Cognitive decline, 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D in the European Male Ageing Study (EMAS)

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ABSTRACT

Background: Although lower levels of vitamin D have been related to poor cognitive functioning and dementia in older adults, evidence from longitudinal investigations is inconsistent. The objective of this study was to determine whether 25-hydroxyvitamin D [25(OH)D] and 1,25-dihydroxyvitamin D [1,25$(OH)_2$D] levels are associated with cognitive decline in ageing men.

Methods: The European Male Ageing Study (EMAS) followed 3,369 men aged 40 to 79 over 4.4 years. 25(OH)D levels at baseline were measured by radioimmunoassay and 1,25$(OH)_2$D levels were obtained with liquid chromatography-tandem mass spectrometry. Cognitive functioning at baseline and follow-up was assessed using the Rey-Osterrieth Complex Figure, Camden Topographical Recognition Memory, and the Digit Symbol Substitution Test.

Results: A total of 2,430 men with a mean (SD) age of 59.0 (10.6) were included in the analyses. At baseline the mean 25(OH)D concentration was 64.6 (31.5) nmol/l, and mean 1,25$(OH)_2$D level was 59.6 (16.6) pmol/l. In age-adjusted linear regression models, high 25(OH)D concentrations were associated with a smaller decline on the DSST ($\beta = 0.007$, $p = 0.020$). Men with insufficient 25(OH)D levels (<50 nmol/l) showed a greater decline on the CTRM compared to men with sufficient (≥75 nmol/l) levels ($\beta = -0.41$, $p = 0.035$). However, these associations disappeared after adjusting for confounders such as depression, BMI, and co-morbidities. There was no indication of a relationship between 1,25$(OH)_2$D and cognitive decline.

Conclusion: We found no evidence for an association between 25(OH)D or 1,25$(OH)_2$D levels and cognitive decline over 4.4 years in this sample of middle-aged and elderly European men.

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INTRODUCTION

Vitamin D inadequacy is a common problem in Europe and the United States, with prevalence estimates increasing with age and ranging from 40-100% in the community-dwelling elderly population[1]. Low vitamin D levels have been reported to be associated with various negative health outcomes, including osteomalacia, cancer, hypertension, and diabetes[2]. Recently, vitamin D has been recognized as a neuroactive steroid, and as such can potentially influence cognitive functioning and decline[3]. The physiological plausibility of this relationship is supported by findings that vitamin D is involved in axonal growth[4], brain calcium metabolism[5], and brain cell differentiation[6]. In addition, vitamin D has been shown to stimulate phagocytosis and clearance of amyloid-β in the brain, one of the primary hallmarks of Alzheimer's disease[7]. In accordance with these possible biological pathways, research has indicated that lower vitamin D levels are associated with a greater risk of developing Alzheimer's disease and all-cause dementia[8]. However, evidence from both animal and human behavioural studies towards the role of vitamin D on cognition has been inconsistent. To date, over thirty cross-sectional studies have investigated the association between cognitive functions and 25-hydroxyvitamin D [25(OH)D], the main measure of vitamin D status. Most but not all of these studies reported a positive relationship between 25(OH)D and cognition[9,10]. However, research in this field has been criticised for insufficient adjustment for confounding variables, small sample sizes, and the use of suboptimal methods for measuring 25(OH)D levels[11]. Moreover, prospective studies have reported mixed results regarding vitamin D and cognitive decline. Although four longitudinal studies have shown a significant association between lower vitamin D levels and declines in global cognition[12-15], and/or specific domains such as executive function[12] and attention[15], three major investigations were unable to find any association[16-18]. There is some evidence to suggest that the effects of vitamin D on cognition are gender-specific, with two studies finding more pronounced associations in women than in men[19,20]. It therefore remains to be determined whether vitamin D affects cognitive change over time in men, and many researchers have called for the development of more well-designed prospective studies on the relationship between vitamin D status and cognitive decline[21,22].

