



Published in final edited form as:

*J Am Diet Assoc.* 2008 December ; 108(12): 2013–2020. doi:10.1016/j.jada.2008.09.004.

## 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 (JHS) 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 (35–80 y), participating in the Diet and Physical Activity Sub-Study of the JHS.

**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.

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**Results**—Four dietary patterns were identified: 1) Fast food 2) Southern 3) Prudent and 4) Juice. Individuals in the Fast food pattern (n=153) had significantly lower serum concentrations of lutein plus zeaxanthin and beta cryptoxanthin; those in the Southern cluster (n=99) had significantly lower serum alpha carotene; and those in the Prudent (n=63) and Juice (n=58) clusters had significantly higher serum alpha carotene and beta cryptoxanthin ( $P < 0.05$ ) relative to those in at least one other cluster (all  $P < 0.05$ ). The Juice cluster also had higher serum alpha tocopherol concentrations relative to the Fast food cluster.

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

### Keywords

food frequency questionnaire; Jackson Heart Study; dietary patterns; antioxidant biomarkers; African Americans

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## INTRODUCTION

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 with disease. 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, E and carotenoids are thought to play an important role in the etiology of several human diseases 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 epidemiological data relating risk factors and cardiovascular disease in African American populations (15). The Jackson Heart Study (JHS) 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 the current study was, primarily, to characterize the dietary patterns of a subset of the JHS participants using a culturally suitable dietary assessment instrument and, secondly, to examine the associations 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 (DPASS) of the JHS, a single-site prospective epidemiological investigation of cardiovascular disease among African Americans from the Jackson, Mississippi metropolitan area. Data collection for the JHS began in late 2000 and was completed in early 2004. The JHS 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 JHS cohort (N=5302) was selected for the JHS DPASS. As participants were enrolled in the JHS, investigators recruited participants for DPASS to include an equal number of men and women from younger (34–64 y) and older (65 y 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 DPASS until each of the enrollment strata were filled. The Institutional Review Board of the University of Mississippi approved the DPASS protocols, and all subjects gave written informed consent for their participation.

### Study Design

The aim of DPASS was to provide data for validation of the diet and physical activity instruments used for the entire cohort of the JHS. The design and data collection methods used for the dietary portion of this sub-study have been described previously (18). Briefly, all JHS 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 DPASS subset were then scheduled to complete four, nonconsecutive, 24 hour recalls over several months. Approximately six months after the initial visit, participants completed the original, 283 item FFQ developed for use with the USDA Lower Mississippi Delta Nutrition Intervention Research Initiative (LMD NIRI) (18). This last FFQ was used to determine dietary patterns for the current 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 LMD NIRI. 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 six months to more than one 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 DPASS 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 macro-nutrient 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  $3000 \times g$  for 10 min at 4 °C. Serum was separated, frozen and stored at  $-70^{\circ}\text{C}$  until analyzed for carotenoids and tocopherols. Analyses of serum were conducted at the end of DPASS data collection, thus samples were stored for approximately 6 months to 3 years. Analyses were performed using high performance liquid chromatography (HPLC), as described by Yeum et al. (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 HPLC system consisting of a 600S controller (Millipore, Milford, MA), Waters 616 pump, Waters 717 autosampler, Waters 996 photodiode array detector and C30 carotenoid column ( $3\mu\text{m}$ ,  $150 \times 4.6\text{mm}$ , 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 HPLC 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 less than 5%. Serum cholesterol 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/ mineral supplement use (Y/N) and use of Vitamin E supplement use of 400 International Units or higher (Y/N) 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  $\text{weight}/\text{height}^2$  ( $\text{kg}/\text{m}^2$ ).

