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Plasma cystathionine and risk of acute myocardial infarction among patients with coronary heart disease: Results from two independent cohorts☆

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ABSTRACT

Background: Cystathionine is a thio-ether and a metabolite formed from homocysteine during transsulfuration. Elevated plasma cystathionine levels are reported in patients with cardiovascular disease; however prospective relationships with acute myocardial infarction (AMI) are unknown. We investigated associations between plasma cystathionine and AMI among patients with suspected and/or verified coronary heart disease (CHD).

Methods: Subjects from two independent cohort studies, the Western Norway Coronary Angiography Cohort (WECAC) (3033 patients with stable angina pectoris; 263 events within 4.8 years of median follow-up) and the Norwegian Vitamin Trial (NORVIT) (3670 patients with AMI; 683 events within 3.2 years of median follow-up) were included.

Results: In both cohorts, plasma cystathionine was associated with several traditional CHD risk factors ($P < 0.001$). Comparing the cystathionine quartile 4 to 1, age and gender adjusted hazard ratios (95% confidence intervals) for AMI were 2.08 (1.43–3.03) and 1.41 (1.12–1.76) in WECAC and NORVIT, respectively. Additional adjustment for traditional risk factors slightly attenuated the risk estimates, which were generally stronger in both cohorts among non-smokers, patients with higher age, and lower BMI or PLP status (P-interaction ≤ 0.04). Risk associations also tended to be stronger in patients not treated with B-vitamins. Additionally, in a subset of 80 WECAC patients, plasma cystathionine associated strongly negatively with glutathione, an important antioxidant and positively with lanthionine, a marker of H_2S production ($P < 0.001$).

Conclusions: Plasma cystathionine is associated with increased risk of AMI among patients with either suspected or verified coronary heart disease, and is possibly related to altered redox homeostasis.

1. Introduction

An increased risk of cardiovascular disease (CVD) has been associated with elevated plasma homocysteine (Hcy) levels [1]. However, supplementation with Hcy-lowering B-vitamin therapy did not reveal any beneficial effects on cardiovascular outcomes in secondary prevention trials [2,3]. The thioether containing amino acid cystathionine is produced from Hcy during the transsulfuration, catalyzed by 5′-pyridoxal phosphate-dependent (PLP) cystathionine β-synthase (CBS), a rate-limiting enzyme mainly present in liver, neural and cardiac tissues (Supplemental Fig. 1). Cystathionine is subsequently metabolized by another PLP-dependent enzyme cystathionine γ-lyase (CSE) to α-ketobutyrate and cysteine, the precursor of glutathione (GSH), the major intracellular antioxidant in the body [4,5]. Additionally, the gaseous transmitter hydrogen sulphide (H_2S) is formed through several non-canonical reactions, catalyzed by CBS and CSE and accompanied by synthesis of thioethers lanthionine and homolanthionine, which have been previously used as indirect markers of H_2S biogenesis [6]. The direction of homocysteine into the cystathionine pathway leads to the

☆ All listed authors take responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation.

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loss of methionine (Met), an essential amino acid required for protein synthesis and methylation reactions [5].

The synthesis of cystathionine catalyzed via CBS is regulated by the availability of Met [5,7], and modified by the changes in the redox status of the cell [8]. Notably, experimental evidence suggests that increased flux through CBS exacerbates outcome of stroke [9]. Others have linked genetically induced cystathioninemia to acute lethal myopathy and redox injury [10]. Interestingly, renal disease patients [11], and diabetic subjects with nephropathy [12] had elevated plasma cystathionine concentrations, which were strongly associated with plasma Hcy levels. In addition, elevated plasma cystathionine levels were found in patients with vascular disease [13], and coronary artery disease (CAD) [1]; and increased levels have been suggested to be associated with impaired vascular function in healthy humans subjected to oral Met loading [14].