The European Male Ageing Study (EMAS) is a multi-centre population cohort study which assessed changes in physical and cognitive functioning of a large group of middle-aged and elderly men over a period of 4.4 years[23]. Cross-sectional analyses of the baseline measurements indicated that 25(OH)D levels were positively associated with cognitive processing speed in this cohort. In addition, it was found that the impact of vitamin D on

cognition may be more pronounced in individuals with 25(OH)D levels below 35 nmol/l and men over the age of 60[24]. In the present study we aim to investigate whether vitamin D status can predict cognitive decline using longitudinal data from EMAS. Secondly, it is our objective to examine a potential interaction between age and vitamin D on cognitive function. Nearly all of the previous longitudinal studies have focused on adults aged 65 and over[12-16,18]. The one study that involved participants in late middle age (45-65 years) found no association between 25(OH)D levels and cognitive decline or risk of dementia[17]. It is therefore possible that maintaining an optimal vitamin D level is particularly important for older adults. As the current study includes elderly as well as middle-aged men, we are able to compare the effects of vitamin D deficiency on cognition across a wide age range. Finally, a unique feature of this study is that levels of 1,25-dihydroxyvitamin D [$1,25(OH)_2D$], the active metabolite of 25(OH)D, were also analysed. This is the first study to examine $1,25(OH)_2D$ as a potential marker of cognitive decline.

METHODS

Participants

Participant recruitment, study design and assessments of the European Male Ageing Study have previously been described in detail[23]. Briefly, 8,416 community-dwelling men aged 40 to 79 years were invited to attend a screening at a local clinic. A short questionnaire was used to gather information on sociodemographic, general health, and lifestyle factors. The 3,369 men who agreed to participate subsequently visited a research clinic to complete several interviewer-assisted questionnaires and undergo physical and cognitive assessments. The participating centres were based in Leuven, Belgium; Manchester, UK; Florence, Italy; Lodz, Poland; Malmö, Sweden; Santiago de Compostela, Spain; Szeged, Hungary; and Tartu, Estonia. Baseline measurements were carried out between 2003 and 2005, with follow-up testing taking place between 2007 and 2009. The average (SD) time between the two assessments was 4.4 (0.3) years. Of the men who took part in the baseline assessments, 2,736 (86.1% of survivors) returned for Phase II testing. Of the other participants, 193 (5.7%) had died and 440 (13.1%) were lost to follow-up. Ethical approval was obtained in agreement with local constitutional requirements. Written informed consent was given by all the participants.

Assessments

Demographic information and details on co-morbidities, smoking, and alcohol consumption were collected using the postal questionnaire. The interviewer-assisted questionnaire included

questions about general health, the Physical Activity Scale for the Elderly[25] (PASE), and Beck's Depression Inventory II[26] (BDI) to assess the presence and severity of depressive symptoms. Reuben's Physical Performance Test[27] (PPT) was employed to measure physical function. Information on prescription and non-prescription medications was obtained by self-report.

Tests of cognitive function

Cognitive testing was carried out at baseline and during follow-up assessments. The EMAS cognitive test battery consisted of tasks measuring components of fluid intelligence. Tests were specifically selected for minimal cultural and linguistic influences and were standardised across centres. The cognitive tasks used were (in order of administration): the Rey-Osterrieth Complex Figure (ROCF) Copy and Recall tests, the Camden Topographical Recognition Memory (CTRM) test, and the Digit Symbol Substitution Test (DSST). Higher scores on each test reflect better cognitive performance of the participant.

In the ROCF Copy task, participants are required to copy an abstract two-dimensional figure as accurately as possible within five minutes. This test provides an indication of executive functioning and overall visuo-constructional ability[28]. In the ROCF Recall component, participants are asked unexpectedly to draw the figure from memory thirty minutes after completing the Copy task. In addition to visuo-constructional skills, this task taps into visual memory abilities. The ROCF scoring criteria used in this study are based on Osterrieth's original test procedure, with a maximum score of 36 for both the Copy and Recall subtests. The CTRM was used to assess visual recognition memory[29] and involves the presentation of photographs of urban scenes followed by a forced-choice recognition component. Each correctly identified image is awarded with one point, with a maximum score of 30. Finally, cognitive processing speed and visual scanning were measured using the DSST[30,31]. In this timed paper-and-pencil subtest from the Wechsler Adult Intelligence Scale III, participants are asked to substitute as many symbols for digits as possible within 60 seconds using a coding table.

