Long-term Nutrient Intake and Early Age-Related Nuclear Lens Opacities

Paul F. Jacques, ScD; Leo T. Chylack, Jr, MD; Susan E. Hankinson, ScD; Patricia M. Khu, MD; Gail Rogers, MA; Judith Friend, MA; William Tung; John K. Wolfe, PhD; Nita Padhye, MD; Walter C. Willett, MD, DrPH; Allen Taylor, PhD

Objective: To assess the relation between usual nutrient intake and subsequently diagnosed age-related nuclear lens opacities.

Subjects: Four hundred seventy-eight nondiabetic women aged 53 to 73 years from the Boston, Mass, area without previously diagnosed cataracts sampled from the Nurses’ Health Study cohort.

Methods: Usual nutrient intake was calculated as the average intake from 5 food frequency questionnaires that were collected during a 13- to 15-year period before the evaluation of lens opacities. The duration of vitamin supplement use was determined from 7 questionnaires collected during this same period. We defined nuclear opacities as a nuclear opalescence grade of 2.5 or higher using the Lens Opacification Classification System III.

Results: The prevalence of nuclear opacification was significantly lower in the highest nutrient intake quintile category relative to the lowest quintile category for vitamin C (P < .001), vitamin E (P = .02), riboflavin (P = .05), folate (P = .009), β-carotene (P = .04), and lutein/zeaxanthin (P = .03). After adjustment for other nutrients, only vitamin C intake remained significantly associated (P = .003 for trend) with the prevalence of nuclear opacities. The prevalence of nuclear opacities was significantly lower (P < .001) in the highest vitamin C intake quintile category relative to the lowest quintile category (odds ratio, 0.31; 95% confidence interval, 0.16-0.58). There were also statistically significant trends of decreasing prevalence of nuclear opacities with increasing duration of use of vitamin C (P = .004 for trend), vitamin E (P = .03 for trend), and multivitamin (P = .04 for trend) supplements, but only duration of vitamin C supplement use remained significantly associated with nuclear opacities after mutual adjustment for use of vitamin E (P = .05 for trend) or multivitamin (P = .02 for trend) supplements. The prevalence of nuclear opacities was significantly lower (P = .004) for women who used a vitamin C supplement for 10 or more years relative to women who never used vitamin C supplements (odds ratio, 0.36; 95% confidence interval, 0.18-0.72). Plasma measures of vitamins C and E taken at the eye examination were also inversely associated with the prevalence of nuclear opacities.

Conclusion: These results provide additional evidence that antioxidant nutrients play a role in the prevention of age-related nuclear lens opacities.

SUBJECTS AND METHODS

SUBJECTS AND STUDY POPULATION

In 1976, 121,700 female nurses aged 30 to 55 years who resided in 11 US states completed a mailed questionnaire on known and suspected risk factors for cancer and heart disease. These women formed the NHS cohort.23 Every 2 years since 1976, these women have been contacted by mail to update information on risk factors and disease status. In 1993, we identified approximately 1707 NHS cohort members aged 53 to 73 years who resided in the Boston, Mass, area; were free of diagnosed cancer other than nonmelanoma skin cancer; had complete dietary data; and had both lenses intact. With a goal of enrolling 600 women into the NVP, all 1707 eligible NHS participants were initially contacted by a letter from the NHS and requested to return an enclosed reply postcard indicating whether they would be willing to participate in the study. To preserve their participation in the NHS, women who did not return the postcard received no further mailings or telephone contact. We received positive responses from 730 (43%) of the women with this one mailing. Six hundred three of these volunteers were ultimately scheduled and examined as part of the NVP from April 16, 1993, through August 4, 1995. Scheduling conflicts (due to work and travel) were the most common reasons for failure to examine the 127 who agreed to participate but were never seen. Informed consent was obtained from all study participants, and all procedures were approved by the Human Investigations Review Committee at the New England Medical Center and the Human Research Committee at the Brigham and Women’s Hospital, both in Boston.

ASSESSMENT OF NUTRIENT INTAKE

Since 1976, the members of the NHS cohort have received biennial questionnaires requesting information on various health and lifestyle issues. A 61-item semiquantitative food frequency questionnaire was initially incorporated into the biennial questionnaire in 1980.24 The food frequency questionnaire queried usual intake during the previous year, with 9 possible response categories, ranging from “never or less than once per month” to “6 or more times per day.” In addition, the 1980 questionnaire collected information on vitamin supplement use in 1980 and duration of vitamin supplement use before 1980. In 1984, 1986, and 1990, revised and expanded versions of the food frequency questionnaire were included in the biennial questionnaire. Every questionnaire since 1980 has included questions on vitamin supplement use. The present version of the food frequency questionnaire includes approximately 130 food items and details of vitamin and mineral supplement use that collectively account for more than 90% of the total absolute intake of the 70 nutrients measured by this instrument.25 The food frequency questionnaire has been extensively validated relative to long-term diet records and biochemical markers of nutrient status.26-28 In addition to the food frequency and vitamin questionnaires collected routinely as part of the NHS, we administered an additional food frequency questionnaire that included questions on vitamin supplement use as part of the NVP (1993-1995).

