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Original Contribution

Selenium Level and Cognitive Function in Rural Elderly Chinese

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Selenium is a trace element associated with antioxidant activity and is considered to be a protective agent against free radicals through enhanced enzyme activity. Studies on selenium and cognitive function or Alzheimer's disease have yielded inconsistent results. A cross-sectional survey of 2,000 rural Chinese aged 65 years or older from two provinces in the People's Republic of China was conducted from December 2003 to May 2005 by use of the Community Screening Instrument for Dementia, the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) Word List Learning Test, the Indiana University Story Recall Test, the Animal Fluency Test, and the Indiana University Token Test. Over 70% of the study participants have lived in the same village since birth. Nail samples were collected and analyzed for selenium contents. Analysis-of-covariance models were used to estimate the association between quintile selenium levels measured in nail samples and cognitive test scores, with adjustment for other covariates. Lower selenium levels measured in nail samples were significantly associated with lower cognitive scores ($p < 0.0087$ for all tests) except the Animal Fluency Test ($p = 0.4378$). A dose-response effect of selenium quintiles was also seen for those significant associations. Results in this geographically stable cohort support the hypothesis that a lifelong low selenium level is associated with lower cognitive function.

aged; Asian continental ancestry group; cognition; selenium

Abbreviations: CERAD, Consortium to Establish a Registry for Alzheimer's Disease; CSID, Community Screening Instrument for Dementia; IU, Indiana University.

Selenium is a trace element associated with the activity of the antioxidant enzyme glutathione peroxidase. It is considered to be a protective agent against free radicals through enhanced enzyme activity. Associations between low levels of selenium and increased risk in various disease indices (cancer, cardiovascular disease, reproduction and neonatal health, and asthma) have been reported (1). The process of biologic aging has also been hypothesized to be linked to deleterious free radical reactions involving inflammation processes and autoimmune reactions (2). The limited studies

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on selenium and cognitive function or Alzheimer's disease, however, have had inconsistent results (3–11).

It has been suggested that long-term exposure to environmental factors dating back as far as childhood may be required to impact brain function and lead to disease such as Alzheimer's disease (12–18). Studying the relation between long-term selenium exposure and cognitive decline is difficult, because populations are often mobile and consume foods that were produced and prepared in different areas of the world. The selenium content in food, especially grain, is highly variable, depending on the selenium content of the soils in which it is grown (19). Moreover, supplements containing selenium are often ingested, particularly by health conscious individuals, further confounding the results. The rural elderly Chinese population represents a unique opportunity for studying the relation between long-term selenium exposure and cognitive function. The rural Chinese are unusually stable, with most living in the same village throughout their entire life and consuming food that is locally grown. In addition, it is rare for these villagers to take dietary supplements. In addition, Chinese scientists have assembled extensive data on selenium distributions in many parts of the country, and hence it is possible to select sites with different selenium levels so that an extended range can be achieved to maximize the statistical power for detecting potential association. In this paper, we report the association between selenium levels and cognitive function in 2,000 rural elderly Chinese.

MATERIALS AND METHODS

Study population

Two thousand Chinese aged 65 years or older from four sites in China were enrolled in this study. Two sites were from the Sichuan Province in southwestern China, and anther two sites were from the Shandong Province in eastern China. Because Chinese scientists have mapped the selenium distribution in many parts of the country, the two provinces were selected because of the various selenium levels within each province. Prior to final site selection, Chinese investigators traveled to several candidate sites and collected demographic information, ensuring that the local elderly population was large enough to provide a sample of 500 elderly subjects. Samples of grain (corn, rice, and wheat), soil, water, and nail clippings from randomly selected individuals at each candidate site were also collected and analyzed for selenium and other trace elements. The goal was to have two sites within one province that differed in selenium levels but were similar in trace element measures and other potential confounders. Sites with known endemic diseases were excluded from consideration.

For each village included in the study, the Chinese investigators and a team of interviewers who were employees of the provincial and county centers for disease control traveled to the area, established temporary headquarters, and conducted a complete census of residents aged 65 years or older in the area. They enrolled eligible residents by going door-to-door, obtaining informed consent before conducting the interview, and collecting biologic samples. The team of laboratory scientists collected samples of water, soil, and food items from five locations within each village for analysis of selenium levels. Five hundred subjects from Qionglai, Sichuan Province, were interviewed from December 2003 to January 2004, 500 subjects from Gaomi, Shandong Province, were interviewed in May 2004, 500 subjects from Jiange, Sichuan Province, were interviewed in October 2004, and the last 500 subjects from Zichuan, Shandong Province, were interviewed in May 2005. There were no refusals. However, a few subjects with hearing problems were not enrolled. The study was approved by the Indiana University Institutional Review Board and the Institute for Environmental Health and Related Safety, Chinese Center for Disease Control and Prevention.

