

# Nutrients and bioactives in green leafy vegetables and cognitive decline

## Prospective study

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## Abstract

### Objective

To increase understanding of the biological mechanisms underlying the association, we investigated the individual relations to cognitive decline of the primary nutrients and bioactives in green leafy vegetables, including vitamin K (phylloquinone), lutein,  $\beta$ -carotene, nitrate, folate, kaempferol, and  $\alpha$ -tocopherol.

### Methods

This was a prospective study of 960 participants of the Memory and Aging Project, ages 58–99 years, who completed a food frequency questionnaire and had  $\geq 2$  cognitive assessments over a mean 4.7 years.

### Results

In a linear mixed model adjusted for age, sex, education, participation in cognitive activities, physical activities, smoking, and seafood and alcohol consumption, consumption of green leafy vegetables was associated with slower cognitive decline; the decline rate for those in the highest quintile of intake (median 1.3 servings/d) was slower by  $\beta = 0.05$  standardized units ( $p = 0.0001$ ) or the equivalent of being 11 years younger in age. Higher intakes of each of the nutrients and bioactives except  $\beta$ -carotene were individually associated with slower cognitive decline. In the adjusted models, the rates for the highest vs the lowest quintiles of intake were  $\beta = 0.02$ ,  $p = 0.002$  for phylloquinone;  $\beta = 0.04$ ,  $p = 0.002$  for lutein;  $\beta = 0.05$ ,  $p < 0.001$  for folate;  $\beta = 0.03$ ,  $p = 0.02$  for  $\alpha$ -tocopherol;  $\beta = 0.04$ ,  $p = 0.002$  for nitrate;  $\beta = 0.04$ ,  $p = 0.003$  for kaempferol; and  $\beta = 0.02$ ,  $p = 0.08$  for  $\beta$ -carotene.

### Conclusions

Consumption of approximately 1 serving per day of green leafy vegetables and foods rich in phylloquinone, lutein, nitrate, folate,  $\alpha$ -tocopherol, and kaempferol may help to slow cognitive decline with aging.

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## Glossary

BMI = body mass index; FFQ = Food frequency questionnaires; MAP = Rush Memory and Aging Project.

Population projections indicate sharp increases in the prevalence of dementia worldwide as the oldest age groups continue to grow in number.<sup>1</sup> Decline in cognitive abilities, the central feature of dementia, is also one of the more feared conditions of aging. The identification of effective prevention strategies for dementia is critical to staving off a public health crisis and for meeting demand for this kind of information, particularly around diet. Among all of the different types of vegetables, green leafy vegetables have been identified as having the strongest protective relations against cognitive decline.<sup>2,3</sup> We examined this relation in a prospective cohort study, and investigated the individual relations to cognitive decline of a number of nutrients and bioactives that are rich in green leafy vegetables: lutein, vitamin K (phyloquinone), nitrate, folate,  $\alpha$ -tocopherol,  $\beta$ -carotene, and the flavonoid kaempferol.

## Methods

### Study population

The analytic sample is drawn from the Rush Memory and Aging Project (MAP), a study of volunteers from more than 40 retirement communities, senior public housing units, and churches and senior centers in the Chicago area. As previously described,<sup>4</sup> MAP is an ongoing open cohort study that began in 1997. Participants are free of dementia at enrollment based on structured clinical neurologic examination<sup>5</sup> and agree to annual clinical evaluations and organ donation after death. Food frequency questionnaires (FFQ) were incorporated into the study beginning in February 2004, at which point 1,545 persons had enrolled in the study, 90 had died, and 149 withdrew, leaving 1,306 participants eligible for these analyses. Of these, 1,068 completed the FFQ and 960 had at least 2 cognitive assessments for the analyses of cognitive change. There were no material differences in characteristics between the larger cohort and the analyzed sample. For example, on average, both were 80 years of age, had 14 years of education, physical activity score of 3, and body mass index (BMI) of 27; 13% had history of myocardial infarction, 74% hypertension, 18% and 19%, respectively, diabetes, and 11% and 10%, stroke. Participants meeting criteria for mild cognitive impairment<sup>6</sup> ( $n = 220$ ) were not excluded except in secondary analyses. The analytic sample was 95% white and 98.5% non-Hispanic.

### Standard protocol approvals, registrations, and patient consents

The institutional review board of Rush University Medical Center approved the study and all participants gave written informed consent.

