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

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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  $60\pm 11$  years were included in the analysis. The  
14 mean 25(OH)D concentration was  $63\pm 31$  nmol/L. In age-adjusted linear regressions higher  
15 levels of 25(OH)D were associated with higher scores on the ROCF-copy ( $\beta$  per 10  
16 nmol/L=0.096; 95%CI 0.049-0.144), CTRM ( $\beta$  per 10 nmol/L=0.075; 95%CI 0.026-0.124)  
17 and DSST ( $\beta$  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 ( $\beta$  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 <sup>1</sup>. 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 <sup>2</sup>. While there are some data to suggest that vitamin D  
31 deficiency may have adverse effects on cognition or behaviour <sup>3,4</sup>, 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 <sup>5</sup>.

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)<sub>2</sub>D), principally in the kidneys <sup>6</sup>.  
40 Serum 25(OH)D levels are typically measured to establish the vitamin D status of an  
41 individual. 1,25(OH)<sub>2</sub>D 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 <sup>3</sup>. 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  $\alpha$ -  
46 hydroxylase, the final enzyme in the vitamin D activation pathway <sup>7</sup>. In vitro experiments  
47 have demonstrated that vitamin D has both neurotrophic and neuroprotective properties <sup>8,9</sup>,  
48 whilst long-term 1,25(OH)<sub>2</sub>D supplementation has been shown to retard hippocampal ageing  
49 in rats <sup>10</sup>. 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 <sup>11;12</sup>, 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 <sup>13</sup>. 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. <sup>14</sup> 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.

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## 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 previously<sup>15</sup>.  
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

101 function (see below). The IAQ included the Physical Activity Scale for the Elderly (PASE)  
102 <sup>16</sup> and the 21-item Beck Depression Inventory (BDI) to assess the presence and severity of  
103 depressive symptoms <sup>17</sup>. In addition, physical function was assessed during the clinic visit  
104 using Reuben's Physical Performance test (PPT) <sup>18</sup>. Height and weight were measured using  
105 standardized instruments.

106

### 107 **Serum 25-hydroxyvitamin D**

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

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### 115 **Tests of cognitive function**

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

122 Copying and delayed reproduction of the ROCF was used as a measure of visual perceptual  
123 abilities and visual memory <sup>19</sup>. The scoring criteria used was based upon Osterreith's original  
124 test procedure which defined 18 units of the drawing, assigning point values of 0 to 2 to each  
125 unit dependent upon the degree to which the units are correctly drawn and placed. Each



126 element of the ROCF test had a maximum score of 36. The CTRM, developed in order to  
127 measure the recognition component of visual memory retrieval, taps into the cortical  
128 component of visual memory<sup>20</sup>. The CTRM involves the presentation of 30 coloured  
129 photographs of outdoor topographical scenes, each for 3 seconds, followed by a three-way  
130 forced recognition component. The CTRM had a maximum score of 30. The DSST is a  
131 subtest adopted from the Wechsler Adult Intelligence Scales and provides a reliable measure  
132 of psychomotor speed and visual scanning<sup>21</sup>. Subjects were asked to make as many correct  
133 symbol-for-digit substitutions as possible within a 1 minute period.

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### 135 **Analysis**

136 Analyses were undertaken using the statistical package Intercooled STATA version 9.2  
137 (StataCorp, College Station, TX, USA). Subjects with missing cognitive and/or 25(OH)D  
138 data were excluded from the analysis. Age (years), age leaving education, and BMI (kg/m<sup>2</sup>)  
139 were analyzed as continuous variables. The BDI score was treated both as a continuous and  
140 categorical variable, with the latter based upon Beck's original cut-off scores<sup>17</sup>. Both the  
141 PASE and PPT scores were treated as continuous variables and also categorized into tertiles.  
142 25(OH)D was examined as a continuous variable and classified into sub-optimal (50-75  
143 nmol/L), insufficiency (25-49 nmol/L) and deficiency (< 25 nmol/L) groups broadly based on  
144 previously recommended cut-points<sup>22,23</sup>.

145 The relationship between 25(OH)D levels and cognitive function was initially evaluated  
146 graphically (while simultaneously adjusting for age) using the LOcally WEighted Scatterplot  
147 Smoothing (LOWESS) technique<sup>24</sup>. This approach, where linear regression is applied  
148 repeatedly to sequential small sections of the covariate-outcome relationship, is primarily  
149 exploratory. By reducing the influence of outliers, this technique provides a smooth fit to the  
150 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  $\beta$  coefficients and 95% confidence intervals (CI).

