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Vitamin B12 and Homocysteine Levels and 6-Year Change in Peripheral Nerve Function and Neurological Signs

Kira Leishear,[1] Luigi Ferrucci,[2] Fulvio Lauretani,[3] Robert M. Boudreau,[1] Stephanie A. Studenski,[4] Caterina Rosano,[1] Rosanna Abbate,[5] Anna M. Gori,[6,7] Anna M. Corsi,[8] Angelo Di Iorio,[9] Jack M. Guralnik,[10] Stefania Bandinelli,[11] Anne B. Newman,[1,4] and Elsa S. Strotmeyer[1]

[1]Department of Epidemiology, Graduate School of Public Health, University of Pittsburgh, Pennsylvania.
[2]Longitudinal Studies Section, Clinical Research Branch, National Institute on Aging, Baltimore, Maryland.
[3]Geriatric Unit, University Hospital of Parma, Italy.
[4]Department of Medicine, Division of Geriatric Medicine, School of Medicine, University of Pittsburgh, Pennsylvania.
[5]Department of Medical and Surgical Critical Care, University of Florence, Italy.
[6]Department of Medical and Surgical Critical Care, Thrombosis Centre, Florence, Italy.
[7]Centro S. Maria agli Ulivi, Fondazione Don Carlo Gnocchi Onlus IRCCS, Impruneta, Florence, Italy.
[8]Tuscany Regional Agency for Health, Florence, Italy.
[9]Department of Medicine and Sciences of Aging, University of Chieti, Italy.
[10]Laboratory of Epidemiology, Demography, and Biometry, National Institute on Aging, Bethesda, Maryland.
[11]Geriatric Rehabilitation Unit, Azienda Santori Firenze, Florence, Italy.

Address correspondence to Elsa S. Strotmeyer, PhD, MPH, Department of Epidemiology, Graduate School of Public Health, University of Pittsburgh, 130 North Bellefield Avenue, Room 515, Pittsburgh, PA 15213. Email: strotmeyere@edc.pitt.edu

Background: Low vitamin B12 and high homocysteine (Hcy) levels are common in older adults and may be associated with worse neurological function. The aim of this study is to determine whether changes in B12 or Hcy levels are associated with longitudinal changes in peripheral nerve function and clinical neurological signs and symptoms.

Methods: Participants aged 60 years and older at baseline (n = 678; 72.2 ± 6.2 years; 43.5% male) were from the InCHIANTI Study. Low B12 (<260 pmol/L) and high Hcy (≥13 μmol/L) were measured at baseline and 3-year follow-up. Neurological function was assessed by peroneal nerve conduction amplitude (compound motor action potential) and velocity, neurological examination, and peripheral neuropathy symptoms at baseline, 3-year, and 6-year follow-up.

Results: At baseline, 43.8% had low B12 levels and 58.6% had high Hcy levels. Over 6 years, 12.4% declined to poor compound motor action potential (<1 mV) and 42.1% declined to poor nerve conduction velocity (<40 m/s). In mixed models analyses, sustained high Hcy was associated with worse compound motor action potential compared with sustained normal Hcy (p = .04), adjusting for demographics, diabetes, and folate level. Participants whose Hcy level became high at follow-up were more likely to become unable to detect monofilament at 6-year follow-up compared with those with sustained normal Hcy (odds ratio: 5.4; 95% CI: 1.5–19.0), adjusting for demographics, diabetes, body mass index, and peripheral arterial disease. There was no association with vitamin B12 level or with symptoms.

Conclusions: High Hcy may be associated with worse sensory and motor peripheral nerve function. Because poor nerve function has been associated with lower strength and physical performance, these results have important implications for disability in older adults.

Key Words: Vitamin B12—Homocysteine—Peripheral nerve function—Neurological signs.

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VITAMIN B12 deficiency affects 5%–20% of older adults, while up to 40% have low B12 levels (1,2). The prevalence of poor B12 increases with age (3). B12 deficiency can cause myelin damage as a result of deficient methylation of myelin basic protein (4). Along with hematological, neuropsychological, and cardiovascular diseases, B12 deficiency has been associated with myeloneuropathy, optic neuropathy, peripheral neuropathy (PN), loss of sensation in peripheral nerves, and weakness in lower extremities (2,5–6).

Because vitamin B6, B12, and folate work synergistically to convert homocysteine (Hcy) to methionine, low B12 or folate levels may result in high Hcy levels. Hyperhomocysteinemia has a prevalence of about 30% in older adults when defined as more than 14 μmol/L (7). Hyperhomocysteinemia has been associated with older age, male sex, white race, smoking,

high coffee consumption, lack of physical activity (PA), cognitive impairment, depression, cardiovascular disease, poor renal function, slower gait speed, lower quadriceps strength, functional decline, and an increased risk of mortality (8–12).