### Cognitive assessments

The annual structured clinical evaluations included a battery of cognitive tests administered by trained and certified technicians. Composite scores from 19 of these tests were used to characterize cognition in 5 cognitive domains (episodic memory, working memory, semantic memory, visuospatial ability, and perceptual speed) as described previously.<sup>6</sup> The composite scores were computed for each cognitive domain and for a global measure of all 19 tests; raw scores for each test were standardized using the baseline population mean and standard deviation, and the standardized scores averaged. The number of annual cognitive assessments for analyzed participants ranged from 2 to 10; 52% of the sample participants had 5 or more cognitive assessments.

### Diet assessment

FFQs were collected at each annual clinical evaluation. However, because we wanted to relate estimated dietary effects on cognitive change prospectively, we limited our analysis to data from the first obtained FFQ. Daily servings of green leafy vegetables and nutrient intake levels were computed from responses to a modified Harvard semiquantitative FFQ that was shown to be a valid and reliable measure of dietary intake in older Chicago community residents.<sup>7</sup> The FFQ ascertained usual frequency of intake of 144 food items over the previous 12 months. The FFQ describes natural portion sizes (e.g., 1 banana) for many food items. For other foods (e.g., fish as main dish), the serving sizes were based on sex-specific mean portion sizes reported by the oldest men and women of national surveys. The 3 green leafy vegetable items and their serving sizes on the FFQ included (1) spinach (1/2 cup cooked), (2) kale/collards/greens (1/2 cup cooked), and (3) lettuce salad (1 cup raw). Dietary intake levels of the nutrients of interest were estimated from responses to all FFQ food items. Supplement intake levels were not analyzed due to the focus on food-derived nutrients and bioactives. Few randomized trials or epidemiologic studies find protective benefit of vitamin supplements on cognitive decline. To compute daily nutrient intake, nutrient levels for the food item portion sizes were multiplied by the frequency of intake and summed over all food items. All nutrients were calorie-adjusted separately for men and women using the regression residual method. The correlation between the baseline level of green leafy vegetable intake and the level on the last obtained FFQ, on average 4.4 years later, was 0.48.

### Covariates

Dietary intake levels of total energy (kcal), ratio of unsaturated (g/d) to saturated fats (g/d), alcohol consumption (g/d of beer, wine, and liquor), and seafood consumption (weekly consumption vs less of tuna sandwich, fish

sandwich, fish as a main dish, shrimp/lobster/crab) were based on responses to the baseline FFQ. Nondietary variables were obtained from structured interview questions and measurements at the participants' annual clinical evaluations. Age (in years) was computed from self-reported birth date and date of the first cognitive assessment in this analysis. Education was based on self-reported years of regular schooling. *APOE* genotyping was performed using high throughput sequencing as previously described.<sup>4</sup> All other covariates were based on data collected at the time of each cognitive assessment and were modeled as time-varying covariates to represent updated information from participants' previous evaluations. A variable for frequency of participation in cognitively stimulating activities was computed as the average frequency rating, based on a 5-point scale, of different activities (e.g., reading, playing games, writing letters, visiting the library).<sup>4,8</sup> Hours per week of physical activity was computed based on the sum of self-reported minutes spent over the previous 2 weeks on 5 activities (walking for exercise, yard work, calisthenics, biking, and water exercise).<sup>9</sup> Smoking history was categorized as never, past, and current smoker. Number of depressive

symptoms was assessed by a modified 10-item version of the Center for Epidemiologic Studies–Depression scale.<sup>10</sup> BMI (weight in kg/height in m<sup>2</sup>) was computed from measured weight and height and modeled as 2 indicator variables, BMI  $\leq 20$  and BMI  $\geq 30$ , with referent of  $20 < \text{BMI} < 30$ . Hypertension history was determined by self-reported medical diagnosis, measured blood pressure (average of 2 measurements  $\geq 160$  mm Hg systolic or  $\geq 90$  mm Hg diastolic), or current use of hypertensive medications. Myocardial infarction history was based on self-reported medical diagnosis or interviewer-recorded use of cardiac glycosides (e.g., lanoxin, digitoxin). Diabetes history was determined by self-reported medical diagnosis or current use of diabetes medications. Interviewers inspected and recorded data on all medications taken within the previous 2 weeks. Clinical diagnosis of stroke was based on neurologic examination and medical history.<sup>11</sup>

### Statistical methods

To examine the relations of green leafy vegetables and nutrients to change in cognitive scores, we used separate linear mixed models with random effects in SAS version 9.3

**Table 1** Baseline characteristics by quintile of green leafy vegetable consumption among 960 Rush Memory and Aging Project participants, 2004–2012

