The association between serum vitamin D levels and physical performance in Dutch elderly: a cross-sectional study
The association between serum vitamin D levels and physical performance in Dutch elderly: a cross-sectional study

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Prologue

In this thesis, written for the bachelor Nutrition and Dietetics at the Amsterdam University of Applied Sciences, we describe our research on vitamin D and muscle function. In a period of 20 weeks we studied the association between 25-hydroxyvitamin D and muscle function, using data of the B-PROOF study from Wageningen University. B-PROOF is a randomized placebo controlled trial which is originally designed to study the effect of vitamin B12 and folic acid on osteoporotic fractures in Dutch elderly. This thesis consists of an overview of relevant literature and a concept article.

We would like to use this opportunity to show our gratitude to our supervisor M.F. Engberink for the positive guidance, relevant feedback and support.

Furthermore, we would like to thank Elske Brouwer and Anouk Vaes, for the warm welcome, positive guiding and useful feedback. Also our gratitude goes out to the B-PROOF team for the pleasant days during our practical work for B-PROOF.

Priya Dewansingh and Saimi van Straalen
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## List of abbreviations and relevant definitions

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<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,25(OH)_2D</td>
<td>Calcitriol</td>
</tr>
<tr>
<td>25(OH)D</td>
<td>Calcidiol</td>
</tr>
<tr>
<td>25-hydroxyvitamin D</td>
<td>Calcidiol</td>
</tr>
<tr>
<td>BMI</td>
<td>Body Mass Index</td>
</tr>
<tr>
<td>B-PROOF</td>
<td>Prevention of Osteoporotic Fractures</td>
</tr>
<tr>
<td>EMC</td>
<td>Erasmus Medical Center</td>
</tr>
<tr>
<td>EUR</td>
<td>Erasmus University Rotterdam</td>
</tr>
<tr>
<td>FFQ</td>
<td>Food Frequency Questionnaire</td>
</tr>
<tr>
<td>Hcy</td>
<td>Homocysteïne</td>
</tr>
<tr>
<td>LAPAQ</td>
<td>LASA physical activity questionnaire</td>
</tr>
<tr>
<td>SPPB</td>
<td>Short Physical Performance Battery</td>
</tr>
<tr>
<td>Vitamin B_12</td>
<td>Cobalamin</td>
</tr>
<tr>
<td>Vitamin D_2</td>
<td>Ergocalciferol</td>
</tr>
<tr>
<td>Vitamin D_3</td>
<td>Cholecalciferol</td>
</tr>
<tr>
<td>VUmc</td>
<td>VU University Medical Center</td>
</tr>
<tr>
<td>WU</td>
<td>Wageningen University</td>
</tr>
</tbody>
</table>
Abstract

Background
It has been suggested that vitamin D might play a functional role in muscle tissue. The precise physiological function and relevance to muscle physiology is still unclear. The aim of this study was to examine whether serum 25(OH)D is related to physical performance.

Methods
The present study had a cross-sectional design. The sample size consisted of 2919 community dwelling elderly aged 65 years and older from the B-PROOF study. Serum 25(OH)D was assessed by blood analysis. Physical performance was assessed using 4 tests: chair stand test, modified Romberg test, hand grip strength and walking speed. Linear regression analysis was used to determine the association with adjustment for several potential confounders.

Results
After adjusting for age, gender, BMI and education, a linear association was found for serum 25(OH)D levels <50 nmol/L. Physical performance was lower in participants with serum 25(OH)D levels <50 nmol/L (β=-1.0 ; p<0.001) compared to participants with serum 25(OH)D levels >75 nmol/L (reference). The category 50-75 nmol/L showed a β=-0.2, but the association was not significantly related to physical performance in comparison with the reference group.

Conclusion
Serum 25(OH)D levels <50 nmol/L are associated with lower physical performance in elderly. Serum vitamin D levels >50 nmol/L do not show a significant association with physical performance. Because 45,5% of the study population had serum 25(OH)D levels <50 nmol/L, prevention strategies could be aimed at in this group to prevent vitamin D deficiency.

Keywords: 25(OH)D · elderly · physical performance
**Introduction**

The amount of hip fractures increases with age, both in women and men. (1) In the Netherlands, the incidence of hip fractures is higher for women than for men. Incidence of hip fractures increases from 2 per 1.000 for women aged 65-69 years to 28 per 1.000 for women aged 85 years and older. In men, in the same age groups, it increases from 1 to 16 per 1.000. (1, 2) Incidence in the Netherlands is comparable to other countries in West-Europe. (3, 4) One of the contributing factors for increasing numbers of hip fractures may be deficiency of vitamin D, because vitamin D deficiency is a major contributor to falls, which is a common cause of fractures. (5, 6)

