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PLASMA TRACE ELEMENTS AND COGNITIVE FUNCTION IN OLDER MEN AND WOMEN:THE RANCHO BERNARDO STUDY

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Abstract: *Objective*. This study examines the sex-specific associations of plasma concentrations of iron, copper, and zinc with cognitive function in older community-dwelling adults. *Design:* Cross-sectional study. *Setting:* 1988-92 follow-up clinic visit. *Participants:* 602 men and 849 women (average age=75 ±8 years) who were community-dwelling and not clinically demented. *Measurements:* Blood samples were assayed for trace elements and 12 cognitive function tests were administered. Sex-specific analyses were adjusted for age, education, alcohol consumption, smoking, exercise, and estrogen use in women. *Results*. Men and women differed significantly in education and alcohol intake (p's<0.001), concentrations of plasma iron, copper and zinc (p's<0.001) and scores on 11 of 12 cognitive function tests (p=0.04 to <0.001). Regression analyses showed significant inverted U-shaped associations in men; *both* low and high iron levels were associated with poor performance on total and long-term recall and Serial 7's (p's=0.018, 0.042 and 0.004, respectively) compared to intermediate concentrations. In women, iron and copper concentrations had inverse linear associations with Buschke total, long and short-term recall and Blessed scores (p's<0.05). Zinc was positively associated with performance on Blessed Items (p=0.008). Analyses comparing cognitive function using categorically defined mineral concentrations yielded similar sex specific results. *Conclusion*. Optimal trace element concentrations may exist for optimal cognitive function in older adults, and these levels may differ by sex and cognitive function domain.

Key words: Cognitive function, copper, iron, trace elements, zinc.

Introduction

The decline in cognitive function with increasing age impairs quality of life (1), increases economic burden (2), and may hasten mortality (3). Researchers from Europe and North America estimate that the prevalence of mild cognitive impairment among older adults exceeds 20% (4-8). As reviewed elsewhere (9), age-related cognitive impairment may be attributable in part to inadequate nutrition, a potentially modifiable risk factor. Even in relatively affluent nations, the prevalence of inadequate nutrition in community-dwelling adults ranges from 5 to 10% (10). Inadequate nutrition often includes deficiencies in vitamins and minerals (10, 11).

Early studies demonstrated adverse effects on brain function resulting from deficiencies in trace elements such as iron, copper and zinc (12-14). In contrast, studies performed in the last 15 years suggest that high concentrations of these minerals may be associated with Alzheimer's disease (15-17). Relatively few studies have reported the association of these minerals with performance on cognitive function tests in relatively healthy older community-dwelling men and women unselected for cognitive impairment. One small clinical trial of supplemental vitamins and minerals reported that individuals with adequate blood-nutrient concentrations performed significantly better on cognitive function tests (18), while another small trial found that high-dose vitamin and mineral supplementation did not improve cognitive performance (19). A large prospective study found that use of supplemental zinc in addition to antioxidants (vitamins A, C, or E), lowered risk of incident cognitive impairment (RR=0.66, 95% CI=0.44-1.00) and risk of cognitive decline (RR=0.71, 95% CI=0.49-1.01) (20). However, a large randomized controlled trial found no effects for supplemental zinc or copper on cognitive function (21). These varied findings reflect the complex nature of the association between trace elements and cognitive function and warrant further evaluation (22).

We report here the sex-specific associations of plasma iron, copper, and zinc concentrations with performance on 12 cognitive function tests in a large sample of older, community-dwelling men and women unselected for cognitive impairment.

Methods

Participants

Between 1972-74, 82% of all residents of the predominantly white, middle-class Southern California community of Rancho Bernardo were surveyed for heart disease risk factors (23). In 1988-1992, a follow-up clinic visit included blood collection and assessment of cognitive function. A total of 909 women and 629 men aged 60 and older participated, representing 80%

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of the surviving cohort. After excluding 52 women and 25 men who declined all cognitive function tests and/or had missing plasma mineral assays and 2 men and 8 women with extreme plasma mineral values, there remained 1451 participants (849 women and 602 men) who form the focus of this report. This study was approved by the UCSD Human Research Protections Program; all participants were ambulatory and gave written informed consent prior to participation.

