Homocysteine, frailty and all-cause mortality in older men: the Health In Men Study

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ABSTRACT

Background: Frailty and hyperhomocysteinaemia are common in the older population. Our objectives were to determine whether elevated homocysteine (tHcy) is associated with frailty and mortality.

Methods: We conducted a prospective cohort study. tHcy was measured by immunoassay in 4248 community-dwelling men aged 70-88 years. Frailty was assessed with the FRAIL scale. Mortality was determined from the death registry.

Results: At baseline, 1117 men (26.3%) had high tHcy (≥ 15 µmol/l) and 685 (16.2%) were frail (i.e. having 3 or more deficits in the FRAIL scale). There were 749 deaths during a follow-up duration of 5.1 ± 1.3 years. In cross-sectional analysis, high tHcy was associated with increased prevalent frailty (OR 1.49, 95% CI 1.22 to 1.81) after adjusting for confounding factors. After a period of 5.3 ± 0.8 years, the longitudinal relationship between high tHcy and frailty was weakened in multivariate analysis (OR 1.25, 95% CI 0.95 to 1.65). When assessing the relationship between tHcy and incident frailty, the odds of being frail at follow-up for men with high tHcy and having zero deficit at baseline (i.e. FRAIL scale=0) were 1.59 (95% CI 0.88 to 2.89) in adjusted analysis. High tHcy also predicted all-cause mortality (HR 1.25, 95% CI 1.06 to 1.48) after adjusting for frailty and other covariates.

Conclusions: Hyperhomocysteinaemia is associated with the prevalence of frailty. It is also predictive of all-cause mortality, independent of frailty. Our results suggest that the association between tHcy and mortality is largely not mediated through the occurrence of frailty.
INTRODUCTION

Frailty is becoming increasingly common as the world’s population ages. It is defined as “a state of excess vulnerability to stressors due to age-related decline in physiologic reserve across multisystems, resulting in reduced ability to maintain or regain homeostasis after a destabilizing event” (1). Differing conceptual approaches have been applied to describe this phenomenon, including incorporation of physical characteristics and function (2), and utilizing a combination of clinical deficits and co-morbidity domain (3). The FRAIL scale was subsequently developed, which incorporates the above two approaches (4-5). Frailty has been reported to independently predict risks of adverse health outcomes including falls, disability, institutionalization, health-related quality of life, and mortality (6-9).

Various factors are thought to mediate the development of frailty, such as advanced age and medical co-morbidities (10). The physiological correlates of frailty have also been explored, with no conclusive evidence of association between biomarkers and frailty to date (11). Homocysteine, a B-vitamin metabolite, is one possible candidate that may underlie the development of the frailty syndrome. This is a sulfur amino acid whose metabolism stands at the intersection of 2 pathways: remethylation, which requires folic acid and B12 coenzymes, and transsulfuration, which requires pyridoxal-5’-phosphate, the B6 coenzyme. Total plasma homocysteine (tHcy) has been shown to be inversely related to the intake and plasma levels of folate and B-vitamins (12), and as such, may be used as a surrogate biochemical marker to reflect their metabolic function (13). At the cellular level, sufficient stores of B-vitamins are essential for “one-carbon” transfer metabolisms, and their deficiencies may result in mitochondrial dysfunction with deleterious changes in cellular function (14). These could conceivably cause muscle weakness and atrophy, leading to sarcopenia with progressive physical decline (15). At the molecular level,
B-vitamin deficiency may be mediated via hyperhomocysteinaemia through mechanisms of oxidative stress(16), or by homocysteinylation(17) which involves covalent binding of tHcy to proteins. These modified proteins or neoantigens can trigger the inflammatory cascade, resulting in vascular endothelium damage and subsequently vascular events, further leading to functional decline and frailty. Homocysteine-induced endothelial dysfunction can occur through different mechanisms, via atherosclerotic plaque formation and increased risk of thromboembolic events(18). All these biological pathways could lead to a multisystem decline due to destabilization of the neuromuscular and metabolic balance. In addition, severe hyperhomocysteinaemia can cause endoplasmic reticulum stress leading to cellular growth arrest and apoptosis(19), and ultimately accelerated ageing(20). This can result in a higher risk of mortality, thus making this biochemical marker an important target for investigating this adverse health outcome in older people. As frailty also predicts survival(21), its influence should not be ignored whilst elucidating the biologic association of tHcy with mortality.