The prevalence of poor peripheral nerve function and neuropathy is high in older adults and increases with age (13,14); more than 25% of older adults have PN or clinically positive tests for neuropathy (15). Diabetes mellitus (DM) is the major risk factor for clinical PN in older adults but accounts for only half of the cases (13). Subclinically, poor peripheral nerve function is associated with lower physical performance and strength in older adults, independent of DM (16,17). Therefore, implications for disability associated with subclinical peripheral nerve function exist and identifying key risk factors for poor peripheral nerve function is critical.

To our knowledge, there have been no cohort studies examining the association between vitamin B12 or Hcy and peripheral nerve function, despite their clear association with aging. We hypothesized that low B12 and high Hcy and sustained low B12 and high Hcy are associated with greater decline in neurological function, compared with sustained normal B12 and Hcy levels. The purpose of this study is to examine whether low B12 or high Hcy levels are associated cross-sectionally or longitudinally with: (i) peripheral nerve function, (ii) clinical neurological signs, and (iii) PN symptoms.

Methods

Study Population

The InCHIANTI Study is an ongoing, population-based longitudinal cohort study examining factors contributing to the decline of mobility in late life in the Chianti region of Italy. Details of the study have been described elsewhere (18). Briefly, in August 1998, 1,616 adults, aged 21–102 years, were selected from the population registry of Greve in Chianti (a rural area; 11,709 inhabitants, with 19.3% 65+ years) and Bagno a Ripoli (Antella village, near Florence; 4,704 inhabitants, with 20.3% 65+ years). The participation rate was 90% (1,453/1,616).

There were 1,203 participants who were 60+ years at baseline, and 1,091 (91%) had B12 or Hcy in addition to at least one neurological measure. At the 3-year follow-up, 70 refused to participate, 10 relocated, 3 could not be located, and 107 died. Of the 892 participants with a 3-year visit, 797 (89%) had B12 or Hcy measured. Of these, 119 did not have a 6-year follow-up visit (18 refused, 6 relocated, and 95 died), leaving 678 participants in our analytic sample. The study was approved by the Ethics Committee of the Italian National Institute of Research and Care of Aging, and informed consent was obtained from all participants.

Assays

Blood samples were collected and stored at −80°C. Serum vitamin B12 levels were measured with a radioligand-binding assay (SimulTrac-SNB Radioassay; ICN Pharmaceuticals). The minimum detectable concentration was 75 ng/L and the intraassay and interassay coefficients of variation were 11.0% and 12.0%, respectively. Plasma Hcy concentrations were measured by a fluorimetric polarized immunoassay method (IMX; Abbott Laboratories) (19). The sensitivity of the IMX Hcy assay was 0.5 μmol/L, and the interassay coefficient of variation was 4.1%. Serum folate, vitamin B6, total cholesterol, creatinine, and inflammatory markers (interleukin 6 and tumor necrosis factor-alpha) were measured with commercial assays, which were previously described (19).

Low B12 was defined as less than 260 pmol/L and normal as more than or equal to 260 pmol/L (20), and high Hcy was defined as more than or equal to13 μmol/L and normal as less than 13 μmol/L (21). B12 and Hcy levels were only measured at baseline and 3-year follow-up and categories of 3-year change included: (i) Stay Normal, (ii) Poor to Normal, (iii) Normal to Poor, and (iv) Stay Poor.

Peripheral Motor Nerve Function

Standard surface electroneurographic studies of the right peroneal nerve were conducted within 3 weeks of the home interview by a trained geriatrician (18). All studies were performed on an electroneurographic-neuro MYTO device (EB Neuro S.p.A, Florence, Italy) using standard electroneurographic-neuro disposable electrodes (22). Nerve conduction amplitude (compound motor action potential [CMAP]) and nerve conduction velocity [NCV] were measured between the fibular head and ankle.

Neurological Examination

Neurological signs within the motor and sensory system and cranial nerves were assessed in the neurological examination (23). The intraclass correlation coefficient for test–retest reliability for each item was more than 0.8. Neurological signs evaluated in relation to low B12 were (i) abnormal touch sensitivity, (ii) abnormal vibration sensitivity, (iii) impaired sense of ankle position, (iv) abnormal deep tendon reflexes, (v) positive Babinski reflex, and (vi) a positive Romberg sign. Participants were excluded from the neurological exam analyses if they had Parkinson's disease, history of stroke, or multiple sclerosis.

