Substantial evidence suggests that fruit and vegetable intake reduces the risk of some cancers and other chronic diseases. While a varied diet containing fruits and vegetables may confer benefits greater than those of any single nutrient, it would be useful to have data on the plasma nutrients most influenced by fruit and vegetable intake. The authors examined the correlation between fruit and vegetable intake as measured by the abbreviated CLUE II food frequency questionnaire and several plasma antioxidants. This study includes 116 male subjects aged 35–72 years who were nonsmokers and nonusers of vitamin supplements and who provided blood samples in the CLUE II Study in Washington County, Maryland. Plasma was assayed for ascorbic acid, beta-carotene, beta-cryptoxanthin, and alpha- and gamma-tocopherol. Lipid- and energy-adjusted partial correlation for the relation with fruit and vegetable intake was $r = 0.64$ for ascorbic acid, $r = 0.44$ for beta-carotene, and $r = 0.50$ for beta-cryptoxanthin. While this study does not address efficacy, the stronger association of ascorbic acid with fruit and vegetable intake seen here may imply that ascorbic acid is an important component of the protective effect seen for fruits and vegetables in numerous epidemiologic studies.

Numerous studies have found a significant inverse relation between cancer risk and intake of fruits and vegetables (1). Although the consumption of whole foods provides a complex nutrient mix that may confer a benefit superior to that of any particular component, it would be useful to understand which nutrients are most associated with a high intake of fruits and vegetables. A number of studies using food frequency questionnaires (FFQs) have examined the relation between dietary estimates of particular nutrients and the corresponding plasma nutrient levels. Very few, however, have examined the plasma nutrient levels simply in relation to reported intake of foods rather than to estimates of nutrients. In other words, what plasma nutrient levels are most influenced by a diet high in fruits and vegetables? This study examines plasma levels of several antioxidants in relation to intake of fruits and vegetables.

**MATERIALS AND METHODS**

Subjects were selected from among participants in the Washington County, Maryland, CLUE II Study, a blood collection campaign conducted by the Johns Hopkins Training Center for Epidemiologic Research and the Washington County Health Department. In 1989, CLUE II recruited residents of Washington County and surrounding counties; most samples were obtained in the fall. CLUE II obtained plasma samples, brief personal data, and a brief food frequency questionnaire. More than 30,000 persons from Washington County and surrounding counties provided samples.

Respondents for this study were selected from counties surrounding Washington County. Subjects were men aged 35–72 years (mean, 53 years) who did not smoke and did not take vitamin supplements. Respondents with an estimated energy intake of less than 1,000 kcal were dropped to exclude persons who may have been ill, were dieting, or had completed the questionnaire incorrectly.

The questionnaire used in the CLUE II Study is a 60-item scannable version of the Block/National Cancer Institute (NCI) questionnaire. The questionnaire contained 10 vegetable items and six fruit items (table 1). Collectively, these foods contribute 70.6 percent of the carotenoid intake in the US diet among men in this age range and 57.8 percent of the dietary vitamin C in the United States, on the basis of the Third National Health and Nutrition Examination Survey (G. Block, unpublished data, 1997). Frequency of consumption of these foods was summed to estimate total fruit and vegetable consumption. (The “GRPFRQ” variables produced by the software were used rather than the portion size-related measures; summary “global” questions were not asked in this FFQ.) Questionnaires were analyzed by using the Block/NCI software (2), and estimates were made of usual dietary intake of nutrients and food groups. Subjects

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Abbreviations: FFQ, food frequency questionnaire; FV, fruit and vegetable consumption; Heme, meat intake; NCI, National Cancer Institute.

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were included in this analysis if their reported dietary intake placed them in either the top or bottom quintile on both fruit and vegetable consumption (FV) and meat intake (Heme). (Heme was obtained for a different analysis, and those results are reported elsewhere (3).) Subjects were selected in groups of four (HiFV + HiHeme, HiFV + LoHeme, LoFV + HiHeme, and LoFV + LoHeme), matched within each group on age and body weight. A total of 29 subjects were selected for each of the four groups, resulting in a sample of 116 men for these analyses.

Venous blood was drawn in heparinized Vacutainers (Becton, Dickinson, & Co., Franklin Lakes, New Jersey), centrifuged, and processed within a few hours. One aliquot was prepared by using 10 percent metaphosphoric acid to stabilize ascorbic acid. All samples were stored at −70°C. The long-term stability of these nutrients, when stored at −70°C to −80°C, has been examined in numerous studies and found to be acceptable (4–6). Masked duplicate samples were sent to each laboratory and included in the assays. In addition, a single pooled blood sample was divided into multiple aliquots and shipped with samples over the course of the study to permit analyses of laboratory drift. Reproducibility of all assays was excellent.