In the Netherlands, about 85% of elderly aged 50 years and older, living in residential cares and nursing homes, have a deficient serum vitamin D level. This is about double the percentage of deficiency in community dwelling elderly. (7-9) Elderly are at risk for vitamin D deficiency because of low sunshine exposure, decreased skin capacity to synthesize vitamin D, decreased absorption of vitamin D and decreased amount or activity of vitamin D receptors in peripheral tissues. (10-13) When deficiency turns severe, it can result in osteomalacia for adults. This disease leads to weak and painful bones caused by decreased bone mineralization. Vitamin D deficiency can also cause loss of bone mass and osteoporosis because of secondary hyperparathyroidism, which is mainly the case with elderly. In addition, severe deficiency of vitamin D can cause muscle weakness and muscle cramps, which can result in falls. (14, 15)

Vitamin D increases calcium absorption from the gut, which stimulates mineralization of bone. However, recently it has been suggested that vitamin D might also play a functional role in many other tissues. One of these tissues is muscle tissue, which has a related function to physical performance. (16-18) In bone and gastrointestinal tract are specific receptors for 1,25(OH)2D. It has been suggested that these specific receptors are also present in skeletal muscle tissue, but this is not commonly agreed. (13, 19, 20) The precise physiological function and relevance to muscle physiology is still unclear. It appears that vitamin D receptors are activated in skeletal muscle resulting in protein synthesis and subsequent muscle cell growth. This mechanism decreases with age, partially explaining declining muscle function. (21)

The most important source of vitamin D is cutaneous synthesis. Vitamin D is synthesized in human skin that is exposed to UV radiation. (22, 23) There are several external and internal factors influencing cutaneous synthesis. External factors are: latitude, season, time of day, ozone amount, cloud amount, aerosol and albedo (reflectivity of surface). (23) Internal factors are: skin type, age, clothing, use of sunscreen, and sometimes a choice of external factors, e.g. when to expose unprotected skin. (23) Another source of vitamin D is dietary intake. Vitamin D is mostly found in animal products such as dairy products and fatty fish. In the Netherlands only margarine and cooking
fats are fortified with vitamin D. (15) Elderly aged 70 years and older need 20 µg vitamin D per day to avoid or solve deficiency. (24-26)

There are different forms of vitamin D. Both cholecalciferol (vitamin D₃) and ergocalciferol (vitamin D₂) are present in human nutrition, but vitamin D₃ is also synthesized in human skin. Humans cannot produce vitamin D₂. (22) (16) The metabolite of vitamin D₂ and D₃ is 25(OH)D (calcidiol). This metabolite is produced in the liver and is the best indicator of vitamin D status in blood. (22) Half-life time is estimated to be 10 to 40 days, and concentration is not under tight homeostatic regulation. (27) Vitamin D supply from cutaneous synthesis, diet and supplementation, metabolism, excretion and sequestration into body tissues is best reflected by serum 25(OH)D. (27) It has been suggested that vitamin D₃ is more effective in raising serum 25(OH)D levels than vitamin D₂. (28)

A possible positive effect of vitamin D supplementation on muscle function can be important to public health, because it may increase muscle strength of elderly. This might lead to better physical performance, fewer falls and decreased amount of fractures. Quality of life can be improved and health care costs can be reduced. Therefore, the main aim of this study is to examine whether vitamin D status is related to physical performance (muscle strength and balance) in elderly aged 65 years and older. We hypothesize that a vitamin D level above 50 nmol/L improves physical performance in elderly aged 65 years and older. (22)
Methods

Study population for data-analyses

For the present study, data was collected within the B-PROOF (PRevention Of Osteoporotic Fractures) study. B-PROOF is a randomized double-blind placebo controlled trial that studies the effects of vitamin B₁₂ and folic acid on the prevention of osteoporotic fractures in elderly. The primary outcome of the trial is time to first osteoporotic fracture. In view of secondary outcomes of the B-PROOF study also physical performance was assessed during baseline measurements.

Figure 1. Recruitment and baseline measurements in participants of the B-PROOF study
Recruitment took place from August 2008 until March 2011. Most participants were recruited via registries of municipalities in the area of the research centres. Inhabitants aged 65 years and older were invited by mail. Approximately 69,000 people were addressed to participate in the B-PROOF study of which 6242 persons responded positive and were screened. This resulted in 3027 eligible participants. Before the start of baseline measurements, 108 participants withdrew consent, mostly for personal reasons like disease and not willing to fill in questionnaires. Furthermore, inhabitants of elderly homes in the area of Rotterdam, Amsterdam and Wageningen were invited to participate, after providing information brochures and information meetings. In addition, elderly who participated in previous studies of the research centres were approached. All participants gave written informed consent before the start of the intervention. In March 2011, 2919 participants (aged 65 years and older) were included for baseline measurements (Figure 1). All participants had an elevated plasma homocysteine concentration (Hcy ≥12 µmol/L). Participants had to be competent to make their own decisions and could not be bedridden or wheelchair bound.

