

eAppendix

Supplementary methods

Measurement of cognitive function

20-59-year-old adults. Computerized cognitive function testing was administered to a random sample (approximately 50%) of all participants aged 20-59 years in NHANES III by a trained examiner in a soundproof room in the mobile examination centres (MECs). Three computerized tests from the Neurobehavioral Evaluation System 2^{S1} were used: (1) a simple reaction time test, which tests motor response speed to a visual stimulus; (2) a symbol-digit substitution test, which tests pattern recognition; and (3) a serial digit learning test, which tests short-term memory. We used the following derived scores, which have been described in detail elsewhere¹⁵: (1) mean reaction time; (2) mean of the two best error-corrected latencies and (3) the summary score.^{S1, 15}

60-90-year-old adults. Cognitive function tests were performed in all 60-90 year-old participants either at the MECs or at the participant's home if they were unable to attend the clinics. Serum calcium and 25(OH)D levels were not available from those participants who performed the tests at home so only the data obtained at MECs were analyzed in this study. A memory recall test measuring the ability to learn new information and the general ability to remember overall ideas was used to test cognitive function in this age group. The detailed description is available at <http://www.cdc.gov/nchs/data/nhanes/nhanes3/cdrom/nchs/manuals/mecint.pdf>. A story describing a real-life situation with six key points was read to participants, who were asked to freely recall the story immediately and later during the interview. Total number of recalled key points (0-12) at the two time points was used in the analyses. This is justified because the

immediate and delayed recall scores correlated strongly with each other ($r=0.862$) and because total number of recalled key points correlated strongly with number of items recalled immediately ($r=0.963$) and later ($r=0.967$).

Measurement of confounders

Age and gender of all participants was recorded at the time of the clinic assessment. Physician's impression of health status at the limited standard medical examination performed in the mobile examination clinics was used as an indicator of general health. Self-reported ethnicity/race was categorized as non-Hispanic white, non-Hispanic black, Mexican-American or Other (including all Hispanics, regardless of race, who were not Mexican-American and all non-Hispanics from racial groups other than white or black).

A binary variable for outdoor activity during the past month was derived from the interview data according to the method described by Scragg and Camargo.^{s2} Body mass index (BMI) was obtained from weight and height assessed in the clinic and data on smoking (pipe/cigars/cigarettes), alcohol use (measured as number of days during which any alcohol was consumed during the last 12 months) and poverty-income ratio was obtained by interview. Data on familiarity with computer games and pre-test caffeine consumption were obtained by interview before testing.

Additional analysis information

The NHANES III survey is not a random sample of the population so traditional statistical methods are not applicable in the data analysis. NHANES III is a stratified multistage

probability sample of 89 survey locations so statistical methods need to account for the clustered nature of the sample and the overrepresentation of some ethnic groups. The svy command in Stata does this by applying appropriate sampling weights (MEC weights in these analyses). These weights incorporate the differential probabilities of selection and include adjustments for noncoverage and nonresponse. In addition, the strata and primary sampling unit pairings from the sample design were used in variance estimation. A more detailed description of recommended statistical methodology is described in^{S3}.

Supplementary results

Change in characteristics across quartiles of serum calcium

Tables 1 and 2 in the main paper show distributions of characteristics in young adults (table 1) and old adults (table 2) across quartiles of calcium. Serum 25(OH)D increased across the quartiles of serum calcium in both age groups. Among the 20-59 year olds, mean age, proportion of individuals with non-white ethnicity and the proportion of those who used Spanish in the test decreased across the quartiles. The proportion of women and those who reported outdoor activity during past month increased across the quartiles of pH-normalized serum calcium. Among the 60-90 year olds, mean alcohol consumption, head of household education years and proportion of women decreased across the quartiles. Mean BMI and proportion of individuals from households with poverty-income ratio below 1 tended to increase across the quartiles. The health status and performance in psychometric tests were similar across quartiles of pH-normalized calcium in both age groups .

Change in characteristics across quartiles of serum 25(OH)D

Supplementary web-tables e1 (young adults) and e2 (older adults) show equivalent results across distributions of 25(OH)D. In both age groups, the proportion of women, non-Hispanic whites, those who reported outdoor activity during past month or had better health status increased across the quartiles of 25(OH)D. Mean performance score in all psychometric tests also increased across the quartiles in both age groups. The proportion of non-Hispanic blacks, smokers and those with poor or fair health status decreased across the quartiles of 25(OH)D. Among 20-59 year olds, mean serum calcium and head of household education years increased over the quartiles, while a decreasing trend was observed with mean age and proportion of those who performed the psychometric tests in Spanish. In the older age group, mean BMI and proportion of Mexican-Americans decreased across the quartiles of 25(OH)D.