Plasma was assayed for ascorbate, beta-carotene, beta-cryptoxanthin, and alpha- and gamma-tocopherol by one of the investigators (E. N.). Plasma ascorbate concentration was determined spectrophotometrically by using 2,4-dinitrophenylhydrazine as chromogen (7), which has been shown to correlate highly with high-pressure liquid chromatography methods (8–11). Plasma carotenoids and vitamin E were determined by reversed-phase high-pressure liquid chromatography (12).

Analysis of variance, t tests, and Pearson and Spearman correlations were used. Variables were examined for normality and skewness and transformed by using log or square root, as appropriate. Pearson correlations using the transformed variables were almost identical to Spearman correlations, so only the latter are reported here. Statistical analyses were performed using PC-SAS version 6.11 (SAS Institute, Inc., Cary, North Carolina).

RESULTS

The characteristics of the participants in this analysis are shown in table 2. Body weight ranged from 120 to 250 pounds (54.48 to 113.5 kg), and mean frequency of fruit and vegetable intake was 2.9 times per day. Analysis of variance including the meat category, the fruit and vegetable category, and their interaction term indicated that meat consumption and the interaction term were not related to any plasma antioxidant (data not shown). Consequently, all analyses in this report related to plasma antioxidant level consider only the fruit and vegetable intake.

Correlations between frequency of FV and plasma antioxidants are shown in table 3. Both carotenoids and ascorbic acid are highly significantly associated with frequency of consumption of fruits and vegetables. However, the correlation with ascorbic acid is considerably higher than that for the carotenoids, both unadjusted and after adjustment for several covariates. This higher correlation of FV with ascorbic acid remained after standardization of the plasma carotenoids by plasma cholesterol. Plasma alpha-tocopherol is positively associated with FV only after standardization with plasma cholesterol, while gamma-tocopherol is significantly negatively correlated with FV. Partial correlations adjusted for age, education, body weight, energy intake, or fat intake did not change this pattern. After adjustment for age and energy intake, the correlation between fruit and vegetable intake and ascorbic acid was 0.64, while lipid-adjusted total carotenoids reached only 0.44. The highest correlation besides that of ascorbic acid was lipid-adjusted beta-cryptoxanthin (which is found largely in oranges and orange juice), at 0.50.

DISCUSSION

Although numerous investigators have examined the relation between serum antioxidant nutrient levels and estimates of antioxidant intake from food frequency questionnaires, few have reported the correlations between serum antioxidants and fruit and vegetable frequency as opposed to nutrient estimates (13–19). Only two studies were of non-smokers (16, 17), and the results presented here correspond well to the carotenoid correlations observed in these earlier reports. Campbell et al. (16) recruited 50 male and 49 female nonsmokers aged 18–37 years, selecting only those in the highest or lowest quintile of FV; 29 percent were supplement users. (Smoking lowers plasma beta-carotene and ascorbic acid levels, and supplement use increases them, irrespective of fruit and vegetable intake. Inclusion of subjects with these behaviors makes it difficult to detect a relation between these plasma nutrients and fruit and vegetable intake.) The 153-item Willett FFQ was self-administered and included 35 veg-

<table>
<thead>
<tr>
<th>TABLE 1. Foods used to rank subjects on fruit and vegetable intake*, Washington County, Maryland, 1989</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fruits and vegetables on the CLUE II questionnaire</td>
</tr>
<tr>
<td>Carrots or mixed vegetables containing carrots</td>
</tr>
<tr>
<td>Spinach</td>
</tr>
<tr>
<td>Broccoli</td>
</tr>
<tr>
<td>Sweet potatoes, yams</td>
</tr>
<tr>
<td>Tomatoes, tomato juice</td>
</tr>
<tr>
<td>Vegetable or tomato soups</td>
</tr>
<tr>
<td>Coleslaw, cabbage, sauerkraut</td>
</tr>
<tr>
<td>Mustard greens, turnip greens, collards</td>
</tr>
<tr>
<td>Green salad</td>
</tr>
<tr>
<td>Any other vegetables, including green beans, corn, peas</td>
</tr>
<tr>
<td>Oranges</td>
</tr>
<tr>
<td>Grapefruit</td>
</tr>
<tr>
<td>Orange juice or grapefruit juice</td>
</tr>
<tr>
<td>Cantaloupe</td>
</tr>
<tr>
<td>Apples, applesauce, pears</td>
</tr>
<tr>
<td>Any other fruit, including bananas, fruit cocktail</td>
</tr>
</tbody>
</table>

