Correlation between Biomarkers of Omega-3 Fatty Acid Consumption and Questionnaire Data in African American and Caucasian United States Males with and without Prostatic Carcinoma

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Abstract

Results from animal studies suggest that omega-3 fatty acids from marine sources are protective against cancer. To determine whether adipose tissue and erythrocyte membrane fatty acid composition could serve as biomarkers of essential fatty acid consumption in subjects with prostate cancer, we compared fish consumption, which was estimated using a food frequency survey, to the omega-3 fatty acid content of adipose tissue and erythrocyte membranes. The study was conducted using 127 men who had undergone a prostate biopsy. All subjects were recruited from a university hospital urology clinic. African Americans comprised 23% of the subjects, and 70% were diagnosed with prostate cancer. We found a correlation of 0.44 with 95% confidence intervals (CIs) = 0.29–0.57 between reported fish consumption and the omega-3 fatty acid eicosapentaenoic acid composition in erythrocyte membranes and 0.38 with 95% CI = 0.21–0.53 when the dietary survey was compared to eicosapentaenoic acid in adipose tissue. The survey/biomarker correlations in cases were not significantly different from the correlations in controls. The study had 90% power to detect a 0.35 difference between correlations. These results suggest that the presence of prostate cancer does not affect the adipose tissue or erythrocyte membrane biomarkers of fatty acid consumption, and that erythrocyte membranes are as useful as biomarkers as is adipose tissue. Our findings corroborate previous studies that found that tissue biomarkers can reflect past fatty acid consumption and support the use of biomarkers in case-control studies using cancer patients.

Introduction

Animal studies suggest that essential fatty acid consumption may modulate the risk of contracting cancer. Rodent feeding experiments have demonstrated a prostate cancer-promoting effect of linoleic acid, an omega-6 fatty acid found in vegetable oils, whereas EPA, an omega-3 fatty acid found almost exclusively in fish oils, appears to inhibit the growth of breast, colon, and prostate cancers (1–9). In humans it is postulated that the high omega-3 fatty acid content of Eskimo diets is responsible for their low rates of cancer and cardiovascular disease (10–14).

The omega-3 fatty acids EPA and DHA and the omega-6 fatty acid linoleic acid are essential fatty acids. Although they are stored in adipose tissue as triglycerides, they cannot be generated endogenously in humans. Studies using varying techniques, including multiple diet recall questionnaires (15) and structurally labeled fatty acid tracers (16), have consistently demonstrated that the composition of stored essential fatty acids in adipose tissue reflects fatty acid composition over a period of years and is unperturbed by short-term variations in diet (17–19).

Several studies have concluded that adipose tissue fatty acid composition correlates with diet questionnaires (15, 20–24), which supports its use as a biomarker of essential fatty acid consumption for epidemiological studies. However, relatively little information is available on the validity of erythrocyte membrane fatty acid composition (25, 26) as a biomarker of fatty acid consumption. Blood specimens can be obtained more quickly and with less discomfort than fat aspirations, and the essential fatty acids incorporated into erythrocyte membranes are less likely to be obscured by large quantities of nonessential fatty acids than are fatty acids in adipose tissue samples.

The purpose of our study was to investigate whether prostate cancer affected the ability of biomarkers to reflect past fatty acid consumption and to determine whether erythrocyte membranes (reflecting fatty acid intake over months) could be substituted for adipose tissue (reflecting fatty acid intake over years) as an estimate of typical essential fatty acid consumption. We compared omega-3 fatty acid composition from adipose tissue and erythrocyte membranes with reported fish consumption from a food frequency questionnaire in prostate cancer and control patients.

1 The abbreviations used are: EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; FAME, fatty acid methyl ester.
Materials and Methods

Subjects. Subjects were recruited from men over age 45 years attending a university hospital urology clinic between July 1989 and December 1991. The men were recruited for a case-control study of prostate cancer risk in African Americans and Caucasians. Patients were referred to the clinic for either an abnormal digital rectal examination or for symptoms of bladder outlet obstruction. Few patients were referred because of an elevated serum prostate-specific antigen level because the test was just coming into common use during the accrual period. To be eligible the subjects had to have had a prostate biopsy within the last year and could not have had a previous cancer diagnosis. Control subjects were confirmed to be free of prostate cancer by prostate biopsy. This requirement lead to the accrual of fewer controls (38) than cases (89) because few urology patients underwent prostate biopsy who were not subsequently diagnosed with prostate cancer. A total of 147 potential subjects were approached, with 86% agreeing to participate. The study protocol was approved by human subjects committees at the University of North Carolina (Chapel Hill, NC) Schools of Medicine and Public Health.

