A randomized controlled trial to the effect of two-year vitamin B12 and folic acid supplementation on physical performance, strength, and falling: additional findings from the B-PROOF study


Submitted for publication
Abstract

Background
Elevated homocysteine concentrations are associated with a decline in physical function in elderly persons. Homocysteine-lowering therapy may slow down this decline.

Objective
This study aimed to examine the effect of a 2-year intervention of vitamin B12 and folic acid supplementation on physical performance, handgrip strength and risk of falling in elderly subjects in a double-blind, randomized placebo-controlled trial.

Design
Participants aged ≥65 years with elevated plasma homocysteine concentrations (12-50 µmol/L (n=2,919)) were randomly assigned to daily supplementation of 500 µg vitamin B12, 400 µg folic acid, and 600 IU vitamin D3, or to placebo with 600 IU vitamin D3. Physical performance (range 0-12) and handgrip strength (kg) were measured at baseline and after 2 years. Falls were reported prospectively on a research calendar. Intention-to-treat (primary) and per-protocol (secondary) analyses were performed. Interaction effects of age, sex, and homocysteine with treatment were tested.

Results
Physical performance level and handgrip strength significantly decreased during the follow-up period, but this decline did not differ between the groups. Moreover, time to first fall and the number of falls per participant were not significantly different (HR: 1.0, 95% CI: 0.9, 1.2, and OR: 1.0, 95% CI: 0.9, 1.1, respectively). Per-protocol analyses identified an interaction effect with age on physical performance.

Conclusion
Two-year supplementation of vitamin B12 and folic acid was neither effective in reducing the age-related decline in physical performance and handgrip strength, nor in the prevention of falling in elderly persons. The effect on physical performance in compliant persons >80 years, should be further tested in future studies.
Introduction

Poor physical function in the elderly is associated with adverse health outcomes, including falls and fractures, a reduced quality of life, nursing home admission and mortality.\textsuperscript{1-5} Circulating homocysteine (Hcy) concentrations increase with age, and elevated concentrations occur in up to 50\% of persons over the age of 60.\textsuperscript{6} In the past decade, the association between Hcy and physical function has been examined in several cross-sectional and longitudinal observational studies. These studies have consistently shown that elevated Hcy concentrations are associated with accelerated decline in mobility and muscle strength.\textsuperscript{7-13} Hcy-induced neurological deterioration or adverse muscle characteristics is thought to account for the observed associations.\textsuperscript{14,15}

Elevated Hcy concentrations can effectively be reduced with vitamin B12 and/or folic acid supplementation.\textsuperscript{16} If the relation between Hcy and physical function is causal, supplementation can be expected to prevent or slow down decline in physical function in the elderly, and this should be tested in randomized controlled trials. One such intervention study investigated the effect of randomly assigned B-vitamin supplementation versus placebo on movement performance in a population of elderly,\textsuperscript{17} and did not demonstrate a difference. However, participants were only followed for 4 months, which might be too short for physical decline to occur. A second intervention study reported no difference in fall rate after 2 years of vitamin B12 and folic acid supplementation versus placebo in a highly selective population of stroke survivors.\textsuperscript{18}

The aim of the current study was to investigate the effect of a 2-year intervention of daily vitamin B12 and folic acid supplementation on changes in physical performance, handgrip strength and the risk of falling in a mildly hyperhomocysteinemic elderly population.

Subjects and Methods

Design and study sample
The B-vitamins in the PRevention Of Osteoporotic Fractures (B-PROOF) study is a double-blind, randomized, placebo-controlled trial. The main outcome of the trial was the incidence of osteoporotic fractures; physical performance, handgrip strength, and falls were pre-specified secondary outcomes. The study design of the B-PROOF study has been published elsewhere.\textsuperscript{19} In short, the intervention comprised daily oral supplementation of 500 µg vitamin B12, 400 µg folic acid and 600 IU vitamin D for a period of 2 years. The placebo tablet contained 600 IU vitamin D only. Vitamin D was added to ensure normal vitamin D concentrations. The tablets were indistinguishable with respect to smell, taste, and appearance. The random allocation sequence was computer-generated. Randomization was carried out by an independent research dietician to a one-by-one allocation ratio in
blocks of 24, after stratification for sex, age (65-80 years, ≥80 years), study location, and baseline Hcy concentration (12-18 µmol, ≥18 µmol). Both the participants and the research team, including examiners and data-analyzers, were blinded for the treatment allocation. Persons were included if they were aged ≥65 years, and had plasma Hcy concentration of 12-50 µmol/L. Persons were excluded if they had a history of cancer in the last 5 years, except non-melanoma skin cancer, if they were bedridden or wheelchair bound, or had a serum creatinine concentration >150 µmol/L. A total of 2,919 participants were included from October 2008 to March 2011. Subsequently, follow-up was completed in March 2013. Measurements were performed at baseline and after 2 years by trained examiners. Furthermore, the participants recorded falls and fractures prospectively on a research calendar. Persons who dropped-out during follow-up were asked if they agreed to continue to complete the calendar and to participate at the 2-year follow-up measurements.

