td>ND</td>
<td>—</td>
</tr>
<tr>
<td>γ-Tocopherol</td>
<td>5,742 (22,956)</td>
<td>2,013 (996)</td>
<td>0.14</td>
</tr>
<tr>
<td>α-Tocopherol</td>
<td>29,199 (67,722)</td>
<td>10,894 (4,034)</td>
<td>0.10</td>
</tr>
<tr>
<td>Total tocopherols</td>
<td>38,082 (90,527)</td>
<td>12,907 (4,523)</td>
<td>0.09</td>
</tr>
<tr>
<td>15-F2t-isoprostane</td>
<td>14,420 (48,223)</td>
<td>131 (54)</td>
<td>0.07</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>3,188 (1,118)</td>
<td>1,78 (3,26)</td>
<td>0.09</td>
</tr>
</tbody>
</table>

Abbreviation: ND, not determined.

*Kendall’s rank correlations were determined with and without adjusting for (partialing out) two covariates: plasma cholesterol and NAF cholesterol levels.

In plasma, only total lycopene and total β-carotene were determined and those values are shown. Correlation coefficients are for calculated total lycopene in NAF with total lycopene in plasma. The level of 15-F2t-isoprostane in NAF was not (initially) adjusted for NAF cholesterol, however, as that was deemed unnecessary. For each NAF micronutrient variable, the statistical modeling was done after preliminary analysis to find the best of 14 covariance structures for its three repeated measures (at 0, 6, and 12 months) based on smallest value of Akaike’s information criterion. Because this was a small study, the focus of the modeling over time was on total tocopherols and total carotenoids.

Results

NAF Yields and Micronutrient Levels. As reported previously, the number of women collecting NAF was about two third of those who attempted the procedure (40). At each time point, subjects were asked to pool five NAF collections for the analyses reported here. The goal of this collection method was to increase volumes of NAF available for analysis, but pooling of collections also can serve to reduce intrindividual variation for each subject, which is large (46). NAF was analyzed for tocopherols, carotenoids, and retinol from 59 women at baseline, 49 at 6 months, and 50 at 12 months. The respective numbers of women providing enough fluid for micronutrient analyses at 0, 6, and 12 months were 16, 17, and 16 in the control arm; 14, 11, and 10 in the low-fat arm; 13, 12, and 13 in the high-fruit-vegetable arm; and 16, 9, and 11 in the combination arm. Although all the NAF could not be recovered from the amber collection vial, amounts obtained ranged 0 to 700 mg based on the weight of the aliquots prepared when thawed. There were two instances of women reporting collection of NAF and yet we were not able to find any NAF in the vial. Four women were able to provide more than 200 mg fluid, and two of those women did so at both 6 and 12 months, resulting in six samples with more than 200 mg fluid. Mean NAF yield for all samples collected was ~40 mg (median, 23 mg).

Data for individual micronutrient levels at baseline in NAF and plasma are shown in Table 1. If it can be assumed that 1 g fluid is approximately equivalent to 1 mL, levels in NAF can be compared with levels in plasma. Levels in NAF were higher than in plasma for some micronutrients (Table 1): 5-fold higher for γ-tocopherol, α-tocopherol, and zeaxanthin and 2-fold higher for β-cryptoxanthin. Lutein levels were of similar magnitude in NAF and plasma. Levels in NAF were slightly higher than in plasma for other carotenoids: 2- to 3-fold for α-carotene and β-carotene and 4-fold for lycopene. As indicated by the SDs and medians, there was wide interindividual variation in levels of all micronutrients measured, with a few women having undetectable levels of several micronutrients in their NAF. Total carotenoids in plasma were significantly lower than those between carotenoids in breast adipose tissue and serum (47).

Changes in 15-F2t-Isoprostane Levels. There was only one significant effect on 15-F2t-isoprostane levels: low-fat/high fruit-vegetable (P = 0.005), the intervention interaction (without any time effect). Hence, mean 15-F2t-isoprostane levels were statistically distinct by individual diet arm at baseline and remained so over the 12-month study (Fig. 1). Mean levels were higher in the combination arm than in the other three arms but there was no statistically significant change over time. A similar result was obtained even if NAF cholesterol was included as an additional covariate. The percentage change from baseline to 12 months in each arm

referred group. Women who had never lactated before this study were coded with a zero for both indicator variables. We refer to those who had lactated in the past as either the short LD group or the long LD group.

