CHAPTER 4

Elevated homocysteine levels are associated with low muscle strength and functional limitations in older persons

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Abstract

Objective

The current study aimed to examine homocysteine in relation to different aspects of physical functioning.

Design, setting and participants

Cross-sectional and longitudinal data (3-years follow-up) from the Longitudinal Aging Study Amsterdam (LASA) were used. The study was performed in persons aged ≥65 years (N=1,301 after imputation).

Measurements

Different measures of physical functioning, including muscle mass, grip strength, functional limitations, and falling were regarded as outcomes. Gender and serum creatinine level were investigated as effect modifiers.

Results

Results were stratified by gender. In men, higher homocysteine levels were associated with lower grip strength (Quartile 4: regression coefficient (B): -3.07 (-4.91, -1.22)), and more functional limitations at baseline (Quartile 4: B: 1.15 (0.16, 2.14)). In women, higher homocysteine levels were associated with more functional limitations after 3 years (Quartile 4: B: 1.19 (0.25, 2.13)). Higher homocysteine levels were not associated with low muscle mass or falling.

Conclusions

These data suggest an inverse association of homocysteine levels with functional limitations in older men and women, and with muscle strength in older men.

Introduction

Plasma homocysteine levels rise with increasing age. Population based studies revealed that 30-50% of the older persons have elevated homocysteine levels.[1] The main determinants of elevated homocysteine levels are low folate, low vitamin B12, and impaired renal function.[2] Elevated homocysteine levels have been associated with adverse cerebro-, and cardiovascular outcomes,[3] cognitive impairment,[4,5] and fractures.[6-8]

The mechanism of action by which homocysteine induces an increased fracture risk remains unclear. It has been suggested that bone mineral density, bone turnover, collagen cross-linking, and osteoclastgenesis are involved.[9-13] Beside these direct effects of homocysteine on bone quality and bone strength, homocysteine might also contribute indirectly to an increased fracture risk via adverse effects on muscle strength, physical functioning, and falling.

Previous studies examined homocysteine in relation to objective performance measures, consistently finding lower physical abilities in persons with elevated homocysteine levels.[9,14-18] Its underlying fundaments such as muscle mass and muscle strength in relation to homocysteine levels have gained limited attention.[15,19] Further, also other aspects of physical functioning, for instance people's subjective appraisal of their physical abilities (i.e. functional limitations),[15,20] or falling remained underexposed.[21,22]

The observed associations between homocysteine and physical functioning might depend on gender. It is known from the literature, that the homocysteine distribution differs between and men and women, and that these differences might be attributable to differences in fat-free mass.[23] Furthermore, the relationship between homocysteine and muscle characteristics might be creatinine-dependent. Muscle mass is a supplier of creatinine, and in turn, high creatinine, as a proxy of decreased renal function, is an important determinant of high homocysteine levels.[2] Hence, the role of creatinine in the associations is complex, and serum creatinine might be an effect modifier. Up till now, most studies did not examine gender as an effect modifier, and none of the studies examined creatinine levels as an effect modifier.

The current study aimed to examine homocysteine levels in relation to the different aspects of physical functioning, e.g. muscle mass, muscle strength, functional limitations, and falling using cross-sectional as well as prospective approaches in a large population-based sample of older persons. In addition, it was examined whether gender and creatinine levels are effect modifiers in the relationship between homocysteine levels and physical functioning.

Methods

Study sample

Data from the Longitudinal Aging Study Amsterdam (LASA) were used. The LASA is an ongoing cohort study of a population-based sample of older people in the Netherlands. Every 3 years data on physical, cognitive, emotional, and social functioning were collected in a main and medical interview. Further information on data collection and sampling have been presented elsewhere.[24]

Details on sample size are presented in Figure 1. Because of blood-sampling availability, 1995-1996 was used as baseline in our study. Cross-sectional and longitudinal associations were examined, using data from baseline and 3-year follow-up. After imputation of missing data, the number of participants was 1,301 in the cross-sectional analyses. Missing data at 3-year follow-up were only imputed in persons who did not decease during follow-up, resulting in n=1,138 in the longitudinal analyses. The study was approved by the Medical Ethics Committee of VU University Medical Center. All respondents signed informed consent.