We used the data from women who completed 5 food frequency questionnaires collected between 1980 and 1993-1995 to calculate the average total nutrient intake (from food and supplements) for each participant. For these analyses, we considered the intakes of vitamin C, vitamin E, folate, riboflavin, and the individual carotenoids. We used 7 reports of vitamin supplement use from 1980 through 1993-1995 to categorize women by duration of vitamin C, vitamin E, and multivitamin supplement use. We assigned 2 years of supplement use to the duration-of-use variable for each report of supplement use between 1980 and 1990. For women reporting supplement use on the food frequency questionnaire collected as part of the NVP, we added the interval between 1990 and the date of the eye examination to the duration-of-use variable. Finally, we added the reported duration of use before 1980 to the duration-of-use variable. We assumed that a woman who started or stopped using supplements during the interval between questionnaires did so halfway through the period.

PLASMA NUTRIENT MEASUREMENT

Fasting plasma samples were obtained at the eye examination for analyses for plasma antioxidant concentrations. Plasma for the vitamin analyses was stabilized by the addition of an equal volume of perchloric acid, 0.35 mol/L, containing EDTA, 0.26 mmol/L, and was centrifuged at 4000g within 30 minutes of venipuncture. Ascorbic acid (reduced vitamin C) was determined on fresh plasma samples by reversed-phase high-performance liquid chromatographic analyses with electrochemical detection.29 Samples for vitamin E and total carotenoids were frozen at −70°C for up to 1 month. Vitamin E (α-tocopherol) was measured by reversed-phase high-performance liquid chromatography by the method of Bieri et al.30 and total carotenoids were measured spectrophotometrically by the method of Roels et al.31

ASSESSMENT OF LENS STATUS

All NVP participants underwent a detailed eye examination using standardized techniques. The examination included an ocular and medical history, a Bailey Lovie test of visual acuity and manifest refraction, an external ocular examination, applanation tonometry, contrast sensitivity function and glare testing, and a slitlamp examination of the anterior segment. The latter included an assessment of the anterior chamber to determine the risk of angle-closure glaucoma. Measurement of intraocular pressure was required to determine if it was safe to complete the eye examination, including dilation. Before a slitlamp examination of the lens was performed, the pupils were dilated to

in that study, the relation between intermediate levels of vitamin C intake and the risk of opacification could not be examined; also, associations with either vitamin E or the carotenoids could not be independently considered. The present study, the Nutrition and Vision Project (NVP), is based on a second, larger subset of women from the NHS cohort that was selected without regard to nutrient intake. This study examines the relation between newly diagnosed age-related nuclear opacities and usual nutrient intake, which was calculated as the average in-
a minimum of 6 mm with phenylephrine hydrochloride and tropicamide. The posterior segment was examined by direct and indirect ophthalmoscopy. The examiner (L.T.C., P.M.K., N.P., and W.T.) had no knowledge of the nutrient status of any of the volunteers.

Color film images were taken with a photographic slit-lamp (Carl Zeiss, Oberkochen, West Germany) and film (Ektachrome 200) to assess the degree of nuclear color and opalescence. The Lens Opacity Classification System III (LOCS III) was used to measure the degree of nuclear opalescence, the degree of light scattering from the nucleus, with possible grades ranging from 0.1 to 6.9. To do the grading, each of 2 individual graders (L.T.C. and J.K.W.) wrote down a score for the photograph on a score sheet. The graders then compared scores and arrived at a consensus score, which was recorded on the third form. Consensus scores typically fell between the 2 individual scores, but were not true averages. All photographs were graded in several sessions within a 2-month period after all photographs and images were obtained. We considered eyes to have nuclear opacities if the nuclear opalescence grade was 2.5 or higher. This threshold represents an early stage of opacification and is not associated with symptoms such as reduced vision.

DEFINING POTENTIAL CONFOUNDERS

Data on known or suspected nonnutritional determinants of cataract risk were obtained from the 1980 through 1990 biennial NHS questionnaires. For the present analyses, we considered confirmed history of diabetes and hypertension (yes or no) as reported on the 1990 or previous questionnaires, cigarette pack-years smoked through 1990 (0, 1-29, and ≥30 pack-years), summertime sunlight exposure (≥8 h/wk) as reported on the 1980 questionnaire, alcohol use based on the average from 3 food frequency questionnaires, and height and weight as reported on the 1980 questionnaire. The latter 2 measures were used to calculate body mass index (calculated as weight in kilograms divided by the square of height in meters).

STATISTICAL METHODS

We estimated the odds ratios (ORs) relating prevalence of nuclear opacities to average nutrient intake and duration of vitamin supplement use from logistic regression with the SAS GENMOD procedure. This procedure allowed the individual eyes to be the unit of observation and requires that information for both eyes is available. This generalized estimating equation approach to estimate logistic regression models adjusts the SEs of the model parameters for the correlated data resulting from repeated measurements on the same individual.

The primary independent variable used to examine the relation between usual nutrient intake and prevalence of nuclear opacities was average nutrient intake, previously described. This variable was classified into quintile categories derived from the entire NVP sample of 603 women. These intake categories were modeled with indicator variables using women in the lowest quintile category as the reference category. To test for trend across quintile categories, we assigned the median intake of each quintile category to everyone with intakes in the category, and then included this quintile median variable as a continuous factor in the logistic regression models. We also used the quintile median variable to test for possible interactions between our nutritional measures and age (<60 or ≥60 years) and smoking (never smokers or current or past smokers). Because of the number of interactions considered, we used P = .01 as the cutoff to indicate the presence of an interaction.

