# CHAPTER 5

# Homocysteine and the methylenetetrahydrofolate reductase 677C→T polymorphism in relation to muscle mass and strength, physical performance, and postural sway

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## Abstract

**Background/Objectives**

Elevated plasma homocysteine has been linked to reduced mobility and muscle functioning in elderly. The relation of methylenetetrahydrofolate reductase (MTHFR) 677C→T polymorphism with these associations has not yet been studied. This study aimed to investigate (1) the association of plasma homocysteine and the MTHFR 677C→T polymorphism with muscle mass, handgrip strength, physical performance, and postural sway; (2) the interaction between plasma homocysteine and the MTHFR 677C→T polymorphism.

**Subjects/Methods**

Baseline data from the B-PROOF study (n=2,919, mean age=74.1 ± 6.5) were used. Muscle mass was measured using dual X-ray absorptiometry, handgrip strength with a handheld dynamometer and physical performance with walking-, chair stands- and balance tests. Postural sway was assessed on a force platform. The data were analyzed using regression analyses with plasma homocysteine levels in quartiles.

**Results**

There was a significant inverse association between plasma homocysteine and handgrip strength (Quartile 4: Regression coefficient B: -1.14; 95% confidence interval: -1.96, -0.32) and physical performance score (Quartile 3: B: -0.53; 95% CI: -0.95, -0.10; and Quartile 4: -0.94; 95% CI: -1.40, -0.48) in women only, independent of serum vitamin B12 and folic acid. No association was observed between the MTHFR 677C→T polymorphism and the outcomes. High plasma homocysteine in the 677CC and 677CT genotypes, but not in the 677TT genotype, was associated with lower physical performance.

**Conclusions**

Elevated plasma homocysteine concentrations are associated with reduced physical performance and muscle strength in older women. There is an urgent need for randomized controlled trials to examine whether lowering homocysteine levels might delay physical decline.

## Introduction

Muscle tissue is progressively lost with aging, contributing to a decline in muscle strength. Muscle weakness and loss of muscle strength are risk factors of reduced physical functioning, increased fall risk, and mortality.[1] Reduced physical functioning, muscle wasting and muscle weakening are thought to be multifactorial. Determinants include lifestyle variables, chronic diseases, genetic variables, inflammatory processes and hormonal changes.[2]

One of the potential determinants is the amino acid homocysteine. Elevated plasma homocysteine concentrations have been associated with an increased fracture risk in independently living elderly.[3-5] Increasing evidence from observational studies suggests that elevated plasma homocysteine concentrations are also associated with a decline in physical functioning in older persons,[6-11] which might be mediated by a reduced muscle strength.[8,11] Evidence of plasma homocysteine being related to a higher risk of falling and reduced balance is less convincing.[3,6,11,12]

In the metabolism of homocysteine, essential vitamins and enzymes include vitamin B12, folic acid and the enzyme methylenetetrahydrofolate reductase (MTHFR). A common genetic variation of the MTHFR gene is the replacement of cytosine by thymine (C-T) at base position 677. This single nucleotide polymorphism induces elevated homocysteine concentrations by a reduced enzymatic activity of MTHFR, particularly in the presence of suboptimal folate intake.[13] The MTHFR 677C→T polymorphism could be one of the factors leading to muscle weakness and performance decline in older persons by its homocysteine-increasing potential. At present, studies on the MTHFR 677C→T polymorphism and muscle characteristics, physical performance and balance are lacking.

In the current study, we examined (1) the associations of plasma homocysteine concentrations and the MTHFR 677C→T polymorphism with muscle mass, handgrip strength, physical performance, and postural sway; (2) the interaction between plasma homocysteine and the MTHFR 677C→T polymorphism.

## Subjects and methods

### Study sample

This study was performed using baseline data of the B-PROOF study. B-PROOF is an acronym for B-vitamins for the PRevention of OsteoporOtic Fractures. The study design, population, and data collection have been described elsewhere in detail.[14] In summary, the B-PROOF study is a multi-center, double-blind, randomized clinical trial on the efficacy of vitamin B12 and folic acid supplementation in the prevention of fractures in persons aged ≥65 years. In total, 2, 919 persons with elevated plasma homocysteine concentrations (≥12 µmol/L) and serum creatinine ≤150 µmol/L were included in the B-PROOF study. For the current study,

persons with incomplete data on confounders (n=91) or with serum vitamin B12 concentrations >800 pmol/L (n=9) were excluded, resulting in a study sample of 2,819 persons. The number of persons included in the analyses differed per outcome measure (Table 1). The Medical Ethics Committee of Wageningen University approved the study and local feasibility was provided by the Medical Ethics Committee of Erasmus MC and VU University Medical Center. All participants provided written informed consent. The B-PROOF study is registered with the Netherlands Trial (NTR1333) and with ClinicalTrials.gov (NCT00696514).