The B-PROOF study was carried out by a consortium of researchers from Wageningen University (WU), VU University Medical Center (VUmc), and Erasmus MC, the Netherlands. The B-PROOF study was approved by the Medical Ethics Committee of WU, and the Medical Ethics Committees of Erasmus MC and VUmc gave additional approval for local feasibility. All participants gave written informed consent. B-PROOF is registered with the Netherlands Trial Register (NTR1333), and the ClinicalTrials.gov (NCT 00696514).

**Outcomes**

Physical performance was assessed with three function tests (walking test, chair stands, tandem stand), resembling the Short Physical Performance Battery. During the walking test, the time needed to walk three meters back and forth as quickly as possible was measured. The chair-stands contained the measurement of the time needed to stand up from and sit down on a chair for five successive times, without using hands. During the tandem stand, the ability to stand with the feet right in front of each other for ten seconds with eyes open was measured. Scores on the walking test (0-4) and the chair stands (0-4) were based on timed quartiles of the study sample. The tandem stand was categorized as follows: unable or able to hold position less than 4 s (score 0), able to hold position for 4-9 s (score 2), able to hold position for at least 10 s (score 4). Total physical performance score was calculated by summing up the scores of the three individual components, and consequently ranged from zero (low physical performance) to twelve (high physical performance).

Handgrip strength (kg) was measured with a hand held dynamometer (Takei TKK 5401, Takei Scientific Instruments CO. Ltd., Tokyo, Japan). Two maximum handgrip strength trials were performed with each hand. Handgrip strength was calculated as the mean of the highest scores of both hands.

Falling was assessed prospectively during the study period. Participants reported falls weekly on the research calendar. Calendar pages were returned to the study centers every
three months. Participants were contacted if calendars were incomplete or unclear. Drop-outs with no further calendar information after drop-out were assigned as lost-to-follow-up. Time to first and time to second fall were regarded as outcomes as well as the number of falls per individual.

**Compliance**
Participants returned their remaining supplement tablets bi-annually. Compliance was assessed by count of the returned tablets. Two different definitions of compliance were used. First, concerning physical performance and handgrip strength, compliance was defined as taking at least 80% of the tablets in the 2-year period between baseline and follow-up. Second, concerning time to falling, 80% was used as cutoff for compliance for the time the participant actually participated (i.e. time to study completion, or time to drop-out).

**Baseline characteristics**
Plasma Hcy, serum holotranscobalamin, methylmalonic acid, vitamin B12, and folate were determined. Details of the determination were described previously. Smoking habits (former, current, no smoker), alcohol use (light, moderate, excessive), physical activity, education level (low, intermediate, high), supplement use, and retrospective falling were assessed with questionnaires. Standing height was measured using a stadiometer, and weight using a calibrated scale (Seca, Deventer, the Netherlands).

**Statistical analyses**
Analyses were performed using IBM SPSS Statistics 20 (IBM, Armonk, New York, United States). Comparisons of baseline characteristics between treatment groups have been made previously. Additional baseline comparisons between the intervention and placebo group were made using a Mann-Whitney U test for physical performance, using a t-test for handgrip strength, and using a Chi-squared test for percentage of retrospective fallers.

The primary analyses were performed according to the intention-to-treat (ITT) principle, in which all participants were included. With respect to physical performance and handgrip strength, linear mixed model analyses were performed to assess the effect of the treatment. An important feature of this model, necessary for longitudinal analyses, is that the dependency of repeated observations within subjects is taken into account. Moreover, all subjects with at least one observation are included, regardless of missing values. Thus, subjects with baseline data who were lost to follow-up, were also included in the analyses. The treatment effect is the mean difference from baseline to follow-up in the treatment group compared with the mean difference from baseline to follow-up in the placebo group, as defined by the treatment-by-time interaction.
With respect to falling, differences in time to first and time to second fall between treatment groups were tested with the crude log-rank test. Moreover, Cox proportional hazards analyses were performed to assess the HR of falling. Person-time was the time to first/second fall, time to lost-to-follow-up, or time to study completion, whichever came first. The assumptions of proportional hazards were assessed by visual judgment of the log-minus-log survival plots, and were not violated. To compare the number of falls per participant across treatment groups while allowing for multiple events, we used the negative binomial model.

