CHAPTER 3

Cross-sectional and longitudinal association between homocysteine, vitamin B12 and physical performance in older persons

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Abstract

Background/Objectives

Decreases in physical performance are associated with multiple negative health outcomes. The objective of this study was to examine whether high plasma homocysteine and low serum vitamin B12 are independent risk factors for lower physical performance, both cross-sectionally and longitudinally.

Subjects/Methods

This study was performed in persons aged ≥65 years of the LASA (Longitudinal Aging Study Amsterdam), an ongoing cohort study. Blood was collected in 1995/1996 (n=1,352). Physical performance was assessed in 1995/1996 and in 1998/1999 using three tests: the walking test, the chair stands test and the tandem stand (n=901-1,155).

Results

After adjustment for confounding, women in the highest quartile of homocysteine had a significantly lower physical performance than did those in the lowest quartile in the cross-sectional analyses (beta: -0.93; se: 0.34; $p<0.01$). This association was borderline statistically significant in the longitudinal analyses (beta: -0.69; se: 0.35; $p=0.05$). After additional adjustment for serum vitamin B12, both associations were statistically significant ($p<0.05$). For vitamin B12 in women, and for homocysteine and vitamin B12 in men, the observed associations were less consistent.

Conclusions

High plasma homocysteine is an independent risk factor for lower physical performance in older women. The association between vitamin B12 and physical performance is less clear.

Introduction

Homocysteine levels increase and vitamin B12 levels decrease with aging.[1-3] In the LASA (Longitudinal Aging Study Amsterdam), a representative sample of Dutch older persons, 46% of men and 29% of women had a high homocysteine level, and 22% of men and 16% of women had a low vitamin B12 level.[4] Homocysteine and B-vitamins have been associated with cardiovascular disease (especially stroke),[5,6] cognitive decline and dementia,[7-9] fractures[10-12] and mortality.[13-15] In addition, elevated homocysteine has been associated with lower physical performance .[16-21]

Physical performance decreases with aging and is associated with multiple negative health outcomes, such as cardiovascular disease,[22] falls and fractures,[23,24] nursing home admission and mortality.[25,26] Five previous studies observed associations between elevated homocysteine and lower physical performance in the general older population.[16-19,21] Only two of those studies examined the longitudinal association,[16,21] showing that elevated homocysteine increased the risk of physical decline. These studies were relatively small (n=499 and n=574, respectively), and the first study was limited to highly functioning men and women with a narrow age range (70-79 years). It is important to validate these findings in a larger sample with a wider age and function range.

To our knowledge, no studies examined the direct association between serum vitamin B12 and physical performance in older persons. As low vitamin B12 levels are associated with neurological and neuropsychiatric disturbances,[27] it may be a potential risk factor for lower physical performance.

The objective of this study was to examine whether high plasma homocysteine and low serum vitamin B12 are independent risk factors for lower physical performance, both cross-sectionally and longitudinally, in a representative sample of Dutch older persons.

Patients and methods

Subjects

The LASA is an ongoing multidisciplinary cohort study on predictors and consequences of changes in physical, cognitive, emotional and social functioning in older persons.[28,29] A random sample of men and women aged ≥55 years, stratified by age, sex, urbanization grade and expected 5-year mortality rate was drawn from the population registers of 11 municipalities in three regions of the Netherlands. In total, 3,107 persons were enrolled in the baseline examination in 1992/1993. For this study, persons who participated in the medical interview in 1995/1996, and were born in or before 1930 (aged ≥65 years as of 1 January 1996), were selected (n=1509). In 1,352 of these persons, blood samples were drawn. Data on homocysteine, physical performance and potential confounders were available for 1,146 persons in the cross-sectional analyses in 1995/1996, and for 901

persons in the longitudinal analyses between 1995/1995 and 1998/1999. In all, 32 persons having serum vitamin B12 levels >800 pmol/L were excluded.[4] This resulted in 1,155 persons having data on serum vitamin B12, physical performance and potential confounders in the cross-sectional analyses in 1995/1996, and 907 persons in the longitudinal analyses between 1995/1996 and 1998/1999. The Medical Ethics Committee of the VU University Medical Center (VUmc) approved this study, and all persons gave informed consent.