	Total, n	Quintile of leafy green vegetable intake				
		Q1	Q2	Q3	Q4	Q5
Mean servings per day		0.09	0.27	0.65	0.90	1.29
Age, y, mean	960	81.1	82.6	81.0	82.4	81.1
Female, %	960	28	28	25	25	21
<i>APOE</i> $\epsilon 4$ (% with 1 or 2 alleles)	825	23	23	21	27	23
Education, y, mean	960	14.0	14.7	15.0	15.8	15.5
Cognitive activities, mean score	959	3.0	3.2	3.3	3.3	3.3
Physical activities, mean	958	2.4	3.1	4.2	4.0	4.0
Smoking, %	960					
Never		49	57	63	52	58
Former		46	40	36	48	40
Current		5	3	1	<1	2
Alcohol consumption, mean g/d	960	3.8	3.2	4.2	4.9	6.1
Cardiovascular conditions, %						
Myocardial infarction	960	18	18	15	14	13
Hypertension	954	78	75	76	77	72
Stroke	870	13	6	10	6	7
Diabetes	960	26	21	16	26	20
Body mass index	927	27.3	27.6	27.1	26.9	26.8
Depressive symptoms, n, mean	959	1.4	1.2	1.0	0.8	1.0
Total energy, kcal/d	960	1,498	1,665	1,762	1,949	1,910

(SAS Institute, Cary, NC). We modeled the nutrient variables obtained from the first FFQ in quintiles with the lowest quintile as the referent category. A test for linear trend was also examined for each nutrient by assigning the median quintile intake level to those in a given quintile and modeling these values as a single categorical variable. The basic-adjusted model included terms for age, sex, education, smoking history, physical activity, participation in cognitive activities, alcohol consumption, total energy intake (green leafy model only), seafood consumption (green leafy model only), the dietary variable of interest, a variable for time, and multiplicative terms between time and each model covariate, the latter providing the covariate estimated effect on cognitive change.

Nonstatic covariates (e.g., cognitive and physical activities, BMI, depressive symptoms, and cardiovascular conditions) were modeled as time-varying variables except in analyses in which they were being investigated as potential effect modifiers in which case only the baseline measure for that covariate was modeled. To account for time-varying covariates, the SAS modeling was conducted on a dataset that contained updated values for all variables at each timepoint. Tests for statistical interaction by potential effect modifiers were computed in the basic-adjusted model by modeling 2-way and 3-way multiplicative terms among the nutrient, time, and the effect modifier, with the 3-way multiplicative term test for interaction set at  $p \leq 0.05$ . We characterized the estimated effect of being in the highest vs lowest quintiles of green leafy vegetable intake as the equivalent age difference in years by computing the ratio of the  $\beta$  coefficients ( $\beta$  [time $\times$ age]/ $\beta$  [time $\times$ quintile 5]) in the basic-adjusted model.

## Results

The sample of 960 MAP participants was on average 81 years of age, 74% female, and had a mean educational level of

14.9 years. Participants had a mean of 4.7 years (range 2–10) of follow-up. Green leafy vegetable intake ranged from a mean 0.09 servings per day for those in the lowest quintile of intake to a mean 1.3 servings per day for the highest quintile. Compared to those participants in the lowest quintile, those in the highest were more likely to be higher educated, male, to participate more frequently in cognitive and physical activities, and to have fewer cardiovascular conditions and depressive symptoms (table 1). Consumption of green leafy vegetables highly correlated with food intakes of nitrate ( $r = 0.78$ ), lutein and phyloquinone ( $r = 0.67$  for each), and  $\beta$ -carotene ( $r = 0.60$ ), and moderately correlated with folate ( $r = 0.47$ ) and kaempferol ( $r = 0.44$ ). Dietary intakes of lutein, phyloquinone, folate, and  $\beta$ -carotene were also highly to moderately correlated with each other (table 2).

On average, the sample declined in global cognitive scores over time at a rate of 0.08 standardized units/y. In age-adjusted models, consumption of green leafy vegetables was positively and significantly associated with slower rate of cognitive decline (table 3). The estimated effect was not changed materially with further adjustment for education, participation in cognitive and physical activities, smoking, alcohol consumption, and seafood consumption in the basic model (figure). When compared to the estimated effect of age on cognitive decline, the top quintile for green leafy vegetable consumption (median of 1.3 servings/d) vs the lowest quintile (median of 0.09 servings/d) was the equivalent of about 11 years younger. There was no evidence that the association was mediated by cardiovascular conditions (table 3). In further analyses, we added variables to the basic-adjusted model for depressive symptoms, low weight (BMI  $\leq 20$ ), and obesity (BMI  $\geq 30$ ), conditions that may be both cause and effects of dementia processes. However, the estimate of effect remained unchanged and statistically significant ( $\beta$  for Q5 vs Q1 = 0.04,  $p < 0.001$ ).

