Association between 25-hydroxyvitamin D levels and cognitive performance in middle-aged and older European men

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David M Lee, Abdelouahid Tajar, Aslan Ulubaev, Neil Pendleton, Terence W O'Neill, et al.. Association between 25-hydroxyvitamin D levels and cognitive performance in middle-aged and older European men. *Journal of Neurology, Neurosurgery and Psychiatry*, 2009, 80 (7), pp.722. 10.1136/jnnp.2008.165720 . hal-00552736

HAL Id: hal-00552736

<https://hal.science/hal-00552736>

Submitted on 6 Jan 2011

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ASSOCIATION BETWEEN 25-HYDROXYVITAMIN D LEVELS AND COGNITIVE PERFORMANCE IN MIDDLE-AGED AND OLDER EUROPEAN MEN

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Short title: ‘Vitamin D and cognitive function in men’

Key words: vitamin D; cognition; ageing; male health; population survey

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1 **BACKGROUND:** Although there is evidence that vitamin D inadequacy may be linked to
2 adverse cognitive outcomes, results have been inconsistent. The aim of our study was to
3 examine the association between 25-hydroxyvitamin D (25(OH)D) levels and cognitive
4 performance in middle-aged and older European men.

5 **METHODS:** This population-based cross-sectional study included 3,369 men aged 40 to 79
6 years from eight centres enrolled in the European Male Ageing Study (EMAS). Cognitive
7 function was assessed using the Rey-Osterrieth Complex Figure test (ROCF), the Camden
8 Topographical Recognition Memory test (CTRM) and the Digit Symbol Substitution test
9 (DSST). Serum 25(OH)D levels were measured by radioimmunoassay. Additional
10 assessments included physical activity, functional performance and mood/depression.
11 Associations between cognitive function and 25(OH)D were explored using locally weighted
12 and linear regression models.

13 **RESULTS:** 3,133 men, mean ($\pm$ SD) age 60 ± 11 years were included in the analysis. The
14 mean 25(OH)D concentration was 63 ± 31 nmol/L. In age-adjusted linear regressions higher
15 levels of 25(OH)D were associated with higher scores on the ROCF-copy (β per 10
16 nmol/L=0.096; 95%CI 0.049-0.144), CTRM (β per 10 nmol/L=0.075; 95%CI 0.026-0.124)
17 and DSST (β per 10 nmol/L=0.318; 95%CI 0.235-0.401) tests. After adjusting for additional
18 confounders, 25(OH)D levels were associated with the DSST test only (β per 10
19 nmol/L=0.152; 95%CI 0.051-0.253). Locally weighted and spline regressions suggested the
20 relationship between 25(OH)D and cognitive function was most pronounced at 25(OH)D
21 concentrations below 35 nmol/L.

22 **CONCLUSION:** In this study lower 25(OH)D levels were associated with poorer
23 performance on the DSST test. Further research is warranted to determine whether vitamin D
24 sufficiency may play a role in preserving cognitive function in older adults.

25

26 INTRODUCTION

27 The essential role of vitamin D on bone health and calcium metabolism has long been
28 recognized ¹. However, a growing body of research has revealed that the vitamin D
29 endocrine system is associated with a broad range of physiological outcomes besides
30 systemic calcium homeostasis ². While there are some data to suggest that vitamin D
31 deficiency may have adverse effects on cognition or behaviour ^{3,4}, there is currently
32 insufficient evidence to draw definitive conclusions. Disagreement remains as to what actual
33 threshold levels of 25(OH)D best describe either deficient or sub-optimal status, although
34 based on currently recommended levels vitamin D ‘inadequacy’ has been reported to be
35 highly prevalent ⁵.

36 Vitamin D is a fat-soluble secosteroid, primarily synthesized in the skin from 7-
37 dehydrocholesterol following solar UV-B exposure and derived to a lesser extent from
38 dietary sources. Vitamin D is hydroxylated to 25(OH)D in the liver and subsequently to the
39 main active molecule, 1,25-dihydroxyvitamin D (1,25(OH)₂D), principally in the kidneys ⁶.
40 Serum 25(OH)D levels are typically measured to establish the vitamin D status of an
41 individual. 1,25(OH)₂D exerts its biological effect both via the nuclear vitamin D receptor
42 (VDR), thereby modulating the expression of numerous target genes, and through binding to
43 cell membrane receptors, initiating rapid non-genomic intracellular signalling ³. The VDR
44 has been found in key areas of the brain known to regulate behaviour, i.e., the cortex,
45 cerebellum and limbic system, and it is often co-localized in the same cells with 1 α -
46 hydroxylase, the final enzyme in the vitamin D activation pathway ⁷. In vitro experiments
47 have demonstrated that vitamin D has both neurotrophic and neuroprotective properties ^{8,9},
48 whilst long-term 1,25(OH)₂D supplementation has been shown to retard hippocampal ageing
49 in rats ¹⁰. Together, such findings suggest that the active form of vitamin D may function as a
50 neuro-steroid.