**Plasma homocysteine concentrations and MTHFR genotype**

Participants were in fasting state, or had taken a light breakfast when blood samples were drawn. Homocysteine was assessed in EDTA plasma using HPLC at Wageningen University (intra-assay coefficient of variation (CV)=3.1%, inter-assay CV=5.9%), LC-MS/MS at Erasmus MC (Waters, Etten-Leur, the Netherlands) (CV=3.1%), or the Architect i2000 RS analyzer at VU University Medical Center (Abbott Laboratories, Abbott Park, IL, USA) (intra-assay CV=2%, inter-assay CV=4%).[14] Cross-calibration of the assays revealed that the outcomes did not differ significantly. DNA was isolated from buffy coats for genotyping. Single nucleotide polymorphisms were determined using Illumina Omni-express array (Illumina Inc., San Diego, CA, USA). MTHFR polymorphism data were available in 2,677 persons.

**The outcomes**

Body composition was measured using dual-energy X-ray absorptiometry (DXA) in a subsample of the study population (n=1,221, 42%) in two of the study centers. The Hologic QDR 4500 Delphi device (Hologic Inc., Waltham, MA, USA; used in VU University Medical Center) or the GE Lunar Prodigy device (GE Healthcare, Madison, WI, USA; used in Erasmus MC) was used. As a measure of skeletal muscle mass (kg), the amount of fat-free soft tissue was calculated as the sum of the fat-free, bone-free mass of the arms and legs.[15]

Muscle strength was operationalized by measuring handgrip strength (kg). Participants were asked to perform two maximum handgrip trials with each hand in standing position using a strain-gauged dynamometer (Takei, TKK 5401, Takei Scientific Instruments Co. Ltd., Tokyo, Japan). Handgrip strength was calculated as the average of the highest scores of both hands.

Physical performance was assessed using three different tests: (1) the walking test, that is, the time needed to walk 3 m, turn around and walk back as quickly as possible, (2) the chair stands test, that is, the time needed to rise from and sit down in a chair for five times without the use of arms, as quickly as possible, and (3) the tandem stand, that is, the ability to stand with one foot in front of the other for 10 s. Quartiles of the time needed to perform the walking test and chair stands were calculated.[16] The categories for walking were: score 0 (unable), score 1 (≥9 s), score 2 (7-8 s), score 3 (6 s), and score 4 (≤5 s); the

categories for the chair stands were: score 0 (unable), score 1 (≥15 s), score 2 (12-14 s), score 3 (10-11 s), and score 4 (≤9 s). The tandem stand score was categorized as follows: score 0 (unable), score 2 (able to hold position for 4-9 s), and score 4 (able to hold position for at least 10 s). A total physical performance score (range 0-12, with a score of 12 representing optimal physical performance) was calculated by summing up the scores of the three different tests. The physical performance tests have been shown to be reliable and valid instruments to measure physical performance: A lower total score has been associated with an increased fall- and fracture risk, frailty, and lower cognitive functioning.[16-19]

Postural sway was measured in a subsample of the study population (n=114) with a portable force platform (AccuSway System, AMTI, Watertown, MA, USA). Participants were instructed to stand quietly on the force platform with their feet comfortably spread for 30 s. This was done twice with eyes open and eyes closed. Medial-lateral and anterior-posterior sway of the center of pressure, as well as total length of the sway patterns, were reported.



**Potential modifiers and confounders**

Gender was regarded as potential modifier, because gender differences have been observed in the plasma homocysteine distribution, which might be caused by differences in fat-free mass.[20] Furthermore, the effect modification by serum creatinine was checked, as creatinine is a marker for muscle mass and renal function, therefore being a major determinant of plasma homocysteine. Potential confounders included gender and creatinine (if no interaction was found), age, years of education, study location, body mass index, current smoking status, alcohol use, physical activity, vitamin B12, and folic acid status.

Serum creatinine (µmol/L) was assessed using the Modular P analyzer (Roche Diagnostics, Indianapolis, IN, USA). Clinically used reference values of creatinine were ≤90 µmol/L in women and ≤104 µmol/L in men.[21] Educational level was assessed by asking participants' highest level of completed education. This was converted into years of education (range 5-18). The study location (Amsterdam/Rotterdam/Wageningen) is the research center at which the participant was included in the trial. Body height was measured using a stadiometer. Body weight was measured on a calibrated balance scale. Body mass index was calculated as weight (kg)/height $(m)^2$. Current smoking status (yes/no) and alcohol use were self-reported. Alcohol use was categorized as 'never', 'light', 'moderate' and 'excessive'.[22] Total physical activity (min/day) was measured with the validated LASA Physical Activity Questionnaire (LAPAQ).[23] In serum, vitamin B12 and folic acid were measured using an electrochemiluminescence immunoassay on a Roche Modular E170 (Roche, Almere, The Netherlands).