In this study, we sought to determine if elevated tHcy is associated with frailty and mortality in later life. We addressed these aims by investigating the cross-sectional and longitudinal relationship between tHcy and frailty (measured by the FRAIL scale) using a large cohort of community-dwelling men aged 70-88 years, as well as the longitudinal relationship between elevated tHcy and all-cause mortality, taking into account the possible mediating effect of frailty.
METHODS

Study design and participants

We conducted a prospective cohort study, using participants from the Health in Men Study (HIMS), which has been described in detail elsewhere(22). In brief, 12203 community-dwelling men aged 65-87 years sampled from the electoral roll of Australia completed a health assessment between 1996 and 1999 (HIMS Wave 1). In 2001-2004, 10940 men were invited to participate in the second phase of this study (HIMS Wave 2) and blood samples were collected from 4249 of them. In 2008-2009 (HIMS Wave 3), 7445 surviving men were mailed a third questionnaire, of which 3274 responded. The Human Research Ethics Committee of the University of Western Australia approved the protocol for HIMS which was conducted in accordance with the Helsinki Declaration for Human Rights.

Outcome of interest

Frailty was assessed during Waves 2 and 3 with a FRAIL scale(4-5). This consists of 5 domains: fatigue, resistance, ambulation, illness, and loss of weight. A score of 1 for each domain indicates its presence, and a score of 0 indicates its absence. Responses to the SF-36 Health Survey(23) during Waves 2 and 3 were used to assess symptoms of fatigue (worn out or feeling tired most of the time), resistance (inability to climb a flight of stairs) and ambulation (inability to walk 100m). A score of 1 was recorded for illness if the participant reported having more than five of the following during Waves 2 and 3 respectively: arthritis, diabetes, angina or myocardial infarction, hypertension, stroke, asthma, chronic bronchitis, emphysema, osteoporosis, colorectal cancer, skin cancer, depression or an anxiety disorder, Alzheimer’s disease or other dementia, or leg ulcers. Participants scored 1 for weight loss if their weight decreased by more than 5%
between Waves 1 and 2, and between Waves 2 and 3. We considered participants to be frail if they scored a total of three or more in these domains (i.e. FRAIL scale ≥ 3). This approach has been validated by analyzing the predictive utility of the scale for all-cause mortality and disability(6, 24).

Records of all hospital admissions and mortality were obtained from the Western Australian Data Linkage System (WADLS)(25), which links together records from the mental health data, cancer registry, death registry and hospital morbidity data.

**Explanatory variables**

The following socio-demographic variables were collected from participants: age (difference in years between date of assessment and date of birth), education (completed high school or better by Wave 1), living circumstance (living alone or in residential aged care facility during Waves 2 and 3) and smoking status (classified as never, former or current smoker during Waves 2 and 3). We identified cardiovascular disease, hypertension, diabetes and dyslipidaemia as potential confounders in the relationship between tHcy and mortality, and hence further elaborated the prevalence of these comorbid diseases from self-reported, clinical and biochemical data available from Waves 1, 2 and 3. Cardiovascular disease was present when the participant reported having a history of angina, myocardial infarction, heart failure, coronary artery bypass grafting, coronary angioplasty, carotid endarterectomy, aortic bypass surgery, peripheral arterial surgery or stroke, or use of medications at the time of assessment for these conditions. Men were considered to have hypertension if they reported having the condition or use of anti-hypertensive medications or had measured blood pressure of equal to or greater than 140/90 mmHg. Men who were diagnosed with diabetes, reported use of glucose-lowering medication, or had a fasting or
non-fasting glucose of ≥7 mmol/l or ≥11 mmol/l respectively, were considered to have diabetes. Men who self-reported the condition or use of lipid-lowering medication, or had fasting low-density lipoprotein of 3.4 mmol/l or higher, high-density lipoprotein less than 0.9 mmol/l, triglycerides of 1.8 mmol/l or higher, or total cholesterol of 5.5 mmol/l or higher were considered to have dyslipidaemia.