51 Although the influence of vitamin D on brain function is biologically plausible there have
52 been few studies exploring the association between vitamin D status and adult cognitive
53 function. Two recent clinic-based observational studies reported that lower serum 25(OH)D
54 levels were associated with poorer cognitive test performance among patients with mild
55 Alzheimer's disease ^{11;12}, while a retrospective study of older adults attending a memory
56 assessment clinic found a positive correlation between 25(OH)D levels and scores on the
57 mini-mental state examination ¹³. Conversely, a cross-sectional analysis of a sub-sample of
58 participants with secondary hyperparathyroidism from the Tromsø study reported no
59 association between 25(OH)D levels and a battery of 14 neuropsychological tests. Overall,
60 these studies were not generalizable to community-dwelling individuals and were limited by
61 small sample sizes and failure to adjust for multiple confounders. To the best of our
62 knowledge there has been only one large, community-based study that has examined the
63 association between vitamin D and cognitive function. McGrath et al. ¹⁴ utilised NHANES
64 III survey data and found no evidence of an association between lower 25(OH)D levels and
65 impaired neurocognitive performance. However, this study failed to adjust for potential
66 confounders, such as education and depression, and used different cognitive tests for different
67 age groups.

68 We used baseline data from the European Male Ageing Study (EMAS), a large population-
69 based study of ageing in middle-aged and older men, to evaluate the association between
70 vitamin D levels and cognition. In addition to standardised measurements of cognitive
71 function, EMAS includes comprehensive lifestyle and functional assessments. This allowed
72 us to adjust our analyses for a wide range of possible confounders of the association between
73 vitamin D and cognition.

74

75

76 **METHODS**

77 **Subjects**

78 The major aims of EMAS are to examine the nature, prevalence and incidence of symptoms
79 associated with physiological ageing, exploring their relationships with endocrine functions
80 and other predisposing risk variables. There are two phases: a cross-sectional survey of a
81 random population sample of middle-aged and older men which was completed in 2005 and a
82 follow-up assessment which is scheduled for completion in 2009. Details regarding
83 recruitment, response rates and assessments in EMAS have been described previously¹⁵.
84 Briefly, non-institutionalized men aged 40-79 years were recruited from municipal or
85 population registers in eight centres: Florence (Italy); Leuven (Belgium); Lodz (Poland);
86 Malmö (Sweden); Manchester (UK); Santiago de Compostela (Spain); Szeged (Hungary);
87 Tartu (Estonia). For the baseline survey, stratified random sampling was used with the aim
88 of recruiting equal numbers of men into each of four age bands (40-49, 50-59, 60-69 and 70-
89 79 years). Subjects were invited by letter to complete a short postal questionnaire and to
90 attend for screening at a local clinic. The study was funded by the European Union and
91 ethical approval for the study was obtained in accordance with local institutional
92 requirements in each centre.

93

94 **Assessments**

95 The short postal questionnaire included items concerning demographic, health and lifestyle
96 information. Subjects were asked about tobacco use (response set = current / past / non-
97 smoker), typical alcohol consumption during the preceding month (response set = every day /
98 5-6 days/week / 3-4 days/week / 1-2 days/week / < once/week / not at all), and the age they
99 left full-time education. Those who agreed to participate subsequently attended a research
100 clinic to complete an interviewer-assisted questionnaire (IAQ) and assessment of cognitive

function (see below). The IAQ included the Physical Activity Scale for the Elderly (PASE)¹⁶ and the 21-item Beck Depression Inventory (BDI) to assess the presence and severity of depressive symptoms¹⁷. In addition, physical function was assessed during the clinic visit using Reuben's Physical Performance test (PPT)¹⁸. Height and weight were measured using standardized instruments.

Serum 25-hydroxyvitamin D

Morning phlebotomy was performed prior to 10 a.m. to obtain a fasting blood sample from all subjects. Processed serum was stored at -80°C prior to analysis and shipped on dry-ice to a single laboratory (University of Leuven) for measurement of 25(OH)D. Serum 25(OH)D levels were determined using a radioimmunoassay (RIA kit: DiaSorin, Stillwater, MN, USA). Intra- and inter-assay coefficients of variation for 25(OH)D were 11% and 9%, respectively. The detection limit of the RIA kit was 3.7 nmol/L 25(OH)D.