Measurements

Homocysteine

Before blood sampling, subjects were allowed to eat toast or drink tea, but were otherwise fasting. EDTA blood samples were stored at -20 °C until determination of total homocysteine levels (µmol/L) in 2001-2002, using the Abbott IMx analyzer (Abbott Park, USA). The inter-assay coefficient of variation was 4%.

Muscle mass

Body composition was measured in a subsample (n=499 at baseline, n=321 after 3 years) using Dual-energy X-ray Absorptiometry (DXA Hologic QDR 2000 scanner, software version V5-70 A, Waltham, MA). The appendicular skeletal muscle mass (kg) was calculated as the sum of the fat-free, bone-free mass of the arms and legs.[25]

Grip strength

Grip strength (kg) was measured using a strain-gauged dynamometer (Takei TKK 5001, Japan). Respondents were asked to perform two maximum grip strength trials with each hand, in standing position with their arms along their body. Grip strength was defined as the average of the highest score of the left and right hand.[26] Grip strength is a good indicator of overall strength, because grip strength is positively correlated with both upper-body and lower-extremity strength in older persons.[27,28]

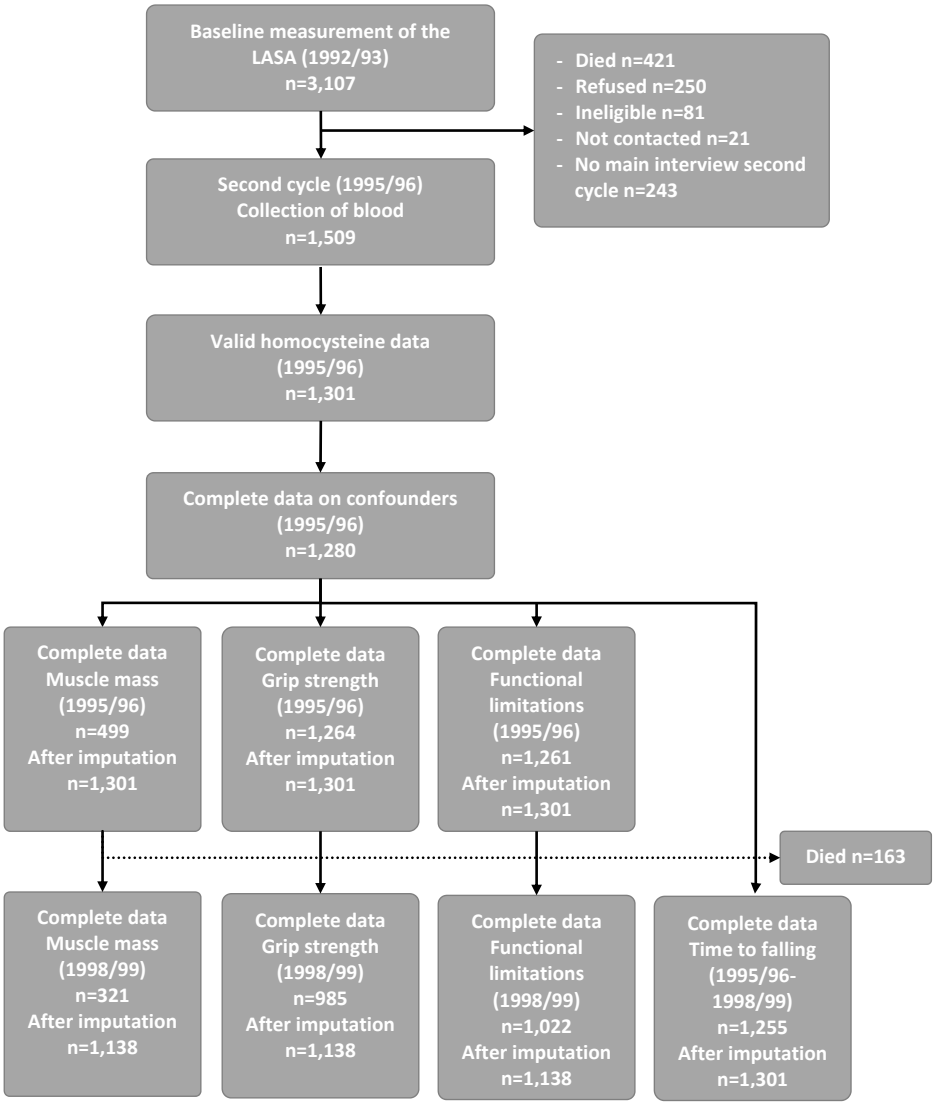


Figure 1 Design of the study sample.

