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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 ± 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

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

114

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 ¹⁹. 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 memory²⁰. 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 scanning²¹. Subjects were asked to make as many correct
133 symbol-for-digit substitutions as possible within a 1 minute period.

134

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/m²)
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 scores¹⁷. 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^{22,23}.

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) technique²⁴. 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 β 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 by 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 (β per 10 nmol/L = 0.096;
202 95% CI 0.049, 0.144), the CTRM test (β per 10 nmol/L = 0.075; 95% CI 0.026, 0.124), and
203 the DSST test (β 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 (β 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: β 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 data²⁵. 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: β per 10 nmol/L = 0.258; 95%

226 CI 0.135 0.381 [60-69 years]; β per 10 nmol/L = 0.215; 95% CI 0.105, 0.326 [over 70 years])
227 and not younger men (DSST test: β per 10 nmol/L = 0.044; 95% CI -0.153, 0.242 [40-49
228 years]; β 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 β 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 β coefficients reached statistical
243 significance.

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251 **DISCUSSION**

252 In this population-based study of European men aged ≥ 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 ~ 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 ¹⁴ 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) ¹⁴, 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)₂D treatment
285 increases choline acetyltransferase activity in rat brain nuclei ²⁶, or they could operate via a
286 neuroprotective pathway, as demonstrated by the stimulation of neurotrophin production ²⁷
287 and modulation of neuronal Ca²⁺ homeostasis ⁹ 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 ²⁸. 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 ³, 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 disease²⁹, increased cancer risk and mortality³⁰,
305 sarcopenia³¹, and diabetes³². Several population-based studies have documented the
306 relationship between cardiovascular and metabolic diseases and cognitive impairment³³⁻³⁵.
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 performance³⁶, 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 adults³⁷, 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^{38;39}. 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^{5;40}. 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 D₃ (cholecalciferol) and vitamin D₂ (ergocalciferol). The form mainly produced in
340 the skin and derived from natural dietary sources is vitamin D₃, while the primary source of
341 vitamin D₂ is multivitamin preparations and some fortified foods⁴¹. There have been
342 conflicting reports whether vitamin D₂ and vitamin D₃ are equally effective at maintaining
343 25(OH)D levels^{42;43}, with another study suggesting that the D₂ form may actually have a
344 negative effect on 25(OH)D₃ status⁴⁴. 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)D₂ + 25(OH)D₃. 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)D₂ or 25(OH)D₃ and
349 cognitive performance. Future studies in animals or in vitro may shed more light on whether

350 or not the D₂ and D₃ 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 ¹⁵, 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 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

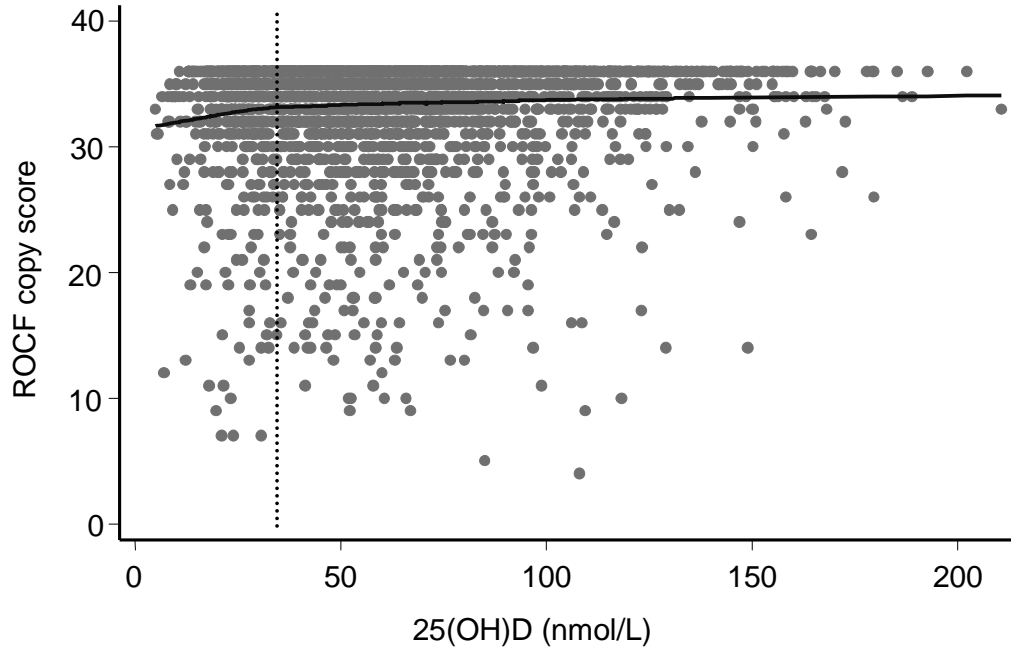
Table 4. Association between cognitive test scores and serum 25(OH)D levels: spline regression analyses