To determine if there was an advantage to the use of multiple measurements of intake relative to the use of a single measurement, we considered the relation between nutrient intake reported at the eye examination (single measurement) and prevalent nuclear opacities. The form of these analyses was otherwise identical to those we used to examine the relation between usual (average) nutrient intake and prevalence of opacities.

To examine the relation of duration of vitamin supplement use to the prevalence of nuclear opacities, categories of duration of vitamin supplement use were modeled with indicator variables, using women who reported no vitamin supplement use as the reference category. A test for trend of prevalence across supplement duration categories was performed by assigning participants the median value of their duration category and then entering this as a continuous variable into the logistic regression model. The P for trend was the resulting P for the associated logistic regression coefficient.

To investigate the relation between plasma nutrient measures and nuclear opacification, the nutrient measures were also modeled using quintile categories with indicator variables. Women in the lowest quintile category served as the reference category. Trend across quintile categories was assessed in the same way as it was for the intake variables.

To help evaluate the independent contribution of each nutrient, all nutrients that were significantly associated with nuclear opacities when entered individually into the logistic regression models (based on a P for trend of <.10) were simultaneously entered into a logistic regression model using the nutrient quintile median variables. This model also included all covariates previously listed. Using a backward selection procedure, nutrients were removed based on level of significance until only those nutrients that were significantly associated with the prevalence of nuclear opalescence (P < .05) remained. Because only 3 types of supplements were considered, the vitamin supplement associations were adjusted for one another in a pairwise manner. Likewise, the plasma associations were also adjusted in a pairwise manner.

Odds ratios for the prevalence of nuclear opacities for the nutrient intake and plasma quintile categories and the supplement duration categories were calculated as the antilogarithm of the logistic regression coefficient for each of these categories. All ORs were adjusted for age and other potential confounders previously described.
trient for the intakes of other nutrients to examine their unique contribution to the risk of lens opacities.

### RESULTS

#### COMPARISON OF PARTICIPANTS AND NONPARTICIPANTS

There were few notable differences between participants and nonparticipants (Table 1). They were similar in age, alcohol consumption, body mass index, reported summertime sunlight exposure, prevalence of hypertension, and vitamin C and multivitamin supplement use between 1980 and 1995. Participants reported fewer pack-years of smoking and were also slightly more likely to have used vitamin E supplements between 1980 and 1995.

#### PARTICIPANT CHARACTERISTICS

Of the 603 women examined as part of the NVP, we excluded 76 who reported a history of cataract to avoid the possibility that prior knowledge of lens opacification might influence nutrient intake and 9 with a confirmed diagnosis of diabetes in 1990 or earlier because of our concern that diabetes might alter the association between nutrition and nuclear opacification. In addition, 33 women with lens photographs that were missing or could not be graded or with questionable nuclear lens data and 7 with missing information for covariates were not included in the analyses. Four hundred seventy-eight remaining women were included in these analyses. The 478 women who were included in the analyses were different from the 85 who were excluded for the following characteristics: women included in the analyses were younger (aged 61 vs 65 years; \(P < .001\)), had a lower average nuclear opalescence score (2.2 vs 2.6; \(P < .001\)), had a lower body mass index in 1980 (24.3 vs 25.3; \(P = .05\)), and consumed more alcohol (7.8 vs 5.1 g/d; \(P = .005\)) than women who were excluded. There were no differences between those included and those excluded from analyses for vitamin C intake, lutein/zeaxanthin intake, pack-years smoked, reported summer sunlight exposure, prevalence of high blood pressure, or use of vitamin C supplements for 10 or more years. Women who were missing data were the same average age as women included in the analyses, and did not differ on any other covariate or nutrient measures for which data were available. The comparison of nuclear lens scores was not possible because all but 7 of these women were missing lens data.

The distribution of LOCS III nuclear opalescence scores is displayed in the Figure for the 956 eyes available for analyses. The mean score and its SD were 2.2 and 0.6, respectively. The median and 5th and 95th percentile values were 2.1, 1.3, and 3.4, respectively. Two hundred forty-nine eyes (26.0%) from 163 women had nuclear opacities (ie, an LOCS III grade \(\geq 2.5\)). Selected characteristics of the women included in the analyses are given in Table 2 according to the presence or absence of nuclear opacities. Women with nuclear opacities were significantly older than women without opacities and were less likely to use vitamin C and vitamin E supplements (\(P < .05\)).

Table 3 displays the quintile values used to define the nutrient intake and plasma categories. The dietary reference intake estimated average requirement (EAR) values for 51- to 70-year-old women and cutoff values for adequate plasma nutrient concentrations are also shown to allow comparisons between the quintile cutoff values and these normative values. This sample of women appears to be generally well nourished with respect to most nutrients considered. The 20th percentile values exceeded the EARs for vitamin C and riboflavin. In other words, more than 80% of the women reported intakes greater than the EARs for these nutrients. Between 60% and 80% of the women had intakes that exceeded the EAR for folate, and between 40% and 60% had intakes that exceeded the EAR for vitamin E. For plasma ascorbic acid and \(\alpha\)-tocopherol, the 20th percentile value was well above the cutoff point for adequacy. There are

