



## A BRIEF ORIGINAL CONTRIBUTION

### Cohort Study of Vitamin C Intake and Cognitive Impairment

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To test the hypothesis that vitamin C protects against cognitive impairment, the authors conducted a cohort study ( $n = 117$ ) in a retirement community in Sydney, Australia. Vitamin C intake was assessed at baseline (1991) with a semiquantitative food frequency questionnaire, and cognitive function was assessed 4 years later (1995). After adjustment for age, sex, smoking, education, total energy intake, and use of psychotropic medications, consumption of vitamin C supplements was associated with a lower prevalence of more severe cognitive impairment (based on scores on the Mini-Mental State Examination; adjusted odds ratio = 0.39, 95% confidence interval 0.18–0.84). There were no associations between vitamin C intake and scores on tests of verbal and category fluency. This study suggests that vitamin C might protect against cognitive impairment. *Am J Epidemiol* 1998;148:45–50.

Alzheimer's disease; ascorbic acid; cohort studies; cognition; dementia

The hypothesis that antioxidant vitamins might protect the brain against Alzheimer's disease has been the subject of much speculation (1–4). Antioxidant vitamins could also protect against vascular dementia, given recent findings that higher vitamin C intakes are associated with reduced risk of ischemic stroke (5). Epidemiologic studies of the relation between antioxidant vitamins and cognitive function have mostly used case-control and cross-sectional designs (6–12). It is impossible in these types of studies to determine the temporal relation between vitamin levels and cognition; cognitive impairment may lead to lower vitamin intake. Furthermore, people with cognitive impairment may underreport their dietary vitamin intake.

Two cohort studies of dietary antioxidants and cognitive impairment have been published recently (13, 14). One of these studies found that vitamin C was associated with better cognitive function (13); the other found no such association (14). In this paper, we report the findings of a small cohort study in which vitamin C intake was assessed 4 years before assessment of cognitive function.

## MATERIALS AND METHODS

The study described in this paper involved a subset of subjects enrolled in the Western Sydney Stroke in the Elderly (SITE) Study, a cohort study designed to identify dietary and hemostatic risk factors for stroke. The SITE Study is described in detail elsewhere (15).

### Study subjects

Subjects for the SITE Study were recruited during 1991 from people living independently in a group of retirement villages in the western suburbs of Sydney, Australia. Of the 311 eligible men and 1,106 eligible women, 225 men (72 percent) and 787 women (71 percent) participated in the baseline assessments of the SITE Study. Exclusion criteria were living in a nursing home or obtaining a Mini-Mental State Examination (16) score less than 24. Potential participants were not excluded on the basis of any preexisting disease. Subjects ( $n = 141$ ) with poor quality dietary data (missing or implausible values on the food frequency questionnaire) were not eligible for inclusion in the analysis described here.

For the present study, our aim was to recruit subjects from the extremes of the vitamin C intake range. We divided subjects into users and nonusers (in 1991) of supplements containing vitamin C and then ranked people within each of these two groups according to their (1991) dietary vitamin C intakes. We identified three groups of subjects: nonusers of supplements with the lowest dietary vitamin C intakes ( $n = 58$ ); nonus-

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Abbreviations: CI, confidence interval; MMSE, Mini-Mental State Examination; SITE, Stroke in the Elderly.

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ers of supplements with the highest dietary vitamin C intakes ( $n = 61$ ); and users of vitamin C supplements with the highest dietary vitamin C intakes ( $n = 60$ ). We hoped to recruit 40 subjects from each of these three groups.

All living subjects were contacted by the study coordinator (M. P.) during mid-1995, and arrangements were made for interviews to be conducted in the subjects' own residences. This included subjects who had moved into nursing homes or hostels for the aged since the baseline interview.

