Current Research

Serum Carotenoid and Tocopherol Concentrations Vary by Dietary Pattern among African Americans

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ABSTRACT

Background Intakes and biochemical concentrations of carotenoids and tocopherols have been associated with chronic diseases.

Objective To describe dietary patterns in Jackson Heart Study participants and to determine if biochemical measurements of antioxidants differ across these.

Design Cross-sectional analysis of data for 373 African-American men and women (age 35 to 80 years), participating in the Diet and Physical Activity Substudy of the Jackson Heart Study.

Methods Dietary intake was assessed with a region specific food frequency questionnaire. Patterns were defined by cluster analysis of food groups, as percent of energy intake.

Results Four dietary patterns were identified: fast food, Southern, prudent, and juice. Individuals in the fast-food pattern (n=153) had significantly lower serum concentrations of lutein plus zeaxanthin and β-cryptoxanthin; those in the Southern cluster (n=99) had significantly lower serum α-carotene; and those in the prudent (n=63) and juice (n=58) clusters had significantly higher serum α-carotene and β-cryptoxanthin (P<0.05) relative to those in at least one other cluster (all P<0.05). The juice cluster also had higher serum α-tocopherol concentrations relative to the fast-food cluster.

Conclusions Diets high in fast foods, snacks, soft drinks, and meat were associated with relatively low concentrations of carotenoids and α-tocopherol. This pattern contained the largest number of participants, and could contribute to the extensive health disparities seen in this region.


Empirical dietary patterns (generated by cluster or factor analysis) have been used to describe dietary behaviors of populations (1,2), associations of dietary behaviors with biochemical parameters and biomarkers (3,4), and as risk factors in the development of several diseases, including cardiovascular disease (5,6), cancer (7,8), diabetes (9), and mortality (10). There are several reasons why researchers consider dietary pattern analyses complementary to traditional single-nutrient analysis. Dietary exposures are highly correlated to each other. People do not eat isolated nutrients, but meals consisting of various foods that have a combination of nutrients. Nutrients work synergistically, and separation of specific effects of single nutrients or foods on disease outcomes can often be difficult. Dietary patterns derived from habitual food consumption represent a combination of foods and nutrients and thus, their synergistic effect on outcomes (11,12).

Suboptimal intakes of antioxidants such as vitamins C and E and the carotenoids are thought to play an important role in the etiology of several diseases in human beings, including heart disease (13). In the United States, the prevalence of heart disease among African Americans is higher than that of whites. Also, relative to other states and the District of Columbia, the state of Mississippi fares poorly in terms of cardiovascular disease mortality (14). Most of the physiological, environmental, and genetic factors that account for this excess cardiovascular disease in African Americans remain uncertain. There are limited prospective epidemiologic data relating risk factors and cardiovascular disease in African-American populations (15). The Jackson Heart Study was therefore initiated to investigate the causes of cardiovascular disease in an all African-American cohort based in Jackson, MS.

Given the important role antioxidant nutrients play in prevention of chronic disease, and the unique opportunity the study of dietary pattern analysis presents in nutritional epidemiology, the aim of our study was, primarily, to characterize the dietary patterns of a subset of the Jackson Heart Study participants using a culturally suitable dietary...
assessments of these patterns with serum carotenoid and tocopherol concentrations. It was hypothesized that a dietary pattern low in fruit and vegetable consumption would be associated with lower concentrations of antioxidant nutrients.

SUBJECTS AND METHODS

Study Population

The men and women in this cross-sectional analysis were participants of the Diet and Physical Activity Sub-Study of the Jackson Heart Study, a single-site prospective epidemiologic investigation of cardiovascular disease among African Americans from the Jackson, MS, metropolitan area. Data collection for the Jackson Heart Study began in late 2000 and was completed in early 2004. The Jackson Heart Study design initially included participants from the Jackson cohort of the Atherosclerosis Risk in Communities study, an additional sample of randomly selected adults in the community, a family component and a structured volunteer sample. A more detailed description of the original study has been published elsewhere (16,17).

