HHS Public Access

Author manuscript

Eur J Nutr. Author manuscript; available in PMC 2022 April 01.

Published in final edited form as:

Eur J Nutr. 2021 April ; 60(3): 1511–1520. doi:10.1007/s00394-020-02353-7.

Serum magnesium concentration and incident cognitive impairment: the Reasons for Geographic and Racial Differences in Stroke Study

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Abstract

Purpose: To examine the prospective association between serum Mg level and the incidence of cognitive impairment.

Methods: A random sub-cohort (n=2,063) from the Reasons for Geographic and Racial Differences in Stroke (REGARDS) cohort was included in this study. Baseline serum Mg concentration was measured using inductively coupled plasma mass spectrometry. According to

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Author contributions

KH contributed to study concept and design, and acquisition of data. CC analyzed and interpreted data, and drafted manuscript. All co-authors contributed to critical revision of manuscript for intellectual content.

Conflicts of interest

None.

the current reference interval of serum magnesium (0.75-0.95 mmol/L), we classified participants below the interval as Level 1 and used it as the referent. The rest of the study population were equally divided into three groups, named Level 2 to 4. Incident cognitive impairment was identified using the Six-Item Screener. Multivariable-adjusted odds ratios (ORs) and corresponding 95% confidence intervals (CIs) were estimated using logistic regression models.

Results: After adjustment for potential confounders, an inverse threshold association between serum Mg level and incident cognitive impairment was observed. Compared to those with hypomagnesemia (Level 1: <0.75 mmol/L), the relative odds of incident cognitive impairment was reduced by 41% in the second level [OR (95% CI) = 0.59 (0.37, 0.94)]; higher serum Mg level did not provide further benefits [Level 3 and 4 versus Level 1: OR (95% CI) = 0.54 (0.34, 0.88) and 0.59 (0.36, 0.96), *P* for linear trend = 0.08].

Conclusions: Findings from this prospective study sugggest that sufficient Mg status within the normal range may be benificial to cognitive health in the US general population.

Introduction

Dementia and cognitive aging are critical public health concerns that lower the quality of life of sufferers and their families, and impose a heavy economic burden on society [1]. Owing to the lack of effective treatment for dementia, efforts are shifting to the primary prevention of early cognitive impairment and identification of modifiable risk factors for dementia such as diet [2].

Magnesium (Mg) is a nutritious mineral, and its ionic form is found in a relatively large concentration in the central nervous system [3]. Because synaptic strength and plasticity in neuronal networks, which are functional substrates of memory encoding [4, 5], depend closely on ion flux across neuronal membrane [6], differences in Mg homeostasis could contribute to the pathophysiology of cognitive aging. In animal models, administration of Mg improved learning and memory in aged animals [7, 8], and Mg deficiency impaired memory function [9]. However, human studies relating Mg to cognitive aging are sparse. Some cross-sectional studies found that Mg levels measured in serum, cerebrospinal fluid, or hair were lower among Alzheimer's disease patients compared with apparently healthy individuals [10-12]. Low serum Mg levels were also associated with poorer global cognitive function [11, 13, 14]. But, there was only one cohort study conducted in the Netherlands that found both low and high serum Mg levels were associated with an increased risk of all-cause dementia [15].

Therefore, we examined the association between baseline serum Mg level and the incidence of cognitive impairment over ten years of follow-up in a US general population using data from the Reasons for Geographic and Racial Differences in Stroke (REGARDS) cohort.

Methods

Study design and population

REGARDS is an ongoing population-based prospective cohort study designed to investigate the incidence of stroke and cognitive impairment in the US. The detailed study design has

been reported elsewhere [16]. Briefly, REGARDS recruited a cohort of 30,239 black and white Americans aged ≥45 years from 2003-7 and has been following them since. After verbally consenting to participate in the study, participants were interviewed by telephone to self-report demographic (age, sex, race, and region), socioeconomic (education and family income), and lifestyle factors (smoking status, alcohol consumption, and depressive symptom). An in-person physical assessment followed 3-4 weeks later, in which blood and urine samples, as well as physical measurements (blood pressure, height, weight, and electrocardiogram), were collected using standardized protocols. Written informed consents were obtained and self-administered questionnaires were left with participants to gather dietary information and medical history. This study was approved by institutional review boards of all REGARDS participating institutions and Columbia University Irving Medical Center (IRB AAAS5777).