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176 **RESULTS**

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

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

186 Table 2 summarizes the influence of factors which could potentially confound the  
187 relationship between 25(OH)D levels and cognitive function. There were significant cross-  
188 sectional age-related decreases in all four cognitive test scores, but there was no association  
189 between 25(OH)D and age ( $\beta = -0.0135$ ; 95% CI -0.114, 0.087). Depression (BDI), BMI,  
190 physical activity (PASE), physical performance (PPT) and smoking were all consistently  
191 associated with both cognitive test scores and 25(OH)D. The CTRM and DSST scores,  
192 together with 25(OH)D levels, were additionally associated with drinking one or more  
193 alcoholic drink per week. As expected, 25(OH)D levels varied markedly by season of  
194 measurement peaking in the summer (mean 85.0 nmol/L) with a nadir in the winter (mean  
195 49.7 nmol/L). Mean serum 25(OH)D also varied significantly by geographical region  
196 (one-way ANOVA,  $P < 0.001$ ), with highest levels observed in northern and western  
197 European centres (72.6 nmol/L), lower levels in southern centres (60.5 nmol/L), and the  
198 lowest levels in eastern European centres (53.9 nmol/L).

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

201 levels were associated with higher scores in the ROCF copy test ( $\beta$  per 10 nmol/L = 0.096;  
202 95% CI 0.049, 0.144), the CTRM test ( $\beta$  per 10 nmol/L = 0.075; 95% CI 0.026, 0.124), and  
203 the DSST test ( $\beta$  per 10 nmol/L = 0.318; 95% CI 0.235, 0.401). However, after additional  
204 adjustments for age left education, depression, BMI, physical activity, physical performance,  
205 smoking, alcohol consumption, season and centre, increasing 25(OH)D was found to be only  
206 associated with higher DSST scores ( $\beta$  per 10 nmol/L = 0.152; 95% CI 0.051, 0.253). No  
207 association was observed between 25(OH)D and the ROCF copy, ROCF recall, and CTRM  
208 scores in fully adjusted models. The association between increasing 25(OH)D levels and  
209 higher DSST scores was also observed when 25(OH)D was classified into sub-optimal (50-  
210 74.9 nmol/L), insufficiency (25-49 nmol/L) and deficiency (< 25 nmol/L) groups. When  
211 subjects who reported taking any form of vitamin D/calcium supplements were excluded  
212 from the regression analyses the results remained the same (data not shown). Excluding the  
213 season and centre covariates from the regression analyses did not significantly affect the  
214 results (DSST test:  $\beta$  per 10 nmol/L = 0.126; 95% CI 0.043, 0.209). Although the ROCF  
215 copy data was left skewed, linear regression performs well in moderately large samples ( $n >$   
216 500) even from very non-Normal data<sup>25</sup>. However, to verify the results of the linear  
217 regression models we inverted the ROCF copy score and considered it as count data. The  
218 transformed data followed a negative binomial distribution (variance larger than the mean,  
219 data over-dispersed). We then carried out a negative binomial regression analysis using the  
220 same covariates as in the linear model and found the same result, i.e., no evidence of an  
221 independent association between the ROCF copy score and 25(OH)D (data not shown).

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

226 CI 0.135 0.381 [60-69 years];  $\beta$  per 10 nmol/L = 0.215; 95% CI 0.105, 0.326 [over 70 years])  
227 and not younger men (DSST test:  $\beta$  per 10 nmol/L = 0.044; 95% CI -0.153, 0.242 [40-49  
228 years];  $\beta$  per 10 nmol/L = 0.097; 95% CI -0.124, 0.317 [50-59 years]). There were no age by  
229 25(OH)D interactions for ROCF copy ( $P_{\text{interaction}} = 0.5$ ), ROCF recall ( $P_{\text{interaction}} = 0.7$ ) and  
230 CTRM ( $P_{\text{interaction}} = 0.3$ ), nor evidence of any significant season by 25(OH)D, centre by  
231 25(OH)D, or season by physical activity interaction effects for any of the four cognitive  
232 outcomes (data not shown).