Physical performance

Physical performance was assessed by three tests: time needed to walk 3 meters along a rope, turn 180 degrees and walk back (walking test); time needed to stand up from and sit down on a chair five times with arms folded across the chest (chair stands test); and the ability to perform the tandem stand (one foot placed behind the other on a straight line) for at least 10 s (tandem stand) (adapted from [25]). The walking test is an indicator of coordination, proximal muscle strength and balance; the chair stands test is primarily an indicator of proximal muscle strength; and the tandem stand is primarily an indicator of balance.[30] For the walking test and chair stands test, scores 1-4 were given according to the quartile of the distribution of time needed. Score 0 was given to those respondents who could not complete the test. The tandem stand was categorized as follows: unable (score 0), able to hold position for 3–9 s (score 2), and able to hold position for at least 10 s (score 4). The total physical performance sum score ranged from 0 (low physical performance) to 12 (high physical performance). The total physical performance score was well associated with falls and fractures,[23] frailty,[31] and cognitive functioning[32] and was shown to be a reliable and valid measure of physical performance.

Biochemistry

Morning blood samples were obtained in Amsterdam, Zwolle and Oss in 1995/1996. Subjects were allowed to have tea and toast, but no dairy products. Dairy products were not allowed in the LASA because these may influence parathyroid hormone and bone turnover (not included in this study). Blood samples were centrifuged and stored at -20^{o} C until determination in 2001/2002. Plasma homocysteine levels were measured using a fluorescence polarization immunoassay on an IMx analyzer (Abbott Laboratories, Abbott Park, IL, USA) at the Laboratory of Clinical Chemistry of the VUmc. The inter-assay coefficient of variation was 4%. Serum vitamin B12 levels were determined with a competitive luminescence immunoassay on an automated ACS 180 System (Bayer Diagnostics, Mijdrecht, The Netherlands) at the Endocrine Laboratory of the VUmc. The inter-assay coefficient of variation was 5%. Serum creatinine levels were measured using the Jaffe alkaline picrate reaction with a Hitachi 747 analyzer (Roche Diagnostics, The Netherlands). Serum 25-hydroxyvitamin D was determined according to a competitive protein binding

assay ((Nichols Diagnostics, San Juan Capistrano, CA, USA) with an inter-assay coefficient of variation of 10%.

Potential effect modifiers

Potential effect modifiers were age and sex. Older age and male gender are associated with higher homocysteine levels.[1,2,4,33] Female gender is associated with lower vitamin B12 levels.[4] These differences may influence the associations with physical performance. Data on age and sex were derived from the population registries.

Potential confounders

Potential confounders that were considered included age and sex (if no interaction was present), region of living, years of education, body mass index, alcohol consumption, smoking, creatinine and serum 25-hydroxyvitamin D. Education level was assessed by asking the respondent for the highest education level completed. This was converted into years of education. Body mass index was calculated as body weight in kilograms divided by height in meters squared. Body weight was measured without clothes and shoes using a calibrated bathroom balance scale; body height was measured using a stadiometer. Alcohol consumption (does not drink, light, moderate, excessive drinking)[34] and smoking (never /stopped smoking >15 years ago, stopped smoking ≤15 years ago, current smoker) were assessed by questionnaires.

Statistical analysis

First, baseline differences between quartiles of homocysteine and vitamin B12 were tested using one-way analysis of variance for continuous variables and Pearson Chi-square test for frequencies, respectively. Second, the effect modifiers age (<75 years versus ≥75 years= median of the population) and sex were tested with multivariable linear regression analysis using homocysteine and vitamin B12, respectively, in quartiles. Third, three different regression models were made: (1) a model adjusted for age and sex (if no interaction was present); (2) a model adjusted for all confounders and (3) a model adjusted for all confounders and vitamin B12 (in case of homocysteine-physical performance analyses) or homocysteine (in case of vitamin B12-physical performance analyses). Potential confounders that did not correlate with both determinant and outcome ($p>0.10$) were not included in the multivariable model. The p-value of the F-test was $p<0.01$ for all models, indicating that the categorical variable significantly contributed to the model. The assumptions of linear regression analyses were checked by making histograms and normal probability plots of the standardized residuals. Multi-collinearity was checked by calculating correlation coefficients between the independent variables. These were sufficiently low to enter all variables ($r<0.4$). In the longitudinal analyses, all models were adjusted for baseline physical performance. Decline in physical performance during 3 years of follow-up was