	ROCF copy score [†]	ROCF recall score [†]	CTRM score [†]	DSST score [†]
	β coefficient (95% CI) – per 10 nmol/L 25(OH)D			
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)

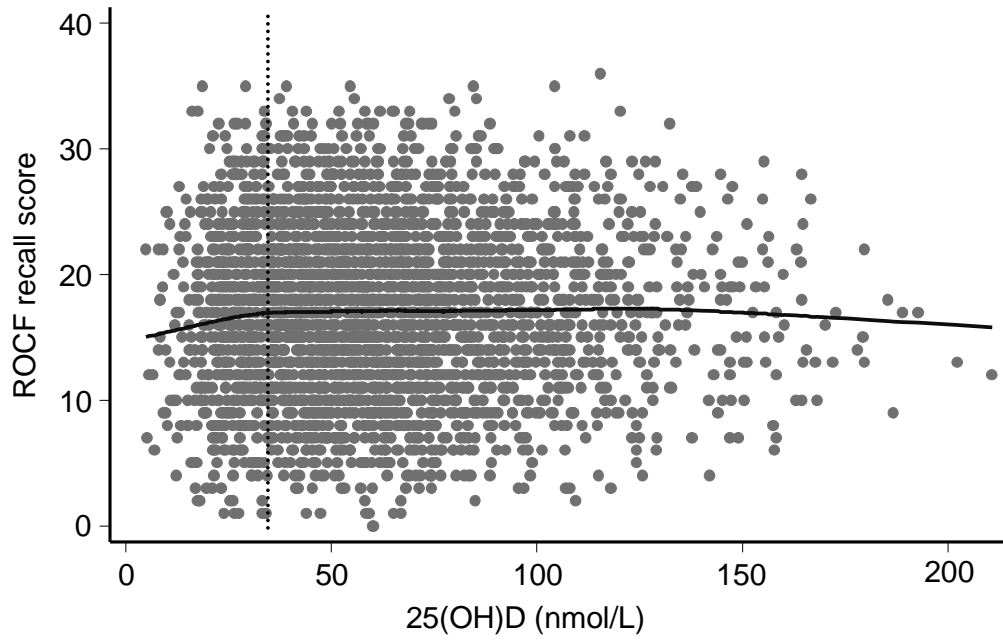
[†]Dependent variables[‡]Adjusted for age[§]Adjusted for age, education, depression, body mass index, physical activity, physical performance, smoking, alcohol consumption, centre and season* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Figure 1.