To analyze baseline levels of circulating minerals, a sub-cohort (n=2,666) of REGARDS participants was randomly selected with a fixed sampling probability of 9% in each stratum jointly classified by age (<55, 55-64, 65-74, 75-84, and ≥85 years), gender (female and male), race (black and white), and region of residence (Stroke Buckle, the rest of Stroke Belt, and non-Stroke-Belt region) [17, 18]. The comparisons of baseline characteristics between the REGARDS entire cohort and the random sub-cohort are shown in Supplemental Table 1. Significant difference was not found.

Laboratory analyses

Urine and fasting blood samples were collected at the baseline in-person physical assessment. Samples were placed in transfer vials, stored in a refrigerator until pick-up by a courier on the same day, shipped overnight with ice packs to a central laboratory, and stored at −80 °C for reprocessing and analysis [19]. Serum Mg and calcium concentrations were measured by inductively coupled plasma mass spectrometry (Perkin Elmer, MA, USA) [20]. Mg samples were analyzed in batches of 30-120 with instrument blanks, NIST 956d quality control samples (CVs of Level 1 to 3=1.3%, 1.4%, and 1.6%), and in-house pooled QC serum (CV=2.0%). Lipid profiles and glucose were measured using colorimetric reflectance spectrophotometry with the Ortho Vitros Clinical Chemistry System 950IRC instrument (Johnson & Johnson Clinical Diagnostics, Rochester, NY) [21].

Assessment of cognitive function

Trained REGARDS interviewers administered a two-level cognitive function assessment longitudinally through telephone contact till April 1, 2015. To assess global cognitive function, the Six-Item Screener (SIS) was administered annually beginning from baseline (December 2003) [22]. The SIS assesses recall of a 3-item word list and temporal orientation of year, month, and day of the week, with scores ranging from 0 to 6. To assess learning, memory and executive function, a three-test battery, including the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) battery, Animal Fluency Test (AFT), and Letter Fluency Test (LFT), was administered every two years during follow-up, beginning in 2006 for CERAD battery and AFT, and 2008 for LFT. CERAD battery included Word List Learning (WLL) and Word List Delayed Recall (WLD) tests that measured new learning and verbal memory of a 10-item list, with scores ranging from 0-30 for WLL and 0-10 for WLD

[23]. AFT and LFT measured semantic fluency and phonemic fluency scored as the number of animals and the number of words beginning with the letter “F” that a participant can name in 60 seconds [24, 25]. In all tests, lower scores indicate poorer cognitive functions or greater impairment. These measures gathered via telephone-based assessment are included in the vascular cognitive impairment harmonization standards and have been widely used in observational and interventional studies of cognitive aging and dementia [26]. The validity of these measures has been verified in quality control in REGARDS [27].

In this study, the primary outcome was incident global cognitive impairment defined as having a SIS score ≤4 at the most recent assessment as of April 1, 2015 [28]. In secondary analyses, we examined the longitudinal associations with domain-specific cognitive functions measured by the three test battery (WLL, WLD, AFT, and LFT).

Other covariates

Important covariates measured at baseline included age, gender, race (black or white), region (Stroke Buckle, the rest of Stroke Belt, or non-Stroke-Belt region), education level (<high school, high school graduate, some college, or college graduate), family income (<$20, $20-34, $35-74, or ≥$75 thousand per year), smoking status (never, past, or current smoker; pack-years of cigarettes), alcohol consumption (never, past, or current drinker; drinks per week), physical activity (none, 1–3, or ≥4 times/week), body mass index (BMI, <25.0, 25.0-29.9, or ≥30.0 kg/m^2), hypertension (yes or no), diabetes (yes or no), dyslipidemia (yes or no), history of heart disease (yes or no), depressive symptom, blood pressure, total cholesterol, high-density lipoprotein (HDL)-cholesterol, low-density lipoprotein (LDL)-cholesterol, triglycerides, glucose, and serum calcium concentration. Demographics, socioeconomics, and lifestyle factors were self-reported. Weight and height were measured by trained professionals based on the standardized protocols and were used to calculate BMI (kg/m^2). Hypertension was defined as any self-reported use of blood pressure control medication or systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg. Diabetes was defined as any self-reported use of glucose control medication or a fasting blood glucose concentration >126 mg/dL or non-fasting glucose >200 mg/dL. Dyslipidemia was defined as any self-reported use of lipid control medication or triglycerides ≥240 mg/dL or LDL-cholesterol ≥160 mg/dL or HDL-cholesterol ≤40 mg/dL. History of heart disease was defined by self-reported myocardial infarction, coronary artery bypass graft, angioplasty, stenting or evidence of myocardial infarction from an electrocardiogram performed during the in-home examination. Depressive symptom, which was indicated by the score of the Center for Epidemiological Studies – Depression four-item version (CESD-4), was evaluated over the telephone [29]. Total intake of Mg (diet plus supplementation) was estimated using Block98 FFQ [16].