**Statistical analyses**

Plasma homocysteine was categorized into gender-specific quartiles. Comparisons of baseline characteristics among quartiles of homocysteine and MTHFR genotype were carried out using Chi-squared tests for categorical variables, analysis of variance for normally distributed continuous variables, or Kruskall-Wallis for skewed continuous variables. Differences between sub-samples were compared using Chi-squared tests, t-tests, or Mann-Whitney U tests. Hardy-Weinberg equilibrium of the genotype distribution was calculated with standard procedures of Chi-squared analyses.

Linear regression analyses were performed. It was tested whether the interaction with serum creatinine and gender was significant ($p<0.10$). Adjustments were made for gender (if no interaction was found) and age. Other confounders were added to the model when they were associated with both plasma homocysteine or MTHFR genotype and the outcome ($p<0.10$). Serum vitamin B12 and folic acid were added to the fully adjusted model. P-values for trends across homocysteine categories were examined by including the quartiles as an ordinal variable. The assumptions of linear regression analyses were verified by evaluating normal probability plots of the standardized residuals. Next, the interaction between plasma homocysteine (quartile 1 and 2 versus quartile 3 and 4) and the MTHFR genotype was examined. Analyses of covariance were performed to calculate adjusted means per category. The data were analyzed using IBM SPSS Statistics 20 (SPSS Inc, Chicago, IL, USA). Significance level of the associations was set at $p<0.05$.

## Results

Baseline characteristics of the study sample are presented in Table 1. Homocysteine quartiles according to gender are presented in Table 2. Persons in the highest homocysteine quartile were older ($p<0.01$), had a lower serum vitamin B12 ($p<0.01$) and folic acid ($p<0.01$), and a higher serum creatinine ($p<0.01$), had lower handgrip strength and physical performance scores ($p<0.01$), had lower education ($p<0.01$) and were more frequently non-alcohol users ($p<0.01$) compared with persons in the lowest homocysteine quartile.

**Table 1** Baseline characteristics of the study sample.

| Characteristic | N | Men | N | Women |
|---|---|---|---|---|
| Age (years)[a] | 1,424 | 73.3 ± 6.1 | 1,395 | 74.9 ± 6.8 |
| Homocysteine (µmol/L) | 1,424 | 14.6 [13.1-16.9] | 1,395 | 14.2 [13.0-16.4] |
| Vitamin B12 (pmol/L) | 1,424 | 259.4 [201.3-329.4] | 1,395 | 268.1 [212.7-348.9] |
| Folic acid (nmol/L) | 1,424 | 18.5 [14.7-23.5] | 1,395 | 19.0 [15.0-24.6] |
| Creatinine (µmol/L) | 1,424 | 90.0 [81.0-101.0] | 1,395 | 73.0 [65.0-83.0] |
| Muscle mass (kg)[a] | 621 | 25.8 ± 3.3 | 566 | 18.1 ± 2.5 |
| Handgrip strength (kg)[a] | 1,406 | 39.0 ± 8.0 | 1,357 | 23.0 ± 5.6 |
| Physical performance (0-12) | 1,413 | 10 [7-11] | 1,367 | 8 [4-10] |
| Postural sway (cm) | | | | |
| - Medial-lateral, eyes open | 35 | 0.34 [0.22-0.48] | 71 | 0.26 [0.21-0.39] |
| - Medial-lateral, eyes closed | 33 | 0.39 [0.26-0.77] | 70 | 0.34 [0.22-0.47] |
| - Anterior-posterior, eyes open | 35 | 0.49 [0.38-0.58] | 71 | 0.41 [0.30-0.52] |
| - Anterior-posterior, eyes closed | 33 | 0.62 [0.48-1.01] | 70 | 0.53 [0.41-0.69] |
| - Total length of sway, eyes open | 35 | 55.3 [35.3-69.6] | 71 | 39.6 [30.6-51.3] |
| - Total length of sway, eyes closed | 33 | 88.2 [46.5-147.1] | 70 | 60.7 [38.4-87.5] |
| Study location (%) | 1,424 | | 1,395 | |
| - Amsterdam | | 20.3 | | 32.4 |
| - Wageningen | | 34.6 | | 24.4 |
| - Rotterdam | | 45.2 | | 43.2 |
| Education (years) | 1,424 | 10 [6-15] | 1,395 | 9 [6-11] |
| Body mass index (kg/m$^2$) | 1,421 | 26.5 [24.7-28.6] | 1,384 | 27.0 [24.5-30.0] |
| Current smoking (%) | 1,424 | 10.7 | 1,395 | 8.5 |
| Physical activity (min/day) | 1,424 | 115.7 [70.7-173.6] | 1,395 | 143.6 [96.4-205.7] |
| Alcohol use (% moderate) | 1,424 | 37.2 | 1,495 | 20.5 |

All variables are presented in median [interquartile range] unless stated otherwise. [a]Results are presented in mean ± standard deviation.