assessed using the Edwards-Nunnally index to determine clinically significant change.[35] The index is adjusted for regression to the mean. On the basis of confidence intervals (CIs), the Edwards-Nunnally index classifies change as improved, stable, or declined. For the analyses, changes in physical performance scores were dichotomized into decline versus no decline (stability or improvement), using a critical value of 1.96 ($p<0.05$). To analyze homocysteine and vitamin B12 in relation to the individual components of physical performance (walking test, chair stands test, tandem stand), cumulative logistic ordinal regression analyses, with the feature of proportional odds, were used.[36,37] The assumption of cumulative logistic ordinal regression was fulfilled when the p-value for the test of parallel lines was >0.05.

Results

The baseline characteristics are presented in Table 1. Persons in the higher quartiles of plasma homocysteine were significantly older, less often female and had higher serum creatinine levels and lower serum vitamin B12 levels. In addition, differences with regard to region of living and body mass index were observed. Persons in the lower quartiles of serum vitamin B12 were significantly older, less often female, had higher plasma homocysteine levels. Furthermore, differences with regard to region of living and alcohol consumption were observed.

In Table 2, mean physical performance scores are presented. There were no statistically significant differences between quartiles of plasma homocysteine ($p=0.20$) or serum vitamin B12 ($p=0.92$) with regard to the interval between the first and second measurement of physical performance (3.0 years for total population). In total, 19.5% of women and 16.5% of men showed decline in physical performance between 1995/1996 and 1998/1999 according to the Edwards-Nunnally index.

The interaction with sex was significant in the cross-sectional and longitudinal analyses for both the analyses on homocysteine and vitamin B12 ($p<0.10$). Therefore, further analyses were stratified by sex. No interaction with age was observed ($p>0.22$).

Table 1 Baseline characteristics according to quartiles of homocysteine[a] and quartiles of vitamin B12[b].

	Quartile 1 homo-cysteine	Quartile 2 homo-cysteine	Quartile 3 homo-cysteine	Quartile 4 homo-cysteine	P-value	Quartile 1 vitamin B12	Quartile 2 vitamin B12	Quartile 3 vitamin B12	Quartile 4 vitamin B12	P-value
Age (yrs)[c]	73.4 ± 5.9	74.8 ± 6.2	76.2 ± 6.7	78.0 ± 6.5	<0.01	76.5 ± 6.7	75.1 ± 6.4	75.1 ± 6.4	75.1 ± 6.7	0.02
Sex (% female)	64.6%	55.1%	45.6%	40.4%	<0.01	44.4%	49.5%	49.4%	60.9%	<0.01
Region										
- Amsterdam	62.1%	48.9%	38.6%	32.6%	<0.01	37.6%	42.5%	46.9%	57.1%	<0.01
- Zwolle	14.1%	32.2%	38.6%	36.0%		31.4%	32.0%	31.6%	22.6%	
- Oss	23.8%	18.9%	23.1%	31.4%		31.0%	25.5%	21.5%	20.3%	
Education (yrs) (5-18)[c]	9.2 ± 3.4	8.8 ± 3.3	9.0 ± 3.3	8.7 ± 3.3	0.38	9.1 ± 3.5	8.9 ± 3.3	8.9 ± 3.0	8.7 ± 3.4	0.46
Body mass index (kg/m^2)[c]	26.9 ± 4.2	26.9 ± 4.1	27.3 ± 4.4	26.3 ± 4.2	0.02	26.7 ± 4.2	26.7 ± 4.3	27.0 ± 4.2	26.9 ± 4.2	0.78
Alcohol use:										
- No	21.2%	24.6%	23.6%	29.6%	0.20	26.0%	23.2%	21.3%	26.4%	0.04
- Light	53.2%	50.5%	52.5%	42.9%		42.8%	55.3%	51.6%	52.4%	
- Moderate	20.6%	19.4%	17.8%	19.8%		23.2%	16.4%	19.7%	17.0%	
- (Very) excessive	4.9%	5.5%	6.1%	7.7%		8.0%	5.1%	7.4%	4.2%	
Smoking:										
- Never, or >15 yrs ago	68.0%	68.9%	65.4%	64.5%	0.60	67.2%	64.6%	66.1%	65.7%	0.96
- ≤15 yrs ago	12.9%	13.2%	16.2%	17.9%		14.5%	17.7%	15.8%	15.1%	
- Current	19.1%	17.8%	18.3%	17.6%		18.3%	17.7%	18.1%	19.2%	
Serum creatinine (µmol/L)[d,e]	82.0 [74.5-91.5]	87.0 [78.0-97.0]	92.0 [81.8-104.3]	101.0 [88.0-119.0]	<0.01	93.0 [81.0-105.0]	89.0 [78.0-102.0]	89.0 [79.0-101.0]	88.0 [79.0-101.8]	0.11
Serum 25(OH)D (nmol/L)[d,e]	51.2 [38.2-67.8]	54.2 [39.8-70.0]	51.6 [34.3-69.5]	46.3 [31.7-65.3]	0.10	51.8 [34.5-70.4]	50.5 [35.4-67.6]	52.4 [37.4-68.9]	51.1 [36.6-68.4]	0.33
Serum vitamin B12 (pmol/L)[d,e]	303.0 [251.0-379.0]	270.0 [227.5-327.0]	256.0 [209.0-313.5]	222.0 [179.5-274.3]	<0.01	183.0 [156.0-201.0]	237.0 [227.0-251.0]	288.0 [276.0-306.3]	386.5 [354.0-440.0]	<0.01
Plasma homocysteine (µmol/L)[d,e]	9.7 [8.5-10.4]	12.3 [11.7-12.9]	15.0 [14.1-15.9]	19.9 [18.2-23.4]	<0.01	16.4 [13.1-20.0]	13.7 [11.2-17.0]	12.8 [10.8-15.8]	12.2 [10.0-14.6]	<0.01

