Differences between Plasma and Adipose Tissue Biomarkers of Carotenoids and Tocopherols

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Abstract

Biomarkers of dietary exposure or nutritional status are sought actively to overcome limitations of traditional dietary methodology. We compared plasma and adipose tissue biomarkers for carotenoids and tocopherols. The data consisted of samples from 91 men and 122 women, ages 45–70 years, from the control group of the European Community Multicentre Study on Antioxidants, Myocardial Infarction, and Cancer of the Breast (EURAMIC) Study. Pearson correlations between plasma and adipose tissue measurements for β-carotene, lycopene, and α-tocopherol adjusted for smoking status displayed low, although significant, correlations of 0.39, 0.24, and 0.39, respectively. The correlation was further stratified by sex. After being corrected for measurement error using deattenuation factors obtained from a reproducibility study, the stratified correlation coefficients were as high as 0.80 for β-carotene in men, 0.62 for lycopene in women, and 0.52 for α-tocopherol in women. In addition, plasma and adipose tissue measurements from the myocardial infarction (MI) subset of the EURAMIC study population were used to evaluate the odds of MI, adjusting for confounders. We found that the concentration of lycopene in plasma was not positively associated significantly with MI (odds ratio, 1.78; P = 0.26). Adipose tissue lycopene, in contrast to reports elsewhere on the total population, showed an inverse association with MI (odds ratio, 0.62; P = 0.15). These results suggest that plasma and adipose carotenoids represent different markers for nutritional status and cannot be used interchangeably in epidemiological and dietary validation studies.

Introduction

Carotenoids are common constituents of fruits and vegetables, the consumption of which is considered to be associated with reduced risk of cardiovascular diseases and cancer in general. The bioavailability of carotenoids is affected by food preparation in the presence of lipids and the functional status of the intestine (1). Of the >600 different carotenoids identified, ~40 of them can be metabolized to retinol. In humans, dependent on the presence of fat in the meal, appreciable quantities of intact carotenoids are absorbed and can be found in circulating plasma and later in adipose tissue stores.

Vitamin E (α-, β-, γ-, and δ-tocopherol and α-, β-, γ-, and δ-tocotrienol) status is often described by its concentration in serum. However, the plasma level of α-tocopherol changes very rapidly after changes in dietary intake within a few days (2, 3). Because serum tocopherol levels reflect short-term intake, multiple independent assessments are necessary to distinguish interindividual tocopherol level reliably (4). On the other hand, adipose α-tocopherol levels have been shown to respond slowly to dietary changes in experimental animals and humans (5, 6). It has been estimated that ~90% of the total body content of tocopherols is found in adipose tissue (7). Tocopherols are also distributed homogeneously over adipose tissue stores in the body (8). Consequently, the tocopherol concentration in adipose tissue could be an alternative biological measure of tocopherol intake.

Many epidemiological studies addressing the relationship between dietary antioxidants and cardiovascular diseases or cancer have been criticized because of the inherent problems in obtaining reliable data on the long-term nutrient exposure of individuals (8–10). Food composition data may be unavailable or inappropriate because food frequency questionnaires tend to result in overreporting of carotenoids. Dietary questionnaires are also difficult to quantitate. Some individuals are poor at estimating portion sizes. Finally, large intraindividual day-to-day variations in diet can constitute an important source of error (11). As a result, several independent measurements may be required to characterize individuals if dietary recalls are used (8).

Biomarkers of dietary intake, especially those providing an integrated exposure measure of nutritional status, can offer an alternative to questionnaire-based diet assessment by assessing nutrient levels directly in tissue. Biochemical analyses are quantitative measures of the nutrient of interest and are not subject to biased reporting or faulty recall. These levels are,
however, affected by both genetic and life-style factors, as well as the interaction with intake of other nutrients (12). Dietary biomarkers also reflect biological parameters that affect consumption of the nutrients of interest during the relevant time period. As a medium, adipose tissue biopsy may have advantages over measurement of plasma levels for fat-soluble substances. Unlike plasma, which for some nutrients may only indicate recent rather than long-term exposure (13), adipose tissue may provide a useful indicator for long-term average intake for many fat-soluble nutrients, which is often of greater interest in epidemiological research (14).