A subset of participants (n=499) from the Jackson Heart Study cohort (N=5,302) was selected for the Diet and Physical Activity Sub-Study. As participants were enrolled in the Jackson Heart Study, investigators recruited participants for Diet and Physical Activity Sub-Study to include an equal number of men and women from younger (age 34 to 64 years) and older (65 years and older) age groups, from lower and higher socioeconomic status, and from lower and higher physical activity groups. All eligible participants were invited to be part of Diet and Physical Activity Sub-Study until each of the enrollment strata were filled. The Institutional Review Board of the University of Mississippi approved the Diet and Physical Activity Sub-Study protocols, and all subjects gave written informed consent for their participation.

Study Design

The aim of Diet and Physical Activity Sub-Study was to provide data for validation of the diet and physical activity instruments used for the entire cohort of the Jackson Heart Study. The design and data collection methods used for the dietary portion of this sub-study have been described previously (18). Briefly, all Jackson Heart Study participants were first asked to complete a short, (158 item) food frequency questionnaire (FFQ) during their initial clinic visit. Blood samples were obtained at this time. Members of the Diet and Physical Activity Sub-Study subset were then scheduled to complete four, nonconsecutive, 24-hour recalls across several months. Approximately 6 months after the initial visit, participants completed the original, 283 item FFQ developed for use with the US Department of Agriculture Lower Mississippi Delta Nutrition Intervention Research Initiative (18). This last FFQ was used to determine dietary patterns for our analysis. This was selected as the most complete measure of long-term usual intake.

Dietary Assessment

The FFQ used in this analysis was developed from 24-hour dietary recall data, previously collected by telephone survey with both African-American and white adults living in the Mississippi Delta region by the Lower Mississippi Delta Nutrition Intervention Research Initiative. The FFQ was developed specifically for a southern population. Serving sizes were adapted for this population and regional foods such as ham hocks, chitterlings, and grits, were included to capture the regional eating patterns. Details regarding development and validation of this regional FFQ are available elsewhere (19-21). As designed, the reference period for the FFQ was the previous 6 months. Actual data collection was frequently delayed due to difficulty in scheduling. Therefore the length of time between baseline blood draw and final FFQ administration ranged from 6 months to more than 1 year.

All dietary data were collected during face-to-face encounters by trained interviewers from the community. The baseline (158 item) FFQ was administered by clinic staff, while the recalls and the 283 item FFQ was administered by registered dietitians. Extensive quality control was conducted. Five percent of the FFQ administrations were audio taped and received a secondary review by Diet and Physical Activity Sub-Study staff. Interviewers were retrained whenever review of these tapes suggested problems with accuracy or completeness. After nutrient analysis, additional quality control was conducted by identification of micro- and/or macronutrient outliers and verification with original data forms.

Laboratory Analyses

On the morning of the baseline interview, participants provided fasting (12-hour) blood samples. These were collected in vacutainer tubes and centrifuged at 3,000 g for 10 minutes at 4°C. Serum was separated, frozen and stored at −70°C until analyzed for carotenoids and tocopherols. Analyses of serum were conducted at the end of Diet and Physical Activity Sub-Study data collection, thus samples were stored for approximately 6 months to 3 years. Analyses were performed using high-performance liquid chromatography, as described by Yeum and colleagues (22,23). After standard lipid extraction with chloroform:methanol (2:1) followed by hexane, samples were analyzed for carotenoids and tocopherols using a reverse-phase high-performance liquid chromatography system consisting of a 600S controller (Millipore, Milford, MA), Waters 616 pump, Waters 717 autosampler, Waters 996 photodiode array detector, and C30 carotenoid column (3 μm, 150x4.6 mm) (YMC, Wilmington, NC). Millennium32 was the operating system. The programmable photodiode array detector was set at 445 and 455 nm for carotenoids and 292 nm for tocopherols. Carotenoids and tocopherols were quantified by determining peak areas in the high-performance liquid chromatography chromatograms, calibrated against known standards. These analyses were conducted at the Human Nutrition Research Center on Aging at Tufts University, Boston, MA. Samples were run against standard references from the National Institute of Standards and Technology for quality control; the intra-assay coefficients of variation for most carotenoids and tocopherols were <5%. Serum choles-
terol concentrations were determined according to methods described previously (24).