25(OH)D and 1,25(OH)$_2$D assays

In the baseline phase, morning phlebotomy was performed before 10 AM to obtain a fasting blood sample from all participants. Once processed, the serum was stored at -80ºC and shipped on dry ice to a central laboratory in Leuven, Belgium. A radio-immunoassay kit was used to determine total serum 25(OH)D levels (RIA kit; DiaSorin, Stillwater, MN, USA). Intra- and interassay coefficients for 25(OH)D levels were 11% and 9%, respectively.

$1,25(OH)_2D$ concentration was measured using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Detailed methodology of the $1,25(OH)_2D$ measurements has previously been described by Vanderschueren and colleagues[32].

Analyses

The data were analysed using the statistical programme Stata version 13.1 (StataCorp LP, College Station, TX, USA). Participants with missing cognitive and/or 25(OH)D data were excluded from the analyses. Continuous cognitive decline on the ROCF Copy and Recall, CTRM, and DSST was analysed as a continuous as well as a categorical variable to allow for a possible non-linear association with cognitive decline as suggested by previous studies[10,19]. There is no established cut-off value for defining vitamin D deficiency in relation to cognition, leading to different 25(OH)D deficiency thresholds being used in studies of cognitive function. This heterogeneity in definitions of vitamin D sufficiency has made it difficult to compare results from different studies in the past. Following recommendations from Annweiler and colleagues[22], we looked at three of the commonly used thresholds. 25(OH)D was classified as deficient (<25 nmol/l), insufficient (25-49 nmol/l), suboptimal (50-74 nmol/l) and sufficient (≥75 nmol/l). $1,25(OH)_2D$ concentration was examined as a continuous variable. For the covariates, age (years), age left education (years), BMI (kg/m^2), BDI score, PASE score, and PPT rating were analysed as continuous variables. Centre, tobacco use (currently smoking vs. non-smoking), alcohol consumption (≥1 day/week vs. <1 day/week), co-morbidities (0,1, or ≥2), and season at which the blood test was taken at baseline (winter (Jan-March), spring (April-June), summer (July-Sept), and autumn (Oct-Dec)) were included as categorical variables.

The associations between 25(OH)D level and cognitive decline were initially evaluated graphically using the Locally Weighted Scatterplot Smoothing (LOWESS) technique[33]. As this outlier-resistant method makes no assumptions about the form of the relationship, the resulting regression lines can provide information about non-linear associations between variables. Age-adjusted multiple linear regressions were then performed with continuous decline on all four cognitive tests as the outcome variables and continuous 25(OH)D, categories of 25(OH)D, or continuous $1,25(OH)_2D$ at baseline as the predictor. Subsequently, models were fitted with further adjustments for education, physical activity and performance, depression, co-morbidities, and lifestyle factors. Interaction terms between age by decade and 25(OH)D or $1,25(OH)_2D$ level were added to the full models to assess differences across age groups in the relationship between cognitive decline and vitamin D

status. Finally, participants taking vitamin D and/or calcium supplements at baseline were excluded and the above analyses repeated using the remaining sample.

RESULTS

Subjects

Of the 2,736 men returning for follow-up assessments, 105 participants were excluded from the analyses due to missing 25(OH)D measurements. Two participants with 25(OH)D levels above 250 nmol/l were removed from the dataset, as these values are higher than the upper limit of the normal range[34]. A total of 199 participants with missing CTRM and DSST data and 527 participants with missing ROCF data were omitted, leaving a sample of 2,430 men in the CTRM and DSST analyses and 2,102 men in the ROCF Copy and Recall models. For the 1,25-dihydroxyvitamin D analyses, 408 men with missing $1,25(OH)_2D$ information, 190 men with missing CTRM and DSST scores, and 517 men with missing ROCF scores were excluded. The final sample consisted of 2,138 participants in the CTRM and DSST analyses and 1,811 participants in the ROCF analyses. Baseline characteristics of the participants are listed in Table 1. Overall, the mean age of the study population (N = 2,430) was 59.0 years, average BMI was 27.6, and 41.9% of the participants had one or more co-morbidities.