**Table 2** Quartiles of homocysteine.

| | Homocysteine (µmol/L) | |
|---|---|---|
| Quartile | Men | Women |
| Quartile 1 | 12.0 to 13.1 | 12.0 to 12.9 |
| Quartile 2 | 13.2 to 14.5 | 13.0 to 14.1 |
| Quartile 3 | 14.6 to 16.8 | 14.2 to 16.3 |
| Quartile 4 | ≥16.9 | ≥16.4 |

**Association of plasma homocysteine with muscle mass, handgrip strength, physical performance and postural sway**

Persons with data on muscle mass (42% of study sample) were significantly younger (p<0.01), had a higher serum folic acid (p=0.01), had a higher handgrip strength (p=0.01) and physical performance level (p<0.01), a lower body mass index (p=0.04), were more physically active (p<0.01), and used more alcohol (p<0.01) compared with persons without data.

Linear regression analysis identified serum creatinine as an effect modifier in the association of plasma homocysteine with muscle mass (p=0.04), whereas gender was not (p=0.60). In persons with normal serum creatinine, a lower mean muscle mass was observed in the second but not in the third or fourth quartile of homocysteine as compared with the first quartile (Table 3). P for linear trend across the four quartiles was not significant (p=0.41). Furthermore, in persons with high serum creatinine concentrations, no significant associations were observed (Table 3).

In the association of plasma homocysteine with handgrip strength, gender was identified as an effect modifier (p=0.03). Women in the fourth quartile of homocysteine had significantly lower handgrip strength as compared with women in the first quartile. P for linear trend was significant (p=0.02) (Table 3). Additional adjustments for serum vitamin B12 and folic acid concentrations did not change the results.

In the association of plasma homocysteine with physical performance, a significant interaction effect between plasma homocysteine and gender was found (p=0.05). Women in both the third and fourth quartile of homocysteine had a significantly lower physical performance score as compared with women in the first quartile. The associations were independent of vitamin B12 and folic acid status (Table 3). P for linear trend was significant (p<0.01). In men, no association was found (Table 3).

Significant associations between plasma homocysteine and postural sway were not observed (data not shown). Moreover, serum vitamin B12 was not associated with the examined outcomes (Quartile 1 vs. Quartile 4 for muscle mass: regression coefficient B: 0.02; 95% confidence interval: -0.43, 0.47; for handgrip strength: B: -0.50; 95% CI: -1.14, 0.14; and for physical performance: B: 0.02; 95% CI: -0.26, 0.29), and neither was serum folic acid (Quartile 1 vs. Quartile 4 for muscle mass: B: -0.08; 95% CI: -0.52, 0.36; for handgrip strength: B: 0.08; 95% CI: -0.56, 0.73; and for physical performance: B: -0.18; 95% CI: -0.45, 0.10).

**Table 3** Regression coefficients and 95% CI as measures of association of homocysteine with muscle mass, and handgrip strength, and physical performance.

| | | Quartile 1<br>B (95% CI) | Quartile 2<br>B (95% CI) | Quartile 3<br>B (95% CI) | Quartile 4<br>B (95% CI) | P for linear trend |
|---|---|---|---|---|---|---|
| Muscle mass | | | | | | |
| Model 1[a] | Normal creatinine | Reference | -0.56 (-1.01, -0.11)* | 0.35 (-0.10, 0.79) | -0.10 (-0.60, 0.41) | 0.41 |
| | High creatinine | Reference | 0.58 (-1.16, 2.33) | -0.54 (-2.17, 1.10) | -0.49 (-2.11, 1.13) | 0.21 |
| Model 2[b] | Normal creatinine | Reference | -0.57 (-1.02, -0.11)* | 0.33 (-0.14, 0.79) | -0.13 (-0.67, 0.41) | 0.52 |
| | High creatinine | Reference | 0.53 (-1.26, 2.33) | -0.64 (-2.35, 1.06) | -0.67 (-2.45, 1.11) | 0.21 |
| Handgrip strength | | | | | | |
| Model 1[c] | Men | Reference | -1.04 (-2.07, -0.02)* | -0.53 (-1.54, 0.48) | -0.77 (-1.86 0.32) | 0.29 |
| | Women | Reference | -0.46 (-1.19, 0.27) | -0.39 (-1.12, 0.35) | -1.03 (-1.80, -0.26)* | 0.02* |
| Model 2[b] | Men | Reference | -1.06 (-2.09, -0.03)* | -0.60 (-1.64, -0.44) | -0.89 (-2.07, 0.29) | 0.23 |
| | Women | Reference | -0.50 (-1.23, 0.24) | -0.45 (-1.20, 0.30) | -1.14 (-1.96, -0.32)* | 0.02* |
| Physical performance | | | | | | |
| Model 1[d] | Men | Reference | 0.06 (-0.30, 0.41) | -0.33 (-0.68, 0.03) | -0.10 (-0.48, 0.28) | 0.24 |
| | Women | Reference | -0.19 (-0.60, 0.22) | -0.50 (-0.92, -0.09)* | -0.88 (-1.31, -0.45)* | <0.01* |
| Model 2[b] | Men | Reference | 0.05 (-0.31, 0.41) | -0.35 (-0.71, 0.02) | -0.13 (-0.54, 0.28) | 0.21 |
| | Women | Reference | -0.20 (-0.61, 0.21) | -0.53 (-0.95, -0.10)* | -0.94 (-1.40, -0.48)* | <0.01* |