The purpose of this study was to examine the relationship between plasma and adipose antioxidants, i.e., α- and β-carotene, retinol, lycopene, and α- and γ-tocopherol, after adjusting for measurement error (12, 15). It also compares the associations between MI2 and antioxidants, which were modeled using both plasma- and adipose tissue-based estimates of exposure.

Materials and Methods

Study Population. This study was conducted as part of the multicenter EURAMIC Study on antioxidants and MI and cancer of the breast. The study design was described in detail earlier (16). Briefly, it was a case-control study encompassing nine European countries and Israel to include subjects with a large variation in antioxidant intake, which increases the potential for confounding. Groups of cases and controls were selected from each of the participating countries. The disease end points of first acute MI in men and early breast cancer in women were chosen. Subjects in the study were men and women, ages 45–60 and 50–75 years, respectively. Controls were subjects without a previous history of the diseases, as described above, recruited from the population in the catchment area and frequency matched to cases by 5-year age intervals. For the purpose of examining the relationship between plasma and adipose carotenoid and tocopherol concentrations, only the controls with both plasma and adipose tissue micronutrient measures in this study were included in the analyses. This avoided any potential influence of disease status on tissue antioxidants.

To account for the measurement error in plasma and adipose tissue antioxidants, a population with repeated measurements from the Netherlands was used to study reproducibility. The details of this study have been described by Kardinal et al. (12). Briefly, a comparable population for the EURAMIC study, consisting of 56 healthy volunteers, 30 men and 26 women, ages 50–70 years, were recruited, and two samples of plasma and adipose tissue for each subject (4 months apart) were collected. These subjects were nonsmokers, did not use vitamin supplements or prescribed medication that might alter the status of antioxidants of interest, had stable food habits, and had not lost weight in excess of 5 kg during the previous year.

Data Collection and Biochemical Analyses. Subcutaneous adipose tissue was taken from the buttocks by needle aspiration (17). Samples were stored at −70°C until analysis, as described previously (16). Nonfasting venous blood samples were drawn in a 10-ml heparin tube and refrigerated until the plasma was separated. Blood was centrifuged within 2 h after drawing, and the separated plasma was stored at −80°C. Concentrations of α-tocopherol, γ-tocopherol, retinol, lycopene, α-carotene, and β-carotene in adipose tissue and plasma were determined by reversed-phase high-performance liquid chromatography and spectrophotometric detection at the TNO Nutrition and Food Research Institute in Zeist (18–20). The coefficients of variation for the analysis of retinol, β-carotene, and α-tocopherol in adipose tissue were 4.3, 7.1, and 4.9%, respectively (12). For determinations in plasma, coefficients of variation were 4.3% for retinol, 7.3% for β-carotene, and 3.2% for α-tocopherol, respectively. Samples were protected from light during these analyses. Quality control was assured by analyzing reference samples in each run and evaluating the within- and between-run analytical variation. Standard Reference Material from the National Institute of Standards and Technology (Gaithersburg, MD) has been tested by the Swiss Vitamin Institute, which has also participated in a Micronutrients Measurements Quality Assurance program for fat-soluble vitamins, carotenoids, and vitamin C with the National Institute of Standards and Technology laboratory. Equal numbers of samples from cases and controls were analyzed in each run. Results of micronutrient assays were validated, and values derived from faulty chromatograms or samples containing an extremely small amount (<10 mg) of fat aspirate were excluded. Information on socioeconomic status, familial history, smoking habits, alcohol intake, and anthropometric measures was also collected by questionnaire for all subjects.

Data Analysis. Plasma and adipose carotenoids and tocopherols had significant nonnormal distributions in the study sample. Because the assumption of normality is central for Pearson correlation and linear regression analyses, most of the micronutrient values were normalized by applying the natural logarithm transformation. For others that could not be normalized by the natural logarithm, the Box-Cox regression algorithm available as part of quality control module in SAS (21) was used to determine the optimum transformation factor. The transformation factors are noted in the footnote of Table 2. Valid data on both plasma and adipose micronutrients were available for 208–211 subjects, depending on the micronutrient in question.

