**Association between blood manganese and cognitive function in a national representative survey**

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**Abstract**

**Background:** Manganese (Mn) is an essential heavy metal required for the normal development of many organ systems, including the brain. However, excessive Mn is a neurotoxicant, and Mn exposure has been associated with motor, behavioral, and cognitive impairment. Since few studies have used pre-clinical tests to examine the link between Mn exposure and cognitive function, we assessed the association between the internal dose of Mn, as estimated by blood Mn concentration, and cognitive function.

**Methods:** This cross-sectional study investigated US citizens (60–80 years old) who participated in the National Health and Nutrition Examination Survey (NHANES) from 2011 to 2014 (sample size, 2,439). The outcome (dependent) variable, cognitive function, was measured using three tests: animal fluency test, digit symbol substitution test (DSST), and the Consortium to Establish a Registry for Alzheimer’s Disease (CERAD) word learning subset. We created a composite cognitive z-score by using the average of the standardized scores of the six cognitive tests (animal fluency test, DSST, CERAD delayed recall, and three trials of the CERAD word list learning test). The exposure (independent) variable, Mn, was measured in blood samples. Linear regression was performed by constructing three models: a univariate model, a model with all covariates, and a model with only significant covariates. The models were adjusted for sex, age, race/ethnicity, education level, total number of people in the family, cigarette smoking, and income (family monthly poverty level category).

**Results:** Median Mn was 8.71 µg/L (interquartile range, 6.94–11.16 µg/L). Blood Mn quartiles were significantly associated with the composite z-score only in the univariate model. However, in linear regression analyses, after adjustment for potential confounders, blood Mn as a continuous variable was not significantly associated with the composite cognitive z-score (μg/L, β = 0.0038, 95% CI −0.004 to 0.012). The mean composite z-score in the first quartile was significantly lower than the means of the second, third, and fourth quartiles. Meanwhile, the mean of the third quartile was the highest, followed by that of the fourth and second quartiles (0.022, 0.012, and 0.007, respectively).

**Conclusions:** We found no consistent association between low blood Mn concentrations in a US population and a decreased cognitive function. Higher blood Mn levels might show different results, and further research is thus needed.

# Introduction

Manganese (Mn) is an essential trace element involved in amino acid, cholesterol, glucose, and carbohydrate metabolism, reactive oxygen species scavenging, bone formation, reproduction, and the immune response [1]. Excessive exposure to Mn, through occupation, environment, or diet, may result in neurotoxicity. Excess Mn accumulates in the basal ganglia, especially in the striatum, globus pallidus, and substantia nigra. Case reports describing Mn toxicity in humans date back to 1837, when a clinical syndrome of Mn neurotoxicity, now known as “manganism”, was described for the first time in workers with excessive occupational exposure to airborne Mn [2]. Mn exposure has been associated with reduced performance on neuropsychological tests, poor eye-hand coordination and hand steadiness, decreased reaction time, lower cognitive flexibility, and poor postural stability [3].

Mn toxicity has been shown to occur in certain occupational settings through inhalation of manganese-containing dust [4]. High Mn occupational exposure has been linked to motor, behavioral, and cognitive impairment [5]. Manganism is a syndrome similar to Parkinson’s disease that is characterized by psychiatric and cognitive deficits and motor impairment [6]. However, a pilot study of farm workers in Costa Rica did not find strong evidence that Mn concentrations were associated with working memory-related brain activity [5].

Contaminated air and water poses a risk of Mn intoxication to the general population, with Mn exposure from environmental sources linked to a higher prevalence of Parkinsonian disturbances [7]. Environmental Mn exposure has also been associated with lower performance on neuropsychological tests measuring a variety of cognitive functions, including significant associations between Mn exposure and cognitive function obtained in the domains of visuospatial memory and verbal skills [8]. The presence of excessive Mn levels in drinking water has been related to worse memory and attention and hyperactive behavior in school-aged children, with consumption of water containing elevated Mn levels exerting adverse effects on cognitive function in 10-year-old children [9].