For each NAF micronutrient model, the predictors were the following: LFE, HFVE, their interaction (a cross-product term); time, its interactions with LFE, HFVE, and LFE*HFVE; and three covariate terms (for NAF cholesterol, and the two LD indicator variables). NAF 15-F2t-isoprostane was not (initially) adjusted for NAF cholesterol, however, as that was deemed unnecessary. For each NAF micronutrient variable, the statistical modeling was done after preliminary analysis to find the best of 14 covariance structures for its three repeated measures (at 0, 6, and 12 months) based on smallest value of Aikake’s information criterion. Because this was a small study, the focus of the modeling over time was on total tocopherols and total carotenoids.
was 101% control, 99% low fat, 230% high fruit-vegetable, and 36% combination. The decrease in the combination arm was statistically significant when the seven samples with very high readings on the ELISA were included, but those data were not used in the final model because these samples were above the limits of the calibration curve and therefore could not be quantified accurately. Other studies have shown that dietary or antioxidant interventions tend to be more effective on decreasing oxidative stress in individuals who have higher initial oxidative stress levels (48, 49), and here, mean baseline levels were highest in the combination arm.

**Changes in Retinol and Tocopherol Levels.** There were no significant time-dependent effects found for either retinol or total tocopherols (after natural log transformation). For retinol, women who were assigned to either high fruit-vegetable intervention had higher levels averaged over time than women without high fruit-vegetable intervention (P = 0.018). This indicates that the randomization did not equalize the interindividual variation in NAF retinol levels. There were, however, no significant differences in the slopes of retinol levels with time either for intervention effect or for any individual intervention arm versus control (Fig. 1).

The analysis of tocopherols was first done using total tocopherols, which was calculated as the sum of α-tocopherol, γ-tocopherol, and δ-tocopherol. The mixed-model ANOVA indicated no significant time effects on total tocopherols (Fig. 1). To confirm that analyzing total tocopherols did not obscure differences in changes of individual α-tocopherol and γ-tocopherol, because those tocopherols were differentially affected by low-fat intake in blood (39), separate ANOVA models were created for each of those two tocopherols. These analyses confirmed that the decreases were not significant over time by low-fat status for mean levels of either α-tocopherol (P = 0.136) or γ-tocopherol (P = 0.089). There was, however, a statistically significant decrease (P = 0.029) over time in the mean level of γ-tocopherol for all study women combined.

**Changes in Carotenoid Levels.** Total carotenoids were calculated from the sum of lutein, zeaxanthin, α-cryptoxanthin, β-cryptoxanthin, trans-lycopene, cis-lycopene, α-carotene, β-carotene, and cis-β-carotene. We observed two significant interaction effects on total carotenoids: low-fat/high fruit-vegetable (P = 0.004) and time*low-fat/high fruit-vegetable (P = 0.011). Therefore, stratified analyses were done. For women without high fruit-vegetable intervention, the time*low-fat interaction was significant (P = 0.002), indicating that the decrease in total carotenoids in the low-fat arm was significantly different versus control (Fig. 2). Among women with high fruit-vegetable intervention, the time*low-fat interaction was not significant (P = 0.121), indicating that changes over time did not differ depending on whether they also had low-fat intake. Levels of total carotenoids increased significantly over time for women with high fruit-vegetable intervention versus those without high fruit-vegetable intervention (e.g., those in the high fruit-vegetable and combination arms versus those in the control and low-fat arms). The differences in the slope (P = 0.020) were highly significant for women with and without high fruit-vegetable intervention. The increase in total carotenoids was achieved by 6 months and remained increased at 12 months (Fig. 2). The individual carotenoids with the greatest increases were α-carotene and β-carotene with 3- and 2-fold increases over baseline, respectively, in either the high fruit-vegetable or combination arms at 6 or 12 months.

Of all the individual carotenoids measured, mean lutein and zeaxanthin levels exhibited the strongest trends for decreases in the low-fat arm. The ratios of levels at 12 months to levels at baseline were 0.41 for lutein and 0.38 for zeaxanthin, whereas the ratios for each of the other individual carotenoids were ≥0.66. Therefore, these two carotenoids were modeled separately. There was a significant time*low-fat interaction effect (P = 0.009) for lutein, so stratified analyses were done. For women with high fruit-vegetable intervention, there was no significant change over time, but for women without high fruit-vegetable intervention, there was a significant difference in the slope of lutein level over time by low-fat status (P = 0.003). This indicated that the decrease in lutein levels in the low-fat arm was significantly different than control. Similar results emerged for zeaxanthin in NAF with no significant change over time for women assigned to high fruit-vegetable intervention and a lower mean zeaxanthin over time for women in the low-fat arm versus the control arm (P = 0.001).

**Discussion**

This study is unique in that the independent and interactive effects of a high fruit-vegetable and low-fat intervention could be examined on micronutrient levels in breast NAF. One of the main findings was that carotenoids in NAF increased with a high fruit and vegetable diet regardless of whether dietary fat intake was concomitantly decreased. There was, however, surprisingly little correlation between levels of micronutrients in NAF and plasma. These findings, if confirmed, could lend insight into distribution of dietary micronutrients into the NAF.