Relationships between plasma and adipose carotenoids and tocopherols were assessed through a combination of correlation analyses, graphic plots, and regression analyses. The regression analyses used the forward selection procedure to identify the variables with significant predictive power in modeling each of the micronutrients. The results suggested that sex and smoking status could be important predictive factors for many of the micronutrients. Correlation analyses conditioned on smoking status were thus stratified by sex in addition to simple pooled analyses.

Deattenuation factors calculated on the basis of plasma and adipose micronutrient measures from the reproducibility study described by Kardinal et al. (12) were used. Within- and between-individual variances were calculated with and without stratification on sex. The deattenuated correlation coefficient \( r' \) was then calculated as follows:

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\rho' = \rho \left( \frac{1 + \frac{W_p}{B_p}}{1 + \frac{W_a}{B_a}} \right) \]

where \( \rho \) is the observed correlation coefficient, \( W_p \) and \( W_a \) are the within-person variances for adipose and plasma micronutrients, respectively, and \( B_p \) and \( B_a \) are the between-person variances for adipose and plasma micronutrients, respectively.

Adipose tissue concentrations may be a function of total fat mass. To account for this, the total stores of both carotenoids and tocopherols were used for approximation in addition to the

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1 The abbreviations used are: MI, myocardial infarction; EURAMIC, European Community Multicentre Study on Antioxidants, Myocardial Infarction, and Cancer of the Breast; OR, odds ratio.
measured concentrations relative to the specimen sample. Each individual’s nonlean body mass was derived from height and weight measurements, according to the method of Womersley and Durnin (22). The resulting figure for total body fat was then multiplied by the concentration of carotenoids and tocopherols in the adipose tissue sample to create an estimated body burden of carotenoids and tocopherols. The correlation of plasma micronutrients with their adipose tissue counterparts was evaluated, both for micronutrients expressed as concentrations per unit of total fatty acids measured and for total estimated body burden of micronutrients carried in fat.

To test for differences in correlation coefficients, a single sample test based on the Z-statistic was used, as described in Kleinbaum et al. (23). The formula for testing two correlation coefficients to be equal ($H_0: \rho_1 = \rho_2$) is as follows:

$$Z = \sqrt{\frac{(1 - r_1^2) + (1 - r_2^2)}{2r_12r_2 - r_1^2r_2(1 - r_1^2 - r_2^2)}}$$

This formula adjusts for the fact that the measured correlations are not independent. Under the assumptions of large sample size, the statistic is approximately normal. The normal probability function (PROBNORM) in SAS (21) was used to compute $P$ for the difference of correlation coefficients.

Finally, to illustrate the use of both plasma and adipose tissue as markers of micronutrient status in an epidemiological study, results drawn from the EUROMIC study pertaining to men in the case-control study of MI were examined. The analyses were restricted to those who had both plasma and adipose tissue lycopene and $\beta$-carotene measures available. Logistic regression models conditioned on age were used to calculate the OR for the contrast between 25th and 75th percentiles using the two different measures. Results for both concentration-based and total body fat burden-based measurements are included.

### Results

#### Population Characteristics

Table 1 shows the characteristics of the main study population. The mean ages for men and women were 52.5 and 61.3 years, respectively. Women were more likely to use vitamin C supplements than were men. The distributions of body mass index and alcohol and cigarette use were very similar between men and women. There were differences in mean concentrations of micronutrients between men and women for all micronutrients examined except plasma $\alpha$-tocopherol and $\beta$-carotene and adipose tissue retinol and $\gamma$-tocopherol.