Clinical information from WADLS were collected from 1990 to the time of blood sampling and the weighted Charlson Comorbidity Index calculated(26). The latter takes into account 17 common medical conditions that predict one-year mortality: myocardial infarction, congestive heart failure, peripheral arterial disease, cerebrovascular disease, dementia, chronic pulmonary disease, connective tissue disease, ulcers, liver disease, diabetes (including diabetes with end organ damage), hemiplegia, renal disease, leukaemia, lymphoma, other tumours, metastatic tumours, and AIDS.

**Biochemical analyses and other measures**

Blood samples were collected during Wave 2 between 0800 and 1030. Plasma was separated from the blood samples within 1 hour of collection and stored at -80°C until assayed. tHcy was measured by fluorescence polarization immunoassay on an IMx analyzer(27) and dichotomized into ‘high tHcy’ (≥ 15 µmol/l) and ‘normal tHcy’ (< 15 µmol/l) as determined by the laboratory’s reference range. The inter-assay coefficient of variation was 4%.

Serum creatinine, glucose, cholesterol, low-density lipoprotein and triglycerides were measured with a Roche Hitachi 917 analyzer (Roche Diagnostics). High-sensitivity C-reactive protein (hsCRP) was measured with assay on a BNII analyzer (Dade Behring, Birmingham, UK).
Estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation(28).

Height, weight and blood pressure were measured by trained research assistants during Wave 2. Height and weight were self-reported during Wave 3. Body mass index (BMI) was calculated from height and weight in kg/m².

**Statistical analysis**

Data were analyzed using Stata release 11.1 (StataCorp, College Station, TX, USA). Descriptive statistics were calculated for the demographic, lifestyle and clinical variables in Waves 2 and 3 according to the FRAIL scale. The association between tHcy and frailty were investigated in three different ways: according to whether tHcy was ≥ 15 µmol/l, per 5-µmol/l increment in tHcy, and by doubling of tHcy concentration (by dividing the natural logarithm of tHcy by the natural logarithm of 2). To determine the cross-sectional and longitudinal relationship between tHcy and frailty, logistic regression analyses were used. Adjustments were made for age, education, living circumstance, smoking and renal function (using eGFR as proxy in this study). A sensitivity analysis was performed to determine whether exclusion of men who had a history of cardiovascular disease would affect the cross-sectional association. Logistic regression analyses were repeated for incident cases of frailty during Wave 3 for those men with zero deficit at baseline (i.e. FRAIL scale =0 during Wave 2). The association between high tHcy and individual components of the FRAIL scale during Wave 3 was tested using multivariate logistic regression analyses.

Cox proportional hazards models and Cuzik’s test for trend were used to explore the association between tHcy and all-cause mortality. Adjustments were made for age, education, living
circumstance, smoking, cardiovascular disease, diabetes, hypertension, dyslipidaemia, Charlson’s comorbidity index, renal function and baseline frailty status. The results were reported as Odds Ratio (OR) or Hazards Ratio (HR) with 95% confidence intervals (95% CI). P-values < 0.05 were considered statistically significant.
RESULTS

A flow chart detailing disposition of the study participants is shown in Figure 1. Men who had died prior to Wave 2 follow-up or did not attend due to various reasons were older in age (p<0.001), more likely to be current or former smokers (p<0.001), and self-report a history of cardiovascular disease (p=0.025), hypertension (p<0.001) and diabetes (p<0.001) during Wave 1, in comparison to those men who subsequently responded in Wave 2. Similarly, men who had died prior to Wave 3 follow-up or had not attended to the questionnaire were older in age (p<0.001) and more likely to be current or former smokers (p<0.001) in Wave 2, compared to those men who had completed the questionnaire in Wave 3. However, there was no statistically significant difference in their Charlson’s comorbidity indices (p=0.155).