**Table 2** Correlations of baseline dietary intake levels of green leafy vegetables, phyloquinone, folate, lutein,  $\beta$ -carotene,  $\alpha$ -tocopherol, nitrate, and kaempferol among 960 Rush Memory and Aging Project participants, 2004–2014

	Phylloquinone	Folate	Lutein	$\beta$ -Carotene	$\alpha$ -Tocopherol	Nitrate	Kaempferol
<b>Phylloquinone</b>	1.0	0.53 <sup>a</sup>	0.96 <sup>a</sup>	0.83 <sup>a</sup>	0.24 <sup>b</sup>	0.89 <sup>b</sup>	0.74 <sup>b</sup>
<b>Folate</b>		1.0	0.52 <sup>a</sup>	0.57 <sup>a</sup>	0.25 <sup>b</sup>	0.51 <sup>b</sup>	0.34 <sup>b</sup>
<b>Lutein</b>			1.0	0.84 <sup>a</sup>	0.21 <sup>b</sup>	0.91 <sup>b</sup>	0.77 <sup>b</sup>
<b><math>\beta</math>-Carotene</b>				1.0	0.20 <sup>b</sup>	0.81 <sup>b</sup>	0.63 <sup>b</sup>
<b><math>\alpha</math>-Tocopherol</b>					1.0	0.21 <sup>b</sup>	0.15 <sup>b</sup>
<b>Nitrate</b>						1.0	0.78 <sup>b</sup>
<b>Kaempferol</b>							1.0
<b>Green leafy vegetables</b>	0.67 <sup>a</sup>	0.47 <sup>a</sup>	0.67 <sup>a</sup>	0.13 <sup>a</sup>	0.19 <sup>b</sup>	0.78 <sup>a</sup>	0.44 <sup>a</sup>

<sup>a</sup>  $p < 0.01$ .

<sup>b</sup>  $p < 0.0001$ .

**Table 3** Estimated effects ( $\beta$ , 95% confidence interval) of consumption of green leafy vegetables and nutrients on global cognitive decline over a mean 4.5 years among 960 participants of the Rush Memory and Aging Project, 2004–2014

Diet variable model	Quintiles of intake					<i>p</i> for linear trend
	Q1	Q2	Q3	Q4	Q5	
<b>Green leafy vegetables</b>						
Median servings per day	0.07	0.21	0.64	0.93	1.14	
<b>Basic-adjusted<sup>a</sup></b>						
$\beta$	Ref	0.04	0.02	0.02	0.05	0.003
95% CI		0.01 to 0.06	0.00 to 0.04	–0.01 to 0.05	0.02 to 0.07	
<b>+Cardiovascular<sup>b</sup></b>						
$\beta$		0.03	0.02	0.01	0.04	
95% CI	Ref	0.01 to 0.06	0.00 to 0.04	–0.02 to 0.04	0.02 to 0.07	0.01
<b>Phylloquinone</b>						
Median $\mu$ /d	71	113	153	205	373	
<b>Basic-adjusted<sup>a</sup></b>						
$\beta$	Ref	0.00	0.02	0.003	0.04	0.0009
95% CI		–0.02 to 0.02	–0.01 to 0.04	–0.02 to 0.03	0.01 to 0.06	
<b>+Cardiovascular<sup>b</sup></b>						
$\beta$		0.00	0.02	0.00	0.04	0.003
95% CI	Ref	–0.02 to 0.03	–0.02 to 0.04	–0.02 to 0.03	0.01 to 0.06	
<b>Folate</b>						
Median $\mu$ /d	173	219	251	287	339	
<b>Basic-adjusted<sup>a</sup></b>						
$\beta$	Ref	0.00	0.01	0.03	0.04	0.0001
95% CI		–0.02 to 0.03	–0.02 to 0.03	0.00 to 0.05	0.02 to 0.07	
<b>+Cardiovascular<sup>b</sup></b>						
$\beta$		0.00	0.00	0.02	0.04	
95% CI	Ref	–0.02 to 0.03	–0.02 to 0.03	0.00 to 0.05	0.01 to 0.06	0.0004
<b>Lutein-zeaxanthin</b>						
Median $\mu$ /d	1,462	2,031	2,854	4,060	7,893	
<b>Basic-adjusted<sup>a</sup></b>						
$\beta$	Ref	–0.01	–0.00	–0.01	0.04	0.0001
95% CI		–0.03 to 0.01	–0.02 to 0.02	–0.03 to 0.01	0.01 to 0.06	
<b>+Cardiovascular<sup>b</sup></b>						
$\beta$		–0.01	–0.01	–0.01	0.04	
95% CI	Ref	–0.03 to 0.01	–0.03 to 0.02	–0.03 to 0.01	0.01 to 0.06	0.0003
<b><math>\beta</math>-Carotene</b>						
Median $\mu$ /d	1,642	2,680	3,582	4,913	7,864	
<b>Basic-adjusted<sup>a</sup></b>						
$\beta$	Ref	–0.02	0.01	–0.00	0.02	0.01