### Measurement of vitamin C intake

The food frequency questionnaire used to assess dietary vitamin C intake is described in detail elsewhere (17). In brief, the instrument was a self-administered semiquantitative food frequency questionnaire developed by the Division of Nutrition of the Commonwealth Scientific and Industrial Research Organisation (Adelaide, Australia). Approximately 4 weeks after completing the baseline food frequency questionnaire, 62 SITE subjects completed a repeat food frequency questionnaire. The intraclass correlation coefficient for vitamin C intake in this repeatability study was 0.8 (17).

### Assessment of cognitive function

Four separate tests were used to assess cognitive function at the follow-up home visit: the Mini-Mental State Examination (MMSE), the Reid brief neuropsychological screen (W. Reid, Concord Hospital (Sydney, Australia), personal communication, 1995), the Animals test of category fluency (18), and the F, A, S test of verbal fluency (18). All tests were scored so that higher scores indicated better cognitive function.

The MMSE comprises 11 questions concerning orientation in place and time, immediate and delayed recall of three words, attention and calculation, language and praxis, and visual construction. Scores can range from zero to 30. The Reid brief neuropsychological screen extends the MMSE by including more complex tasks. For example, the recall task involves remembering seven words, and the delayed recall interval is 10 minutes. The Reid screen is scored from zero to 80. For the test of category fluency, subjects have 60 seconds to name as many animals as possible; the score is simply the number of different animals named. The test of verbal fluency involves asking subjects to name as many words as possible beginning with the letters F, A, and S. Subjects have 60 seconds for each letter. A total verbal fluency score is calculated by summing the number of words mentioned for each letter.

Tests of cognitive function were performed by the study coordinator (M. P.). He was trained in the use of the tests by an experienced neuropsychologist (Dr. W. Reid, Aged and Extended Care Division, Concord Hospital, Sydney). The study coordinator was blinded to subjects' vitamin C intakes while the tests were administered.

### Potentially confounding factors

The potential confounders that we considered were age, sex, smoking history (ever smoking versus never smoking), educational achievement (primary school, secondary school, a trade or technical certificate, or university degree), total energy intake (in kilocalories per day, as calculated from the food frequency questionnaire), use of psychotropic medications, and history of stroke or Parkinson's disease. Except for information on psychotropic medication use, data on these variables were gathered in the 1991 baseline questionnaires. Current use of psychotropic medications (mainly benzodiazepines, antidepressants, or neuroleptics) was recorded at the 1995 follow-up interview, at the same time as assessment of cognitive function.

### Statistical analysis

Mean scores on the Reid brief neuropsychological screen, the Animals test of category fluency, and the F, A, S test of verbal fluency were compared across vitamin C intake groups using analysis of covariance, controlling for age, sex, smoking history, educational achievement, use of psychotropic medications, and total energy intake.

MMSE scores were heavily right-skewed, with 37 percent of subjects scoring the maximum of 30 points. Hence, differences in group MMSE scores were analyzed using the Kruskal-Wallis nonparametric test.

Ordinal regression based on the cumulative odds model was also used to assess the relation between MMSE score and vitamin C intake (19). Subjects were divided into five groups based on their MMSE scores:  $<24$  ( $n = 7$ ); 24 or 25 ( $n = 8$ ); 26 or 27 ( $n = 18$ ); 28 or 29 ( $n = 41$ ); and 30 ( $n = 43$ ). Odds ratios from the ordinal regression models estimated the probability of having a particular level of cognitive impairment (or worse) in comparison with the probability of less severe cognitive impairment. Ordinal regression models assume that odds ratios are not dependent on the particular cutpoints chosen. This proportional odds assumption held ( $p > 0.05$ ) for the models used in this analysis. Odds ratios were adjusted for age, sex, smok-

ing history, educational achievement, use of psychotropic medications, and total energy intake.

The study was designed to have 80 percent power ( $\alpha = 5$  percent) to detect a difference between vitamin C intake groups of 2.8 points on the MMSE scale.