### Statistical Analyses

DPASS participants without serum samples for antioxidant analysis ( $n=39$ ), without an FFQ ( $n=1$ ), with more than 10% of questions blank on the FFQ ( $n=3$ ), or with energy intake estimates outside the plausible range ( $\leq 600$  kcal or  $\geq 4000$  kcal,  $n=27$ ) were excluded from these analyses. This resulted in a sample size of 429 participants. Prior to analysis, the 283 food items on the Delta NRI FFQ were sorted into 33 food groups (Appendix A), based on similarity or difference in nutrient content and general usage. 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 (SAS version 9.01, 2002–2003, SAS Institute Inc., Cary, North Carolina). 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  $> 5$  SD 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 3 to 8 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 prior to analysis. Means and standard deviations for energy contributions for the 33 food groups were calculated by cluster. Nutrient intakes, socio-demographic and health characteristics were assessed across clusters using generalized linear models (with Tukey-Kramer's adjustment for multiple comparisons) for continuous data and the  $\chi^2$  test for variables expressed as proportions. For each of the clusters, means  $\pm$  standard errors were calculated for serum alpha and beta carotene, lutein plus zeaxanthin, beta cryptoxanthin, lycopene, alpha, gamma and delta tocopherols after adjusting for several covariates. For analyses with gamma and delta tocopherol, we excluded 7 and 112 participants respectively, with serum concentrations below detectable levels. Alpha for all analyses was set at the 0.05 level.

## RESULTS

More than 60% of the study participants were women. The average age was 61 y with a range from 35 to 80 y. Women had a slightly higher age adjusted BMI (31.9 vs. 29.6 kg/m<sup>2</sup>,  $P < 0.05$ ) 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: 1) Fast food, Snacks, Soft drinks & Meat; 2) Cereal, Milk & Fruit; 3) Corn products & Bread; and 4) Fruit Juice (Table 1). These describe foods which 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 1) Fast food; 2) Prudent; 3) Southern and 4) 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 participants in the Juice cluster had the highest BMI, 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 (Y/N), serum cholesterol concentration and vitamin/mineral supplement use (Y/N), no significant differences were observed across the clusters for concentrations of serum beta 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 beta cryptoxanthin (than the Prudent or Juice clusters). The Southern cluster had lower serum alpha carotene concentrations than the Prudent or Juice clusters.

Analyses for serum tocopherol biomarkers were also adjusted for Vitamin E supplement use of 400 International Units or higher (Y/N), along with the previously mentioned covariates. Serum alpha 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 gamma and delta 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, 1) Fast food; 2) Prudent; 3) Southern and 4) Juice. The Fast food cluster was characterized by high intake of fast foods, salty snacks, non diet soft drinks and meat, and was associated with significantly lower serum concentrations of several carotenoids and alpha 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 “Healthy” 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 alpha carotene and beta 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 grits, cornbread, corn muffins, prepared corn meal, and hush puppies. It was associated with lower concentrations of serum alpha 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 Epidemiological Follow-up Study data, Tseng et al. (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 et al. (32) also detected a “Southern” pattern among post-menopausal women in the Breast Cancer Detection Demonstration Project. Foods with positive loadings for this pattern included cooked greens, fried fish and corn products like 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 beta carotene and beta cryptoxanthin. Fruit juices are a predominant source of beta 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 fruit juices. This pattern was positively associated with serum carotenoids, while a “Western” pattern, with higher intake of meat, sweets and desserts, was negatively associated with serum carotenoids (35). Pryer et al. (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 alpha and beta 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). This 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. However, 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 interviewers 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. While 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 six months, studies have shown that patterns reported in FFQs tend to be reproducible over time, as 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

In summary, 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 alpha tocopherol. In contrast, relatively few of these participants reported the healthier, 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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**Table 1**

Percentage energy contribution from selected food groups across the 4 dietary patterns identified among the participants of the Diet and Physical Activity Sub-Study<sup>1,2,3,4</sup>