Symptoms Related to PN

Participants were asked (i) "at present, do you ever experience pain in the left foot? . . . right foot?" and (ii) "at present, do you ever experience a sensation of coldness in the left foot? . . . right foot?"

Potential Confounders

Potential confounders associated with vitamin B12 or Hcy and neurological function were included as covariates in the multivariable analyses. Demographic and health information were assessed at baseline. DM was diagnosed using the criteria from the American Diabetes Association: self-reported physician diagnosis, fasting glucose more than or equal to 126 mg/dL, or diabetes medication (24). Chronic health conditions (eg, hypertension, peripheral arterial disease, congestive heart failure, myocardial infarction, stroke, thyroid disease) were adjudicated using self-reported history, physical examination (eg, blood pressure), and medical records (18). Smoking status was assessed by self-report. Alcohol intake (g/d) was estimated using the European Prospective Investigation into Cancer and Nutrition food frequency questionnaire (25). To assess PA, participants were asked if they performed any sport or recreational activity for more than or equal to 3 months in the last year (26) and to specify the type and frequency. PA was categorized as (i) sedentary (hardly any PA, mostly sitting), (ii) light (light exercise 2–4 h/wk), and (iii) moderate–high (moderate or intense exercise ≥1 h/wk). Body mass index was calculated as weight (kg)/height^2 (m^2).

Statistical Analyses

Differences in demographics, lifestyle factors, body composition, chronic health conditions, and cardiovascular risk factors were tested by change in B12 and Hcy levels, using Pearson χ^2 tests or Fisher exact test for categorical variables and analysis of variance, Kruskal–Wallis, or *t* tests for continuous variables.

Logistic regression was performed at baseline for neurological signs and symptoms. Multinomial logistic regression was used to model change in signs and symptoms, using change in B12 and Hcy levels, separately as independent variables.

For nerve conduction, cross-sectional analyses were performed at baseline, using separate multivariable linear regression models for each independent variable (baseline low B12 and high Hcy) with each outcome (baseline CMAP and NCV). Multivariable linear regression was used for longitudinal change, separately for change in B12/Hcy for outcomes: (i) 6-year CMAP change and (ii) 6-year NCV change. Mixed linear modeling was performed separately for CMAP and NCV (using data from baseline, 3-year, and 6-year follow-up) with change in B12 and Hcy modeled individually as the independent variables.

The models were built progressively in order, adjusting for potential confounders: demographics (age, sex, clinic site), DM, body mass index, lifestyle factors (smoking status, alcohol intake, activity level), cardiovascular risk factors (hypertension, cardiovascular disease, peripheral arterial disease, myocardial infarction, stroke), thyroid disease, creatinine, use of hypoglycemic medications, inflammatory markers (interleukin 6, tumor necrosis factor-alpha), and folate and vitamin B6 levels. Vitamin B12 and Hcy were considered as confounders when not the predictor in the models. Mini-Mental State Examination was additionally included for the neurological signs and symptoms. Demographics and DM were included in all models; other variables were removed if $p > .10$. Sensitivity analyses were conducted removing participants with DM and excluding those who changed categories for B12 or Hcy but only had a small amount of overall change (eg, B12 within 50 pmol/L; Hcy within 1 μmol/L). Multicollinearity for independent variables was assessed using the variance inflation factor; no variance inflation factor was more than 2. All analyses were conducted with SAS, version 9.2 (SAS Institute Inc., Cary, NC).

There were no differences among neurological outcomes between those with B12 and Hcy data at baseline and those without B12 and Hcy data at baseline. Compared with participants with baseline and 6-year follow-up, participants with only baseline data were more likely to be older (77.7 ± 7.7 years vs 72.2 ± 6.2 years; $p < .0001$), have DM (15.3% vs 9.0%; $p = .006$), high Hcy (73.6% vs 56.4%; $p < .0001$), and worse CMAP (5.1 ± 3.1 vs 6.8 ± 3.2 mV; $p < .0001$) and NCV (43.4 ± 3.9 vs 44.5 ± 4.0 m/s; $p = .0002$) at baseline.

Results

Baseline characteristics by change in B12 and Hcy categories are shown in Tables 1 and 2, respectively. Men were more likely to have low B12 at follow-up. Higher alcohol consumption and Hcy were associated with having low B12 at either time compared with having normal B12 at both times. Participants with low B12 at baseline had lower folate levels compared with having sustained normal B12. High Hcy was associated with older age, male sex, higher alcohol consumption, higher interleukin 6 and tumor necrosis factor-alpha levels, and lower B6, B12, and folate levels.