Cognitive assessment

Cognitive assessment was conducted in face-to-face interviews by use of the Community Screening Instrument for Dementia (CSID), the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) Word List Learning Test, the CERAD Word List Recall Test (20), the Indiana University (IU) Story Recall Test, the Animal Fluency Test (21), and the IU Token Test. The CSID was developed as a screening tool for dementia in populations with various cultural backgrounds and literacy levels. Details of the instrument have been published elsewhere (22). The CSID consists of two parts: 1) an interview with the study participant measuring cognitive function and 2) an interview with a close relative to gather information on daily functioning and cognitive decline. Cognitive assessment items were selected to measure the following functions in a short interview: memory, abstract thinking, judgment, other disturbances of higher cortical function (aphasia, apraxia, agnosia, constructional difficulty), and personality changes and daily functioning at work and home and in social relationships. The CSID has demonstrated both good 2-week test/retest reliability and interrater reliability, as well as good validity in detecting dementia in various populations (22, 23). CSID scores range from 0 to 30.

The CERAD Word List Learning Test is one of the measures from the CERAD neuropsychological assessment battery that was designed to assess cognitive skills in the elderly. It consists of a 10-item, three-trial word list in which free recall is taken after each learning trial and after a brief delay (approximately 5 minutes). The score is the total number of words recalled across the three learning trials (range: 0–30) and at delay (range: 0–10). The task of the IU Story Recall Test was created by the research team to be suitable to the Chinese culture and the rural population. The examiner reads the story out loud to the subject, who attempts to recall it verbatim immediately and again after a brief delay. The story has 14 units of information that are gist scored (range: 0–14). The story was tested in 1,500 elderly Chinese in a previous pilot study, and it was found to be acceptable to the villagers and produced a normally distributed range of scores (24). The Animal Fluency Test is a measure of executive function in which a subject names as many animals as possible in 60 seconds. The IU Token Test is a brief measure of language comprehension and working memory (25).

A sheet of paper has an array of circles and squares that vary in size (small and large) and color (red, black, yellow, and green). The examiner reads aloud a series of 12 commands that ask the subject to point to or touch the shapes in various combinations and orders. Commands that are correctly executed on the first exposure receive 2 points. If an error occurs, the command is repeated, and the subject receives 1 point for a correct response or no points for another failure. The score is the number correct across all 12 commands (range: 0–24). The validity of the CSID, the CERAD Word List Learning Test and Recall Test, and the Animal Fluency Test has been established previously in the Chinese population and elsewhere (26).

The questionnaires were harmonized, translated into Chinese, and back translated into English. To avoid potential bias, this process was accomplished by using lay persons from outside the research team from Beijing and Indiana University who were not familiar with the goals of the interview.

Intensive training sessions for the interviewers were held prior to the start of the first site, and refresher training was held prior to interviewing at each of the other three sites. High interrater reliability was achieved after each interviewer-training course using volunteers from the community as study subjects.

Selenium measures

Nail samples from all study subjects were collected at the time of interview and stored in clean plastic bags labeled with subject identification numbers. In addition, approximately 10 percent of the subjects were randomly sampled to provide a 10-ml venous blood sample, of which 0.10 ml was used for selenium analysis. Five samples from each type of food, water, and soil were collected from the houses of five participants, located in the east, west, south, north, and center of each village to cover the geographic spread of the village. The five samples of the same type were then combined in the laboratory to provide selenium measurement in each food item for the village. The method of fluorometric determination of the trace amount of selenium with 2,3-diaminonaphthalene is described in detail elsewhere (27) and was used to determine trace amounts of selenium in blood, nail, food, water, and soil samples. Quality control in the laboratory was maintained by using certified reference materials and by interlaboratory comparisons. Standard samples with selenium levels equaling 0.134 μg/g, 0.24 μg/g, 0.49 μg/g, and 0.58 μg/g were used throughout the entire analysis process, and the relative measured differences between our laboratory measures and the standard referent material values were 6.1 percent, 9.8 percent, 5.5 percent, and 2.2 percent, respectively, all within the range of acceptable values. A subsample ($n = 22$) was also analyzed in another laboratory, and the average relative difference between the two laboratory measures was 6.0 percent, again within the range of acceptable values.