Crude analyses were performed, and analyses adjusted for age, sex, study center, baseline Hcy, and variables that differed between groups at baseline, that is baseline holotranscobalamin. The analyses of the number of falls were additionally adjusted for participation time, that is time to lost-to-follow-up, or time to study completion. Interaction of age (below and above 80 years), sex, and baseline Hcy concentration (below and above 18 µmol) with the treatment effect were studied. A p-value of ≤0.10 for interaction was considered as a justification for stratified analyses. All analyses were repeated according to the per-protocol (PP) principle, in which only compliant participants were included. Significance level of the effect was set at p<0.05.

Results

Of the 2,919 included participants, 1,461 were assigned to the intervention group, and 1,458 to the placebo group. The drop-out rate was 14.5%. Among the drop-outs, 144 participants (34%) agreed to complete the follow-up measurements, and 84 drop-outs (20%) still completed the research calendar (Figure 1). Baseline comparisons between the intervention and placebo group are presented in Table 1.

A significant 2-year decline in physical performance score was observed in both the intervention (p<0.01) and placebo group (p<0.01). Linear mixed models did not show a significant treatment effect, indicating that the decline did not differ between treatment groups (treatment effect: 0.1; 95% CI: -0.1, 0.3) (Table 2). No interactions with age, sex, and baseline Hcy concentration were observed. The PP analyses, with 84% of the participants included, showed similar results (Table 2). In the PP analyses evidence for interaction with age was observed (p=0.10). Stratified analyses showed that in persons ≤80 years (n=2,107) the decline in physical performance score was not significantly different between treatment groups (treatment effect: 0.0; 95% CI: -0.2, 0.3). In persons >80 years (n=340), a treatment effect of 0.6 was observed (95% CI: 0.0, 1.1), which was close to statistical significance (Figure 2). Results were similar after adjustments for age, sex, study center, baseline Hcy, and baseline holotranscobalamin (data not shown).
Randomization and start intervention
N=2,919

Intervention group
N=1,461

Baseline data on
- Physical performance N=1,441
- Handgrip strength N=1,442

Drop-out N=222
- Deceased N=16
- No longer motivated or personal event N=42
- No longer capable, too burdensome N=118
- Perceived side effects N=14
- Reason unknown N=32
- Agreed follow-up measurement N=82
- Agreed to continue fall calendar N=47

Follow-up data on
- Physical performance N=1,266
- Handgrip strength N=1,304
- Falling N=1,461

Included in the intention-to-treat analyses
- Physical performance N=1,454*
- Handgrip strength N=1,453*
- Falling N=1,461
* With data on baseline and/or follow-up

Placebo group
N=1,458

Baseline data on
- Physical performance N=1,434
- Handgrip strength N=1,446

Drop-out N=200
- Deceased N=26
- No longer motivated or personal event N=40
- No longer capable, too burdensome N=92
- Perceived side effects N=13
- Reason unknown N=29
- Agreed follow-up measurement N=62
- Agreed to continue fall calendar N=37

Follow-up data on
- Physical performance N=1,280
- Handgrip strength N=1,309
- Falling N=1,459

Included in the intention-to-treat analyses
- Physical performance N=1,452*
- Handgrip strength N=1,455*
- Falling N=1,459
* With data on baseline and/or follow-up

Figure 1 Flow chart of the B-PROOF study sample.
Table 1 Baseline characteristics of the 2,919 participants of the B-PROOF study according to treatment group.

