

Long-term Nutrient Intake and 5-Year Change in Nuclear Lens Opacities

Paul F. Jacques, ScD; Allen Taylor, PhD; Suzen Moeller, PhD; Susan E. Hankinson, ScD; Gail Rogers, MA; William Tung, BS; José Ludovico, MD; Walter C. Willett, MD, DrPH; Leo T. Chylack, Jr, MD

Objective: To determine if usual nutrient intake is related to a 5-year change in the amount of lens nuclear opacification assessed by computer-assisted image analysis.

Design: A sample of 408 Boston, Mass–area women from the Nurses' Health Study aged 52 to 74 years at baseline participated in a 5-year study related to nutrition and vision. Usual nutrient intake was calculated as the average intake from 5 food frequency questionnaires that were collected over a 13- to 15-year period before the baseline evaluation of lens nuclear density. Duration of vitamin supplement use before baseline was determined from 7 questionnaires collected during this same period. We assessed the degree of nuclear density (opacification) using computer-assisted image analysis of digital lens images with amount of nuclear density measured as a function of average pixel gray scale, ranging from 0 (clear) to 255 (black).

Results: Median (range) baseline and follow-up nuclear densities were 44 (19 to 102) and 63 (32 to 213). The median (range) 5-year change in nuclear density was 18 (–29 to 134) and was positively correlated with the amount of opacification at baseline (Spearman correlation coefficient = 0.35; $P < .001$). Geometric mean 5-year change in nuclear density was inversely associated with the intake of riboflavin (P trend = .03) and thiamin (P trend = .04) and duration of vitamin E supplement use (P trend = .006).

Conclusion: Our results suggest that long-term use of vitamin E supplements and higher riboflavin and/or thiamin intake may reduce the progression of age-related lens opacification.

Arch Ophthalmol. 2005;123:517-526

Author Affiliations: From the Jean Mayer USDA Human Nutrition Research Center on Aging (Drs Jacques, Taylor, and Moeller, and Ms Rogers), School of Nutrition Science and Policy (Drs Jacques and Taylor), Tufts University, Boston, Mass; Department of Ophthalmology and Visual Sciences, University of Wisconsin, Madison (Dr Moeller); The Channing Laboratory, Department of Medicine, Harvard Medical School and Brigham and Women's Hospital (Drs Hankinson and Willett), Departments of Nutrition (Dr Willett) and Epidemiology (Drs Hankinson and Willett), Harvard School of Public Health, Center for Ophthalmic Research, Brigham and Women's Hospital (Mr Tung and Drs Ludovico and Chylack), and Harvard Medical School (Dr Chylack), Boston.
Financial Disclosure: None.

AGE-RELATED CATARACT IS the clinical manifestation of opacification of the eye lens. Lens opacification results from the disruption of the transmission of light through the lens and is most likely a consequence of modifications to the lens proteins from decades of accumulated damage.^{1,2} Animal and in vitro studies suggest that oxidation is responsible for much of the damage to lens constituents and that antioxidants might protect the lens against formation of cataract.^{3,4} These observations are also consistent with epidemiological studies, which

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indicate a possible role for nutritional antioxidants in prevention of age-related cataract.^{1,2} However, there is still much uncertainty regarding the role of specific antioxidant nutrients in the development of cataract.

We have previously demonstrated in the Nutrition and Vision Project, a subset of wom-

en from the Nurses' Health Study (NHS) cohort⁵ who reside in the Boston, Mass, area, that the prevalence of newly diagnosed age-related nuclear opacities was inversely associated with usual, long-term vitamin C intake.⁶ In the present study, we used computer-assisted image analysis methods to examine the change in nuclear lens opacification during a 5-year follow-up period in the same group of women. The method that we used to measure change in nuclear opacification is highly reproducible and provides a sensitive procedure for measuring progression of nuclear density.^{7,8}

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.⁵ 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 52 to 74 years who resided in the Boston 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 Nutrition and Vision Project, 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 women (43%) with this 1 mailing. Six hundred three of these volunteers were ultimately scheduled and examined as part of the Nutrition and Vision Project between April 1993 and August 1995. Scheduling conflicts (because of work and travel) were the most common reasons for failure to examine the 127 who agreed to participate but were never seen. Approximately 5 years after the baseline examinations (November 1998–November 2000), follow-up eye examinations were conducted on 451 of the original participants. Participants with bilateral cataract extraction or missing baseline images ($n = 26$) were ineligible and not invited to the follow-up examination. One hundred twenty-six of the eligible participants did not complete a follow-up examination for the following reasons: lack of interest ($n = 79$), illness or death ($n = 27$), relocation outside the Boston area ($n = 13$), inability to contact, and scheduling conflicts ($n = 7$). Informed consent was obtained from all study participants and all procedures were approved by the Human Investigations Review Committee at the Tufts–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 received biennial questionnaires requesting information on a variety of health and lifestyle issues. A 61-item semiquantitative food frequency questionnaire was initially incorporated into the biennial questionnaire in 1980.⁹ The food frequency questionnaire queried usual intake over 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.¹⁰ The food frequency questionnaire has been extensively validated relative to both long-term diet records^{9,10} and biochemical markers of nutrient status.^{11–13} 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 Nutrition and Vision Project (1993–1995).