The prevalence of low B12 decreased slightly from baseline (44%) to 3-year follow-up (39%), whereas the prevalence of high Hcy decreased considerably from 59% to 46% at follow-up. More than half of the participants had low B12 at one time (53%) with 30% having low levels at both times. Only 37% had normal Hcy at both times, whereas 41% had high Hcy at both times, and 18% improved to normal Hcy at the 3-year follow-up visit.

Cross-sectionally at baseline, participants with high Hcy had lower NCV compared with normal Hcy (44.1 ± 3.7 vs 44.8 ± 4.2 m/s; $\beta = -.71$; $p = .04$), but after adjusting for confounders, the association was no longer significant ($\beta = .40$; $p = .22$).

The prevalence of poor CMAP (<1 mV) and NCV (<40 m/s) (27) increased at each time point, with a substantial increase in poor NCV at the 6-year follow-up to more than 50%. Those who had high Hcy at both times or became high at follow-up had significantly lower NCV compared with those with sustained normal levels (Table 3). There were no

Table 1. Baseline Characteristics by Change in Vitamin B12

	Stay Normal (n = 313)	Low to Normal (n = 89)	Normal to Low (n = 60)	Stay Low (n = 202)	p Value
Age (y)	71.7 ± 5.9	72.4 ± 7.1	71.8 ± 5.6	73.1 ± 6.4*	.19
Male (%)	33.6	43.8	56.7†	54.5*	<.0001
Diabetes mellitus (%)	15.0	5.6‡	14.3	9.6	.06
Former smoker (%)	24.6	25.8	23.3	29.7	.58
Alcohol use (g/d)	11.5 ± 16.2	17.3 ± 21.9‡	19.0 ± 19.1†	20.8 ± 27.1*	.0002
Physical activity level					.47
Sedentary (%)	15.1	14.6	18.3	11.4	
Light (%)	45.3	52.8	43.3	43.8	
BMI (kg/m^2)	27.8 ± 4.2	27.6 ± 4.1	27.4 ± 3.3	27.6 ± 4.2	.92
PAD (%)	6.3	11.0	7.3	6.4	.50
Creatinine (mg/dL)	0.40 ± 0.50	0.34 ± 0.50	0.29 ± 0.46	0.35 ± 0.48	.31
IL-6 (pg/mL)	3.2 ± 2.1	3.2 ± 2.3	3.3 ± 2.0	3.6 ± 2.5	.57
TNF-α (pg/mL)	4.8 ± 3.0	4.6 ± 2.0	4.7 ± 2.5	5.1 ± 3.9	.81
Folate (nmol/L)	8.1 ± 4.8	6.6 ± 3.6‡	7.8 ± 3.8	6.8 ± 3.4*	.0006
Vitamin B6 (nmol/L)	32.5 ± 27.3	28.3 ± 14.2	28.7 ± 15.7	25.4 ± 13.1*	.06
Hcy (μmol/L)	13.7 ± 3.9	15.0 ± 5.3‡	14.9 ± 3.9†	16.9 ± 7.8*,§	<.0001
Vitamin B12 (pmol/L)	480.2 ± 295.2	204.1 ± 40.2‡	348.6 ± 147.6†,‖	177.7 ± 53.2*,§,¶	<.0001
Change in B12 (pmol/L)	8.5 ± 320.0	148.6 ± 170.2‡	−135.9 ± 153.5†,‖	−1.4 ± 51.3§,¶	<.0001

Notes: p < .05 for * = Stay Low versus Stay Normal; † = Normal to Low versus Stay Normal; ‡ = Low to Normal versus Stay Normal; § = Stay Low versus Low to Normal; ‖ = Normal to Low versus Low to Normal; ¶ = Stay Low versus Normal to Low. Low B12 = <260 pmol/L; BMI = body mass index; Hcy = homocysteine; IL-6 = interleukin 6; PAD = peripheral arterial disease; TNF-α = tumor necrosis factor-alpha.

significant differences in motor nerve function by change in B12 (data not shown). About 12% of the participants declined to poor CMAP. Less than half (49%) had normal NCV at both baseline and 6-year follow-up. There were 42% who had normal NCV at baseline and declined to poor NCV at follow-up (mean decline: 7.1 ± 3.3 m/s).

In the multivariable mixed models, no significant association was found between change in B12 and changes in CMAP or NCV. However, high Hcy at baseline and 3-year follow-up was associated with worse CMAP across visits compared with sustained normal Hcy (p = .04), after adjusting for demographics, DM, and folate level (Table 4). Change in Hcy was not significantly associated with NCV change after adjustments or NCV longitudinal trend. Sex and DM attenuated the association. There was no association between B12 or Hcy and PN symptoms, cross-sectionally or longitudinally.