* These items comprise foods that contribute the following proportions of US nutrient intake of carotenoids: 70.6% (65.4% from the 14 foods excluding “Any other vegetables” and “Any other fruit”) and of dietary vitamin C: 57.8% (44.8% from the 14 foods excluding “Any other vegetables” and “Any other fruit”). (Block, unpublished data, 1997).
etetable items and 24 fruit items. Lipid- and energy-adjusted correlations between total fruit and vegetable intake and the average of two measurements of plasma beta-carotene and cryptoxanthin were 0.45 and 0.47, respectively, for men and women combined. (Results were not reported separately by gender.) Michaud et al. (17) analyzed data from 110 male nonsmokers from the Health Professionals Follow-up Study. The study questionnaire contained 131 food items (including 31 vegetables and 15 fruits). Supplement use was not addressed, but was presumably present for some participants. Plasma carotenoids were adjusted for lipids, body mass index, and age; fruit and vegetable estimates were based on the average of two FFQs and two 1-week diet records. For men, correlations were 0.35 and 0.36 for beta-carotene and cryptoxanthin, respectively. Thus, our results of 0.38 and 0.50 for these two plasma carotenoids are consistent with previous data on nonsmokers.

Other studies of fruit and vegetable intake and plasma nutrients examined correlations with serum carotenoids and included both smokers and supplement users (18, 19). Tucker et al. (18) reported on the relation between total fruit and vegetable intake, as estimated by the 126-item Willett FFQ, in participants in the Framingham Heart Study. Ten percent of the 201 men were smokers, and 11.9 percent used beta-carotene supplements. Among men, after adjustment for energy and other risk factors, correlations were $r = 0.25$ for alpha- and beta-carotene, 0.16 for beta-cryptoxanthin, 0.17 for lycopene, and 0.14 for lutein-zeaxanthin. Resnicow

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### TABLE 3. Spearman correlations and partial correlations between fruit/vegetable frequency of consumption and several plasma antioxidants for 116 men aged 35–72 years, Washington County, Maryland, 1989

<table>
<thead>
<tr>
<th></th>
<th>Ascorbic acid*</th>
<th>Total carotene**</th>
<th>Lipid-adjusted total carotene*</th>
<th>Lipid-adjusted $\beta$-carotene**</th>
<th>Lipid-adjusted cryptoxanthin*</th>
<th>Lipid-adjusted lutein-zeaxanthin*</th>
<th>$\alpha$-toc††,‡</th>
<th>Lipid-adjusted $\alpha$-toc†</th>
<th>Gamma-toc***</th>
<th>Lipid-adjusted gamma-toc†</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Unadjusted correlation</strong></td>
<td>0.59</td>
<td>0.34</td>
<td>0.40</td>
<td>0.35</td>
<td>0.38</td>
<td>0.43</td>
<td>0.46</td>
<td>0.06</td>
<td>0.26</td>
<td>−0.25</td>
</tr>
<tr>
<td>Adjusted for</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>0.59</td>
<td>0.37</td>
<td>0.43</td>
<td>0.34</td>
<td>0.36</td>
<td>0.43</td>
<td>0.47</td>
<td>0.03</td>
<td>0.22</td>
<td>−0.26</td>
</tr>
<tr>
<td>Education</td>
<td>0.58</td>
<td>0.33</td>
<td>0.40</td>
<td>0.35</td>
<td>0.38</td>
<td>0.41</td>
<td>0.45</td>
<td>0.07</td>
<td>0.27</td>
<td>−0.24</td>
</tr>
<tr>
<td>Body weight</td>
<td>0.61</td>
<td>0.35</td>
<td>0.42</td>
<td>0.36</td>
<td>0.38</td>
<td>0.43</td>
<td>0.47</td>
<td>0.06</td>
<td>0.26</td>
<td>−0.25</td>
</tr>
<tr>
<td>Dietary energy intake</td>
<td>0.62</td>
<td>0.34</td>
<td>0.41</td>
<td>0.36</td>
<td>0.39</td>
<td>0.44</td>
<td>0.49</td>
<td>0.06</td>
<td>0.28</td>
<td>−0.26</td>
</tr>
<tr>
<td>Dietary fat intake</td>
<td>0.60</td>
<td>0.34</td>
<td>0.40</td>
<td>0.34</td>
<td>0.37</td>
<td>0.42</td>
<td>0.46</td>
<td>0.05</td>
<td>0.25</td>
<td>−0.24</td>
</tr>
<tr>
<td>Age and energy intake</td>
<td>0.64</td>
<td>0.37</td>
<td>0.44</td>
<td>0.36</td>
<td>0.38</td>
<td>0.46</td>
<td>0.50</td>
<td>0.03</td>
<td>0.24</td>
<td>−0.28</td>
</tr>
</tbody>
</table>