In men: Quartile 1=12.0 - 13.1 µmol/L, Quartile 2=13.2 - 14.5 µmol/L, Quartile 3=14.6 - 16.8 µmol/L, and Quartile 4 ≥16.9 µmol/L; In women: Quartile 1=12.0 - 12.9 µmol/L, Quartile 2=13.0 - 14.1 µmol/L, Quartile 3=14.2 - 16.3 µmol/L, and Quartile 4 ≥16.4 µmol/L; [a] Corrected for age, gender, study location, alcohol use, physical activity, and years of education; [b] Additionally corrected for serum vitamin B12 and folic acid; [c] Corrected for age, study location, alcohol use, smoking, serum creatinine, and years of education; [d] Corrected for age, study location, alcohol use, physical activity, serum creatinine, and years of education; *p<0.05; B= regression coefficient.

**Association of MTHFR genotype with muscle mass, handgrip strength, physical performance and postural sway**

Next, we investigated whether the MTHFR 677C→T genotype was associated with muscle mass, handgrip strength, physical performance, and postural sway. The genotype distribution deviated from the Hardy-Weinberg equilibrium (p<0.01). Among persons with 677TT genotype, plasma homocysteine concentrations were significantly higher (p<0.01) and serum folic acid concentrations significantly lower (p<0.01). The median plasma homocysteine concentration [interquartile range] was 14.2 [12.9-16.2] µmol/L in the 677CC genotype, 14.4 [13.0-16.6] µmol/L in the 677CT genotype, and 15.0 [13.4-17.4] µmol/L in the 677TT genotype. The median serum folic acid concentration [interquartile range] was 19.4 [15.4-24.9] nmol/L in the 677CC genotype, 18.4 [14.7-23.8] nmol/L in the 677CT genotype and 16.5 [13.5-22.0] nmol/L in the 677TT genotype. The other characteristics were similar across the three genotypes. Significant associations with muscle mass, handgrip strength, physical performance (Table 4), or postural sway (data not shown), as examined by linear regression analyses, were not found.

**Table 4** Regression coefficients and 95% CI as measures of association of MTHFR genotype with homocysteine, muscle mass, handgrip strength, and physical performance.

| | 677CC genotype B (95% CI) | 677CT genotype B (95% CI) | 677TT genotype B (95% CI) | P for linear trend |
|---|---|---|---|---|
| Homocysteine[a] | Reference | 0.22 (-0.05, 0.50) | 0.80 (0.39, 1.20)* | <0.01 |
| Folic acid[b] | Reference | -0.71 (-1.31, -0.10) | -2.32 (3.19, -1.44) | <0.01 |
| Muscle mass[c] | Reference | 0.21 (-0.14, 0.56) | 0.37 (-0.14, 0.88) | 0.11 |
| Handgrip strength[a] | Reference | 0.28 (-0.24, 0.79) | 0.31 (-0.43, 1.05) | 0.28 |
| Physical performance[a] | Reference | -0.14 (-0.35, 0.08) | -0.01 (-0.32, 0.30) | 0.58 |

[a] Corrected for age, gender, alcohol use, smoking, and years of education; [b] Corrected for age, gender, and years of education; [c] Corrected for age, gender, alcohol use, study location, and years of education; *p<0.05, B= regression coefficient.