Cognition and vitamin D status in EMAS

The mean cognitive scores at baseline are shown in Table 1. Independent *t*-tests indicated that participants who returned for follow-up measurements had higher cognitive scores than those who were lost to follow-up (all $p < 0.001$). Linear regressions showed that older age, fewer years of education, higher scores on Beck's Depression Inventory, lower physical activity and performance, and smoking were associated with greater decline on one or more cognitive tasks in the study sample (Table 2). Kruskal-Wallis tests revealed that change on all four cognitive test scores over time differed significantly between the centres (all $p < 0.001$).

The average (SD) baseline 25(OH)D concentration was 64.4 (32.0) nmol/l and mean serum $1,25(OH)_2D$ was 59.6 (16.6) pmol/l. Deficient, insufficient, and suboptimal vitamin D levels were common in this population with only 31.0% of all participants having sufficient 25(OH)D concentrations. Men with low 25(OH)D levels tended to have a higher BMI, show more depressive symptoms, have lower physical activity and performance scores, be more likely to smoke, consume alcohol less than 1 day a week, and have more co-morbidities (Table 2). Kruskal-Wallis tests were conducted to assess 25(OH)D and $1,25(OH)_2D$ by season and geographical region. 25(OH)D levels were significantly associated with season of measurement ($H(3) = 368.74$, $p < 0.001$), as were $1,25(OH)_2D$ concentrations ($H(3) = 57.1$, p

<0.001). Furthermore, both mean serum 25(OH)D level (H(7) = 268.69, p <0.001) and $1,25(OH)_2D$ level (H(7) – 226.91, p <0.001) varied significantly by centre. The highest levels of 25(OH)D were observed in Belgium (76.8 nmol/l), and lowest concentrations were found in Estonia (47.8 nmol/l).

Table 1 Baseline characteristics of the EMAS participants included in the analyses (n = 2,430)

Variable	Mean (SD) or %
Age (years)	59.0 (10.6)
Age left education (years)	21.0 (7.4)
BDI score	6.5 (6.0)
PASE score	201.9 (88.9)
PPT score	24.2 (2.4)
BMI (kg/m^2)	27.6 (4.0)
Current smoker (%)	19.8
Alcohol consumption ≥1 day/week (%)	58.0
Vitamin D and/or calcium supplementation (%)	0.5
Co-morbidities (%)	
No co-morbidities	58.2
1 co-morbidity	26.1
≥ 2 co-morbidities	15.8
Cognitive tests	
ROCF Copy score	33.8 (3.9)
ROCF Recall score	17.6 (6.4)
CTRM score	23.1 (4.5)
DSST score	28.7 (8.3)
25(OH)D status (%)	
Deficient (<25 nmol/l)	6.5
Insufficient (25 – 49 nmol/l)	31.1
Suboptimal (50 – 74 nmol/l)	31.4
Sufficient (≥75 nmol/l)	31.0

Abbreviations: BDI, Beck's Depression Inventory; PASE, Physical Activity Scale for the Elderly; PPT, Physical Performance Test; BMI, Body Mass Index; 25(OH)D, 25-hydroxyvitamin D; SD, Standard Deviation

Table 2 Determinants of both cognitive decline and baseline vitamin D levels: linear regression analyses[ł]