Other inclusion and exclusion criteria of the B-PROOF study are listed in Table 1. The WU Medical Ethics Committee approved the study protocol, and the Medical Ethics committees of Erasmus Medical Center EMC and VU University Medical Center (VUmc) gave approval for local feasibility.

### Table 1. Inclusion and exclusion criteria B-PROOF study

<table>
<thead>
<tr>
<th>Inclusion criteria</th>
<th>Exclusion criteria:</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Men and women, aged 65 years and older</td>
<td>• Immobilization: bedridden, wheelchair bound</td>
</tr>
<tr>
<td>• Compliance for tablet intake &gt;85%, 4-6 weeks prior to start of trial</td>
<td>• Cancer diagnosis within last 5 year, except skin cancer as basal cell carcinoma and squamous cell carcinoma</td>
</tr>
<tr>
<td>• Competent to make own decisions</td>
<td>• Serum creatinine &gt;150 µmol/L</td>
</tr>
<tr>
<td>• Elevated homocysteine level (≥12 µmol/L and ≤50 µmol/L)</td>
<td>• Current or recent (&lt; 4 months) use of supplements with very high doses of vitamin B12 (intramuscular injections) or folic acid (&gt;300 µg)</td>
</tr>
<tr>
<td></td>
<td>• Participation in other intervention studies</td>
</tr>
</tbody>
</table>

### Blood serum

Blood samples were used to determine serum 25(OH)D levels. Blood was collected in the morning, at the research centres in a fasting state or after consuming a small breakfast. For detailed breakfast guidelines see Appendix 1 (written in Dutch). Blood samples were drawn by a skilled nurse to obtain plasma, serum anduffy coats.

Serum 25(OH)D was measured by isotope dilution - online solid-phase extraction liquid chromatography- tandem mass spectrometry (ID-XLC-MS/MS). Serum 25(OH)D was released from its binding protein(s) and a deuterated internal standard (25(OH)D3-d6) was added. The inter-assay
The coefficient of variation was 9% at the level of 25 nmol/L and 6% at the level of 63 nmol/L. Creatine levels were assessed using enzymatic colorimetric Roche CREA plus assay. Within 4 hours, the plasma was kept frozen at -80°C for later analysis.

**Physical performance tests**

Four physical performance tests were used to assess muscle strength and balance. Participants were scored by performance of tests. The range of score was 0-16 points, 4 points per test, and participant was assigned 0 points if a test was not performed. Range of scores were based on quartiles of the baseline data. Scoring on physical performance tests is well associated with falls, cognitive function, visual impairment and frailty and has shown to be a valid measurement method of physical performance in elderly. (29-32) The four standardized tests were:

**Chair stand test**

Participants were asked to stand up and sit down 5 times as quickly as possible, while their hands were folded across their chest. Time taken to complete the test was recorded in seconds. If the test was completed in 13 seconds or more, the participants scored 1 point, 2 points were assigned if the test was completed between 11-12 seconds, 3 points for completing in 9-10 seconds and 4 points for 8 seconds and faster.

**Modified Romberg test**

Participants were asked to maintain balance in 4 different positions for 10 seconds. The positions were: hip wide position, side-by-side position, semi-tandem position and full tandem position. Each position was performed first with eyes open and then closed, both for 10 seconds. If participants lost balance, the time was stopped. The amount of time they succeeded in performing the test was recorded in seconds. Participants who performed a semi-tandem position were assigned 1 point. Participants who could perform a tandem position with eyes open for 2 seconds or less scored 2 points. If the tandem position with eyes open was maintained for 3-9 seconds, 3 points were scored and maximum of 4 points were scored if tandem position with eyes open was maintained 10 seconds. The tandem test performed with eyes open was chosen for this study, because literature was not clear about using the test with eyes open or closed for analysis. (33, 34)

**Hand grip strength**

Hand grip strength was measured in kilograms, using the strain-gauged dynamometer (Takei, TKK 5401, Takei Scientific Instruments Co. Ltd., Japan, inter observer CV = 5%). Both hands were measured twice to define an average. If the participant achieved 22.9 kg or less, a score of 1 point
was assigned, between 22.1-28.3 kg 2 point were scored, 3 points between 28.4-38.1 kg and 4 points for 38.2 kg or more were scored.

*Walking speed*

Participants were asked to walk besides a cord (3 m), turn 180°, and walk back. Participants were not allowed to run, but were supposed to walk as fast as they can. Time was measured in seconds. If the test was completed in 8 seconds or more, the participants scored 1 point, 2 points were assigned if completed between 6-7 seconds, 3 points for 5 seconds and 4 points for 4 seconds or faster.