Food Group	Dietary Pattern			
	Fast food (n=153)	Southern (n=99)	Prudent (n=63)	Juice (n=58)
Fast Food	4.8 ± 3.8 <sup>b</sup>	2.7 ± 2.2 <sup>a</sup>	2.1 ± 1.8 <sup>a</sup>	3.1 ± 2.4 <sup>a, b</sup>
Salty Snacks	4.8 ± 4.7 <sup>b</sup>	2.0 ± 2.7 <sup>a</sup>	1.9 ± 2.7 <sup>a</sup>	2.4 ± 4.1 <sup>a</sup>
Non diet Soft Drinks	3.9 ± 4.7 <sup>b</sup>	2.5 ± 3.4 <sup>a</sup>	0.8 ± 1.4 <sup>a</sup>	1.6 ± 3.1 <sup>a, b</sup>
Meat	3.7 ± 2.5 <sup>b</sup>	2.8 ± 2.3 <sup>a</sup>	1.9 ± 1.6 <sup>a</sup>	2.7 ± 2.1 <sup>a</sup>
Corn Products	4.0 ± 2.5 <sup>a</sup>	11.9 ± 4.7 <sup>c</sup>	5.7 ± 4.1 <sup>b</sup>	4.0 ± 3.2 <sup>a</sup>
Bread	6.7 ± 3.5 <sup>a</sup>	9.5 ± 4.3 <sup>b</sup>	6.3 ± 3.5 <sup>a</sup>	6.2 ± 4.5 <sup>a</sup>
Hot Cereal	1.1 ± 1.6 <sup>a</sup>	1.5 ± 2.1 <sup>a</sup>	9.9 ± 5.3 <sup>b</sup>	1.9 ± 3.5 <sup>a</sup>
Milk & Dairy	4.6 ± 3.8 <sup>a</sup>	4.5 ± 3.7 <sup>a</sup>	6.7 ± 5.4 <sup>b</sup>	4.0 ± 3.2 <sup>a</sup>
Fruit	2.9 ± 2.8 <sup>a</sup>	3.1 ± 2.6 <sup>a, b</sup>	6.0 ± 3.2 <sup>c</sup>	4.2 ± 3.4 <sup>b, c</sup>
Fruit Juice	3.1 ± 2.6 <sup>a</sup>	3.9 ± 3.2 <sup>a</sup>	6.9 ± 3.9 <sup>b</sup>	15.3 ± 6.1 <sup>c</sup>
Rice or Pasta	8.1 ± 5.0 <sup>c</sup>	6.2 ± 3.8 <sup>a</sup>	7.4 ± 4.8 <sup>b</sup>	8.1 ± 5.1 <sup>b, c</sup>
Processed Meat	3.3 ± 2.3 <sup>a, b</sup>	3.9 ± 3.2 <sup>b</sup>	2.0 ± 1.9 <sup>a</sup>	2.7 ± 2.0 <sup>b</sup>
Dairy Desserts	3.2 ± 3.2 <sup>b</sup>	2.8 ± 3.2 <sup>a, b</sup>	1.8 ± 2.3 <sup>a</sup>	4.0 ± 3.2 <sup>a, b</sup>
Vegetables	1.6 ± 1.1 <sup>a</sup>	1.8 ± 1.1 <sup>a, b</sup>	2.2 ± 1.4 <sup>b</sup>	2.1 ± 1.4 <sup>a, b</sup>
Baked Desserts	5.6 ± 4.4 <sup>a</sup>	5.2 ± 4.3 <sup>a</sup>	3.3 ± 2.8 <sup>a</sup>	3.6 ± 2.3 <sup>a</sup>
Poultry	4.4 ± 2.8 <sup>a</sup>	4.9 ± 3.4 <sup>a</sup>	5.2 ± 3.8 <sup>a</sup>	4.5 ± 3.1 <sup>a</sup>
Cold Cereal	2.6 ± 3.2 <sup>a</sup>	2.9 ± 3.2 <sup>a</sup>	2.9 ± 3.2 <sup>a</sup>	3.5 ± 4.3 <sup>a</sup>
Potato	2.9 ± 2.3 <sup>a</sup>	3.1 ± 2.5 <sup>a</sup>	2.3 ± 1.9 <sup>a</sup>	2.4 ± 2.0 <sup>a</sup>
Nuts & Seeds	2.8 ± 4.2 <sup>a</sup>	1.9 ± 2.9 <sup>a</sup>	2.2 ± 3.0 <sup>a</sup>	2.3 ± 3.5 <sup>a</sup>
Beans & Legumes	2.0 ± 1.5 <sup>a</sup>	2.0 ± 1.3 <sup>a</sup>	2.2 ± 1.5 <sup>a</sup>	1.8 ± 1.4 <sup>a</sup>

<sup>1</sup> 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).