Collectively, these studies strongly suggest that plasma cystathionine may be associated with atherosclerotic CVD; however, the prospective relation between plasma cystathionine and acute myocardial infarction (AMI) risk in larger populations with long-time follow-up is unknown. We investigated the associations between plasma cystathionine and the risk of subsequent AMI, using data from two large independent cohort studies consisting of patients with either suspected or verified coronary heart disease (CHD).

2. Methods

2.1. Study cohorts

The present study consisted of patients from two large independent cohorts: the Western Norway Coronary Angiography Cohort (WECAC) [15] and the Norwegian Vitamin Trial (NORVIT) [3] and both have been described previously. In brief, WECAC comprised 4164 adult participants who were undergoing elective coronary angiography for suspected stable angina pectoris (SAP) between 2000 and 2004. Of these, 2573 (61.8%) were enrolled in the Western Norway B-vitamin Intervention Trial (WENBIT), a secondary prevention study to investigate the effect of Hcy-lowering B-vitamins on all-cause mortality and cardiovascular events [2]. NORVIT included 3749 patients who were hospitalized with AMI during the time period from 1998 to 2002, and underwent identical study treatment as the patients in WENBIT. Subjects with missing baseline data on plasma cystathionine were excluded, leaving a total of 3033 and 3670 patients in the WECAC and NORVIT eligible for the final analyses, respectively (Fig. 1). In addition, among WECAC patients, 2623 had provided urine samples at baseline.

The study protocol was in accordance with the Declaration of Helsinki, and was approved by the regional ethics committee and the Norwegian Data Inspectorate. Written informed consent was provided by all patients.

2.2. Baseline data

Information about patient's lifestyle and medical history were obtained from self-administered questionnaires, and was validated against hospital records when available [3,15]. In both cohorts, smoking status was defined according to self-reports and plasma cotinine (≥85 nmol/L) at baseline [15]. In the WECAC, diabetes was defined by fasting plasma glucose levels >7 mmol/L or non-fasting glucose >11.1 mmol/L or glycated hemoglobin >6.5% according to the American Diabetes Association guidelines [16]. Left ventricular ejection fraction (LVEF) was determined by ventriculography or echocardiography performed during cardiac catheterization. The angiographic extent of CAD was scored as 0–3 according to the number of significantly stenotic coronary arteries. Among NORVIT patients, we did not have information on plasma glucose or glycated hemoglobin, hence, diabetes was defined according to pre-existing diagnoses. The parameters LVEF, and extent of CAD were not available for the NORVIT study.

2.3. Follow-up and study end points

WECAC patients were followed-up from enrollment throughout the year 2006, whereas patients included in the NORVIT were followed until suffering from first AMI or through December 31, 2004. Information on study outcomes was collected from the Cardiovascular Disease in Norway project (CVDNOR; https://cvdnor.b.uib.no/) [17], recording all patients being discharged with a CVD diagnosis from any Norwegian hospitals during 1994–2009. The primary endpoint was total AMI, including both fatal and non-fatal events, and was classified according to the International Statistical Classification of Disease, Tenth Revision system (ICD-10; codes I21-I22).