We used the data from women who completed 5 food frequency questionnaires collected between 1980 and 1993 through 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, thiamin, niacin, and the individual carotenoids. We used

7 reports of vitamin supplement use from 1980 through 1993 to 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 Nutrition and Vision Project, 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 time of the eye examination for analyses of plasma antioxidant concentrations. Plasma for the vitamin C analyses was stabilized by the addition of an equal volume of 0.35M perchloric acid containing 0.26mM EDTA and was centrifuged at $\times 4000g$ within 30 minutes of venipuncture. Ascorbic acid (reduced vitamin C) measurements were determined on fresh plasma samples by reverse-phase high-performance liquid chromatography analyses with electrochemical detection.¹⁴ Samples for vitamin E and total carotenoids were frozen at $-70^{\circ}C$ for up to 1 month. Vitamin E (α -tocopherol) was measured on a reverse-phase high-performance liquid chromatograph by the method of Bieri et al,¹⁵ and total carotenoids were measured spectrophotometrically by the method of Roels et al.¹⁶

ASSESSMENT OF LENS STATUS

All Nutrition and Vision Project participants received a detailed eye examination using standardized techniques.⁶ The examination included an ocular history and medical history, Bailey Lovie test of visual acuity and manifest refraction, 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 a minimum of 6 mm with phenylephrine and tropicamide. The posterior segment was examined by direct and indirect ophthalmoscopy. The examiner had no knowledge of the nutrient status of any of the volunteers.

We assessed the degree of nuclear opacification (density) using computer-assisted image analysis of digital lens images.^{7,8} Scheimpflug, digitalized, black-and-white images of the lens nucleus were taken with a Nidek EAS 1000 camera (Nidek, Tokyo, Japan). Baseline and follow-up images were graded in nonsequential portions of the same grading sessions. A single grader determined nuclear opalescence using the Nidek EAS 1000 digital image analysis software (version 1.23E). The amount of nuclear opacification was assessed as nuclear density measured as a function of a standard gray scale ranging from 0 (clear) to 255 (black) and reported as pixel density units (pdu). This method is highly reproducible. In a control set of 70 eyes that were graded twice, 80% of the scores were identical and 100% of scores were within ± 3 pdu on regrading.

DEFINING POTENTIAL CONFOUNDERS

Data on known or suspected nonnutritional determinants of cataract risk were obtained from the 1980 through 1994 biennial

Table 1. Nutrient Quintiles Used to Define Intake and Plasma Nutrient Categories

Nutrient	Normative Value*	Quintile Values			
		20	40	60	80
Intake					
Vitamin C, mg	60	140	182	241	362
Vitamin E, mg	12	6.7	10.1	20.3	90.8
Riboflavin, mg	0.9	1.65	2.12	2.87	4.39
Thiamin, mg	1.1	1.24	1.59	2.21	3.94
Niacin, mg	14	21.2	25.8	32.2	42.5
α-Carotene, mg	NA	0.4	0.6	0.8	1.2
Beta carotene, mg	NA	3.0	4.0	5.1	6.6
β-Cryptoxanthin, mg	NA	0.04	0.06	0.09	0.1
Lutein/zeaxanthin, mg	NA	2.4	3.3	4.3	5.6
Lycopene, mg	NA	5.7	7.4	9.4	12.5
Total carotenoids, mg	NA	12.8	16.4	19.9	24.4
Plasma concentration					
Ascorbic acid, mg/dL	0.40	0.92	1.14	1.32	1.51
α-Tocopherol, mg/dL	0.43	0.99	1.21	1.38	1.76
Total carotenoids, μmol/L	NA	1.7	2.2	2.7	3.3

Abbreviations: EAR, Dietary Reference Intake Estimated Average Requirement; NA, not applicable.

SI conversions: To convert ascorbic acid to micromoles per liter, multiply by 56.78; α-tocopherol to milligrams per deciliter, 23.22.

*Normative values for plasma ascorbic acid¹⁸ and plasma α-tocopherol.¹⁹ Normative intake levels were determined from the EAR for intake for 51- to 70-year-old women.^{20,21} The EAR is the intake value that is estimated to meet the requirement of half the healthy individuals in a life-stage and sex group and is used to assess the adequacy of intakes of population groups. Normative values are not available for carotenoids.

nial NHS questionnaires and used to adjust analyses for potential confounding. We considered confirmed history of diabetes mellitus (yes/no) and current smoking (yes/no) as reported on the NHS questionnaires collected before the baseline examination (from the 1992 questionnaire for participants examined before June 1, 1994, and from the 1994 questionnaire for those examined after this date). We also adjusted for reported summertime sunlight exposure (≥ 8 h/wk) reported on the 1980 questionnaire, alcohol use at baseline, and body mass index (BMI) (calculated as weight in kilograms divided by square of height in meters) using height reported in 1976 and weight reported on the NHS questionnaire collected before the baseline examination (from the 1992 questionnaire for participants examined before June 1, 1994, and from the 1994 questionnaire for those examined after this date).