Continued

**Table 3** Estimated effects ( $\beta$ , 95% confidence interval) of consumption of green leafy vegetables and nutrients on global cognitive decline over a mean 4.5 years among 960 participants of the Rush Memory and Aging Project, 2004–2014 (continued)

Diet variable model	Quintiles of intake					<i>p</i> for linear trend
	Q1	Q2	Q3	Q4	Q5	
<b>95% CI</b>		-0.05 to 0.00	-0.01 to 0.03	-0.02 to 0.02	-0.00 to 0.04	
<b>+Cardiovascular<sup>b</sup></b>						
<b><math>\beta</math></b>		-0.02	0.02	0.00	0.02	
<b>95% CI</b>	Ref	-0.04 to 0.01	-0.01 to 0.04	-0.02 to 0.03	-0.00 to 0.05	0.01
<b><math>\alpha</math>-Tocopherol</b>						
<b>Median mg/d</b>	4.3	5.1	5.7	6.4	8.1	
<b>Basic-adjusted<sup>a</sup></b>						
<b><math>\beta</math></b>	Ref	-0.01	0.00	0.02	0.03	0.0008
<b>95% CI</b>		-0.04 to 0.01	-0.02 to 0.03	-0.01 to 0.04	0.00 to 0.05	
<b>+Cardiovascular<sup>b</sup></b>						
<b><math>\beta</math></b>		-0.01	0.00	0.02	0.02	
<b>95% CI</b>	Ref	-0.03 to 0.01	-0.02 to 0.02	-0.01 to 0.04	0.00 to 0.05	0.007
<b>Nitrate</b>						
<b>Median mg/d</b>	58	106	141	184	287	
<b>Basic-adjusted<sup>a</sup></b>						
<b><math>\beta</math></b>	Ref	-0.01	0.01	0.01	0.04	0.0003
<b>95% CI</b>		-0.03 to 0.02	-0.01 to 0.04	-0.01 to 0.04	0.01 to 0.06	
<b>+Cardiovascular<sup>b</sup></b>						
<b><math>\beta</math></b>		-0.00	0.02	0.01	0.04	0.0005
<b>95% CI</b>	Ref	-0.02 to 0.03	-0.00 to 0.04	-0.01 to 0.04	0.01 to 0.06	
<b>Kaempferol</b>						
<b>Median mg/d</b>	0.6	1.4	2.8	4.7	9.5	
<b>Basic-adjusted<sup>a</sup></b>						
<b><math>\beta</math></b>	Ref	0.01	-0.00	0.02	0.04	0.001
<b>95% CI</b>		-0.01 to 0.04	-0.03 to 0.02	-0.01 to 0.04	0.01 to 0.06	
<b>+Cardiovascular<sup>b</sup></b>						
<b><math>\beta</math></b>		0.02	0.00	0.02	0.04	
<b>95% CI</b>	Ref	-0.01 to 0.04	-0.02 to 0.02	-0.00 to 0.04	0.02 to 0.07	0.0007

Abbreviation: CI = confidence interval.