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

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251 **DISCUSSION**

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

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

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

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

281 Although experimental models in animals and in vitro studies point to the biological  
282 plausibility of low 25(OH)D levels resulting in impaired cognitive performance, the  
283 mechanisms by which vitamin D affects adult cerebral functions remain unknown. Possible  
284 positive effects could be direct, as suggested by the observation that 1,25(OH)<sub>2</sub>D treatment  
285 increases choline acetyltransferase activity in rat brain nuclei <sup>26</sup>, or they could operate via a  
286 neuroprotective pathway, as demonstrated by the stimulation of neurotrophin production <sup>27</sup>  
287 and modulation of neuronal Ca<sup>2+</sup> homeostasis <sup>9</sup> by vitamin D. It is also plausible that  
288 additional factors not included in this analysis, such as parathyroid hormone (PTH) and  
289 calcium levels, may be involved in a more complex relationship with cognitive function.  
290 Evidence for such a relationship comes from the Tromsø health study, where a subset of  
291 subjects with secondary hyperparathyroidism were found to perform less well than normal  
292 controls on cognitive tests assessing working memory capacity and speed of information  
293 processing <sup>28</sup>. Although their data derives from individuals with an overt endocrine disorder,  
294 the Tromsø investigators found no evidence of any independent associations between  
295 25(OH)D and cognitive performance. They did observe, however, that low serum 25(OH)D  
296 was significantly associated with a higher depression score. The relationship between  
297 vitamin D and mood/depression has yet to be substantiated <sup>3</sup>, although we did find an inverse  
298 association between 25(OH)D levels and the BDI score (see Table 2).

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

301 possible that low serum 25(OH)D is merely a ‘risk marker’ and mechanistically separated  
302 from the biological processes resulting in declining cognitive performance in older adults.  
303 Low levels of 25(OH)D have been shown to be associated with a number of unfavourable  
304 health outcomes including, cardiovascular disease <sup>29</sup>, increased cancer risk and mortality <sup>30</sup>,  
305 sarcopenia <sup>31</sup>, and diabetes <sup>32</sup>. Several population-based studies have documented the  
306 relationship between cardiovascular and metabolic diseases and cognitive impairment <sup>33-35</sup>.  
307 When we additionally adjusted for self-reported cardiovascular disease and diabetes in our  
308 regression models the associations between 25(OH)D and DSST remained unchanged (data  
309 not shown). Conversely, cognitive impairment itself may contribute to lower levels of  
310 25(OH)D by reducing the level of outdoor physical activity and hence exposure to sunlight.  
311 Interestingly, there is increasing evidence supporting the association between vitamin D  
312 deficiency and decreased physical performance <sup>36</sup>, though cause and effect has yet to be  
313 differentiated. This raises the possibility that the positive association between 25(OH)D and  
314 psychomotor processing speed seen in our study may, in part, reflect improved physical  
315 motor speed. In an attempt to better control for this confounder in our data, we included only  
316 the score from the seven timed elements from the PPT (in place of the total score) in our  
317 regression models. However, the relationship between 25(OH)D and the DSST score  
318 remained unchanged, suggesting that the association may be more to do with neuronal  
319 processing speed than muscle/motor speed. Overall, we attempted to adjust for potential  
320 confounders in our analysis but cannot exclude the possibility that some of our findings may  
321 be due to unmeasured factors and/or residual confounding.

322 There remains uncertainty as to what level of vitamin D indicates a sub-optimal or deficient  
323 status. There is strong evidence that 25(OH)D levels below 12.5 nmol/L can result in bone  
324 diseases such as rickets in infants and osteomalacia in adults <sup>37</sup>, although to the best of our  
325 knowledge no previous studies have assessed cognitive function in subjects with such overt



326 hypovitaminosis D. Serum 25(OH)D concentrations above 50 nmol/L are generally defined  
327 as sufficient, although recent studies have suggested that serum 25(OH)D levels below 75  
328 nmol/L are sub-optimal for health<sup>38;39</sup>. Nonetheless, our data showing that 8 % of subjects  
329 have 25(OH)D levels below 25 nmol/L (deficiency) and 33 % have levels between 25 and 50  
330 nmol/L (insufficiency), broadly agrees with previous reports from Europe<sup>5;40</sup>. In terms of  
331 any relationship between a given threshold level of 25(OH)D and reduced cognitive  
332 performance, our data suggest that cognitive function is increasingly negatively associated  
333 with 25(OH)D at levels below around 35 nmol/L. However, in spline regression models  
334 adjusted for age and other covariates (see Table 4) none of the associations between  
335 25(OH)D (either above or below 35 nmol/L) and cognitive function were statistically  
336 significant. This may in part be due to insufficient power to detect the relatively modest  
337 changes in, for example the DSST score, with varying levels of 25(OH)D.