Collection of Samples. A trained nurse drew 7 ml of venous blood for erythrocyte membrane fatty acid analysis into a citrated tube. The technique described by Handelman et al. (21) was used to obtain the fat sample from one of three sites: the upper arm, buttock, and/or abdomen. We found the upper arm to be the most convenient site for the patients and the nurses, and over one-half of the specimens were obtained from that site. The fatty acid composition of different biopsy sites has been shown to be similar (28). A 25-gauge needle containing 1–2 ml of 2% lidocaine with epinephrine was used to infiltrate the aspiration site. A 15-gauge needle attached to a vacutainer adapter containing an evacuated blood collection tube was inserted under the skin into the anesthetized s.c. fat pad, and a small fat sample was aspirated into an evacuated blood collection tube. Both adipose tissue and blood specimens were placed in labeled freezer tubes and stored at −70°C until analyzed. The erythrocyte membranes were prepared for storage by centrifuging to remove the plasma, WBC, and platelets from the blood sample before freezing. Acceptable adipose tissue samples were obtained from 112 participants. Approximately one-third of the subjects (48) were enrolled into the study before obtaining a pathological diagnosis. Among the remaining subjects, the mean time from diagnosis to obtaining erythrocyte membrane and adipose tissue samples was 93 days with a median of 38.5 days.

Biochemical Analysis. The samples were analyzed weekly, five at a time, in duplicate throughout the study accrual time. Because only numerical identifiers were used, the analyst was unaware of the disease status of the subjects. The reliability of the laboratory measures were assessed with both internal and external standards. Heptadecanoic acid was added to each tissue sample as an internal standard before the extract was blown dry. The solution was washed twice with 2 ml saturated NaCl. The extract was then transferred to a clean tube, and 20 µl of MeOH added as an internal standard before the extract was blown dry. The FAMEs were then suspended in 0.2 ml undecane. Free fatty acid concentrations were assayed as methyl esters by using capillary gas chromatography.

The fatty acids were extracted from erythrocyte membranes using a modification of the Bligh and Dyer method (30). An aliquot of erythrocyte suspension (0.4 ml) was vortexed in 2 ml methanol for at least 20 s to ensure an even suspension. Then 1 ml chloroform was added while vortexing for another 20 s. The extract was washed with 1 ml of 0.9% NaCl after another 2 ml chloroform were added. The phases were then separated by centrifugation for 5 min at 600 g. The lower (chloroform) phase was transferred to a clean tube, and the extract was blown dry under nitrogen at 50°C. The extract was then transferred to a clean tube and analyzed using the same procedure used with the adipose tissue samples.

Both adipose tissue and erythrocyte membrane FAMEs were analyzed on a Perkin Elmer Cetus Sigma 300 Gas chromatograph (Norwalk, CT), with helium as the carrier gas at 2 ml/min and by using a splitless injection. The esters were separated on a DB225 column (30 m × 0.25-mm, inner diameter), held for 2 min at 140°C, and then programmed up to 220°C for 17 min. The injector and detector temperatures were 220 and 250°C, respectively. The peaks were analyzed on a Shimadzu CR601 integrator (Columbia, MD). Individual fatty acids were identified using the retention times of standard fatty acid preparations (Nu Check Prep, Elysisn, MN).

Survey Analysis. Omega-3 fatty acid intake was measured using an abbreviated form of the Health Habits and History Questionnaire (HHHQ) (31), modified by the addition of four questions on the frequency and specific types of fish consumed. Seafood is the primary source of EPA and DHA in the diet. The questionnaire was administered by trained research nurses. Food frequency information was obtained during a face-to-face interview on the same day as the biological samples, and dietary information was elicited from the previous 12 months. Three subjects had inadequate survey data and were dropped from the analysis. The fish consumption questions for the remaining 124 subjects were correlated with erythrocyte membrane and adipose tissue biochemical data. Although information was collected on specific types of fish consumed, we did not adjust for the omega-3 fatty acid content of the consumed fish in the analysis.