Mn concentrations in airborne particles are higher in areas with heavy traffic, from a contribution of vehicle emissions in relation to non-exhaust sources such as road dust resuspension, brake dust, and tire wear [10] and to exhaust due to the potential combustion of the gasoline additive methylcyclopentadienyl Mn tricarbonyl (MMT) [11]. Indeed, children exposed to elevated airborne Mn in an area close to a ferromanganese alloy plant in Brazil had greater IQ impairment of verbal skills and lower neuropsychological performance in tests of executive function of inhibition responses, strategic visual formation, and verbal working memory [12].

Alzheimer’s disease (AD) is the most common cause of dementia in the elderly population. Long-term exposure to environmental contaminants, including Mn, induces neuroinflammation and neuropathology, paving the way for AD development [13]. A Chinese study found an increased association between plasma amyloid-beta peptides with elevated Mn, suggesting that high Mn levels may be an essential pathogenic factor in the progression of AD [14].

Few studies have used pre-clinical tests to assess the association between low-dose background levels of Mn and cognitive function to better understand AD. Accordingly, we used three different pre-clinical assessments to investigate the association between Mn and cognitive function. The present results will serve as a basis for assessing the association between the internal dose of Mn and AD. Our findings may contribute to and influence policies and regulations regarding the recommended Mn levels in the diet, ambient air, and certain occupations such as mining and welding.

# Methods

This is a cross-sectional study of men and women who took part in the National Health and Nutrition Examination Survey (NHANES) from 2011 to 2014. The NHANES sample represents the total noninstitutionalized civilian US population residing in the 50 states and the District of Columbia. The sample design comprised multi-year, stratified, clustered four-stage samples, with data releases in 2-year cycles. The first stage comprised the selection of primary sampling units (PSUs) from a frame of all US counties. The first-stage PSUs were mostly counties, although adjacent counties were sometimes combined to keep the PSUs above a certain minimum size. The second selection stage included a sample of smaller area segments, comprising census blocks or a combination of blocks. The third stage comprised dwelling units, including noninstitutional group quarters such as dormitories. The fourth stage consisted of persons within occupied dwelling units or households. All eligible members within a household were listed and a subsample of individuals was selected based on sex, age, race, Hispanic origin, and income [15]. Participants aged 60 years and older were eligible. Participants who did not understand or read English, Spanish, Korean, Vietnamese, or traditional or simplified Mandarin or Cantonese or participants who needed a proxy informant were not administered the assessments.

Cognitive function is a broad term that refers to mental processes involved in the acquisition of knowledge, manipulation of information, and reasoning. Cognitive functions include the domains of perception, memory, learning, attention, decision-making, and language abilities. Cognitive function can be measured using three assessments:

1. **Animal fluency test:** examines categorical verbal fluency, a component of executive function. Participants are asked to name as many animals as possible in 1 minute. One point is given for each named animal. A score under 15 indicates cognitive impairment (threshold of 15) [16].
2. **DSST:** relies on processing speed, sustained attention, and working memory. The exercise is conducted using a paper form that has a key at the top containing nine numbers paired with symbols. Participants have 2 minutes to copy the corresponding symbols into the 133 boxes adjoining the numbers. The score is the total number of correct matches. A score under 25 indicates cognitive impairment [16].
3. **CERAD word learning subset:** assesses immediate and delayed learning ability for new verbal information. The test comprises three consecutive learning trials and a delayed recall test. For the learning trials, participants are instructed to read aloud 10 unrelated words, one at a time, as they are presented. Immediately following the presentation of the words, participants recall as many words as possible. In each of the three learning trials, the order of the 10 words is changed. The maximum score possible on each trial is 10. A score under 10 indicates cognitive impairment [16].

Confounding variables, including sex, smoking, socioeconomic status (SES), and age, were included in multivariate models. The rationale for the inclusion of these confounders is discussed in Supplementary Material 1.

Approximately 10% of recorded interviews were independently reviewed over the course of the data collection cycles to clarify inconsistent responses, evaluate the quality of the data, and finalize the data set. Moreover, the NHANES is a national representative survey, which improves validity by reducing sample bias. Whole blood Mn specimens were processed, stored, and shipped for analysis to the Division of Laboratory Sciences, National Center of Environmental Health, and Centers for Disease Control and Prevention. Whole blood Mn concentrations were determined using inductively coupled plasma mass spectrometry.