Functional limitations

Functional limitations were assessed using a 6-item questionnaire about the degree of difficulties in climbing stairs, using transportation, cutting their own toenails, (un)dressing, rising from a chair, and walking outside for five minutes (0= no difficulty, to 4= unable). The items were derived from the validated OECD questionnaire.[29] The total score on functional limitations ranged from 0-24, with a score of 0 when no difficulties were reported and a score of 24 when respondents could not perform any of the activities.

Falling

Falls have been recorded prospectively in the period between 1995-1996 and 1998-1999. Respondents were asked to record fall events weekly on a calendar. If the respondents were not able to complete the calendar, did not return it even after a reminder, or completed it incorrectly, then the participants or proxies were contacted by telephone.

Potential effect modifiers and confounders

Potential effect modifiers were: gender and creatinine. In case no interaction effect was present, gender and creatinine were added to the models as confounders. Other potential confounders were: body mass index, region of living, level of education, alcohol consumption, smoking, and serum vitamin B12 concentration.

Creatinine (µmol/L) was analysed using the Jaffe alkaline picrate reaction with a Hitachi 747 analyzer (Roche Diagnostics, The Netherlands). Body weight and height were used to calculate the body mass index as mass (kg) divided by the length (m) squared. Region of living was outlined as living in the west, northeast, or south of the Netherlands. The highest level of education completed was measured by self-report and expressed in years of education. Alcohol consumption (no, light, moderate or excessive drinking) and smoking (never, former or current smoking) were self-reported.[30] Serum vitamin B12 (pmol/L) was measured using a competitive immunoassay luminescence on the automated ACS 180 System (Bayer Diagnostics, The Netherlands). The selected confounders are known to be associated with homocysteine and aspects of physical functioning.[2,31]

Statistical analyses

Analyses were performed using IBM SPSS statistics 20. Baseline characteristics were presented as means (standard deviation) for normally distributed variables, as median (interquartile range) for skewed variables, and as percentages for categorical variables. Multiple imputations (n=10, model type: predictive mean matching) were applied to missing data, except for missing data due to mortality. The missing-at-random assumption was tested using t-tests, and was not violated. Linear regression analyses were used to analyze muscle mass, muscle strength, and functional limitations, and Cox-proportional hazards was used for prospective falling (time to first fall, with deceased respondents included in the analyses until time of drop-out). The pooled results of the imputed datasets were presented. Quartile-based analyses were conducted by adding the quartiles of homocysteine as dummies in the regression models. The first quartile of homocysteine was considered the reference group. Effect modification by gender and/or creatinine was tested by adding the product terms of homocysteine and gender, and homocysteine and creatinine, respectively, to the model ($p<0.10$). Clinically based cutoff values of ≤104 µmol/L for men and ≤90 µmol/L for women were considered as normal, levels above those cut-offs were considered as high.[32] Stratified analyses were performed in case of effect

modification. Histograms and normal probability plots of the standardized residuals were made to verify the assumptions of linear regression analyses. The assumption of Cox-proportional hazards models was checked using log-minus-log plots, and was not violated. Further, linearity of the associations was checked. If the regression coefficients of the homocysteine dummies did not increase or decrease linearly, the association was considered non-linear. P-values for trends were examined by including quartiles of homocysteine as an ordinal variable. Non-pooled cubic spline regression functions were made using R version 2.15.0 to investigate non-linear associations further. Spline regression models are piecewise polynomial functions that join smoothly at points called knots. In contrast to categorical models that assume a constant association within categories, in spline models all data points are used, providing a better estimate of the relationships. Spline models were tested with 3 to 5 knots.