Procedures

A self-administered questionnaire assessed age, education, smoking status (never/past/ current), alcohol consumption ≥3 times/week (no/yes), and exercise frequency ≥3 times/week (no/yes). Current estrogen use by women, was queried and validated by examining pill containers and prescriptions brought to the clinic for that purpose. A fasting (12-16h) venous blood sample was drawn using a trace element-free plastic tube containing 2 drops of 2% sodium oxalate, centrifuged within 2 hours and frozen at -70°C for later measurement of trace elements.

A trained interviewer administered a battery of cognitive function tests with established reliability and validity (24, 25) to individual participants. Unless specified, higher scores on the following tests indicate better cognitive performance:

The Buschke-Fuld Selective Reminding Test (26) assesses short and long-term storage, retention, and total recall of 10 spoken words. Higher scores on the short-term test indicate poorer performance.

The Heaton Visual Reproduction Tests (27) adapted from the Wechsler Memory Scale (28) assesses memory for geometric forms. Three stimuli of increasing complexity are presented, one at a time, for 10 seconds each. The participant reproduces the figures immediately (to assess short-term memory), after 30 minutes of unrelated testing (to assess long-term memory), and copies the stimulus figures to assess visual-spatial impairments. Three scores are obtained: immediate recall, delayed recall, and copying.

The Mini-Mental State Examination (29, 30) assesses orientation, registration, attention, calculation, language, and recall. Scores range from 0 to 30. Two items were analyzed separately: counting backward from 100 by sevens (Serial 7's) and spelling the word "world" backward. For both items, the maximum possible score is 5.

Items from the Blessed Information-Memory-Concentration Test (25) assess concentration by having the subject name the months of the year backward and assess memory by asking subjects to recall a five-part name and address following a 10-minute delay. The maximum possible score is 7.

Trails B from the Halstead-Reitan Neuropsychological Test Battery (31), tests visuomotor tracking and attention. The subject scans a page continuously to identify numbers and letters in a specified sequence while shifting from number to letter sets. A maximum of 300 seconds is allowed; performance is rated by the time required to finish the test. Higher scores indicate poorer performance.

In Category Fluency (32), the subject names as many animals as possible in 1 minute. The score is the number of animals named correctly. Repetitions, variants (e.g., dogs after producing dog) and intrusions (e.g., apple) are not counted.

Between 1993 and 1997, frozen plasma was shipped on dry ice for mineral analysis at the Grand Forks Human Nutrition Research Center. The samples were diluted 1:4 with distilled deionized water before analysis. Serachem Clinical Chemistry Control Serum Concentration 1 (Instrumentation Laboratory Co., Lexington, MA) and UTAK Normal Range Control Serum (UTAK Laboratories, Valencia, CA), prepared the same as the plasma samples, were used for quality assurance/quality control. The inductively coupled plasma (ICP) atomic emission spectrometer (Thermo Optek Corp., Franklin, MA) was used for rapid, simultaneous assay of plasma samples for iron, copper, and zinc. Results and performance were essentially the same as those using the atomic absorption spectrometer (AAS, Perkin-Elmer Corporation, Norwalk, CT), which measures only one element at a time. Comparisons of assay values from 20 random samples with both techniques indicated less than 3-5% differences between the ICP and AAS results, which approximates the expected day-to-day variability in the analyses (D. Milne, personal communication, May 5, 1993).

Statistical analysis

Means and standard deviations were calculated for continuous variables including trace elements, which were approximately normally distributed. Rates were calculated for categorical variables. Comparisons between women and men were made using t-tests for continuous data and chi-square tests for categorical data.

Cognitive function scores were rescaled such that higher scores indicated better performance and converted to z scores to bring all scores to the same scale. Separate regression models with each cognitive function z score as the dependent variable were examined using the three trace elements (intercorrelations <0.20) as independent continuous variables in the same model. Because of known sex differences in cognitive function (33), analyses were sex-specific; potential confounders (age, education, smoking, alcohol consumption, exercise, and current estrogen use) were included only if their inclusion changed beta estimates by >10% for at least one mineral. Linear and quadratic terms for trace elements were examined; quadratic terms were included in models only if they were statistically significant. A positive quadratic beta estimate indicates a U-shaped risk curve, while a negative quadratic estimate indicates an inverted U-shaped curve. Graphs of cognitive function z scores by mineral concentrations were created based on beta estimates obtained from regression analyses. As recommended elsewhere for exploratory analyses, no adjustment was made for multiple comparisons and exact p-values are shown (34).