<table>
<thead>
<tr>
<th></th>
<th>Intervention group</th>
<th>Placebo group</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N=1,461</td>
<td>N=1,458</td>
<td></td>
</tr>
<tr>
<td><strong>Age (years)</strong>&lt;sup&gt;a&lt;/sup&gt;</td>
<td>74.0 ± 6.6</td>
<td>74.2 ± 6.4</td>
<td>0.60</td>
</tr>
<tr>
<td><strong>Sex (% women)</strong>&lt;sup&gt;b&lt;/sup&gt;</td>
<td>50.4</td>
<td>49.7</td>
<td>0.71</td>
</tr>
<tr>
<td><strong>Homocysteine (µmol/L)</strong>&lt;sup&gt;c&lt;/sup&gt;</td>
<td>14.3 [13.0-16.5]</td>
<td>14.5 [13.0-16.7]</td>
<td>0.46</td>
</tr>
<tr>
<td><strong>Holotranscobalamin (pmol/L)</strong>&lt;sup&gt;c&lt;/sup&gt;</td>
<td>63.0 [45.0-84.0]</td>
<td>65.0 [48.0-86.0]</td>
<td>0.03*</td>
</tr>
<tr>
<td><strong>Methylmalonic acid (µmol/L)</strong>&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.23 [0.18-0.31]</td>
<td>0.22 [0.18-0.30]</td>
<td>0.25</td>
</tr>
<tr>
<td><strong>Vitamin B12 (pmol/L)</strong>&lt;sup&gt;c&lt;/sup&gt;</td>
<td>266 [204-343]</td>
<td>267 [213-341]</td>
<td>0.27</td>
</tr>
<tr>
<td><strong>Folate (nmol/L)</strong>&lt;sup&gt;c&lt;/sup&gt;</td>
<td>18.9 [14.8-24.5]</td>
<td>18.8 [14.9-24.7]</td>
<td>0.50</td>
</tr>
<tr>
<td><strong>Creatinine (µmol/L)</strong>&lt;sup&gt;a&lt;/sup&gt;</td>
<td>84.1 ± 18.0</td>
<td>83.9 ± 18.6</td>
<td>0.73</td>
</tr>
<tr>
<td><strong>Height (cm)</strong>&lt;sup&gt;a&lt;/sup&gt;</td>
<td>169.2 ± 9.3</td>
<td>169.4 ± 9.4</td>
<td>0.63</td>
</tr>
<tr>
<td><strong>Weight (kg)</strong>&lt;sup&gt;a&lt;/sup&gt;</td>
<td>77.8 ± 13.3</td>
<td>77.9 ± 13.3</td>
<td>0.99</td>
</tr>
<tr>
<td><strong>Current smoker (%)</strong>&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.7</td>
<td>9.5</td>
<td>0.97</td>
</tr>
<tr>
<td><strong>Alcohol use</strong>&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
<td>0.46</td>
</tr>
<tr>
<td>- Light (%)</td>
<td>66.8</td>
<td>68.0</td>
<td></td>
</tr>
<tr>
<td>- Moderate (%)</td>
<td>29.0</td>
<td>28.5</td>
<td></td>
</tr>
<tr>
<td>- Excessive (%)</td>
<td>4.2</td>
<td>3.5</td>
<td></td>
</tr>
<tr>
<td><strong>Physical activity (min/day)</strong>&lt;sup&gt;c&lt;/sup&gt;</td>
<td>131 [86-193]</td>
<td>126 [81-190]</td>
<td>0.17</td>
</tr>
<tr>
<td><strong>Education</strong>&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
<td>0.79</td>
</tr>
<tr>
<td>- Low (%)</td>
<td>53.6</td>
<td>52.4</td>
<td></td>
</tr>
<tr>
<td>- Intermediate (%)</td>
<td>21.1</td>
<td>21.1</td>
<td></td>
</tr>
<tr>
<td>- High (%)</td>
<td>25.4</td>
<td>26.5</td>
<td></td>
</tr>
<tr>
<td><strong>B12 and/or folic acid supplement use (%) yes</strong>&lt;sup&gt;b&lt;/sup&gt;</td>
<td>15.8</td>
<td>15.3</td>
<td>0.76</td>
</tr>
<tr>
<td><strong>Vitamin D supplement use (%) yes</strong>&lt;sup&gt;b&lt;/sup&gt;</td>
<td>19.7</td>
<td>18.3</td>
<td>0.64</td>
</tr>
<tr>
<td><strong>Retrospective falls (%) yes</strong>&lt;sup&gt;b&lt;/sup&gt;</td>
<td>32.6</td>
<td>32.5</td>
<td>0.96</td>
</tr>
<tr>
<td><strong>Physical performance (0-12)</strong>&lt;sup&gt;c&lt;/sup&gt;</td>
<td>9 [6-10]</td>
<td>9 [6-11]</td>
<td>0.24</td>
</tr>
<tr>
<td><strong>Handgrip strength (kg)</strong>&lt;sup&gt;a&lt;/sup&gt;</td>
<td>31.0 ± 10.6</td>
<td>30.8 ± 10.6</td>
<td>0.71</td>
</tr>
<tr>
<td><strong>Study center</strong>&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
<td>0.91</td>
</tr>
<tr>
<td>- WU (%)</td>
<td>29.2</td>
<td>29.6</td>
<td></td>
</tr>
<tr>
<td>- VUmc (%)</td>
<td>26.4</td>
<td>26.8</td>
<td></td>
</tr>
<tr>
<td>- Erasmus MC (%)</td>
<td>44.4</td>
<td>43.6</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Presented as mean ± SD, difference tested using t-test; <sup>b</sup>Presented as percentages, differences tested using Chi-squared test; <sup>c</sup>Presented as median [IQR], differences tested using Mann-Whitney U test; *p<0.05; VUmc= VU University Medical Center, WU= Wageningen University.
Table 2 The effect of the intervention on physical performance and handgrip strength, as derived from linear mixed models.

