Vitamin D is not associated with incident dementia or cognitive impairment: an 18-y follow-up study in community-living old men[1–3]

Erika Olsson,[4] Liisa Byberg,[5]* Brita Karlström,[4] Tommy Cederholm,[4] Håkan Melhus,[6] Per Sjögren,[4] and Lena Kilander[7]*

Departments of [4]Public Health and Caring Sciences/Clinical Nutrition and Metabolism, [5]Surgical Sciences, Orthopedics, [6]Medical Sciences, Clinical Pharmacogenomics and Osteoporosis, and [7]Public Health and Caring Sciences/Geriatrics, Uppsala University, Uppsala, Sweden

ABSTRACT

Background: Vitamin D has been implicated as being important for maintaining cognitive function in old age. Results from longitudinal studies examining the association of vitamin D with incident dementia and cognitive impairment have been inconsistent.

Objective: We investigated the relation between vitamin D, assessed in 3 different ways, and the risk of dementia.

Design: We measured plasma 25-hydroxyvitamin D [25(OH)D] with the use of high-performance liquid chromatography–mass spectrometry, assessed dietary vitamin D intake with the use of 7-d dietary records, and created a vitamin D–synthesis genetic risk score (GRS) at baseline (1991–1995) in a cohort of 1182 Swedish men (mean age: 71 y). In a maximum of 18 y (median: 12 y) of follow-up, 116 men developed Alzheimer disease, 64 men developed vascular dementia, and 250 men developed all-cause dementia. An additional 80 men declined in cognitive function as assessed with the use of the Mini-Mental State Examination. Adjusted HRs and ORs were calculated with the use of Cox and logistic regressions.

Results: The mean ± SD plasma 25(OH)D concentration was 68.7 ± 19.1 nmol/L. Plasma 25(OH)D, dietary vitamin D intake, and vitamin D–synthesis GRS were not associated with any cognitive outcomes (crude and adjusted HRs and ORs were ∼1.0 for all continuous exposures). The adjusted HR for all-cause dementia was 0.88 (95% CI: 0.59, 1.31) in men with plasma 25(OH)D concentrations ≤50 compared with >75 nmol/L. The adjusted HR for all-cause dementia was 0.92 (95% CI: 0.63, 1.32) for the lowest compared with highest tertiles of vitamin D intake. The adjusted HR for the continuous GRS for all-cause dementia was 1.04 (95% CI: 0.91, 1.19).

Conclusion: In this cohort study, we show that there is no association between baseline vitamin D status and long-term risk of dementia or cognitive impairment over an 18-y period of time.

Am J Clin Nutr 2017;105:936–43.

Keywords: Alzheimer disease, cognitive impairment, cohort, dementia, Mendelian randomization, vascular dementia, vitamin D, 25-hydroxyvitamin D

INTRODUCTION

Vitamin D has been implicated as being important for maintaining cognitive function in old age. Vitamin D may have neuroprotective properties by decreasing inflammation and oxidative stress (1, 2). Vitamin D receptors have been shown to be present in brain regions that are responsible for memory development and cognitive functions (3, 4) and may also be involved in plaque clearance (5, 6). Vitamin D is produced in the skin by UVB sunlight, and a smaller amount of vitamin D is provided by the diet. Plasma 25-hydroxyvitamin D [25(OH)D][8] is the main biomarker of vitamin D status. There has been an ongoing debate regarding the cutoffs for vitamin D deficiency and the optimum value for physical and mental health, with no global consensus (7–9).

Several cross-sectional studies and a few observational studies with short follow-up have observed an association between low vitamin D concentrations and Alzheimer disease (AD), vascular dementia, and cognitive impairment (10, 11). However, results from long-term studies have been more inconsistent (12–21). In cross-sectional studies and studies with short follow-ups, reverse causation with cognitive impairment that leads to poor nutrition and an absence of sun exposure may induce incorrect conclusions. In addition, with consideration that AD has a prodromal phase over decades (22) and vascular dementia progresses slowly over time, studies with longer follow-ups may be preferable for valid conclusions.

To circumvent the issue that observational associations may be influenced by reverse causation or by an inadequate control of confounders, the Mendelian randomization approach offers an opportunity to study the unconfounded association between vitamin D and dementia. One study that used the Mendelian

[1] Supported by the Swedish Research Council (grant no. K2013-69x-21414-04-3) and by Uppsala County Council.

[2] The funding agencies did not have any role in the conduct of the study; collection, management, analysis, or interpretation of the data; or preparation, review, or approval of the manuscript.

[3] Supplemental Figure 1 and Supplemental Tables 1 and 2 are available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at http://ajcn.nutrition.org.

*To whom correspondence should be addressed. E-mail: erika.olsson@pubcare.uu.se (E Olsson), liisa.byberg@surgsci.uu.se (L Byberg).

[8] Abbreviations used: AD, Alzheimer disease; *CYP2R1*, vitamin D 25-hydroxylase gene; *DHCR7*, 7-dehydrocholesterol reductase gene; *GC*, vitamin D binding protein; GRS, genetic risk score; MMSE, Mini-Mental State Examination; 25(OH)D, 25-hydroxyvitamin D.

Received July 6, 2016. Accepted for publication January 12, 2017.

First published online February 15, 2017; doi: 10.3945/ajcn.116.141531.

Supplemental Material can be found at:
http://ajcn.nutrition.org/content/suppl/2017/02/15/ajcn.116.141531.DCSupplemental.html

randomization approach showed no association between genetic variants that are related to vitamin D concentrations and cognitive function (23), but to our knowledge, there have been no previous studies on vitamin D and incident dementia.

The aim of this study was to investigate whether vitamin D as measured via plasma 25(OH)D, vitamin D intake, and the genetic risk score (GRS) for vitamin D synthesis with the use of a Mendelian randomization approach is associated with incident AD, vascular dementia, all-cause dementia, and performance in the Mini-Mental State Examination (MMSE) in Swedish elderly men (mean age: 71 y) who were followed for a median of 12 y.