* All correlations in this column, $p < 0.0001$.
** All correlations in this column, $p < 0.001$.
*** All correlations in this column, $p < 0.01$.
†† All correlations in this column, $p < 0.05$.
‡‡ All correlations in this column, $p > 0.10$.
α-toc, alpha-tocopherol.
et al. (19) studied fruit and vegetable intake and plasma carotenoids in 775 African-American men and women in Atlanta, Georgia. Smokers and vitamin supplement users were included. A modification of the full-length Block/NCI questionnaire was used, which contained 36 fruit and vegetable items. Correlations were \( r = 0.34 \) for alpha-carotene, 0.31 for beta-carotene, 0.26 for beta-cryptoxanthin, and 0.21 for lutein. In a subset of 68 persons who completed three 24-hour recalls, correlations between the 36-item fruit and vegetable questionnaire and these serum carotenoids were much higher (\( r = 0.52, 0.46, 0.43, \) and 0.30, respectively). Other studies have examined serum nutrient relations with individual foods (14, 15) or have conducted small feeding studies with subjects, many of whom were vitamin supplement users (20).

To our knowledge, only one other study has examined both plasma carotenoids and ascorbic acid in relation to fruit and vegetable intake. In France, Drewnowski et al. (13) studied a community-based sample of 837 subjects, of whom 23.1 percent of the women and 41.6 percent of the men were current smokers. Supplement use was not reported. Data were collected by using a dietary history interview. Correlations with energy-adjusted fruit and vegetable intake were \( r = 0.36 \) for serum beta-carotene and 0.29 for ascorbic acid.

In our study, ascorbic acid was considerably more highly associated with fruit and vegetable intake than were the carotenoids. Thus, it is possible that ascorbic acid is as important as or more important than carotenoids in conferring the protective benefit of fruits and vegetables. Unless studies examine plasma ascorbic acid in addition to other plasma antioxidants, conclusions regarding the active agent may be misleading. Interestingly, both this study and that of Michaud et al. (17) found beta-cryptoxanthin to be more highly correlated with fruit and vegetable intake than was beta-carotene (although others have not observed this (18, 19)). In this context, it should be noted that the major contributors of beta-cryptoxanthin are oranges and orange juice. Thus, if ascorbic acid is high, beta-cryptoxanthin may also be high. Without a measurement of plasma ascorbic acid, it may be difficult to attribute effects to the proper nutrient.

This study does not directly address the potential efficacy of ascorbic acid or other nutrients in affecting disease prevention. That would require epidemiologic studies that obtain a wide range of plasma nutrients and precursors of endogenous antioxidant systems. The stronger association of ascorbic acid with fruit and vegetable intake seen here may imply that ascorbic acid is an important component of the protective effect seen for fruits and vegetables in numerous epidemiologic studies. However, it is also possible that ascorbic acid appeared to be more strongly associated than carotenoids because of differences in storage or metabolism or in the difficulties of measurement. Ascorbic acid is water soluble, with major stores in muscle tissue, and the rate of utilization depends on numerous factors, including body weight, smoking, vigorous exercise, exposure to stressors, and, possibly, gender. Carotenoids are lipid soluble, with storage in fatty tissue, and utilization also depends on smoking and body weight, although possibly to a lesser extent. It is possible that had carotenoids been measured in adipose tissue, correlations with fruit and vegetable intake would have been higher.

The inverse association of gamma-tocopherol with fruit and vegetable intake is not well understood. In an unsupplemented diet, vegetable oils and salad dressings are the main sources of both tocopherols, although vegetables do provide some alpha-tocopherol. Supplementation with alpha-tocopherol is known to suppress gamma-tocopherol levels, and these data suggest an inverse relation between alpha- and gamma-tocopherol, even in an unsupplemented diet. Some studies suggest that gamma-tocopherol is a more potent antioxidant than alpha-tocopherol in some assay conditions, but the inverse relation between gamma-tocopherol and fruit and vegetable intake seen here seems inconsistent with a beneficial effect of gamma-tocopherol.