Statistical Analyses. Estimates of fish consumption from the modified HHHQ were quantified and ranked. Correlation between the ranked quantified fish consumption and ranked omega-3 fatty acid measurements from adipose tissue and from erythrocyte membranes, respectively, were calculated for all study participants and for subgroups by race and disease status. Spearman’s ranked correlation coefficients were used because of the skewed distribution of the data. Fisher’s z transformations were used to transform the correlation coefficients so that they acquired normal distribution properties and the resultant z values were used to calculate CIs for the correlation coefficients. Differences in correlation coefficients for adipose tissue and erythrocyte membrane for cases and controls and for African Americans and Caucasians were evaluated using the transformed z values and P values were calculated by using z tests (32).
Table 1  Spearman correlation coefficients for erythrocyte membrane compared with food frequency questionnaire estimates of essential fatty acid consumption by race and disease status

<table>
<thead>
<tr>
<th>Fatty acid consumption</th>
<th>n</th>
<th>C20:5(3)EPA</th>
<th>P*</th>
<th>C22:6(3)DHA</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>124</td>
<td>0.44</td>
<td>0.41</td>
<td>C22:6(3)DHA</td>
<td></td>
</tr>
<tr>
<td>Cases</td>
<td>88</td>
<td>0.49</td>
<td>0.42</td>
<td>0.47</td>
<td>0.11</td>
</tr>
<tr>
<td>Controls</td>
<td>36</td>
<td>0.36</td>
<td>0.19</td>
<td>C22:6(3)DHA</td>
<td></td>
</tr>
<tr>
<td>African American</td>
<td>29</td>
<td>0.28</td>
<td>0.23</td>
<td>0.39</td>
<td>0.90</td>
</tr>
<tr>
<td>Caucasian</td>
<td>95</td>
<td>0.50</td>
<td>0.41</td>
<td>0.41</td>
<td>0.41</td>
</tr>
</tbody>
</table>

* P values represent a test for differences between two correlations.

Table 2  Spearman correlation coefficients for adipose tissue compared with food frequency questionnaire estimates of essential fatty acid consumption by disease status and race

<table>
<thead>
<tr>
<th>Fatty acid consumption</th>
<th>n</th>
<th>C20:5(3)EPA</th>
<th>P*</th>
<th>C22:6(3)DHA</th>
<th>P</th>
</tr>
</thead>
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<tr>
<td>Overall</td>
<td>109</td>
<td>0.38</td>
<td>0.32</td>
<td>C22:6(3)DHA</td>
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<tr>
<td>Cases</td>
<td>76</td>
<td>0.43</td>
<td>0.58</td>
<td>0.34</td>
<td>0.64</td>
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<tr>
<td>Controls</td>
<td>33</td>
<td>0.33</td>
<td>0.42</td>
<td>C22:6(3)DHA</td>
<td></td>
</tr>
<tr>
<td>African American</td>
<td>25</td>
<td>0.15</td>
<td>0.11</td>
<td>0.06</td>
<td>0.12</td>
</tr>
<tr>
<td>Caucasian</td>
<td>84</td>
<td>0.49</td>
<td>0.41</td>
<td>0.41</td>
<td>0.41</td>
</tr>
</tbody>
</table>

* P values represent a test for differences between two correlations.

Results

The mean age of the subjects was 68 years with a range from 46 to 86 years. Of the 127 subjects, 89 were diagnosed with prostate cancer and 38 were controls. Twenty-nine subjects were African Americans and 98 were Caucasians. Duplicate samples were analyzed for each participant. The paired t test comparing the two samples did not demonstrate a significant difference. The P values were 0.16 and 0.44 for EPA and DHA in erythrocyte membranes and 0.29 and 0.26 in adipose tissue, respectively.

Table 1 shows the correlation between estimates of fish consumption derived from the questionnaire and relative amounts of omega-3 and omega-6 fatty acids measured in erythrocyte membranes. The compositions of the fatty acids were expressed as a percentage of total fatty acids. The overall correlations with the survey data for EPA (r = 0.44) and DHA (r = 0.41) were both substantial and essentially equivalent with 95% CIs of 0.29–0.57 and 0.25–0.54, respectively.

When the correlations were stratified by disease status, subjects with prostate cancer had higher correlations between erythrocyte membrane essential fatty acid composition and survey fish consumption data than did subjects without prostate cancer. The differences between the correlations in cases and controls were not statistically significant for either EPA (r = 0.42) or DHA (r = 0.11). The correlation for EPA in Caucasians (r = 0.50) was much higher than that in African Americans (r = 0.28), but the difference did not reach statistical significance (P = 0.23).