Results were adjusted for confounders. Additional adjustments for vitamin B12 were performed in separate models, because on the one hand this may be necessary to investigate the independency of the associations from vitamin B12, but on the other hand it may lead to overadjustment. Significance level was set at $p<0.05$.

Results

Table 1 Baseline characteristics of study population.

	Complete dataset		Imputed dataset	
	N	Mean ± SD	N	Mean ± SD
Homocysteine (µmol/L)[b]	1,280	13.5 [11.1-16.9]	1,301	13.6 [11.1-17.0]
Vitamin B12 (pmol/L)[b]	1,204	264.5 [213.0-329.8]	1,301	263.0 [213.0-328.0]
Creatinine (µmol/L)[b]	1,280	90 [79-103]	1,301	90 [79-103]
Age (years)[a]	1,280	75.6 ± 6.6	1,301	75.6 ± 6.6
Gender (% female)	1,280	51.5	1,301	51.4
Body Mass Index (kg/m^2)[a]	1,280	26.9 ± 4.2	1,301	26.9 ± 4.2
Region (% Amsterdam)	1,280	45.6	1,301	45.3
Years of education[a]	1,280	8.9 ± 3.3	1,301	8.9 ± 3.3
Alcohol (% moderate)	1,280	18.3	1,301	19.4
Smoking (% current)	1,280	18.3	1,301	18.2
Muscle mass (kg)[b]	499	17.7 [14.5-21.6]	1,301	15.4 [13.2-18.5]
Grip strength (kg)[a]	1,264	27.4 ± 10.0	1,301	27.2 ± 10.0
Functional limitations[b]	1,261	1.0 [0-5]	1,301	1.0 [0-5]
Falling (% yes)	1,255	55.6	1,301	58.0

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

Table 1 shows the characteristics of the study sample, based on both the complete and the imputed data. The number of deceased persons between baseline and 3-year follow-up was 163. The average age was 75.6 ± 6.6 years.

Gender was identified as an effect modifier in the associations with muscle mass (p=0.01), and muscle strength (p=0.07), but not in the associations with functional limitations (p=0.38) and falling (p=0.12). No effect modification by serum creatinine was demonstrated (p=0.25 for muscle mass, p=0.61 for muscle strength, p=0.11 for functional limitations, and p=0.27 for falling). For consistency across all analyses, all analyses were stratified by sex. Sex-specific quartiles of homocysteine were used in the analyses (Table 2).

Table 2 Quartiles of homocysteine.

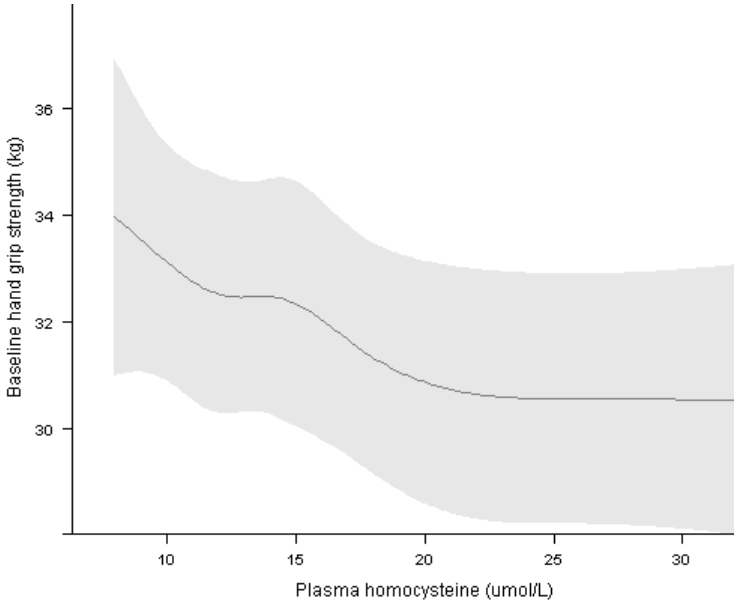
	Homocysteine (µmol/L)	
	Men N=632	Women N=669
Quartile 1	≤12.01	≤10.37
Quartile 2	12.02 to 14.45	10.38 to 12.71
Quartile 3	14.46 to 17.74	12.72 to 15.71
Quartile 4	≥17.75	≥15.72