Statistical analyses

According to the current reference interval of serum magnesium (0.75-0.95 mmol/L) [30, 31], we considered participants who had a serum magnesium level below the interval as the referent (Level 1) in all analyses. The rest of the participants were equally divided into three groups, named Level 2 to 4, because only 5% of the study population had serum magnesium concentrations above the reference interval (> 0.95 mmol/L).

Baseline characteristics of the study population were summarized using mean values with standard deviations for continuous variables and proportions for categorical variables. Analysis of variance, Kruskal-Wallis tests, or chi-squared tests were used to compare participants' characteristics across serum Mg levels, as appropriate. Logistic regression models were used to examine the association between serum Mg level and incident cognitive impairment in three sequential models. Model 1 was adjusted for age, gender, race, interactions of age-gender, age-race and gender-race, and region. Model 2 was additionally adjusted for education level, family income, smoking status, alcohol consumption, physical activity, and BMI. Model 3 was further adjusted for medical histories (hypertension, diabetes, dyslipidemia, and heart disease), clinical measurements (CESD-4 score, systolic blood pressure, total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, and glucose), and serum calcium that have been shown to interact with Mg [32]. In addition, interactions with some pre-specified factors, including age, gender, race, and serum calcium that has been shown to interact with Mg [32], were examined in model 3.

Sensitivity analyses were performed to test the robustness of the findings. First, to reduce the possibility of reverse causality, all cases of incident cognitive impairment within the first 4 years after serum Mg measurement were excluded [15]. Second, participants with only 1 follow-up SIS measure were excluded. Third, because hypomagnesemia may be clinically diagnosed with serum Mg concentration <0.70 mmol/L, we used this cut-off point in a sensitivity analysis. Participants who had a serum Mg level <0.70 mmol/L were the referent and the rest were equally divided into three groups (Level 2 to 4).

The associations between baseline serum Mg level and the repeated assessments of WLL, WLD, AFT, and LFT were examined using linear mixed models with the adjustment for covariates in model 3. Analyses of AFT and LFT also included covariates to adjust for whether participants who were identified in review of tape recordings received assistance from someone in their home environment or were given a disallowed prompt by the interviewer. Similar to prior REGARDS reports, no random effects accounting for time between tests were included [33]. Covariance structure was chosen based on the lowest Bayesian information criterion (BIC).

P values ≤0.05 were considered statistically significant. All analyses were conducted in SAS version 9.4 (SAS Institute, Cary, NC, USA).

Results

Of the 2,666 participants in the random sub-cohort, 223 did not have data on serum Mg, 193 reported cognitive impairment at baseline, 116 reported stroke at baseline, and 72 underwent only one Six-Item Screener cognitive assessment, which leaves a total of 2,062 participants in the present study (Figure 1).

The baseline characteristics of this study population are shown in Table 1. The mean participant age at baseline was 64 years (standard deviation 9 years) with 55% women and 38% black. The distribution of serum Mg concentration was slightly left skewed with a median being 0.82 mmol/L (inter-quartile range=0.77-0.88 mmol/L). Participants with

higher serum Mg level were more likely to be male and white, have higher levels of education, family income, alcohol consumption, and physical activity, and have normal weight. They were also less likely to have hypertension and type 2 diabetes. In addition, they were more likely to have lower blood pressure, higher total cholesterol and LDL cholesterol, lower glucose, and higher serum calcium levels.