**Homocysteine-MTHFR genotype interaction**

Subsequently, we investigated the homocysteine-gene interaction for handgrip strength and physical performance. The interaction term of plasma homocysteine times MTHFR genotype was added to the model, and was significant for physical performance (p=0.04). No interaction effect was found for handgrip strength. Figure 1 shows the mean physical performance scores per genotype stratified by plasma homocysteine concentration after adjustment for confounding. Physical performance scores were significantly lower in persons with a high plasma homocysteine concentration in both the 677CC (p<0.01) and 677CT genotype (p<0.01), but not in the 677TT genotype (Figure 1).

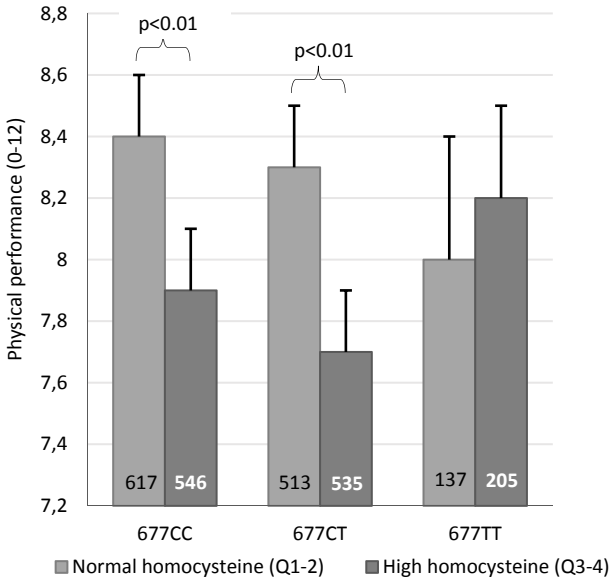




**Figure 1** Adjusted mean physical performance scores for normal and high homocysteine concentrations presented according to MTHFR genotype.
Estimated marginal means derived from Analysis of covariance. Results are adjusted for age, gender, study location, alcohol use, physical activity, serum creatinine, and years of education. Numbers of subject are presented in the bars. High homocysteine concentrations is defined as levels >14.5 µmol/L in men and >14.1 µmol/L in women.

## Discussion

This study demonstrates that in a population of older persons, higher plasma homocysteine concentrations were associated with lower handgrip strength and physical performance score in women. Higher plasma homocysteine concentrations were observed in persons with the 677TT genotype of the MTHFR gene, but this polymorphism was not associated with muscle mass, strength, physical performance or postural sway. Persons with high plasma homocysteine levels in combination with the 677CC or 677CT genotype, respectively, had lower physical performance scores, but this was not found in persons with the combination of high plasma homocysteine and the 677TT genotype.

Prior studies suggested a reduced physical functioning and accelerated mobility decline in older persons with elevated plasma homocysteine concentrations.[6-11] The results of the current study consolidate this suggestion, as well as the suggestion that elevated plasma homocysteine is associated with reduced muscle strength.[8,11] In addition, the observed gender interactions are comparable with results from an earlier study on homocysteine and physical performance from the Longitudinal Aging Study Amsterdam.[11]

Furthermore, in persons with high creatinine we did not find a statistically significant association with plasma homocysteine; in persons with low creatinine, only a statistically significant association was observed for the second quartile of homocysteine. The latter finding may be a coincidental one, as it does not seem biologically plausible that only the second quartile would be statistically significant. The lack of associations between plasma homocysteine and muscle mass is also congruent with our previous findings from the Longitudinal Aging Study Amsterdam.[24]

Previous empirical support for the relation between MTHFR polymorphisms and physical performance is limited. The MTHFR 677C→T polymorphism in relation to the frailty syndrome was studied in the Women's Health and Aging Study in >300 older women.[25] Frailty was defined by the presence of three or more frailty criteria including shrinking, muscle weakness, poor endurance, low physical activity, and low walking speed. The MTHFR 677C→T polymorphism was not associated with frailty in older women.[25] In contrast, in a study among Caucasian families, other polymorphisms in the MTHFR gene were associated with lean body mass.[26] To our knowledge, our study is the first that analyzed MTHFR 677C→T polymorphism in relation to physical performance and muscle function in a large sample of older persons.

We showed that the MTHFR 677C→T polymorphism was not related to physical outcomes in older persons, in contrast to plasma homocysteine concentration itself. According to the principles of Mendelian randomization,[27] our finding that MTHFR 677C→T polymorphism is associated with elevated plasma  levels, but is not associated with physical outcomes, might indicate that the association between plasma homocysteine and physical outcomes is not a causal relationship. This suggestion is supported by the homocysteine-gene interactions, that is, high plasma homocysteine concentrations in the 677TT genotype were not associated with physical performance, in contrast to high plasma homocysteine concentrations in the 677CC and 677CT genotype. If homocysteine itself is a causal factor, an association would be expected in all genotypes whenever plasma homocysteine concentrations are elevated, and especially in the 677TT genotype that is associated with higher plasma homocysteine concentrations. However, our results remain inconclusive, as the results in the 677TT genotype might have been compromised by a small sample size. Also, the effect of MTHFR on plasma homocysteine elevation was only modest, for example, 40% of the persons with the 667TT genotype were in the first or second quartile of homocysteine.