### Pooled Correlation

Crude and deattenuated correlation coefficients for plasma and adipose tissue adjusted for smoking status micronutrients are presented in Table 2. With all control subjects considered, there were significant associations between plasma and adipose tissue measures for $\beta$-carotene ($r = 0.39$), lycopene ($r = 0.24$), and $\alpha$-tocopherol ($r = 0.39$). A significant inverse correlation between plasma retinol and adipose tissue $\alpha$-carotene was also noted ($r = -0.22$). After deattenuation, the correlation coefficients increased to 0.52, 0.41, and 0.51 for $\beta$-carotene, lycopene, and $\alpha$-tocopherol, respectively. Retinol concentrations in plasma and adipose tissue were not significantly correlated.

### Stratified Correlation Analyses

In addition to the results from forward selection regression models (not shown), the graphic plot illustrated in Fig. 1 for the correlation between plasma and adipose tissue lycopene suggests that sex was an influential factor for the correlation (slope) examined. The correlation analyses were therefore stratified by sex. Overall, $\beta$-carotene concentrations in plasma and adipose tissue were correlated significantly in both men and women. The significant correlation coefficients of plasma and adipose tissue $\beta$-carotene were 0.33 for women and 0.59 for men. The deattenuated correlation coefficient reached as high as 0.80 for men. Plasma and adipose tissue lycopene concentrations were statistically significantly correlated for both sexes as well.
correlation coefficients were 0.62 and 0.43 for women and men, respectively. Plasma \( \alpha \)-tocopherol was significantly correlated with adipose tissue \( \alpha \)-tocopherol and poorly correlated with \( \gamma \)-tocopherol. The deattenuated correlation coefficients for plasma and adipose tissue \( \alpha \)-tocopherol were 0.52 women and 0.44 for men when adjusted for smoking status.

**Adipose Tissue Concentration and Body Fat Burden.** Although it may be hypothesized that the total body fat burden of a micronutrient better reflects long-term exposure or uptake of fat-soluble micronutrient compared with the simple expression of concentration, it was unknown which of these indices would correlate better with plasma micronutrient measurements. Table 3 contrasts the correlations between the plasma and adipose tissue measures when adipose tissue micronutrients are expressed in terms of concentration and in terms of body fat burden. All correlations observed for direct comparison of like nutrients were higher for concentration-based micronutrient measurements compared with body fat burden-based measurements. The difference in correlation coefficients obtained using burden- rather than concentration-based adipose tissue micronutrient measures for \( \beta \)-carotene was statistically significant (based on Fisher’s Z-test). Nonsignificant differences in correlations were seen in lycopene, retinol, and \( \alpha \)-tocopherol.

**Comparison of ORs.** The results for both plasma and adipose tissue as markers of micronutrient status in an epidemiological study are presented in Table 4. Data pertain to males with and without MI. With plasma measurement, \( \beta \)-carotene was in-
versely but lycopene positively associated with MI. None of these associations was statistically significant. The adipose tissue β-carotene concentration showed a marginally significant inverse association with MI (OR, 0.33; \( P = 0.06 \)), whereas the adipose tissue lycopene concentration displayed a milder inverse association with MI (OR, 0.62; \( P = 0.16 \)). When the total body fat burden of these two micronutrients was used to examine the associations with MI, more significant associations were seen for both micronutrients. The OR for MI associated with total body fat β-carotene burden was 0.31 and was statistically significant (\( P < 0.01 \)). The total body fat lycopene burden was inversely associated with MI, with \( P \) decreasing from 0.16 to 0.14 when body fat burden was used instead of the simple adipose tissue lycopene concentration.

**Discussion**

Plasma and adipose tissue carotenoids and tocopherols have been used as dietary biomarkers and nutritional status indices of these micronutrients. This study examined whether there is a difference between plasma and adipose tissue measures. We found significant but weak correlations for β-carotene, lycopene, and α-tocopherol between the plasma and the adipose tissue measures with pooled \( r = 0.39, 0.24, \) and 0.39, respectively. Noticeable differences were seen between men and women. However, the differences were not systematic enough for general conclusions about physiological distinctions by sex across all nutrients. Nevertheless, the differences seen did suggest that the correlation examined should be stratified by sex. After stratification, the correlation coefficient reached as high as 0.80 for β-carotene in men and 0.62 for lycopene in women for this study population. For all nutrients, however, the measures remained sufficiently dissimilar as to question their interchangeability.