Muscle mass

At baseline, no associations between quartiles of homocysteine and muscle mass were found (Regression coefficient (B) and 95% confidence interval (95% CI) in men: Quartile (Q)2: -0.07 (-0.83, 0.69); Q3: -0.06 (-0.72, 0.61); Q4: 0.17 (-0.61, 0.95); in women: Q2: -0.04 (-0.57, 0.48); Q3: -0.02 (-0.64, 0.60); Q4: 0.17 (-0.42, 0.76). The median absolute change in muscle mass over the 3-year follow-up period was +1.4 kg. Also in the longitudinal models, quartiles of homocysteine were not associated with muscle mass (data not shown). When vitamin B12 was added to the fully adjusted model, the results did not change substantially.

Grip strength

Cross-sectional analyses showed that in men, the second and fourth quartile of homocysteine were associated with significantly lower grip strength compared with the first quartile. When additional adjustments were made for vitamin B12, the association became stronger (Table 3). The p-value for trend in men was <0.01. In women, no statistically significant observations were observed (Table 3). The p-value for trend in women was 0.48. Figure 2 shows the spline functions of the association between homocysteine and baseline grip strength in men and women.



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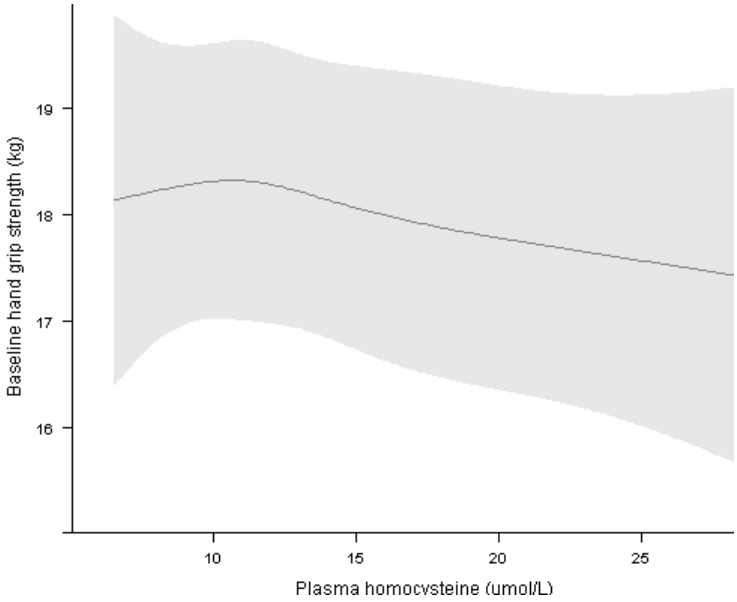


B

Figure 2 Spline functions of the association between homocysteine and handgrip strength, adjusted for age, serum creatinine, body mass index, region, years of education, alcohol use, and smoking. A: men; B: women.

The median change in grip strength over the 3-year follow-up period was -3.4 kg. Longitudinal analyses revealed no statistically significant associations between quartiles of homocysteine and grip strength in both men and women (Table 3).

Table 3 Relationship between homocysteine and grip strength.