At baseline, high Hcy was not associated with abnormal touch sensitivity (monofilament) after adjusting for covariates. Body mass index and PA attenuated the association. In the longitudinal analysis, participants with normal Hcy at baseline and high Hcy at follow-up were more likely to

Table 2. Baseline Characteristics by Change in Homocysteine

	Stay Normal (n = 245)	High to Normal (n = 119)	Normal to High (n = 32)	Stay High (n = 273)	p Value
Age (y)	70.1 ± 5.2	71.5 ± 6.1‡	73.6 ± 6.3†	74.0 ± 6.4*,§	<.0001
Male (%)	24.5	52.1‡	50.0†	56.8*	<.0001
Diabetes mellitus (%)	10.8	7.7	21.9‖	12.0	.15
Former smoker (%)	21.2	29.4	15.6	31.9*	.02
Alcohol use (g/d)	10.3 ± 14.9	19.8 ± 23.8‡	11.3 ± 17.8‖	20.2 ± 25.2*	<.0001
Physical activity level					.03
Sedentary (%)	11.9	7.6	15.6	18.8*,§	
Light (%)	50.4	45.4	34.4	43.9	
BMI (kg/m^2)	27.8 ± 4.1	27.6 ± 3.7	27.4 ± 4.5	27.5 ± 4.2	.75
PAD (%)	7.4	6.3	10.0	6.9	.91
Creatinine (mg/dL)	0.35 ± 0.48	0.34 ± 0.48	0.34 ± 0.48	0.40 ± 0.51	.69
IL-6 (pg/mL)	2.9 ± 1.8	3.4 ± 2.3‡	3.6 ± 2.7†	3.6 ± 2.4*	.0005
TNF-α (pg/mL)	4.2 ± 2.1	5.5 ± 2.9‡	4.4 ± 2.5	5.1 ± 2.9*	<.0001
Folate (nmol/L)	8.2 ± 4.8	7.8 ± 4.3	7.8 ± 5.1	6.6 ± 3.4*,§	<.0001
Vitamin B6 (nmol/L)	34.6 ± 27.7	32.5 ± 21.2	21.1 ± 11.2†,‖	24.5 ± 13.0*,§	<.0001
Vitamin B12 (pmol/L)	397.3 ± 288.6	333.3 ± 248.2‡	291.2 ± 157.5†	298.7 ± 221.2*	<.0001
Hcy (μmol/L)	10.9 ± 1.4	14.9 ± 2.2‡	11.7 ± 1.1†,‖	18.7 ± 6.4*,§,¶	<.0001
Change in Hcy (μmol/L)	−0.6 ± 1.5	−3.6 ± 2.7‡	3.5 ± 3.3†,‖	−0.4 ± 5.9§,¶	<.0001

Notes: p < .05 for * = Stay High versus Stay Normal; † = Normal to High versus Stay Normal; ‡ = High to Normal versus Stay Normal; § = Stay High versus High to Normal; ‖ = Normal to High versus High to Normal; ¶ = Stay High versus Normal to High; high Hcy: ≥13 μmol/L; BMI = body mass index; Hcy = homocysteine; IL-6 = interleukin 6; PAD = peripheral arterial disease; TNF-α = tumor necrosis factor-alpha.

Table 3. Motor Nerve Function by Change in Homocysteine

	Stay Normal (n = 205)	High to Normal (n = 94)	Normal to High (n = 19)	Stay High (n = 196)	p Value
Compound motor action potential (mV)					
Baseline	6.8 ± 3.2	6.4 ± 3.1	6.4 ± 3.8	6.4 ± 3.3	.63
3 y	4.1 ± 2.8	4.2 ± 3.4	4.4 ± 3.8	3.7 ± 2.7	.70
6 y	3.8 ± 2.4	4.1 ± 2.6	3.7 ± 2.8	3.8 ± 2.2	.92
Nerve conduction velocity (m/s)					
Baseline	45.1 ± 4.1	44.4 ± 3.5	42.4 ± 4.7*	44.0 ± 3.8†	.004
3 y	44.5 ± 4.2	44.3 ± 4.9	41.8 ± 4.2*	42.6 ± 4.7†,‡	.001
6 y	40.4 ± 3.8	39.6 ± 3.9	36.9 ± 5.3*	39.2 ± 3.8†,§	.001

Notes: $p < .05$ for * = Normal to High versus Stay Normal; † = Stay High versus Stay Normal; ‡ = Stay High versus High to Normal; § = Stay High versus Normal to High; high homocysteine = ≥13 μmol/L.

become unable to detect monofilament at 6-year follow-up compared with those with normal Hcy at both times (odds ratio: 5.4; 95% confidence interval: 1.5–19.0), after adjusting for demographics, DM, body mass index, and peripheral arterial disease. Results were consistent in our sensitivity analyses.