*Potential confounders*

Possible confounders were age, gender, body mass index (BMI), level of education, alcohol use, smoking, chronic diseases, physical activity, season of blood collection and serum creatine. BMI was calculated as weight (kg)/length (m)^2. Smoking was categorized in never, former and current smoker. Classification of alcohol use was based on number of glasses per week. Alcohol consumption was categorized as 0, 1-3, 4-7 or more than 8. Data about alcohol usage and smoking were based on self-reports. Education level was in years of education, and categorized into three categories: low (≤9 years), intermediate (10–12 years), and high level (>12 years). Physical activity was assessed by using LASA Physical Activity Questionnaire (LAPAQ). This is a validated questionnaire to measure physical activity in elderly people (35). Activities included walking, cycling, gardening, sports and light and heavy household activities. Frequency and duration of each activity during the last two weeks were assessed. Physical activity was calculated in minutes/day and kcal/day. Season of blood collection was categorized into two periods, summer (April-September) and winter (October-March). Through blood collection, serum creatinine was assessed. Dietary vitamin D and calcium intake were assessed using a Food Frequency Questionnaire (FFQ).

*Statistical analyses*

*Baseline characteristics*

Based upon the effects of vitamin D on fracture risks the institute of medicine considers 25(OH)D levels below 50 nmol/L as being insufficient for elderly. (36) The level of 75 nmol/L is based on the guidelines of the Endocrine Society. (37) Based on these guidelines serum 25(OH)D was divided into three groups: <50, 50-75, and >75 nmol/L for statistical analysis. Population characteristics are reported as mean with standard deviation (± SD), number or percentages and were calculated across the different levels of vitamin D. Chi-squared test was used to compare gender over serum 25(OH)D categories. Analysis of variance was performed on age, BMI, education, physical activity, season of blood collection and creatine. Smoking status and alcohol consumption were compared with the
Kruskal Wallis test. In this way, the study population was described and potential confounding factors could be identified.

Statistical analysis
Multiple linear regression analysis were used for cross-sectional analysis to determine an association between serum vitamin D and physical performance. Serum 25(OH)D was divided into three groups: <50, 50-75, and >75 nmol/L as the reference group. Normality assumptions of the crude model were tested by histograms and probability plots. Potential confounders added to the crude model are: age, gender, BMI, level of education, season of blood collection and serum creatine. Changes in the regression coefficient (β) that were caused by confounders of ≥10% were retained in the model. All analysis were performed using SPSS statistics 19.0 and a p-value <0.05 was considered statistically significant.
Results

Study population

In total, 2919 participants completed baseline measurements and were included in the present analysis. The mean age of the study population was 77.3 ± 7.0 years for women and 75.8 ± 6.2 years for men. The mean serum 25(OH)D level was 53.9 nmol/L for women (±24.8; p=0.433) and 57.4 nmol/L (±24.7) for men. Serum 25(OH) D levels lower than 50 nmol/L is defined as vitamin D deficiency, which was present in 45.4% of the participants. Characteristics of the study population are presented in table 2.

Table 2. Characteristics of participants of the B-PROOF study

<table>
<thead>
<tr>
<th></th>
<th>&lt;50 nmol/L</th>
<th>50-75 nmol/L</th>
<th>&gt;75 nmol/L</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender, Men</td>
<td>N=1325</td>
<td>N=997</td>
<td>N=597</td>
<td></td>
</tr>
<tr>
<td>Age, years</td>
<td>612 (46.2)</td>
<td>528 (53.0)</td>
<td>319 (53.4)</td>
<td>0.001</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>75.1 ± 7.1</td>
<td>73.5 ± 6.0</td>
<td>72.8 ± 5.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>&gt;75 nmol/L</td>
<td>27.5 ± 4.3</td>
<td>27.1 ± 3.6</td>
<td>26.4 ± 3.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Education, Years</td>
<td>≤9</td>
<td>728 (54.9)</td>
<td>541 (54.3)</td>
<td>278 (46.6)</td>
</tr>
<tr>
<td></td>
<td>10-12</td>
<td>269 (20.3)</td>
<td>216 (21.7)</td>
<td>130 (21.8)</td>
</tr>
<tr>
<td></td>
<td>≥13</td>
<td>328 (24.8)</td>
<td>240 (24.1)</td>
<td>189 (31.7)</td>
</tr>
<tr>
<td>Smoking status</td>
<td>≤9</td>
<td>468 (35.3)</td>
<td>329 (33.0)</td>
<td>192 (32.2)</td>
</tr>
<tr>
<td></td>
<td>Current smoker</td>
<td>144 (10.9)</td>
<td>89 (8.9)</td>
<td>48 (8.0)</td>
</tr>
<tr>
<td></td>
<td>Former smoker</td>
<td>713 (53.8)</td>
<td>579 (58.1)</td>
<td>357 (59.8)</td>
</tr>
<tr>
<td>Alcohol consumption, glasses/week</td>
<td>≤9</td>
<td>239 (18)</td>
<td>399 (10)</td>
<td>68 (10.7)</td>
</tr>
<tr>
<td></td>
<td>1-3</td>
<td>1022 (77.2)</td>
<td>550 (85.2)</td>
<td>497 (83.3)</td>
</tr>
<tr>
<td></td>
<td>4-7</td>
<td>62 (4.7)</td>
<td>44 (4.4)</td>
<td>30 (5.7)</td>
</tr>
<tr>
<td></td>
<td>≥8</td>
<td>2 (0.2)</td>
<td>4 (0.4)</td>
<td>2 (0.3)</td>
</tr>
<tr>
<td>Physical activity, min/day</td>
<td>148.6 ± 99.2</td>
<td>165.7 ± 112.9</td>
<td>171.9 ± 104.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Season of blood collection</td>
<td>Summer</td>
<td>235(18.1)</td>
<td>318(33.0)</td>
<td>225(38.9)</td>
</tr>
<tr>
<td></td>
<td>Winter</td>
<td>1064(81.9)</td>
<td>646(67.0)</td>
<td>353(61.1)</td>
</tr>
</tbody>
</table>