## RESULTS

Of the 179 subjects selected for this study, 117 (65 percent) participated. Thirty-three subjects had died since the baseline interview, 24 subjects declined the invitation to participate, four subjects could not be contacted, and one subject was blind and unable to complete the MMSE and Reid tests. Loss to follow-up was somewhat higher in the group with a high dietary intake of vitamin C (39 percent lost: 11 deaths and 13 additional nonresponders) than in the low-dietary-intake group (31 percent lost: 12 deaths and six other nonresponders) and the vitamin C supplement group (33 percent lost: 10 deaths and 10 other nonresponders).

The 117 subjects included in the study comprised 40 in the low dietary vitamin C group, 37 in the high dietary vitamin C group, and 40 in the vitamin C supplement group. Relevant baseline characteristics of the study subjects are shown in table 1. Supplement users and those with high dietary vitamin C intakes were more likely to be female and to have a university degree than were those with low dietary vitamin C intake. Supplement users were less likely than nonusers to be smokers. The correlation coefficient between total energy intake and dietary vitamin C intake was 0.3. Approximately 25 percent of each vitamin C intake group was using psychotropic medications in 1995.

There were no differences across vitamin C intake

groups in mean scores on the tests of category and verbal fluency or the Reid brief neuropsychological screen (see table 2).

MMSE scores were heavily skewed and could not be normalized through transformation, because the most frequent score was the maximum score of 30. Hence, MMSE data were analyzed using a nonparametric test, as well as by treating MMSE as an ordered categorical variable. Using the nonparametric Kruskal-Wallis test, there was some evidence of an association between MMSE score and vitamin C intake group ( $p = 0.07$ ).

The results from the ordinal regression models with MMSE score entered as a five-level ordinal variable are shown in table 3. In model 1, vitamin C supplement use and dietary vitamin C intake (continuous variable) were included as separate variables. Users of supplements had a lower prevalence of cognitive impairment (adjusted odds ratio = 0.39, 95 percent confidence interval (CI) 0.18–0.84). In model 2, the two vitamin C sources were combined and subjects were categorized into quartiles. Higher total vitamin C intake was associated with a lower prevalence of cognitive impairment (test for trend:  $p = 0.05$ ).

Analyses were repeated using logistic regression modeling with cognitive impairment dichotomized in the standard way (MMSE score  $<24$  vs.  $\geq 24$ ). The adjusted odds ratio for vitamin C supplement use was 0.27 (95 percent CI 0.02–3.30), and the adjusted odds ratio per 100 mg/day of dietary vitamin C intake was 0.90 (95 percent CI 0.39–2.06) (data not shown).

Seventeen subjects reported a history of stroke or Parkinson's disease at the baseline interview in 1991. In the remaining 100 subjects, adjusted odds ratios for the five-level cognitive impairment variable were 0.35

**TABLE 1. Selected baseline characteristics of a subset of participants in the SITE\* Study, by vitamin C intake group, Sydney, Australia, 1991**

Characteristic	Range	Group 1: low dietary intake of vitamin C (n = 40)	Group 2: high dietary intake of vitamin C (n = 37)	Group 3: vitamin C supplement use (n = 40)
Mean age (years)	69–91	79 (4)†	77 (4)	79 (4)
Mean dietary intake of vitamin C (mg/day)	11–389	53 (13)	296 (33)	207 (82)
Mean energy intake (kcal/day)	940–4,974	1,756 (687)	2,198 (588)	1,896 (589)
% female		68	76	83
Level of education (%)				
Primary school		25	15	18
Secondary school		55	54	52
Trade or technical certificate		13	18	8
University degree		7	13	22
% ever smokers		35	38	33

\* SITE, Stroke in the Elderly.

† Numbers in parentheses, standard deviation.