Previous studies suggested that the 677TT genotype induces an elevated plasma homocysteine concentration, particularly when the folic acid concentration is low.[13] We did not find such an interaction between genotype and serum folic acid in this study population (data not shown). This may have been caused by the fact that mean serum folic acid concentration was relatively high, and only a small subgroup was folic acid-depleted. Serum

folic acid and vitamin B12 could not explain our findings, because both B-vitamins were not associated with the examined outcomes.

The physical performance score was 0.94 points lower in the highest quartile compared to the lowest homocysteine quartile. Two previous studies showed that a change score of physical performance of 1 point can be considered as clinically important in older persons.[28,29] Although this meaningful change score reflects a change over time instead of a difference between groups, it can be used to indicate the relevance of our finding. Similar studies on handgrip strength have not been performed.

This study has some limitations. First, genotype data were not in Hardy-Weinberg equilibrium. This could be explained by the screening for an elevated plasma homocysteine concentration (≥12 µmol/L) at the start of the study: Approximately 50% of elderly who had provided informed consent were excluded because of too low plasma homocysteine concentrations. As the 677TT genotype is associated with elevated homocysteine levels, this could have resulted in an overrepresentation of persons with the 677TT genotype. Second, as a consequence of this screening, the range of plasma homocysteine was limited (≥12 µmol/L), which might have reduced the power to show associations. This may have resulted in an underestimation of the reported associations. Third, selection bias may have attenuated the results on muscle mass, as persons who were willing to undergo a DXA measurement were, in general, healthier and more physically capable than those who did not want a DXA, because they had to be able to visit the research institution for an additional examination. Further, the use of two different DXA devices might have introduced a systematic bias. Because of the lack of a phantom for cross-calibrating DXA devices with regard to total body composition, we minimized this bias by adjusting for study location.

This study demonstrated that elevated plasma homocysteine concentrations are a determinant for reduced physical performance and handgrip strength in older women. Genetic analyses of the MTHFR polymorphism suggest that high plasma homocysteine may be a marker rather than a direct cause of low physical performance in older persons. Further support from randomized controlled trials is needed. As B-vitamin supplementation has been shown to be effective to reduce homocysteine levels,[30] it would be relevant to study whether B-vitamin supplementation can delay physical decline among the elderly.



**Conflict of Interest Statement**

The authors declare no conflict of interest.

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## References


1 Visser M, Schaap LA. Consequences of sarcopenia. Clin Geriatr Med 2011;27(3):387-399.

2 Marcell TJ. Sarcopenia: causes, consequences, and preventions. J Gerontol A Biol Sci Med Sci 2003;58(10):M911-M916.

3 Dhonukshe-Rutten RAM, Pluijm SMF, de Groot LCPG, Lips P, Smit JH, van Staveren WA. Homocysteine and vitamin B12 status relate to bone turnover markers, broadband ultrasound attenuation, and fractures in healthy elderly people. J Bone Miner Res 2005;20(6):921-929.

4 McLean RR, Jacques PF, Selhub J et al. Homocysteine as a predictive factor for hip fracture in older persons. N Engl J Med 2004;350(20):2042-2049.

5 van Meurs JBJ, Dhonukshe-Rutten RAM, Pluijm SMF et al. Homocysteine levels and the risk of osteoporotic fracture. N Engl J Med 2004;350(20):2033-2041.

6 Gerdhem P, Ivaska KK, Isaksson A et al. Associations between homocysteine, bone turnover, BMD, mortality, and fracture risk in elderly women. J Bone Miner Res 2007;22(1):127-134.

7 Kado DM, Bucur A, Selhub J, Rowe JW, Seeman T. Homocysteine levels and decline in physical function: MacArthur Studies of Successful Aging. Am J Med 2002;113(7):537-542.

8 Kuo HK, Liao KC, Leveille SG et al. Relationship of homocysteine levels to quadriceps strength, gait speed, and late-life disability in older adults. J Gerontol A Biol Sci Med Sci 2007;62(4):434-439.

9 Rolita L, Holtzer R, Wang C, Lipton R, Derby C, Verghese J. Homocysteine and Mobility in Older Adults. J Am Geriatr Soc 2010;58:545-550.

10 Soumare A, Elbaz A, Ducros V, Tavernier B, Alperovitch A, Tzourio C. Cross-sectional association between homocysteine and motor function in the elderly. Neurology 2006;67(6):985-990.