METHODS

This cohort study used data from the Uppsala Longitudinal Study of Adult men (24, 25). In 1970, all men (n = 2841) who were born between 1920 and 1924 and were living in the municipality of Uppsala, Sweden, were invited to complete a health survey regarding cardiovascular disease risk factors and 2322 men (mean age: 50 y) participated. The men were regularly re-examined, and the current analyses were based on the third investigation in 1991–1995 (baseline) when the men were 69–74 y old (mean age: 71 y). The participation rate was 73%; 1221 of 1681 survivors who were living in Uppsala, and nearly all (n = 1194) of them had valid measurements of plasma 25(OH)D. We excluded participants with a dementia diagnosis or stroke with aphasia before baseline and those who were diagnosed with these conditions within 2 y after baseline (n = 12), which left 1182 men in our study (**Supplemental Figure 1**). The investigation was approved by the Regional Ethical Review Board at Uppsala University, and all participants gave their written informed consent.

Vitamin D exposures

Plasma 25(OH)D

Baseline plasma samples from 1991 to 1995 (at a participant mean age of 71 y) were drawn after an overnight fast and stored at −70°C for a maximum of 15 y until analysis (24). HPLC-atmospheric pressure chemical ionization-mass spectrometry (HPLC–mass spectrometry) (Vitas) was used to determine plasma 25(OH)D (26, 27). 25(OH)D is stable in stored plasma (28), and the CV for the interassay analysis was 7.6% at 47.8 nmol/L and 6.9% at 83.0 nmol/L (28).

Dietary vitamin D intake

A validated, precoded, optically readable food record was filled in during 7 consecutive days (29, 30) by 1117 of 1182 participants (in 1991–1995; mean age: 71 y) after having been given instructions by a dietitian or a nurse on how to complete the food record. To calculate daily energy and nutrient intakes, a computerized program and information from the Swedish National Food Administration were used. Four participants with reported energy intakes <800 kcal/d and 1 participant with a reported intake >4000 kcal/d were excluded because of possible extreme reported intakes (31), and thus, a total of 1101 participants were eligible for the analyses of vitamin D intake. The use of vitamin D–containing multivitamin supplements was registered (n = 37), and the contribution of supplementary vitamin D was added to dietary intake to obtain total vitamin D intake.

GRS

A GRS was created by adding the number of alleles that were associated with higher 25(OH)D concentrations in 2 single-nucleotide polymorphisms in genes that are involved with vitamin D with no described pleiotropic effects; i.e., rs12785878 in the 7-dehydrocholesterol reductase gene [*DHCR7*; the T allele is associated with higher 25(OH)D] and rs12794714 in the vitamin D 25-hydroxylase gene [*CYP2R1*; the G allele is associated with higher 25(OH)D] (32, 33). We had data on single-nucleotide polymorphisms for 1087 participants (in 1991–1995; mean age: 71 y).

Outcomes

Dementia diagnoses

The MMSE was administered at baseline (mean age: 71) and at a follow-up examination in 2003–2005 (mean age: 82 y). Participants with low scores were recommended to make an appointment at the Memory Clinic, Uppsala University Hospital, at which the vast majority of all incident cases of dementia were captured. To ensure complete follow-up, 2 experienced geriatricians, independently of each other, further reviewed all participants' medical records from Uppsala University Hospital and Uppsala Primary Care between 1991 and the end of 2009 to identify incident cases of AD, vascular dementia, and all-cause dementia. In case of disagreement, a third geriatrician reviewed the case (34). AD was defined according to the National Institute of Neurological and Communication Disorders and Stroke and Alzheimer's Disease and Related Disorders Association criteria (35) and the Diagnostic and Statistical Manual of Mental Disorders (Fourth Edition) criteria (36). Vascular dementia was diagnosed according to the criteria of Chui et al. (37), and cases with concomitant AD and vascular dementia were allocated to this group. Cases of dementia without neuroimaging or sufficient clinical details in medical records to set a specific subtype diagnosis were classified as unspecified dementia (34). All-cause dementia included AD, vascular dementia, frontotemporal dementia (38), Lewy body dementia (39), unspecified dementia, and Parkinson disease with dementia (38, 40).

Cognitive impairment

As a secondary outcome, we defined cognitive impairment according to the performance on the MMSE (41). The MMSE was administered to 863 of 1182 participants at baseline in 1991–1995 (mean age: 71 y). In 2003–2005, all men (mean age: 82 y) who were still alive and living in Uppsala (n = 952) were invited for a new survey. At follow-up, 488 men performed the MMSE, and MMSE results from both occasions were available in 408 men. Cognitive impairment was defined as a decline in the MMSE ≥3 points over the 12-y period or an MMSE score <25 points at follow-up. Thus, we ended up with the following 4 outcomes: AD, vascular dementia, all-cause dementia, and cognitive impairment.

Covariates

Baseline measurements of systolic and diastolic blood pressures in the supine position, blood glucose, serum cholesterol, and body weight and height were undertaken. Hypertension was defined as systolic blood pressure ≥140 mm Hg, diastolic blood pressure ≥90 mm Hg, or treatment with an antihypertensive

medication. Hypercholesterolemia was defined as a serum cholesterol concentration ≥6.5 mmol/L or treatment with cholesterol-lowering medications. Diabetes was defined as a fasting plasma glucose concentration >7 mmol/L or treatment with a diabetes medication. BMI (in kg/m^2) was calculated as weight divided by the square of height. Information on smoking status (never, former, or current); educational level [stratified as low (elementary school only; 6–7 y), medium (high school), or high (college or university education)]; health status; and physical activity level [stratified as sedentary, moderate, regular, or athletic (42)] were retrieved via a questionnaire and interviews. Alcohol consumption was categorized as abstainers, consumers, or daily consumers. The season of blood collection was defined as summer (May 1 to October 31) or winter (November 1 to April 30).

Statistical analysis

Plasma 25(OH)D concentrations and dietary vitamin D were analyzed in relation to the outcomes both as continuous variables and in predefined categories (≤50, >50–75, and >75 nmol/L) for 25(OH)D concentrations and as tertiles (≤4.7, >4.7–6.5, and >6.5 μg/d) for dietary vitamin D with the use of the highest group as the reference. The GRS was analyzed as a continuous variable. Cox proportional hazards regression was used to estimate the association between exposures and AD, vascular dementia, and all-cause dementia. The time at risk was calculated from the date of the baseline examination until the dementia diagnosis, death (n = 700), or the end of follow-up (31 December 2009). Stroke cases with aphasia were censored in the Cox regression analysis (n = 26). Logistic regression was used to estimate the association between exposures and cognitive impairment.

To investigate potential nonlinear trends between plasma and dietary vitamin D and dementia outcomes, restricted cubic splines with 3 knots placed at the 10th, 50th, and 90th percentiles of the distribution (43) were calculated with 50 nmol/L as the reference point for plasma 25(OH)D concentrations and 5 μg/d as the reference point for dietary vitamin D.