We merged the 2011–2012 and 2013–2014 NHANES survey cycles (19,931 participants). After excluding participants younger than 60 years of age and participants without Mn data (missing), we obtained a total sample size of 2,439 (Figure 1). Power calculation was conducted based on published measurements of one of the metrics of cognitive function, CERAD. Based on a population average CERAD of 79.8 (standard deviation, 9.4) [17], with an anticipated decrease of 10% in the mean CERAD for exposed individuals, the statistical power would be greater than 99% with a sample size of 2,439 and alpha of 0.5. With a more conservative 1% decrease in the mean CERAD score, the power would be 84%, meaning that there would be a 16% probability of a type II error occurring. Consequently, there is sufficient statistical power (β > 0.80) with a sample size of 2,439 participants.

SAS version 9.2 was used for statistical analyses. Because of the wide range of cognitive functions in the elderly population, individual cognitive tests are subject to floor and ceiling effects. To minimize such effects, we created a composite cognitive z-score by using the average of the standardized scores of the six cognitive tests (animal fluency test, DSST, CERAD delayed recall, and three trials of the CERAD word list learning test). Graphical tests of the residues were conducted to test the normality assumption of the composite z-score. Descriptive statistics for our study population, including proportions, medians, and interquartile ranges (IQRs), were calculated. Univariate linear regression of the association between blood Mn and composite z-score was performed (Model 1). Afterward, multivariate linear regression was applied to assess the association between the blood Mn and composite z-score adjusted for sex, age, race/ethnicity, education level, total number of people in the family, cigarette smoking, and income (family monthly poverty level category) (Model 2). Only those covariates that were significant in Model 2 were included in Model 3. We evaluated the association between continuous blood Mn and composite z-scores, in addition to quartiles of blood Mn and composite z-scores. Sensitivity analyses were conducted by comparing the different multivariate linear regression models to assess how inclusion or exclusion affected outcomes.

# **Results**

Data from 2,439 participants meeting our inclusion criteria were analyzed (Figure 1). The percentages of men and women in the study were similar (49.4% males, 50.6% females). Median age was 69 years with an IQR of 6.93–11.17. All study participants had blood Mn levels above the limit of detection (1.06 μg/L). Median blood Mn for the entire study population was 8.71 μg/L with an IQR of 6.94–11.16 (Table 1). Blood Mn was inversely correlated with age: the mean Mn values were 9.55 μg/L from 60 to 66 years, 9.74 μg/L from 67 to 73 years, and 8.94 μg/L from 74 to 80 years. The demographic characteristics of participants with blood Mn measurements are presented in Table 1.

[[Insert Table 1 here.]]

There was a significant difference in blood Mn concentrations between males and females (t2340.886 = −5.593, p = 0.002), with higher mean blood Mn concentrations in females than in males (9.85 and 8.95, respectively). The effect size was small (Cohen’s d = 0.23). Using the Pearson correlation coefficient, blood Mn concentrations were significantly inversely correlated with age (r = −0.066, p = 0.001). In addition, blood Mn concentrations were significantly associated with race/ethnicity: the mean Mn of Non-Hispanic Asians was significantly higher than that of all other races (Mexican American, other Hispanic, Non-Hispanic White, and Non-Hispanic Black). The R-squared value was small (R-squared = 0.066). Mn concentrations did not significantly differ according to education level, total number of people in the family, cigarette smoking, and family monthly poverty level category.

Linear regression was performed, with adjustment for sex, age, race/ethnicity, education level, total number of people in the family, cigarette smoking, and income (family monthly poverty level category). Table 2 presents the results of blood Mn as a continuous variable in relation to cognitive function (composite z-score), with three models. Model 1, a univariate linear regression performed on Mn and the composite z-score, was not statistically significant (μg/L, β = 0.0038, 95% CI −0.004 to 0.012). Model 2 was a multivariate linear regression adjusted for all explanatory variables (sex, age, race/ethnicity, education level, total number of people in the family, cigarette smoking, and income [family monthly poverty level category]). The association between Mn and the composite z-score in Model 2 was also not significant (μg/L, β = −0.006, 95% CI −0.013 to 0.0004). Model 3 was a multivariate linear regression adjusted for only significant explanatory variables (sex, age, race/ethnicity, education level, and income [family monthly poverty level category]). The association between Mn and the composite z-score in Model 3 was again not significant (μg/L, β = −0.006, 95% CI −0.0135 to 0.0006). Education level, total number of people in the family, and income (family monthly poverty level category) were, for convenience, converted from ordinal to rational scales after similar results were obtained.