Values are means ± SD, number (percentage)
Vitamin D level was positively associated with physical activity ($p < 0.001$) and negatively with age ($p < 0.001$), BMI ($p < 0.001$) and alcohol intake ($p = 0.005$).

Table 3. Multiple linear regression analysis of serum 25(OH)D as a predictor of physical performance

<table>
<thead>
<tr>
<th></th>
<th>&lt;50 nmol/L</th>
<th>50-75 nmol/L</th>
<th>&gt;75 nmol/L</th>
<th>$p$-trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 1</td>
<td>-1.3 (-1.6, -1.0)*</td>
<td>-0.3 (-0.6, 0.0)</td>
<td>Reference</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Model 2</td>
<td>-0.6 (-0.9, -0.4)*</td>
<td>-0.2 (-0.4, 0.1)</td>
<td>Reference</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Model 3</td>
<td>-1.0 (-1.4, -0.6)*</td>
<td>-0.2 (-0.6, 0.2)</td>
<td>Reference</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data expressed as $\beta$ (95% confidence intervals).

Model 1: Not adjusted
Model 2: Adjusted for age and gender
Model 3: Adjusted for age, gender, BMI and education in years

* $p<0.001$

Serum 25(OH)D and physical performance

After adjustment for age, gender, BMI and education, a serum vitamin D level below 50 nmol/L was significantly associated with a lower physical performance, meaning that participants with serum 25(OH)D below 50 nmol/L had a 1.0 lower score on the 16-point scale, compared to the reference group (Figure 2). The association between serum 25(OH)D and physical performance is presented in Table 3. Linear trends were assessed across vitamin D categories by entering the median values within the categories into the model as linear covariate.

Significant differences were observed between the categories serum 25(OH)D <50 nmol/L and the reference group for all models. No change in $\beta$ was observed when adding the possible confounders: physical activity, season of blood collection (summer and winter) and creatinine ($\mu$mol/L) into the model.
Discussion

The present cross-sectional analyses in 2919 elderly aged above 65 years showed a strong significant inverse association between serum 25(OH)D below 50 nmol/L and physical performance compared to the serum 25(OH)D category above 75 nmol/L (reference group), after adjusting the model for age, gender, BMI and education years. Participants with a serum 25(OH)D below 50 nmol/L scored on average -1.0 point compared to the reference group.

The results do partly confirm the hypothesis which states that a serum vitamin D level above 50 nmol/L improves physical performance in elderly aged 65 years and older. In this study, also participants with a serum 25(OH)D above 50 nmol/L seemed to have lower physical performance score, but this was not significant. The results are in agreement with the results found in a study of Wicherts et al. a cross-sectional study with 1234 participant. This study observed a significant inverse association between serum 25(OH)D below 50 nmol/L and a non-significant association with serum levels above 50 nmol/L compared to the serum 25(OH)D levels above 75 nmol/L (reference group). (34)

The Pro.V.A. study is a cross-sectional study with 2694 participants. Similar results were found in this study, only this study focused on the difference in gender. Men in the Pro.V.A. study had more preserved muscle strength than the women of the same age, therefore women could already be below strength and speed thresholds at which vitamin D might have a significant impact. Despite differences in physical performance, the pattern of association between 25(OH)D levels and physical performance is similar in both genders. (38)