Baseline characteristics	ROCF Copy change score	ROCF Recall change score	CTRM change score	DSST change score	Baseline 25(OH)D level (nmol/l)	Baseline $1,25(OH)_2D$ level (pmol/l)
			β-coefficient (95% CI)			
Age (years)	-0.018 (-0.033; -0.004)*	-0.049 (-0.072; -0.026)*	-0.011 (-0.026; 0.003)	-0.048 (-0.077; -0.031)*	0.084 (-0.035; 0.202)	-0.058 (-0.123; 0.006)
Age left education (years)	0.023 (0.002; 0.044)*	0.021 (-0.013; 0.054)	-0.002 (-0.022; 0.019)	-0.016 (-0.042; 0.010)	-0.162 (-0.333; 0.009)	-0.049 (-0.143; 0.046)
BDI score	-0.028 (-0.053; -0.003)*	0.004 (-0.037; 0.044)	-0.017 (-0.042; 0.008)	-0.038 (-0.069; -0.006)*	-0.771 (-0.978; -0.564)*	0.055 (-0.060; 0.170)
BMI (kg/m^2)	0.029 (-0.009; 0.068)	0.036 (-0.025; 0.098)	-0.033 (-0.071; 0.004)	-0.041 (-0.089; 0.007)	-0.986 (-1.300; -0.672)*	-0.321 (-0.491; -0.150)*
PASE score	-0.000 (-0.002; 0.002)	0.005 (0.002; 0.008)*	-0.000 (-0.002; 0.002)	0.000 (-0.002; 0.002)	0.029 (0.013; 0.045)*	0.015 (0.006; 0.024)*
PPT rating	0.149 (0.080; 0.218)*	0.284 (0.174; 0.394)*	0.006 (-0.060; 0.073)	-0.080 (-0.16; 0.005)	1.29 (0.729; 1.843)*	-0.252 (-0.555; 0.051)
Current smoker						
No	Reference	Reference	Reference	Reference	Reference	Reference
Yes	0.007 (-0.395; 0.409)	-0.648 (-1.287; -0.008)*	0.249 (-0.133; 0.631)	-0.070 (-0.554; 0.414)	-9.981 (-13.171; -6.791)*	-2.392 (-4.140; -0.645)*
Alcohol consumption						
<1 day/week	Reference	Reference	Reference	Reference	Reference	Reference
≥1 day/week	0.039 (-0.277; 0.355)	-0.072 (-0.575; 0.431)	-0.001 (-0.306; 0.303)	0.232 (-0.154; 0.619)	9.058 (6.529; 11.586)*	1.905 (0.516; 3.293)
Co-morbidities						
No co-morbidities	Reference	Reference	Reference	Reference	Reference	Reference
1 co-morbidity	0.120 (-0.264; 0.503)	0.362 (-0.248; 0.973)	-0.045 (-0.414; 0.324)	-0.125 (-0.592; 0.343)	-2.672 (-5.754; 0.411)	0.337 (-1.350; 2.025)
≥2 co-morbidities	-0.024 (-0.488; 0.439)	0.161 (-0.577; 0.899)	-0.125 (-0.583; 0.332)	-0.550 (-1.131; 0.030)	-6.809 (-10.634; -2.984)*	-1.993 (-4.076; 0.089)

* $p < .05$

ł Age-adjusted where applicable

Abbreviations: BDI, Beck's Depression Inventory; BMI, Body Mass Index; PASE, Physical Activity Scale for the Elderly; PPT, Physical Performance Test; ROCF, Rey-Osterrieth Complex Figure; CTRM, Camden Topographical Recognition Memory; DSST, Digit Symbol Substitution Test; 25(OH)D, 25-hydroxyvitamin D; $1,25(OH)_2D$, 1,25-dihydroxyvitamin D

25(OH)D, 1,25(OH)$_2$D and cognitive decline

Age-adjusted LOWESS plots revealed no clear associations between 25(OH)D level at baseline and cognitive decline on the four tasks (see Figure 1). Results from the multiple linear regression models exploring the relationship between continuous 25(OH)D and cognitive decline are displayed in Table 3. In age-adjusted models, higher 25(OH)D concentrations were associated with a smaller decline in DSST performance over time. However, this association was not maintained when models were fully adjusted for covariates. Age-adjusted multiple regression analyses of categorical 25(OH)D status indicated that participants with vitamin D deficiency showed a greater decline on the DSST than participants with sufficient vitamin D levels. In addition, vitamin D insufficiency was associated with greater cognitive decline on the CTRM compared with sufficient vitamin D status. Both associations again disappeared when models were adjusted for additional covariates (see Table 4). There were no significant associations between 1,25(OH)$_2$D and cognitive decline in either age-adjusted or fully adjusted models (Table 3). There was no evidence for a significant interaction between 25(OH)D or 1,25(OH)$_2$D level and age in any of the models (all p >0.05). The results were unchanged when participants reporting vitamin D or calcium supplements intake were excluded from the regression analyses.