Table 3 presents the results of blood Mn as a categorical variable in relation to cognitive function (composite z-score). As before, three models were derived: univariate, all variables, and significant variables alone. The first quartile of Mn was the lowest (referent, ≤15.75 μg/L), whereas the fourth quartile was the highest (47.25–63 μg/L). In Model 1, the mean of the composite z-score in the first quartile was significantly lower than that in the second, third, and fourth quartiles. The mean composite z-score of the third quartile was the highest, followed by the fourth and second (0.022, 0.012, and 0.007, respectively). In Models 2 and 3, the association between the categorical Mn and composite z-score was not significant.

# **Discussion**

In this study of US adults aged 60–80 years, the results did not show a consistent or significant dose-response association between blood Mn concentrations and cognitive function assessed by a composite cognitive z-score comprising the average of the standardized scores of six cognitive tests. Various attempts were made to control for potential confounding factors in our analysis. However, our univariate linear regression analysis did show a significant positive association between categorical blood Mn and the composite z-score of cognitive function (Model 1, Table 3).

There are a number of potential explanations for the lack of a consistent significant association. This study assessed background low-dose exposure in the US population. Median blood Mn was 8.71 μg/L with an IQR of 6.94–11.16 (Table 1), significantly lower than the median blood Mn concentration reported in Santos-Burgoa et al. [18] (15 μg/L). Santos-Burgoa et al. reported an association between the blood Mn concentration and an increasing risk of deficient cognitive performance in the Mexican adult population. The fact that the median blood Mn in our sample was nearly half that of the Mexican population studied could be one of the reasons why our results showed no significant association between blood Mn levels and cognitive function. Our findings suggest that there is no significant association between the internal dose of Mn and cognitive function at such low concentrations.

There was a significant difference in Mn between males and females (t2340.886 = −5.593, p = 0.002). Mean Mn was higher in females than in males (9.85 and 8.95, respectively). These findings are similar to those reported in other studies of Mn-exposed adults [19]. The higher blood Mn levels observed in females suggest sex-dependent metabolic differences in Mn homeostasis. A study by Finley and Davis [20] demonstrated that women who consume adequate dietary amounts of Mn appear to absorb more Mn than males. This result might also be due to the lower iron levels in women, which are associated with increased Mn absorption [20]. Our results of blood Mn correlated with age showed that the mean Mn levels were 9.55 μg/L from 60 to 66 years, 9.74 μg/L from 67 to 73 years, and 8.94 μg/L from 74 to 80 years. Data from other studies have also demonstrated a decrease in Mn blood levels with age [21]. Concerns have been raised that this is because the homeostatic mechanisms regulating Mn excretion are not fully developed in children and younger age groups [22]. Mn concentrations were significantly associated with race/ethnicity: the mean Mn in Non-Hispanic Asians was higher than that of all other races (Mexican American, other Hispanic, Non-Hispanic White, and Non-Hispanic Black). These racial/ethnic differences may be explained by a combination of differences in occupational exposure, dietary exposure, and metabolic differences. These results are reinforced by the results obtained by Jain and Choi [23]. It is very likely that Non-Hispanic White and Non-Hispanic Black individuals eliminate Mn from the body at a faster rate than Non-Hispanic Asian people. It should also be kept in mind that Non-Hispanic Asian and other Hispanic individuals are a very diverse group of people comprising quite a few ethnicities. Mn concentrations did not significantly differ according to education level, total number of people in the family, cigarette smoking, and income (family monthly poverty level category). Similar results were found in the study by Da Silva et al. [21]. In the NHANES 2011–2012 [23], non-smokers had numerically but nonsignificantly higher levels of blood Mn. Smokers had lower blood Mn concentrations, possibly because of certain constituents in smoke that induce enzymes, such as CYP1A2, that may accelerate the excretion of Mn [23].