Estimates of essential fatty acid consumption from adipose tissue fatty acid composition were also positively and significantly correlated with estimates from food frequency questionnaires (Table 2). We obtained an adequate adipose tissue specimen from 112 subjects, 109 of whom had also completed diet questionnaires. With a few exceptions (notably DHA in controls), all adipose tissue correlations for the omega-3 fatty acids EPA and DHA were lower than comparable erythrocyte membrane correlations. The overall adipose tissue correlations were 0.38 for EPA and 0.32 for DHA with 95% CIs of 0.21–0.53 and 0.14–0.48, respectively.

The adipose tissue stratified correlations demonstrated similar results to the erythrocyte membrane fatty acid correlations in Table 1. The presence of prostate cancer did not significantly affect the correlations. Correlations for both EPA and DHA were substantially lower for African Americans than for Caucasians. The racial differences in the correlations were not statistically significant, possibly due to the relatively small number of African American subjects. The study had 90% power to detect a 0.35 difference between two correlations but only 50% power to detect a 0.25 difference.

Table 3 compares the correlations between omega-3 fatty acids measured in adipose tissue to those from erythrocyte membrane specimens. Adipose tissue and erythrocyte membrane biomarkers of essential fatty acid consumption are well correlated, with little difference between cases and controls. The African American/Caucasian differences seen in Tables 1 and 2 remain, even in correlations between the two biological specimens.

Discussion

In this paper, we report results that support the use of erythrocyte membrane fatty acids as biomarkers of dietary omega-3 fatty acid consumption in men. Erythrocyte membrane measures were significantly correlated with self-reported dietary intakes of omega-3 fatty acids as assessed using a food frequency questionnaire. Surprisingly, the correlations for erythrocyte membrane were higher than for the adipose tissue correlations, which have been the standard biomarker for essential fatty acids.

Our study did not have the power to detect small but meaningful differences between subgroups of our population. Nevertheless, our results suggest that disease status had little effect on the correlation between either fat or erythrocyte membrane biomarkers and the survey data. Erythrocyte membrane and adipose tissue essential fatty acid correlations with diet did not differ significantly by disease status or by race, implying that the assay can provide a useful biomarker in case control studies. Our study had 90% power to find a relatively large (0.35) difference between correlations, but smaller differences in correlation between racial groups or between cases and controls were much less likely to be detected.

Our study population does not reflect the general population because it consists of men who had undergone a prostate biopsy in a urology clinic. However, the subjects were not selected with reference to their fatty acid consumption, and there is no reason to suspect that the correlation between reported fatty acid consumption and consumption estimated using biomarkers is different in the study population than that of the general population.

Our correlation results are consistent with research by Hunter et al. (24), comparing food frequency and food record data with adipose tissue fatty acid composition. Their Spearman correlation coefficient for EPA comparing a food frequency questionnaire and adipose tissue was 0.47. Tjonneland et al. (33) reported EPA and DHA Spearman correlation coefficients...
for two 7-day diet records and adipose tissue fatty acid composition from 23 Danish males as 0.28 and 0.36, respectively. London et al. (34) found a correlation for the sum of EPA and DHA content of 0.43 between adipose tissue and a food frequency questionnaire for 115 American women. We found biomarker/dietary survey correlations for EPA and DHA of 0.38 and 0.32 in adipose tissue, respectively, and of 0.44 and 0.41 in erythrocyte membranes, respectively.

A major methodological difficulty in studying the relationship between consumption and prostate cancer risk is the use of self-report dietary assessment questionnaires. Dietary survey methods used to rank individuals in epidemiological studies are subject to misclassification error, possibly masking small but meaningful differences in the consumption of specific fatty acids (35). Different species of fish contain variable amounts of omega-3 fatty acids. For example, sole, perch, and flounder have almost no omega-3 fatty acid, whereas lake trout has about as much omega-3 fatty acid as tuna. Both the HHHQ and Willett questionnaires contain too few items to assess high versus low omega-3 fatty acid containing types of fish. The picture is further complicated by intraspecies variation in fatty acid content. Depending on factors such as water temperature and season, different species of salmon vary almost 7-fold in EPA content (36). Assessing the omega-3 fatty acid consumption of middle-aged and elderly males may be particularly difficult because these individuals often do not prepare food and may not be aware of the specific species of fish they are consuming.

Biomarkers can present a more objective measure of dietary exposure. However, requiring adipose tissue samples may greatly complicate subject recruitment and retention. The use of erythrocyte membranes offers advantages over adipose tissue biopsy in that the procedure is less invasive, and blood samples are technically easier to analyze for omega-3 fatty acids.