---

**Table 1. Characteristics of Participants and Nonparticipants**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Nonparticipants (n = 1114)</th>
<th>Participants (n = 603)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean, y</td>
<td>60</td>
<td>61</td>
</tr>
<tr>
<td>Alcohol intake, mean, g</td>
<td>7.1</td>
<td>7.5</td>
</tr>
<tr>
<td>BMI in 1980, mean†</td>
<td>24.8</td>
<td>24.5</td>
</tr>
<tr>
<td>Pack-years smoked through 1990, mean, y‡</td>
<td>20.6</td>
<td>16.7</td>
</tr>
<tr>
<td>Outdoors in the summer ‡</td>
<td>87.3</td>
<td>87.9</td>
</tr>
<tr>
<td>Diagnosis of high blood pressure before 1990</td>
<td>18.0</td>
<td>21.1</td>
</tr>
<tr>
<td>Supplement users 1980-1995</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin C</td>
<td>38.3</td>
<td>42.3</td>
</tr>
<tr>
<td>Vitamin E‡</td>
<td>28.6</td>
<td>34.8</td>
</tr>
<tr>
<td>Multivitamin</td>
<td>60.5</td>
<td>64.5</td>
</tr>
</tbody>
</table>

*Data are given as the percentage of subjects unless otherwise indicated.
†BMI indicates body mass index (calculated as weight in kilograms divided by the square of height in meters).
‡The difference between participants and nonparticipants was statistically significant (\(P < .05\)).

---

**Figure** Distribution of Lens Opacity Classification System III nuclear opalescence scores for 956 eyes available for analyses.
Table 2. Characteristics of Study Participants by Presence of Nuclear Opacities

<table>
<thead>
<tr>
<th>Variable</th>
<th>Nuclear Opacity†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Present (n = 163)</td>
</tr>
<tr>
<td>Age, mean, y‡</td>
<td>65</td>
</tr>
<tr>
<td>Alcohol intake, mean, g</td>
<td>7.7</td>
</tr>
<tr>
<td>BMI in 1980, mean§</td>
<td>24.4</td>
</tr>
<tr>
<td>Pack-years smoked through 1990, mean, y</td>
<td>17.8</td>
</tr>
<tr>
<td>Outdoors in the summer ≥8 h/wk in 1980</td>
<td>91.4</td>
</tr>
<tr>
<td>Diagnosis of high blood pressure before 1990</td>
<td>22.7</td>
</tr>
</tbody>
</table>

Table 3. Nutrient Quintiles Used to Define Intake and Plasma Nutrient Categories

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Normative Values*</th>
<th>Quintile Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20</td>
<td>40</td>
</tr>
<tr>
<td>Intake level, mg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin C</td>
<td>60</td>
<td>140</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>12</td>
<td>6.7</td>
</tr>
<tr>
<td>Riboflavin</td>
<td>0.9</td>
<td>1.65</td>
</tr>
<tr>
<td>Folate</td>
<td>320</td>
<td>284</td>
</tr>
<tr>
<td>α-Carotene</td>
<td>NA</td>
<td>0.4</td>
</tr>
<tr>
<td>β-Carotene</td>
<td>NA</td>
<td>3.0</td>
</tr>
<tr>
<td>β-Cryptoxanthin</td>
<td>NA</td>
<td>0.04</td>
</tr>
<tr>
<td>Lutein/zeaxanthin</td>
<td>NA</td>
<td>2.4</td>
</tr>
<tr>
<td>Lycopene</td>
<td>NA</td>
<td>5.7</td>
</tr>
<tr>
<td>Total carotenoids</td>
<td>NA</td>
<td>12.8</td>
</tr>
<tr>
<td>Plasma level, µmol/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>23</td>
<td>52</td>
</tr>
<tr>
<td>α-Tocopherol</td>
<td>10</td>
<td>23</td>
</tr>
<tr>
<td>Total carotenoids</td>
<td>NA</td>
<td>1.7</td>
</tr>
</tbody>
</table>

*Data are given as the percentage of participants unless otherwise indicated.
†At least one opacity in the nuclear region.
‡The difference between women with and without opacities was statistically significant (P < .05).
§BMI indicates body mass index (calculated as weight in kilograms divided by the square of height in meters).

Prevalence of nuclear opacification with increasing intake were seen for vitamin C (P = .003), riboflavin (P = .03), and folate (P = .005). No significant trends were observed for vitamin E (P = .06), β-carotene (P = .08), or lutein/zeaxanthin (P = .08) intake, but the prevalence of nuclear opacification was significantly lower in the highest β-carotene quintile category (P = .04), the upper 2 vitamin E quintile categories (P = .02 and .03, respectively), and the upper 4 lutein/zeaxanthin quintile categories (P = .03, .03, .04, and .02, respectively) than in the lowest quintile category for each of these nutrients. None of the interactions between nutrient intake and either age or smoking were statistically significant.

To determine if the observed associations between risk of nuclear opacification and intakes of each nutrient were independent of the other nutrients, we mutually adjusted the relations between odds of nuclear opalescence and intake of vitamin C, vitamin E, riboflavin, folate, β-carotene, β-cryptoxanthin, and lutein/zeaxanthin using a backward selection procedure. Only vitamin C intake remained significantly associated with nuclear opalescence (P = .003) after stepwise removal of nonsignificant nutrients. Moreover, none of the other nutrients remained significantly associated with the prevalence of nuclear opacities in models that included these nutrients in a pairwise adjustment with vitamin C, whereas vitamin C remained significantly associated with nuclear opalescence in all of the pairwise models.