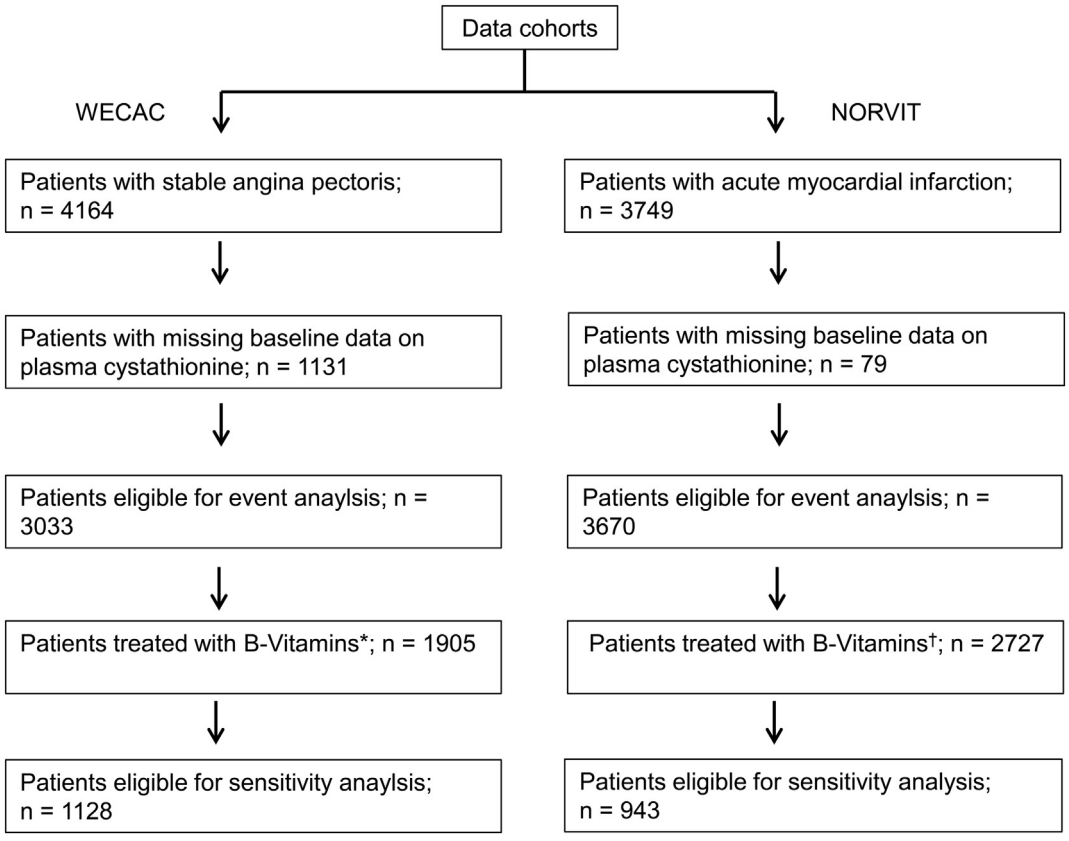

Fig. 1. Flow chat. Flow-diagram showing patient selection from the two study cohorts. NORVIT indicates the Norwegian Vitamin Trial; WECAC, Western Norway Coronary Angiography Cohort. *Excluding patients not randomized to Western Norway B-vitamin Intervention Trial (WENBIT) and group in WENBIT given placebo. †Excluding group given placebo.

2.4. Biochemical analyses

Details on the routines of collection, and biochemical analyses have been previously reported [3,15]. Study specific blood samples obtained from WECAC were immediately stored at −80 °C, whereas samples from NORVIT were sent by mail to the central laboratory, resulting in a delay of maximum 2 days, before separation and storage at −80 °C.

Routine blood analyses were carried out on fresh blood samples at each recruiting hospital, whereas study-specific analyses were performed at Bevital AS, Bergen, Norway (www.bevital.no) by laboratory personnel blinded to the clinical outcomes of patients. Plasma cystathionine and total homocysteine (tHcy) were measured by gas chromatography–tandem mass spectrometry (GC–MS/MS) method (detailed description in Supplemental file). Serum, total cholesterol and c-reactive protein (CRP) were estimated as previously described [15]. Among 2952 patients in WECAC, serum troponin T (cTnT) concentration was obtained and measured by using high-sensitive assay on Modular E170 (Roche Diagnostics). The detection limit was 3 ng/L. Additionally, in a subset of 80 WECAC patients, which was randomly selected based on plasma cystathionine levels with a low-cystathionine group (median [IQR] = 0.10 [0.02]) and high-cystathionine group (median [IQR] = 1.35 [1.28] (n = 40 each), plasma concentrations of GSH and lanthionine, or homolanthionine were analyzed by HPLC and GC–MS/MS, respectively. In NORVIT, we did not have data on plasma cTnT, GSH, lanthionine or homolanthionine levels.