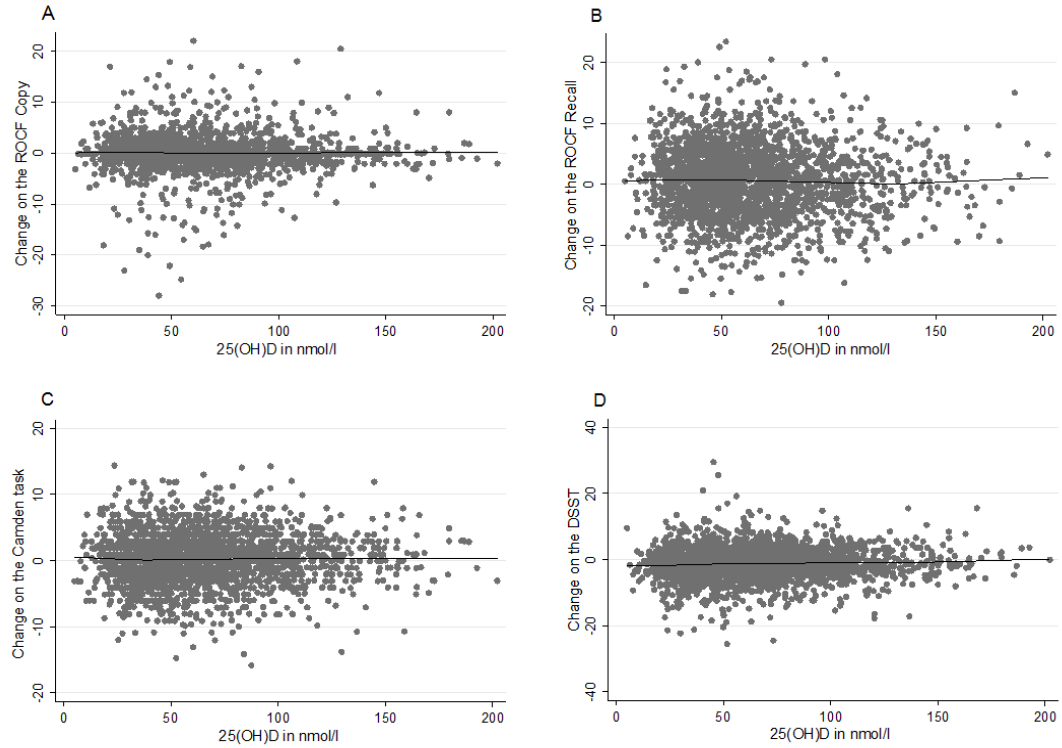

Figure 1 Age-adjusted LOWESS plots of cognitive decline and 25(OH)D level.

Table 3 Linear regression models for baseline 25(OH)D level, baseline 1,25(OH)$_2$D level, and decline in cognitive scores

Model	ROCF Copy	ROCF Recall	CTRM	DSST
		β-coefficient (95% CI)		
25(OH)D				
Model 1[a]	-0.001 (-0.005; 0.004)	-0.005 (-0.013; 0.003)	-0.003 (-0.002; 0.008)	0.007 (0.001; 0.013)*
Model 2[b]	0.001 (-0.007; 0.008)	-0.010 (-0.021; 0.001)	0.004 (-0.003; 0.011)	0.003 (-0.007; 0.012)
1,25(OH)$_2$D				
Model 1[a]	-0.002 (-0.012; 0.009)	0.012 (-0.004; 0.028)	-0.007 (-0.017; 0.003)	0.005 (-0.008; 0.017)
Model 2[b]	0.005 (-0.007; 0.017)	0.010 (-0.008; 0.028)	-0.009 (-0.020; 0.003)	0.009 (-0.004; 0.023)

*p <0.05

[a]Adjusted for age

[b]Adjusted for age, education, co-morbidities, centre, season, depressive symptoms, and lifestyle factors

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; 1,25(OH)$_2$D, 1,25-dihydroxyvitamin D; ROCF, Rey-Osterrieth Complex Figure; CTRM, Camden Topographical Recognition Memory; DSST, Digit Symbol Substitution Test