Through April 1, 2015, 255 participants developed cognitive impairment. A significant inverse threshold association between serum Mg level and incident cognitive impairment was observed (Table 2). Compared to those with hypomagnesemia (Level 1), the multivariable-adjusted odds ratios (ORs) [95% confidence intervals (CIs)] in Level 2 to 4 were 0.73 (0.49, 1.09), 0.64 (0.42, 0.96), and 0.71 (0.48, 1.04) (*P* for linear trend = 0.13) after adjustment for demographics (model 1). Further adjustment for socioeconomics and lifestyle factors (model 2) did not substantially changed the results [Level 2 to 4 versus Level 1: OR (95% CI) = 0.63 (0.41, 0.98), 0.61 (0.39, 0.95), and 0.69 (0.45, 1.05), *P* for linear trend = 0.16]. In the final model that additionally adjusted for medical history and clinical measurements (model 3), the inverse threshold association was more pronounced [Level 2 to 4 versus Level 1: OR (95% CI) = 0.59 (0.37, 0.94), 0.54 (0.34, 0.88), and 0.59 (0.36, 0.96), *P* for linear trend = 0.08]. The inverse association was not significantly modified by age, gender, race, or serum calcium level (all *P* for interaction >0.05, Table 3).

In sensitivity analyses, the observed association was not substantially changed, but was attenuated when excluding 43 cases within the first 4-year follow-up [Level 2 to 4 versus Level 1: OR (95% CI) = 0.56 (0.34, 0.94), 0.58 (0.34, 0.97), 0.62 (0.37, 1.04), *P* for linear trend = 0.28] or excluding 129 participants with only 1 follow-up SIS measurement [Level 2 to 4 versus Level 1: OR (95% CI) = 0.62 (0.37, 1.02), 60 (0.35, 0.96), 0.67 (0.40, 1.12), *P* for linear trend = 0.30], presumably due to reduced statistical power. Total intake of Mg was not associated with incident cognitive impairment (data not shown). In addition, there were only 165 participants (24 incident cognitive impairment cases) having serum Mg concentration <0.70 mmol/L. Using <0.70 mmol/L as the referent, the ORs of incident cognitive impairment in level 2-4 are 0.87 (0.47, 1.59), 0.65 (0.34, 1.21), and 0.65 (0.34, 1.24). The inverse association was attenuated presumably due to reduced statistical power.

We did not find a significant association between serum Mg level and the three test battery measures (Table 4). Serum Mg level was not associated with verbal learning and memory measured by WLL and WLD, semantic fluency measured by AFT, or phonemic fluency measured by LFT test after adjustment for potential confounders in model 3.

Discussion

In this biracial US cohort, we observed a lower incidence of global cognitive impairment, as measured by the Six Item Screener, in those with adequate baseline serum Mg status. However, we observed no association between serum Mg and other measures of domain-specific cognitive function.

Evidence from longitudinal cohort studies that investigated the long-term neurotrophic effects of Mg is sparse [15]. Our findings are generally consistent with the other cohort

study conducted in the Netherlands, which found both low and high serum Mg levels were associated with an increased risk of all-cause dementia [15]. In our study, we found that, compared to those with hypomagnesemia, individuals with higher Mg level had significantly lower incidence of cognitive impairment measured by a global test. However, because of the limited number of participants with hypermagnesemia, we were not able to examine whether Mg concentration above the reference interval was associated with cognitive impairment incidence. Therefore, a U-shaped dose-response relationship may be observable in populations with more extreme serum Mg levels than this study population. In addition, lack of a U-shaped association in the present study may be due to the different study outcome. All-cause dementia that includes Alzheimer's disease and other neurodegenerative diseases was examined in the Dutch study, while cognitive impairment defined based on an objective test, not a clinical diagnosis of Alzheimer's disease, was examined in the present study. More longitudinal studies are warranted to confirm the nature of the relationship of Mg level with cognitive aging. But, given the limited data in human studies, the present study certainly provides important evidence that sufficient Mg status within the normal range may be beneficial to cognitive aging.