The socio-demographic, clinical and biochemical characteristics of the study population during Waves 2 and 3 according to FRAIL scale are shown in Table 1. tHcy levels were available for 4248 men, aged between 70 and 88 years, during Wave 2. 1117 men (26.3%) had high tHcy (≥15 µmol/l) and the mean (± SD) tHcy concentration for the Wave 2 cohort was 13.4 ± 5.6 µmol/l. 4227 men had complete data for frailty and were thus the focus of our cross-sectional analysis. 685 (16.2%) of these men were frail (i.e. having 3 or more deficits). After a follow-up period of 5.3 ± 0.8 years (Wave 3), 237 men (34.6%) who were frail at baseline died, compared to 498 men (14.0%) who were non-frail (i.e. having less than 3 deficits) (p<0.001). Of those participants who responded to Wave 3, 1824 had complete data for frailty, comorbidities and tHcy levels at baseline. They were similar in age, smoking and comorbidity status compared to the rest of the Wave 3 cohort who were not included in our longitudinal analysis. 461 men (25.3%) during Wave 3 were frail, out of which 131 (28.4%) had high tHcy.
Men aged 65 years and older were randomly selected from electoral roll and invited to participate in HIMS (N=19352)

7149 men were excluded from study, out of town, unwell, untraceable or did not respond

12203 men attended clinic and completed Wave 1 questionnaire in 1996-1999

1418 men died before follow-up in Wave 2; 5200 men were unable to attend, unwell, untraceable or did not respond

4263 men attended clinic and completed Wave 2 questionnaire in 2001-2004
1322 men completed Wave 2 questionnaire by postage only
4249 of the above had blood samples collected from them

3340 men died before invitation to follow-up in Wave 3

7445 men were invited to participate in Wave 3

126 men died after invitation; 4045 men were unable to attend, unwell, untraceable or did not respond

3274 men completed Wave 3 questionnaire in 2008-2009, of which 1824 men had complete data on frailty components, comorbidities and plasma homocysteine levels

Figure 1 Study flow diagram of Health In Men Study (HIMS)
In univariate cross-sectional logistic regression analyses (Table 2), high tHcy was associated with increased odds of being frail (OR 2.11, 95% CI 1.78 to 2.51). The association persisted after adjusting for age, education, living circumstance, smoking and eGFR (OR 1.49, 95% CI 1.22 to 1.81). When men with a history of cardiovascular disease were excluded from the models, the associations persisted in univariate (OR 2.03, 95% CI 1.56 to 2.63) and multivariate (OR 1.43, 95% CI 1.06 to 1.93) analyses. When modeled as continuous variables, elevated tHcy continued to be associated with prevalent frailty.

In longitudinal logistic regression analyses (Table 2), high tHcy was associated with increased odds of being frail after a period of 5.3 ± 0.8 years (OR 1.66, 95% CI 1.30 to 2.12). The association was weakened after adjusting for age, education, living circumstance, smoking and eGFR (OR 1.25, 95% CI 0.95 to 1.65). When assessing the longitudinal relationship between tHcy and incident frailty, only 809 men with FRAIL scale = 0 during Wave 2 were included in the analyses. The odds of being frail at follow-up for these men with high tHcy were 1.89 (95% CI 1.11 to 3.22). After adjusting for potential confounders, the odds were reduced to 1.59 (95% CI 0.88 to 2.89). The association between high tHcy and individual components of the FRAIL scale during Wave 3 was tested using multivariate logistic regression analyses. High tHcy at baseline predicted the ambulation (OR 1.32, 95% CI 1.01 to 1.72) component. There was no statistically significant association with the fatigue (OR 1.21, 95% CI 0.96 to 1.53), resistance (OR 1.07, 95% CI 0.84 to 1.37), illness (OR 1.07, 95% CI 0.77 to 1.48) and weight loss (OR 1.10, 95% CI 0.85 to 1.42) components.