[a] Homocysteine: quartile 1: 5.7-11.1 µmol/L; quartile 2: 11.1-13.5; quartile 3: 13.6-17.0; quartile 4: 17.0-95.1 µmol/L; [b] Vitamin B12: quartile 1: 86.0-212.0 pmol/L; quartile 2: 213.0-263.0; quartile 3: 264.0-327.0; quartile 4: 328.0-705.0 pmol/L; [c] Mean ± standard deviation; [d] Median [interquartile range]; [e] Normal values: serum creatinine: 60-110 µmol/L in adults; serum 25(OH)D: 25-100 nmol/L; serum vitamin B12: 156-672 pmol/L; plasma homocysteine: 8-18 µmol/L in men, 6-19 µmol/L in postmenopausal women; 25(OH)D= 25-hydroxyvitamin D.

Table 2 Physical performance in total population, women and men.

	Total population	Women	Men
Total physical performance in 1995/96[a]	7.4 ± 3.2	6.8 ± 3.3	7.9 ± 3.0
- Walking test[b]	2 [1-4]	2 [1-3]	3 [2-4]
- Chair stands test[b]	2 [1-3]	2 [1-3]	2 [1-3]
- Tandem stand[b]	4 [2-4]	4 [0-4]	4 [2-4]
Total physical performance in 1998/99[a]	6.5 ± 3.3	6.1 ± 3.3	7.0 ± 3.2
- Walking test[b]	2 [1-3]	1 [1-2]	2 [1-3]
- Chair stands test[b]	2 [1-3]	1 [1-2]	2 [1-3]
- Tandem stand[b]	4 [0-4]	4 [0-4]	4 [2-4]

[a] Mean ± standard deviation; [b] Median [interquartile range].

In women, the second (beta: -0.60; p=0.04) and fourth (beta: -0.93; p<0.01) quartiles of homocysteine were significantly associated with lower physical performance as compared with the first quartile after adjustment for age, region, body mass index, smoking, alcohol consumption and serum 25-hydroxyvitamin D (Table 3). After additional adjustment for serum vitamin B12, only the fourth quartile of homocysteine (beta: -0.82; p=0.02) remained significantly associated with lower physical performance. In the longitudinal analyses, a significantly lower physical performance was observed for women in the fourth quartile of homocysteine (beta: -0.82; p=0.03) after adjustment for baseline performance, other confounders and serum vitamin B12. In addition, women in the fourth quartile of homocysteine had a two-fold higher risk on decline in physical performance (odds ratio (OR): 2.0; 95% CI: 1.0, 4.0 in the fully adjusted model; OR: 2.0; 95% CI: 1.0, 4.4 after additional adjustment for serum vitamin B12; data not shown). In men, no statistically significant associations were observed for homocysteine in the cross-sectional and longitudinal analyses.