Assessment of Intake Based on Multiple and Single Measurements

We examined the effect of characterizing intake with multiple measurements of intake over an extended period rela-
tive to one cross-sectional measurement of intake obtained at the eye examination. The Spearman rank correlation coefficient between the average and single time point measures was 0.80 for vitamin C intake. When the measurement of intake was limited to a single cross-sectional measurement, the ORs (95% confidence intervals) for nuclear opalescence in the second to fifth quintile categories of vitamin C intake relative to the first category were as follows: 0.56 (0.28-1.10), 0.38 (0.21-0.69), 0.29 (0.14-0.62), and 0.34 (0.19-0.59). The P for trend was .003. These ORs and confidence intervals are similar to those based on the average of 5 measurements of vitamin C intake (Table 6).

### DURATION OF VITAMIN SUPPLEMENT USE AND PREVALENCE OF OPACITIES

Table 7 displays the relation between duration of vitamin supplement use and the prevalence of nuclear opacification. The prevalence of nuclear opacities was 64% or 43% lower among women who used vitamin C or multivitamin supplements, respectively, for 10 or more years. There was a statistically significant trend of decreasing prevalence of nuclear opalescence with increasing duration for users of either supplement. The prevalence of nuclear opacities among women who used vitamin E supplements for 5 to 9 years and 10 or more years was less than 50% of the prevalence among those who did not use vitamin E supplements, but the number of users in these duration categories was small and the ORs were not significantly lower than 1.0. However, the trend across duration categories was statistically significant (P = .03). After mutual adjustment of the vitamin supplement associations for one another, only the association between nuclear opalescence and vitamin C supplements remained largely unaffected and statistically significant (Table 8). None of the interactions between vitamin supplement use and either age or smoking were statistically significant.

### PLASMA NUTRIENTS AND PREVALENCE OF OPACITIES

There was a significant inverse association between plasma ascorbic acid measured at baseline and the prevalence of nuclear opacities (Table 9). The OR relating the prevalence of opacities in the fourth ascorbic acid quintile category to that in the lowest category was statistically significant, but the OR in the highest quintile category was only marginally significant. For plasma α-tocopherol, the OR in the highest quintile category was significantly less than 1, but the inverse trend across quintile categories was only marginally significant. On the mutual adjustment of these 2 plasma vitamins, neither remained significantly associated with prevalent nuclear opacification. Total plasma carotenoids were unrelated to the prevalence of nuclear opacities. None of the interactions between these plasma nutrients and age or smoking were statistically significant.

---

**Table 4. Spearman Rank Correlation Coefficient Matrix for Vitamins and Total Carotenoids**

<table>
<thead>
<tr>
<th>Intake</th>
<th>Vitamin E</th>
<th>Riboflavin</th>
<th>Folate</th>
<th>Total Carotenoids</th>
<th>Ascorbic Acid</th>
<th>α-Tocopherol</th>
<th>Total Carotenoids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin C</td>
<td>0.62</td>
<td>0.64</td>
<td>0.65</td>
<td>0.46</td>
<td>0.37</td>
<td>0.29</td>
<td>0.14</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>...</td>
<td>0.69</td>
<td>0.65</td>
<td>0.20</td>
<td>0.32</td>
<td>0.53</td>
<td>0.12†</td>
</tr>
<tr>
<td>Riboflavin</td>
<td>...</td>
<td>...</td>
<td>0.76</td>
<td>0.27</td>
<td>0.26</td>
<td>0.30</td>
<td>0.15</td>
</tr>
<tr>
<td>Folate</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>0.42</td>
<td>0.33</td>
<td>0.31</td>
<td>0.19</td>
</tr>
<tr>
<td>Total carotenoids</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>0.10</td>
<td>0.08†</td>
<td>0.19</td>
</tr>
<tr>
<td>Plasma concentration</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>0.29</td>
<td>0.28</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>0.33</td>
</tr>
<tr>
<td>α-Tocopherol</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>0.33</td>
</tr>
</tbody>
</table>

*All correlations are statistically significant (P < .05) unless otherwise indicated.

†These correlations are not statistically significant (P > .05).

**Table 5. Spearman Rank Correlation Coefficient Matrix for Individual and Total Carotenoids**

<table>
<thead>
<tr>
<th>Intake</th>
<th>β-Carotene</th>
<th>β-Cryptoxanthin</th>
<th>Lutein/Zeaxanthin</th>
<th>Lycopene</th>
<th>Total Carotenoids</th>
<th>Plasma Concentration (Total Carotenoids)</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-Carotene</td>
<td>0.83</td>
<td>0.30</td>
<td>0.48</td>
<td>0.31</td>
<td>0.62</td>
<td>0.15</td>
</tr>
<tr>
<td>β-Cryptoxanthin</td>
<td>...</td>
<td>0.41</td>
<td>0.77</td>
<td>0.42</td>
<td>0.81</td>
<td>0.20</td>
</tr>
<tr>
<td>Lutein/zeaxanthin</td>
<td>...</td>
<td>...</td>
<td>0.33</td>
<td>0.25</td>
<td>0.38</td>
<td>0.16</td>
</tr>
<tr>
<td>Lycopene</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>0.40</td>
<td>0.76</td>
<td>0.18</td>
</tr>
<tr>
<td>Total carotenoids</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>0.84</td>
<td>0.13</td>
</tr>
</tbody>
</table>