<sup>2</sup> Mean ± Standard Deviation

<sup>3</sup> Energy contributions from selected food groups in each cluster do not total 100% because not all 33-food groups are included

<sup>4</sup> Values in the same row with different superscript letters (a, b, c) are significantly different, (P < 0.05, after Tukey-Kramer adjustment for multiple comparisons)

**Table 2**

Sample characteristics and daily nutrient intakes (as measured by food frequency questionnaire) by dietary pattern among participants of the Diet and Physical Activity Sub-Study<sup>1</sup>

Characteristics	Dietary Pattern			
	Fast food (n=153)	Southern (n=99)	Prudent (n=63)	Juice (n=58)
<b>Sample Characteristics</b>				
Age <sup>2, 3</sup> , y (n=373)	57.8 ± 0.74 <sup>a</sup>	64.6 ± 0.93 <sup>b</sup>	62.7 ± 1.17 <sup>b</sup>	61.0 ± 1.21 <sup>a, b</sup>
Women <sup>4</sup> , %, (n= 373)	57.5 <sup>a</sup>	52.5 <sup>a</sup>	74.6 <sup>b</sup>	58.6 <sup>a</sup>
Body Mass Index <sup>2, 5</sup> , kg/m <sup>2</sup> , (n=352)	31.7 ± 0.56 <sup>a, b</sup>	29.4 ± 0.72 <sup>a</sup>	30.0 ± 0.88 <sup>a, b</sup>	33.4 ± 0.90 <sup>b</sup>
Smoking Status <sup>4</sup> , %, (n=373)				
- Never	62.8	60.6	76.2	69.0
- Former	25.5	29.3	19.1	25.9
- Current	11.8	10.1	4.8	5.2
Education <sup>4</sup> , %, (n=370)				
- <12 y	15.8	26.8	12.7	8.6
- High School Diploma/GED <sup>6</sup>	20.4	26.8	20.6	12.1
- Vocational or some college	15.8	12.4	17.5	22.4
- Associates degree or higher	48.0	34.0	49.2	56.9
Vitamin/Mineral Supplement Use <sup>4</sup> , % (n=373)	58.8	60.6	73.0	53.5
Vitamin E Supplement Use <sup>4, 7</sup> , % (n=373)	21.0	18.2	28.6	24.1
<b>Nutrients (n =373)</b>				
Energy <sup>2, 8</sup> , kcal/d	2157 ± 53 <sup>b</sup>	1869 ± 66 <sup>a</sup>	1733 ± 82 <sup>a</sup>	1824 ± 85 <sup>a</sup>
Fat <sup>2, 9</sup> , % energy	36.4 ± 0.41 <sup>b</sup>	36.1 ± 0.50 <sup>b</sup>	30.7 ± 0.63 <sup>a</sup>	30.9 ± 0.64 <sup>a</sup>
Trans Fats <sup>2, 9</sup> , g	5.33 ± 0.13 <sup>b</sup>	5.24 ± 0.16 <sup>b</sup>	4.22 ± 0.20 <sup>a</sup>	4.34 ± 0.21 <sup>a</sup>
Saturated Fat <sup>2, 9</sup> , % energy	11.4 ± 0.15 <sup>b</sup>	10.9 ± 0.18 <sup>b</sup>	9.20 ± 0.23 <sup>a</sup>	9.21 ± 0.24 <sup>a</sup>
Carbohydrate <sup>2, 9</sup> , % energy	49.1 ± 0.54 <sup>a</sup>	50.1 ± 0.66 <sup>a</sup>	54.6 ± 0.82 <sup>b</sup>	55.2 ± 0.84 <sup>b</sup>
Dietary Fiber <sup>2, 9</sup> , g	16.3 ± 0.34 <sup>a, b</sup>	15.5 ± 0.42 <sup>a</sup>	21.0 ± 0.52 <sup>c</sup>	17.5 ± 0.54 <sup>b</sup>
Protein <sup>2, 9</sup> , % energy	14.9 ± 0.21 <sup>a</sup>	14.4 ± 0.25 <sup>a</sup>	15.9 ± 0.32 <sup>a</sup>	14.8 ± 0.32 <sup>a</sup>