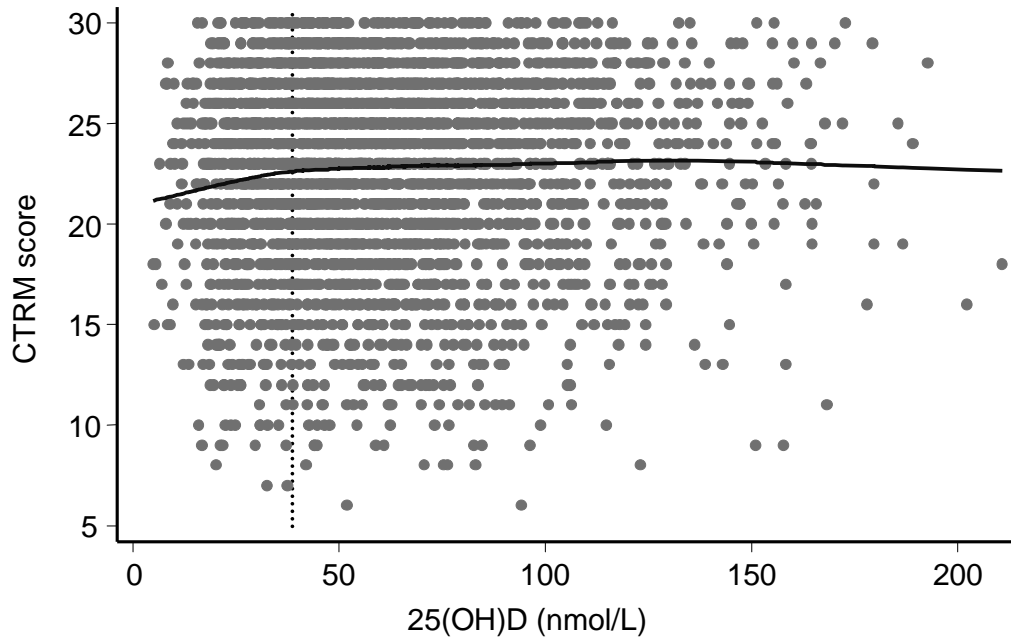
A



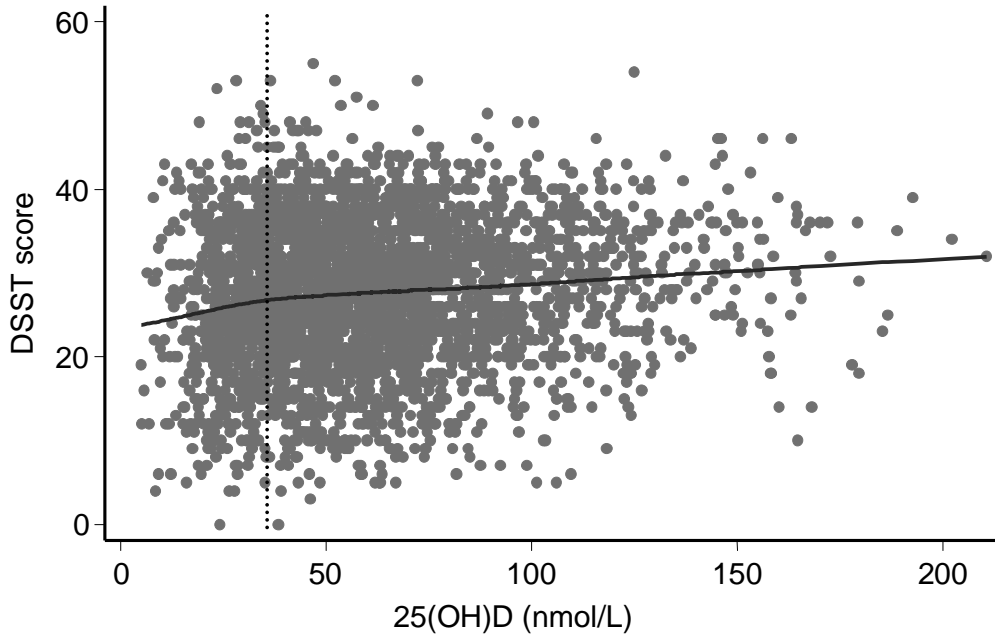
B



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.

References

1. Lips P. Vitamin D deficiency and secondary hyperparathyroidism in the elderly: consequences for bone loss and fractures and therapeutic implications. *Endocr.Rev.* 2001;**22**:477-501.
2. Holick MF. Evolution and function of vitamin D. *Recent Results Cancer Res.* 2003;**164**:3-28.
3. McCann JC, Ames BN. Is there convincing biological or behavioral evidence linking vitamin D deficiency to brain dysfunction? *FASEB J.* 2008;**22**:982-1001.
4. Kalueff AV, Tuohimaa P. Neurosteroid hormone vitamin D and its utility in clinical nutrition. *Curr.Opin.Clin.Nutr.Metab Care* 2007;**10**:12-9.
5. Zittermann A. Vitamin D in preventive medicine: are we ignoring the evidence? *Br.J.Nutr.* 2003;**89**:552-72.
6. Jones G, Strugnell SA, DeLuca HF. Current understanding of the molecular actions of vitamin D. *Physiol Rev.* 1998;**78**:1193-231.
7. Eyles DW, Smith S, Kinobe R, Hewison M, McGrath JJ. Distribution of the vitamin D receptor and 1 alpha-hydroxylase in human brain. *J.Chem.Neuroanat.* 2005;**29**:21-30.
8. Kalueff AV, Minasyan A, Keisala T, Kuuslahti M, Miettinen S, Tuohimaa P. The vitamin D neuroendocrine system as a target for novel neurotropic drugs. *CNS.Neurol.Disord.Drug Targets.* 2006;**5**:363-71.