Among those participants who had data for tHcy levels during Wave 2, 749 (17.6%) men subsequently died during a mean follow-up duration of 5.1 ± 1.3 years (range 0.1 to 7.2 years). Men who died were older (p<0.001), had more co-morbidities (p<0.001) and had higher tHcy
levels (15.1 ± 8.1 µmol/l versus 13.0 ± 4.8 µmol/l, p<0.001) than those who were alive by the end of the study. There was a graded association between tHcy and all-cause mortality, as shown in Figure 2 (z=8.9, p<0.001). This association was tested with multivariate Cox proportional hazards models (Table 3). After adjusting for age, education, living circumstance, smoking, cardiovascular disease, diabetes, hypertension, dyslipidaemia, Charlson co-morbidity index, renal function and frailty status at baseline, high tHcy continued to predict all-cause mortality (HR 1.25, 95% CI 1.06 to 1.48). When tHcy was included as quantitative variables, the associations remained significant.
Table 1  Demographic, lifestyle and clinical characteristics of the study population (HIMS Wave 2 and 3) according to FRAIL scale

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<tr>
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<tbody>
<tr>
<td></td>
<td>FRAIL scale ≥ 3</td>
<td>FRAIL scale &lt; 3</td>
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<tr>
<td></td>
<td>(n=685)</td>
<td>(n=3542)</td>
</tr>
<tr>
<td>Age, years&lt;sup&gt;a&lt;/sup&gt;</td>
<td>78.0 (4.0)</td>
<td>76.3 (3.5)</td>
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<tr>
<td>Completed high school or better, n(%)</td>
<td>274 (40.2)</td>
<td>1767 (50.0)</td>
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<tr>
<td>Lived alone or in residential aged care facility, n(%)</td>
<td>151 (22.1)</td>
<td>562 (15.9)</td>
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<tr>
<td>Smoking, n(%)</td>
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<td></td>
</tr>
<tr>
<td>Never smoked</td>
<td>157 (23.1)</td>
<td>1251 (35.4)</td>
</tr>
<tr>
<td>Former smoker</td>
<td>475 (69.8)</td>
<td>2120 (59.9)</td>
</tr>
<tr>
<td>Current smoker</td>
<td>49 (7.1)</td>
<td>167 (4.7)</td>
</tr>
<tr>
<td>Cardiovascular disease, n(%)</td>
<td>395 (58.0)</td>
<td>1423 (40.2)</td>
</tr>
<tr>
<td>Diabetes, n(%)</td>
<td>178 (26.1)</td>
<td>506 (14.3)</td>
</tr>
<tr>
<td>Hypertension, n(%)</td>
<td>621 (91.2)</td>
<td>3111 (87.9)</td>
</tr>
<tr>
<td>Dyslipidaemia, n(%)</td>
<td>494 (72.5)</td>
<td>2496 (70.6)</td>
</tr>
<tr>
<td>Charlson’s Index Score ≥ 5, n(%)</td>
<td>74 (10.9)</td>
<td>95 (2.7)</td>
</tr>
<tr>
<td>BMI, n(%)</td>
<td></td>
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<tr>
<td>&lt;18.5 kg/m&lt;sup&gt;2&lt;/sup&gt;</td>
<td>6 (0.9)</td>
<td>21 (0.6)</td>
</tr>
<tr>
<td>18.5-24.9 kg/m&lt;sup&gt;2&lt;/sup&gt;</td>
<td>213 (31.3)</td>
<td>1213 (34.3)</td>
</tr>
<tr>
<td>25.0-29.9 kg/m&lt;sup&gt;2&lt;/sup&gt;</td>
<td>314 (46.1)</td>
<td>1833 (51.8)</td>
</tr>
<tr>
<td>≥30.0 kg/m&lt;sup&gt;2&lt;/sup&gt;</td>
<td>148 (21.7)</td>
<td>471 (13.3)</td>
</tr>
<tr>
<td>tHcy, µmol/l&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.0 (6.3)</td>
<td>13.0 (4.7)</td>
</tr>
<tr>
<td>hsCRP, mg/L&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.8 (8.1)</td>
<td>3.5 (6.6)</td>
</tr>
<tr>
<td>eGFR, ml/min/1.73m&lt;sup&gt;2a&lt;/sup&gt;</td>
<td>66.4 (19.0)</td>
<td>71.4 (14.2)</td>
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<sup>a</sup> Abbreviations: BMI, body mass index; tHcy, plasma total homocysteine; hsCRP, high-sensitivity C-reactive protein; eGFR, estimated glomerular filtration rate.
Table 2: Univariate and multivariate logistic regression analyses of associations between elevated homocysteine (tHcy) and frailty (FRAIL scale ≥ 3) during HIMS Wave 2 and 3