<sup>1</sup> Cluster names were based on relative energy contributions of food groups

<sup>2</sup> Mean ± Standard Error. Values in the same row with different superscript letters (a, b) or (a, b, c) are significantly different, (P < 0.05, after Tukey-Kramer adjustment for multiple comparisons)

<sup>3</sup> Adjusted for sex

<sup>4</sup> Homogeneity across strata tested with  $\chi^2$  test showed P < 0.05 for education, but not for smoking or vitamin use

<sup>5</sup> Adjusted for age, sex, energy intake and physical activity levels

<sup>6</sup> GED: General Educational Development certificate

<sup>7</sup> Vitamin E supplement use 400 International Units and higher

<sup>8</sup> Adjusted for age and sex

<sup>9</sup> Adjusted for age, sex, and energy intake



**Table 3**Serum antioxidant concentrations, by dietary pattern, in Diet and Physical Activity Sub-Study participants <sup>1,2,3,4,5</sup>

Antioxidant Concentrations	Dietary Pattern			
	Fast food	Southern	Prudent	Juice
<b>Carotenoids (μg/dl)</b>				
Alpha carotene	3.3 ± 0.28 <sup>a, b</sup>	3.1 ± 0.32 <sup>a</sup>	4.3 ± 0.40 <sup>b</sup>	4.1 ± 0.41 <sup>b</sup>
Beta carotene	32.7 ± 2.81 <sup>a</sup>	34.2 ± 3.47 <sup>a</sup>	39.6 ± 4.31 <sup>a</sup>	48.1 ± 4.47 <sup>a</sup>
Lutein plus Zeaxanthin	16.4 ± 0.61 <sup>a</sup>	19.1 ± 0.75 <sup>a, b</sup>	19.7 ± 0.94 <sup>a, b</sup>	19.4 ± 0.97 <sup>b</sup>
Beta Cryptoxanthin	8.5 ± 0.59 <sup>a</sup>	9.8 ± 0.72 <sup>a, b</sup>	12.1 ± 0.90 <sup>b</sup>	12.1 ± 0.93 <sup>b</sup>
Lycopene	69.4 ± 3.04 <sup>a</sup>	64.4 ± 3.75 <sup>a</sup>	78.1 ± 4.67 <sup>a</sup>	76.6 ± 4.84 <sup>a</sup>
<b>Tocopherols (μg/dl)</b>				
Alpha Tocopherol	1305 ± 45 <sup>a</sup>	1361 ± 56 <sup>a, b</sup>	1487 ± 70 <sup>a, b</sup>	1472 ± 72 <sup>b</sup>
Gamma Tocopherol	243 ± 14.1 <sup>a</sup>	243 ± 17 <sup>a</sup>	263 ± 21.4 <sup>a</sup>	214 ± 22 <sup>a</sup>
Delta Tocopherol	19.4 ± 3.66 <sup>a</sup>	21.4 ± 4.67 <sup>a</sup>	17.2 ± 6.31 <sup>a</sup>	28.1 ± 6.49 <sup>a</sup>

<sup>1</sup> Clusters names were based on relative energy contributions of food groups.

<sup>2</sup> For Carotenoids: Values are Mean ± Standard Error, adjusted for age (per 10 y), sex, BMI, energy intake, current smoking (Y/N), serum cholesterol concentration and vitamin/mineral supplement use (Y/N). n=371

<sup>3</sup> For Tocopherols: Values are Means ± Standard Error, adjusted for age (per 10 y), sex, BMI, energy intake, current smoking (Y/N), serum cholesterol concentration, vitamin/mineral supplement use (Y/N) and Vitamin E supplement use of 400 International Units or higher (Y/N). For alpha tocopherol, n=372; for gamma tocopherol, n=365; for delta tocopherol, n=261