Covariates included in our main model (model 2) were selected on the basis of directed acyclic graphs (44). For plasma 25(OH)D, model 1 included age and the season of blood collection. Model 2 further included BMI, education, and physical activity. Model 3 included covariates in model 2 and smoking, diabetes, hypertension, hypercholesterolemia (as categorical variables), vitamin D supplements, and alcohol intake (yes or no).

For vitamin D intake, model 1 included age and energy intake. Model 2 further included BMI, education, physical activity, and smoking. Model 3 included covariates in model 2 and diabetes, hypertension, and hypercholesterolemia (as categorical variables).

For the GRS, model 1 included crude data, and model 2 included age, season of blood collection, BMI, education, and physical activity.

Covariates with missing information (physical activity: n = 88; weight: n = 1; height: n = 4; BMI: n = 5; smoking status: n = 68; alcohol consumption: n = 84; and hypertension: n = 1) were imputed with the use the technique of the last-value carried

TABLE 1
Baseline characteristics (n = 1182)[1]

	Plasma 25(OH)D, nmol/L			
	≤50 (n = 183)	>50–75 (n = 562)	>75 (n = 437)	Total cohort (n = 1182)
Age, y	70.9 (70.4–71.5)[2]	71 (70.6–71.4)	71.2 (70.7–71.5)	71.0 (70.7–71.5)
BMI (n = 1177), kg/m^2	26.7 ± 3.9[3]	26.4 ± 3.4	26.0 ± 3.3	26.3 ± 3.4
Plasma 25(OH)D, nmol/L	40.9 ± 8.3	62.7 ± 6.9	88.1 ± 12.5	68.7 ± 19.1
Education, n (%)				
Low	99 (54)	333 (59)	245 (56)	677 (57)
Medium	64 (35)	143 (26)	132 (30)	339 (29)
High	20 (11)	86 (15)	60 (14)	166 (14)
Leisure time physical activity (n = 1094), n (%)				
Low	7 (4)	25 (5)	12 (3)	44 (4)
Medium	54 (33)	194 (37)	134 (33)	382 (35)
High	101 (62)	305 (58)	262 (64)	668 (61)
Smoking (n = 1114), n (%)				
Never	67 (40)	182 (34)	138 (34)	387 (35)
Former	76 (45)	273 (51)	206 (51)	555 (50)
Current	26 (15)	83 (15)	63 (15)	172 (15)
Diabetes, n (%)	25 (14)	57 (10)	42 (10)	124 (11)
Hypertension, n (%)	142 (78)	407 (72)	329 (75)	878 (74)
Hypercholesterolemia, n (%)	46 (25)	195 (35)	119 (27)	360 (30)
Vitamin D supplement users (n = 1119), n (%)	2 (1)	19 (4)	18 (4)	39 (3)
Alcohol intake (n = 1098), n (%)				
Abstainers	26 (16)	71 (14)	37 (9)	134 (12)
Modest and daily users	141 (84)	454 (86)	369 (91)	964 (88)
Plasma cystatin C (n = 953), mg/L	1.19 (1.1–1.3)	1.19 (1.1–1.4)	1.23 (1.1–1.4)	1.20 (1.1–1.4)
Plasma PTH (n = 1166), pmol/L	4.35 (3.4–6.4)	3.98 (3.0–5.0)	3.87 (2.8–5.1)	3.99 (3.0–5.3)

[1] PTH, parathyroid hormone; 25(OH)D, 25-hydroxyvitamin D.
[2] Median; range in parentheses (all such values).
[3] Mean ± SD (all such values).

forward on the basis of information from previous examinations. Missing information on supplement use was regarded as no use. Statistical analyses were performed with Stata/SE 11.0 software (StataCorp LP).

RESULTS

In a total follow-up of 18 y (median: 12 y and 14,093 person-years at risk), we identified 116 cases of AD, 64 cases of vascular dementia, and 250 cases of all-cause dementia. Eighty of 488 men who participated in the MMSE at follow-up were classified as cognitively impaired (16.4%). Baseline characteristics by 25(OH)D categories are shown in **Table 1**. Plasma 25(OH)D concentrations varied with the season; the mean 25(OH)D concentration was 65.2 nmol/L for samples that were taken between May and October and was 73.0 nmol/L for samples that were taken between November and April. All analyses of plasma vitamin D were adjusted for season of blood sampling.

25(OH)D and cognition

The mean ± SD plasma 25(OH)D concentration in men at baseline without incident dementia during follow-up was 68.4 ± 19.3 nmol/L. The corresponding concentrations were as follows: for cases of incident AD cases, 69.1 ± 19.4 nmol/L; for cases of vascular dementia, 69.3 ± 19.0 nmol/L; and for cases of all-cause dementia, 69.9 ± 18.5 nmol/L. In men without cognitive impairment and in men with cognitive impairment, mean plasma 25(OH)D concentrations were 69.3 ± 18.2 and 70.8 ± 20.2 nmol/L, respectively. Twenty individuals (1.7%) had 25(OH)D concentrations <30 nmol/L. Four of these 20 men developed dementia during follow-up. A total of 183 individuals (15.5%) had concentrations <50 nmol/L. There were no associations between 25(OH)D and incident AD, vascular dementia, all-cause dementia, or MMSE impairment for the continuous variable or for the categorized variables (**Table 2**). No nonlinear associations between 25(OH)D and any of the outcomes could be observed on the basis of restricted cubic-spline curves (**Figure 1**), which conformed with the results from categorical variables of 25(OH)D.

Dietary and total intake of vitamin D and cognition

Thirty-seven participants took multivitamin supplements that contained 5–10 μg vitamin D/d at baseline. With the addition of this amount to baseline dietary intake, median total vitamin D intake, including that from vitamin D supplements, was 5.5 μg/d (range: 4.3–7.1 μg/d). Total median intakes were 5.5 μg/d (range: 4.4–7.2 μg/d) in men without incident dementia and 5.5 μg/d (range: 4.3–6.8 μg/d) in men with incident all-cause dementia. Total vitamin D intake in tertiles and as a continuous exposure was not associated with incident AD, vascular dementia, all-cause dementia, or cognitive impairment during follow-up (**Figure 2**, **Table 3**).