This study has several strengths and limitations. One of the main strengths is the large sample size, consisting of an equal number of men and women. Also the number of potential confounders (age, gender, BMI, education, season of blood collection, creatinine and physical activity) that were investigated is a positive aspect of this study. Different potential confounders were added to the model to determine if they would influence the regression model. Age could be considered a confounder because the ability to synthesize vitamin D decreases with age. Frail elderly people may not be able to synthesize sufficient vitamin D in the skin. (39) Additionally, elderly are more likely to have a low energy intake and with that a lower vitamin D intake compared to younger persons. (40) Gender could be a confounder because men are less likely to have vitamin D deficiency. Differences can be caused by men having more preserved muscle strength compared to women. Therefore, women are more likely to score below speed and strength thresholds, at which vitamin D might have a significant influence. BMI was add as another confounder. In studies of obese adolescents, elevated BMI has been correlated with vitamin D deficiency. (41, 42) The study of Wortsman et al. had 19 obese subjects and assessed whether obesity alters the cutaneous production of vitamin D3 (cholecalciferol) or the intestinal absorption of vitamin D2. The study showed adipose tissue can
‘trap’ cholecalciferol, which might result in lower serum 25(OH)D levels. (43) Season of blood collection is investigated as a potential confounder. Though more blood was collected in the winter period, additional adjustment for season of blood collection did not change the model. It can be hypothesized that less active participants are weaker and go less often outside into the sunlight, so a smaller amount of vitamin D is synthesized in their skin. Another strength is that physical performance was measured by 4 different tests. A maximum score of 16 points could be achieved, where in other studies only a maximum of 8 or 12 points could be scored. (33) The regression analysis of this study could therefore be considered more sensitive in comparison. However, the values of these different studies cannot be compared directly because of different tests being used.

The main limitation of this study is that participants were relative healthy. Participants were only included if they were ambulatory, because they had to travel to the research centres and to perform physical activity tests. Furthermore, only participants of 65 years and older were included, so this study can only be generalized to apparently healthy elderly aged above 65 years. Another limitation is that blood collection took place in the period October 2008 till March 2011. This resulted in baseline measurements not being obtained at the same time of the season. More blood was collected in the winter period (72.6%) than during summer (27.4%). In this period, 25(OH)D levels are usually lower because of less sun exposure. Therefore, the distribution of 25(OH)D levels is affected. The third limitation in this study is that linear regression analysis is performed on the total physical performance tests. These tests are not analysed separately. For this reason it is not possible to determine which test showed a strong association or no association at all. The LAPAQ is a questionnaire that measures physical activity in elderly people. The questionnaire is valid when information is gathered by a face-to-face interview. (35) In this study, participants received the LAPAQ by mail to fill in the form. They were asked to bring the forms to the research center where it was discussed with an interviewer. This may have influenced the validity of the questionnaire, because participants could overestimate their physical activity pattern. Finally, this study has a cross-sectional design and therefore reverse causation is possible. To confirm an effect, a randomized controlled trial is warranted.

Several studies described the meaningful change for the effect of vitamin D on physical performance. These several studies examined different tests independently or as Short Physical Performance Battery (SPPB). In these studies, the maximum score was 12 points, while in this study the maximum score was 16 points. Furthermore, this study cannot describe a change, because of a cross-sectional design. However, this does not imply that the result ($\beta=-1.0; p<0.001$) is not relevant for the population. The 4 physical performance tests represent tasks and activities in the daily life of elderly. A significant association in the <50 nmol/L category might imply an association in daily life.
Other studies with a randomized controlled trial design should study the effect and meaningful change over time of vitamin D and physical performance.

For this study, vitamin D deficiency has been defined as serum 25(OH)D levels below 50 nmol/L, but no generally accepted criteria for vitamin D deficiency is set yet. Until now, proposals for adequate vitamin D were based on levels of Parathyroid hormone (PTH), bone mineral and fracture risk and not on physical performance or muscle strength. Research shows a serum 25(OH)D level of at least 40 nmol/L appears to be desirable for an optimal lower extremity function, whereas levels of 90-100 nmol/L appear to be beneficial. (44)

The general public should be made more aware of the high prevalence of vitamin D deficiency and insufficiency amongst elderly. Awareness can be increased by communication to elderly about vitamin D by physicians, hospitals and nursing homes, dieticians and other relevant parties. More effort should be put in early detection and treatment by supplementation. This will result in lower health care costs, because vitamin D status can be considered a predictive value for mortality, nursing home admissions and fractures. (45-47) Deficiency could be prevented by supplementation. The Dutch health council (Gezondheidsraad) advises elderly of 70 years and older to take 20 µg vitamin D supplements per day. Elderly under 70 years are advised 10 µg vitamin D supplements per day. (48) In the Netherlands, only margarine is fortified with vitamin D, whereas in other non-European countries it is common to fortify other food products. (49) The efficiency of food fortification with vitamin D could be evaluated, especially with regard to 25(OH)D status in elderly.

In conclusion, 25(OH)D levels lower than 50 nmol/L are associated with lower physical performance in elderly. Serum vitamin D levels above 50 nmol/L did show an association with physical performance, but this was not significant. Large part of the study population had 25(OH)D levels below 50 nmol/L. This is common in Dutch elderly and should be prevented by early detection and supplementation.
References

7. Manders M. Nutritional care in old age, the effect of supplementation on nutritional status and performances. 2006. (Available by: Fight malnutrition)


Richtlijnen voor een licht verteerbaar ontbijt

Het onderzoeksteam vraagt u om de *avond die vooraf gaat aan de bloedafname* niet meer te eten na 20:00 uur. Tussen 20:00 uur en 23:00 uur mag u nog alles drinken met uitzondering van alcoholische dranken. Na 23:00 uur mag u alleen water drinken. *Tijdens de dag van de bloedafname* is het toegestaan tot een uur voor de bloedafname een licht verteerbaar ontbijt te nuttigen, maar natuurlijk kunt u ook besluiten niet te ontbijten.