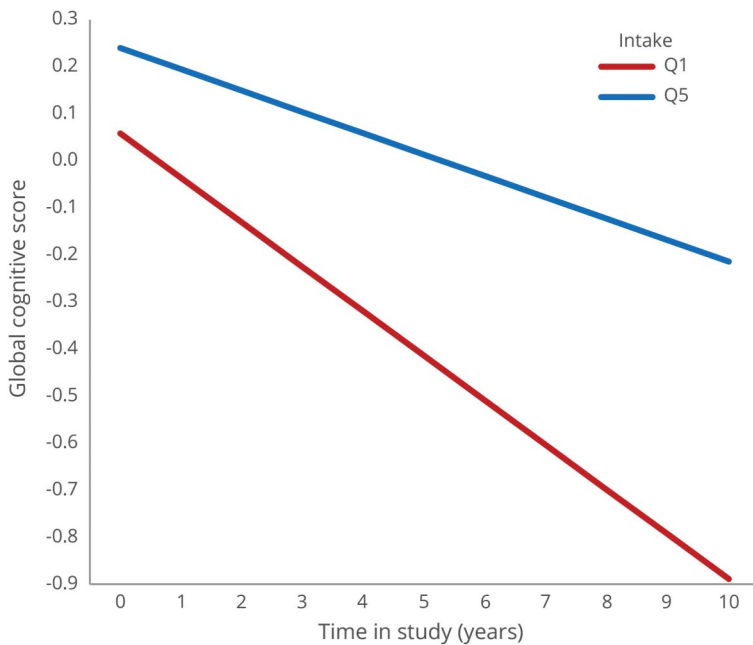
<sup>a</sup> Basic-adjusted model (based on 954 participants with complete information on all covariates) includes age, sex, education, participation in cognitive activities, physical activity, smoking, total energy intake, alcohol consumption, and for green leafy vegetable model only, seafood consumption.

<sup>b</sup> +Cardiovascular model (based on 856 participants with complete information on all covariates) includes covariates from the basic-adjusted model plus history of myocardial infarction, hypertension, stroke, and diabetes.

We next investigated the relations to cognitive change of food sources of folate, phylloquinone, lutein, nitrate,  $\alpha$ -tocopherol, and kaempferol. Food intakes of these nutrients and bioactives were each positively and significantly associated with slower rates of cognitive decline. The effect estimates for these

nutrients and bioactives did not change appreciably in models adjusted for the primary risk factors of cognitive change, nor did they appear to be mediated by cardiovascular conditions (table 3). The effect estimates also did not change in analyses with additional adjustment for depressive symptoms, low

**Figure** Rate of decline in global cognitive score for the top and the lowest quintile of intake of green leafy vegetables



Rate of decline in global cognitive score for the top quintile of intake of green leafy vegetables (median 1.3 servings/d) and the lowest quintile of intake (median 0.09 servings/d) based on mixed models adjusted for age, sex, education, energy intake, participation in cognitive activities, physical activity, smoking, and alcohol consumption in 960 Rush Memory and Aging Project participants, 2004–2014.

weight, and obesity in the basic-adjusted model ( $\beta$ ,  $p$  value for Q5 vs Q1 were folate  $\beta = 0.04$ ,  $p = 0.003$ ; phyloquinone  $\beta = 0.04$ ,  $p = 0.004$ ; lutein  $\beta = 0.04$ ,  $p = 0.004$ ;  $\alpha$ -tocopherol  $\beta = 0.03$ ,  $p = 0.02$ ; nitrate  $\beta = 0.04$ ,  $p = 0.006$ ; kaempferol  $\beta = 0.03$ ,  $p = 0.01$ ). The exception was  $\beta$ -carotene, which was modified and no longer statistically significant ( $\beta = 0.01$ ,  $p = 0.24$ ). We also considered whether these observed associations could be due to confounding by other nutrients shown in previous studies to protect against cognitive decline. For all of these nutrients and bioactives, except  $\beta$ -carotene, which was no longer statistically significant (0.02,  $p = 0.16$ ), the effect estimates changed little with additional adjustment for dietary intakes of  $\alpha$ -tocopherol and the ratio of unsaturated to saturated fats in the basic-adjusted models (Q5 vs Q1 were folate  $\beta = 0.04$ ,  $p = 0.002$ ; phyloquinone  $\beta = 0.03$ ,  $p = 0.006$ ; lutein  $\beta = 0.03$ ,  $p = 0.005$ ).

Next, we examined whether the strong protective relation of green leafy vegetable consumption to cognitive decline could be accounted for by dietary intakes of the nutrients and bioactives in separate basic-adjusted models. The estimate of the green leafy vegetable effect was modified and no longer statistically significant with inclusion of phyloquinone ( $\beta = 0.02$ ,  $p = 0.13$ ), lutein ( $\beta = 0.01$ ,  $p = 0.22$ ), or folate ( $\beta = 0.02$ ,  $p = 0.10$ ), suggesting that these nutrients were the source of the effect on cognitive decline.

In secondary analyses, we investigated whether the observed relations of green leafy vegetable consumption and dietary intakes of folate, phyloquinone, and lutein were modified by age (>80 y/ $\leq$ 80 y), sex, APOE  $\epsilon 4$  status, or presence/absence

of mild cognitive impairment. However, there was no evidence (all tests of statistical interaction  $p > 0.10$ ) that the observed relations differed by level of any of these factors.