STATISTICAL METHODS

We related average nutrient intake and duration of vitamin supplement use to the mean 5-year change in nuclear density with the SAS MIXED procedure.¹⁷ This procedure allowed the individual eyes to be the unit of observation and adjusted the standard errors of the model parameters for the correlated data resulting from repeated measurements on the same individual. The measure of change in nuclear density was skewed and, because of 1 extreme outlier (-29 pdu), was not readily normalized using typical data transformations. Therefore, before transforming the data, we modified the extreme outlier by adding 14 to the value to bring it to -15 pdu, which was 1 unit below the next lowest value, and thus retaining its original relative ranking. We then added 20 to every value of nuclear density change and then applied a logarithmic transformation. We chose a constant of 20 so that not only would all values be positive but also the lowest values would be sufficiently greater than 1, allowing the data to normalize appropriately after application of the logarithmic transformation. After estimating the means on the transformed scale, we calculated the geometric means by applying an inverse transformation and subtracting the constant.

The primary independent variables used to examine the relation between usual nutrient intake and increase in nuclear density were average nutrient intake as described earlier. This variable was classified into quintile categories derived from the entire Nutrition and Vision Project sample of 603 women. The quintile values used to determine the categories are presented in **Table 1**. The Dietary Reference Intake Estimated Average Requirement values for 51- to 70-year-old women^{20,21} are also shown in Table 1 to allow comparisons between the intake quintile cutoff values and these normative values. The relation of duration of vitamin supplement use to increase in nuclear density was examined using the following duration categories: never, 1 to 4 years, 5 to 9 years, and 10 or more years.

To test for linear trend across intake 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 statistical models. Similarly, for duration of vitamin supplement use, we assigned each participant the median duration value of their respective duration category and then entered this median duration variable as a continuous factor into the statistical models. The *P* value for trend was the resulting *P* value for the associated model coefficient. We also used the intake quintile category median and supplement duration category median variables to test for possible interactions between our nutritional measures and baseline nuclear density, age, history of diabetes mellitus, and smoking at baseline. Because of the number of interactions considered ($n = 60$), we used a Bonferroni correction to assess the presence of significant interactions, which resulted in a *P* value cutoff of .001.

To investigate the relation between plasma nutrient measures and increased nuclear density, the plasma nutrient measures were also modeled using quintile categories, which are presented in Table 1 with cutoff values for adequate plasma nutrient concentrations^{18,19} to allow comparisons between the quintile cutoff values and these normative values. Linear trend across quintile categories and potential interactions were assessed in the same manner as described for the intake and supplement duration variables.

Table 2. Characteristics of Women Included and Excluded From Analyses*

Variable	Included (n = 408)	Excluded (n = 195)†
Age, y, mean (95% CI)	62 (61-62)	62 (61-63)
Nuclear density at baseline-left eye, pdu, mean (95% CI)	44.9 (43.9-46.1)‡	47.1 (45.4-48.9)
Nuclear density at baseline-right eye, pdu, mean (95% CI)	46.7 (45.5-47.8)‡	49.2 (47.4-51.1)
Nonsmokers in 1993	94.8	92.8
Diabetes mellitus in 1992-1994	2.7‡	9.2
Cataract at baseline	9.8‡	18.5
New cataract at follow-up§	29.2‡	51.2
Vitamin C supplement use 1980-1995	45.7‡	34.9
Vitamin E supplement use 1980-1995	36.0	29.2
Multivitamin supplement use 1980-1995	67.2	66.2

Abbreviations: CI, confidence interval; pdu, pixel density units.

*Values are expressed as percentages unless otherwise indicated.

†Of those excluded from analyses, 152 did not participate in the follow-up and 43 had incomplete data.

‡Difference between women included and excluded was statistically significant ($P < .05$).

§Reported history of cataract diagnosis between baseline and follow-up.

Comparison based on 408 women included in analyses vs 43 seen at follow-up but not included in analyses because of incomplete data.

RESULTS

COMPARISON OF WOMEN INCLUDED AND EXCLUDED FROM ANALYSES

In addition to the 152 original participants who did not return to the follow-up examination, 43 women who attended the examination had incomplete information for items that were used in these analyses. Therefore, analyses were conducted on the remaining 408 women. There were a number of notable differences between the 408 women who had complete data for these analyses and the 195 who were excluded from analyses because of loss to follow-up or incomplete data (**Table 2**). Although the 2 groups were similar in age, smoking status, and use of vitamin E and multivitamin supplements, women included in these analyses were significantly less likely to have a history of diabetes mellitus and cataract at baseline and were more likely to use vitamin C supplements than women excluded from these analyses. Women who were included in the follow-up analyses also had a significantly lower amount of nuclear opacification at baseline in both the left and right eyes than women who were excluded from analyses. Women included in analyses were also significantly less likely to have had a diagnosis of cataract between the baseline and follow-up examinations than women who were seen at follow-up but who were excluded because of incomplete data.

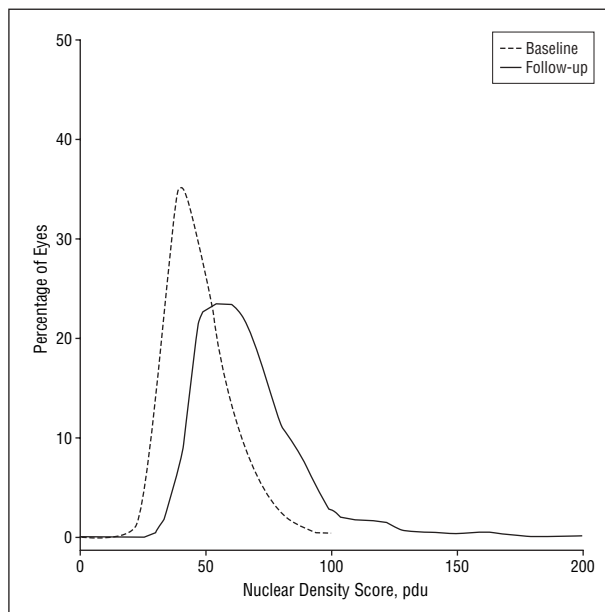


Figure 1. Distribution of nuclear density scores at baseline and follow-up for 816 eyes available for analyses. pdu indicates pixel density units.