2.5. Statistical analysis

Plasma baseline variables are reported as median (interquartile range, IQR) or counts (percentages) as appropriate. Patient baseline characteristics across plasma cystathionine quartiles were investigated by linear median or logistic regression for continuous and categorical data, respectively. Differences in metabolite concentrations of GSH, lanthionine, and homolanthionine by cystathionine groupings were tested with independent samples t-test.

Survival across quartiles of plasma cystathionine was calculated using the Kaplan-Meier method and differences were evaluated by the log-rank test. The risk association between plasma cystathionine and AMI was estimated using Cox regression models. The hazard ratios (HRs) and 95% confidence intervals (CI) were reported according to quartiles of plasma cystathionine, and per 1 standard deviation (SD) increment of logarithmically transformed plasma cystathionine. The simple model (model 1) included age (continuous) and gender (male/female). The multivariate model (model 2) additionally included the categorical variables current smoking, hypertension, diabetes mellitus, as well as BMI (continuous). The extended multivariate model (model 3) consisted of co-variates of model 2 with the addition of previous cardiovascular diseases, lipid parameters total cholesterol, high-density lipoprotein cholesterol (HDL-C), triglycerides, and CRP (WECAC) or previous cardiovascular diseases and total cholesterol (NORVIT). Since previous studies have reported elevated cystathionine levels among patients with reduced renal function [11], we additionally included estimated glomerular filtration rate (eGFR) in the extended multivariate model 3. Additional adjustments for fasting status and medications at baseline (WECAC) or medications (NORVIT) had no significant influence on the risk associations. Hence, these variables were not included in the final model (data not shown). The assumption of proportionality was verified by inspection of survival plots and calculating Schoenfeld residuals.

Subgroup analyses were performed according to established CHD risk factors in both cohorts, and interactions were tested by adding product terms to the models. Because B-vitamin treatment may influence circulating cystathionine concentrations [18], we performed additional sensitivity analyses for both cohorts after excluding patients treated with B-vitamins. In addition, a potential effect modification according to B-vitamin status in total cohorts or according to treatment with folic acid or vitamin B6 was studied among WENBIT and NORVIT patients.

Statistical analyses were performed with the use of the software programs PASW Statistics 21 (SPSS IBM, NY, USA) and R (R Development Core Team, version 3.2.1). In all statistical models, two-sided P value $P \leq 0.05$ was considered statistically significant.

3. Results

3.1. Baseline characteristics

Baseline characteristics of the two study cohorts are presented in Supplemental Tables 1 and 2. The median (IQR) cystathionine levels at baseline were 0.26 (0.19) and 0.31 (0.25) μmol/L among WECAC and NORVIT patients, respectively.

In both cohorts, patients in the upper cystathionine quartiles were substantially older, and had more frequently a history of hypertension, diabetes, and established CVD, but were less likely to be current smokers than those in the lower quartiles. Further, higher plasma cystathionine was positively related to serum creatinine and inversely to eGFR. As expected, there was also a strong positive association between plasma cystathionine and tHcy.

In addition, among WECAC patients, plasma cystathionine showed a strong inverse association with LVEF and a positive association with serum cTnT levels. Patients with higher cystathionine were more likely to have triple-vessel disease at baseline, higher levels of CRP, triglycerides and lower levels of HDL-C. Furthermore, plasma cystathionine displaced a strong positive association with urine cystathionine, with or without correction for urinary creatinine.