Our findings are supported by evidence from laboratory studies. Mg is essential for synaptic conduction and is required for normal functions of the nervous system by serving as a structure stabilizer for nucleic acid and proteins and a cofactor for a number of enzymes [34]. Although not fully understood, the potential neurotrophic effects of Mg on cognitive impairment are likely to be explained by its influences on the strength and pattern of synaptic transmission [35]. Mg-ion modulates synaptic strength by regulating the probability of transmitter release (*e.g.*, glutamate release at presynaptic terminals)[36] and by controlling the initial postsynaptic depolarization through the regulation of hyper-polarization (AHP) amplitude and duration [37]. In addition, long-lasting elevation of Mg-ion levels within physiological range increases the capacity of synapses to be highly plastic by enhancing the N-methyl-D-aspartate subtype of gluta- mate receptor (NMDA-R) responses to excitatory amino acids and calcium influx [38] and by facilitating the expression of long-term potentiation (LTP) of synaptic plasticity [7, 39, 40]. Moreover, long-lasting elevation of Mg-ion causes functional improvements at synapses of the aging brain. In aged Mg-ion-treated rats, the number of functional synaptic connections was significantly increased in hippocampus compared to the aged controls [7]. Because the strength and pattern of synaptic transmission are widely believed to code memory traces [4, 41], their susceptibility to changes in Mg-ion homeostasis suggests that cognitive abilities would also be modulated by altering Mg-ion levels. In an animal study, rats treated with Mg-ion showed significant improvements of learning abilities, working memory as well as short- and long-term memory [7], suggesting that even a small change in brain Mg-ion homeostasis is capable of altering cognitive performances. Furthermore, other hypothesized pathways are oxidative stress and chronic inflammation [42, 43]. Mg deficiency has been found to increase the production of free oxygen radicals and stimulate the excessive production and release of pro-inflammatory molecules, which potentially increases the risk of cognitive impairment [44, 45].

One limitation of the study is that serum Mg level was measured only once at baseline. While a single biomarker measurement can successfully predict cognitive impairment in

prospective epidemiological studies [15], we acknowledge that repeated measurements better reflect the long-term exposure and reduce intra-individual variation. Indeed, the single–exposure limitation is likely to attenuate the observed association between serum Mg level and incident cognitive impairment as well as other domain-specific cognitive tests, so the large effect size we observed might underestimate the true association. In addition, similar to other observational studies, the possibility of residual confounding and confounding from unknown or unmeasured factors cannot be completely ruled out. However, our analyses were adjusted for a variety of potential confounders suggested in literature, thus our results should not be substantially biased.

The use of serum Mg biomarker is a major advantage of this study. Mg intake can be estimated from diet. However, dietary measurement instruments such as food frequency questionnaires may not be able to capture environmental exposure such as Mg content variations in water. Thus, biomarkers at the individual level are preferred measures of mineral status. Serum Mg concentration is relatively stable over time and correlates well with intracellular free Mg-ion, a physiologically active form of the element Mg, [46] and thus it is the most frequently used measure of Mg status [47].

Conclusion

This prospective cohort study provides human data suggesting that sufficient Mg status may prevent or slow down age-related cognitive impairment. Further studies are needed to establish whether this association is causal, and to identify the possible optimal range of Mg level for cognitive health.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

The authors thank the other investigators, the staff, and the participants of the REGARDS study for their valuable contributions. A full list of participating REGARDS investigators and institutions can be found at http://www.regardsstudy.org.

Funding

This study is supported by a research grant from the NIH (R01AG056111). The REGARDS research project is supported by a National Institute of Neurological Disorders and Stroke, National Institutes of Health, and Department of Health and Human Services cooperative agreement U01-NS-041588. Additional support was provided by Centers for Disease Control and Prevention cooperative agreement U01-DP-006302. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute of Neurological Disorders and Stroke, the National Institutes of Health, the Centers for Disease Control and Prevention, or the Department of Health and Human Services.