Tests of cognitive function

To assess cognitive function subjects were asked to complete a battery of three neuropsychological tests; the Rey-Osterrieth Complex Figure (ROCF), the Camden Topographical Recognition Memory (CTRM) and the Digit-Symbol Substitution (DSST) test. These tests assess the domains of visuo-constructional ability, memory, recognition, and speed of information processing, and were selected on the basis that they could be standardized across centres independent of culture and language.

Copying and delayed reproduction of the ROCF was used as a measure of visual perceptual abilities and visual memory¹⁹. The scoring criteria used was based upon Osterreith's original test procedure which defined 18 units of the drawing, assigning point values of 0 to 2 to each unit dependent upon the degree to which the units are correctly drawn and placed. Each

element of the ROCF test had a maximum score of 36. The CTRM, developed in order to measure the recognition component of visual memory retrieval, taps into the cortical component of visual memory²⁰. The CTRM involves the presentation of 30 coloured photographs of outdoor topographical scenes, each for 3 seconds, followed by a three-way forced recognition component. The CTRM had a maximum score of 30. The DSST is a subtest adopted from the Wechsler Adult Intelligence Scales and provides a reliable measure of psychomotor speed and visual scanning²¹. Subjects were asked to make as many correct symbol-for-digit substitutions as possible within a 1 minute period.

Analysis

Analyses were undertaken using the statistical package Intercooled STATA version 9.2 (StataCorp, College Station, TX, USA). Subjects with missing cognitive and/or 25(OH)D data were excluded from the analysis. Age (years), age leaving education, and BMI (kg/m²) were analyzed as continuous variables. The BDI score was treated both as a continuous and categorical variable, with the latter based upon Beck's original cut-off scores¹⁷. Both the PASE and PPT scores were treated as continuous variables and also categorized into tertiles. 25(OH)D was examined as a continuous variable and classified into sub-optimal (50-75 nmol/L), insufficiency (25-49 nmol/L) and deficiency (< 25 nmol/L) groups broadly based on previously recommended cut-points^{22,23}.

The relationship between 25(OH)D levels and cognitive function was initially evaluated graphically (while simultaneously adjusting for age) using the LOcally WEighted Scatterplot Smoothing (LOWESS) technique²⁴. This approach, where linear regression is applied repeatedly to sequential small sections of the covariate-outcome relationship, is primarily exploratory. By reducing the influence of outliers, this technique provides a smooth fit to the data so that relationships and thresholds can be more readily identified. The associations

151 between cognitive test scores and factors that could potentially confound the relationship
152 between cognitive function and 25(OH)D were explored using linear regression, with
153 adjustments made for age. Multiple linear regression models were then used to examine the
154 association between 25(OH)D levels and cognitive function, with the cognitive test scores as
155 dependent variables. Adjustments were made for factors that showed a significant
156 association ($P < 0.05$) with 25(OH)D status and cognitive function in age-adjusted models.
157 Regression models were additionally adjusted for centre and season of the year (winter [Jan-
158 March], spring [April-June], summer [July-Sept], autumn [Oct-Dec]), to account for seasonal
159 effects on 25(OH)D. Effect modification by age was also assessed by inclusion of interaction
160 terms between 25(OH)D and age (by decade) in the regression models.

161 To further explore the relationship between cognitive function and 25(OH)D we used a
162 spline or piecewise regression approach whereby the model fits linear segments to different
163 ranges of the data. Breakpoints, the level of 25(OH)D where the slope of the linear function
164 changes, were estimated from the LOWESS plots. The *mkspline* function within STATA
165 allows the regression function to be continuous at all points including the breakpoint. All
166 regression results are expressed as β coefficients and 95% confidence intervals (CI).

RESULTS

A total of 138 subjects with missing vitamin D data (16 missing blood samples, 122 assay failures) and 98 subjects with incomplete cognitive data were excluded from this analysis. The baseline characteristics of the remaining 3,133 men are shown in Table 1.

The results from the LOWESS analyses exploring the association between 25(OH)D and cognitive performance (while adjusting for age) are shown in Figure 1. Only the LOWESS plot of 25(OH)D versus the DSST score was suggestive of an overall positive association (Figure 1D). However, at 25(OH)D levels of approximately 35nmol/L and under, a more pronounced positive relationship between 25(OH)D and all four cognitive test scores was apparent.

Table 2 summarizes the influence of factors which could potentially confound the relationship between 25(OH)D levels and cognitive function. There were significant cross-sectional age-related decreases in all four cognitive test scores, but there was no association between 25(OH)D and age ($\beta = -0.0135$; 95% CI -0.114, 0.087). Depression (BDI), BMI, physical activity (PASE), physical performance (PPT) and smoking were all consistently associated with both cognitive test scores and 25(OH)D. The CTRM and DSST scores, together with 25(OH)D levels, were additionally associated with drinking one or more alcoholic drink per week. As expected, 25(OH)D levels varied markedly by season of measurement peaking in the summer (mean 85.0 nmol/L) with a nadir in the winter (mean 49.7 nmol/L). Mean serum 25(OH)D also varied by significantly by geographical region (one-way ANOVA, $P < 0.001$), with highest levels observed in northern and western European centres (72.6 nmol/L), lower levels in southern centres (60.5 nmol/L), and the lowest levels in eastern European centres (53.9 nmol/L).