<table>
<thead>
<tr>
<th></th>
<th>Intervention group</th>
<th>Placebo group</th>
<th>Treatment effect&lt;sup&gt;a&lt;/sup&gt; (95% CI)</th>
<th>SE</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline estimated mean</td>
<td>Follow-up estimated mean</td>
<td>2-year change</td>
<td>Baseline estimated mean</td>
<td>Follow-up estimated mean</td>
</tr>
<tr>
<td>Physical performance score</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intention-to-treat</td>
<td>8.0</td>
<td>7.6</td>
<td>-0.4</td>
<td>8.1</td>
<td>7.6</td>
</tr>
<tr>
<td>Per-protocol</td>
<td>8.3</td>
<td>7.9</td>
<td>-0.4</td>
<td>8.3</td>
<td>7.9</td>
</tr>
<tr>
<td>Handgrip strength (kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intention-to-treat</td>
<td>30.9</td>
<td>29.6</td>
<td>-1.3</td>
<td>30.9</td>
<td>29.5</td>
</tr>
<tr>
<td>Per-protocol</td>
<td>31.7</td>
<td>30.5</td>
<td>-1.3</td>
<td>31.7</td>
<td>30.2</td>
</tr>
</tbody>
</table>

<sup>a</sup>The treatment effect is the mean difference from baseline to follow-up in the intervention group compared with the mean difference from baseline to follow-up in the placebo group, as determined by the treatment-by-time estimate; CI= confidence interval, SE= standard error.
Regarding handgrip strength, a significant 2-year decline in both treatment groups was observed (p<0.01 for both groups). The decline was not significantly different between groups (treatment effect: 0.1; 95% CI: -0.2, 0.4) (Table 2). No interaction effects were observed. PP results were similar (Table 2), and also adjustments for confounders did not change the findings (data not shown).

Total follow-up time for falling was 2,914.7 person-years in the intervention group and 2,940.7 person-years in the placebo group. In the intervention group, 1,747 falls occurred in 683 fallers (fall rate: 59.9/100 person-years) versus 1,663 falls in 681 fallers (fall rate: 56.6/100 person-years) in the placebo group. Both time to the first fall and time to the second fall were not significantly different between the intervention and placebo group (log rank p=0.63 and p=0.23, respectively) (Figure 3 for time to first fall). Also, Cox proportional hazards models did not show statistically significant differences between treatment groups with respect to time to first fall (HR: 1.0; 95% CI: 0.9, 1.2) and time to second fall (HR: 1.1; 95% CI: 0.9, 1.3). For time to first fall, a significant interaction was observed with Hcy concentration (p=0.05), but stratified analyses did not show significant effects in persons with Hcy ≤18 or >18 µmol/L (data not shown). In addition, negative binomial regression showed that the intervention had no significant effect on the number of falls per participant (OR: 1.0; 95% CI: 0.9, 1.1). Similar results were observed in the PP analyses, and after adjustment for confounders (data not shown).

**Figure 2** Two-year decline in physical performance scores according to treatment group among participants >80 years, as derived from linear mixed models (per-protocol analysis).
Discussion

Two-year daily supplementation of vitamin B12 and folic acid in hyperhomocysteinemic persons >65 years was not effective in reducing the age-related decline in physical performance and handgrip strength. In addition, the intervention had no effect on the risk of falling.