Food frequency questionnaire

A food frequency questionnaire was administered during the interview in which participants were asked for their average daily intakes of various grains, vegetables, meat, seafood, fruit, nuts, cooking oil, tea, and water. The questionnaire had been developed and validated for use in Chinese populations (28–30). The daily selenium intake was derived from food frequency questionnaires with selenium levels analyzed from local food and water samples (31, 32).

Apolipoprotein E genotype

Blood spots on filter paper were collected from all study participants at the end of the interview. The genotype for apolipoprotein E (gene symbol, *APOE*) was determined by eluting DNA from a dried blood spot (33), followed by *Hha*I digestion of amplified products (34).

Collection of other risk factors

Information collected on the other risk factors during the interview includes age, gender, whether the participant attended school and years of schooling, marital status, household composition, participant's birthplace and migration history, alcohol consumption and smoking history, and history of cancer, Parkinson's disease, diabetes, hypertension, stroke, heart attack, head injury, and bone fracture. Participants' height, weight, and blood pressure (two times) were also measured during the interview. Body mass index was derived from height and weight measurements. The average of the two blood pressure measures was used in our analyses.

Statistical analysis

Pearson's correlation coefficients were used to estimate correlations between nail selenium contents and selenium intake derived from the food frequency questionnaire and local food samples and between the selenium levels measured in nail and blood samples. To best capture the association between selenium levels and cognitive function to include potential nonlinear relations, we divided the study population into quintiles according to nail selenium levels. In addition to selenium levels, the following variables were considered to be potential confounding factors possibly related to both cognitive function and selenium levels: age at interview, gender, education (whether the participant attended school), marital status, household composition, alcohol consumption and tobacco smoking, body mass index derived from weight and height measures, systolic and diastolic blood pressure measures, and the *APOE* genotype (ε4 carriers vs. noncarriers).

Analysis of variance models were used to compare differences in continuous variables, and chi-squared tests were used to compare differences in categorical variables across the five quintile groups defined by nail selenium levels. Multivariate analysis of covariance was used to first examine the association between selenium quintiles and all six cognitive test scores. Following the significance of the multivariate analysis of covariance test, analysis of covariance models were used with each individual cognitive score as outcome variables. A composite cognitive z score was created by using the average of standardized scores of the six

cognitive tests (35, 36). The Wald-test statistic in mixed-effect models was used to detect significant correlation among cognitive scores from participants within the same site. With a nonsignificant correlation structure, regression models or analysis of variance models were conducted to identify variables associated with cognitive outcomes univariately. Analysis-of-covariance models were used with standardized cognitive test scores, including the composite z scores as the dependent variables and the quintile selenium levels as the independent variables adjusting for age, gender, education, and other factors that were found to be related to either the selenium levels or the cognitive scores. To ensure that the associations between selenium and cognitive scores were not due to cardiovascular disease or cancer, we repeated the analysis-of-covariance models, excluding those subjects with a history of heart attack, stroke, or cancer.

RESULTS

Description of participants

In table 1, we present the characteristics of study participants by quintile of selenium levels measured in nail samples. Age, marital status, history of cancer, and *APOE* genotype were not significantly different among the five groups defined by selenium quintiles.

Selenium distribution and correlation in selenium measures

The selenium distribution from the four study sites is presented in table 2. Overall, the four study sites provided an extended range of selenium distribution as designed, with overlapping selenium levels from participants across the four sites. Nail selenium levels were significantly correlated with the selenium levels measured in blood ($r = 0.60$, $p < 0.0001$). The selenium intake derived from food frequency questionnaire and selenium measures of local food samples also correlated significantly with selenium measures in nail samples ($r = 0.51$, $p < 0.0001$) and in blood samples ($r = 0.46$, $p < 0.0001$). Vitamin E was also measured in the blood samples, but it was not correlated with the blood selenium level ($r = -0.04$, $p = 0.5520$).