The results from the regression models exploring the association between 25(OH)D and cognition are summarized in Table 3. In age adjusted linear regressions increasing 25(OH)D

levels were associated with higher scores in the ROCF copy test (β per 10 nmol/L = 0.096; 95% CI 0.049, 0.144), the CTRM test (β per 10 nmol/L = 0.075; 95% CI 0.026, 0.124), and the DSST test (β per 10 nmol/L = 0.318; 95% CI 0.235, 0.401). However, after additional adjustments for age left education, depression, BMI, physical activity, physical performance, smoking, alcohol consumption, season and centre, increasing 25(OH)D was found to be only associated with higher DSST scores (β per 10 nmol/L = 0.152; 95% CI 0.051, 0.253). No association was observed between 25(OH)D and the ROCF copy, ROCF recall, and CTRM scores in fully adjusted models. The association between increasing 25(OH)D levels and higher DSST scores was also observed when 25(OH)D was classified into sub-optimal (50-74.9 nmol/L), insufficiency (25-49 nmol/L) and deficiency (< 25 nmol/L) groups. When subjects who reported taking any form of vitamin D/calcium supplements were excluded from the regression analyses the results remained the same (data not shown). Excluding the season and centre covariates from the regression analyses did not significantly affect the results (DSST test: β per 10 nmol/L = 0.126; 95% CI 0.043, 0.209). Although the ROCF copy data was left skewed, linear regression performs well in moderately large samples ($n > 500$) even from very non-Normal data²⁵. However, to verify the results of the linear regression models we inverted the ROCF copy score and considered it as count data. The transformed data followed a negative binomial distribution (variance larger than the mean, data over-dispersed). We then carried out a negative binomial regression analysis using the same covariates as in the linear model and found the same result, i.e., no evidence of an independent association between the ROCF copy score and 25(OH)D (data not shown).

Additional regression models demonstrated a significant age by 25(OH)D interaction effect with DSST as the outcome ($P_{\text{interaction}} = 0.01$). In fully adjusted linear regressions stratified by age decade, with 25(OH)D as a continuous variable, higher DSST scores were found to be associated with 25(OH)D only among older men (DSST test: β per 10 nmol/L = 0.258; 95%

CI 0.135 0.381 [60-69 years]; β per 10 nmol/L = 0.215; 95% CI 0.105, 0.326 [over 70 years])
and not younger men (DSST test: β per 10 nmol/L = 0.044; 95% CI -0.153, 0.242 [40-49
years]; β per 10 nmol/L = 0.097; 95% CI -0.124, 0.317 [50-59 years]). There were no age by
25(OH)D interactions for ROCF copy ($P_{\text{interaction}} = 0.5$), ROCF recall ($P_{\text{interaction}} = 0.7$) and
CTRM ($P_{\text{interaction}} = 0.3$), nor evidence of any significant season by 25(OH)D, centre by
25(OH)D, or season by physical activity interaction effects for any of the four cognitive
outcomes (data not shown).

The LOWESS plots (see Figure 1) suggested that the relationship between 25(OH)D and the
four cognitive outcomes may not be best explored by a single linear function and that the
slope of the relationships changed at around 30-40 nmol/L 25(OH)D. We used spline
regression models, therefore, to better describe the 25(OH)D – cognitive function relationship
(see Table 4). The breakpoint at which the slope of the linear relationship was allowed to
change was estimated to be 35 nmol/L 25(OH)D. Spline models adjusted only for age
confirmed that the strength of the association between 25(OH)D and cognitive function (as
assessed by the relative magnitude of the β coefficients) was greater at 25(OH)D levels $\leq$ 35
nmol/l as compared to higher levels. Although the same pattern was evident in spline models
adjusted for age and the other covariates, none of the β coefficients reached statistical
significance.

DISCUSSION

In this population-based study of European men aged ≥ 40 years we observed a significant, independent association between a slower information processing speed (as assessed by the DSST test) and lower levels of 25(OH)D. The association appeared strongest among those with a 25(OH)D level less than ~ 35 nmol/L.