9. Brewer LD, Thibault V, Chen KC, Langub MC, Landfield PW, Porter NM. Vitamin D hormone confers neuroprotection in parallel with downregulation of L-type calcium channel expression in hippocampal neurons. *J.Neurosci.* 2001;**21**:98-108.
10. Brewer LD, Porter NM, Kerr DS, Landfield PW, Thibault O. Chronic 1alpha,25-(OH)₂ vitamin D₃ treatment reduces Ca²⁺ -mediated hippocampal biomarkers of aging. *Cell Calcium* 2006;**40**:277-86.
11. Wilkins CH, Sheline YI, Roe CM, Birge SJ, Morris JC. Vitamin D deficiency is associated with low mood and worse cognitive performance in older adults. *Am.J.Geriatr.Psychiatry* 2006;**14**:1032-40.
12. Oudshoorn C, Mattace-Raso FU, van d, V, Colin EM, van der Cammen TJ. Higher serum vitamin D₃ levels are associated with better cognitive test performance in patients with Alzheimer's disease. *Dement.Geriatr.Cogn Disord.* 2008;**25**:539-43.
13. Przybelski RJ, Binkley NC. Is vitamin D important for preserving cognition? A positive correlation of serum 25-hydroxyvitamin D concentration with cognitive function. *Arch.Biochem.Biophys.* 2007;**460**:202-5.
14. McGrath J, Scragg R, Chant D, Eyles D, Burne T, Obradovic D. No association between serum 25-hydroxyvitamin D₃ level and performance on psychometric tests in NHANES III. *Neuroepidemiology* 2007;**29**:49-54.
15. Lee DM, O'Neill TW, Pye SR, Silman AJ, Finn JD, Pendleton N, Tajar A, Bartfai G, Casanueva F, Forti G, Giwercman A, Huhtaniemi IT, Kula K, Punab M, Boonen S, Vanderschueren D, Wu FC. The European Male Ageing Study (EMAS): design, methods and recruitment. *Int.J.Androl* 2008.

16. Washburn RA, Smith KW, Jette AM, Janney CA. The Physical Activity Scale for the Elderly (PASE): development and evaluation. *J.Clin.Epidemiol.* 1993;**46**:153-62.
17. Beck AT, Steer RA, Brown GK. *Manual for the Beck Depression Inventory-II*. San Antonio, TX: Psychological Corporation, 1996.
18. Reuben DB, Siu AL. An objective measure of physical function of elderly outpatients. The Physical Performance Test. *J.Am.Geriatr.Soc.* 1990;**38**:1105-12.
19. Osterrieth PA. Le test de copie d'une figure complexe. *Arch Psychol* 1944;**30**:206-356.
20. Warrington EK. *The Camden memory tests manual*. Hove: Psychology Press, 1996.
21. Uiterwijk JM. *WAIS-III-NL/V*. Lisse: Swets & Zeitlinger, 2001.
22. Lips P. Which circulating level of 25-hydroxyvitamin D is appropriate? *J.Steroid Biochem.Mol.Biol.* 2004;**89-90**:611-4.
23. Holick MF. Vitamin D deficiency. *N.Engl.J.Med.* 2007;**357**:266-81.
24. Cleveland WS. Robust Locally Weighted Fitting and Smoothing Scatterplots. *Journal of the American Statistical Association* 1979;**74**:829-36.
25. Lumley T, Diehr P, Emerson S, Chen L. The importance of the normality assumption in large public health data sets. *Annu.Rev.Public Health* 2002;**23**:151-69.
26. Sonnenberg J, Luine VN, Krey LC, Christakos S. 1,25-Dihydroxyvitamin D3 treatment results in increased choline acetyltransferase activity in specific brain nuclei. *Endocrinology* 1986;**118**:1433-9.