Als u bijvoorbeeld om 8:30 uur voor de bloedafname wordt verwacht, dan dient u het lichte ontbijt vóór 7:30 uur genuttigd te hebben en u mag alleen water drinken in de tussentijd. Indien u medicijnen gebruikt kunt u deze uiteraard gewoon innemen.

Bij het ontbijt voorafgaande aan de bloedafname is het toegestaan om de volgende producten te nuttigen:

- 1-2 Boterhammen, beschuit, roggebrood of koek, eventueel besmeerd met een klein beetje halvarine of margarine, eventueel belegd met:
  - Zoet beleg zoals jam, stroop of hagelslag
  - Kaas
- Een kommetje magere of halfvolle yoghurt of melk, eventueel met havermout
- Een glas magere of halfvolle melk of karnemelk
- Thee

Bij dit ontbijt is het **NIET** toegestaan de volgende producten te nuttigen:

- Koffie
- Pindakaas
- Fruit of vruchtenappèn
- Vleeswaren (alle soorten) en ei
- Ontbijtgranen producten zoals cornflakes, All-bran, krokante muesli, Cruesli
- Producten die verrijkt zijn met vitamine B12 of foliumzuur (dit staat vermeld op de verpakking van het product).

Indien u nog vragen hebt over deze richtlijnen, dan kunt u contact opnemen met Miranda Hillen of Janneke van Wijngaarden, telefoonnummer 0317-485869.
## APPENDIX 2. Literature schedule