Factors associated with cognitive function

The mean cognitive scores by selenium quintiles are presented in table 3. All scores except those of the Animal Fluency Test showed significant differences by selenium quintiles. Wilks' lambda test in multivariate analysis of variance yielded significant differences among the five selenium quintiles ($p < 0.0001$). Using the composite z score as the outcome variable, we found that increasing age, female gender, no school attendance, nondrinkers, nonsmokers, lower body mass index, and lower diastolic blood pressure were univariately associated with lower cognitive function (table 1). No significant correlation among individuals within the same site was detected by use of mixed-effect models with each cognitive score as an outcome variable ($p > 0.1143$ for all cognitive scores). Therefore, subsequent analysis of covariance models, including all significant variables identified in univariate analyses, showed that marital status, household composition, alcohol, cancer, hypertension, and heart attack were not significantly associated with any of the cognitive scores. Results of the final analysis of covariance models for two outcome variables, the CSID score and the composite z score, are presented in table 4. Selenium levels accounted for an additional 3.6 percent of the variance in CSID scores, 2.6 percent in the IU Story Recall Test, 0.7 percent in CERAD Word List Learning Test scores, 0.6 percent in the CERAD Word List Recall Test, 2.1 percent in the IU Token Test, and 1.8 percent in the composite z score after adjustment for all the other covariates included in table 4. Adjusted cognitive scores by selenium quintiles based on the final analysis of covariance models controlling for other covariates for all cognitive outcomes are presented in table 5. *APOE* ε4 carriers had significantly lower CSID ($p = 0.0135$) and IU Token Test ($p = 0.0251$) scores. Increasing age, female gender, no education, and lower body mass index were significantly associated with lower cognitive scores in all models. Increasing selenium quintiles were associated with better cognitive scores for all cognitive scores except those from the Animal Fluency Test (table 5). The estimated difference in CSID scores between participants in the highest and lowest quintiles in nail selenium levels is 0.54 (standard deviation), while the effect of an increase of 10 years in age on the CSID score was estimated to be 0.45 (standard deviation) (table 4). Similar results were obtained after excluding subjects who reported having cancer, stroke, and heart attack from the analyses.

Significant positive associations were found between selenium intake and cognitive scores ($p < 0.0001$ for all scores) after adjustment for age, gender, education, smoking, body mass index, cancer, and *APOE* genotypes. When the same models were conducted in the 200 individuals with blood samples, decreasing blood selenium levels were significantly associated with lower CSID scores ($p < 0.0001$), lower IU Token Test scores ($p = 0.0238$), and marginally associated with lower composite z scores ($p = 0.0603$).

DISCUSSION

In this cross-sectional survey of cognitive function in rural elderly Chinese, we found that decreasing selenium levels measured in nail samples are associated with lower cognitive scores when controlling for age, gender, education, body mass index, and *APOE* status. The effect of the lowest selenium quintile compared with the highest quintile on the CSID score is equivalent to an increase of 10 years in age in this cohort. The amounts of variance explained by selenium level in the CERAD Word List Learning Test and Recall Test scores were lower than for the other scores, because the total variance explained by these models was lower than for those of the other test scores. The explained percentages are similar to reports of other factors associated with cognitive function in the elderly (37–39), and there are currently no similar data on selenium with which we can compare our results. The stability of this rural population

TABLE 1. Demographic characteristics of participants in the selenium study by quintiles of selenium level in nail samples, People's Republic of China, December 2003–May 2005