Often, investigators in major studies do not obtain plasma ascorbic acid because of the belief that it is too difficult to process and too labile to be feasible. This study shows that this is not the case. The CLUE II Study obtained blood samples from 32,808 respondents in a period of 6 months. Samples were obtained in multiple sites across Washington County, including temporary interviewing locations such as in mobile trailers. Blood samples were transported to a central site as whole blood, and processing was done centrally, usually within 6 hours of collection. Ascorbic acid is stable in whole blood for several hours (21), and after centrifugation, the processing of samples for ascorbic acid involves only the preparation of one additional tube containing a stabilizing agent (in our case, metaphosphoric acid). Ascorbic acid in plasma prepared in this way has been shown to be stable at –70°C over a period of several years.

In addition, investigators sometimes fail to include ascorbic acid because of the belief that blood levels represent only the previous few hours or that fasting blood is essential. Again, this appears not to be the case. Most participants in this study were not fasting at the time the blood was drawn, and the correlations shown are with dietary estimates from a questionnaire that asked about average intake in the previous year. These data suggest that plasma ascorbic acid is not as labile or as difficult to process in large studies as has been feared and should be included when studies assess antioxidant status.

A strength of this study is that the effect of fruit and vegetable intake on plasma nutrients could be examined without the effect modification by smoking (22, 23) and without confounding by supplement use (24). In addition, it is notable that the plasma correlations shown here are with reported frequency of consumption of fruits and vegetables, not with dietary estimates of nutrient intake or with grams of intake estimated using reported portion size. Thus, the observed correlations are not influenced by possible inaccuracies in the nutrient database for carotenoids or by problems with portion size estimation. Furthermore, this approach provides data that are directly relevant to the bulk of epidemiologic literature; that body of literature has typi-
cally been based on frequency rather than on portion-based servings and has tended to find stronger etiologic associations with fruit and vegetable intake rather than with specific nutrient estimates.

While the list of fruits and vegetables on the CLUE II questionnaire is not long (10 vegetable items and six fruit items), it encompasses the major sources of these nutrients in the US diet, including eight of the top 10 sources of carotenoids and seven of the top 10 sources of vitamin C. Not counting the two “any other fruit” and “any other vegetable” items, the remaining 14 items represent more than two thirds of all the mentions of fruits and vegetables in the Third National Health and Nutrition Examination Survey database among men in this age group (Block, unpublished data, 1997). If the “any other…” items are considered, then, of course, the list represents the great majority of all fruits and vegetables consumed in the United States. Eight of the 14 specific foods on the questionnaire are major dark green or deep yellow vegetables or fruits. Thus, while the higher correlation of ascorbic acid with fruit and vegetable intake seen here is with this particular list of fruits and vegetables, it should be noted that the list actually encompasses a higher proportion of carotenoids in the US diet (70.6 percent) than of vitamin C (57.8 percent).

As in the study by Campbell et al. (16), subjects were selected for this research by virtue of being either in the upper or the lower quintile of the distribution of frequency of fruit and vegetable intake. This approach tends to result in correlations that are higher than might be observed in studies that include the middle ranges of intake. However, the approach may also make it possible to see relations between intake and plasma most clearly, unobscured by the greater misclassification found in the middle ranges of intake. Estimates at the top and bottom of a frequency-of-consumption distribution are easiest for respondents to report and are reported with less error than estimates in the middle ranges. For example, it is easy and reasonably accurate to say “I eat carrots almost every day” or “I eat carrots only once a year.” What is more difficult, and thus measured with more error, is deciding whether carrots are eaten once a month or twice a month. Thus, we believe that our sample selection approach gives a more accurate picture of the plasma nutrients that may be represented by questionnaires asking about fruits and vegetables.

In summary, this study has found that while both carotenoids and ascorbic acid are elevated in those with higher fruit and vegetable intakes, ascorbic acid is considerably more highly correlated with fruit and vegetable intake than are the carotenoids. Thus, it is possible that raising ascorbic acid levels may be an important mechanism by which fruit and vegetable consumption confers protective benefits. The study has also demonstrated the feasibility of obtaining plasma vitamin C measures in large-scale epidemiologic studies. Epidemiologic studies should include measures of plasma or serum ascorbic acid, in addition to other nutrients, to fully understand etiology and mechanisms.

REFERENCES