**TABLE 2. Mean scores on tests of cognitive function administered in 1995, according to vitamin C intake reported in 1991, Sydney, Australia**

Test	Range of scores	Group 1: low dietary intake of vitamin C*	Group 2: high dietary intake of vitamin C	Group 3: vitamin C supplement use
<i>Unadjusted mean score</i>				
Category fluency	0–26	12.1 (0.8)†	11.0 (0.8) ( <i>p</i> = 0.35)‡	12.0 (0.8) ( <i>p</i> = 0.95)
Verbal fluency	7–91	31.6 (2.2)	30.2 (2.3) ( <i>p</i> = 0.67)	31.9 (2.2) ( <i>p</i> = 0.92)
Reid brief neuropsychological screen	23–77	65.0 (1.4)	66.9 (1.5) ( <i>p</i> = 0.37)	68.1 (1.4) ( <i>p</i> = 0.14)
<i>Adjusted mean score§</i>				
Category fluency		12.3 (0.7)	10.8 (0.8) ( <i>p</i> = 0.19)	12.0 (0.7) ( <i>p</i> = 0.74)
Verbal fluency		32.1 (2.2)	30.4 (2.3) ( <i>p</i> = 0.62)	31.2 (2.1) ( <i>p</i> = 0.78)
Reid brief neuropsychological screen		65.6 (1.4)	66.4 (1.5) ( <i>p</i> = 0.71)	68.0 (1.4) ( <i>p</i> = 0.24)

\* By design, no subjects in the low and high dietary vitamin C groups used vitamin C supplements.

† Numbers in parentheses, standard error.

‡ The *p* values shown are for the comparison between mean values in groups 1 and 2 or groups 1 and 3.

§ Adjusted by analysis of covariance for age (years), sex, educational achievement (four-level variable), smoking history (ever vs. never), total energy intake (kcal/day), and use of psychotropic medications.

(95 percent CI 0.15–0.81) for vitamin C supplement use and 0.79 (95 percent CI 0.54–1.15) per 100 mg/day of dietary vitamin C intake (data not shown).

Table 4 shows odds ratios from ordinal regression models for associations between potential confounders and cognitive impairment. The associations found all pointed in the expected directions: Older age, use of psychotropic medications, and higher energy intake were associated with increased prevalence of cognitive

impairment, while female sex, more education, and smoking were associated with reduced prevalence.

## DISCUSSION

We found that a high vitamin C intake was associated with a lower prevalence of cognitive impairment, as measured by the MMSE. Two other cohort studies of vitamin C and cognitive function have been pub-

**TABLE 3. Odds ratios from ordinal regression models for the association between vitamin C intake as reported in 1991 and cognitive impairment\* as measured in 1995, Sydney, Australia**

Vitamin C intake	No. of subjects	Unadjusted odds ratio	95% confidence interval	Adjusted odds ratio†	95% confidence interval
<b>Model 1</b>					
Supplements (yes/no)	40	0.47	0.23–0.96	0.39	0.18–0.84
Diet (per 100 mg/day)		0.82	0.61–1.10	0.88	0.63–1.22
<b>Model 2</b>					
Quartile of total daily intake (mg)					
<62	29	1.00‡		1.00‡	
62–284	30	0.51	0.20–1.29	0.58	0.12–1.52
285–706	29	0.41	0.16–1.07	0.59	0.21–1.64
>706	29	0.34	0.13–0.87	0.32	0.18–0.88
Test for trend		<i>p</i> = 0.07		<i>p</i> = 0.05	

\* Cognitive impairment was entered into the models as a five-level variable based on Mini-Mental State Examination score: <24 (*n* = 7), 24–25 (*n* = 8), 26–27 (*n* = 18), 28–29 (*n* = 41), or 30 (*n* = 43).

† Adjusted for age (years), sex, educational achievement (four-level variable), smoking history (ever vs. never), total energy intake (kcal/day), and use of psychotropic medications.

‡ Referent.