The potential effect of measurement error is particularly important for studies addressing relatively weak associations (24), which are common in nutritional epidemiology. To address this problem, the correlations examined in this study were adjusted for errors in the measurement of plasma and adipose tissue micronutrients, using deattenuation factors derived from an independent sample population of similar characteristics with repeated plasma and adipose tissue micronutrient measurements. The correlations were attenuated as little as 18% for α-tocopherol measurements in women and as much as 130% for α-carotene measurements in men. Of the micronutrients examined, the smallest improvements from deattenuation occurred for α-tocopherol and β-carotene. This may be attributable to greater accuracy in measuring these two nutrients, greater stability in the concentrations of these nutrients within an individual over time, or both. The micronutrients α-tocopherol and β-carotene may, therefore, be the best among the examined micronutrients for use as dietary biomarkers in nutritional epidemiological studies. Retinol in the body is under tight homeostatic control, which may explain why the correlation between plasma and adipose tissue retinol seen was minimal.

The concept of total body nutrient stores has been introduced in very few other studies. Our study found a difference for some carotenoids and tocopherols between the concentration expressed as units of aspirated fat tissue and the concentration expressed as total body fat stores. Using nonsmokers as an example, the plasma measure of β-carotene was significantly more closely correlated with adipose tissue β-carotene than with total fat β-carotene burden. Other nutrients examined did not show a significant difference for the correlation between the two measurement expressions. However, we noticed apparent differences when examining the association between MI and carotenoids measured in plasma, in adipose tissue as concentration, and as total body fat stores. Using β-carotene and lycopene as examples, we demonstrate that the associations between MI and β-carotene were highly significant for the body fat stores measurement but not significant for the plasma measurement. MI was marginally associated with β-carotene concentration in adipose tissue. In addition, MI was found to be positively associated with plasma lycopene but inversely associated with the measures in adipose tissue and total body stores. It appears that micronutrient measures in different tissues represent very different aspects of micronutrient status in body.

This study examined the difference between two measures of carotenoids and tocopherols, which are used as biomarkers for nutritional status. We have found that they are dissimilar enough to suggest that they measure two different exposures. We were unable to determine the causes of the differences in the correlations between men and women.

The evaluation of the association between MI and carotenoids in plasma and adipose tissue suggested that adipose tissue measurements, especially when expressed as total body fat stores, was associated with MI better than the plasma measurements. These results, however, do not provide information on whether an adipose tissue nutrient concentration or total body fat burden of the same nutrient is a better predictor of nutritional status. The stronger association could be simply an indicator of exhausted adipose storage of β-carotene or lycopene due to the existing MI status because this is a case-control study. Additional studies will be needed to answer that question.

In conclusion, biochemical measurements are considered to be independent of systematic endogenous errors common to other methods such as food frequency questionnaires or dietary records (25), especially in a multicountry design like the EURAMIC study. However, biochemical measurements do not provide a perfect measure of intakes because factors other than diet may influence the concentration of nutrients in blood and tissue. In addition, the distribution and storage of nutrients in blood and tissues could differ substantially, depending on the hydrophilic nature of the ingested nutrient as well as its interactions with other molecules. For fat-soluble vitamins, which are stored in adipose tissue, adipose tissue may thus be a better marker of nutritional status than plasma. A number of studies (26–31) have examined the relationship between dietary intake and plasma nutrient measurements and shown similar crude levels of association between plasma and adipose tissue fat-soluble micronutrients. These findings suggest that adipose tissue and plasma biomarkers are not interchangeable markers of nutritional status for epidemiological or dietary validation studies.

**Acknowledgments**

We thank Cary Crogan at the University of North Carolina at Chapel Hill for work on statistical analyses and Drs. Steven H. Zeisel and Robert Millikan for editorial comments on the manuscript.

**References**


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