Vitamin D–synthesis GRS and cognition

The GRS was associated with plasma 25(OH)D concentrations (**Supplemental Tables 1** and **2**); the mean concentration for 25(OH)D was 57.5 ± 18.3 nmol/L for the lowest GRS and was

TABLE 2
Plasma 25(OH)D and associations with incident Alzheimer disease, vascular dementia, all-cause dementia, and cognitive impairment[1]

Endpoint and plasma 25(OH)D	Events, *n*	Model 1	Model 2	Model 3
Alzheimer disease[2]				
Continuous	116	1.00 (0.99, 1.01)	1.00 (0.99, 1.01)	1.00 (0.99, 1.01)
≤50 nmol/L	17	1.16 (0.65, 2.06)	1.20 (0.68, 2.14)	1.19 (0.67, 2.12)
>50–75 nmol/L	57	1.12 (0.75, 1.68)	1.13 (0.75, 1.70)	1.11 (0.73, 1.67)
>75 nmol/L	41	Referent	Referent	Referent
Vascular dementia[2]				
Continuous	64	1.00 (0.99, 1.01)	1.00 (0.99, 1.01)	1.00 (0.99, 1.01)
≤50 nmol/L	9	0.92 (0.43, 1.97)	0.89 (0.41, 1.91)	0.89 (0.41, 1.93)
>50–75 nmol/L	28	0.81 (0.47, 1.38)	0.76 (0.44, 1.31)	0.73 (0.42, 1.27)
>75 nmol/L	27	Referent	Referent	Referent
All-cause dementia[2]				
Continuous	250	1.00 (1.00, 1.01)	1.00 (1.00, 1.01)	1.00 (1.00, 1.01)
≤50 nmol/L	32	0.88 (0.59, 1.32)	0.88 (0.59, 1.31)	0.86 (0.58, 1.30)
>50–75 nmol/L	119	0.93 (0.71, 1.22)	0.92 (0.70, 1.21)	0.90 (0.68, 1.19)
>75 nmol/L	99	Referent	Referent	Referent
Cognitive impairment[3]				
Continuous	80	1.01 (0.99, 1.02)	1.00 (0.99, 1.02)	1.00 (0.99, 1.02)
≤50 nmol/L	8	0.62 (0.27, 1.43)	0.63 (0.27, 1.46)	0.63 (0.27, 1.48)
>50–75 nmol/L	39	0.90 (0.54, 1.51)	0.90 (0.53, 1.53)	0.87 (0.51, 1.50)
>75 nmol/L	33	Referent	Referent	Referent

[1] Model 1 was adjusted for age and season of blood collection. Model 2 was adjusted as for model 1 and for BMI, education, and physical activity. Model 3 was adjusted as for model 2 and for smoking, diabetes, hypertension, hypercholesterolemia, use of vitamin D supplements, and alcohol intake. HRs were calculated with the use of Cox proportional hazards regression, and ORs were calculated with the use of logistic regression. 25(OH)D, 25-hydroxyvitamin D.

[2] All values are HRs; 95% CIs in parentheses.

[3] All values are ORs; 95% CIs in parentheses. Total *n* = 488. For 25(OH)D concentrations ≤50 nmol/L, *n* = 66; >50–75 nmol/L, *n* = 235; and >75 nmol/L, *n* = 187.

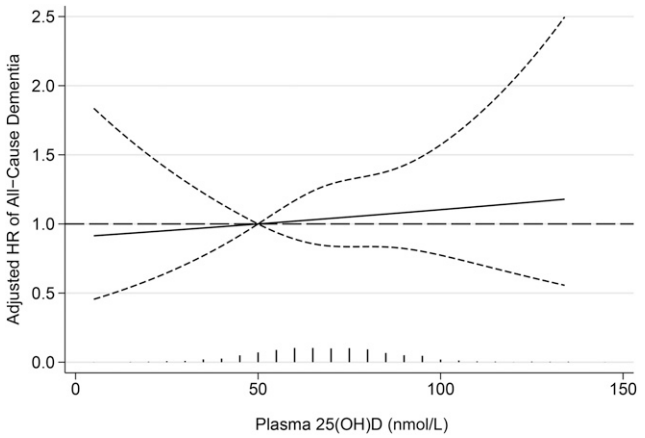

FIGURE 1 HRs (solid line) and 95% CIs (dotted lines) of all-cause dementia according to plasma 25(OH)D concentrations (n = 1182). Values were estimated with the use of a restricted cubic-spline Cox proportional hazards regression–analysis model with a concentration of 50 nmol/L as the reference and were adjusted for age, season of blood collection, BMI, education, and physical activity. Vertical bars represent the distribution of plasma 25(OH)D. 25(OH)D, 25-hydroxyvitamin D.

70.9 ± 22.4 nmol/L for the highest GRS. However, the risk score was not associated with any of the outcomes (**Table 4**).

DISCUSSION

In this population of ∼71-y-old men at baseline, no associations were shown between vitamin D and incident AD, vascular dementia, all-cause dementia, or cognitive decline over a total of 18 y of follow-up. To our knowledge, this is the first study to have assessed both plasma 25(OH)D and vitamin D intake as well as genetic variants for vitamin D synthesis with the use of the Mendelian randomization approach in relation to long-term incident dementia diagnoses. Incident dementia cases were identified from medical records that covered all types of care units, including a Memory Clinic, and were not restricted to diagnoses in hospitalized patients only. In addition, the performance on the MMSE was used to capture cases of milder cognitive impairment.

Previous research has shown no evidence that vitamin D status plays a major role in dementia or cognitive impairment in a 2-y follow-up of very elderly people with a high comorbidity burden (21). The results from previous longitudinal studies with a follow-up >5 y and dementia as the outcome have not been clear cut (13–16). Some of these studies identified dementia cases through hospital and death registries with an obvious risk of bias because low vitamin D concentrations are associated with comorbidities and, thus, greater risk of hospitalization (13, 14, 16). An American study, which was comparable with the current study in terms of study size, the mean age at baseline, the analysis method for 25(OH)D, and the identification of dementia diagnosis showed, unlike in our study, an association between low vitamin D concentrations and increased risks of all-cause dementia and AD (15). The study included white and black American men and women, had a larger proportion of subjects with 25(OH)D concentrations <50 nmol/L (29% compared with 15.5% in the current study), and had a shorter follow-up period (5.6 y compared with 12 y in the current study) (15). Moreover, a substantial number of participants were excluded because of missing information on dementia status. However, in the current study, we had no loss to follow-up in the ascertainment of dementia diagnoses because all participants' medical records were scrutinized.