Trace element variables were also categorized into low, medium, and high levels using classification and regression tree (CART) analysis to determine optimal cut-offs (S-PLUS, Insightful Corp., version 6.1 for Windows, 2002). CART models consisted of a cognitive function z score as the outcome variable with each trace element entered as an independent variable. The CART routine recursively partitions each trace element variable using binary splits to divide the data into optimally homogeneous subgroups. The lowest and highest binary splits were used as cut-offs to define three categories (low/deficient, intermediate, or high/excess) for each trace element. For inverse U-shaped associations, the low and high categories were compared against the intermediate category. For negative linear associations, the high category was compared to the low category.

All significance tests were two-tailed with p≤ 0.05 considered significant. Unless specified, analyses were performed with SAS (Cary, North Carolina, version 9.1 for Windows, 2002-3).

Results

Age ranged from 60-94 (mean=74.9±8.2) years in men and from 60-98 (mean=74.7±7.7) years in women (p=0.64). The majority (78% of men, 63% of women) had attended some college or more (p<0.001). Comparisons on other characteristics, mineral concentrations and cognitive function scores are shown in Table 1. As shown, rates of alcohol intake and exercise were significantly higher in men (p<.001) as were mean plasma concentrations of iron, copper and zinc (p's<.001). Cognitive function scores were normally distributed and differed significantly by sex for 11 of the 12 tests (see Table 1, p <0.04 for Blessed, p's<0.001 for all other tests).