	Quintiles of selenium level in nail samples (μg/g)					*p* value*	*p* value for correlation with *z* score†
	Quintile 1 (≤0.232) (*n* = 393)	Quintile 2 (0.233–0.361) (*n* = 406)	Quintile 3 (0.362–0.441) (*n* = 390)	Quintile 4 (0.442–0.552) (*n* = 406)	Quintile 5 (≥0.553) (*n* = 405)		
Mean age, years (SD‡)	72.0 (5.6)	72.2 (5.6)	71.8 (5.5)	71.9 (5.4)	71.6 (5.7)	0.6195	<0.001
Female (%)	52.9	44.1	47.4	58.4	65.2	<0.0001	<0.001
Ever attended school (%)	27.5	35.7	36.4	35.5	36.3	0.0025	<0.001
Marital status (%)						0.4913	<0.0001
Married	60.8	62.1	65.6	63.3	61.0		
Widowed	38.7	36.5	32.6	35.5	38.3		
Other	0.5	1.5	1.8	1.2	0.7		
Household composition (%)						0.0034	<0.001
Live with spouse	54.1	50.1	48.5	49.3	52.6		
Live with children	26.8	28.0	30.9	28.5	22.8		
Live with spouse and children	2.3	5.5	8.5	8.2	6.0		
Live alone	16.8	16.4	12.1	14.1	18.6		
Consume alcohol (%)	60.1	51.2	41.3	32.8	32.8	<0.001	<0.001
Mean drinks/week (SD)	5.1 (7.6)	5.6 (8.5)	5.5 (8.9)	4.8 (9.2)	4.6 (9.1)	0.4518	<0.001
Smoking (%)						<0.001	<0.001
Current smoker	20.8	24.4	34.6	36.5	30.1		
Former smoker	19.8	17.8	18.5	14.8	14.3		
Mean pack-years, 1,000s (SD)	3.0 (5.3)	4.9 (7.1)	5.0 (7.0)	3.9 (6.1)	4.2 (7.6)	<0.001	<0.001
Body mass index, kg/m^2 (SD)	21.0 (2.7)	21.1 (3.0)	21.8 (3.6)	22.6 (4.0)	23.2 (3.6)	<0.001	<0.001
Systolic blood pressure, mmHg (SD)	134.0 (20.8)	144.0 (23.1)	148.6 (25.4)	150.3 (26.3)	151.6 (24.6)	<0.001	0.1421
Diastolic blood pressure, mmHg (SD)	80.1 (12.3)	84.0 (12.3)	84.5 (13.2)	84.6 (13.2)	84.9 (11.7)	<0.001	0.037
History of (%)							
Cancer	0	0.7	0.5	0.7	1.2	0.2948	0.0388
Parkinson's disease	0	1.00	1.8	0.3	1.5	0.0318	0.7236
Diabetes	1.5	1.2	2.3	3.2	4.7	0.014	0.2658
Hypertension	10.4	10.3	15.6	20.7	27.2	<0.001	0.0129
Stroke	0.5	1.5	2.6	4.9	5.9	<0.001	0.3565
Heart attack	3.6	1.0	1.8	4.7	6.2	<0.001	0.0388
Head injury	9.2	5.7	5.1	3.7	4.9	0.0149	<0.001
Fracture	1.5	0.7	2.8	3.0	4.7	0.005	0.3313
APOE‡ genotype (%)						0.1061	0.0841
ε4 carriers (ε2/ε4, ε3/ε4, ε4/ε4)	14.5	18.7	18.7	17.5	13.3		
No ε4 (ε2/ε2, ε2/ε3, ε3/ε3)	85.5	81.3	81.3	82.5	86.7		
Selenium intake, μg/day (SD)	10.4 (5.0)	14.1 (10.9)	18.6 (12.7)	25.2 (19.0)	39.4 (28.6)	<0.0001	<0.0001
Blood selenium, ng/ml (SD)§	56.7 (19.6)	107.8 (36.7)	119.3 (21.9)	132.2 (32.7)	140.2 (26.7)	<0.0001	0.0516

* *p* values comparing the distribution of participants' characteristics by nail selenium quintiles.
† *p* values for the associations between participants' characteristics and the composite *z* score.
‡ SD, standard deviation; *APOE*, gene symbol for apolipoprotein E.
§ Quintile 1: *n* = 46; quintile 2: *n* = 36; quintile 3: *n* = 36; quintile 4: *n* = 47; quintile 5: *n* = 36.

and the high correlations among different selenium measurements suggest that our results reflect the effect of lifelong selenium exposure on cognitive function.

In two cross-sectional studies that reported on the relation between serum selenium levels of antioxidants and cognitive scores, selenium's effect did not reach statistical

TABLE 2. Selenium distribution at the four study sites, People's Republic of China, December 2003–May 2005

	Site 1 (Qionglai) ($n = 500$)	Site 2 (Gaomi) ($n = 500$)	Site 3 (Jiange) ($n = 500$)	Site 4 (Zichuan) ($n = 500$)
Mean nail selenium, μg/g (SD*)	0.434 (0.138)	0.405 (0.086)	0.211 (0.057)	0.605 (0.163)
Estimated selenium intake, μg/day (SD)	18.90 (9.86)	11.66 (4.72)	9.75 (4.47)	46.73 (24.36)
Blood selenium, ng/ml (SD) ($n = 200$)	121.16 (34.52)	117.38 (22.48)	71.88 (5.52)	147.44 (17.42)
Selenium quintiles, %				
Quintile 5 (≥0.553 μg/g)	15.2	5.8	0.2	59.8
Quintile 4 (0.442–0.552 μg/g)	30.4	23.6	0.4	26.8
Quintile 3 (0.362–0.441 μg/g)	24.2	40.8	1.2	11.8
Quintile 2 (0.233–0.361 μg/g)	25.8	28.6	25.2	1.6
Quintile 1 (≤0.232 μg/g)	4.4	1.2	73.0	0

* SD, standard deviation.

significance after adjustment for other risk factors (8, 40). However, it is known that the serum selenium level reflects short-term dietary intake and is also heavily influenced by supplemental selenium use. The Third National Health and Nutrition Examination Survey, for example, showed that subjects in the lowest selenium quintile also had the lowest education, lowest income, highest proportion of non-White race, and lowest percentage taking supplement. The relation between antioxidant supplements including selenium and cognitive test performance was examined in the Monongahela Valley Independent Elders Survey (12), and no significant differences in cognitive test performance between antioxidant users and nonusers were found after adjustment for other covariates. However, the survey did not examine selenium intake or biomarkers for selenium. A longitudinal follow-up of the Etude du Vieillissement Arteriel cohort reported a significant protective plasma selenium level on cognitive decline (9). In addition, the Duke Epidemiology Study of the Elderly reported a significant association between antioxidant use and less cognitive decline (11).