We also examined whether any of the observed associations could be due to inaccurate reporting of dietary intake by those who had cognitive impairment at baseline. We reanalyzed the basic-adjusted models for green leafy vegetable consumption and intakes of each nutrient after eliminating 220 individuals who were clinically assessed as having mild cognitive impairment at baseline. The effect estimates changed minimally and remained statistically significant ( $\beta$ ,  $p$  value for Q5 vs Q1 were green leafy vegetables,  $\beta = 0.06$ ,  $p < 0.001$ ; folate  $\beta = 0.05$ ,  $p = 0.001$ ; phyloquinone  $\beta = 0.03$ ,  $p = 0.005$ ; lutein  $\beta = 0.04$ ,  $p = 0.003$ ;  $\beta$ -carotene  $\beta = 0.03$ ,  $p = 0.04$ ). In further analyses, we reanalyzed the data after excluding 144 participants whose green leafy vegetable consumption either increased (top 10%) or decreased (bottom 10%) over the study period. Restricting the sample to those with more stable consumption patterns over time had minimal effect on the observed associations ( $\beta$ ,  $p$  value for Q5 vs Q1 in the basic-adjusted models were green leafy vegetables,  $\beta = 0.05$ ,  $p < 0.001$ ; folate  $\beta = 0.04$ ,  $p = 0.005$ ; phyloquinone  $\beta = 0.04$ ,  $p = 0.003$ ; lutein  $\beta = 0.03$ ,  $p = 0.01$ ).

## Discussion

In this prospective study of an older US community population, the consumption of green leafy vegetables was linearly associated with slower cognitive decline. The rate of decline among those who consumed 1–2 servings per day was the

equivalent of being 11 years younger compared with those who rarely or never consumed green leafy vegetables. Investigation of the nutrients for which green leafy vegetables are a rich or primary source indicated that higher food intakes of folate, phyloquinone, and lutein were each linearly associated with slower cognitive decline and appeared to account for the protective relation of green leafy vegetables to cognitive change. These associations were independent of a number of demographic, behavioral, health, and nutrient risk factors as well as the presence of cognitive impairment. A weaker association was observed with dietary intake of  $\beta$ -carotene.

The study findings are supported by 2 large prospective studies<sup>2,3</sup> that examined the relations of different types of vegetables on cognitive decline. In both studies, the consumption of green leafy vegetables, including spinach, kale, collards, and lettuce, had the strongest association with slowed cognitive decline. In this study, we attempted to identify what individual dietary components in green leafy vegetables may be the underlying protective mechanisms. Many prospective studies report protective relations against dementia with dietary intakes of folate<sup>12–16</sup> and  $\beta$ -carotene,<sup>17–20</sup> although the evidence is by no means consistent for either folate<sup>21–24</sup> or  $\beta$ -carotene.<sup>17,25–27</sup> One randomized trial of 3-year supplementation with folic acid in individuals with biochemical evidence of marginal folate status found significantly slower cognitive decline compared with placebo.<sup>28</sup> The effect of  $\beta$ -carotene supplementation on cognitive decline was also tested in randomized trials,<sup>29,30</sup> with protective benefit demonstrated only after 18 years of supplementation.<sup>29</sup> There has been limited investigation of lutein and phyloquinone. One cross-sectional study reported a positive correlation between dietary intake levels of phyloquinone and Mini-Mental State Examination scores.<sup>31</sup> Plasma levels of lutein were associated with decreased risk of incident dementia in the 3-City Bordeaux study,<sup>32</sup> and a small cross-sectional study of octogenarians and centenarians found that brain and serum concentrations of lutein were associated with higher cognitive scores.<sup>33</sup> However, no effect of lutein (10 mg)/zeaxanthin (2 mg) supplementation on cognitive function was found in the Age-Related Eye Disease Study 2 (AREDS2) trial of older persons at risk of late age-related macular degeneration.<sup>34</sup> The negative trial results should not be interpreted as being inconsistent with the epidemiologic studies because the trial was not designed to test whether lutein supplementation is beneficial for cognition in individuals who have low or marginal lutein status.