Discussion

High Hcy was associated with poor motor and sensory peripheral nerve function (CMAP and inability to detect 4-g monofilament touch). This is the first study, to our knowledge, that examined the association between Hcy and peripheral nerve function and that considered change in B12 and Hcy and change in peripheral nerve function. Although sensory neuropathy is known to be associated with B12 deficiency (28), we found an association between high Hcy and both motor and sensory nerve function. These findings are important because worse nerve function may lead to impaired physical function and disability in older adults (14,16,29).

We found no association between B12 or Hcy and PN symptoms. The participants were asked "at present" whether they had the symptoms, and PN is often asymptomatic or symptoms may only be present early (30). Furthermore, the symptoms may have been too general, and PN symptoms such as deep aching or burning pain were not assessed. We did not find an association between low B12 and neurological signs. This is an important finding because older adults may not have clinically recognized signs of deficiency, and thus, low B12 levels may go unnoticed.

There was a high prevalence of low B12 and high Hcy, which was higher than previously found (2,7). In Italy, there is no mandatory folic acid fortification, whereas there has been in the United States since 1998 (1). Because folic acid can lower Hcy, we expected a higher prevalence of elevated Hcy compared with the United States. In addition, less than 4% of participants took supplemental vitamins at baseline (31). Also, alcohol consumption is common in Italian older adults (32) and is related to higher Hcy (33). The high prevalence of poor B12 and Hcy levels may also be attributed to using a higher B12 and a lower Hcy cut-point. We used these cut-points because although B12 deficiency is a known cause of PN, little is known about how low B12 or moderate hyperhomocysteinemia affect peripheral nerve function, and prevalences of both are high among older adults. The prevalence of high Hcy decreased from baseline to 3-year follow-up. Lifestyle factors such as smoking, low PA, and excessive coffee and alcohol consumption can cause hyperhomocysteinemia (34). Participants and their physicians received the test results and may have changed diet or lifestyle habits accordingly. It is possible that the prevalence of high Hcy of those without follow-up data did not decline over time as those with baseline only data were more likely to have high Hcy levels at baseline (74%) compared with those with follow-up data (56%). Thus, the decrease in prevalence could be due to survival bias.

The prevalence of poor CMAP and NCV was also high and increased at each time point, with a considerable increase in poor NCV at 6-year follow-up. We expected NCV to decline before CMAP because CMAP is related to nerve axonal damage and NCV is related to nerve demyelination, which is thought to occur before axonal degeneration because myelin covers and protects the axons (35).

One limitation was having few participants who declined from normal to poor B12 or Hcy. Also, B12 and Hcy levels were not tested at the 6-year follow-up, and methylmalonic acid testing was not done. Methylmalonic acid is highly sensitive and specific at determining poor B12 status, whereas serum B12 is not very sensitive or specific (9), and the inadequacy of determining poor B12 status may possibly explain the null findings with B12. There was no objective sensory nerve function assessed. We did not adjust for multiple comparisons in our analyses which likely would weaken our associations. Importantly, our study included

Table 4. Compound Motor Action Potential (mV) Across All Visits and Change in Homocysteine

Change in Homocysteine	β	p Value
High to normal	−.16	.46
Normal to high	−.22	.62
Stay high	−.41	.04

Reference Group = Stay Normal.
Note: Adjusted for age, sex, site, diabetes, and folate level.

older adults who lived in Italy, and our findings may not be generalizable to other populations due to differences in diet, supplement practices, and folic acid fortification. Because participants with only baseline data had higher Hcy and worse nerve function at baseline, the models may underestimate the effect of the association if dropouts were more likely to have progressed PN. In addition, those who had poor CMAP or NCV at baseline did not have much room to decline because they had very low levels. Thus, looking at continuous change in nerve function may not be appropriate for those who already had poor nerve function at baseline.

A strength of this analysis was that it was longitudinal and included three time points for the outcomes. The longitudinal analysis was critical because we did not find any cross-sectional associations, and we saw the impact of sustained high Hcy on peripheral nerve function. We had a large cohort of older men and women. Measuring motor nerve function with nerve conduction is highly sensitive and reproducible and is considered state of the art (36). We had measures of both motor and sensory nerve function. Monofilament detection, while not as sensitive, is very specific and has high clinical significance because it is an inexpensive and quick test that can be done in an exam room (37). Previous work has shown high creatinine levels are associated with high Hcy (33); however, we adjusted for creatinine in our models and found no difference.