**TABLE 4. Odds ratios from an ordinal regression model for associations between selected variables and cognitive impairment,\* Sydney, Australia, 1991–1995**

Variable†	Un-adjusted OR‡	95% CI‡	Adjusted OR§	95% CI
Age (per 5 years)	1.87	1.25–2.78	1.84	1.21–2.79
Female sex	0.55	0.26–1.18	0.50	0.19–1.33
Smoker (ever)	0.81	0.41–1.63	0.60	0.27–1.35
Post-high school education	0.63	0.30–1.34	0.65	0.29–1.48
Energy intake (per 1,000 mg/day)	1.42	0.82–2.47	1.46	0.78–2.73
Current use of psychotropic medications	3.05	1.40–6.65	3.39	1.53–7.54

\* Cognitive impairment was entered into the model as a five-level variable based on Mini-Mental State Examination score: <24 ( $n = 7$ ), 24–25 ( $n = 8$ ), 26–27 ( $n = 18$ ), 28–29 ( $n = 41$ ), or 30 ( $n = 43$ ).

† Data for all variables were gathered in 1991, except for use of psychotropic medications and Mini-Mental State Examination score, which were assessed in 1995.

‡ OR, odds ratio; CI, confidence interval.

§ Adjusted for vitamin C supplement use, dietary vitamin C intake, and all other variables in the table.

lished recently (13, 14). Perrig et al. followed 446 Swiss subjects for 22 years and found that a high serum level of vitamin C was associated with better performance on tests of memory (13). On the other hand, Kalmijn et al. found no association between dietary vitamin C intake and cognitive decline in 352 Dutch men followed for 5 years (14). Several cross-sectional and case-control studies of cognitive impairment have found that cognitive impairment is associated with lower levels of serum vitamin C (6–8, 11). However, only one previous study has found that low vitamin C intake is associated with cognitive impairment (11).

A protective effect of vitamin C is biologically plausible. Antioxidant vitamins protect against free radical oxidation, and free radicals might be involved in the etiology of the two major diseases that cause cognitive impairment in older people: Alzheimer's disease and vascular dementia. Oxidative damage to neuronal cell membranes and mitochondrial DNA could lead to Alzheimer's disease (20), while oxidation of low density lipoproteins could increase the risk of vascular dementia (21).

We found an association of vitamin C intake with MMSE score but not with our other three measures of cognitive function. The MMSE is an established screening test for dementia in older people, with high sensitivity and specificity (22). The other tests that we used have not been as carefully validated as the MMSE; thus, our failure to find an association with these tests could be due to measurement error.

Our study had several limitations. Our ability to control for confounding factors was restricted mainly to variables measured in the original SITE Study, a study of risk factors for cardiovascular disease. However, we controlled for three of the major risk factors

for cognitive decline: age, educational achievement, and smoking history. We also controlled for baseline total energy intake, which was recently found to be associated with cognitive impairment (23), and use of psychotropic medications at the time of cognitive testing. Exclusion of subjects with baseline stroke or Parkinson's disease made no difference in our results.

Although the SITE Study excluded subjects with moderate or severe baseline cognitive impairment, some subjects may have had mild cognitive impairment. If people with mild cognitive impairment were less likely to report vitamin C intake than cognitively intact individuals, we would have overestimated the size of the association between vitamin C and cognitive impairment. On the other hand, we may have missed associations between vitamin C intake and tests of cognitive function because of the limited statistical power of our small study, due to its small sample size and only 4 years' follow-up.

The response rate at follow-up was 65 percent; the major contributor to this relatively low figure was the death of 18 percent of the study subjects. Mortality was similar in the three vitamin C intake groups, but refusal to participate was lowest in the group with low dietary vitamin C intake. If refusal to participate was associated with poorer cognitive function, as in the study by Kalmijn et al. (14), then our low response rate may have led to overestimation of the size of the association between vitamin C intake and cognitive impairment.

Further epidemiologic research on vitamin C and cognitive impairment should include large cohort studies with long follow-up times and neurologic assessments to determine causes of cognitive impairment. The best test of the hypothesis that vitamin C prevents cognitive decline in older people would be a randomized trial of vitamin C supplementation.

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