Previous studies exploring the relationship between vitamin D and cognitive performance in adults have produced inconsistent findings, with only one other large, population-based study having specifically examined the relationship between vitamin D and cognition in community-dwelling individuals. Using data from the NHANES III survey, McGrath et. al.¹⁴ concluded that serum 25(OH)D levels were not associated with neurocognitive performance in adults. One of the neuropsychological tests they used (Symbol Digit Substitution) assesses broadly the same cognitive domain as the DSST, i.e., speed of information processing. However, their study included both men and women, and the age group assessed using the symbol digit substitution test was significantly younger (20-59 years) than our sample. They did observe an association between vitamin D status and learning and memory tasks in their oldest group (60-90 years)¹⁴, but presented no data on speed of information processing in this group. Interestingly, when we stratified our regression analyses by age decade the association between increased 25(OH)D and a higher DSST score was only significant among the older men, i.e., 60-69 years and 70-79 years. Although the lack of an association between 25(OH)D and DSST among men under 60 years of age may have been due to insufficient power in the stratified analyses, it is intriguing to speculate that any 'potential' beneficial effect of 25(OH)D on psychomotor processing speed may be more pronounced in older men.

It was notable that the only significant association in fully adjusted regression models was observed between 25(OH)D and the DSST. Although we are unable to specifically explain

this, the DSST appears robust to ceiling effects, as opposed to the ROCF copy score and to a lesser extent the CTRM score, and this may in part have enhanced the tests sensitivity at upper levels in our cognitively-intact, generally healthy sample. To further explore potential differential relationships across cognitive domains would require more sensitive and exhaustive neuropsychological test batteries than our study schedule allowed.

Although experimental models in animals and in vitro studies point to the biological plausibility of low 25(OH)D levels resulting in impaired cognitive performance, the mechanisms by which vitamin D affects adult cerebral functions remain unknown. Possible positive effects could be direct, as suggested by the observation that 1,25(OH)₂D treatment increases choline acetyltransferase activity in rat brain nuclei ²⁶, or they could operate via a neuroprotective pathway, as demonstrated by the stimulation of neurotrophin production ²⁷ and modulation of neuronal Ca²⁺ homeostasis ⁹ by vitamin D. It is also plausible that additional factors not included in this analysis, such as parathyroid hormone (PTH) and calcium levels, may be involved in a more complex relationship with cognitive function. Evidence for such a relationship comes from the Tromsø health study, where a subset of subjects with secondary hyperparathyroidism were found to perform less well than normal controls on cognitive tests assessing working memory capacity and speed of information processing ²⁸. Although their data derives from individuals with an overt endocrine disorder, the Tromsø investigators found no evidence of any independent associations between 25(OH)D and cognitive performance. They did observe, however, that low serum 25(OH)D was significantly associated with a higher depression score. The relationship between vitamin D and mood/depression has yet to be substantiated ³, although we did find an inverse association between 25(OH)D levels and the BDI score (see Table 2).

In the context of the cross-sectional nature of the data presented here, any association between 25(OH)D and cognitive function can be interpreted in a number of ways. It is

possible that low serum 25(OH)D is merely a ‘risk marker’ and mechanistically separated from the biological processes resulting in declining cognitive performance in older adults. Low levels of 25(OH)D have been shown to be associated with a number of unfavourable health outcomes including, cardiovascular disease²⁹, increased cancer risk and mortality³⁰, sarcopenia³¹, and diabetes³². Several population-based studies have documented the relationship between cardiovascular and metabolic diseases and cognitive impairment³³⁻³⁵. When we additionally adjusted for self-reported cardiovascular disease and diabetes in our regression models the associations between 25(OH)D and DSST remained unchanged (data not shown). Conversely, cognitive impairment itself may contribute to lower levels of 25(OH)D by reducing the level of outdoor physical activity and hence exposure to sunlight. Interestingly, there is increasing evidence supporting the association between vitamin D deficiency and decreased physical performance³⁶, though cause and effect has yet to be differentiated. This raises the possibility that the positive association between 25(OH)D and psychomotor processing speed seen in our study may, in part, reflect improved physical motor speed. In an attempt to better control for this confounder in our data, we included only the score from the seven timed elements from the PPT (in place of the total score) in our regression models. However, the relationship between 25(OH)D and the DSST score remained unchanged, suggesting that the association may be more to do with neuronal processing speed than muscle/motor speed. Overall, we attempted to adjust for potential confounders in our analysis but cannot exclude the possibility that some of our findings may be due to unmeasured factors and/or residual confounding.