Vitamin D may not be a major player in the β-amyloid aggregation, tauopathy, and cerebrovascular disease that underlie the 2 major dementia disorders AD and vascular dementia but may be important through other mechanisms for maintaining neuronal networks at a high age. In a nondemented elderly population, impaired cognitive performance is multifactorial and may be linked to low socioeconomic status and impaired motivation because of depression or chronic pain. The majority of other studies on brain health with serum vitamin D measurements as the exposure have assessed performance with the use of the screening instrument MMSE and have tested executive function with the use of the Trail Making Test A and B (10, 20). In our cohort, vitamin D status did not predict poor results in the MMSE, which was defined as a decline of ≥3 points or by scoring <25 points at follow-up. The results from other prospective studies on the MMSE with follow-up of >3 y have been diverse. Lower concentrations of 25(OH)D were shown to be associated with a worse outcome on the MMSE or tests on executive function in some studies (45–49) but not in other studies (16, 50). However, the MMSE may be seen as a blunt instrument, and our results do not preclude that vitamin D is associated with a milder cognitive decline such as impaired episodic memory. In addition, we used cognitive impairment as a secondary outcome because the change in the MMSE between 2 examinations could only be assessed in participants who were healthy enough to attend both examinations (i.e., the healthy cohort effect).

Median vitamin D intake was 5.5 μg/d in our cohort. This intake was below the Nordic recommendations of 10 μg/d for this age group (Nordic Nutrition Recommendations 2012), which was set to maintain serum 25(OH)D concentration at ∼50 nmol/L in the majority of the population. Low-fat milk (≤1.5% fat) has been fortified with vitamin D since its introduction in Sweden in 1967 (51). Although reported intake

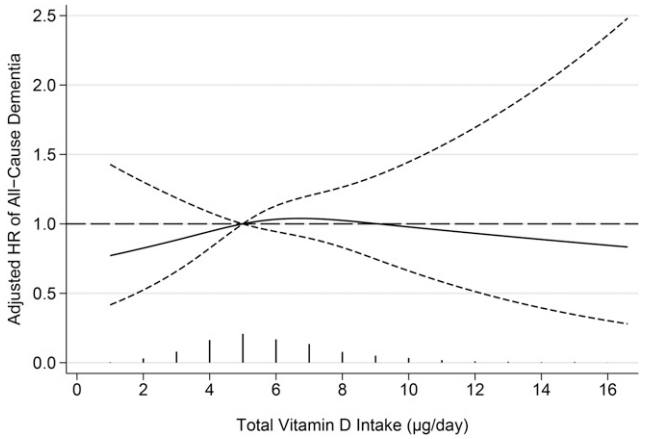

FIGURE 2 HRs (solid line) and 95% CIs (dotted lines) of all-cause dementia according to total vitamin D intake (n = 1101) including vitamin D from the diet and from vitamin D–containing supplements. Values were estimated with the use of a restricted cubic-spline Cox proportional hazards regression–analysis model with an intake of 5 μg/d as the reference and were adjusted for age, BMI, education, physical activity, energy intake. and smoking. Vertical bars represent the distribution of total vitamin D intake.

TABLE 3
Total vitamin D intake and risk associations for Alzheimer disease, vascular dementia, all-cause dementia, and cognitive impairment[1]

Endpoint and total vitamin D intake	Events, *n*	Model 1	Model 2	Model 3
Alzheimer disease[2]				
Continuous	113	0.97 (0.88, 1.07)	0.98 (0.88, 1.08)	0.97 (0.88, 1.08)
Tertile 1	41	1.01 (0.60, 1.72)	0.97 (0.57, 1.64)	0.97 (0.57, 1.65)
Tertile 2	32	0.82 (0.50, 1.34)	0.79 (0.48, 1.30)	0.79 (0.48, 1.30)
Tertile 3	40	Referent	Referent	Referent
Vascular dementia[2]				
Continuous	60	1.07 (0.94, 1.22)	1.08 (0.95, 1.23)	1.07 (0.93, 1.22)
Tertile 1	23	0.73 (0.36, 1.51)	0.74 (0.35, 1.53)	0.78 (0.37, 1.62)
Tertile 2	17	0.68 (0.34, 1.34)	0.69 (0.35, 1.36)	0.68 (0.34, 1.36)
Tertile 3	20	Referent	Referent	Referent
All-cause dementia[2]				
Continuous	237	1.01 (0.94, 1.08)	1.01 (0.94, 1.08)	1.00 (0.94, 1.08)
Tertile 1	88	0.93 (0.65, 1.35)	0.92 (0.63, 1.32)	0.94 (0.65, 1.35)
Tertile 2	71	0.81 (0.58, 1.14)	0.80 (0.57, 1.13)	0.79 (0.56, 1.11)
Tertile 3	79	Referent	Referent	Referent
Cognitive impairment[3]				
Continuous	77	1.02 (0.90, 1.15)	1.02 (0.90, 1.16)	1.03 (0.91, 1.17)
Tertile 1	22	0.75 (0.36, 1.55)	0.76 (0.37, 1.58)	0.74 (0.35, 1.53)
Tertile 2	29	1.10 (0.59, 2.04)	1.08 (0.58, 2.01)	1.00 (0.53, 1.89)
Tertile 3	26	Referent	Referent	Referent

[1] Model 1 was adjusted for age and energy intake. Model 2 was adjusted as for model 1 and for BMI, education, physical activity, and smoking. Model 3 was adjusted as for model 2 and for diabetes, hypertension, and hypercholesterolemia. HRs were calculated with the use of Cox proportional hazards regression, and ORs were calculated with the use of logistic regression.
[2] All values are HRs; 95% CIs in parentheses (unless otherwise indicated).
[3] All values are ORs; 95% CIs in parentheses (unless otherwise indicated). Total, $n = 473$; tertile 1, $n = 148$; tertile 2, $n = 157$; and tertile 3, $n = 168$.

was not adequate according to the recommendations, only 15.5% in our cohort had plasma 25(OH)D concentrations ≤50 nmol/L. Plasma vitamin D status was weakly correlated with dietary intake of vitamin D (data not shown), which was in line with results from other Swedish studies that showed that lifestyle factors, such as intake or individual sun exposure, explain only 25% of the variance of plasma vitamin D (52). In previous studies, higher vitamin D intake has been associated with lower risk of developing AD but not with risk of developing non-AD dementias (53), whereas the association between intake and cognitive tests has been inconsistent (54). However, in the current study, no association between vitamin D intake and cognitive function or dementia was shown. The Women's Health Initiative trial is the only long-term randomized clinical trial in this area in which vitamin D_3 (10 μg) with calcium (1000 mg) or a placebo was given to 2034 women over a mean period of 7.8 y. No effect on incident cognitive impairment was reported (55).