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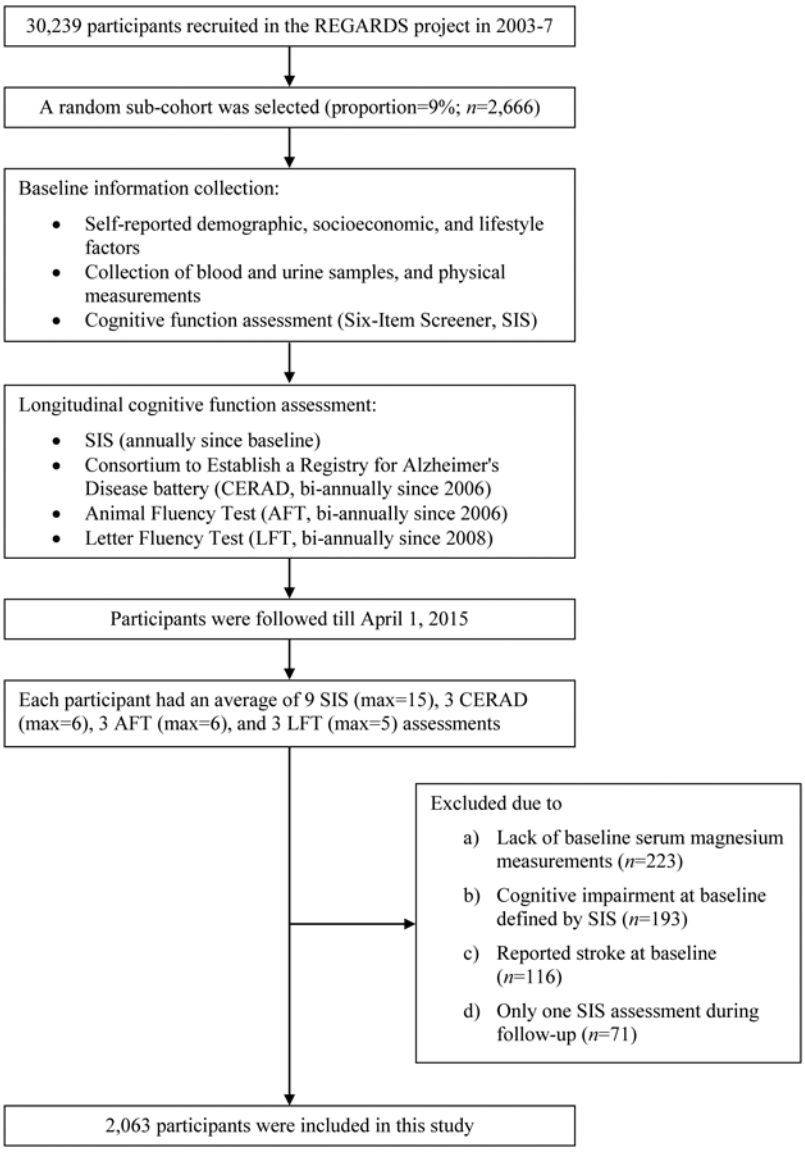

Figure 1.
Flow chart of study sampling.

Table 1.

Baseline characteristics of the study population by serum magnesium levels (n=2,063).[a]

Characteristics	Levels of serum magnesium (mmol/L)				Total	*P* value
	Level 1 (<0.75)	Level 2 (0.75 - <0.81)	Level 3 (0.81 - <0.87)	Level 4 (≥0.87)		
n	389	509	551	614	2.062	--
Demographics						
Age (year)	64.0±9.4	64.0±9.4	63.6±8.9	64.5±8.5	64.1±9.0	0.28
Female (%)	65.3	56.2	54.0	48.7	55.1	<0.01
Black (%)	48.3	41.7	35.1	30.1	37.7	<0.01
US region (%)						0.11
Stroke Buckle	24.4	20.6	20.7	20.4	21.3	
The rest of Stroke Belt	35.7	37.1	34.1	31.1	34.2	
Non-Stroke-Belt region	39.9	42.2	45.2	48.5	44.5	
Socioeconomic status						
Education level (%)						<0.01
Less than high school	13.6	11.0	8.7	7.0	9.7	
High school graduate	26.2	24.6	22.1	22.5	23.6	
Some college	29.6	24.8	28.5	28.1	27.7	
College graduate and above	30.6	39.7	40.7	42.4	39.1	
Family income (%)						<0.01
Less than $20k	21.1	14.7	11.6	11.7	14.2	
$20k-$34k	25.5	22.6	24.3	21.3	23.2	
$35k-$74k	23.1	32.6	35.2	35.5	32.4	
$75k and above	17.0	15.5	18.0	20.2	17.9	
Missing	13.4	14.5	10.9	11.2	12.4	
Lifestyle factors						
Smoking status						
Never (%)	48.8	43.6	48.1	45.0	46.2	0.57
Past (%)	37.5	41.5	37.0	41.4	39.5	
Current (%)	13.6	14.9	14.9	13.7	14.3	
Pack-years of cigarettes	9.8±17.2	12.3±19.7	13.2±23.5	12.7±20.6	12.2±20.6	0.13
Alcohol consumption						
Never (%)	34.5	28.7	30.1	27.0	29.7	0.03
Past (%)	19.5	18.5	15.3	15.8	17.0	
Current (%)	46.0	52.9	54.6	57.2	53.4	
Drinks per week	2.3±6.1	2.1±7.4	2.3±8.3	2.6±6.2	2.3±7.1	0.011
Physical activity (%)						<0.01
None	40.7	30.6	33.3	27.6	32.4	
1-3 times per week	31.9	38.0	36.2	37.5	36.2	
≥4 times per week	27.5	31.4	30.6	34.9	31.5	
BMI group (%)						<0.01