<table>
<thead>
<tr>
<th></th>
<th>Cross-sectional analyses</th>
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<th>Longitudinal analyses</th>
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<tbody>
<tr>
<td></td>
<td>Univariate</td>
<td>Adjusted&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Univariate</td>
<td>Adjusted&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td></td>
<td>OR (95% CI)</td>
<td>OR (95% CI)</td>
<td>OR (95% CI)</td>
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<tr>
<td>tHcy ≥ 15 µmol/l&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.11 (1.78 to 2.51)</td>
<td>1.49 (1.22 to 1.81)</td>
<td>1.66 (1.30 to 2.12)</td>
<td>1.25 (0.95 to 1.65)</td>
</tr>
<tr>
<td>Per 5-µmol/l increment in tHcy</td>
<td>1.38 (1.28 to 1.49)</td>
<td>1.20 (1.11 to 1.29)</td>
<td>1.29 (1.14 to 1.45)</td>
<td>1.12 (1.00 to 1.27)</td>
</tr>
<tr>
<td>Doubling of tHcy</td>
<td>2.38 (2.00 to 2.84)</td>
<td>1.67 (1.37 to 2.05)</td>
<td>1.69 (1.33 to 2.16)</td>
<td>1.22 (0.92 to 1.61)</td>
</tr>
</tbody>
</table>

Abbreviations: OR, Odds ratio; 95% CI, 95% confidence interval.
<sup>a</sup>Odds ratio presented for high tHcy (≥ 15 µmol/l) in comparison with normal tHcy (< 15 µmol/l).
<sup>b</sup>Adjusted for age, education, living circumstance, smoking and renal function (eGFR).
DISCUSSION

Our study has demonstrated an association between elevated tHcy and prevalent frailty, independent of age and other known confounding factors. We also demonstrated that elevated tHcy levels are predictive of all-cause mortality, independent of frailty status and of other covariates. Although tHcy is likely to play some role in the development of frailty in older men, frailty is unlikely to be a major mediator of the association between tHcy and all-cause mortality.

This study, to our knowledge, is the first to investigate the relationship between tHcy and frailty in a large cohort of community-dwelling older men. Previous studies have explored the relationship between B-vitamins and metabolites with frailty, which resulted in mixed findings. Investigators of the Italian InCHIANTI study(29) found that low folate intake was independently associated with frailty (OR 1.84; 95% CI 1.14 to 2.98). Biochemical marker levels were, however, not analyzed or correlated. Using cross-sectional data from the combined Women’s Health and Ageing Study (WHAS I and II), Michelon et al(30) found a higher prevalence of vitamin B12 deficiency among the frail community-dwelling older women compared to the non-frail, but no apparent association between frailty and serum levels of B-vitamins. Semba et al(31) analyzed the relationship prospectively using a subset of this cohort (WHAS I) and concluded that there was no association between the B-vitamins and incident frailty after 3 years of follow-up. Analyses from the combined WHAS cohort were further race-stratified by Matteini et al(32) and investigations limited to Caucasian women. There were higher proportions of vitamin B12 deficiency and elevated methylmalonic acid (MMA) among the Caucasian women compared to the African American women, and these biomarkers were subsequently found to be related to frailty in Caucasian women (OR 0.69, 95% CI 0.49 to 0.99 for quantitative vitamin B12 levels; and OR 1.34, 95% CI 1.00 to 1.80 for MMA). 54 (9.8%) of the Caucasian women presented with
Figure 2    Univariate Cox proportional hazards model exploring total plasma homocysteine (tHcy) levels and association with all-cause mortality. The tHcy scale of 8 to 22 µmol/l relates to the range between 5th and 95th percentiles of values (n=3823). tHcy is entered as restricted cubic spline. Reference value for hazard ratio is 15 µmol/l. Dashed lines denote 95% confidence interval.
Table 3 Univariate and multivariate Cox proportional hazards models of associations between elevated homocysteine (tHcy) and all-cause mortality after 5.1±1.3 years