Table 4 Linear regression models for baseline categorical 25(OH)D status and decline in cognitive scores

	Deficient (N = 157)	Insufficient (N = 755)	Suboptimum (N = 764)	Sufficient (N = 754)
		β-coefficient (95% CI)		
Model 1[a]				
ROCF Copy	0.10 (-0.59; 0.79)	0.12 (-0.28; 0.51)	0.12 (-0.28; 0.52)	Reference
ROCF Recall	0.61 (-0.48; 1.71)	0.48 (-0.14; 1.11)	0.35 (-0.27; 0.98)	Reference
CTRM	0.24 (-0.40; 0.89)	-0.41 (-0.79; -0.03)*	0.00 (-0.37; 0.38)	Reference
DSST	0.84 (-1.66; -0.02)*	-0.48 (-0.96; 0.04)	-0.22 (-0.70; 0.26)	Reference
Model 2[b]				
ROCF Copy	-0.04 (-0.82; 0.74)	-0.21 (-0.68; 0.26)	0.00 (-0.43; 0.44)	Reference
ROCF Recall	0.90 (-0.31; 2.11)	0.21 (-0.51; 0.93)	0.08 (-0.60; 0.76)	Reference
CTRM	0.43 (-0.30; 1.16)	-0.27 (-0.72; 0.18)	0.21 (-0.21; 0.62)	Reference
DSST	-0.11 (-1.01; 0.80)	-0.08 (-0.63; 0.48)	-0.08 (-0.60; 0.43)	Reference

* $p < 0.05$

[a] Adjusted for age

[b] Adjusted for age, education, co-morbidities, centre, season, depressive symptoms, and lifestyle factors

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; ROCF, Rey-Osterrieth Complex Figure; CTRM, Camden Topographical Recognition Memory; DSST, Digit Symbol Substitution Test

DISCUSSION

In this multi-centre prospective study of middle-aged and elderly European men, we found no evidence for any association between vitamin D status and subsequent cognitive decline in several domains, including processing speed, visual memory, and executive functioning.

Contrary to our expectations, we found no interaction effects of age and 25(OH)D concentration on cognition. These results differ from our previous cross-sectional findings that indicated a positive association between 25(OH)D levels and processing speed, particularly in men over the age of 60 years [24]. Although decline on the DSST was associated with both continuous 25(OH)D level and deficient vitamin D status in the age-adjusted models in the present analyses, this association was not maintained when fully adjusted for confounding variables. Similarly, the association between decline on the CTRM and insufficient 25(OH)D status was significant in age-adjusted but not fully adjusted models. No significant associations were found between $1,25(OH)_2D$ and cognitive decline.

Our findings are consistent with results from the Osteoporotic Fractures in Men Study (MrOS), which suggested that slight associations between 25(OH)D and decline in global cognition or executive function over 4.6 years in elderly men were mainly caused by confounding variables such as educational level[18]. Previous studies with insufficient adjustment for covariates may therefore have overestimated the strength of the relationship between vitamin D and cognition. Another possibility is that the mixed findings of positive associations[12-15] and null results[16-18] in earlier longitudinal investigations are due to gender differences, as some studies have suggested that the beneficial cognitive effects of high vitamin D levels are more pronounced in women than in men[19,20]. This would explain the lack of significant findings in both EMAS and the MrOS cohort. However, a large-scale observational study of elderly women was also unable to find significant associations between vitamin D and cognitive decline over 6 years[16]. There is thus no conclusive evidence that vitamin D status affects cognition more in women than in men.