Characteristics	Levels of serum magnesium (mmol/L)				Total	*P* value
	Level 1 (<0.75)	Level 2 (0.75 - <0.81)	Level 3 (0.81 - <0.87)	Level 4 (≥0.87)		
<25.0 kg/m^2	16.7	20.4	25.6	27.9	23.3	
25.0-29.9 kg/m^2	34.2	37.9	37.8	40.7	38.1	
≥30.0 kg/m^2	49.1	41.7	36.5	31.4	38.6	
Medical history						
Hypertension (%)	70.0	58.7	54.6	48.7	56.7	<0.01
Diabetes (%)	38.3	21.8	15.2	9.9	19.6	<0.01
Dyslipidemia (%)	54.2	54.4	58.8	57.9	56.6	0.34
History of heart disease (%)	13.8	14.3	15.0	13.8	14.3	0.93
Clinical measurements						
CESD-4 score (points)	1.3±2.3	1.1±1.9	0.9±1.6	1.1±2.0	1.1±2.0	0.14
Systolic blood pressure (mmHg)	129.2±16.3	127.5±15.9	126.4±15.6	124.9±16.3	126.7±16.1	<0.01
Diastolic blood pressure (mmHg)	77.0±9.8	76.7±8.7	76.5±9.2	75.5±10.1	76.3±9.5	<0.01
Total cholesterol (mg/dL)	188.1±39.9	189.7±39.2	192.6±39.3	195.8±37.6	192.0±39.0	<0.01
HDL-cholesterol (mg/dL)	52.0±16.7	52.2±16.6	51.7±15.9	51.5± 15.1	51.8±16.0	0.43
LDL-cholesterol (mg/dL)	108.1±34.0	111.5±32.9	115.1±34.4	118.8±33.2	114.0±33.8	<0.01
Triglycerides (mg/dL)	144.7±127.4	128.6±75.5	128.7±71.4	129.5±77.2	131.9±87.4	0.16
Glucose (mg/dL)	115.9±47.5	106.2±40.8	99.3±22.2	95.3±18.4	102.9±33.5	<0.01
Serum calcium (mmol/L)	2.41±0.20	2.47±0.15	2.50±0.15	2.56±0.13	2.49±0.17	<0.01

Abbreviations: BMI, body mass index; CESD-4, the Center for Epidemiological Studies-Depression: 4-item version; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

[a]Results are presented by means ± standard deviations or proportions. *P* values are tested for any difference across serum magnesium levels using analysis of variance, Kruskal-Wallis test, or chi-squared test as appropriate.

Table 2.

Associations (odds ratio and 95% confidence interval) between baseline serum magnesium and incident cognitive impairment as measured by the Six Item Screener (*n*=2,063).[a-d]

	Levels of serum magnesium (mmol/L)				*P* for linear trend
	Level 1 (<0.75)	**Level 2 (0.75 - <0.81)**	**Level 3 (0.81 - <0.87)**	**Level 4 (≥0.87)**	
Median (inter-quartile range)	0.71 (0.68-0.73)	0.78 (0.77-0.80)	0.84 (0.83-0.85)	0.91 (0.88-0.93)	--
Number of cases/participants	62/389	63/509	57/551	73/614	--
Model 1	1 (Referent)	0.73 (0.49, 1.09)	0.64 (0.42, 0.96)	0.71 (0.48, 1.04)	0.13
Model 2	1 (Referent)	0.63 (0.41, 0.98)	0.61 (0.39, 0.95)	0.69 (0.45, 1.05)	0.16
Model 3	1 (Referent)	0.59 (0.37, 0.94)	0.54 (0.34, 0.88)	0.59 (0.36, 0.96)	0.08

Abbreviations: BMI, body mass index; CESD-4, the Center for Epidemiological Studies-Depression: 4-item version.

[a]All models were constructed by using logistic regression models. According to the current reference interval of serum magnesium (0.75-0.95 mmol/L), we classified participants below the interval as Level 1 and used it as the referent. The rest of the study population were equally divided into three groups, named Level 2 to 4.