DISTRIBUTION OF NUCLEAR DENSITY SCORES

The distribution of nuclear density scores at baseline and follow-up are displayed in **Figure 1** for the 816 eyes available for analyses. Selected percentile values for baseline and follow-up nuclear density scores and the 5-year change in nuclear density are presented in **Table 3**. The geometric mean increase in nuclear density was 18.4 pdu in the overall sample, and the increase was moderately correlated with baseline nuclear density (**Figure 2**). The Spearman correlation coefficient relating 5-year change in nuclear density scores to baseline nuclear density was 0.35 ($P < .001$). The geometric mean increase in nuclear density was 14.8 pdu for women with baseline nuclear density values in the lowest tertile category (< 39 pdu), 17.7 pdu for women with baseline nuclear density values in the middle tertile category (between 39 and 49 pdu), and 21.7 pdu for women with baseline nuclear density values in the highest tertile category (≥ 50 pdu) (P trend $< .001$).

NUTRIENT INTAKE AND INCREASED NUCLEAR DENSITY

Table 4 presents the relations between the 5-year increase in nuclear density and usual nutrient intake (from both food and supplements) measured over a 13- to 15-year period. All geometric mean increases in nuclear density are adjusted for age at baseline, smoking at baseline, history of diabetes mellitus, BMI, summertime sunlight exposure, alcohol intake, years of follow-up, and baseline nuclear density. Higher riboflavin intake (P trend = .03) and higher thiamin intake (P trend = .04) were significantly associated with a smaller increase in nuclear density, although the associations were weak and inconsistent across quintile categories of both riboflavin and thiamin intake. The geometric mean change in the lowest quintile category of riboflavin intake was 4.2 pdu (27%)

Table 3. Distribution of Nuclear Density Scores at the Baseline and Follow-up Examinations Based on 816 Eyes

Nuclear density, pdu*	Selected Percentiles			Range
	25th	50th	75th	
Baseline	37	44	53	19 to 102
Follow-up	52	63	76	32 to 213
5-y change	13	18	24	-15 to 134

Abbreviation: pdu, pixel density units.

*Full range of possible values for nuclear density scores is 0 to 255.

higher than the change in the highest quintile category. The geometric mean change in the lowest quintile category of thiamin intake was 2.8 pdu (18%) higher than the change in the highest quintile category. Thiamin and riboflavin intakes were very highly correlated (Spearman correlation coefficient = 0.10), and on mutual adjustment for these 2 nutrients, neither remained significantly associated with change in nuclear density.

We evaluated interactions between the intakes of each nutrient in Table 4 and age, history of diabetes mellitus, smoking status, and baseline nuclear density score. There were no significant interactions ($P < .001$) with age, history of diabetes mellitus, or smoking status, but significant interactions were observed between baseline nuclear density and vitamin E, riboflavin, niacin, and thiamin intakes. To examine the nature of these interactions, we stratified the analyses for these 4 nutrients by tertile categories of baseline nuclear density (**Table 5**). For all 4 nutrients, the association between intake and increased nuclear density was stronger among women with higher baseline nuclear density.

For vitamin E intake, the difference in the geometric mean increase in nuclear density between the lowest and highest intake quintile categories was 1.8 pdu for women in the lowest baseline nuclear density tertile category (P trend = .95), -0.9 pdu for women in the middle baseline nuclear density tertile category (P trend = .52), and 6.6 pdu for women in the highest baseline nuclear density tertile category (P trend = .06). For the women with the highest baseline nuclear density, the difference represents a 35% greater increase in nuclear density among those with the lowest vitamin E intake category compared with women in the highest intake category.

The difference in the geometric mean nuclear density change between the lowest and highest categories of riboflavin intake was 0.6 pdu for women in the lowest baseline nuclear density tertile category (P trend = .76), 2.5 pdu for women in the middle baseline nuclear density tertile category (P trend = .68), and 11.0 pdu for women in the highest baseline nuclear density tertile category (P trend = .02). The latter difference represents a 67% greater increase in nuclear density among those with the lowest riboflavin intakes compared with women with the highest intakes.