*All correlations are statistically significant (P < .05).
Results from the NVP provide further evidence that antioxidant nutrients are associated with risk of age-related lens opacification. Total vitamin C intake from diet and supplements was associated with a lower prevalence of nuclear opalescence. Although the vitamin C intake in the lowest intake quintile category ranged up to 140 mg/d, which is nearly twice the recommended daily allowance of 75 mg/d for women, there was a signifi-
cant 48% lower odds of nuclear opacities for usual intakes between 140 and 180 mg/d, a 53% lower odds for intakes between 180 and 240 mg/d, and a 66% lower odds for intakes between 240 and 360 mg/d. There was little additional lowering of the odds of nuclear opalescence at higher intakes. These data are consistent with the prior observation that human eye tissues saturate at intakes of vitamin C between 200 and 300 mg/d. The intake data are also supported by our plasma measures that suggest a lower prevalence of nuclear opacification with increasing plasma ascorbic acid concentrations.

We also observed an inverse association between nuclear opacities and duration of vitamin C supplement use. This corroborates earlier work demonstrating that a significantly lower risk of opacities is not seen until vitamin C supplements are used for 10 or more years. This observation should not be interpreted to suggest that higher supplemental levels of vitamin C would be required to alter cataract risk for the reasons previously noted. Together, the data relating vitamin C intake and vitamin C supplement use to nuclear opacities argue that intake of vitamin C in excess of the recommended daily allowance may play a useful role in maintaining lens health, but that vitamin C consumption of more than 300 mg/d may provide little added benefit to the lens.

Our results also suggest that women with lutein/zeaxanthin intake above 2.4 mg/d may have a lower risk of nuclear cataract. Lutein and zeaxanthin are the predominant carotenoids found in the human eye lens. However, this association was not clearly independent of the relation between vitamin C and nuclear opacities. The association that we observed before adjusting for vitamin C intake is consistent with the results from the Beaver Dam Eye Study, which showed a strong inverse association between past lutein intake and incidence of nuclear cataract and a 30% to 40% reduction in risk of incident nuclear opacities for persons with serum lutein concentrations in the highest tertile category relative to those in the lowest tertile category. Two recent reports from the NHS and the Health Professionals Follow-up

Table 8. Relation Between the Prevalence of Nuclear Lens Opacities and the Duration of Vitamin Supplement Use After Mutual Adjustment for Use of Other Supplements

<table>
<thead>
<tr>
<th>Supplement</th>
<th>Adjusted for This Supplement</th>
<th>Variable</th>
<th>0†</th>
<th>1-4</th>
<th>5-9</th>
<th>≥10</th>
<th>P for Trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin C</td>
<td>Vitamin E</td>
<td>OR‡</td>
<td>1.00</td>
<td>0.64</td>
<td>0.85</td>
<td>0.40</td>
<td>.05</td>
</tr>
<tr>
<td></td>
<td>Multivitamin</td>
<td>OR‡</td>
<td>1.00</td>
<td>0.69</td>
<td>0.78</td>
<td>0.40</td>
<td>.02</td>
</tr>
<tr>
<td></td>
<td>Vitamin E</td>
<td>OR‡</td>
<td>1.00</td>
<td>0.96</td>
<td>0.57</td>
<td>0.91</td>
<td>.79</td>
</tr>
<tr>
<td></td>
<td>Multivitamin</td>
<td>OR‡</td>
<td>1.00</td>
<td>0.89</td>
<td>0.51</td>
<td>0.56</td>
<td>.10</td>
</tr>
</tbody>
</table>

Table 9. Relation Between the Prevalence of Nuclear Lens Opacities and Plasma Nutrient Concentrations

<table>
<thead>
<tr>
<th>Nutrient Variable</th>
<th>Nutrient Quintile Category†</th>
<th>P for Trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin C (ascorbic acid)</td>
<td>OR§</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>95% CI</td>
<td>0.33-1.29</td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>93</td>
</tr>
<tr>
<td>Vitamin E (α-tocopherol)</td>
<td>OR§</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>95% CI</td>
<td>0.36-1.32</td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>97</td>
</tr>
<tr>
<td>Total carotenoids</td>
<td>OR§</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>95% CI</td>
<td>0.75-2.88</td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>88</td>
</tr>
</tbody>
</table>

*The definition of a nuclear lens opacity and explanations of all abbreviations are given in the first footnote of Table 6.
†Referent category.
‡Adjusted for age at examination, pack-years smoked through 1990, history of hypertension through 1990, body mass index in 1980, summer sunlight exposure in 1980, and usual alcohol intake between 1980 and the date of the examination.
§Adjusted for age at examination, pack-years smoked through 1990, history of hypertension through 1990, body mass index in 1980, summer sunlight exposure in 1980, and usual alcohol intake between 1980 and the date of the examination.
Study\textsuperscript{20} demonstrated that women and men with lutein/zeaxanthin intakes of approximately 4 to 6 mg/d had reduced rates of cataract extraction.