Supplementary web-table e3 shows the multivariable associations of calcium and 25(OH)D with cognitive function in older and younger adults, in the subgroup with complete data on all covariables including smoking and alcohol. These results are described in the main paper.

eTable 1. Age- and gender-adjusted characteristics of the younger adults (20-59 years) in the NHANESIII study according to quartiles of circulating 25(OH)D. Data are given as mean (95% confidence interval)

Characteristic	n	1 st (8.7-42.9 nmol/l)	2 nd (43.2-59.7 nmol/l)	3 th (59.9-79.6 nmol/l)	4 th (79.9-243.6 nmol/l)	<i>P</i> for trend
Age (years)	11649	38.0 (37.2-38.9)	38.1 (37.4-38.8)	37.4 (36.8-38.1)	35.7 (35.1-36.2)	0.05
Head of household education (years)	11649	12.6 (12.3-12.9)	12.9 (12.3-13.4)	13.1 (12.8-13.5)	13.3 (13.0-13.6)	0.006
Physician's impression of health (%)	11433					0.001
Excellent	5642	26	24	25	26	
Very good	2682	28	24	25	24	
Good	2329	30	26	23	21	
Fair	397	40	28	18	15	
Poor	42	38	36	21	5	
No data	341	26	29	26	19	
Outdoor activity during last month (%)	10227	39 (36-42)	50 (48-53)	61 (59-63)	70 (68-72)	<0.001
Serum normalized calcium (mmol/l)	10425	1.23 (1.23-1.24)	1.23 (1.23-1.24)	1.24 (1.23-1.24)	1.24 (1.23-1.25)	0.001
BMI (kg/m ²)	11649	29.0 (25.9-32.1)	28.7 (26.0-31.4)	39.0 (24.0-54.0)	25.8 (24.7-26.9)	0.003
Mean of two best error-corrected latencies in SDST (sec)	4869	2.8 (2.7-2.9)	2.7 (2.6-2.8)	2.5 (2.5-2.6)	2.6 (2.5-2.7)	<0.001
Mean reaction time in SRTT (msec)	4929	238 (233-243)	236 (231-240)	233 (228-239)	231 (226-235)	0.01

Total score in SDLT	4760	5.5 (4.9-6.1)	4.8 (4.3-5.3)	3.7 (3.3-4.1)	4.1 (3.7-4.5)	<0.001
Used Spanish in test (%)	4932	5 (3-7)	6 (4-9)	3 (2-5)	3 (2-5)	0.02
Women (%)	11649	35 (32-38)	43 (42-49)	51 (49-53)	55 (53-60)	<0.001
Current smoker (%)	5590	74 (70-77)	65 (61-69)	62 (58-66)	60 (55-65)	<0.001
# of days used alcohol during past 12 months	5671	98.9 (90.6-107.1)	89.1 (79.1-99.0)	91.9 (84.9-99.0)	95.2 (88.7-101.7)	0.94
Ethnicity (%)	11649					<0.001
Non-hispanic white	3900	8	17	29	47	
Non-hispanic black	3638	47	29	16	8	
Mexican-American	3568	21	29	30	20	
Other	543	21	32	25	22	
Poverty-income ratio below 1 (%)	11649	20 (17-24)	14 (12-17)	12 (10-14)	9 (7-11)	<0.001

eTable 2. Age- and gender-adjusted characteristics of the older adults of the NHANESIII study according to quartiles of circulating 25(OH)D.
Data are given as mean (95% confidence interval)

Characteristic	n	1 st (8.7-46.2 nmol/l)	2 nd (46.4-62.2 nmol/l)	3 th (62.4-80.6 nmol/l)	4 th (80.9-400.1 nmol/l)	<i>P</i> for trend
Age (years)	4831	71.4 (70.6-72.2)	71.1 (70.4-71.9)	71.1 (70.5-71.6)	70.6 (70.1-71.0)	0.09
Head of household education (years)	4831	11.0 (10.6-11.4)	11.1 (10.7-11.5)	11.4 (11.1-11.7)	11.3 (10.8-11.8)	0.45
Physician's impression of health (%)	4831					0.002
Excellent	636	18	25	29	29	
Very good	1116	21	26	26	28	
Good	1744	25	25	26	25	
Fair	1016	31	26	22	20	
Poor	186	46	21	18	15	
No data	133	26	28	20	26	
Outdoor activity during last month (%)	4310	40 (35-46)	52 (48-57)	63 (58-67)	73 (68-77)	<0.001
Serum normalized calcium (mmol/l)	4310	1.22 (1.22-1.23)	1.23 (1.22-1.24)	1.24 (1.23-1.24)	1.24 (1.23-1.24)	0.12
BMI (kg/m ²)	4310	27.6 (27.2-28.0)	27.6 (27.2-28.0)	26.6 (26.1-27.1)	25.9 (25.6-26.3)	0.01
Combined recalled items (max 12)	4831	7.7 (7.4-8.0)	7.9 (7.7-8.1)	8.1 (7.9-8.3)	8.0 (7.8-8.3)	0.005
Women (%)	4831	25 (22-29)	40 (37-43)	455 (42-48)	54 (51-57)	<0.001