In our population, selenium had a consistent, dose-response relation with cognitive performance, such that higher selenium levels were associated with better cognition. The largest effects were seen for the CSID, which is a multifactorial cognitive screening test, and the IU Token Test, which measures working memory (an executive function) and language comprehension. New learning measures, such as the CERAD Word List Learning Test task and the IU Story Recall Test, were also clearly related to selenium levels. The one test that did not appear to have a relation with selenium levels was the Animal Fluency Test. This test is unique in the battery in tapping a nonmemory, executive ability. It may be that selenium exerts its effects primarily through working and secondary memory mechanisms. Alternatively, it may be that the rural lifestyle produces such a wide and regular exposure to farm animals that the task features of the Animal Fluency Test are altered in subtle ways that cause it to be less of an ''executive function'' measure. However, the fact that the mean scores from this sample are very much in keeping with those reported for other elderly samples in both rural (26, 41) and urban settings (42) argues against this explanation.

Selenium is recognized as an important dietary micronutrient in humans and is hypothesized to impact the aging

TABLE 3. Mean cognitive scores by quintiles of selenium levels measured in nail samples, People's Republic of China, December 2003–May 2005

	Quintiles of selenium level in nail samples (μg/g)					*p* value
	Quintile 1 ($n = 393$)	Quintile 2 ($n = 406$)	Quintile 3 ($n = 390$)	Quintile 4 ($n = 406$)	Quintile 5 ($n = 405$)	
CSID* score (SD*)	23.96 (3.47)	24.96 (3.49)	25.81 (3.44)	25.83 (3.58)	26.15 (3.35)	<0.001
IU* Story Recall Test (SD)	4.68 (2.32)	4.86 (2.72)	5.11 (2.87)	5.67 (3.03)	6.28 (3.12)	<0.001
Animal Fluency Test (SD)	12.67 (4.50)	12.84 (4.99)	12.50 (4.91)	12.90 (5.24)	12.77 (4.92)	0.8063
CERAD* Word List Learning Test (SD)	12.59 (3.90)	12.68 (3.90)	12.98 (3.82)	13.16 (3.96)	13.81 (4.06)	<0.001
CERAD Word List Recall Test (SD)	4.49 (1.87)	4.35 (1.84)	4.71 (1.97)	4.74 (1.92)	4.88 (2.02)	<0.001
IU Token Test (SD)	14.32 (5.47)	15.39 (5.35)	16.22 (5.07)	16.73 (5.25)	17.35 (4.88)	<0.001
Composite *z* score (SD)	−0.19 (0.72)	−0.10 (0.72)	0.01 (0.72)	0.08 (0.78)	0.19 (0.77)	<0.001

* CSID, Community Screening Instrument for Dementia; SD, standard deviation; IU, Indiana University; CERAD, Consortium to Establish a Registry for Alzheimer's Disease.

TABLE 4. Association between selenium quintiles in nail samples and cognitive test scores, with adjustment for other covariates, People's Republic of China, December 2003–May 2005*