This is the first study performed in a large sample of elderly, with moderate long term follow-up duration. The results are in line with previous findings of Lewerin et al.\textsuperscript{17} In that study, movement performance as indicated by a postural-locomotor-manual test, did not improve after 4 months of treatment with 0.5 mg vitamin B12, 0.8 mg folic acid, and 3 mg vitamin B6 versus placebo in elderly participants (n=209, mean age: 76 years (range 70-93 years), mean Hcy: 17.2 µmol/L). However, interaction effects with age were not examined in that study. In the randomized controlled trial of Sato et al., no differences in fall incidence between the intervention and placebo group were reported after 2-year supplementation of 1.5 mg vitamin B12 and 5 mg folic acid among a highly selective sample of stroke survivors (n=628, mean Hcy: 19.9 µmol/L).\textsuperscript{18} Those findings are consistent with our results too.

In a subgroup of persons >80 years who were compliant in taking the supplement, a tendency towards a preventive effect on decline in physical performance was observed. The number of persons per treatment group in this analysis was relatively small (162 in the
intervention and 178 in the placebo group). Although the results were not statistically significant, they might be clinically relevant. Previous studies showed that a change in physical performance score of 0.3-0.8 points may be considered as minimally clinically relevant among the elderly, and a change of 0.4-1.5 points as substantially clinically relevant.\textsuperscript{21,22} We suggest that our observed change of 0.6 points might be even more relevant for persons >80 years as compared to persons ≤80 years, since baseline physical performance scores in this subgroup were significantly lower as compared to persons ≤80 years. Because the interaction effect with age was only observed in secondary analyses on a PP base, the subgroup effect on physical performance can be considered as a hypothesis that needs to be tested in further studies.

Recently, we have shown that B-vitamin supplementation was effective in the prevention of fractures in the same >80 years subgroup as the subgroup of the currently observed borderline effect on physical performance, that is persons >80 years who were compliant in taking the supplement.\textsuperscript{20} The mechanism of action by which supplementation reduces the fracture risk remains uncertain. It has been suggested that bone mineral density, collagen cross-linking, osteoclast activity and/or methylation capacity may be involved.\textsuperscript{23} An alternative or complementary intermediate might be physical function. To test this hypothesis, we added physical performance scores to the fracture analyses in a post hoc analysis. In the compliant 80+ subgroup with data on physical performance, the HR for fractures in the intervention group was 0.2 and did not change after adjustment for physical performance scores. This indicates that physical performance cannot be considered as a mediator in the reported effect on fractures.

Both the intervention and placebo tablets contained 600 IU vitamin D. In a meta-analysis of 13 studies, positive effects of vitamin D supplementation on muscle function have been demonstrated among elderly persons.\textsuperscript{24} Another meta-analysis observed a reduced fall risk and rate of falls with vitamin D supplementation among the elderly.\textsuperscript{25} Vitamin D receptors have been located in many tissues, such as muscle tissue and brain tissue.\textsuperscript{26,27} Age-related decline in vitamin D concentration and reduction of activity and expression of vitamin D receptors have been associated with reduced muscle cell function and are suggested to affect neuromuscular control and coordination.\textsuperscript{28,29} The presence of vitamin D supplementation in both treatment arms has most likely reduced the contrast between the groups and may have attenuated the results, although vitamin D and vitamin B12/folic acid supplementation are assumed not to have similar mechanistic actions.

Another limitation might be that the participants in the current study were only mildly hyperhomocysteinemic, which may have diminished the effect of supplementation. Although we only observed an interaction effect of baseline Hcy with treatment for the outcome falling, with no significant effects in subsequent stratified analyses, the results might be different in severe hyperhomocysteinemia. The strength of this study is its randomized controlled study design, as well as the large number of included participants.
In addition, the compliance to the treatment was good, and the applied doses of vitamin B12 and folic acid were sufficient to lower homocysteine levels.\textsuperscript{20}

In conclusion, this study showed no overall effect of daily 2-year supplementation of vitamin B12 and folic acid on physical performance, handgrip strength, and falling among elderly persons with mild hyperhomocysteinemia. In compliant persons >80 years, a tendency towards a preventive effect on decline in physical performance was observed. It should be noted that more cancer diagnoses were observed in the intervention group as compared to placebo, as reported previously.\textsuperscript{20} Although the current results emphasize the need for further research among the oldest old, caution is therefore warranted.

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Conflict of interest
PL and NMVS declare to have received an unconditional grant of Merck and Co for vitamin D assessment in Longitudinal Aging Study Amsterdam and PL received personal fees from Merck and Co and Bristol-Myers Squibb. The other authors all state they have no conflict of interest to declare.
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