For thiamin, the difference in the geometric mean increases in nuclear density in the lowest and highest intake categories was 0.3 pdu for women in the lowest baseline nuclear density tertile category (P trend = .75), 1.5 pdu for women in the middle tertile category of baseline nuclear

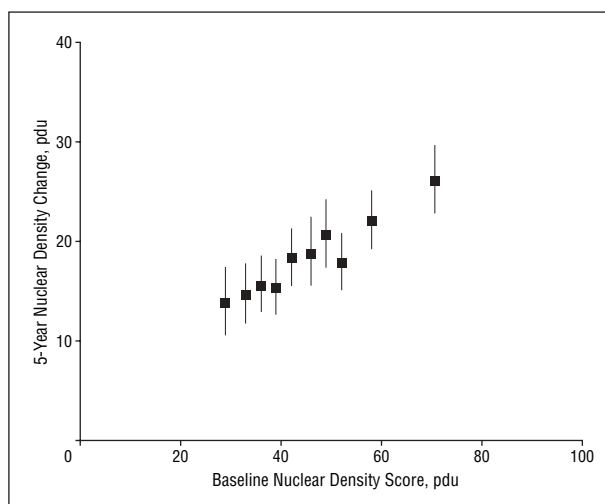


Figure 2. Mean 5-year change in nuclear density scores according to decile category of baseline nuclear density value. pdu indicates pixel density units.

density (P trend = .68), and 8.4 pdu for women in the highest baseline nuclear density tertile category (P trend = .03), with this latter difference representing a 55% increase in nuclear density for those with the lowest thiamin intakes.

Finally, for niacin, the difference in the geometric mean nuclear density change between the lowest and highest categories of intake was 0 pdu for women in the lowest baseline nuclear density tertile category (P trend = .99), 1.1 pdu for women in the middle baseline nuclear density tertile category (P trend = .55), and 10.7 pdu for women in the highest baseline nuclear density tertile category (P trend = .04). For women with the highest baseline nuclear density, this difference represents a 57% greater increase in nuclear density for women in the lowest niacin intake category compared with women in the highest intake category.

DURATION OF VITAMIN SUPPLEMENT USE AND INCREASED NUCLEAR DENSITY

Duration of vitamin E supplement use was inversely associated with the 5-year increase in nuclear density (P trend = .006) (**Table 6**). The increase in nuclear density among women who never used vitamin E supplements was 42% higher than the increase among those who used vitamin E supplements for 10 or more years.

There were no interactions between duration of use of any of the vitamin supplements and age, history of diabetes mellitus, or smoking status, but a significant in-

Table 4. Relation Between 5-Year Change In Nuclear Density and Total Nutrient Intake From Food and Supplements*

Nutrient	Nutrient Quintile Categories†					P Trend
	1	2	3	4	5	
Vitamin C						
Mean	18.7	18.8	17.1	16.5	16.9	.21
95% CI	14.5-23.4	14.6-23.5	13.1-21.6	12.4-21.1	12.8-21.5	
Sample size	79	82	75	85	87	
Vitamin E						
Mean	18.5	16.5	17.8	18.9	16.0	.15
95% CI	14.2-23.3	12.5-21.1	13.7-22.3	14.8-23.5	12.0-20.5	
Sample size	81	77	79	84	87	
Riboflavin						
Mean	19.8	17.1	18.6	20.0	15.6	.03
95% CI	15.5-24.7	13.1-21.6	14.6-23.2	15.5-25.0	11.8-19.9	
Sample size	80	83	84	82	79	
Thiamin						
Mean	18.4	18.3	18.2	18.9	15.6	.04
95% CI	14.1-23.2	14.3-22.8	14.1-22.9	14.7-23.7	11.7-20.0	
Sample size	80	81	79	89	79	
Niacin						
Mean	20.8	17.2	18.4	20.0	16.7	.08
95% CI	16.2-26.0	13.1-21.7	14.5-22.8	15.5-25.1	12.7-21.2	
Sample size	84	70	86	87	81	
α-Carotene						
Mean	18.3	17.8	17.9	18.5	16.8	.40
95% CI	14.2-22.8	13.6-22.5	13.7-22.7	14.2-23.2	12.7-21.3	
Sample size	80	81	75	84	88	
Beta carotene						
Mean	18.1	18.8	17.5	19.0	15.8	.15
95% CI	14.0-22.7	14.6-23.5	13.4-22.1	14.7-23.8	11.9-20.2	
Sample size	79	76	88	83	82	
β-Cryptoxanthin						
Mean	18.5	18.4	16.9	16.6	17.9	.56
95% CI	14.3-23.1	14.3-22.9	12.9-21.5	12.5-21.3	13.6-22.7	
Sample size	80	84	77	84	83	
Lutein/zeaxanthin						
Mean	18.5	18.7	17.9	18.0	16.8	.23
95% CI	14.3-23.3	14.3-23.6	13.8-22.4	13.8-22.6	12.7-21.4	
Sample size	73	90	84	82	79	
Lycopene						
Mean	16.2	19.3	18.7	18.1	16.8	.85
95% CI	12.2-20.7	15.0-24.2	14.5-23.4	14.0-22.5	12.7-21.4	
Sample size	82	78	83	90	75	
Total carotenoids						
Mean	18.3	18.0	18.3	18.0	16.1	.20
95% CI	14.1-23.0	13.7-22.7	14.2-22.9	13.9-22.6	12.0-20.6	
Sample size	76	89	73	94	76	

Abbreviation: CI, confidence interval.

*Geometric 5-year change in mean nuclear density measured as pixel density units adjusted for age at examination, smoking at baseline, body mass index at baseline, summertime sunlight exposure in 1980, usual alcohol intake at baseline, baseline nuclear density value, and length of follow-up.