338 Although we have referred to 25(OH)D throughout, vitamin D exists in two common forms;  
339 vitamin D<sub>3</sub> (cholecalciferol) and vitamin D<sub>2</sub> (ergocalciferol). The form mainly produced in  
340 the skin and derived from natural dietary sources is vitamin D<sub>3</sub>, while the primary source of  
341 vitamin D<sub>2</sub> is multivitamin preparations and some fortified foods<sup>41</sup>. There have been  
342 conflicting reports whether vitamin D<sub>2</sub> and vitamin D<sub>3</sub> are equally effective at maintaining  
343 25(OH)D levels<sup>42;43</sup>, with another study suggesting that the D<sub>2</sub> form may actually have a  
344 negative effect on 25(OH)D<sub>3</sub> status<sup>44</sup>. The immunoassay used in our study has the same  
345 reported sensitivity to both forms of 25(OH)D (data from manufacturers manual), so the  
346 reported 25(OH)D levels here are the sum of 25(OH)D<sub>2</sub> + 25(OH)D<sub>3</sub>. As we have no  
347 information as to the relative abundance of each form within samples, we are unable to draw  
348 any conclusions as to the different associations, if any, between 25(OH)D<sub>2</sub> or 25(OH)D<sub>3</sub> and  
349 cognitive performance. Future studies in animals or in vitro may shed more light on whether

350 or not the D<sub>2</sub> and D<sub>3</sub> forms of vitamin D are differentially associated with brain function  
351 and/or development.

352 The main strengths of our study are that it is based on a large population-based sample and  
353 used uniform methods to not only assess vitamin D status, but also potential confounders  
354 such as depressed mood, physical activity and physical function. In addition, the battery of  
355 cognitive tests was specifically selected on the basis that they could be standardized across  
356 different centres and applied to individual subjects independent of language and culture.  
357 Methodological limitations inherent to the EMAS study have been described in detail  
358 previously <sup>15</sup>, although a number of specific factors need be considered in interpreting the  
359 results presented here. The overall response rate for participation in the study was 41%.  
360 Those who participated may have differed with respect to levels of cognitive function and  
361 also vitamin D status than those who did not participate and some caution, therefore, is  
362 needed in interpreting these data. The main findings, however, were based on an internal  
363 comparison of responders and therefore any selection factors were unlikely to have had any  
364 important effect on these data. Our analysis was restricted to vision-based tests of cognitive  
365 function. This was primarily to avoid language and cultural effects inherent in many text  
366 based tests, but also due to the practical constraints of a large, population-based study.  
367 Although serum 25(OH)D levels remain the accepted metric of vitamin D status, it is  
368 questionable whether the single 25(OH)D measurement performed in our study reflects each  
369 subject's long-term vitamin D status. The single 25(OH)D value would be an indicator of  
370 sun exposure and dietary intake over the past few weeks, rather than years, thereby increasing  
371 random measurement error. The net result of this would be to reduce the reported  
372 associations between 25(OH)D and cognitive function toward the null rather than produce  
373 spurious associations. Finally our results were obtained from a predominantly Caucasian  
374 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.

388

389

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400

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403

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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 <sup>2</sup> )	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 ( $\geq 75.0$ 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 ( $\geq 1$ day/week)	56.7
Vitamin D/Ca <sup>2+</sup> 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)
	$\beta$ coefficient (95% CI) <sup>†</sup>				
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 <sup>2</sup> )	-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)*

<sup>†</sup>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 <sup>†</sup>	ROCF recall score <sup>†</sup>	CTRM score <sup>†</sup>	DSST score <sup>†</sup>
	β coefficient (95% CI)			
25(OH)D (per 10nmol/L) <sup>‡</sup>	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) <sup>§</sup>	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) <sup>§</sup>				
<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)*