**Assessment of Covariates**

Information on age, smoking status, physical activity, and education level was collected by questionnaire at either the initial home interview or at the time of the participant’s clinic visit. Vitamin and/or mineral supplement use (yes or no) and use of vitamin E supplement use of 400 IU or higher (yes or no) was obtained from FFQ. Participant height and weight were measured in an exam gown with no shoes by trained technicians at the clinic visit. Anthropometric procedures were conducted at clinic visit as previously described (16). Body mass index (BMI) was calculated as kg/m².

**Statistical Analyses**

Diet and Physical Activity Sub-Study participants without serum samples for antioxidant analysis (n=39), without an FFQ (n=1), with >10% of questions blank on the FFQ (n=3), or with energy intake estimates outside the plausible range (≤600 kcal or ≥4,000 kcal, n=27) were excluded from these analyses. This resulted in a sample size of 429 participants. Before analysis, the 283 items on the Delta Nutrition Intervention Research Initiative FFQ were sorted into 33 food groups (see the **Figure**), based on similarity or difference in nutrient content and general use. The percentage of energy contributed from each food group was calculated and used in the cluster analysis. This was done to avoid biased grouping due to variation in body size and energy requirement.
Cluster analysis was performed using the FASTCLUS procedure in SAS (version 9.1, 2002-2003, SAS Institute Inc, Cary, NC). Cluster seeds were first assigned by the program at approximate locations. The Euclidean distance from each subject to each cluster center was calculated, and the subject was assigned to the nearest cluster center. The seeds were then replaced within the revised clusters, and the distance calculations and assignments were repeated in an iterative process until no further changes occurred. Individuals with energy contributions that were >5 standard deviations from the mean of any food group variable were removed from the clustering analysis, as has been done previously (8,25). Applying these criteria, 56 outliers were identified and removed. A series of cluster analyses, with three to eight clusters specified, was performed to identify the most meaningful set of patterns. The four-cluster solution was selected as most interpretable.

Serum measures of carotenoids and tocopherols were log transformed before analysis. Means and standard deviations for energy contributions for the 33 food groups were calculated by cluster. Nutrient intakes, sociodemographic and health characteristics were assessed across clusters using generalized linear models (with Tukey-Kramer’s adjustment for multiple comparisons) for continuous data and the χ2 test for variables expressed as proportions. For each of the clusters, means±standard errors were calculated for serum α- and β-carotene; lutein plus zeaxanthin; β-cryptoxanthin; lycopene; and α-, γ-, and δ-tocopherols after adjusting for several covariates. For analyses with γ- and δ-tocopherol, we excluded seven and 112 participants, respectively, with serum concentrations below detectable levels. α for all analyses was set at the .05 level.

### RESULTS

More than 60% of study participants were women. The average age was 61 years with a range of 35 to 80 years. Women had a slightly higher age adjusted BMI (31.9 vs 29.6, P<0.05) with a significantly higher percentage of women than men reporting taking dietary supplements (66% vs 54%; P<0.05). Four distinct dietary patterns emerged from the cluster analysis: fast food, snacks, soft drinks, and meat; cereal, milk, and fruit; corn products and bread; and fruit juice (Table 1). These describe foods that contribute most uniquely to the cluster, using the criteria that intakes were significantly higher than for at least two of the three other clusters, and that mean intakes were at least 20% greater than any other cluster.

For brevity and consistency with recently published studies, we will call these clusters fast food, prudent, Southern, and juice. Participants in the fast-food cluster were younger than those in two other clusters (Table 2). The highest percentage of women was in the prudent cluster. Although par-