†See Table 1 for nutrient intake quintile values.

teraction was observed between baseline nuclear density and duration of multivitamin supplement use ($P < .001$). To examine the interaction, we stratified the analyses relating change in nuclear density and duration of multivitamin supplement use according to tertile categories of baseline nuclear density. In spite of the highly significant interaction, there was no clear association seen in any of the baseline nuclear density categories after stratification as evidenced by the lack of any statistical trends. The P values for the test for trends in the lowest to highest baseline nuclear density categories were .21, .84, and .56, respectively.

PLASMA NUTRIENTS AND INCREASED NUCLEAR DENSITY

There were no significant associations between the 5-year increase in nuclear density and plasma antioxidant nutrient concentrations (**Table 7**). However, there was a significant interaction between baseline nuclear density and carotenoid concentrations ($P < .001$). After stratifying the association by tertile categories of baseline nuclear density, there were no discernible trends or significant associations in any of the baseline nuclear density categories. The P values for trend were .43 for women in

Table 5. Geometric Mean 5-Year Change in Nuclear Density in the Lowest and Highest Nutrient Intake Quintile Categories for Nutrients Demonstrating Significant Interactions With Baseline Nuclear Density: Stratified by Baseline Nuclear Density

Nutrient	Baseline Nuclear Density by Intake Quintile Category								
	<39 pdu			39-49 pdu			≥50 pdu		
	1st	5th	P Trend	1st	5th	P Trend	1st	5th	P Trend
Vitamin E	15.7	13.9	.95	16.7	17.6	.52	25.4	18.8	.06
Riboflavin	14.0	13.4	.76	19.9	17.4	.68	27.5	16.5	.02
Thiamin	13.9	13.6	.75	18.9	17.4	.68	23.7	15.3	.03
Niacin	14.0	14.0	.99	18.0	16.9	.55	29.4	18.7	.04

Abbreviation: pdu, pixel density units.

Table 6. Relation Between 5-Year Change in Nuclear Density and Duration of Vitamin Supplement Use*

Supplement	Duration of Supplement Use				P Trend
	None†	1-4 y	5-9 y	≥10 y	
Vitamin C					.58
Mean	17.7	18.5	19.2	16.6	
95% CI	13.9-21.9	14.4-23.0	14.2-24.8	12.2-21.6	
Sample size	221	96	40	50	
Vitamin E					.006
Mean	17.9	18.1	16.6	12.6	
95% CI	14.1-22.2	14.2-22.5	11.8-22.1	8.2-17.6	
Sample size	261	87	31	29	
Multivitamin					.93
Mean	18.2	17.1	18.5	17.8	
95% CI	14.2-22.8	13.1-21.6	14.3-23.2	13.8-22.3	
Sample size	134	86	73	115	

Abbreviation: CI, confidence interval.

*Geometric 5-year change in mean nuclear density measured as pixel density units adjusted for age at examination, smoking at baseline, body mass index at baseline, summertime sunlight exposure in 1980, usual alcohol intake at baseline, nuclear density at baseline, and length of follow-up.

†No use of supplement or use of supplement for less than 1 year.

Table 7. Relation Between 5-Year Change in Nuclear Density and Plasma Nutrient Concentrations*

Nutrient	Nutrient Quintile Categories†					P Trend
	1	2	3	4	5	
Vitamin C (ascorbic acid)						.18
Mean	17.8	18.1	17.9	18.7	15.3	
95% CI	13.7-22.4	13.8-22.9	13.8-22.4	14.4-23.6	11.3-19.8	
Sample size	71	80	85	79	84	
Vitamin E (α-tocopherol)						.68
Mean	17.8	17.5	17.0	18.7	16.8	
95% CI	13.7-22.5	13.3-22.2	12.8-21.7	14.5-23.4	12.8-21.4	
Sample size	79	73	78	83	84	
Total carotenoids						.09
Mean	16.5	18.8	16.9	18.3	19.9	
95% CI	12.6-20.8	14.5-23.6	12.8-21.6	14.0-23.1	15.4-24.9	
Sample size	85	74	74	77	86	

Abbreviation: CI, confidence interval.

*Geometric 5-year change in mean nuclear density measured as pixel density units adjusted for age at examination, smoking at baseline, body mass index at baseline, summertime sunlight exposure in 1980, usual alcohol intake at baseline, nuclear density at baseline, and length of follow-up.

†See Table 1 for nutrient intake quintile values.

the lowest baseline nuclear density tertile category, .79 for women in the middle baseline nuclear density tertile category, and .09 for women in the highest baseline nuclear density tertile category.

COMMENT

Our results provided added support for a protective association between intake of certain nutrients and nuclear

opacification. Use of vitamin E supplements for 10 or more years was associated with a slower increase in nuclear opacification during the 5-year follow-up. We also observed that higher riboflavin and thiamin intakes were associated with a smaller 5-year increase in nuclear opacification. Higher niacin intake was also associated with a smaller increase in nuclear opacification among women with higher baseline opacification. Further, our data support 2 of the assumptions regarding the development of cataract. First, the lens nuclear changes appeared to be largely unidirectional in nature, and second, the rate of change in these opacities was dependent on the degree of opacification.