Linear regression performed on blood Mn as a continuous variable in relation to cognitive function (composite z-score) was not significant in any of the three models (univariate, all variables, and only significant variables) (Table 2). However, categorical blood Mn in relation to the composite z-score was significant in the univariate linear regression (Model 1, Table 3). The mean composite z-score was significantly lowest in the first quartile. These findings did not follow a clear dose-response relationship because the mean of the third quartile was the highest, followed by the fourth and then the second (0.022, 0.012, and 0.007, respectively). These findings are similar to those of other studies, where lower IQ scores were significantly associated with blood Mn concentrations greater than 11.2 μg/L or lower than 8.2 μg/L [24]. Hence, this similar finding highlights the association of both low and elevated blood Mn levels with cognitive deficits, illustrating a relationship between Mn exposure and cognitive function even at low-dose background exposures.

The association between sex and cognitive function was statistically significant (μg/L, β = −0.2573, 95% CI −0.3137 to −0.2009). These findings are in agreement with those of earlier studies. A study that examined sex differences in the cognitive function of patients with chronic schizophrenia found that male schizophrenic patients had more severe cognitive deficits than female patients in immediate and delayed memory, but not in language, visuospatial, and attention indices [25]. As with age (μg/L, β = −0.0451, 95% CI −0.0494 to −0.0407), the association was significant. A score indicating possible impairment has a worse prognosis in the very elderly population than in the younger elderly population [26]. In addition, education level and income (family monthly poverty level category) were also significantly associated with cognitive function (μg/L, β = 0.1926, 95% CI 0.1679–0.2172; and μg/L, β = 0.0942, 95% CI 0.06008–0.1283, respectively). The higher the levels of education and income, the higher the cognitive function scores. Studies have indicated that residence in areas of low SES (availability of community resources, access to health care, and attitudes and beliefs about health practices and stress) increases the risk of morbidity and mortality for many health outcomes [27].

In the third CERAD trial, univariate linear regression with categorical blood Mn was significant (p = 0.0171). However, after adjustment for potential confounders (sex, age, SES, smoking), the association was no longer significant (p = 0.2576). We found collinearity between blood Mn and race/ethnicity because, when we eliminated race/ethnicity, the association was closer to significance (p = 0.08). When we eliminated sex in addition to race/ethnicity, the association was highly significant (p = 0.0061). Therefore, there is multicollinearity among Mn, sex, and race/ethnicity, which can explain the lack of a significant association between blood Mn and cognitive function. We did not find this clear collinearity in the other cognitive tests. In the third CERAD trial, participants recall as many words as possible immediately following the presentation of the words, as they also do in the first two trials. Thus, it is unclear why multicollinearity is strong in this specific trial.

Another reason why the majority of the results were not significant, in addition to low levels of blood Mn (median, 8.71 μg/L), is that there is only one blood sample of Mn for each participant in the NHANES. Blood Mn samples might show variability depending on the sampling time during the day, alcohol consumption, and other variables. In addition, different biomarkers, such as air, nail, and hair Mn, may present different results [28]. Reliance on a single biological sample to assess the internal dose introduces a significant exposure misclassification that would likely diminish the results toward the null. Exposure misclassification is likely to be non-differential and therefore would not have biased our study findings.

There are several strengths of our study. First, our analysis is based on a large representative sample of the base population, which minimizes selection bias. Second, we created a composite cognitive z-score by using the average of the standardized scores of six cognitive tests, which provided a more complete representation of cognitive function and minimized the floor and ceiling effects of single cognitive tests. Third, exposure was assessed by blood Mn as an estimate of the internal dose of Mn. However, a single biological sample does not accurately reflect long-term exposure, and multiple samples would have given a more accurate representation of the true internal dose of Mn. One of the study limitations was that the cognitive z-score limited our explanation of the practical meaning of each specific test. To prevent information bias, the assessments were administered by trained interviewers, and two interviewers transcribed the verbatim responses from the audio recordings and scored the CERAD-WL and animal fluency assessments. Most of the DSSTs were also independently scored by two interviewers. In addition, the audio recordings of the assessments were reviewed to evaluate the consistency of the interviewer's instructions and to determine test score accuracy. Nevertheless, this study, due to its cross-sectional design, restricted us from assessing the temporal relationships of the associations. Recall bias may explain the nonsignificant results of the association between cigarette smoking and cognitive function because the association was not significant in the second model in both Tables 1 and 2.