	Standardized CSID† score				Standardized composite *z* score			
	Entire sample ($n = 1,995$)‡		Subsample ($n = 1,860$)§		Entire sample ($n = 1,995$)‡		Subsample ($n = 1,860$)§	
	Parameter estimate (SE†)	*p* value	Parameter estimate (SE)	*p* value	Parameter estimate (SE)	*p* value	Parameter estimate (SE)	*p* value
Age (in years)	−0.045 (0.004)	<0.001	−0.045 (0.004)	<0.001	−0.040 (0.003)	<0.001	−0.040 (0.003)	<0.001
Sex (female vs. male)	−0.434 (0.052)	<0.001	−0.426 (0.053)	<0.001	−0.229 (0.039)	<0.001	−0.236 (0.039)	<0.001
Education (attended school vs. no school)	0.378 (0.046)	<0.001	0.373 (0.048)	<0.001	0.369 (0.034)	<0.001	0.360 (0.035)	<0.001
Smoking								
Current smoker	0.028 (0.052)	0.594	0.043 (0.053)	0.425	0.024 (0.039)	0.528	0.041 (0.039)	0.293
Former smoker	0.050 (0.058)	0.391	0.102 (0.061)	0.096	0.117 (0.043)	0.007	0.142 (0.045)	0.002
Nonsmoker	Referent		Referent		Referent		Referent	
Body mass index	0.032 (0.006)	<0.001	0.031 (0.006)	<0.001	0.029 (0.004)	<0.001	0.028 (0.004)	<0.001
Cancer (yes vs. no)	0.412 (0.236)	0.081			0.434 (0.175)	0.013		
APOE† (ε4 carriers vs. noncarriers)	−0.131 (0.051)	0.011	−0.132 (0.053)	0.012	−0.062 (0.038)	0.105	−0.051 (0.039)	0.186
Selenium quintiles (μg/g)								
Quintile 5 (≥0.553)	0.535 (0.062)	<0.001	0.523 (0.064)	<0.001	0.276 (0.046)	<0.001	0.268 (0.047)	<0.001
Quintile 4 (0.442–0.552)	0.466 (0.061)	<0.001	0.448 (0.063)	<0.001	0.200 (0.045)	<0.001	0.178 (0.046)	<0.001
Quintile 3 (0.362–0.441)	0.420 (0.061)	<0.001	0.411 (0.062)	<0.001	0.106 (0.045)	0.019	0.100 (0.046)	0.029
Quintile 2 (0.233–0.361)	0.205 (0.060)	<0.001	0.186 (0.061)	0.002	0.024 (0.045)	0.592	0.019 (0.045)	0.680
Quintile 1 (≤0.232)	Referent		Referent		Referent		Referent	

* Results of analysis of covariance models by standardized cognitive test scores.

† CSID, Community Screening Instrument for Dementia; SE, standard error; *APOE*, gene symbol for apolipoprotein E.

‡ Five study participants were excluded from the models: One did not have a body mass index measure, and another four participants were smokers who had missing information regarding their classification into "current" or "former smoker" categories.

§ The subsample excluded participants with a history of heart attack, stroke, or cancer.

process. The selenium content in foods varies greatly depending on the selenium content of the soil where plants are grown, while up to 10-fold differences in selenium contents can be found in the same food item (43). Dietary selenium is found to be highly bioavailable (44), and its elimination in humans was shown to be in three phases, with the last phase lasting as long as 200 days (45). Detailed information on selenium absorption, metabolism, and excretion can be found elsewhere (46, 47). Many studies have examined selenium contents in toenails and found that selenium levels in toenails are highly reproducible in a 1-year period (48–51), and toenails are generally regarded as useful biomarkers for

TABLE 5. Adjusted mean differences in standardized cognitive test scores by selenium quintiles in nail samples, with adjustment for age, gender, education, smoking, body mass index, cancer, and *APOE** genotypes, People's Republic of China, December 2003–May 2005

	Quintiles by nail selenium level					*p* value
	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	
CSID* score (SE*)	Referent	0.20 (0.06)	0.42 (0.06)	0.47 (0.06)	0.54 (0.06)	<0.0001
IU* Story Recall Test (SE)	Referent	0.02 (0.07)	0.06 (0.07)	0.26 (0.07)	0.44 (0.07)	<0.0001
Animal Fluency Test (SE)	Referent	−0.04 (0.06)	−0.13 (0.07)	−0.01 (0.07)	−0.05 (0.07)	0.2880
CERAD* Word List Learning Test (SE)	Referent	−0.04 (0.07)	0.01 (0.07)	0.08 (0.07)	0.21 (0.07)	0.0029
CERAD Word List Recall Test (SE)	Referent	−0.12 (0.07)	0.04 (0.07)	0.07 (0.07)	0.11 (0.07)	0.0107
IU Token Test (SE)	Referent	0.13 (0.06)	0.24 (0.06)	0.35 (0.06)	0.42 (0.06)	<0.0001
Composite *z* score (SE)	Referent	0.02 (0.04)	0.11 (0.05)	0.20 (0.05)	0.28 (0.05)	<0.0001

* *APOE*, gene symbol for apolipoprotein E; CSID, Community Screening Instrument for Dementia; SE, standard error; IU, Indiana University; CERAD, Consortium to Establish a Registry for Alzheimer's Disease.

long-term exposure (52). There are also studies demonstrating excellent correlation in the selenium measured between toenail and fingernail samples ($r = 0.919$), and both toenail and fingernail samples showed identical correlations with selenium levels measured in blood samples (53).