In line with our results on plasma and dietary vitamin D, we observed no association between the genetic variants that are involved in vitamin D synthesis and any of the outcomes. There have been few studies in this area especially on incident dementia. Previous studies have observed little evidence for an association between genotypes that are associated with 25(OH)D and cognitive function, and few studies have examined the genes that are thought to affect vitamin D synthesis directly (20, 32). No clear associations were shown when several genetic variants were examined, including the vitamin D receptor gene [which has not been associated with circulating 25(OH)D] and the vitamin D 25-hydroxylase gene [*CYP2R1*; which has been associated with higher 25(OH)D] in relation to different cognitive tests (23). Another study observed that variants that are associated with higher 25(OH)D in the vitamin D binding protein (*GC*) gene were associated with poorer word-recall scores (56). However, the *GC* gene may have pleiotropic effects that render it unsuitable as a formal instrument for 25(OH)D (32).

The current study has several strengths as follows: the long follow-up period; the identification of incident dementia

TABLE 4
Genetic risk score (continuous) and associations with incident Alzheimer disease, vascular dementia, all-cause dementia, and cognitive impairment ($n = 1087$)[1]

Endpoint	Events, *n*	Model 1	Model 2
Alzheimer disease[2]	108	0.98 (0.81, 1.19)	0.96 (0.79, 1.18)
Vascular dementia[2]	58	1.06 (0.81, 1.39)	1.08 (0.82, 1.42)
All-cause dementia[2]	234	1.04 (0.91, 1.18)	1.04 (0.91, 1.19)
Cognitive impairment[3]	77	1.03 (0.80, 1.32)	1.03 (0.80, 1.34)

[1] Model 1 was unadjusted. Model 2 was adjusted for age, season of blood collection, BMI, education, and physical activity. HRs were calculated with the use of Cox proportional hazards regression, and ORs were calculated with the use of logistic regression.
[2] All values are HRs; 95% CIs in parentheses (unless otherwise indicated).
[3] All values are ORs; 95% CIs in parentheses (unless otherwise indicated). $n = 471$.

without a loss to follow-up, which was performed via a review of individual medical records and was not restricted to hospital registries; and the use of 3 different measurements of vitamin D exposure. In addition, we used the HPLC–mass spectrometry method for the analysis of 25(OH)D, which is considered the gold-standard method (57).

The limitations of the study include the lack of repeated measurements of plasma 25(OH)D and dietary vitamin D intakes, the lack of information on sun exposure as a covariate, and the inclusion of men only. In this overall healthy cohort, there were very few participants with low concentrations of 25(OH)D; however, this factor is in line with previous studies in community-dwelling people (58, 59) in Sweden. Future studies may want to focus on a population in whom vitamin D deficiency is more prevalent. Although repeated blood sampling and dietary assessment improve the precision of exposure information, a long follow-up period may be an advantage and may limit the risk of reverse causation when studying dementia, which has a long induction period. Furthermore, plasma 25(OH)D has been shown to be stable over time (Pearson's correlation coefficient: 0.7) (60). For total vitamin D intake, we also included vitamin D from multivitamin supplements. Because only 37 men reported taking such multivitamin supplements, we did not investigate the role of vitamin D supplements per se in relation to dementia. The study size may have been too small to assess weak associations with the use of the Mendelian randomization analysis, which generally demands the use of large study populations. However, associations have previously been identified with the use of the Mendelian randomization approach in the current cohort (61).

In conclusion, the current study does not confirm an association between vitamin D that is measured via plasma 25(OH)D, dietary vitamin D intake, or genetic risk scoring and long-term risk of AD, vascular dementia, all-cause dementia, or cognitive impairment in elderly Swedish men. Randomized controlled trials are needed to study possible beneficial effects of vitamin D in frail elderly with severe deficiency.

The authors' responsibilities were as follows—EO: had primary responsibility for the final manuscript; EO, LB, PS, and LK: analyzed the data and performed the statistical analysis; HM and LK: collected the data; and all authors: designed the research, wrote the manuscript, contributed to and agreed with the current findings, and read and approved the final version of the manuscript. None of the authors reported a conflict of interest related to the study.