After adjustment for confounding, no statistically significant results were observed in the analyses using serum vitamin B12 as a determinant of physical performance, neither in men nor in women (Table 4). With regard to decline in physical performance, only a statistically significant result for men in the second quartile of vitamin B12 was observed (OR: 0.4; 95% CI: 0.2, 1.0 in the fully adjusted model; OR: 0.4; 95% CI: 0.2, 1.0 after additional adjustment for plasma homocysteine; data not shown).

Table 3 Cross-sectional and longitudinal association between homocysteine in quartiles and physical performance.

		Cross-sectional		Longitudinal	
	Homo-cysteine	Unstandardized Beta (SE)	P-value	Unstandardized Beta (SE)	P-value
Women					
Model 1	Q1	Reference	Reference	Reference	Reference
	Q2	-0.61 (0.30)	0.04	-0.35 (0.29)	0.23
	Q3	-0.72 (0.32)	0.02	-0.15 (0.31)	0.62
	Q4	-1.20 (0.34)	<0.01	-0.46 (0.34)	0.18
Model 2	Q1	Reference	Reference	Reference	Reference
	Q2	-0.60 (0.30)	0.04	-0.48 (0.29)	0.10
	Q3	-0.50 (0.32)	0.11	-0.25 (0.32)	0.43
	Q4	-0.93 (0.34)	<0.01	-0.69 (0.35)	0.05
Model 3	Q1	Reference	Reference	Reference	Reference
	Q2	-0.55 (0.30)	0.07	-0.54 (0.30)	0.07
	Q3	-0.43 (0.33)	0.19	-0.34 (0.33)	0.30
	Q4	-0.82 (0.36)	0.02	-0.82 (0.37)	0.03
Men					
Model 1	Q1	Reference	Reference	Reference	Reference
	Q2	-0.01 (0.35)	0.99	-0.11 (0.35)	0.75
	Q3	-0.57 (0.34)	0.10	0.03 (0.35)	0.94
	Q4	- 0.55 (0.34)	0.11	0.58 (0.35)	0.10
Model 2	Q1	Reference	Reference	Reference	Reference
	Q2	0.07 (0.35)	0.85	-0.12 (0.36)	0.73
	Q3	-0.49 (0.34)	0.15	-0.01 (0.36)	0.98
	Q4	-0.41 (0.35)	0.24	0.53 (0.37)	0.15
Model 3	Q1	Reference	Reference	Reference	Reference
	Q2	-0.01 (0.34)	0.98	-0.16 (0.36)	0.66
	Q3	-0.63 (0.34)	0.07	-0.06 (0.36)	0.87
	Q4	-0.71 (0.36)	0.05	0.39 (0.38)	0.30

Model 1: Adjusted for age (and baseline physical performance in longitudinal analyses); Model 2: Additionally adjusted for region, body mass index, smoking, alcohol use and serum 25-hydroxyvitamin D; Model 3: Additionally adjusted for serum vitamin B12; SE= standard error, Q= quartile.

Table 4 Cross-sectional and longitudinal association between vitamin B12 in quartiles and physical performance.