<table>
<thead>
<tr>
<th>Table 1. Percentage of energy contribution from selected food groups across the four dietary patterns identified among the participants of the Diet and Physical Activity Sub-Study of the Jackson Heart Study</th>
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<tbody>
<tr>
<td><strong>Dietary Pattern</strong></td>
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<tr>
<td><strong>Food group</strong></td>
</tr>
<tr>
<td>Fast food</td>
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<tr>
<td>Salty snacks</td>
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<tr>
<td>Nonalcoholic soft drinks</td>
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<td>Meat</td>
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<td>Corn products</td>
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<tr>
<td>Bread</td>
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<tr>
<td>Hot cereal</td>
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<tr>
<td>Milk and dairy</td>
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<tr>
<td>Fruit</td>
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<td>Fruit juice</td>
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<tr>
<td>Rice or pasta</td>
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<tr>
<td>Processed meat</td>
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<tr>
<td>Dairy desserts</td>
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<td>Vegetables</td>
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<tr>
<td>Baked desserts</td>
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<tr>
<td>Poultry</td>
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<tr>
<td>Cold cereal</td>
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<tr>
<td>Potato</td>
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<tr>
<td>Nuts and seeds</td>
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<td>Beans and legumes</td>
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*a* Diet pattern cluster names are based on relative energy contributions of food groups (significantly greater than for at least two other clusters with means at least 20% greater than for any other cluster).

*b* Energy contributions from selected food groups in each cluster do not total 100% because not all 33 food groups are included.

**xyz**Values in the same row with different superscript letters (x,y,z) are significantly different at P<0.05, after Tukey-Kramer adjustment for multiple comparisons.


participants in the juice cluster had the highest BMIs, this was significantly different only from individuals from the Southern cluster. Those with the Southern pattern had the lowest levels of education. Smoking status and supplement use did not vary significantly across cluster.

The fast-food cluster had the highest energy intake and, along with the Southern cluster, the highest intakes of fat, saturated fat, and trans-fatty acids. The juice and the prudent clusters had the highest percentages of carbohydrate intake. The prudent cluster had the highest intake of fiber. Protein intakes did not differ significantly across clusters.

After adjusting for age, sex, BMI, energy intake, current smoking (yes or no), serum cholesterol concentration and vitamin/mineral supplement use (yes or no), no significant differences were observed across the clusters for concentrations of serum β-carotene or lycopene (Table 3). The fast-food cluster had the lowest levels of serum lutein plus zeaxanthin (significantly lower than the Juice cluster) and β-cryptoxanthin (than the prudent or juice clusters).

The Southern cluster had lower serum α-carotene concentrations than the prudent or juice clusters.

Analyses for serum tocopherol biomarkers were also adjusted for vitamin E supplement use of 400 IU or higher (yes or no), along with the previously mentioned covariates. Serum α-tocopherol concentrations for the juice cluster were higher than those for the Fast-food cluster. There were no significant differences across the clusters for serum γ- and δ-tocopherol concentrations.

**DISCUSSION**

Dietary pattern analysis is emerging as a valid alternative method of examining diet-disease relationships. However, data on dietary patterns of regional and ethnically specific populations are limited. In this study, the dietary patterns of African Americans in the southern United States were assessed with a regionally specific FFQ and associations with serum carotenoids and tocopherols were examined. Four dietary patterns were identified in
this population: fast food, prudent, Southern, and juice. The fast-food cluster was characterized by high intake of fast foods, salty snacks, nondiet soft drinks, and meat, and was associated with significantly lower serum concentrations of several carotenoids and α-tocopherol. This was the most common dietary pattern (41% of participants) of the four identified in this population. Individuals consuming this pattern had significantly higher energy, saturated and trans-fat intakes than those in other patterns. Similar intake patterns have been reported by several other researchers and have often been labeled as junk food or Western (9,26).

In contrast, the prudent pattern, which provided higher fiber and lower total fat intake relative to others, contained relatively few (17%) individuals, the majority women. Findings of a healthful or prudent pattern with these characteristics have been reported by numerous other studies (27-31), and one of these also reported a higher percentage of women (30). This pattern had significantly higher serum α-carotene and β-cryptoxanthin relative to those in at least two other clusters (P<0.05).