In conclusion, our results do not show a consistent or significant association between blood Mn concentrations and cognitive function as measured by the animal fluency test, DSST, CERAD delayed recall, and three trials of the CERAD word list learning test. With the sufficient power of the analysis, we are fairly certain that the nonsignificant results are not due to a type II error (16% chance of a type II error with 2,439 participants to detect a 1% decrease in cognition scores). While our findings are not consistent with earlier studies showing an association between Mn and cognitive function, our study uses biomarker data in addition to the assessment of cognitive function with multiple pre-clinical tests. Higher levels of blood Mn might grant different results, and further research is thus needed.

**AUTHOR CONTRIBUTIONS**

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Dr. Nitza Barkan was involved in the statistical analysis of the study data, helping with the linear regression, sensitivity analyses, and interpretation of the results.

**CONFLICTS OF INTEREST**

The authors have stated explicitly that there are no conflicts of interest in connection with this article.

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**Figure Legend**

Figure 1. Study population from the combined 2011–2012 and 2013–2014 NHANES, with inclusion criteria

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Table 1. Demographics of the study population

|  |  |  |
| --- | --- | --- |
|  |  | **Manganese (μg/L)** |
| **Characteristics** | **N (%)** | **Median, (IQR)** | **p** |
| **Overall manganese** | 2,439 (100) | 8.71 (6.94–11.16) |  |
| **Sex**MaleFemale | 1,204 (49.4)1,235 (50.6) | 8.32 (6.62–10.58)9.10 (7.25-11.49) | 0.002 |
| **Age, years**60–80\* | 2,439 (100) | 69 (6.93–11.17) | 0.001 |
| **Race/ethnicity**Mexican AmericanOther HispanicNon-Hispanic WhiteNon-Hispanic BlackNon-Hispanic Asian | 204 (8.5)255 (10.6)1112 (46.4)588 (24.5)239 (10.0) | 9.09 (7.56–11.27)9.32 (7.58–11.49)8.57 (6.85–10.76)7.59 (6.18–9.72)11.66 (9.63–14.09) | 0.000 |
| **Education level**Less than 9th grade9th–11th grade (includes 12th grade with no diploma)High-school graduate/GED or equivalentSome college or AA degreeCollege graduate or above | 384 (15.8)337 (13.8)567 (23.3)633 (26.0)515 (21.1) | 8.65 (6.96–10.71)8.53 (6.75–11.30)8.44 (6.82–10.92)8.63 (6.90–10.84)9.19 (7.34–11.70) | 0.088 |
| **Total number of people in the family**1234 | 706 (31.9)1049 (47.4)284 (12.8)174 (7.9) | 8.58 (6.71–10.93)8.67 (6.95–10.96)8.65 (6.97–11.14)8.71 (7.02–11.72) | 0.022 |
| **Cigarette smoking**Lifetime smoking of at least 100 cigarettesYesNo | 1,214 (49.8)1,224 (50.2) | 8.48 (6.82–10.91)8.95 (7.07–11.29) | 0.600 |
| **Income****Family monthly poverty level category**Monthly poverty level index ≤ 1.30Monthly poverty level index 1.30–1.85Monthly poverty level index > 1.85 | 783 (34.6)324 (14.3)1,155 (51.1) | 8.80 (7.03–11.24)8.70 (6.73–10.96)8.59 (6.93–11.02) | 0.449 |

\*Detailed in results.