Current smoker (%)	2565	31 (25-38)	26 (22-31)	18 (14-22)	21 (17-26)	0.002
# of days used alcohol during past 12 months	1396	181.1 (143.2-219.0)	171.6 (137.8-205.5)	161.2 (139.8-182.7)	169.6 (145.4-193.7)	0.48
Ethnicity (%)	4831					<0.001
Non-hispanic white	2848	17	23	29	32	
Non-hispanic black	910	46	25	18	11	
Mexican-American	950	31	30	21	18	
Other	123	29	31	20	21	
Poverty-income ratio below 1 (%)	4831	12 (10-14)	9 (7-12)	9 (7-11)	8 (5-11)	0.08

eTable 3. Association of circulating pH-normalized calcium and 25(OH)D levels with psychometric test scores (both in z-scores) in those with data on smoking and alcohol use.

Score	Age (years)	n	SD	Model 1 ^a Mean difference in test score (SD) per 1 SD change in serum levels (95%CI)	Model 2 ^b	Model 3 ^c
pH-normalized serum calcium						
Mean reaction time ^d	20-59	1413	48.8	0.00 (-0.08 to 0.07)	0.00 (-0.08 to 0.07)	0.00 (-0.08 to 0.07)
Best SDST score ^d	20-59	1546	1.1	0.01 (-0.04 to 0.07)	0.01 (-0.04 to 0.07)	0.02 (-0.04 to 0.07)
Total SDLT score ^d	20-59	1526	4.8	-0.02 (-0.09 to 0.06)	-0.02 (-0.09 to 0.05)	-0.02 (-0.09 to 0.05)
Recalled items	60-90	920	2.7	-0.04 (-0.12 to 0.05)	-0.02 (-0.11 to 0.06)	-0.02 (-0.11 to 0.06)
Serum 25(OH)D						
Mean reaction time ^d	20-59	1560	49.3	-0.03 (-0.06 to 0.01)	-0.02 (-0.06 to 0.02)	-0.02 (-0.06 to 0.02)

Best SDST score ^d	20-59	1546	1.1	0.00 (-0.03 to 0.03)	0.01 (-0.02 to 0.04)	0.01 (-0.02 to 0.04)
Total SDLT score ^d	20-59	1526	4.8	-0.01 (-0.06 to 0.05)	0.00 (-0.05 to 0.06)	0.01 (-0.04 to 0.05)
Recalled items	60-90	1026	2.8	-0.01 (-0.07 to 0.06)	-0.02 (-0.08 to 0.05)	-0.01 (-0.07 to 0.05)

SDLT serial-digit learning test; SDST symbol-digit substitution test

^aModel 1 is adjusted for age, race/ethnicity and gender

^bModel 2 as Model 1 plus outdoor activity during past month, physician's impression of health status, BMI and poverty-income ratio

^cModel 3 as Model 2 plus smoking and alcohol use

^dadditional adjustment for language used in test, examiner, familiarity with computer games and caffeine consumption within 3 hours

SUPPLEMENTARY REFERENCES

- (S1) Baker EL, Letz R, Fidler A. A computer-administered neurobehavioral evaluation system for occupational and environmental epidemiology. Rationale, methodology, and pilot study results. *J Occup Med*. 1985;27(3):206-212.
- (S2) Scragg R, Camargo CA, Jr. Frequency of leisure-time physical activity and serum 25-hydroxyvitamin D levels in the US population: results from the Third National Health and Nutrition Examination Survey. *Am J Epidemiol*. 2008;168(6):577-586.
- (S3) Landis JR, Lepkowski JM, Eklund SA, Stehouwer SA. A statistical methodology for analyzing data from a complex survey: the first National Health and Nutrition Examination Survey. *Vital Health Stat 2*. 1982;(92):1-52.