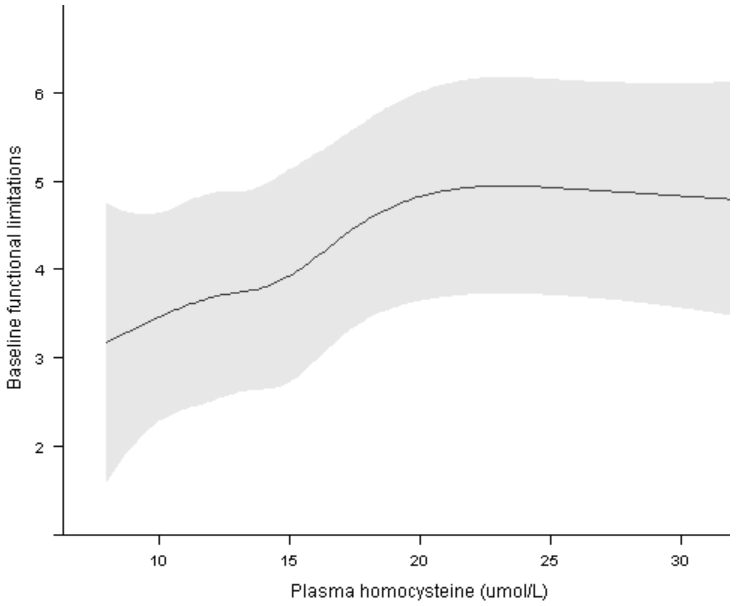
		Grip strength cross-sectional data		Grip strength 3-year follow-up[a]	
	Homo-cysteine	Men B (95% CI)	Women B (95% CI)	Men B (95% CI)	Women B (95% CI)
Model 1	Q1	Reference	Reference	Reference	Reference
	Q2	-1.83	0.14	-1.75	-0.85
		(-3.48, -0.19)*	(-0.88, 1.16)	(-4.32, 0.81)	(-3.24, 1.54)
	Q3	-1.18	-0.26	-1.29	0.63
		(-2.84, 0.48)	(-1.30, 0.78)	(-4.00, 1.41)	(-1.99, 3.25)
	Q4	-2.43	-0.32	-1.21	1.15
		(-4.23, -0.63)*	(-1.42, 0.78)	(-4.77, 2.35)	(-1.82, 4.12)
Model 2	Q1	Reference	Reference	Reference	Reference
	Q2	-1.89	0.19	-1.78	-0.87
		(-3.53, -0.26)*	(-0.84, 1.22)	(-4.33, 0.78)	(-3.25, 1.52)
	Q3	-1.43	-0.19	-1.49	0.60
		(-3.09, 0.23)	(-1.26, 0.88)	(-4.20, 1.23)	(-2.03, 3.23)
	Q4	-3.07	-0.21	-1.80	1.10
		(-4.91, -1.22)*	(-1.37, 0.95)	(-5.56, 1.95)	(-1.82, 4.01)

Results of regression models. See Table 2 for cut-offs for the homocysteine quartiles. Model 1: Adjusted for age, serum creatinine, Body Mass Index, region, years of education, alcohol use, and smoking; Model 2: Additional adjusted for vitamin B12; [a] Adjusted for baseline grip strength; *p<0.05. B= unstandardised regression coefficient, CI= confidence interval, Q= quartile.

Functional limitations

The cross-sectional analyses revealed that in men, the fourth quartile of homocysteine was significantly associated with more functional limitations as compared to the first quartile of homocysteine after adjustment for vitamin B12 (Table 4). The P-value for trend in men was 0.05. The findings are shown by the spline functions of the association of homocysteine with functional limitations (Figure 3). In women, the association did not reach statistical significance. P for trend was 0.08 (Figure 3, Table 4).

In men, the longitudinal analyses did not indicate a significant association. In women, the longitudinal analyses showed that the fourth quartile of homocysteine was associated with more functional limitations 3 years later as compared with the first quartile. This association became stronger after adjustment for vitamin B12 (Table 4). The P-value for trend was 0.02.



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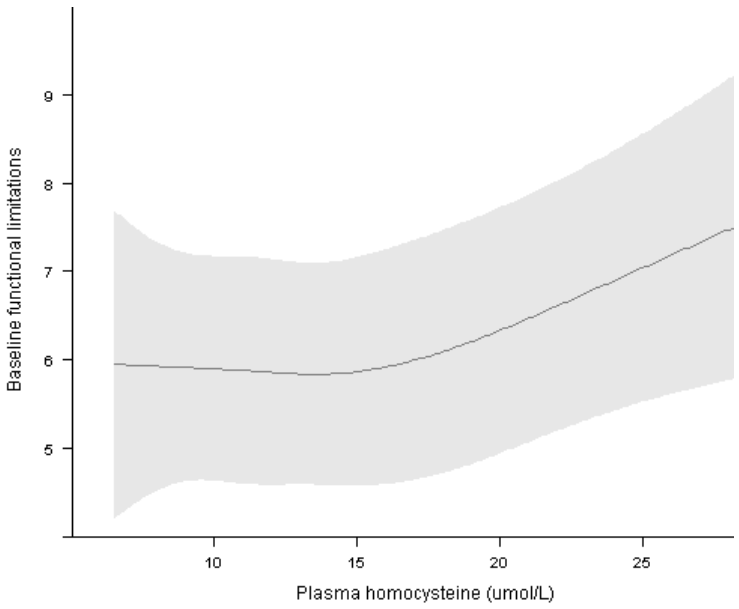