The association with vitamin E supplement use is fairly consistent with results of earlier longitudinal studies of nuclear opacities. Vitamin E supplement use and higher plasma vitamin E concentrations were related to a decreased risk of nuclear opacification in the Longitudinal Study of Cataract,²² and there was an inverse association between plasma vitamin E concentrations and incidence of nuclear opacities in the Beaver Dam Eye Study cohort.²³ However, in the full NHS cohort no association was observed between vitamin E intake or vitamin E supplement use and overall cataract extraction or extraction of nuclear cataract.²⁴

Although limited, the available evidence relating higher riboflavin intake and status to a lower risk of nuclear opacification is fairly consistent.¹ Riboflavin intake was reported to be inversely associated with prevalent nuclear lens opacities at baseline in the Beaver Dam cohort,²⁵ the Lens Opacities Case-Control Study,²⁶ and the Blue Mountains Eye Study.²⁷ We also observed an inverse association between riboflavin and prevalence of nuclear opacities at baseline in our Nutrition and Vision Project cohort,⁶ and riboflavin intake showed a weak inverse relation with cataract extraction in the full NHS cohort but only when the contribution from vitamin supplements was excluded.²⁸ In the latter study, extraction of nuclear cataract was not considered separately. In addition to associations between nuclear opacities and riboflavin intake, Leske et al²⁹ also reported an inverse association between riboflavin status measured in erythrocytes and nuclear cataract in the Lens Opacities Case-Control Study.

In spite of the fairly consistent evidence relating higher riboflavin intake to a reduced risk of nuclear opacities, it was difficult in many of these studies to separate the potential influence of riboflavin on risk of nuclear opacities from that of other nutrients, such as niacin and thiamin^{25,27} or vitamin C.⁶ As in the present study, niacin and thiamin intakes were also inversely associated with prevalence of nuclear opacities in the Beaver Dam cohort²⁵ and the Blue Mountains Eye Study,²⁷ but the independent contribution of these nutrients and riboflavin could not be determined because of the high correlation between these nutrients. The relation between riboflavin and nuclear opacities was also examined in the Linxian cataract studies.³⁰ In the general population trial, use of a supplement containing riboflavin (3 mg) and niacin (40 mg) for up to 6 years was associated with a 55% lower prevalence of nuclear cataract among participants aged 65 to 74 years, but the combination of both riboflavin and niacin

does not allow us to identify which nutrient provided the protection.

We observed that the amount of nuclear opacification at baseline affects both the degree of change in nuclear density over time and the strength of the association between that change and nutrient intake. The fact that we only observed associations between nutrient intake and change in opacification among those with a greater amount of opacification at baseline does not necessarily imply that higher nutrient intakes are unrelated to change in nuclear density among those with very early grades of opacification. Rather, it is possible that the failure to see significant associations among women with the lower amounts of opacification at baseline is a consequence of the reduced statistical power due to the smaller increases in opacification seen in these women during the 5-year follow-up.

We failed to observe any association between increase in amount of nuclear opacification and vitamin C intake. This is in contrast to cross-sectional analyses in this same sample of women in which we observed a striking inverse association between vitamin C intake and prevalence of early lens opacities.⁶ There is no clear explanation for this inconsistency. The longitudinal analyses are usually less prone to certain types of bias and therefore results from such studies are commonly believed to provide more valid evidence for causal associations. However, in the present study, bias may have limited our ability to observe associations with vitamin C intake, particularly vitamin C supplement use. As presented in Table 2, there was a relationship between inclusion in these analyses and both vitamin C supplement use and the degree of nuclear opacification assessed in 3 ways: baseline nuclear density, baseline prevalence of cataract, and development of cataract during follow-up. Among women not included in these analyses, the prevalence of having a baseline nuclear density value greater than the median was twice as likely among those who did not use vitamin C supplements than among those who did. However, among women included in these analyses, the prevalence of a baseline nuclear density greater than the median was only 12.5% greater among women who did not use vitamin C supplements compared with women who did (data not shown). Thus, the women who did not use vitamin C supplements and had a greater risk of increased nuclear density were somehow systematically excluded from our analyses. This might produce some attenuation of a relation between vitamin C intake and nuclear opacification.

Apart from this potential explanation for the discrepancies between the baseline and longitudinal analyses in our study sample, we propose an alternative explanation based on a hypothesis that different nutrients might influence the process of lens opacification at different stages. Our earlier cross-sectional studies examined very early opacities, whereas the current study largely examines the progression of these early opacities. As noted earlier, we have little power to detect the smaller changes largely seen among women with mainly clear lenses at baseline. It may be possible that the influence of vitamin C is on very early stages of nuclear opacification, perhaps protecting the lens crystallins from some initial in-

sult, whereas vitamin E and possibly riboflavin or other nutrients act at a later stage in opacification, perhaps protecting the lens membranes against damage. Acting at separate stages in cataract development could explain the differences that we observed between the baseline and follow-up analyses in this sample of women. The possibility that there are distinct stages in the development of cataract is supported by recent data suggesting that when damaged, the association of lens crystallins with the fiber cell membranes is enhanced.³¹ Earlier retrospective studies that have focused largely on more advanced stages of nuclear opacification did not report associations with vitamin C intake but reported associations with intake of other nutrients such as vitamin E or riboflavin,^{25,27} whereas studies that included mostly cases of early nuclear opacities³² or mixtures of early and more advanced opacities²⁶ reported associations between vitamin C intake and nuclear opacities. Previously published longitudinal studies of nuclear opacities and antioxidant nutrients^{22,23,33} report inverse associations between the progression of nuclear opacities and use of vitamin E supplements, vitamin E intake, or vitamin E status but not the use of vitamin C supplements or vitamin C intake. However, as in the present study, these earlier studies are based on approximately 5 years of follow-up. Therefore, based on our observations that the degree of progression is dependent on the initial level of opacification, it can be inferred that most of the progression in these longitudinal studies is likely to be among those with a greater degree of opacification at baseline owing to the relatively short period of follow-up.