There remains uncertainty as to what level of vitamin D indicates a sub-optimal or deficient status. There is strong evidence that 25(OH)D levels below 12.5 nmol/L can result in bone diseases such as rickets in infants and osteomalacia in adults³⁷, although to the best of our knowledge no previous studies have assessed cognitive function in subjects with such overt

hypovitaminosis D. Serum 25(OH)D concentrations above 50 nmol/L are generally defined as sufficient, although recent studies have suggested that serum 25(OH)D levels below 75 nmol/L are sub-optimal for health^{38;39}. Nonetheless, our data showing that 8 % of subjects have 25(OH)D levels below 25 nmol/L (deficiency) and 33 % have levels between 25 and 50 nmol/L (insufficiency), broadly agrees with previous reports from Europe^{5;40}. In terms of any relationship between a given threshold level of 25(OH)D and reduced cognitive performance, our data suggest that cognitive function is increasingly negatively associated with 25(OH)D at levels below around 35 nmol/L. However, in spline regression models adjusted for age and other covariates (see Table 4) none of the associations between 25(OH)D (either above or below 35 nmol/L) and cognitive function were statistically significant. This may in part be due to insufficient power to detect the relatively modest changes in, for example the DSST score, with varying levels of 25(OH)D.

Although we have referred to 25(OH)D throughout, vitamin D exists in two common forms; vitamin D₃ (cholecalciferol) and vitamin D₂ (ergocalciferol). The form mainly produced in the skin and derived from natural dietary sources is vitamin D₃, while the primary source of vitamin D₂ is multivitamin preparations and some fortified foods⁴¹. There have been conflicting reports whether vitamin D₂ and vitamin D₃ are equally effective at maintaining 25(OH)D levels^{42;43}, with another study suggesting that the D₂ form may actually have a negative effect on 25(OH)D₃ status⁴⁴. The immunoassay used in our study has the same reported sensitivity to both forms of 25(OH)D (data from manufacturers manual), so the reported 25(OH)D levels here are the sum of 25(OH)D₂ + 25(OH)D₃. As we have no information as to the relative abundance of each form within samples, we are unable to draw any conclusions as to the different associations, if any, between 25(OH)D₂ or 25(OH)D₃ and cognitive performance. Future studies in animals or in vitro may shed more light on whether

or not the D₂ and D₃ forms of vitamin D are differentially associated with brain function and/or development.

The main strengths of our study are that it is based on a large population-based sample and used uniform methods to not only assess vitamin D status, but also potential confounders such as depressed mood, physical activity and physical function. In addition, the battery of cognitive tests was specifically selected on the basis that they could be standardized across different centres and applied to individual subjects independent of language and culture. Methodological limitations inherent to the EMAS study have been described in detail previously¹⁵, although a number of specific factors need be considered in interpreting the results presented here. The overall response rate for participation in the study was 41%. Those who participated may have differed with respect to levels of cognitive function and also vitamin D status than those who did not participate and some caution, therefore, is needed in interpreting these data. The main findings, however, were based on an internal comparison of responders and therefore any selection factors were unlikely to have had any important effect on these data. Our analysis was restricted to vision-based tests of cognitive function. This was primarily to avoid language and cultural effects inherent in many text based tests, but also due to the practical constraints of a large, population-based study. Although serum 25(OH)D levels remain the accepted metric of vitamin D status, it is questionable whether the single 25(OH)D measurement performed in our study reflects each subject's long-term vitamin D status. The single 25(OH)D value would be an indicator of sun exposure and dietary intake over the past few weeks, rather than years, thereby increasing random measurement error. The net result of this would be to reduce the reported associations between 25(OH)D and cognitive function toward the null rather than produce spurious associations. Finally our results were obtained from a predominantly Caucasian European population and should be extrapolated beyond this setting with care.

375

376 **CONCLUSION**

377 In this population-based study of middle-aged and older European men lower levels of
378 serum 25(OH)D were significantly associated with slower psychomotor processing speed as
379 measured by the DSST test. This relationship may be more pronounced among men with
380 25(OH)D levels below 35 nmol/L and in age-stratified analyses appeared to be restricted to
381 older men. Although the magnitude of the association between 25(OH)D and processing
382 speed was comparatively small, if cognitive function can be improved by a simple
383 intervention such as vitamin D supplementation, this would have potentially important
384 implications for population health. In light of our findings, and the fact that vitamin D
385 inadequacy is common among adults, further prospective studies are warranted to determine
386 whether vitamin D supplementation could aid in minimizing ageing-related declines in
387 specific cognitive domains.

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389

390 **ACKNOWLEDGEMENTS**

391 The European Male Ageing Study is funded by the Commission of the European
392 Communities Fifth Framework Program “Quality of Life and Management of Living
393 Resources” Grant QLK6-CT-2001-00258. Additional support was also provided by the
394 Arthritis Research Campaign. The authors wish to thank the men who participated in the
395 eight countries, the research/nursing staff in the eight centres: C Pott, Manchester, E Wouters,
396 Leuven., M Nilsson, Malmö, M del Mar Fernandez, Santiago de Compostela, M
397 Jedrzejowska, Lodz, H-M Tabo, Tartu, A Heredi, Szeged for their data collection, C
398 Moseley, Manchester for data entry and project co-ordination, and E Van Herck, Leuven for
399 performing the 25(OH)D assays.