REFERENCES

1. Pérez-López FR, Chedraui P, Fernández-Alonso AM. Vitamin D and aging: beyond calcium and bone metabolism. Maturitas 2011;69:27–36.
2. Amer M, Qayyum R. Relation between serum 25-hydroxyvitamin D and C-reactive protein in asymptomatic adults (from the continuous National Health and Nutrition Examination Survey 2001 to 2006). Am J Cardiol 2012;109:226–30.
3. Annweiler C, Schott AM, Berrut G, Chauvire V, Le Gall D, Inzitari M, Beauchet O. Vitamin D and ageing: neurological issues. Neuropsychobiology 2010;62:139–50.
4. Buell JS, Dawson-Hughes B. Vitamin D and neurocognitive dysfunction: preventing "D"ecline? Mol Aspects Med 2008;29:415–22.
5. Mizwicki MT, Menegaz D, Zhang J, Barrientos-Duran A, Tse S, Cashman JR, Griffin PR, Fiala M. Genomic and nongenomic signaling induced by 1alpha,25(OH)2-vitamin D3 promotes the recovery of amyloid-beta phagocytosis by Alzheimer's disease macrophages. J Alzheimers Dis 2012;29:51–62.
6. Dursun E, Gezen-Ak D, Yilmazer S. A novel perspective for Alzheimer's disease: vitamin D receptor suppression by amyloid-beta and preventing the amyloid-beta induced alterations by vitamin D in cortical neurons. J Alzheimers Dis 2011;23:207–19.
7. Ross AC, Manson JE, Abrams SA, Aloia JF, Brannon PM, Clinton SK, Durazo-Arvizu RA, Gallagher JC, Gallo RL, Jones G, et al. The 2011 report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine: what clinicians need to know. J Clin Endocrinol Metab 2011;96:53–8.
8. Spiro A, Buttriss JL, Vitamin D. An overview of vitamin D status and intake in Europe. Nutr Bull 2014;39:322–50.
9. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, Murad MH, Weaver CM. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab 2011; 96:1911–30.
10. Annweiler C, Montero-Odasso M, Llewellyn DJ, Richard-Devantoy S, Duque G, Beauchet O. Meta-analysis of memory and executive dysfunctions in relation to vitamin D. J Alzheimers Dis 2013;37:147–71.
11. van der Schaft J, Koek HL, Dijkstra E, Verhaar HJ, van der Schouw YT, Emmelot-Vonk MH. The association between vitamin D and cognition: a systematic review. Ageing Res Rev 2013;12: 1013–23.
12. Breitling LP, Perna L, Muller H, Raum E, Kliegel M, Brenner H. Vitamin D and cognitive functioning in the elderly population in Germany. Exp Gerontol 2012;47:122–7.
13. Afzal S, Bojesen SE, Nordestgaard BG. Reduced 25-hydroxyvitamin D and risk of Alzheimer's disease and vascular dementia. Alzheimers Dement 2014;10:296–302.
14. Knekt P, Saaksjarvi K, Jarvinen R, Marniemi J, Mannisto S, Kanerva N, Heliovaara M. Serum 25-hydroxyvitamin D concentration and risk of dementia. Epidemiology 2014;25:799–804.
15. Littlejohns TJ, Henley WE, Lang IA, Annweiler C, Beauchet O, Chaves PH, Fried L, Kestenbaum BR, Kuller LH, Langa KM, et al. Vitamin D and the risk of dementia and Alzheimer disease. Neurology 2014;83:920–8.
16. Schneider AL, Lutsey PL, Alonso A, Gottesman RF, Sharrett AR, Carson KA, Gross M, Post WS, Knopman DS, Mosley TH, et al. Vitamin D and cognitive function and dementia risk in a biracial cohort: the ARIC Brain MRI Study. Eur J Neurol 2014;21:1211–8, e69–70.
17. Bartali B, Devore E, Grodstein F, Kang JH. Plasma vitamin D levels and cognitive function in aging women: the Nurses' Health Study. J Nutr Health Aging 2014;18:400–6.
18. Shen L, Ji HF. Vitamin D deficiency is associated with increased risk of Alzheimer's disease and dementia: evidence from meta-analysis. Nutr J 2015;14:76.
19. Annweiler C, Dursun E, Feron F, Gezen-Ak D, Kalueff AV, Littlejohns T, Llewellyn DJ, Millet P, Scott T, Tucker KL, et al. 'Vitamin D and cognition in older adults': updated international recommendations. J Intern Med 2015;277:45–57.
20. Brouwer-Brolsma EM, de Groot LC. Vitamin D and cognition in older adults: an update of recent findings. Curr Opin Clin Nutr Metab Care 2015;18:11–6.
21. Graf CE, Rossi C, Giannelli SV, Nobari BH, Gold G, Herrmann FR, Zekry D. Vitamin D is not associated with cognitive status in a cohort of very old hospitalized patients. J Alzheimers Dis 2014;42(Suppl 3):S53–61.
22. Bateman RJ, Xiong C, Benzinger TL, Fagan AM, Goate A, Fox NC, Marcus DS, Cairns NJ, Xie X, Blazey TM, et al. Clinical and biomarker changes in dominantly inherited Alzheimer's disease. N Engl J Med 2012;367:795–804.
23. Jorde R, Mathiesen EB, Rogne S, Wilsgaard T, Kjaergaard M, Grimnes G, Schirmer H. Vitamin D and cognitive function: the Tromso Study. J Neurol Sci 2015;355:155–61.
24. Zethelius B, Berglund L, Sundstrom J, Ingelsson E, Basu S, Larsson A, Venge P, Arnlov J. Use of multiple biomarkers to improve the prediction of death from cardiovascular causes. N Engl J Med 2008;358:2107–16.
25. Byberg L, Melhus H, Gedeborg R, Sundstrom J, Ahlbom A, Zethelius B, Berglund LG, Wolk A, Michaelsson K. Total mortality after changes in leisure time physical activity in 50 year old men: 35 year follow-up of population based cohort. BMJ 2009;338:b688.
26. Melhus H, Snellman G, Gedeborg R, Byberg L, Berglund L, Mallmin H, Hellman P, Blomhoff R, Hagstrom E, Arnlov J, et al. Plasma 25-hydroxyvitamin D levels and fracture risk in a community-based cohort of elderly men in Sweden. J Clin Endocrinol Metab 2010; 95:2637–45.