Alternatively, it could be argued that the testing interval of 4.4 years in the current study was too short to uncover small effects of 25(OH)D on cognitive deterioration. This especially concerns middle-aged participants, as they are less likely to demonstrate rapid and marked cognitive decline than older adults. Biological risk factors are sometimes present for a substantial period of time before they have an impact on cognition. For example, neurofibrillary tangles and senile plaques associated with Alzheimer's disease start to accumulate around the age of thirty in some individuals[35] while clinical changes generally do not appear until old age. Similarly, it is possible that chronic vitamin D deficiency in midlife only leads to increased risk of cognitive decline several years or even decades later. As other longitudinal studies spanning three to five years did report a relationship between cognitive decline and serum 25(OH)D[13-15], however, we expected that the time frame of our investigation would have been sufficient to detect such associations. As our study sample

was relatively young, cognitive deterioration may have been smaller than in studies of elderly individuals, making it more difficult to detect associations with vitamin D levels. However, interaction terms for 25(OH)D or 1,25$(OH)_2$D levels, age by decade, and cognition showed that even in the oldest age group (≥70 years) there was no significant relationship between vitamin D and cognitive decline. It is therefore improbable that the null findings are caused entirely by the young age of this population compared to other longitudinal studies. A final explanation may be that, although the tasks used here assessed a wide range of cognitive functions, other domains such as language skills are more strongly affected by vitamin D. Previous research, however, has found no association between vitamin D concentration and verbal memory[21] or verbal fluency[36], whereas executive function[12,14,37] and processing speed[24,37] were correlated with serum 25(OH)D levels in several studies.

Consistent with our results, there is at present little evidence from clinical studies that increasing vitamin D levels improves cognition. Although animal models have suggested that vitamin D supplementation has a positive effect on brain energy metabolism and cognitive decline[38], this finding has not yet been confirmed by clinical trials involving healthy human adults. A study of 128 young adults receiving daily capsules of 5000 IU vitamin D or placebo found no difference in cognitive functioning between the groups after 6 weeks of supplementation[39]. This study was possibly underpowered due to its small sample size and relatively short administration of supplements. However, another investigation of 4,142 older women also found that taking one tablet of 400 IU vitamin D and 1000 mg of calcium carbonate per day for 7.8 years did not lead to slower cognitive decline or lowered risk of dementia compared to a placebo[40]. In both studies, the majority of the participants had sufficient vitamin D levels at baseline. It is possible that clinical trials focusing on individuals with deficient vitamin D status at baseline would produce different results. Further investigations using vitamin D supplements without additional nutrients in diverse populations are therefore needed to conclusively determine the role of serum 25(OH)D in cognitive functioning. Nevertheless, our current findings suggest that an increase in vitamin D concentration is unlikely to affect cognitive decline with age.

Strengths and weaknesses

A main strength of the European Male Ageing Study is the broad range of multi-disciplinary data collected. Due to extensive physiological assessments we were able to adjust for multiple critical confounders such as education, general health, depression, and BMI. Furthermore, this is the first longitudinal study to include middle-aged as well as elderly men, which allowed us

to examine the effects of vitamin D across different age groups. In contrast with previous studies, we also used multiple thresholds to identify participants with deficient, insufficient, suboptimal, or sufficient vitamin D status. As there is not yet an established cut-off value for vitamin D deficiency with regards to cognition, investigations like the present one can provide valuable information for future clinical trials[22]. Finally, this is the first investigation of vitamin D and cognition to include measurements of $1,25(OH)_2D$. It is acknowledged that interpretations of the data are limited by the single measurement of vitamin D concentrations, as these may not reflect 25(OH)D status at Phase II. Finally, the ROCF Copy showed a ceiling effect and may lack sensitivity for assessing cognitive deterioration in this relatively healthy population. It is also probable that practice effects influenced performance on the ROCF Recall and CTRM in Phase II, leading to potential underestimations of cognitive decline. Repeated testing is not expected to have affected results on the DSST, however, as this task is only minimally if at all subject to learning effects.

Conclusion

In this cohort of middle-aged and older European men, we found no indication of a relationship between 25(OH)D or $1,25(OH)_2D$ levels and cognitive decline. Weak associations between 25(OH)D and processing speed or visual memory were explained by other factors such as co-morbidities and adverse lifestyle factors. The current findings are in line with several prospective studies and human clinical trials that have failed to find associations between vitamin D and cognitive decline. Further studies of longer duration and including both genders may be needed to clarify the relationship between 25(OH)D status and cognition. Based on our results, however, vitamin D levels do not appear to be markedly associated with cognitive functioning in ageing men.

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