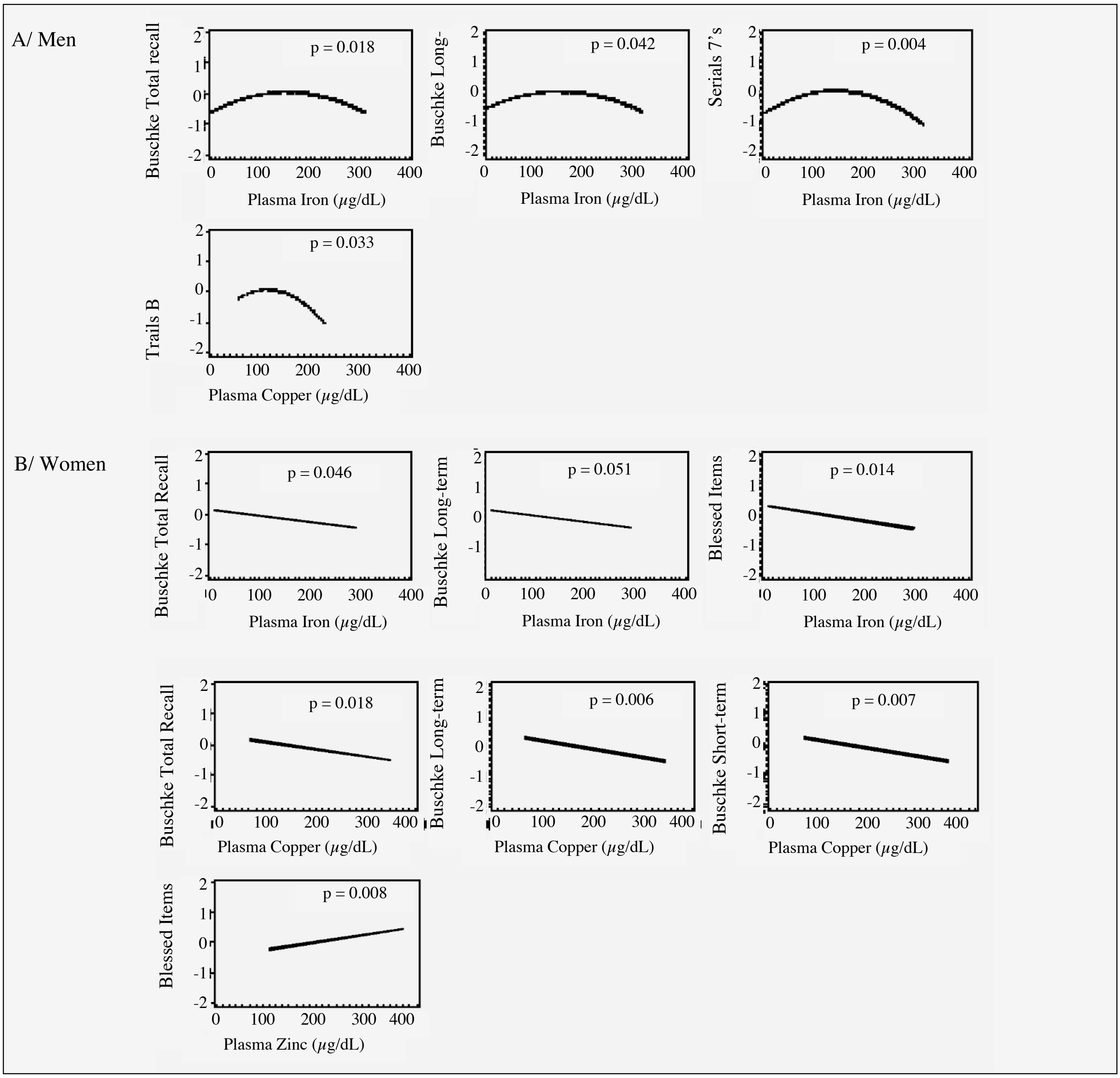
Linear regression analyses were performed with cognitive function z scores as the dependent variables in separate models and all three minerals as continuous independent variables, along with confounding covariates of age, education, alcohol use, current smoking, exercise, and estrogen use (women only). Models contained both linear and quadratic terms for each mineral with significant results shown graphically for men in Figure 1A, and women in Figure 1B. For men, all associations were quadratic and inverse U-shaped. After adjustment for covariates, significant quadratic associations were found between iron and Buschke total (p=0.018) and long-term (p=0.042) recall and Serial 7's (p=0.004) such that both very low and very high iron concentrations were related to poorer cognitive performance. A significant negative quadratic association was also found between plasma copper and Trails B (p=0.033). Zinc was not significantly associated with any cognitive function scores in men. For women, all associations were linear. After adjustment for covariates, plasma iron had significant inverse associations with Buschke total and long-term recall (p=0.046 and p=0.051) and Blessed score (p=0.014). Copper also had significant inverse associations with Buschke total (p=0.018), long-term (p=0.006), and short-term (p=0.007) recall. Zinc was *positively* associated with Blessed score (p=0.008). Stratified analyses showed similar associations in estrogen users and non-users (data not shown). For both women and men, no significant interactions were found between pairs of trace elements or between each mineral and the other covariates.

Table 1
Characteristics of women and men; Rancho Bernardo, California 1988-1992

Characteristics	Women (n=849)	Men (n=602)	p-value
	%	%	
Education (some college)	63	78	<0.001
Current smokers	9	7	0.21
Alcohol (≥ 3 times/ week)	38	55	<0.001
Exercise (≥ 3 times/ week)	65	75	<0.001
Current estrogen user	35	---	---
	Mean (SD)	**Mean (SD)**	
Age (years)	74.7 (7.7)	74.9 (8.2)	0.641
Iron (μg/dL)	108.2 (34.8)	121.5 (40.1)	<0.001
Copper (μg/dL)	123.5 (28.1)	101.5 (17.5)	<0.001
Zinc (μg/dL)	75.3 (12.5)	80.8 (14.5)	<0.001
Buschke Total Recall	39.3 (9.0)	34.5 (9.5)	<0.001
Buschke Long-Term Recall	33.3 (12.2)	26.6 (12.6)	<0.001
Buschke Short-Term Recall†	6.1 (4.2)	7.9 (4.5)	<0.001
Heaton Immediate Recall	9.1 (3.4)	9.8 (3.9)	<0.001
Heaton Delayed Recall	6.3 (3.8)	7.3 (4.5)	<0.001
Heaton Copying	15.0 (2.1)	15.1 (2.2)	0.82
Mini-Mental State Exam	27.2 (2.1)	26.5 (3.2)	<0.001
Serial 7's	3.9 (1.3)	4.3 (1.1)	<0.001
"World" Backward	4.8 (0.6)	4.7 (0.9)	<0.001
Blessed Items	6.0 (1.3)	5.9 (1.6)	0.04
Trails, part B†	143.8 (67.4)	131.0 (64.2)	<0.001
Category Fluency	17.2 (4.8)	18.3 (5.3)	<0.001