27. Wang Y, Chiang YH, Su TP, Hayashi T, Morales M, Hoffer BJ, Lin SZ. Vitamin D(3) attenuates cortical infarction induced by middle cerebral arterial ligation in rats. *Neuropharmacology* 2000;**39**:873-80.
28. Jorde R, Waterloo K, Saleh F, Haug E, Svartberg J. Neuropsychological function in relation to serum parathyroid hormone and serum 25-hydroxyvitamin D levels. The Tromso study. *J.Neurol.* 2006;**253**:464-70.
29. Holick MF. Vitamin D: importance in the prevention of cancers, type 1 diabetes, heart disease, and osteoporosis. *Am.J.Clin.Nutr.* 2004;**79**:362-71.
30. Grant WB. The likely role of vitamin D from solar ultraviolet-B irradiance in increasing cancer survival. *Anticancer Res.* 2006;**26**:2605-14.
31. Visser M, Deeg DJ, Lips P. Low vitamin D and high parathyroid hormone levels as determinants of loss of muscle strength and muscle mass (sarcopenia): the Longitudinal Aging Study Amsterdam. *J.Clin.Endocrinol.Metab* 2003;**88**:5766-72.
32. Scragg R, Sowers M, Bell C. Serum 25-hydroxyvitamin D, diabetes, and ethnicity in the Third National Health and Nutrition Examination Survey. *Diabetes Care* 2004;**27**:2813-8.
33. Breteler MM, Claus JJ, Grobbee DE, Hofman A. Cardiovascular disease and distribution of cognitive function in elderly people: the Rotterdam Study. *BMJ* 1994;**308**:1604-8.
34. Muller M, Grobbee DE, Aleman A, Bots M, van der Schouw YT. Cardiovascular disease and cognitive performance in middle-aged and elderly men. *Atherosclerosis* 2007;**190**:143-9.

35. Verhaegen P, Borchelt M, Smith J. Relation between cardiovascular and metabolic disease and cognition in very old age: cross-sectional and longitudinal findings from the berlin aging study. *Health Psychol.* 2003;**22**:559-69.
36. Houston DK, Cesari M, Ferrucci L, Cherubini A, Maggio D, Bartali B, Johnson MA, Schwartz GG, Kritchevsky SB. Association between vitamin D status and physical performance: the InCHIANTI study. *J.Gerontol.A Biol.Sci.Med.Sci.* 2007;**62**:440-6.
37. Scharla SH. Prevalence of subclinical vitamin D deficiency in different European countries. *Osteoporos.Int.* 1998;**8 Suppl 2**:S7-12.
38. Bischoff-Ferrari HA. The 25-hydroxyvitamin D threshold for better health. *J.Steroid Biochem.Mol.Biol.* 2007;**103**:614-9.
39. Heaney RP. The case for improving vitamin D status. *J.Steroid Biochem.Mol.Biol.* 2007;**103**:635-41.
40. van der Wielen RP, Lowik MR, van den BH, de Groot LC, Haller J, Moreiras O, van Staveren WA. Serum vitamin D concentrations among elderly people in Europe. *Lancet* 1995;**346**:207-10.
41. Holick MF. High prevalence of vitamin D inadequacy and implications for health. *Mayo Clin.Proc.* 2006;**81**:353-73.
42. Trang HM, Cole DE, Rubin LA, Pierratos A, Siu S, Vieth R. Evidence that vitamin D3 increases serum 25-hydroxyvitamin D more efficiently than does vitamin D2. *Am.J.Clin.Nutr.* 1998;**68**:854-8.
43. Holick MF, Biancuzzo RM, Chen TC, Klein EK, Young A, Bibuld D, Reitz R, Salameh W, Ameri A, Tannenbaum AD. Vitamin D2 is as effective as vitamin D3 in maintaining

circulating concentrations of 25-hydroxyvitamin D. *J.Clin.Endocrinol.Metab* 2008;**93**:677-81.

44. Armas LA, Hollis BW, Heaney RP. Vitamin D2 is much less effective than vitamin D3 in humans. *J.Clin.Endocrinol.Metab* 2004;**89**:5387-91.