<table>
<thead>
<tr>
<th></th>
<th>Univariate HR (95% CI)</th>
<th>Model 1 HR (95% CI)</th>
<th>Model 2 HR (95% CI)</th>
<th>Model 3 HR (95% CI)</th>
</tr>
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<tbody>
<tr>
<td>tHcy ≥ 15 µmol/l&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.75 (1.51 to 2.03)</td>
<td>1.50 (1.29 to 1.74)</td>
<td>1.30 (1.10 to 1.54)</td>
<td>1.25 (1.06 to 1.48)</td>
</tr>
<tr>
<td>Per 5-µmol/l increment in tHcy</td>
<td>1.14 (1.11 to 1.17)</td>
<td>1.12 (1.09 to 1.16)</td>
<td>1.11 (1.06 to 1.15)</td>
<td>1.11 (1.07 to 1.16)</td>
</tr>
<tr>
<td>Doubling of tHcy</td>
<td>1.89 (1.65 to 2.17)</td>
<td>1.60 (1.38 to 1.86)</td>
<td>1.41 (1.18 to 1.67)</td>
<td>1.37 (1.15 to 1.65)</td>
</tr>
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</table>

Abbreviations: HR, Hazards ratio; 95% CI, 95% Confidence interval.
Model 1: adjusted for age.
Model 2: adjusted for age, education, living circumstance, smoking, cardiovascular disease, diabetes, hypertension, dyslipidaemia, Charlson co-morbidity index, renal function (eGFR).
Model 3: adjusted for age, education, living circumstance, smoking, cardiovascular disease, diabetes, hypertension, dyslipidaemia, Charlson co-morbidity index, renal function (eGFR), frailty status at baseline.
<sup>d</sup>Hazards ratio presented for high tHcy (≥ 15 µmol/l) in comparison with normal tHcy (< 15 µmol/l).
hyperhomocysteinaemia (defined as > 13.9 µmol/l in the study), and there was no association between tHcy and frailty in adjusted analysis (OR 1.08; 95% CI 0.72 to 1.62). This is the only study to date that had explored the relationship between tHcy and frailty, and as the authors noted, should be extended to other races and gender to better elucidate the metabolic pathways of frailty. It may also have lacked statistical power to detect small to moderate effect changes.

Our finding that men with low and very high BMIs (<18.5 and ≥ 30 kg/m², respectively) showed increased frailty was consistent with a previous study conducted by Hubbard et al that utilized the English Longitudinal Study of Ageing (ELSA) cohort(35). Sarcopenia, arbitrarily defined as a loss of muscle protein mass and function, plays a predominant role in the pathogenesis and development of frailty(15). An increase in body fat mass may obscure the loss of muscle tissue, a condition termed as “sarcopenic obesity” which is related to physical disability(36). Hence, the weight loss component of our FRAIL scale may be of less significance in the operational definition of sarcopenia and frailty. About 19.8% of our participants met our weight loss criterion, compared with 43.2% for the fatigue domain and 29.9% for the resistance domain, both of which were measurements of physical health and function.