		Cross-sectional		Longitudinal	
	Vitamin B12	Unstandardized Beta (SE)	P-value	Unstandardized Beta (SE)	P-value
Women					
Model 1	Q1	-0.56 (0.33)	0.09	0.23 (0.32)	0.47
	Q2	-0.47 (0.31)	0.13	0.20 (0.30)	0.51
	Q3	0.13 (0.31)	0.67	0.12 (0.30)	0.70
	Q4	Reference	Reference	Reference	Reference
Model 2	Q1	-0.40 (0.33)	0.22	0.15 (0.33)	0.65
	Q2	-0.39 (0.31)	0.21	0.14 (0.30)	0.64
	Q3	0.21 (0.31)	0.51	0.05 (0.31)	0.88
	Q4	Reference	Reference	Reference	Reference
Model 3	Q1	-0.02 (0.36)	0.97	0.52 (0.35)	0.14
	Q2	-0.24 (0.31)	0.45	0.28 (0.31)	0.36
	Q3	-0.28 (0.31)	0.38	0.12 (0.31)	0.70
	Q4	Reference	Reference	Reference	Reference
Men					
Model 1	Q1	0.34 (0.33)	0.31	0.68 (0.34)	0.05
	Q2	-0.08 (0.34)	0.81	0.58 (0.35)	0.10
	Q3	-0.18 (0.34)	0.60	0.25 (0.35)	0.47
	Q4	Reference	Reference	Reference	Reference
Model 2	Q1	0.39 (0.33)	0.24	0.64 (0.35)	0.07
	Q2	-0.03 (0.34)	0.92	0.54 (0.35)	0.13
	Q3	-0.18 (0.34)	0.60	0.23 (0.35)	0.51
	Q4	Reference	Reference	Reference	Reference
Model 3	Q1	0.68 (0.34)	0.05	0.64 (0.36)	0.08
	Q2	0.05 (0.34)	0.89	0.54 (0.36)	0.13
	Q3	-0.12 (0.34)	0.72	0.23 (0.35)	0.51
	Q4	Reference	Reference	Reference	Reference

Model 1: Adjusted for age (and baseline physical performance in longitudinal analyses); Model 2: Additionally adjusted for region; Model 3: Additionally adjusted for homocysteine; SE= standard error, Q= quartile.

Table 5 shows the adjusted cumulative OR for the cross-sectional analyses between homocysteine and vitamin B12, respectively, and the individual physical performance tests. Women in the fourth quartile of homocysteine were 2.53 times more likely to score one point lower on the walking test and 1.86 times more likely to score one point lower on the chair stands test, as compared with women in the first quartile. In addition, women in the lowest quartile of vitamin B12 were 1.55 times more likely to score one point lower on the walking test. No statistically significant associations were observed in men.

Table 5 Cross-sectional association between homocysteine, vitamin B12 and individual physical performance tests.

	Walking test (score 0-4)	Chair stands test (score 0-4)	Tandem stand (score 0, 2, 4)
Women			
Homocysteine[a]			
Q1	Reference	Reference	Reference
Q2	1.44 (0.98, 2.13)	1.09 (0.75, 1.58)	1.47 (0.93, 2.33)
Q3	1.23 (0.81, 1.87)	1.23 (0.82, 1.84)	1.34 (0.83, 2.19)
Q4	2.53 (1.59, 4.01)	1.86 (1.21, 2.87)	1.27 (0.76, 2.10)
Vitamin B12[b]			
Q1	1.55 (1.01, 2.36)	1.32 (0.88, 1.98)	1.10 (0.68, 1.79)
Q2	1.14 (0.76, 1.70)	1.38 (0.94, 2.03)	1.26 (0.79, 2.00)
Q3	1.01 (0.68, 1.51)	0.89 (0.61, 1.32)	0.84 (0.52, 1.35)
Q4	Reference	Reference	Reference
Men			
Homocysteine[a]			
Q1	Reference	Reference	Reference
Q2	1.05 (0.65, 1.70)	0.99 (0.63, 1.55)	0.89 (0.46, 1.73)
Q3	1.44 (0.90, 2.31)	1.23 (0.79, 1.93)	1.33 (0.71, 2.48)
Q4	1.39 (0.86, 2.25)	1.36 (0.86, 2.15)	1.40 (0.75, 2.61)
Vitamin B12[b]			
Q1	0.88 (0.56, 1.36)	0.96 (0.63, 1.47)	0.74 (0.43, 1.29)
Q2	0.94 (0.60, 1.48)	1.20 (0.78, 1.85)	0.92 (0.53, 1.60)
Q3	1.06 (0.68, 1.65)	1.45 (0.95, 2.24)	0.91 (0.52, 1.59)
Q4	Reference	Reference	Reference

Data represent adjusted cumulative OR for lower physical performance. [a] Adjusted for age, region, body mass index, smoking, alcohol use and serum 25-hydroxyvitamin D; [b] Adjusted for age and region.

Discussion

Women in the highest quartile of homocysteine had a significantly lower physical performance than did those in the lowest quartile both in the cross-sectional and in the longitudinal analyses. In addition, women in the highest quartile of homocysteine had a two-fold higher risk on decline in physical performance. For vitamin B12 in women, and for homocysteine and vitamin B12 in men, the observed associations were less consistent.