11 van Schoor NM, Swart KMA, Pluijm SMF et al. Cross-sectional and longitudinal association between homocysteine, vitamin B12 and physical performance in older persons. Eur J Clin Nutr 2012;66(2):174-181.

12 Sato Y, Honda Y, Iwamoto J, Kanoko T, Satoh K. Effect of folate and mecobalamin on hip fractures in patients with stroke: a randomized controlled trial. JAMA 2005;293(9):1082-1088.

13 Holmes MV, Newcombe P, Hubacek JA et al. Effect modification by population dietary folate on the association between MTHFR genotype, homocysteine, and stroke risk: a meta-analysis of genetic studies and randomised trials. Lancet 2011;378(9791):584-594.

14 van Wijngaarden JP, Dhonukshe-Rutten RAM, van Schoor NM et al. Rationale and design of the B-PROOF study, a randomized controlled trial on the effect of supplemental intake of vitamin B12 and folic acid on fracture incidence. BMC Geriatr 2011;11:80.

15 Visser M, Fuerst T, Lang T, Salamone L, Harris TB. Validity of fan-beam dual-energy X-ray absorptiometry for measuring fat-free mass and leg muscle mass. Health, Aging, and Body Composition Study--Dual-Energy X-ray Absorptiometry and Body Composition Working Group. J Appl Physiol 1999;87(4):1513-1520.

16 Guralnik JM, Simonsick EM, Ferrucci L et al. A short physical performance battery assessing lower extremity function: association with self-reported disability and prediction of mortality and nursing home admission. J Gerontol 1994;49(2):M85-M94.

17 Puts MTE, Lips P, Deeg DJH. Static and dynamic measures of frailty predicted decline in performance-based and self-reported physical functioning. J Clin Epidemiol 2005;58(11):1188-1198.

18 Stel VS, Smit JH, Pluijm SMF, Lips P. Balance and mobility performance as treatable risk factors for recurrent falling in older persons. J Clin Epidemiol 2003;56(7):659-668.

19 van Schoor NM, Smit JH, Pluijm SMF, Jonker C, Lips P. Different cognitive functions in relation to falls among older persons. Immediate memory as an independent risk factor for falls. J Clin Epidemiol 2002;55(9):855-862.

20 Dierkes J, Jeckel A, Ambrosch A, Westphal S, Luley C, Boeing H. Factors explaining the difference of total homocysteine between men and women in the European Investigation Into Cancer and Nutrition Potsdam study. Metabolism 2001;50(6):640-645.

21 Junge W, Wilke B, Halabi A, Klein G. Determination of reference intervals for serum creatinine, creatinine excretion and creatinine clearance with an enzymatic and a modified Jaffe method. Clin Chim Acta 2004;344(1-2):137-148.

22 Garretsen H. Probleemdrinken, prevalentiebepaling, beinvloedende factoren en preventiemogelijkheden, theoretische overwegingen en onderzoek in Rotterdam. 2003. Lisse: Swets & Zeitlinger.

23 Stel VS, Smit JH, Pluijm SMF, Visser M, Deeg DJH, Lips P. Comparison of the LASA Physical Activity Questionnaire with a 7-day diary and pedometer. J Clin Epidemiol 2004;57(3):252-258.

24 Swart KM, van Schoor NM, Heymans MW, Schaap LA, den HM, Lips P. Elevated homocysteine levels are associated with low muscle strength and functional limitations in older persons. J Nutr Health Aging 2013;17(6):578-584.

25 Matteini AM, Walston JD, Bandeen-Roche K et al. Transcobalamin-II variants, decreased vitamin B12 availability and increased risk of frailty. J Nutr Health Aging 2010;14(1):73-77.

26 Liu X, Zhao LJ, Liu YJ, Xiong DH, Recker RR, Deng HW. The MTHFR gene polymorphism is associated with lean body mass but not fat body mass. Hum Genet 2008;123(2):189-196.

27 Davey Smith G, Ebrahim S. 'Mendelian randomization': can genetic epidemiology contribute to understanding environmental determinants of disease? Int J Epidemiol 2003;32(1):1-22.

28 Kwon S, Perera S, Pahor M et al. What is a meaningful change in physical performance? Findings from a clinical trial in older adults (the LIFE-P study). J Nutr Health Aging 2009;13(6):538-544.

29 Perera S, Mody SH, Woodman RC, Studenski SA. Meaningful change and responsiveness in common physical performance measures in older adults. J Am Geriatr Soc 2006;54(5):743-749.

30 Homocysteine Lowering Trialists' Collaboration. Dose-dependent effects of folic acid on blood concentrations of homocysteine: a meta-analysis of the randomized trials. Am J Clin Nutr 2005;82(4):806-812.