Dietary carotenoids have been reported to be less strongly correlated with adipose tissue levels than with plasma levels. Plasma levels likely reflect absorption from the diet to a relatively greater degree, with the subsequent distribution and destruction of micronutrients making more of a contribution to adipose tissue levels (50). In breast adipose tissue, correlation coefficients for carotenoid levels with serum ranged roughly 0.3 to 0.5, which is somewhat stronger than that observed here for NAF and plasma (47). Micronutrients in NAF will likely reflect both distribution of micronutrients to the breast as well as secretion or diffusion of micronutrients into the fluid. Micronutrients in the ducal fluid in turn can be degraded by lipid peroxidation products, and levels of 5-F2t-isoprostane (Table 1) and cholesterol oxides are very high in the NAF (51). This is likely to affect negatively on the cells lining the breast ducts, making replenishment of antioxidant micronutrients by diet important.

It has been suggested previously that a low-fat diet may compromise intakes of tocopherols, which are found in vegetable oils (52, 53). In the Nutrition and Breast Health Study, it also was published previously that plasma levels of γ-tocopherol (but not α-tocopherol) decreased significantly in both low-fat arms (39). The lack of a significant decrease in NAF tocopherol levels indicates the possibilities that tocopherol is relatively slower to deplete in NAF than plasma in response to decreased intakes, breast stores of tocopherols are less affected than those of plasma by diet, or that the large interindividual variation in NAF requires larger sample sizes for significant differences to be evident. This is in accord with the relatively weaker adipose tissue-plasma correlations for tocopherols than for carotenoids (54). The aim of this study, however, was not to modify tocopherol intakes or levels.

The high fruit-vegetable intervention in this study was specifically designed to increase the amount and variety of carotenoid intakes. The magnitude of the increase in NAF carotenoids was ~2-fold relative to baseline by 6 months, which is similar to that observed in plasma from these women for total carotenoids (39). It is important to note that the concomitant counseling for a decreased fat intake had no significant effect on the increase in total carotenoid levels with the high fruit-vegetable intervention, indicating that these carotenoids were similarly bioavailable from a high fruit-vegetable diet when fat intake was or was not decreased.
The significant effects of fat intake in the absence of simultaneous high fruit-vegetable intervention were also interesting. It might have been expected that the low-fat goal might interfere with absorption of carotenoids resulting in lower levels in the combination arm than the high fruit-vegetable arm, but this was not observed. The only statistically significant decrease in carotenoids was observed in the low-fat arm, and lutein and zeaxanthin in NAF were decreased the most by the low-fat intervention in this study (see Results). Lutein bioavailability, in particular, is affected negatively by fat intake more than that of a-carotene or β-carotene using carotenoid suppletion (55).

In summary, tocopherol and retinol levels, which were not targeted by any intervention, were not significantly changed. The high fruit-vegetable intervention, regardless of fat intake, increased total carotenoid levels in NAF. This did not affect levels of 15-F2t-isoprostane, although there was a trend for decreased levels in the combination arm. The low-fat intervention, without an increase in fruits and vegetables, decreased carotenoid levels in NAF. These results indicate that a high fruit-vegetable diet can be useful to increase carotenoids in breast NAF, which may be useful for prevention of breast cancer because increased carotenoid levels in plasma have been associated with decreased breast cancer risk (29-31).

Acknowledgments
We thank the women who generously took the time to participate in the Nutrition and Breast Health Study. Janice B. Depper and Kathleen M. Poore were dietitians for the study. Elizabeth Wesenberg, Jennifer Redd, and Vera Maranci were study coordinators at various times during the conduct of the study and taught women the methods for breast fluid expression.

References
49. Thompson HJ, Heimendinger J, Sedlacek S, et al. 8-Isoprostane F2α excretion is reduced in women by increased vegetable and fruit intake. Am J Clin Nutr 2005;82:768–76.
Effects of High Fruit-Vegetable and/or Low-Fat Intervention on Breast Nipple Aspirate Fluid Micronutrient Levels

Zora Djuric, Gang Chen, Jianwei Ren, et al.


Updated version

Access the most recent version of this article at:
http://cebp.aacrjournals.org/content/16/7/1393

Cited articles

This article cites 53 articles, 14 of which you can access for free at:
http://cebp.aacrjournals.org/content/16/7/1393.full#ref-list-1

E-mail alerts

Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions

To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions

To request permission to re-use all or part of this article, use this link http://cebp.aacrjournals.org/content/16/7/1393.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.