A less commonly seen dietary pattern, the Southern pattern, was reported by 27% of the sample, and was characterized by a high contribution of energy from fats, cornbread, corn muffins, prepared corn meal, and hush puppies. It was associated with lower concentrations of serum α-carotene, relative to at least two other clusters. Two other studies in the literature have reported such a pattern (27,32). Using principal components analysis with the nationally representative National Health and Nutrition Examination Survey Epidemiologic Follow-up Study data, Tseng and colleagues (27) identified a pattern with high loadings for cornbread and grits. Individuals with a high score for this pattern tended to be African Americans living in the South. The researchers noted that they were able to identify this pattern because the dietary data specifically included these items and further, because these food groups were not collapsed for analysis. Velie and colleagues (32) also detected a Southern pattern among postmenopausal women in the Breast Cancer Detection Demonstration Project. Foods with positive loadings for this pattern included cooked greens, fried fish, and corn products such as muffins and corn bread. Their Southern pattern was associated with a lower risk for invasive breast cancer.

The fourth pattern in this population (reported by 16%) was characterized by a relatively high intake of fruit juice. Consistently, this group had relatively high serum concentrations of β-carotene and β-cryptoxanthin. Fruit juices are a predominant source of β-cryptoxanthin (33,34).

Few studies have examined associations between dietary patterns and serum antioxidant nutrients. In a validity study conducted within the Health Professionals Follow-up Study, researchers derived a prudent pattern from factor analysis, defined by fruit, vegetables, and juice. This pattern was positively associated with serum carotenoids, whereas a Western pattern, with higher intake of meat, sweets, and desserts, was negatively associated with serum carotenoids (35). Pryer and colleagues (36) using a national representative sample of older men and women in the United Kingdom reported a “healthy” dietary pattern associated with higher nutrient biomarkers, including α- and β-carotene, folate, and vitamin C.

Greater consumption of fruit and vegetables has been associated with lower risk of several chronic diseases, including cancer and cardiovascular disease (37-40).
risk reduction has been attributed to various mechanisms, including the presence of antioxidant nutrients (41), phytochemicals (42), fiber (43), and displacement of saturated fat (44). It is, therefore, of particular concern that, in this region of the United States where rates of heart disease and many cancers are higher than in other parts of the country, so few participants reported a prudent pattern.

Dietary pattern analysis is a useful tool to describe the eating behavior of populations. It should be noted that pattern analysis involves subjective decisions, including which variables to include in food groups, the nature of input variables, and the number of clusters reported. Despite this apparent subjectivity, results show remarkably good consistency across studies (39). Actual patterns will differ across groups, and an important consideration is to be sure data are sufficiently disaggregated to identify these differences, such as the Southern pattern seen here. Additional limitations include reporting error. For example, juice drinks, which are less expensive than 100% juice, may be perceived and reported as juice by some participants. Although we asked separately for fruit drinks, and our interviewees were trained and sensitive to this potential error, it remains possible that some of the fruit juice in our juice pattern was actually fruit drink. However, the expected association with carotenoids supports the likelihood that fruit juice was consumed.

The lag time that was taken to complete the FFQ relative to the original design could introduce additional limitations. Whereas this should not affect the identification of dietary patterns, this placed the dietary data farther away from the blood draw than planned for many participants. Seasonality may be of concern with respect to this timing. However, this would be likely to attenuate results toward the null. Further, although the reference time for the FFQ was the previous 6 months, studies have shown that patterns reported in FFQs tend to be reproducible over time, because they represent long-term dietary intake (45). In contrast to expectation, results from the National Health Interview Survey suggested that carotenoid intakes do not differ significantly throughout the year, reflecting the year round availability of carotenoid-rich foods (46).

CONCLUSIONS
Using data from a regionally specific FFQ, we derived four dietary patterns in this population of southern African Americans. The fast-food cluster was the most common dietary pattern observed, had the lowest energy contribution from fruit and vegetables and was associated with lower concentrations of serum carotenoids and α-tocopherol. In contrast, relatively few of these participants reported the more healthful, prudent pattern, which tended, along with the juice pattern, to have the highest concentrations of serum carotenoids. This distribution of dietary patterns and associated antioxidant concentrations warrants targeted interventions to improve patterns of dietary intake and increase intake of antioxidant rich foods in this African-American population, which is currently at high risk for the development of diet-related chronic disease.

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