In this study, the correlations of erythrocyte membrane and dietary questionnaire omega-3 fatty acids were consistently higher than that for adipose tissue. In the analysis of omega-3 fatty acids in adipose tissue, measurement error may account for the correlations that are lower than those using erythrocyte membranes. Because omega-3 fatty acids are such a small proportion of total fatty acids found in adipose that it is difficult to measure them accurately. Another problem with using tissue biopsies to validate dietary intake reports is the variation in tissue metabolism. The tissue measurements of fatty acid proportions reflect not only dietary consumption but also the modification of fatty acids through: (a) desaturation and chain elongation by the liver and other tissues; and (b) selective fatty acid acylation into specific phospholipids.

The correlations of both erythrocyte membrane and adipose tissue fatty acids with dietary data were somewhat lower for controls and for African Americans. Among Caucasians, 68% completed >12 years of school, compared to only 7% of African Americans, and the lower educational levels may have affected the ability of the African American subjects to accurately complete the questionnaire. It is possible that cultural differences in food choices or food preparation may account for the differences. For example, the lower correlation could be explained if African Americans ate similar amounts of fish to Caucasians, but the fish consumed by African Americans was consistently lower in omega-3 fatty acids.

Where erythrocyte membranes were compared to adipose tissue, the lower correlations for African Americans were unexpected. The small numbers of African Americans may have yielded unstable correlation estimates, or the lower correlations between biochemical estimates of essential fatty acid consumption could be due to racial differences in the transportation, storage, or mobilization of essential fatty acids. Although the differences are not statistically significant, the EPA correlation difference between African Americans, \( r = 0.16 \) (95% CI = –0.23–0.52) and Caucasians, \( r = 0.49 \) (95% CI = 0.31–0.64), is quite large.

A possible source of bias in case control studies using biomarkers of fatty acid consumption is systematic changes in tissue fatty acid composition due to metabolic changes caused by the malignancy. A study could erroneously find higher omega-3 fatty acid consumption among controls only because the cases that had poor correlation between omega-3 fatty acid consumption and the measured tissue composition and not because of true differences in consumption between cases and controls. Disproportionate weight loss among the cancer cases may also affect fatty acid composition, although neither a 20% weight loss nor a 10% weight gain has been shown to affect EPA or DHA levels in adipose tissue (37). Although the number of controls in this study was not large, this study did not demonstrate a trend toward lower correlation between biomarker and questionnaire data in patients with prostate cancer.

In summary, we found significant correlations between a dietary history of omega-3 fatty acid consumption and erythrocyte membrane fatty acid composition, both in prostate cancer patients and in control subjects. The presence of prostate cancer did not affect the correlations, but the correlations were lower in African Americans than in Caucasians. The lack of large differences in the correlations between cases and controls provides support for the use of erythrocyte membranes as a biomarker of essential fatty acid consumption in place of adipose tissue, which is more difficult to both obtain and analyze. Furthermore, these findings support the use of biomarkers in case control studies where samples are taken from cancer patients, as well as from control subjects without cancer. Because of the ease with which erythrocyte membranes can be assayed, we believe that assaying erythrocyte membrane fatty acid composition may be the most feasible method to estimate omega-3 fatty acid consumption.

Acknowledgments
The authors would like to thank Victor Schoenbach, Ph.D. for assisting with manuscript preparation.

Appendix

<table>
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<tr>
<th>Subgroup</th>
<th>Range</th>
<th>Mean</th>
<th>Median</th>
</tr>
</thead>
<tbody>
<tr>
<td>African Americans</td>
<td>0–8.1</td>
<td>2.6</td>
<td>2.1</td>
</tr>
<tr>
<td>Caucasians</td>
<td>0–9.5</td>
<td>1.9</td>
<td>1.5</td>
</tr>
<tr>
<td>Cases</td>
<td>0–8.1</td>
<td>1.8</td>
<td>1.4</td>
</tr>
<tr>
<td>Controls</td>
<td>0–9.5</td>
<td>2.8</td>
<td>2.5</td>
</tr>
<tr>
<td>Total</td>
<td>0–9.5</td>
<td>2.1</td>
<td>1.6</td>
</tr>
</tbody>
</table>

References


Correlation between biomarkers of omega-3 fatty acid consumption and questionnaire data in African American and Caucasian United States males with and without prostatic carcinoma.

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