These nutrients for which green leafy vegetables are a rich source may have independent mechanisms of action that synergistically protect the brain. Serum carotenoid levels were associated with less severe periventricular white matter lesions, particularly in older smokers.<sup>35</sup> In addition, lutein has been shown to reduce phospholipid peroxidation in human erythrocytes,<sup>36</sup> and to attenuate oxidative stress and mitochondrial dysfunction<sup>37</sup> and neuroinflammation.<sup>38</sup> Folate was reported to inhibit tau phosphorylation and APP, PS1, and A $\beta$  protein levels that underlie Alzheimer disease pathogenesis,

and to increase methylation potential and DNA methyltransferase activity.<sup>39</sup>

Confidence in the validity of the study findings is supported by a number of strengths. The prospective study design and high follow-up rates decrease the potential for biased results. The outcome of change in cognitive abilities was measured by in-person administration of a large battery of tests conducted annually for up to 10 years, thus providing a sensitive measure of decline that is unlikely to be biased by baseline levels of attained cognitive ability.<sup>40</sup> Dietary intake levels were assessed by a validated comprehensive FFQ shown to be unbiased by age and cognitive abilities.<sup>7</sup> Further, the results did not change when cognitively impaired individuals were excluded from the analyses and there was no evidence of effect modification by age.

The study findings were based on an observational study and as such, confounding bias can never be ruled out as an alternative explanation of the observed relations. Further, results of the study may not be generalizable to younger adults or to nonwhite or Hispanic populations.

Consumption of green leafy vegetables may help to slow decline in cognitive abilities with older age, perhaps due to the neuroprotective actions of lutein, folate,  $\beta$ -carotene, and phyloquinone. The addition of a daily serving of green leafy vegetables to one's diet may be a simple way to contribute to brain health.

### Author contributions

All authors participated in the design (M.C.M., D.A.B.), funding (M.C.M., D.A.B.), data collection (M.C.M., L.L.B., D.A.B.), statistical analyses (M.C.M., Y.W., D.A.B., L.L.B., B.D.-H., S.L.B.), writing (M.C.M., D.A.B., L.L.B., B.D.-H., S.L.B.), or final approval of the manuscript (M.C.M., Y.W., L.L.B., D.A.B., B.D.-H., S.L.B.).

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### Disclosure

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# Nutrients and bioactives in green leafy vegetables and cognitive decline

## Prospective study

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### Study question

How does the consumption of green leafy vegetables affect the risk of aging-related cognitive decline?

### Summary answer

Eating one serving of green leafy vegetables daily may slow aging-related cognitive decline.

### What is known and what this article adds

Some studies have suggested that green leafy vegetables may offer protective effects against aging-related cognitive decline. This study provides corroborating evidence for the protective effects of green leafy vegetables rich in lutein, vitamin K, nitrate, folate,  $\alpha$ -tocopherol,  $\beta$ -carotene, and kaempferol.

### Participants and setting

This study examined 960 participants (ages 58–99 years) from the Rush Memory and Aging Project, an open cohort study that began recruiting Chicago-area residents beginning in 1997 and introduced food frequency questionnaires in 2004.

### Design, size, and duration

The study analyzed the results of annual clinical evaluations that included a battery of 19 cognitive tests covering episodic memory, working memory, semantic memory, visuospatial ability, and perceptual speed. Each patient's dietary intake of green leafy vegetables was determined based on the first completed food frequency questionnaire. The questionnaire's 3 green leafy vegetable categories were spinach, kale/collards/greens, and lettuce. Estimated nutrient intakes were calorie-adjusted with reference to sex. The study analyzed available data from 2004 to 2012.

### Primary outcomes

The primary outcome was change in cognitive abilities, as determined by annual testing of a battery of 19 cognitive tests for up to 10 years of follow-up.

Quintile	Median servings per day of green leafy vegetables	Adjusted $\beta$ (95% CI)
Q1	0.07	Ref
Q2	0.21	0.04 (0.01 to 0.06)
Q3	0.64	0.02 (0.00 to 0.04)
Q4	0.93	0.02 (–0.01 to 0.05)
Q5	1.14	0.05 (0.02 to 0.07)

### Main results and the role of chance

The mean daily green leafy vegetable intake ranged from 0.09 servings in the lowest quintile (Q1) to 1.29 servings in the highest quintile (Q5). The average global cognitive score declined at a rate of 0.08 standardized units per year. An age-adjusted model showed that greater green leafy vegetable consumption was significantly associated with slower cognitive decline, with the difference between Q5 and Q1 ( $\beta$  0.05; 95% confidence interval 0.02–0.07;  $p$  for linear trend = 0.003) being equivalent to an 11-year age difference.

### Bias, confounding, and other reasons for caution

This was an observational study, so confounding bias cannot be ruled out.

### Generalizability to other populations

The results of this study may not be generalizable to younger adults or to nonwhite or Hispanic populations.

### Study funding/potential competing interests

This study was funded by the NIH and the US Department of Agriculture. The authors report no competing interests. Go to [Neurology.org/N](https://doi.org/10.1212/WNL.0000000000004815) for full disclosures.

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