This study partly confirms the results of five earlier studies that observed an association between homocysteine and physical performance in the general older population.[16-19,21] In addition, this study confirms the presence of a longitudinal association between homocysteine and physical performance of two earlier studies.[16,21] In the first three studies, the association between homocysteine and physical performance was

observed in a combined sample of men and women.[16-18] The results of our study extend the findings of Kado et al.[16] by identifying sex as an effect modifier, and by studying men and women with a much wider age range (65-88 years in our study versus 70-79 years in the study of Kado et al.). However, in contrast to our results, sex was not identified as an effect modifier in the study by Rolita et al.[21]

In one of the studies, the analyses on homocysteine and physical performance were not adjusted for potential confounding variables.[19] In three studies, the analyses were adjusted for potential confounders including serum vitamin B12.[16-18] For comparability reasons, we also chose to adjust for serum vitamin B12, but this was done in a separate model. Both vitamin B12 and homocysteine are part of the methionine-homocysteine cycle, and vitamin B12 is an important determinant of plasma homocysteine concentration. Adjustment for vitamin B12 may lead to over-adjustment on one hand, but is also necessary to establish whether the association between homocysteine and physical performance is independent from vitamin B12 status. In our study, adjustment for vitamin B12 made the longitudinal association between homocysteine and physical performance somewhat stronger. This may be explained by the fact that elevated homocysteine levels are not always associated with low vitamin B12 levels, as other determinants of homocysteine may be more important (folate status, renal function, genetic factors, etc.). Homocysteine and vitamin B12 are not highly correlated (in our study Pearson r: -0.39, $p<0.01$), and may have different effects on physical performance. This was also shown in our study by additionally examining the direct associations between vitamin B12 and physical performance. No consistent associations were observed for vitamin B12. Again, this indicates that other determinants of homocysteine may be more important.

The observed association between homocysteine and physical performance in women was strongest for the walking test and chair stands test. The walking test is an indicator of coordination, proximal muscle strength and balance; the chair stands test is an indicator of proximal muscle strength; the tandem stand is an indicator of balance.[30] Physical performance, especially walking speed, has been associated with multiple negative health outcomes.[22-26]

Several mechanisms may explain the observed association between elevated homocysteine and physical performance. First, it has been shown that elevated homocysteine is associated with lower calf muscle density in persons having peripheral arterial disease.[20] The authors propose several potential explanations: one of them being that homocysteine can induce inflammatory responses. Inflammatory cytokines may alter muscle homeostasis by inhibiting repair after muscle tissue injury and by promoting muscle proteolysis. In a post hoc analysis, we added C-reactive protein to the fully adjusted model. This did not change the results (data not shown), suggesting that C-reactive protein does not explain the relationship between plasma homocysteine/serum vitamin B12 and physical performance. Second, elevated homocysteine may lead to neurological problems. Elevated

homocysteine is associated with an increase in white matter hyperintensities, which have been correlated with lower extremity function.[17,38] The above explanation is supported by our observation that elevated homocysteine is related to the chair stands test, a measure for proximal muscle strength. Third, elevated homocysteine may lead to impaired cognitive function or dementia[7-9] and fractures,[10-12] which all may contribute to lower physical performance. More research is necessary to elucidate the possible mechanism.

It would be very interesting to examine in a randomized controlled trial whether physical decline may be delayed by lowering homocysteine levels. Homocysteine levels can be decreased by supplementation with folic acid (20-25% reduction) and vitamin B12 (additional 7% reduction).[39] If not causally related, elevated homocysteine may also be used a predictor for physical decline in older women.

The strengths of this study are the large, population-based study sample, the possibility to examine effect modifiers, and to adjust for confounders, as well as the possibility to study the association between homocysteine/vitamin B12 and physical performance both cross-sectionally and longitudinally. Limitations are that the measurements of homocysteine and vitamin B12 were not repeated, that folic acid and methylmalonic acid (a measure of B12 bioavailability) were only assessed in a small subgroup and that vitamin B6 was not assessed and could therefore not be included in this study.

In conclusion, elevated homocysteine levels are associated with decreased physical performance in older women. The association between vitamin B12 and physical performance is less clear.

Conflict of interest

The authors declare no conflict of interest.

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3