<sup>†</sup>Dependent variables

<sup>‡</sup>Adjusted for age

<sup>§</sup>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

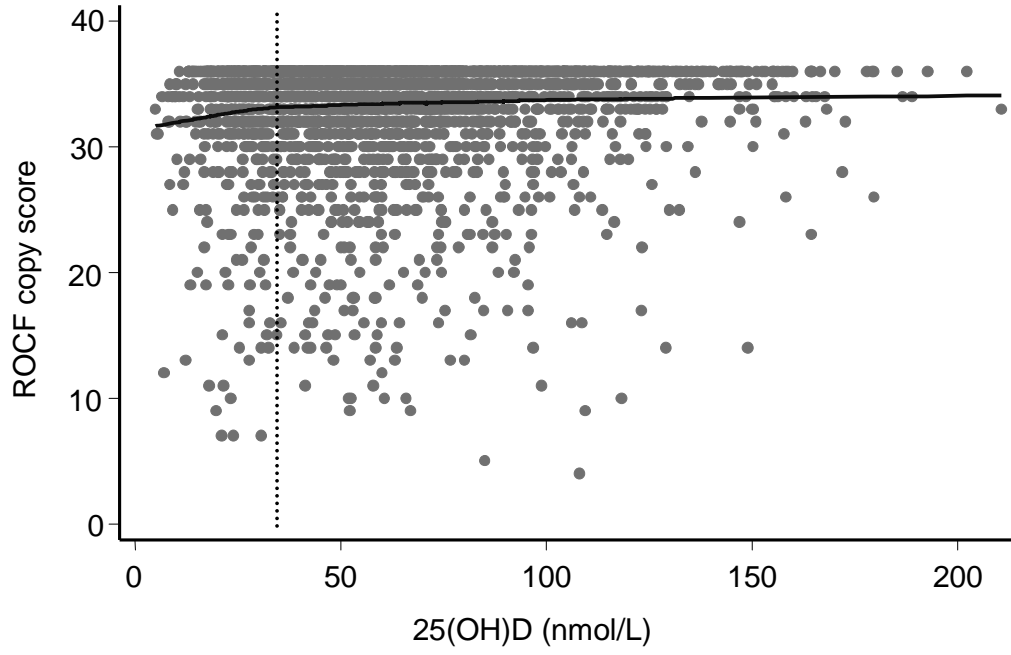
	ROCF copy score <sup>†</sup>	ROCF recall score <sup>†</sup>	CTRM score <sup>†</sup>	DSST score <sup>†</sup>
	$\beta$ coefficient (95% CI) – per 10 nmol/L 25(OH)D			
Spline model 1 <sup>‡</sup>				
25(OH)D $\leq$ 35 nmol/L	0.556 (0.207, 0.906)**	0.693 (0.192, 1.194)**	0.588 (0.228, 0.948)**	1.171 (0.562, 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 <sup>§</sup>				
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)

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

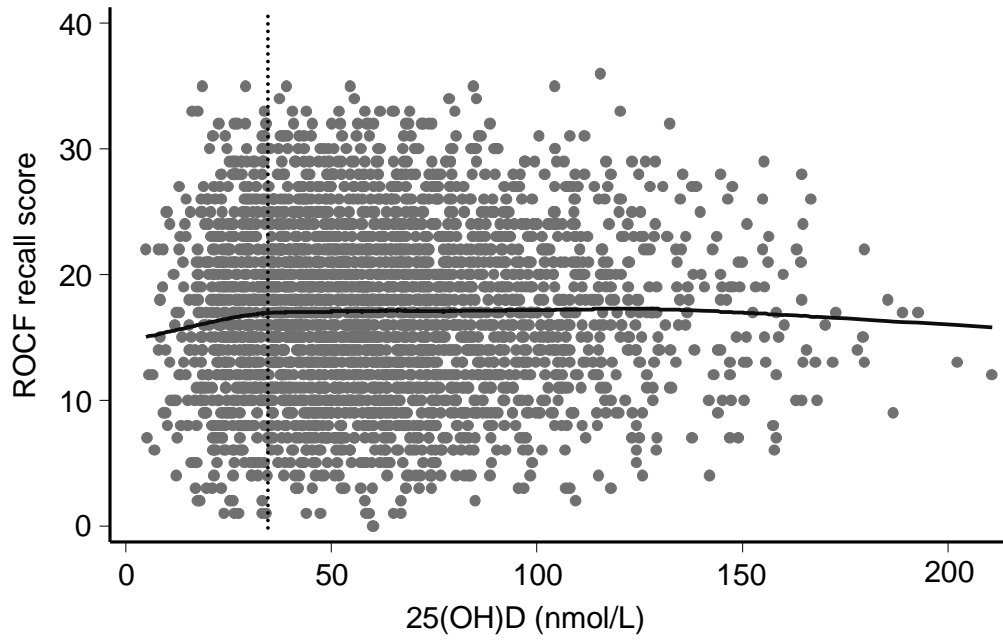


Figure 1.