Suggestions from the current study that carotenoid intake was unrelated to change in nuclear density differs from much of the evidence from both retrospective and longitudinal studies, which suggests that carotenoids, particularly lutein and zeaxanthin, may protect the lens from opacification. For example, the Beaver Dam Eye Study showed a strong inverse association between past lutein intake and incidence of nuclear cataract²³ 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.³³ Baseline analyses from our sample of women suggested an inverse association between the consumption of lutein and zeaxanthin and the prevalence of nuclear opacities.⁶ Furthermore, women in the full NHS cohort, as well as men in the Health Professionals Follow-up Study, with higher lutein and zeaxanthin intakes had a lower risk of extraction of nuclear cataract.^{34,35} We also failed to see any significant associations between plasma total carotenoid concentrations and change in nuclear opacification. However, lutein, the only carotenoid found in the lens in measurable quantities³⁶ and the carotenoid most commonly associated with nuclear^{6,23,33-35} and other^{34,35} opacities, accounts for only about one quarter of circulating total carotenoids.³⁷

The clinical meaning of our results may not be readily apparent because the use of digitalized image analysis to assess nuclear density is fairly recent and limited.^{8,38} Therefore, the best means of quantifying the possible influence of nutrition on change in nuclear density scores based on this method is to consider the differences in rate of change

over time among those with higher and lower nutrient intakes. For example, if we assume that the rate of change in nuclear density remains constant over time, then, using the data from Table 6, the annual increase in nuclear density for a woman who did not use vitamin E supplements would be 3.4 pdu per year and the increase among those who used vitamin E supplements for 10 or more years would be 2.4 pdu per year. Therefore, it would take a woman who was a long-term vitamin E supplement user, on average, about 17 months to achieve the same increase in nuclear density (ie, 3.4 pdu) that a woman who did not use supplements achieved in 12 months. In other words, assuming a linear change over time, long-term vitamin E supplement users would delay progression of nuclear density by approximately 5 months for each year of follow-up. This difference would become quite substantial over a longer period. Moreover, these estimates are conservative since we know that the rate of change in nuclear density increases as the degree of nuclear density increases. Thus, the initial slowing of the increase of nuclear density among supplement users would result in even greater differences between those with higher and lower nutrient intakes over time.

Interpretation of results from the present study is subject to some caveats. We have considered the relations between an increase in nuclear density and 9 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. Only the association with vitamin E supplement use would remain statistically significant after any conservative adjustments for multiple comparisons. We applied an adjustment to the tests for interaction because of the number of tests performed to limit the number of potential spurious interactions. While we controlled for the most likely known or suspected determinants of increased nuclear density 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 or lifestyle factors. As noted earlier, the differential loss of participants at the follow-up examination might have introduced some bias, particularly with respect to vitamin C.

In summary, the results of the present study provide added support for a relation between nutrient intake and nuclear opacification. Our observation that vitamin E intake is associated with a reduction in nuclear opacification is consistent with other longitudinal studies, strengthening the hypothesized role for this specific nutrient in nuclear cataract formation, and the associations with riboflavin, thiamin, and niacin should serve to focus added effort on examining the role of these nutrients in the development of nuclear cataract.

Submitted for Publication: June 3, 2003; final revision received August 16, 2004; accepted August 16, 2004.

Correspondence: Allen Taylor, PhD, 711 Washington St, Boston, MA 02111 (allen.taylor@tufts.edu).

Funding/Support: This project was supported by agreement 58-1950-9-001 from the US Department of Agriculture, Washington, DC; grants 98-01023 and 92-37200-

7704 from the National Research Initiative Competitive Grants Program; Brigham Surgical Group, Boston, Mass; research grants EY-09611, EY-13250, EY-14183, and CA-40356 and training grant T32 AG00209 from the National Institutes of Health, Bethesda, Md; and grants from Roche Vitamins and Fine Chemicals Division, Parsippany, NJ; Kemin Foods, Des Moines, Iowa; and the Florida Department of Citrus, Lakeland.

Disclaimer: Any opinions, findings, conclusion, or recommendations expressed in this publication are those of the authors and do not necessarily reflect the views or policies of the US Department of Agriculture, nor does mention of trade names, commercial products, or organizations imply endorsement by the US government.

Acknowledgment: We wish to acknowledge the invaluable assistance of project staff and the many others whose effort supported this project. We would particularly like to thank Sheila Crosby, Karen Corsano, BA, Kate Saunders, Laura Bury, Patricia Khu, MD, Judith Friend, MS, John K. Wolfe, PhD, Nita Padhye, MD, Mini Balaram, MD, Rosaline Bowen, BA, Ester Epstein, BA, Tom Nowell, BS, and Gayle Petty, BS. We are indebted to the nurses who participated in the study for their continuing contributions and cooperation. Finally, we would like to acknowledge the support of Frank E. Speizer, MD, overall principal investigator for the Nurses' Health Study.

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