<sup>4</sup> Mean ± Standard Error. Values in the same row with different superscript letters (a, b) are significantly different, (P<0.05) after Tukey-Kramer adjustment for multiple comparisons)

<sup>5</sup> To convert serum concentrations from μg/dl to SI units (μmol/L), divide by molecular weight (g/mole) and multiply by 10. Molecular weights for Carotenoids: alpha carotene=537, beta carotene=537, lutein=568, zeaxanthin=568, beta cryptoxanthin=552, lycopene=537. Molecular weights for Tocopherols: alpha tocopherol=431, gamma tocopherol=417, delta tocopherol=403

## Appendix A

### Food groupings used in dietary patterns analyses in the Diet and Physical Activity Sub-Study

Food Group	Food Items
Alcohol	Beer, wine, liquor, mixed drinks, other alcoholic beverages
Baked Desserts	Cakes, pies, doughnuts, sweet rolls, cereal bars, pop tarts, cookies, muffins
Beans and Legumes	Beans (dried and mixed bean preparations), soy products
Bread	Bread (all types), crackers (all types), stuffing, other grain products
Cold Cereal	Ready to eat cold cereal, oats, bran, granola
Condiments	Mustard, relish, basil, turmeric, tarragon, garlic, garlic powder, parsley, salt, pepper, other spices
Corn and Corn products	Grits, cornbread, corn muffins, prepared corn meal, hush puppies, corn tortillas
Dairy Desserts	Puddings, cheesecakes, ice-creams, frozen yogurt, ice-milk
Eggs	Egg and egg preparations (regular and egg beaters)
Fast food	Food from fast food restaurants (hamburgers, chicken, fish, french fries, onion rings, fast food desserts etc.)
Fish	Fish and shell fish preparations
Fruit	Fruit (citrus and non citrus)
Fruit Juice	Fruit Juices (citrus and non citrus, sweetened and unsweetened, fortified and unfortified)
Fruit Drinks	Fruit drinks (fortified and unfortified)
Hot cereal	Oatmeal, cream of wheat, other hot breakfast cereal
Margarine & Butter	Butter (regular, unsalted, light, fat free and spreads), margarine (regular, light, stick or spread)
Meat	Beef, Pork and Lamb preparations (all cuts)
Milk and Dairy	Milk and chocolate milk (whole, 1 or 2% fat and skim), cheese or cottage cheese (regular, low fat and fat free), yogurt (regular, low fat and fat free), cream (heavy, light and half & half)
Miscellaneous Fats	Non dairy creamer, gravy, spray oils, lard, cream cheese, sour cream
Miscellaneous Foods	Meal replacement foods e.g. Ensure <sup>1</sup> and Slimfast <sup>2</sup>
Non Diet Soft Drinks	Non diet soft drinks
Nuts and Seeds	Almonds, walnuts, sunflower seeds, pecans, pistachios, cashews, coconuts, peanut, peanut butter (including peanut butter sandwich)
Oils and Salad Dressing	Vegetable oils, salad dressings (regular, light and fat free), mayonnaise
Organ Meats	Liver, venison, ham hocks, neck bones, other organ meats
Potato	Potato and potato preparations
Poultry	Chicken and turkey preparations (regular and dark meat)
Processed Meat and Poultry	Processed meats and poultry, including breakfast type (regular, lean and extra lean)
Rice and Pasta	Rice and mixed rice preparations, pasta and pasta preparations, tortillas, burritos, tacos
Salty Snacks	Potato and corn chips, popcorn, pretzels
Soups	Soups (water and cream based)
Sugar and Candy	Jams, jellies, syrup, chocolate, non chocolate candy, sugar, gelatin, sherbet
Tea and Coffee	Coffee (regular and decaf), Tea (regular, decaf and green)
Vegetables	Orange vegetables, tomato and tomato products, green leafy vegetables, cruciferous vegetables, other vegetables including onions, lettuce, radish, mixed greens, peppers, string beans, plantains, turnips, etc.

<sup>1</sup> Abbott Laboratories, Abbott Park, IL, U.S.A.

<sup>2</sup> Unilever, United States Inc, West Palm Beach, FL, USA