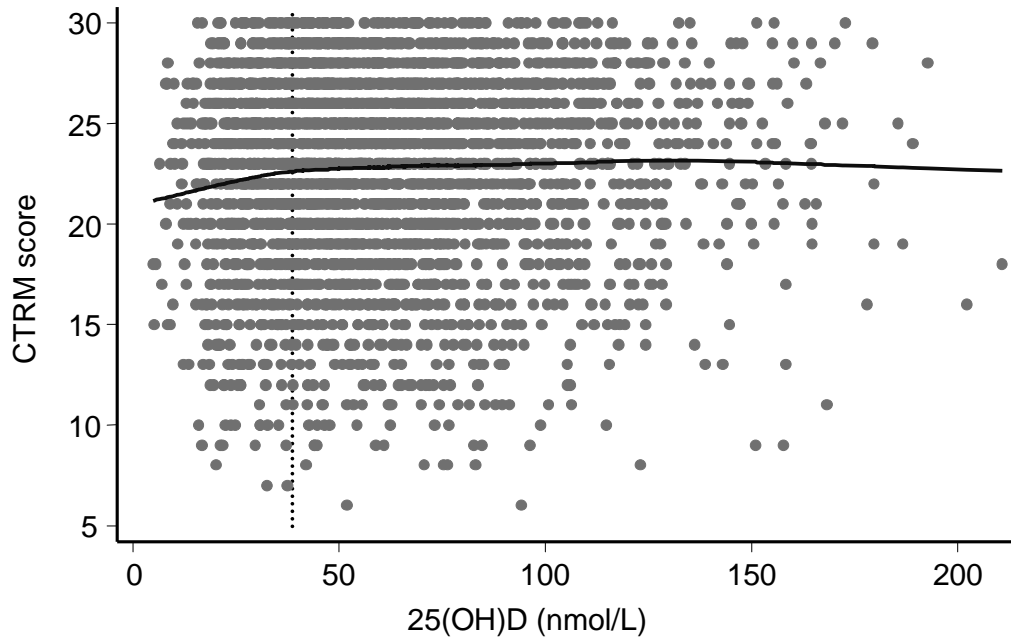
A



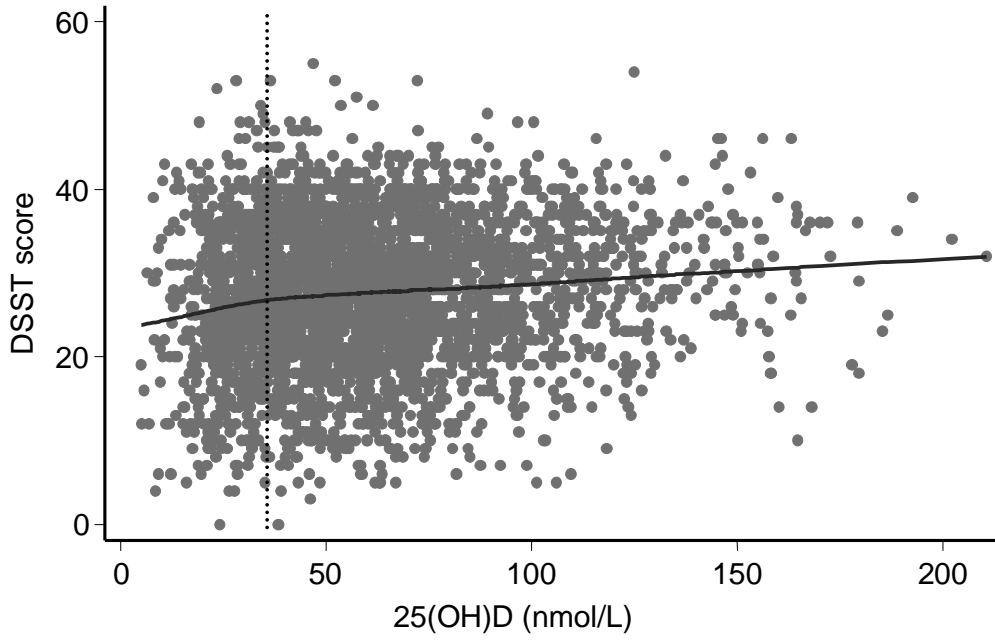
B



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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