<table>
<thead>
<tr>
<th>Study name, auteur, year</th>
<th>Population</th>
<th>Design</th>
<th>Method</th>
<th>Physical performances</th>
<th>Results</th>
<th>Discussion</th>
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</thead>
<tbody>
<tr>
<td>Genderm P RK, Obrant KJ et al. (1)</td>
<td>n=1044 ambulatory, independently living, woman of 75 years old.</td>
<td>Cross-sectional</td>
<td>(chemiluminescence)</td>
<td>-Modified Romberg - balance test - speed walk (30 M, 1 turn) - muscle strength</td>
<td>25OHD levels below 50 nmol/L and 75 nmol/L correlated with inferior balance ($r=0.15$, $p&lt;0.001$), gait speed ($r=0.17$, $p&lt;0.001$), and activity level ($r=0.15$, $p&lt;0.001$). And levels below 50 nmol/l associated with increased risk of fracture HR=2.04(1.04-4.04).</td>
<td>The mean 25OHD level was high compared to other studies. In women with 25OHD levels below 50nmol/l a high incidence of fractures was found. Association between 25OHD levels and bone quality was not found. Increased fracture risk may be due to the association between low 25OHD and fall-associated variables. Group women: considered healthy, 25% refrained from participating due to illness.</td>
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<tr>
<td>Houston DK, Tooze JA, Hausman DB et al. (2)</td>
<td>n=368 Community-dwelling men and women aged 70-89 years.</td>
<td>Cross-sectional</td>
<td>Radioimmunooassay (RIA)</td>
<td>-short physical performance battery (SPPS) including: progressively more challenging standing balance tasks held for 10 seconds each, time to complete five repeated chair stands, and the faster of two 400-m walks to assess usual gait speed.</td>
<td>Vitamin D deficiency at baseline was associated with significantly slower 400-m walk speed (0.79 vs. 0.86 m/s, $p=0.003$). Individuals who were vitamin D deficient at baseline but not follow-up had a significant improvement in SPPB scores(SE:+0.55, $p=0.01$). Change SPPB over 12 months did not differ by baseline.</td>
<td>Participants were recruited to be at risk of disability (selection bias, not generalizable to general population); Recruitment throughout the year, residual confounding by season, not to be ruled out. No information why 25OHD status improved. Strength of study: 25OHD and physical performance were collected at baseline, 6-month and 12 month follow up allowing examination between change in 25OHD and physical performance.</td>
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<td>Dhesi JK, Bearne LM, Moniz C et al. (3)</td>
<td>n=60 Visitors of the falls clinic, and 20 healthy, age matched volunteers as control.</td>
<td>Cross-sectional</td>
<td>IDS Gamma-B 25OH immunoassay with I125 25OH vitamin D label. CV≤5 %</td>
<td>-Aggregate Functional Performance Time (50-ft walk and rising from a 42-cm-high chair, and walking 50 ft and an ascent and descent of 13 stairs) -isometric quadriceps strength and activation -Postural stability -Psychomotor function—choice reaction time</td>
<td>Within subjects who fall, those with 25OH &lt; 12 ug/liter had slower functional performance (51.0 vs. 31.8, t= 4.97, $p&lt;0.05$), weaker quadriceps (223 vs. 271, $t=2.35$, $p=0.02$) slower reaction times(166 vs.0.98, t= 2.86. $p=0.05$).</td>
<td>PHT, but not 25OHD, was found to be an independent variable for muscle strength. Some participants had fractures at baseline. So it is difficult to say if improvement was not caused by the reduction of pain as the fracture healed.</td>
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<tr>
<td>Study</td>
<td>Design</td>
<td>Subjects</td>
<td>Methods</td>
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<td>Allali F, El Aichaoui S, Khazani H et al. (4)</td>
<td>Cross-sectional</td>
<td>n=415 women aged 24 to 77</td>
<td>Chemiluminescence and intra- and interassay coefficient of variation</td>
<td>No significant association between vitamin D and physical performance was found. During the summer season, vitamin D deficiency is very common in adult healthy Moroccan women. Hypovitaminosis D was observed in: -80% of premenopausal women -92% of menopausal women. Socio-demographic and nutritional data were assessed indirectly using a questionnaire. All the subjects were volunteers, so the sample is not representative for the general population.</td>
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<tr>
<td>Mastaglia S.R. SM, Muzio D, et al. (5)</td>
<td>Cross-sectional</td>
<td>n=54 Women aged over 65 years</td>
<td>Radioimmunoassay and intra-assay coefficients of variation</td>
<td>No significant association between vitamin D and physical performance was found. During the summer season, vitamin D deficiency is very common in adult healthy Moroccan women. Hypovitaminosis D was observed in: -80% of premenopausal women -92% of menopausal women. Socio-demographic and nutritional data were assessed indirectly using a questionnaire. All the subjects were volunteers, so the sample is not representative for the general population.</td>
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<td>Boersma D, Demontiero O, Mohtasham Amiri Z et al. (6)</td>
<td>Cross-sectional</td>
<td>n= 145 (73-88 y), at least one fall in past 6 months</td>
<td>Chemiluminescence and intra-assay coefficient of variation</td>
<td>Falls, based on self-report (recall bias). Institutionalized and cognitively impaired subjects were excluded. Two groups high risk of falling.</td>
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<td>Zamboni M, Zoico E, Tosoni P et al. (7)</td>
<td>Cross-sectional</td>
<td>n=269, 68-75 years</td>
<td>Handheld dynamometer and knee strength</td>
<td>Not an RCT. This study failed to observe relation between 25(OH)D, muscle strength and reported disability in men. Small number of men compared to women.</td>
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<tr>
<td>Study</td>
<td>Sample Size</td>
<td>Design</td>
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<td>Toffanello ED, Perisinotto E, Sergi G et al. (8)</td>
<td>n=2694 Italian population, 65 years</td>
<td>Cross-sectional</td>
<td>RIA, intra-assay and interassay coefficients of variation: 8.1%-10.2%</td>
<td>No differences in 25(OH)D for tandem test and quadriceps strength. Significant linear associations for TCS test (women:p&lt;0.0001, men: 0.03), gait speed, 6mW test (&lt;0.001 both genders) and handgrip test (women:p&lt;0.0009, men: 0.0001).</td>
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<tr>
<td>Wicherts IS, van Schoor NM, Boeke AJ et al. (9)</td>
<td>n=1234 Man and women between 55-85 years old.</td>
<td>Cross-sectional and longitudinal design (3-yr follow-up) within LASA</td>
<td>Competitive protein-binding assay. Interassay coefficient of variation was 10%.</td>
<td>Nearly 50% of the study population had low serum 25(OH)D levels. Significant after adjustment for age, gender, and physical activity. Chair stands and tandem stand: significantly related to 25(OH)D, up to 10 ng/ml; walking test significantly related up to 20 ng/ml.</td>
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<td>Ceglia L, Chiu GR, Harris SS et al. (10)</td>
<td>n=1115 Adult men, aged 30-79. Black, Hispanic and white, randomly selected</td>
<td>Populatio n based, observational survey.</td>
<td>competitive binding protein (CPB) assay without prior chromatography</td>
<td>In an age adjusted model, 25(OH)D was related to physical function (p=0.03). In race/ethnic- and multivariate adjusted models, 25(OH)D was not related.</td>
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<td>Verreault R, Semba RD, Volpato S et al. (11)</td>
<td>n=938 moderately to severely disabled women</td>
<td>Prospecti ve cohort study, 3 year follow up.</td>
<td>radio receptor assay</td>
<td>Only women included. Large aged differences. Lots of non-Caucasian participants. Young participants with more muscle strength.</td>
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(39.4±24.1, 56.5±37.5, p<0.001). In women, strength of dominant arm and leg sign. associated with 25(OH)D (r=0.19, p<0.05, r=0.17, p<0.05).
Databases that were included for search were: Cochrane and MEDLINE. The included articles had a randomized controlled, cross-sectional or cohort design. Only articles written in English were included.

Key words: 25-hydroxyvitamin D, elderly, muscle strength.
Reference