This analysis shows that high Hcy may be related to worse sensory and motor peripheral nerve function. These results have important implications for motor functioning and disability in older adults. Several researchers have identified a link between PN and impaired balance and falls (38,39). Vitamins B6, B12, and folic acid can effectively lower Hcy, and supplements are readily available, adequately absorbed, and highly tolerated in older adults (40). However, previous clinical trials have not examined the effect of B vitamins on peripheral nerve function. Future work should examine if vitamin B6, B12, and folic acid can lower Hcy levels and improve peripheral nerve function in older adults.

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References

1. Varela-Moreiras G, Murphy MM, Scott JM. Cobalamin, folic acid, and homocysteine. *Nutr Rev*. 2009;67(suppl 1):S69–S72.
2. Baik HW, Russell RM. Vitamin B12 deficiency in the elderly. *Annu Rev Nutr*. 1999;19:357–377.
3. Bernard MA, Nakonezny PA, Kashner TM. The effect of vitamin B12 deficiency on older veterans and its relationship to health. *J Am Geriatr Soc*. 1998;46:1199–1206.
4. Weir DG, Scott JM. Brain function in the elderly: Role of vitamin B-12 and folate. *Br Med Bull*. 1999;55:669–682.
5. Klee GG. Cobalamin and folate evaluation: Measurement of methylmalonic acid and homocysteine vs vitamin B(12) and folate. *Clin Chem*. 2000;46:1277–1283.
6. Saperstein DS, Barohn RJ. Peripheral neuropathy due to cobalamin deficiency. *Curr Treat Options Neurol*. 2002;4:197–201.
7. Selhub J, Jacques PF, Wilson PW, Rush D, Rosenberg IH. Vitamin status and intake as primary determinants of homocysteinemia in elderly population. *JAMA*. 1993;270:2693–2698.
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;62:434–439.
9. Carmel R, Green R, Rosenblatt DS, Watkins D. Update on cobalamin, folate, and homocysteine. *Hematology Am Soc Hematol Educ Program*. 2003:62–81.
10. Kuo HK, Sorond FA, Chen JH, Hashmi A, Milberg WP, Lipsitz LA. The role of homocysteine in multisystem age-related problems: A systematic review. *J Gerontol A Biol Sci Med Sci*. 2005;60:1190–1201.
11. Gonzalez S, Huerta JM, Fernandez S, Patterson AM, Lasheras C. Homocysteine increases the risk of mortality in elderly individuals. *Br J Nutr*. 2007;97:1138–1143.
12. 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;113:537–542.
13. Baldereschi M, Inzitari M, Di Carlo A, Farchi G, Scafato E, Inzitari D. Epidemiology of distal symmetrical neuropathies in the Italian elderly. *Neurology*. 2007;68(18):1460–1467.
14. Resnick HE, Vinik AI, Heimovitz HK, Brancati FL, Guralnik JM. Age 85+ years accelerates large-fiber peripheral nerve dysfunction and diabetes contributes even in the oldest-old: The Women's Health and Aging Study. *J Gerontol A Biol Sci Med Sci*. 2001;56:M25–M31.
15. Gregg EW, Sorlie P, Paulose-Ram R, et al. Prevalence of lower-extremity disease in the U.S. adult population ≥40 years of age with and without diabetes: 1999–2000 National Health and Nutrition Examination Survey. *Diabetes Care*. 2004;27:1591–1597.
16. Strotmeyer ES, de Rekeneire N, Schwartz AV, et al. The relationship of reduced peripheral nerve function and diabetes with physical performance in older white and black adults: The Health, Aging, and Body Composition (Health ABC) Study. *Diabetes Care*. 2008;31:1767–1772.
17. Strotmeyer ES, de Rekeneire N, Schwartz AV, et al. Sensory and motor peripheral nerve function and lower-extremity quadriceps strength: The Health, Aging, and Body Composition Study. *J Am Geriatr Soc*. 2009;57:2004–2010.
18. Ferrucci L, Bandinelli S, Benvenuti E, et al. Subsystems contributing to the decline in ability to walk: Bridging the gap between epidemiology and geriatric practice in the InCHIANTI study. *J Am Geriatr Soc*. 2000;48(12):1618–1625.
19. Gori AM, Corsi AM, Fedi S, et al. A proinflammatory state is associated with hyperhomocysteinemia in the elderly. *Am J Clin Nutr*. 2005;82(2):335–341.
20. Tucker KL, Hannan MT, Qiao N, et al. Low plasma vitamin B12 is associated with lower BMD: The Framingham Osteoarthritis Study. *J Bone Miner Res*. 2005;20:152–158.
21. Jacques PF, Selhub J, Bostom AG, Wilson PW, Rosenberg IH. The effect of folic acid fortification on plasma folate and total homocysteine concentrations. *N Engl J Med*. 1999;340:1449–1450.
22. Lauretani F, Bandinelli S, Bartali B, et al. Axonal degeneration affects muscle density in older men and women. *Neurobiol Aging*. 2006;27:1145–1154.
23. Ferrucci L, Bandinelli S, Cavazzini C, et al. Neurological examination findings to predict limitations in mobility and falls in older persons without a history of neurological disease. *Am J Med*. 2004;116(12):807–815.
24. Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Report of the expert committee on the diagnosis and classification of diabetes mellitus. *Diabetes Care*. 2003;26(suppl):S5–S20.