400

401 **Conflict of Interest:** The authors have no financial arrangements or conflict of interest to
402 disclose concerning this manuscript.

403

404 **Sponsor's Role:** None.

405

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GRAPHICS

Table 1. Baseline characteristics (n = 3,133)

Variable	Mean (SD)
Age (years)	59.9 (11.0)
25(OH)D (nmol/L)	62.5 (31.4)
Age left education (years)	20.9 (7.7)
Beck Depression Inventory	6.8 (6.4)
Body Mass Index (kg/m ²)	27.7 (4.1)
Physical Activity Scale for the Elderly	197 (92)
Reuben's Physical Performance Test	23.9 (2.7)
Rey-Osterrieth Complex Figure (copy)	33.3 (4.5)
Rey-Osterrieth Complex Figure (recall)	17.0 (6.6)
Camden Topographical Recognition Memory	22.7 (4.7)
Digit Symbol Substitution Test	27.5 (8.8)
	%
25(OH)D status:	
Sufficient (≥ 75.0 nmol/L)	28.8
Sub-optimal (50.0 – 74.9 nmol/L)	30.5
Insufficiency (25.0 – 49.9 nmol/L)	32.8
Deficiency (< 25.0 nmol/L)	7.9
Beck Depression Inventory band (score):	
Normal (0 – 10)	78.1
Mild - Borderline (11 – 20)	18.0
Moderate - Extreme (21+)	3.9
Current smoker	20.9
Alcohol consumption (≥ 1 day/week)	56.7
Vitamin D/Ca ²⁺ supplementation	0.7

SD = standard deviation.

Table 2. Determinants of cognitive test scores and 25(OH)D levels: linear regression analyses

	ROCF copy score	ROCF recall score	CTRM score	DSST score	25(OH)D (nmol/L)
	β coefficient (95% CI) [†]				
Age (years)	-0.127 (-0.142, -0.113)*	-0.228 (-0.247, -0.208)*	-0.163 (-0.177, -0.149)*	-0.415 (-0.439, -0.391)*	-0.0135 (-0.114, 0.087)
Age left education (years)	0.097 (0.077, 0.116)*	0.129 (0.101, 0.157)*	0.060 (0.039, 0.080)*	0.199 (0.165, 0.233)*	-0.127 (-0.272, 0.017)
BDI score	-0.044 (-0.068, -0.021)*	-0.088 (-0.122, -0.055)*	-0.071 (-0.095, -0.047)*	-0.177 (-0.217, -0.136)*	-0.815 (-0.985, -0.644)*
BDI category:					
Normal (0 – 10)	Reference	Reference	Reference	Reference	Reference
Mild - Borderline (11 – 20)	-0.218 (-0.610, 0.173)	-0.732 (-1.293, -0.172)*	-0.849 (-1.252, -0.446)*	-1.743 (-2.427, -1.058)*	-9.641 (-12.51, -6.774)*
Moderate - Extreme (21+)	-1.585 (-2.361, -0.809)*	-2.264 (-3.375, -1.154)*	-1.433 (-2.231, -0.636)*	-4.050 (-5.405, -2.695)*	-13.93 (-19.61, -8.249)*
Body Mass Index (kg/m ²)	-0.059 (-0.096, -0.022)*	-0.041 (-0.093, 0.012)	-0.037 (-0.075, 0.001)	-0.115 (-0.179, -0.050)*	-0.811 (-1.081, -0.541)*
PASE score tertiles:					
Lower	Reference	Reference	Reference	Reference	Reference
Mid	0.809 (0.419, 1.200)*	1.358 (0.796, 1.919)*	1.283 (0.882, 1.685)*	1.836 (1.148, 2.523)*	5.148 (2.244, 8.052)*
Upper	0.592 (0.176, 1.008)*	1.166 (0.569, 1.764)*	1.096 (0.669, 1.524)*	1.381 (0.649, 2.113)*	7.072 (3.972, 10.17)*
PPT total tertiles:					
Lower	Reference	Reference	Reference	Reference	Reference
Mid	1.155 (0.783, 1.526)*	0.899 (0.363, 1.434)*	1.243 (0.860, 1.625)*	3.035 (2.396, 3.673)*	6.433 (3.677, 9.188)*
Upper	1.285 (0.909, 1.661)*	1.089 (0.547, 1.632)*	1.409 (1.021, 1.796)*	4.553 (3.907, 5.199)*	7.302 (4.514, 10.09)*
Current smoker					
No	Reference	Reference	Reference	Reference	Reference
Yes	-0.735 (-1.112, -0.359)*	-1.151 (-1.687, -0.615)*	-1.190 (-1.575, -0.805)*	-2.502 (-3.158, -1.847)*	-10.95 (-13.69, -8.207)*
Alcohol ($\geq$ 1day/week)					
No	Reference	Reference	Reference	Reference	Reference
Yes	0.257 (-0.047, 0.562)	0.180 (-0.255, 0.615)	1.014 (0.704, 1.324)*	2.159 (1.630, 2.687)*	8.521 (6.307, 10.74)*