27. Michaëlsson K, Baron JA, Snellman G, Gedeborg R, Byberg L, Sundström J, Berglund L, Arnlöv J, Hellman P, Blomhoff R, et al. Plasma vitamin D and mortality in older men: a community-based prospective cohort study. Am J Clin Nutr 2010;92:841–8.
28. Hollis BW. Measuring 25-hydroxyvitamin D in a clinical environment: challenges and needs. Am J Clin Nutr 2008;88:507S–10S.
29. Nydahl M, Gustafsson IB, Mohsen R, Becker W. Comparison between optical readable and open-ended weighed food records. Food Nutr Res 2009 Feb 20 (Epub ahead of print; DOI: 10.3402/fnr.v53i0.1889).
30. Becker W. Food habits and intake in Sweden 1989. Uppsala (Sweden): The Swedish National Food Administration; 1994.
31. Willett W. Nutritional Epidemiology. 3rd ed. New York: Oxford University Press; 2013.
32. Vimaleswaran KS, Cavadino A, Berry DJ, Jorde R, Dieffenbach AK, Lu C, Alves AC, Heerspink HJ, Tikkanen E, Eriksson J, et al. Association of vitamin D status with arterial blood pressure and hypertension risk: a mendelian randomisation study. Lancet Diabetes Endocrinol 2014;2:719–29.
33. Wang TJ, Zhang F, Richards JB, Kestenbaum B, van Meurs JB, Berry D, Kiel DP, Streeten EA, Ohlsson C, Koller DL, et al. Common genetic determinants of vitamin D insufficiency: a genome-wide association study. Lancet 2010;376:180–8.
34. Rönnemaa E, Zethelius B, Lannfelt L, Kilander L. Vascular risk factors and dementia: 40-year follow-up of a population-based cohort. Dement Geriatr Cogn Disord 2011;31:460–6.
35. McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. Neurology 1984;34:939–44.
36. American Psychiatric Association. Diagnostic and statistic manual of mental disorders: DSM-IV. Washington (DC): American Psychiatric Association; 1994.
37. Chui HC, Victoroff JI, Margolin D, Jagust W, Shankle R, Katzman R. Criteria for the diagnosis of ischemic vascular dementia proposed by the State of California Alzheimer's Disease Diagnostic and Treatment Centers. Neurology 1992;42:473–80.
38. Neary D, Snowden JS, Gustafson L, Passant U, Stuss D, Black S, Freedman M, Kertesz A, Robert PH, Albert M, et al. Frontotemporal lobar degeneration: a consensus on clinical diagnostic criteria. Neurology 1998;51:1546–54.
39. McKeith IG. Consensus guidelines for the clinical and pathologic diagnosis of dementia with Lewy bodies (DLB): report of the Consortium on DLB International Workshop. J Alzheimers Dis 2006;9:417–23.
40. Olsson E, Karlstrom B, Kilander L, Byberg L, Cederholm T, Sjogren P. Dietary patterns and cognitive dysfunction in a 12-year follow-up study of 70 year old men. J Alzheimers Dis 2015;43:109–19.
41. Folstein MF, Folstein SE, McHugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res 1975;12:189–98.
42. Byberg L, Zethelius B, McKeigue PM, Lithell HO. Changes in physical activity are associated with changes in metabolic cardiovascular risk factors. Diabetologia 2001;44:2134–9.
43. Harrell J, Frank E. Regression modeling strategies; with applications to linear models, logistic regression, and survival analysis. New York: Springer-Verlag; 2001.
44. Textor J, Hardt J, Knuppel S. DAGitty: a graphical tool for analyzing causal diagrams. Epidemiology 2011;22:745.
45. Llewellyn DJ, Lang IA, Langa KM, Muniz-Terrera G, Phillips CL, Cherubini A, Ferrucci L, Melzer D. Vitamin D and risk of cognitive decline in elderly persons. Arch Intern Med 2010;170:1135–41.
46. Slinin Y, Paudel M, Taylor BC, Ishani A, Rossom R, Yaffe K, Blackwell T, Lui LY, Hochberg M, Ensrud KE. Association between serum 25(OH) vitamin D and the risk of cognitive decline in older women. J Gerontol A Biol Sci Med Sci 2012;67:1092–8.
47. Toffanello ED, Coin A, Perissinotto E, Zambon S, Sarti S, Veronese N, De Rui M, Bolzetta F, Corti MC, Crepaldi G, et al. Vitamin D deficiency predicts cognitive decline in older men and women: the Pro.V.A. Study. Neurology 2014;83:2292–8.
48. Wilson VK, Houston DK, Kilpatrick L, Lovato J, Yaffe K, Cauley JA, Harris TB, Simonsick EM, Ayonayon HN, Kritchevsky SB, et al. Relationship between 25-hydroxyvitamin D and cognitive function in older adults: the Health, Aging and Body Composition Study. J Am Geriatr Soc 2014;62:636–41.
49. Moon JH, Lim S, Han JW, Kim KM, Choi SH, Kim KW, Jang HC. Serum 25-hydroxyvitamin D level and the risk of mild cognitive impairment and dementia: the Korean Longitudinal Study on Health and Aging (KLoSHA). Clin Endocrinol (Oxf) 2015;83:36–42.
50. Slinin Y, Paudel ML, Taylor BC, Fink HA, Ishani A, Canales MT, Yaffe K, Barrett-Connor E, Orwoll ES, Shikany JM, et al. 25-Hydroxyvitamin D levels and cognitive performance and decline in elderly men. Neurology 2010;74:33–41.
51. Redogörelse för verksamheten vid statens institut för folkhälsan 1966-1971. [Report from the National Institute of Public Health 1966-1971.] Vår föda 1972;24:125 (in Swedish).
52. Snellman G, Melhus H, Gedeborg R, Olofsson S, Wolk A, Pedersen NL, Michaelsson K. Seasonal genetic influence on serum 25-hydroxyvitamin D levels: a twin study. PLoS One 2009;4:e7747.
53. Annweiler C, Rolland Y, Schott AM, Blain H, Vellas B, Herrmann FR, Beauchet O. Higher vitamin D dietary intake is associated with lower risk of Alzheimer's disease: a 7-year follow-up. J Gerontol A Biol Sci Med Sci 2012;67:1205–11.
54. Andreeva VA, Whegang-Youdom S, Touvier M, Assmann KE, Fezeu L, Hercberg S, Galan P, Kesse-Guyot E. Midlife dietary vitamin D intake and subsequent performance in different cognitive domains. Ann Nutr Metab 2014;65:81–9.
55. Rossom RC, Espeland MA, Manson JE, Dysken MW, Johnson KC, Lane DS, LeBlanc ES, Lederle FA, Masaki KH, Margolis KL. Calcium and vitamin D supplementation and cognitive impairment in the women's health initiative. J Am Geriatr Soc 2012;60:2197–205.
56. Alfred T, Ben-Shlomo Y, Cooper R, Hardy R, Deary IJ, Elliott J, Harris SE, Hypponen E, Kivimaki M, Kumari M, et al. Genetic variants influencing biomarkers of nutrition are not associated with cognitive capability in middle-aged and older adults. J Nutr 2013;143:606–12.
57. Snellman G, Melhus H, Gedeborg R, Byberg L, Berglund L, Wernroth L, Michaelsson K. Determining vitamin D status: a comparison between commercially available assays. PLoS One 2010;5:e11555.
58. Ramnemark A, Norberg M, Pettersson-Kymmer U, Eliasson M. Adequate vitamin D levels in a Swedish population living above latitude 63 degrees N: the 2009 Northern Sweden MONICA study. Int J Circumpolar Health 2015;74:27963.
59. Gerdhem P, Ringsberg KA, Obrant KJ, Akesson K. Association between 25-hydroxy vitamin D levels, physical activity, muscle strength and fractures in the prospective population-based OPRA Study of Elderly Women. Osteoporos Int 2005;16:1425–31.
60. Platz EA, Leitzmann MF, Hollis BW, Willett WC, Giovannucci E. Plasma 1,25-dihydroxy- and 25-hydroxyvitamin D and subsequent risk of prostate cancer. Cancer Causes Control 2004;15:255–65.
61. Gao H, Fall T, van Dam RM, Flyvbjerg A, Zethelius B, Ingelsson E, Hagg S. Evidence of a causal relationship between adiponectin levels and insulin sensitivity: a Mendelian randomization study. Diabetes 2013;62:1338–44.