We also observed inverse associations between the risk of nuclear opalescence and several other nutrient measures, including vitamin E intake, duration of vitamin E supplement use, plasma vitamin E concentrations, and duration of multivitamin supplement use. Multivitamin and vitamin E supplement use was related to the risk of nuclear opacification in the Longitudinal Study of Cataract,\textsuperscript{16} and there was an inverse association between plasma vitamin E concentrations and incidence of nuclear opacities in the Beaver Dam cohort.\textsuperscript{17} However, as with lutein/zeaxanthin intake, it is not certain that the relations between nuclear opacification and vitamin E or multivitamin supplements in the present study were independent of the vitamin C relationship.

Our data clearly demonstrate the difficulties in determining and interpreting the independent contribution of different nutrients or types of supplements to the risk of nuclear opacities. The strong correlation between the intakes of most nutrients and between the plasma measures in this study indicates the need to consider the interrelationships between nutrients. Intakes of many nutrients are correlated because they are found together in many foods and supplements. Our ability to accomplish the mutual adjustment may be limited by the high correlation between some nutrients and types of supplements. For example, the correlation coefficient for the measures of usual vitamin C and vitamin E intake was 0.62, and 79% of women who consumed vitamin E supplements for 10 or more years also consumed vitamin C supplements for 10 or more years. Two thirds of the long-term vitamin C and vitamin E supplement users also took multivitamins for 10 or more years. Even though we tried to assess the independent contribution of the different nutrients and types of vitamin supplements, we may not have been completely effective because of the large overlap in their intake and use. Therefore, we may be limited in our ability to identify the separate contributions of the different antioxidant nutrients to the lower prevalence of opacities, but we can be fairly certain that 1 or more of these antioxidant nutrients are associated with a lower risk of nuclear opacities.

We undertook the present study with the hypothesis that intake based on multiple dietary assessments should be more strongly associated with the prevalence of nuclear opacities than intake based on one dietary assessment. Surprisingly, we observed similar associations between the risk of nuclear opacities and vitamin C intake measured either as the average of 5 values over 13 to 15 years or as a single value from the end of this interval. The apparent reason for the similarity of the relation is that women tended to maintain their intake levels over time in our sample. For example, of women in the lowest quintile category for the average intake measurement, 69% were also in the lowest category for the single measurement and 89% were in the lowest 2 quintile categories for the single measurement. Of women in the highest intake quintile category for the average intake measurement, 82% were also in the highest category for the single intake measurement and 92% were in the highest 2 quintile categories for the single measurement.

Interpretation of results from the present study is subject to some additional caveats. We considered the relations between nuclear opalescence and 10 measures of nutrient intake, 3 measures of plasma nutrient concentrations, and 3 measures of vitamin supplement use. Therefore, we must consider the possibility that some of these associations are spurious. However, the associations with vitamin C intake and supplement use would remain statistically significant even under the most conservative adjustments for multiple comparisons. While we controlled for the most likely known or suspected determinants of cataract risk, it is also possible that we have not adequately controlled for some of these or that the observed associations between these antioxidant nutrients and nuclear opacities might be the result of confounding by other unmeasured nutrients, such as flavonoids or nonnutritional factors. However, for the association between nuclear opacification and vitamin C to be a consequence of unidentified confounding by other unmeasured lifestyle factors, one must presume a potent unknown risk factor for lens opacification that also closely parallels vitamin C intake and vitamin C supplement use. It is difficult to conceive of potential dates that would produce such strong confounding.

Another potential limitation is the retrospective nature of the study. However, the nutrient intake data were collected prospectively and well before any of the women knew that they had lens opacities (since we excluded women diagnosed as having cataract). Furthermore, most of the women had early opacities and so should not have experienced any visual symptoms. In fact, visual acuity did not differ between those with and those without opacities in this study (data not shown). Despite the early nature of these opacities, our observations should be relevant to more advanced opacities since it has been demonstrated that approximately 50% of opacities graded as 2 or higher using LOCS III show significant progression during a 5-year period.\textsuperscript{43} Approximately 50% of our sample had LOCS III scores of 2 or greater.