Despite demonstrating that high tHcy levels predicted the ambulation component of the FRAIL scale, we were unable to definitively exclude reverse causality where hyperhomocysteinaemia may be a consequence of poor physical health. To further eliminate the possibility of tHcy being a marker for a vascular event causing frailty(33), we performed a sensitivity analysis after excluding all men with a history of cardiovascular disease. The association between tHcy and frailty in the cross-sectional analysis persisted, suggesting a possible direct effect of tHcy in the pathogenesis of the frailty syndrome in ageing men. The hypothesis of tHcy-induced inflammation as a mechanism of physical decline(34) was tested when hsCRP was added to the
fully adjusted model for frailty. The effect estimates were altered minimally (data not shown), implying that the relationship between tHcy and frailty in our cohort may be independent of the inflammatory pathway.

Frailty is known to be a dynamic condition, as frail older individuals can become non-frail, and pre-frail older individuals are more likely than non-frail individuals to transit to the full frailty syndrome(2). We did not establish strong associations between tHcy and frailty in our longitudinal analyses, and it is possible that we might have missed transitions of the frailty state that occurred during shorter intervals than our follow-up duration. We refined our analysis by excluding men who were pre-frail (i.e. having 1 or 2 deficits) and frail at baseline, and derived a stronger relationship between high tHcy and incident frailty in those men with zero deficit at baseline. Statistical power was reduced with the smaller number of men, and hence the wider confidence interval.

Our finding that elevated tHcy predicted all-cause mortality complements and extends those of prior epidemiological studies(37-41) and suggests that lowering tHcy levels may potentially reduce mortality risk, regardless of the frailty status. On the other hand, a meta-analysis of large randomized trials has not indicated a beneficial effect of B-vitamin therapies on vascular events or mortality in people at risk of or with established cardiovascular disease(42). It has been argued that these findings may be limited by study methodology and patient selection(43-44), hence supporting the need for further observational and clinical trials with a view to developing appropriate primary preventive strategies. To address the possibility of reverse causality due to pre-existing ill health that might have led to elevated tHcy levels, we repeated the analyses after excluding those men who died within 6 months from baseline. The association with all-cause
mortality persisted (OR 1.24, 95% CI 1.04 to 1.47), suggesting that tHcy might be a risk factor rather than a biomarker of this adverse outcome.

The strengths of this study include our large sample size of population-based community-dwelling men at baseline, with a wide range of tHcy concentrations and high prevalence of hyperhomocysteinaemia to investigate our hypotheses. The focus on this well-established cohort of older men aged 70 years and above was highly relevant in the study of frailty and mortality, with these men being at the highest risk for these adverse health outcomes, and from whom a wealth of clinical information was available. However, limitations include our reliance on self-reported weight data at Wave 3 which could possibly lead to an under- or over-estimation of its value, and hence misclassification bias of the FRAIL scale. The problems in using the SF-36 health survey among older adults have been discussed previously (45), and the measurement of frailty based on its components might be subjected to potential recall bias and day-to-day variation. We did not have access to B-vitamin concentrations for our cohort, and thus were unable to exclude the possibility of effect modification by prevailing folate concentrations. 60 (8.8%) men who were frail at baseline reported taking B-vitamin supplements at the time of assessment, compared to 181 (5.1%) men who were non-frail (p<0.001). We repeated our cross-sectional analysis after excluding those men who took B-vitamin supplements and found that the effect estimate of high tHcy on frailty remained essentially unchanged (OR 1.55, 95% CI 1.26 to 1.90). The self-selection of study participants might have biased our findings towards lower tHcy and less frailty compared to the non-respondents, hence limiting the generalisability of our study results to the total population in Australia. As we have pointed out, men who did not respond or had died prior to the follow-up were older in age, more likely to be smokers or ex-smokers, and had more comorbidities compared to those who had responded. This is likely to move our results
towards the null hypothesis and lead to an underestimation of the association between elevated tHcy and increased risk of frailty. Interpretation of our results will need to take this caveat into consideration.

In conclusion, hyperhomocysteinaemia is associated with the prevalence of frailty. Our attempt to demonstrate a longitudinal relationship did not yield a significant correlation between tHcy and incident frailty. Hyperhomocysteinaemia is also predictive of all-cause mortality, independent of the baseline frailty status. Our results suggest that the association between tHcy and mortality is largely not mediated through the occurrence of frailty.
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