Table 2. Blood Mn levels (continuous) in relation to cognitive function (composite z-score): NHANES 2011–2014

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Model 1** | **Model 2** | **Model 3** |
| **Variables** | **β (95%CI)** | **p** | **β (95%CI)** | **p** | **β (95%CI)** | **p** |
| **Mn as continuous variable (μg/L)** | 0.0038 (−0.004 to 0.012) | 0.3585 | −0.006 (−0.013 to 0.0004) | 0.0656 | −0.006 (−0.0135 to 0.0006) | 0.0731 |
| **Sex**MaleFemale |  |  | −0.2714 (−0.3304 to −0.2125)referent | <0.0001referent | −0.2573 (−0.3137 to −0.2009)referent | <0.0001referent |
| **Age** |  |  | −0.0451 (−0.0495 to −0.04076) | <0.0001 | −0.0451 (−0.0494 to −0.04073) | <0.0001 |
| **Race/ethnicity**Mexican AmericanOther HispanicNon-Hispanic WhiteNon-Hispanic BlackNon-Hispanic Asian |  |  | −0.0063 (−0.1502 to 0.1375)−0.1378 (−0.2711 to −0.0046)0.1963 (0.0859 to 0.3068)−0.1064 (−0.224 to 0.011)referent | 0.93080.04260.00050.0759referent | 0.0025 (−0.141 to 0.1461)−0.1274 (−0.260 to 0.005)0.2173 (0.109 to 0.325)−0.089 (−0.204 to 0.026)referent | 0.97260.0598<0.00010.1308referent |
| **Cigarette smoking****Lifetime smoking of at least 100 cigarettes**NoYes |  |  | 0.04797 (−0.0109 to 0.1069)referent | 0.1108 |  |  |
| **Education level** |  |  | 0.193 (0.1681–0.2178) | <0.0001 | 0.1926 (0.1679–0.2172) | <0.0001 |
| **Total number of people in the family** |  |  | −0.0075 (−0.0289 to 0.0139) | 0.4916 |  |  |
| **Income**Family monthly poverty level category |  |  | 0.0947 (0.0606–0.1288) | <0.0001 | 0.0942 (0.06008–0.1283) | <0.0001 |

Table 3. Blood Mn levels (categorical) in relation to cognitive function (composite z-score): NHANES 2011–2014

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Model 1** | **Model 2** | **Model 3** |
| **Variables** | **β (95%CI)** | **p** | **β (95%CI)** | **p** | **β (95%CI)** | **p** |
| **Mn as categorical variable (μg/L)**First (≤15.75)Second (15.75–31.5)Third (31.50–47.25)Fourth (47.25–63) | referent0.1274 (0.03409– 0.2208)0.1417 (0.0487–0.234)0.1324 (0.039–0.225) | referent0.00750.00280.0055 | referent0.036 (−0.0439 to 0.1159)0.048 (−0.032 to 0.1283)0.027 (−0.055 to 0.111) | referent0.37710.24140.5109 | referent0.0342 (−0.0456 to 0.1141)0.0471 (−0.033 to 0.127)0.0289 (−0.054 to 0.1122) | referent0.40020.25030.4950 |
| **Sex**MaleFemale |  |  | −0.2621 (−0.321 to −0.2031)referent | <0.0001referent | −0.248 (−0.304 to −0.191)referent | <0.0001referent |
| **Age** |  |  | −0.044 (−0.0492 to −0.0404) | <0.0001 | −0.044 (−0.049 to −0.0404) | <0.0001 |
| **Race/ethnicity**Mexican AmericanOther HispanicNon-Hispanic WhiteNon-Hispanic BlackNon-Hispanic Asian |  |  | 0.0095 (−0.1351 to 0.1542)−0.123 (−0.2575 to 0.0108)0.2179 (0.106–0.329)−0.073 (−0.193 to 0.045)referent | 0.89680.07170.00010.2266referent | 0.018 (−0.125 to 0.162)−0.112 (−0.246 to 0.0208)0.238 (0.129–0.348)−0.056 (−0.174 to 0.061)referent | 0.80180.0980<0.00010.3462referent |
| **Cigarette smoking**Lifetime smoking of at least 100 cigarettesNoYes |  |  | 0.047 (−0.011 to 0.106)referent | 0.1151referent |  |  |
| **Education level** |  |  | 0.191 (0.166–0.216) | <0.0001 | 0.191 (0.166–0.216) | <0.0001 |
| **Total number of people in the family** |  |  | −0.007 (−0.0291 to 0.013) | 0.4825 |  |  |
| **Income**Family monthly poverty level category |  |  | 0.0955 (0.0613–0.1297) | <0.0001 | 0.095 (0.0609–0.129) | <0.0001 |