B

Figure 3 Spline functions of the association between homocysteine and baseline functional limitations, adjusted for age, serum creatinine, body mass index, region, years of education, alcohol use, and smoking. A: men; B: women.

Table 4 Relationship between homocysteine and functional limitations.

		Functional limitations cross-sectional data		Functional limitations 3-year follow-up[a]	
	Homo-cysteine	Men B (95% CI)	Women B (95% CI)	Men B (95% CI)	Women B (95% CI)
Model 1	Q1	Reference	Reference	Reference	Reference
	Q2	0.38	0.54	-0.02	0.23
		(-0.50, 1.26)	(-0.40, 1.48)	(-0.85, 0.82)	(-0.55, 1.00)
	Q3	0.28	0.22	-0.34	0.23
		(-0.61, 1.17)	(-0.75, 1.18)	(-1.18, 0.51)	(-0.57, 1.02)
	Q4	0.69	0.89	-0.67	1.03
		(-0.28, 1.66)	(-0.14, 1.91)	(-1.67, 0.34)	(0.15, 1.91)*
Model 2	Q1	Reference	Reference	Reference	Reference
	Q2	0.42	0.49	-0.01	0.29
		(-0.45, 1.29)	(-0.46, 1.44)	(-0.85, 0.82)	(-0.50, 1.08)
	Q3	0.46	0.14	-0.29	0.32
		(-0.42, 1.35)	(-0.86, 1.13)	(-1.14, 0.56)	(-0.49, 1.13)
	Q4	1.15	0.76	-0.52	1.19
		(0.16, 2.14)*	(-0.31, 1.84)	(-1.55, 0.51)	(0.25, 2.13)*

Results of regression models. See Table 2 for cut-offs for the homocysteine quartiles. Model 1: Adjusted for age, serum creatinine, Body Mass Index, region, years of education, alcohol use, smoking; Model 2: Additional adjusted for vitamin B12; [a] Additional adjusted for baseline functional limitations; *p<0.05; B= unstandardised regression coefficient, CI= confidence interval, Q= quartile.

Falling

During the 3-year follow-up period, 698 persons fell at least once (54%). No association of quartiles of homocysteine with time to first fall was found (Hazard ratio and 95% CI in men Q2: 1.16 (0.83, 1.62); Q3: 1.31 (0.93, 1.86); Q4: 1.37 (0.93, 2.02); in women Q2: 1.04 (0.78, 1.39); Q3: 1.00 (0.73, 1.36); Q4: 1.17 (0.83, 1.65).

Discussion

This study showed that in older men and women, elevated homocysteine levels are associated with lower grip strength in men. Furthermore, higher homocysteine levels were associated with more functional limitations in men, and with more functional limitations over a 3-year period in women. The results did not reveal an association of homocysteine levels with muscle mass or falling. No interaction effect with serum creatinine levels was observed.

The results are consistent with results from previous studies. With respect to muscle strength, results from the NHANES study among over 1,600 persons >60 years showed an

inverse association between homocysteine and peak quadriceps strength.[15] The authors suggested that muscle strength acted as a mediator in the inverse association between homocysteine and reduced physical performance. The same study also reported an association between homocysteine and disability in activities of daily living.[15] In contract to objective performance tests that measure physical functioning at a specific moment, functional limitation questionnaires measure functioning averaged over a longer period of time in different circumstances.[33] Although they might be influenced by personal factors, they come closer to true daily functioning as compared with performance tests.