25. Pisani P, Faggiano F, Krogh V, Palli D, Vineis P, Berrino F. Relative validity and reproducibility of a food frequency dietary questionnaire for use in the Italian EPIC centres. *Int J Epidemiol*. 1997;26: 152–160.
26. Elosua R, Bartali B, Ordovas JM, Corsi AM, Lauretani F, Ferrucci L. Association between physical activity, physical performance, and inflammatory biomarkers in an elderly population: The InCHIANTI study. *J Gerontol A Biol Sci Med Sci*. 2005; 60(6):760–767.
27. Maser RE, Nielsen VK, Dorman JS, Drash AL, Becker DJ, Orchard TJ. Measuring subclinical neuropathy: Does it relate to clinical neuropathy? Pittsburgh epidemiology of diabetes complications study-V. *J Diabet Compl*. 1991;5(1):6–12.
28. Smith AG, Singleton JR. The diagnostic yield of a standardized approach to idiopathic sensory-predominant neuropathy. *Arch Intern Med*. 2004;164:1021–1025.
29. Resnick HE, Vinik AI, Schwartz AV, et al. Independent effects of peripheral nerve dysfunction on lower-extremity physical function in old age: The Women's Health and Aging Study. *Diabetes Care*. 2000;23:1642–1647.
30. Franse LV, Valk GD, Dekker JH, Heine RJ, van Eijk JT. 'Numbness of the feet' is a poor indicator for polyneuropathy in Type 2 diabetic patients. *Diabet Med*. 2000;17:105–110.
31. Bartali B, Frongillo EA, Bandinelli S, et al. Low nutrient intake is an essential component of frailty in older persons. *J Gerontol A Biol Sci Med Sci*. 2006;61(6):589–593.
32. Buja A, Scafato E, Sergi G, et al. Alcohol consumption and metabolic syndrome in the elderly: results from the Italian longitudinal study on aging. *Eur J Clin Nutr*. 2010;64(3):297–307.
33. Jacques PF, Bostom AG, Wilson PW, Rich S, Rosenberg IH, Selhub J. Determinants of plasma total homocysteine concentration in the Framingham Offspring cohort. *Am J Clin Nutr*. 2001;73:613–621.
34. De Bree A, Verschuren WM, Blom HJ, Kromhout D. Lifestyle factors and plasma homocysteine concentrations in a general population sample. *Am J Epidemiol*. 2001;154:150–154.
35. Arezzo JC, Zotova E. Electrophysiologic measures of diabetic neuropathy: Mechanism and meaning. *Int Rev Neurobiol*. 2002;50:229–255.
36. England JD, Gronseth GS, Franklin G, et al. Distal symmetric polyneuropathy: A definition for clinical research: Report of the American Academy of Neurology, the American Association of Electrodiagnostic Medicine, and the American Academy of Physical Medicine and Rehabilitation. *Neurology*. 2005;64:199–207.
37. Feng Y, Schlosser FJ, Sumpio BE. The Semmes Weinstein monofilament examination as a screening tool for diabetic peripheral neuropathy. *J Vasc Surg*. 2009;50:675–692.
38. Richardson JK. The clinical identification of peripheral neuropathy among older persons. *Arch Phys Med Rehabil*. 2002;83:1553–1558.
39. Sorock GS, Labiner DM. Peripheral neuromuscular dysfunction and falls in a elderly cohort. *Am J Epidemiol*. 1992;136:584–591.
40. Clarke R, Armitage J. Vitamin supplements and cardiovascular risk: Review of the randomized trials of homocysteine-lowering vitamin supplements. *Semin Thromb Hemost*. 2000;26(3):341–348.