CART analysis defined the low, intermediate and high categories, respectively, as <40, ≥40 and ≤215, and >215 μg/dL for iron; <90, ≥90 and ≤215, >215 μg/dL for copper; and <55, ≥55 and ≤100, and >100 for zinc. Results of regression analyses with trace elements as categorical independent variables are shown in Table 2. Based on the patterns of associations observed (Figure 1), cognitive function scores of men in the high and low groups were compared to those of the intermediate group while for women, the cognitive function scores of the high and intermediate groups were compared with those of the low group. As shown, compared to men with intermediate iron concentrations, men with low and high iron had poorer performance on Buschke total (ß=-1.05 in the low group, p<0.001; ß=-0.53 in the high group, p=0.072), and long-term recall (ß=-0.94 in the low group, p<0.001; ß=-0.69 in high group, p=0.020), and on Serial 7's (ß=-1.05 in the low group, p=0.001; ß=-0.51 in high group, p=0.133). There were no other significant cognitive function differences by categorically defined iron, zinc or copper. Table 2 also shows that compared to women with low copper concentrations, women with high copper concentrations performed worse on Buschke long-term (ß=-0.77, p=0.031), short-term (ß=-0.80, p=0.032) and total recall (ß=-0.63, p=0.076). Compared to women with low iron

Figure 1
Associations of trace elements with cognitive function in (A) men and (B) women; linear and quadratic curves generated from regression analyses adjusted for the other minerals, age, education, alcohol use, current smoking, exercise, and current estrogen use (women only).



concentrations, women with higher concentrations of iron had poorer performance on Buschke total (ß=-.55) and long-term recall (ß=-.51) and Blessed (ß=-0.60), although these differences were not statistically significant (p>0.10). Likewise, women with low zinc scored lower on the Blessed (ß=-0.31) than those with high zinc, but this association was not significant (p=0.313).

Discussion

In this study, significant associations were found between trace elements and cognitive function in older adults. Men and women had significantly different concentrations of iron, copper, and zinc and different patterns of associations between these minerals and cognitive function scores. Analyses using trace minerals as continuous variables showed that for men,

Table 2
Associations of categorical trace mineral concentrations with cognitive function scores†

MEN	ß	p	WOMEN	ß	p
1) Total Recall			1) Total Recall		
Low Iron, vs. intermediate	-1.05	<.001	Intermediate Iron, vs. low	.02	.953
High Iron, vs. intermediate	-.53	.072	High Iron, vs. low	-.55	.228
2) Long-Term Recall			2) Long-Term Recall		
Low Iron, vs. intermediate	-.94	<.001	Intermediate Iron, vs. low	.07	.826
High Iron, vs. intermediate	-.69	.020	High Iron, vs. low	-.51	.266
3) Serial 7's			3) Blessed Items		
Low Iron, vs. intermediate	-1.05	.001	Intermediate Iron, vs. low	-.60	.097
High Iron, vs. intermediate	-.51	.133	High Iron, vs. low	-.77	.118
4) Trails B			4) Total Recall		
Intermediate Copper, vs. low	-.05	.489	Intermediate Copper, vs. low	-.01	.915
			High Copper, vs. low	-.63	.076
			5) Long-Term Recall		
			Intermediate Copper, vs. low	-.04	.756
			High Copper, vs. low	-.77	.031
			6) Short-Term Recall		
			Intermediate Copper, vs. low	.01	.979
			High Copper, vs. low	-.80	.032
			7) Blessed Items		
			Low Zinc, vs. high	-.31	.313
			Intermediate Zinc, vs. high	-.10	.578

†Results of regression models; associations adjusted for age, education, alcohol use, current smoking, exercise, and current estrogen use (women only). The low, intermediate, and high categories were defined as: <40, ≥40 and ≤215, and >215 μg/dL for iron; <90, ≥90 and ≤215, >215 μg/dL for copper; and <55, ≥55 and ≤100, and >100 for zinc. In men, low and high categories were compared to the intermediate category.