In animal studies, selenium deficiency has been shown to increase protein oxidation in mice and to shorten the lifespan in transgenic *Drosophila* (54, 55). Selenium's effect on aging has also been investigated in terms of DNA damage (56). In many previous studies, selenium exposures were measured from either supplement use or blood samples, both reflecting relatively short-term intake and possibly being confounded by supplement ingestion. It is known that the early life environment and its effect in childhood and adolescence are linked to many adult chronic diseases, such as heart disease, stroke, hypertension, diabetes mellitus, and chronic obstructive lung disease (57). Environmental factors can affect brain maturation in childhood and adolescence and may have an impact on later-life cognitive decline. The areas of the brain that take the longest to mature during childhood are the same areas of the brain that show the earliest signs of Alzheimer's disease (58). In addition, animal studies on rats demonstrated that the brain has a unique feature in that it stores selenium (59). Therefore, after the animal is placed on a low selenium diet, the activity of glutathione peroxidase in the brain does not decrease as fast as observed in the liver (60). This suggests that long-term exposure to selenium may be needed to impact brain function later in life. The brain's unique selenium metabolism may also make it more difficult to show the effect of short-term selenium exposure on brain function than on other organs. Because the majority of our participants were lifelong residents of the same towns and villages, selenium measures in the participants reflect lifelong exposure, enhancing our power for detecting a selenium effect.

Selenium levels in various cohorts differ by the geographic locations of the study population (61). Although US cancer studies report mean nail selenium levels of 0.8 μg/g in control subjects, European cohorts include many control groups with nail selenium levels around 0.5 μg/g, overlapping with the selenium range in our cohort. It is worth noting that the selenium levels reported in cohorts from developed countries may also be influenced by dietary supplements and, hence, may not be reflective of lifelong exposure.

The effect of *APOE* in Alzheimer's disease and cognitive function has been of particular interest in Asian populations, because the frequency of ε4 is lower in these populations than in most but not all European and North American populations. The ε4 allele frequency in our cohort is 8.8 percent, higher than the 6.4 percent allele frequency reported in Singapore (62), 7.4 percent in Hong Kong (63), and 4.9 percent in Taiwan (64), but lower than the 11.0 percent in the Shanghai cohort (65). Significantly lower cognitive performance in ε4 carriers was found in the CSID and the IU Token Test scores in our cohort. Various studies have examined the ε4 effect on neuropsychological tests measuring different domains. Although the ε4 allele has been reported to be associated with memory-dominated functions, the association with tests concentrating on language, visuospatial, for example, has been inconsistent, providing evidence that ε4 may impact different brain regions and brain functions (66, 67).

In our cohort, a lower body mass index was associated with lower cognitive scores. Although body mass index has been associated with a variety of common medical disorders and mortality, the relation between body mass index and cognitive function or the risk of Alzheimer's disease has been inconsistent, with some studies suggesting that low body mass index increases the risk of Alzheimer's disease and poor cognitive function (68, 69), while others suggest the opposite (70). The differences may be due to the variation in time lapse between body mass index measurements and outcome measures in various studies, since the onset of dementia may affect body mass index (71). The differences in body mass index results may also be attributed to differences in cohort composition in body mass index, assuming that an optimal body mass index range exists. Hence, cohorts with most participants below this optimal point would be more likely to identify low body mass index as a risk factor, while cohorts with a body mass index range above the optimal would find high body mass index to be a risk factor.

Our study has a number of strengths. Selenium levels were measured in nail samples, dietary intakes, and blood samples, increasing measurement validity. Our study design ensures an extensive range of selenium exposure in the cohort. In addition, the majority of our study participants were lifelong residents of the same towns where they were interviewed, and the participants were known not to take vitamin supplements; hence, the ascertained selenium levels can be inferred as lifelong exposure to selenium without the influence of supplements.

Because lower selenium was previously reported to be associated with increased risk of coronary heart disease and cancer, there was the possibility that selenium's effect on cognitive function could be impacted by participants suffering coronary heart disease or cancer. However, in our study, the association between selenium levels and cognitive scores remained unchanged after excluding subjects with heart attack, stroke, and cancer, indicating that selenium's effects on coronary heart disease and cancer are an unlikely explanation for our findings.

Our result showing higher numbers of participants with diabetes, hypertension, stroke, and heart attack with the increase of selenium levels was surprising given previous reports on selenium's protective effects on coronary heart disease. One potential explanation could be that selenium's effects on coronary heart disease and cancer in relation to mortality have left fewer participants living with these diseases. This possibility merits further examination in our planned follow-up of this cohort.

This study also has important limitations. The reported association was found in a cross-sectional examination of selenium levels and cognitive function. Although the stability of this population makes a reciprocal effect of low cognitive function on selenium levels unlikely, longitudinal evaluation of the cohort will help to establish whether selenium levels affect the rate of cognitive decline associated with aging.

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