[†]Adjusted for age where applicable * $P < 0.05$

ROCF = Rey-Osterrieth Complex Figure, CTRM = Camden Topographical Recognition Memory, DSST = Digit Symbol Substitution Test, BDI = Beck Depression Inventory, PASE = Physical Activity Scale for the Elderly, PPT = Reuben's Physical Performance Test.

Table 3. Association between cognitive test scores and serum 25(OH)D levels: linear regression analyses

	ROCF copy score [†]	ROCF recall score [†]	CTRM score [†]	DSST score [†]
	β coefficient (95% CI)			
25(OH)D (per 10nmol/L) [‡]	0.096 (0.049, 0.144)**	0.041 (-0.027, 0.109)	0.075 (0.026, 0.124)**	0.318 (0.235, 0.401)**
25(OH)D (per 10nmol/L) [§]	0.064 (-0.011, 0.138)	-0.021 (-0.163, 0.121)	-0.001 (-0.146, 0.144)	0.152 (0.051, 0.253)**
25(OH)D categories (nmol/L) [§]				
<i>Sufficiency</i> (≥ 75.0)	Reference	Reference	Reference	Reference
<i>Sub-optimal</i> (50.0 – 74.9)	-0.411 (-0.869, 0.047)	-0.116 (-0.574, 0.343)	-0.143 (-0.752, 0.465)	-0.759 (-1.313, -0.204)*
<i>Insufficiency</i> (25.0 – 49.9)	-0.329 (-0.729, 0.071)	0.260 (-0.660, 1.180)	0.084 (-0.874, 1.043)	-0.768 (-1.822, 0.287)
<i>Deficiency</i> (< 25.0)	-0.614 (-1.320, 0.092)	-0.462 (-1.374, 0.451)	-0.125 (-1.304, 1.054)	-1.404 (-2.681, -0.127)*

[†]Dependent variables

[‡]Adjusted for age

[§]Adjusted for age, education, depression, body mass index, physical activity, physical performance, smoking, alcohol consumption, centre and season

* $P < 0.05$, ** $P < 0.01$

ROCF = Rey-Osterrieth Complex Figure, CTRM = Camden Topographical Recognition Memory, DSST = Digit Symbol Substitution Test

Table 4. Association between cognitive test scores and serum 25(OH)D levels: spline regression analyses

	ROCF copy score [†]	ROCF recall score [†]	CTRM score [†]	DSST score [†]
	β coefficient (95% CI) – <i>per 10 nmol/L 25(OH)D</i>			
Spline model 1 [‡]				
25(OH)D $\leq$ 35 nmol/L	0.556 (0.207, 0.906)**	0.693 (0.192, 1.194)**	0.588 (0.228, 0.948)**	1.171 (0.562, 1.780)***
25(OH)D $\geq$ 35 nmol/L	0.059 (0.004, 0.114)*	-0.011 (-0.090, 0.068)	0.034 (-0.023, 0.091)	0.250 (0.154, 0.346)***
Spline model 2 [§]				
25(OH)D $\leq$ 35 nmol/L	0.192 (-0.349, 0.733)	0.323 (-0.023, 0.669)	0.150 (-0.229, 0.530)	0.398 (-0.451, 1.247)
25(OH)D $\geq$ 35 nmol/L	0.054 (-0.030, 0.138)	-0.046 (-0.201, 0.109)	-0.012 (-0.172, 0.148)	0.134 (-0.001, 0.275)

[†]Dependent variables[‡]Adjusted for age[§]Adjusted for age, education, depression, body mass index, physical activity, physical performance, smoking, alcohol consumption, centre and season* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Figure 1.

A

B

C

D

— LOWESS fit • Scatter

FIGURE LEGEND

Figure 1. Relationship between 25(OH)D and cognitive function: LOWESS plots adjusted for age (A = Rey-Osterrieth Complex Figure copy, B = Rey-Osterrieth Complex Figure recall, C = Camden Topographical Recognition Memory, D = Digit-Symbol Substitution). The vertical dotted line represents the estimated 25(OH)D level at which the slope of the vitamin D – cognition relationship changes.

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