The epidemiological evidence for a relation between antioxidant vitamins and nuclear opacification is promising. Most recent studies report an association between nuclear opacities and one or more antioxidant nutrients. However, these studies do not uniformly demonstrate relations between any particular antioxidant nutrient and risk of nuclear opacification. There are many possible reasons for the lack of consistency between studies of vitamins and lens opacities. Important causes of discordance between studies might be differences in the study samples and methods. For example, Leske and co-workers\textsuperscript{16} recently reported that multivitamin supplements, vitamin E supplements, and higher plasma vitamin E concentrations were associated with a lower risk of increased nuclear opacification during a 5-year follow-up period in the Longitudinal Study of Cataract. However, these investigators did not see any relation between vitamin C supplement use and nuclear opacification. This is in contrast to results from the NVP and from an earlier study\textsuperscript{17} performed in a subset of par-
participants from the NHS cohort. However, in the Longitudinal Study of Cataract,
only 10% of the participants were vitamin C supplement users, while in the NVP
sample, approximately 40% used vitamin C supplements and one quarter of these users consumed the
supplements for 10 or more years. Also, the Longitudinal Study of Cataract combined changes in existing and
newly diagnosed nuclear opacities. The opacities in our study were newly diagnosed and mostly early grade. It
is possible that vitamin C has a differential impact on de-
velopment and progression of nuclear opacities. This
might also explain the discrepant findings between our
study and the expanded reanalysis of the relation be-
tween vitamin C supplement use and cataract extrac-
tion in the complete NHS cohort. In the original analy-
ysis, duration of vitamin C supplement use was strongly
associated with a reduced rate of cataract extraction in
this cohort. However, the later expanded analysis, which
included a longer follow-up and more age-eligible women,
failed to show any relation between duration of vitamin C
supplement use and rate of cataract extraction in the
full eligible group of women, but it did suggest a lower
risk with long-term vitamin C supplement use among sub-
sets of younger women and those who never smoked. The
association between vitamin C intake and incident nuclear
opacities was not statistically significant in the entire Bea-
er Dam Eye Study cohort, but there were significant in-
verse associations reported in subgroups, such as per-
sons with a low glycosylated hemoglobin level, those with
hypertension, and heavy smokers. Such data suggest that
dissimilar associations seen between vitamin C and opaci-
fies in various cohorts might also result from different
health and behavioral characteristics of the partici-
pants. There was also no association between vitamin E
intake and incident nuclear opacities in the Beaver Dam
Eye Study, although higher plasma vitamin E concen-
trations were associated with a reduced incidence in this
cohort, suggesting that methodological differences in
the same population might produce varying results. In
contrast to the inconsistencies for vitamins C and E, our
results relating lutein/zeaxanthin intake to opacifica-
tion are consistent with results from the Beaver Dam,
the NHS, and the Health Professionals Follow-up Study
cohorts.

In summary, the inverse association observed in this
study between vitamin C intake and the prevalence of
nuclear opacification provides added support for a pro-
tective role of antioxidant nutrients against formation of
nuclear opacities. Given some limitations of our study,
we cannot rule out possible inverse associations be-
tween risk of nuclear opacities and other nutrients, in-
cluding lutein/zeaxanthin and vitamin E. Although our
data add more weight to the accumulated evidence that
antioxidant nutrients play a role in the development of
age-related opacities, our results also are not com-
pletely consistent with the results of earlier epidemi-
ological studies. We have attempted to raise some pos-
sible reasons for the discordance in results. Consistency
of an association is one of the most important criteria in
assessing the potential causal nature of a relationship.44
If future epidemiological studies are to play a credible
role in addressing the causal nature of the relation be-
tween antioxidant nutrients and age-related lens opaci-
ties, these studies will need to address the reasons for the
lack of consistency.

Accepted for publication December 21, 2000.

From the Jean Mayer US Department of Agriculture Human Nutrition Research Center on Aging (Drs Jacques and Taylor) and the School of Nutrition Science and Policy (Drs Jacques and Taylor), Tufts University; the Departments of Ophthalmology (Dr Chylack) and Medicine (Drs Hankinson and Willett), Harvard Medical School, Brigham and Women’s Hospital, and the Channing Laboratory (Drs Hankinson and Willett); the Center for Ophthalmic Research (Drs Chylack, Khu Wolf, Padhye, Ms Friend, and Mr Tung); and the Departments of Epidemiology (Drs Hankinson and Willett) and Nutrition (Dr Willett), Harvard School of Public Health, Boston, Mass.

This study was supported by agreement 58-1950-9-
001 from the US Department of Agriculture; grants 98-
01023 and 92-37200-7704 from the National Research Initiative Competitive Grant Program; the Brigham Surgical Group, Boston, Mass; research grant EYE-09611 from the National Eye Institute, Bethesda, Md; research grant CA40356 from the National Institutes of Health, Bethesda; and a grant from the Florida Department of Citrus, Lake-
land.

We thank the project staff for their assistance and the
many others whose effort supported this project, especially
Laura Bury, Rosaline Bowen, Ester Epstein, Mini Bal-
aram, MD, Sheila Crosby, Karen Corsano, Kate Saunders,
Suzen Mueller, Tom Nowell, and Gayle Petty; the nurses
who participated in the study for their continuing contrib-
utions and cooperation; and Frank E. Speizer, MD, over-
all principal investigator for the Nurses’ Health Study, for
his support.

Any opinions, findings, conclusion, or recommenda-
tions expressed in this publication are those of the authors
and do not necessarily reflect the view of the US Depart-
ment of Agriculture.

Corresponding author and reprints: Allen Taylor, PhD, Laboratory for Nutrition and Vision, US Department of Agriculture Human Nutrition Research Center on Aging, Tufts University, 711 Washington St, Boston, MA 02111.

REFERENCES

1. Taylor A. Nutritional and environmental influences on risk for cataract. In: Tay-
lor A, ed. Nutritional and Environmental Influences on the Eye. Boca Raton, Fla:
CRC Press LLC; 1999:53-93.
2. Jacques PF. The potential preventive effects of vitamins for cataract and age-
3. Robertson JM, Donner AP, Trevithick JR. Vitamin E intake and risk of cataracts
5. Jacques PF, Chylack LT Jr. Epidemiologic evidence of a role for the antioxidant
S552-S555.
6. Leske MC, Chylack LT Jr, Wu S. The Lens Opacities Case-Control Study: risk
7. The Italian-American Cataract Study Group. Risk factors for age-related cotti-
cular, nuclear, and posterior subcapsular cataracts. Am J Epidemiol. 1991;133:
541-553.