both very low and very high concentrations of iron and copper were associated with poorer cognitive performance on tests of long-term memory, calculation, and visuomotor attention, compared to intermediate concentrations. Zinc was not associated with cognitive function in men. In women, high iron and copper concentrations were associated with poorer short and long-term recall whereas lower zinc concentrations were related to poorer performance on tests of concentration. Observed associations were independent of age, education, alcohol consumption, cigarette smoking, exercise, and in women, estrogen use. Analyses with categorically defined trace elements yielded similar results although there were fewer significant differences possibly due to the compromised power of categorical analyses.

The higher iron and zinc concentrations in men are consistent with their higher meat consumption (13). The difference in association between iron and cognition for men and women may indicate that men require more iron than women, and the lower iron concentrations may reflect deficient levels for men but not for women. The higher average copper concentrations in women were largely due to higher concentrations among women taking postmenopausal estrogen, compatible with previous reports showing higher copper concentrations in women taking oral contraceptives (35, 36). Estrogen use was not associated with cognitive function in this cohort (37). The distribution of copper values may partially explain the difference in association between copper and cognition in men and women; fewer women had very low copper concentrations and the adverse association was not observed at the lower level but was observed at the higher levels. Other research suggests that iron, copper, and zinc homeostasis are essential for optimal brain function (22, 38, 39). For example, iron deficiency may reduce dopamine neurotransmission, leading to learning deficits (12, 39). Copper deficiency may lead to poor neurotransmission because of its essential role in formation and maintenance of myelin (40), and zinc deficiency could impair neuropsychological function by reducing synaptic responses in the hippocampus or limiting DNA synthesis and repair (14). On the other hand, excess iron may induce oxidative stress by releasing cytotoxic oxygen free radicals and leading to lipid peroxidation, cell membrane damage, and cell death (38, 39, 41). Excessive accumulation of copper (17, 42) and zinc (22) may also lead to oxidative stress and neurodegeneration.

The curvilinear association of iron and copper with cognitive function observed in men in the present study is compatible with these mechanisms and supports the concept of homeostasis. For women, the positive linear and inverse linear associations support the concept that high or low mineral concentrations may adversely affect cognitive function, although no clear U-shaped risk curve was observed for individual minerals. Sex-specific patterns of association in the present study may help to explain the discordance between the results of two small clinical trials, one of which found improved performance (18) in a combined study of women and men supplemented with relatively small doses of iron, copper, and zinc, while the other trial which supplemented only women with high doses of the same minerals and found worsening performance on cognitive function tests (19). Large studies have not reported the role of sex in the association between trace elements and cognition (20, 21). This is the first study to examine these associations separately. The present study has several limitations. The study sample was white, relatively well educated and middle-class, thus results may not generalize to other ethnic groups or social classes. However, the homogeneous nature of this cohort reduces confounding effects of education and socioeconomic status on cognitive function and nutritional status. Few individuals (5%-15%) in this cohort had extremely low or high concentrations of trace elements, thus possibly obscuring associations between extreme levels of trace elements and cognitive impairment. In this study, 72 statistical tests comparing scores on each cognitive function test with each trace element separately in men and women (12 tests x 3 trace elements x 2 sexes = 72) were performed. By chance, significance would be expected in 1 in 20 or almost 4 tests. However, in this study, 11 tests were statistically significant, three times the number expected by chance. Because of the cross-sectional design, we cannot establish whether low or high trace-element concentrations were the cause or consequence of cognitive impairment, although previous studies have shown that mineral supplements increased blood mineral concentration (18) and changed cognitive performance (13, 14, 18). Assessments occurred at one point in time in this study, but mineral status and cognitive performance likely reflect cumulative biological processes over a lifetime. Longitudinal studies are needed to examine changes in cognitive performance with changes in trace element concentrations over time and between men and women.

Both deficiencies and excesses of trace elements may be potentially preventable causes of cognitive impairment. This study found significant associations between trace elements and cognitive function; it is the first study to demonstrate sex-specific differences in associations, possibly due to sex differences in mineral concentrations and nutritional requirements. Other studies longitudinally examining sex-specific associations are needed to confirm these results.

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