Regarding muscle mass, a study using data from the Hordaland Study did not show an association between homocysteine levels and total body lean mass in over 5,000 subjects.[34] Those findings are also similar to our findings. Further, previously, no association was found between homocysteine and recurrent falling.[21] This suggestion was supported by one intervention trial, aimed to reduce homocysteine levels among stroke survivals, showing identical fall rates in the intervention and placebo group.[22]

Gender was identified as an effect modifier in the association with handgrip strength and muscle mass. In line with this, our previous study with physical performance as outcome reported that the association with homocysteine was sex-dependent.[18] In contrast, the previous findings from the NHANES study did not show effect modification by gender in the association of homocysteine and quadriceps strength. Also the association with functional limitations was not modified by gender in that study, and this finding is also supported by the current data.

The change in muscle mass over the 3-year period remained within the range of the measurement error (3%), while muscle strength decreased substantially. This apparently paradoxical difference might be explained by the fact that a decline in muscle strength might not only be a result of muscle atrophy, but also as a result of other age-related alterations, for instance alterations in neurologic function, or contractile properties.[35]

Muscle function might be affected by homocysteine levels in several ways. First of all, high homocysteine levels have been association with neurological dysfunction. Recently, results from the InCHIANTI study among persons aged 60 years and older indicated that homocysteine levels ≥13 μmol/L were associated with worse peripheral nerve function, both sensory and motor function.[36] The authors indicate that this might have important implications for disability, since poor nerve function has been associated with lower strength and performance. Second, the association between homocysteine levels and muscle function might be mediated by creatine. Creatine-phosphate is an important energy source in muscle during high-energy activities. Its synthesis is closely linked to the homocysteine metabolism: Creatine synthesis accounts for about 45% of the methyl group donations of methionine with subsequent homocysteine formation.[37] Creatine synthesis might be reduced as a result of reduced availability of methyl compounds with increasing homocysteine levels or decreasing B-vitamin status. In older persons, decreases in total

creatine and creatine-phosphate are observed.[38] Further, increased serum creatine by creatine supplementation enhances the potential to perform high intensity exercise and increase fat free mass.[38] It would be interesting to focus on the precise mechanism at work in future research.

Vitamin B12 and folic acid are essential cofactors in the remethylation of homocysteine to methionine. Subsequently, low vitamin B12 status induces homocysteine elevation. Since low vitamin B12 status precedes homocysteine elevation, vitamin B12 cannot be an intermediate in the pathway of homocysteine and muscle function. Adjustments were made for vitamin B12 status to study whether the associations between homocysteine and physical functioning were independent from vitamin B12 status. After adjustment for vitamin B12 the observed association became somewhat stronger for grip strength in men, and functional limitations in men and women. An explanation might be that vitamin B12 shows only a modest correlation with more sensitive measures for vitamin B12 bioavailability, such as holotranscobalamin, methylmalonic acid, and homocysteine.[39] Unfortunately, folate and methylmalonic acid data were only available in a small sub-sample. The association of B-vitamins with muscle mass and function and its treatment effects needs further investigation. Positive findings might delay the onset and progression of loss of muscle strength and functional limitations.

The major strength of the current study is that cross-sectional as well as longitudinal data from a large population-based study were used. Several clinical and sub-clinical aspects of physical functioning were studied, with the opportunity to carefully control for confounding factors, and to examine effect modification. Missing values were imputed to efficiently make the use of all available data and increase the power to show an association. Moreover, multiple imputation accounts for the uncertainty within the imputed data.[40] Data from persons who died during follow-up were not imputed. Because homocysteine is associated with mortality in women,[41] the results might be underestimated. However, fall rates in the year before baseline were similar among deceased persons and survivors (p=0.76, data not shown). Other limitations are the single measurement of homocysteine levels, and the lack of adequate B-vitamin markers.

In summary, the results of this large population-based study showed that in older persons elevated homocysteine levels are associated with low muscle strength in men and functional limitations in men and women.

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Conflict of interest

The authors declare that they have no conflict of interest.

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