**Supplementary Material 1. Consideration of confounders**

### **Sex**

The literature indicates an association between sex and cognitive function. For example, a study examining sex differences in the relationship of hypertension with cognitive function in older adults found significant main effects of sex for Visual Memory Span-Tapping Forward (F = 6.17, p < 0.02) and Visual Memory Span-Tapping Backward (F = 3.95, p < 0.05). In all instances, women performed more poorly than men. A separate study that examined sex differences in the cognitive function of patients with chronic schizophrenia found that male schizophrenic patients had more severe cognitive deficits than female patients in immediate and delayed memory, but not in language, visuospatial, and attention indices [2]. Sex is also associated with Mn exposure, with Riojas-Rodriguez et al. [3] finding a significant inverse association between postnatal exposure to manganese and IQ in 7- to 9-year-old females, but not in males.

### **Smoking**

Based on the results of several case-control studies in the elderly population, smoking has a protective effect on the risk of Alzheimer’s disease (AD) [4, 5]. Other studies were inconclusive regarding the effect of smoking on cognitive function [6]. More recently, prospective studies have shown that smoking increases the risk of cognitive decline and dementia in elderly people. According to a meta-analysis that assessed the association of smoking with dementia and cognitive decline in 19 prospective studies with at least 12 months follow-up, current smokers at baseline, relative to never smokers, had risks of 1.79 (95% CI 1.43–2.23) for incident AD, 1.78 (95% CI 1.28–2.47) for incident vascular dementia, and 1.27 (95% CI 1.02–1.60) for any dementia. The authors concluded that elderly smokers have increased risks of dementia and cognitive decline [7]. A study that examined cigarette smoking in relation to cognitive performance in middle-aged people found that current smokers scored significantly worse on the Verbal Learning Test and on the Stroop Color Word Test. Multiple linear regression analyses showed that current smokers had reduced psychomotor speed (β = −0.159, p = 0.0003) and reduced cognitive flexibility (β = −0.133, p = 0.008) compared with never smokers [8]. Smoking is also associated with Mn exposure. A study that examined Mn levels during pregnancy and at birth and the association with smoking found a negative relationship between cigarette smoking and blood Mn levels in the second trimester [9].

### **Socioeconomic Status**

Research has shown that low individual SES, as defined by educational attainment and social class based on occupation, is a significant risk factor for a number of negative health outcomes, including cognitive and functional impairment [10]. Recently, greater attention has been paid to community-level SES because researchers have hypothesized that area characteristics, such as availability of community resources, access to health care, and attitudes and beliefs about health practices and stress, could account for the observed variation in the risk of morbidity and mortality. Studies have indicated that residence in areas of low SES increases the risk of morbidity and mortality for many health outcomes [11]. One study that aimed to determine if people living in communities with higher socioeconomic deprivation are at increased risk of cognitive and functional impairment, even after controlling for the effects of individual SES, found an adjusted OR of cognitive impairment of 2.3 (95% CI 1.8–3.0) for those living in the areas of greatest deprivation compared to those living in the most affluent areas, after controlling for age, sex, social class, and education (p < 0.001) [12]. The association between blood Mn and SES is described in a study that examined exposure to Mn and health effects on the general population and found that blood Mn concentrations were significantly related to the point source in the community. Air and water quality in areas with low SES were worse than in areas with high SES and with a greater risk of exposure to Mn [13].

### **Age**

Based on the literature, there is an association between age and cognitive function. According to a study that examined the change in cognitive function in a population sample of elderly people, a score indicating possible impairment carried a worse prognosis in very elderly individuals than in younger elderly individuals [14]. Another study examined longitudinal changes in cognitive functioning for a sample of aging twins, some of whom developed dementia while others did not. Individuals who were diagnosed with dementia at a mean age of 85 years had achieved lower scores on most tests 20 years before diagnosis and experienced greater declines in vocabulary and forward digit span over time than those surviving to a comparable age without dementia [15]. Age is also associated with Mn, as reported in a study by Dorman et al. [16]; sex and age did not affect Mn delivery to the striatum, a known target site for neurotoxicity in humans, but did influence Mn concentrations in other tissues.