[b]Model 1 was adjusted for age, gender, race (black or white), interactions of age-gender, age-race and gender-race, and region.

[c]Model 2 was additionally adjusted for education level, family income, smoking status, alcohol consumption, physical activity, and BMI.

[d]Model 3 was additionally adjusted for medical histories (hypertension, diabetes, dyslipidemia, and heart disease,) and clinical measurements (CESD-4 score, systolic blood pressure, total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, glucose, and serum calcium concentration).

Table 3.

Associations (odds ratio and 95% confidence interval) between baseline serum magnesium and incident cognitive impairment stratified by pre-specified factors (n=2,063).[a]

	serum magnesium [mean (SD)]	number of cases/participants	Levels of serum magnesium (mmol/L)				*P* for linear trend
			Level 1 (<0.75)	Level 2 (0.75 - <0.81)	Level 3 (0.81 - <0.87)	Level 4 (≥0.87)	
Age							
<65 years	0.82 (0.08)	65/1,122	1 (Referent)	0.49 (0.22, 1.10)	0.26 (0.10, 0.65)	0.29 (0.12, 0.74)	0.03
≥65 years	0.82 (0.08)	190/941	1 (Referent)	0.72 (0.41, 1.27)	0.76 (0.43, 1.35)	0.74 (0.41, 1.32)	0.39
P for interaction	--	--			0.10		
Gender							
Female	0.81 (0.08)	123/1,137	1 (Referent)	0.50 (0.26, 0.93)	0.45 (0.23, 0.87)	0.51 (0.26, 0.99)	0.29
Male	0.83 (0.08)	132/926	1 (Referent)	0.76 (0.36, 1.60)	0.75 (0.35, 1.59)	0.82 (0.38, 1.76)	0.33
P for interaction	--	--			0.77		
Race							
Black	0.81 (0.08)	104/778	1 (Referent)	0.40 (0.20, 0.81)	0.27 (0.12, 0.61)	0.32 (0.15, 0.69)	<0.01
White	0.83 (0.08)	151/1,285	1 (Referent)	0.83 (0.43, 1.60)	0.80 (0.41, 1.54)	0.83 (0.42, 1.65)	0.84
P for interaction	--	--			0.28		
Serum calcium level							
<2.49 mmol/L	0.80 (0.08)	119/1,028	1 (Referent)	0.71 (0.38, 1.31)	0.34 (0.16, 0.70)	0.47 (0.23, 0.97)	0.02
≥2.49 mmol/L	0.84 (0.08)	136/1,035	1 (Referent)	0.54 (0.25, 1.17)	0.80 (0.40, 1.62)	0.76 (0.38, 1.54)	0.95
P for interaction	--	--			0.37		

[a]All models were constructed by using logistic regression models with adjustment for the covariates listed in model 3, Table 2.

Table 4.

Associations [mean difference (95% confidence interval)] between baseline serum magnesium levels and the three test battery.[a]

	Levels of serum magnesium (mmol/L)				*P* for linear trend
	Level 1 (<0.75)	**Level 2 (0.75 - <0.81)**	**Level 3 (0.81 - <0.87)**	**Level 4 (≥0.87)**	
Word List Learning (WLL, *n*=1,780)	0 (Referent)	0.08 (−0.51, 0.67)	0.43 (−0.15, 1.01)	0.15 (−0.47, 0.76)	0.56
Word List Delayed Recall (WLD, *n*=1,780)	0 (Referent)	0.08 (−0.18, 0.33)	0.10 (−0.15, 0.35)	0.12 (−0.15, 0.38)	0.57
Animal Fluency Test (AFT, *n*=1,971)	0 (Referent)	−0.17 (−0.83, 0.48)	0.21 (−0.44, 0.86)	0.04 (−0.65, 0.73)	0.62
Letter Fluency Test (LFT, *n*=2,022)	0 (Referent)	−0.18 (−0.85, 0.49)	−0.09 (−0.75, 0.57)	−0.13 (−0.82, 0.57)	0.48

Abbreviations: CI, confidence interval.

[a]All models were constructed by using linear mixed models with the adjustment for the covariates listed in model 3, Table 2. Analyses of AFT and LFT also included covariates to adjust for whether participants who were identified in review of tape recordings received assistance from someone in their home environment or were given a disallowed prompt by the interviewer.