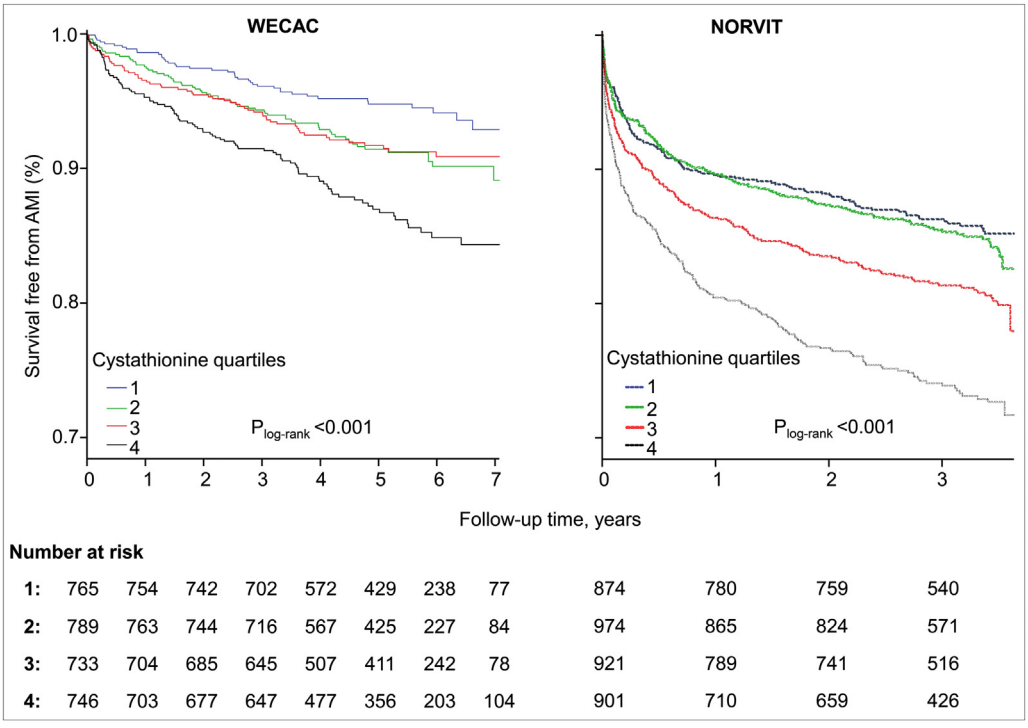

Fig. 2. Event-free survival. Kaplan–Meier curves showing event-free survival according to quartiles of plasma cystathionine among patients in WECAC (left panel) and NORVIT (right panel). The x axis is truncated at 7 and 3.5 years for patients in WECAC and NORVIT, respectively. AMI indicates acute myocardial infarction.

Table 1
The association between plasma cystathionine and incident AMI.

	Quartiles of plasma cystathionine				P_{trend}	Per 1-SD[a]
	1	2	3	4		
In WECAC						
Events (%)	41(5.4)	67 (8.5)	60 (8.2)	96 (12.9)		
Unadjusted	1	1.62 (1.09–2.39)	1.57 (1.05–2.33)	2.56 (1.78–3.69)	<0.001	1.43 (1.29–1.59)
Model 1[b]	1	1.48 (1.01–2.19)	1.35 (0.91–2.02)	2.08 (1.43–3.03)	<0.001	1.35 (1.21–1.50)
Multivariate						
Model 2[c]	1	1.53 (0.97–2.12)	1.30 (0.87–1.95)	1.92 (1.31–2.79)	0.005	1.31 (1.15–1.45)
Model 3[d]	1	1.44 (0.97–2.13)	1.25 (0.83–1.88)	1.83 (1.24–2.69)	0.006	1.29 (1.14–1.45)
In NORVIT						
Events (%)	124 (14.2)	148 (15.2)	176 (19.1)	235 (26.1)		
Unadjusted	1	1.09 (0.86–1.38)	1.39 (1.11–1.76)	2.02 (1.62–2.51)	<0.001	1.34 (1.25–1.43)
Model 1[b]	1	0.99 (0.78–1.26)	1.09 (0.87–1.39)	1.41 (1.12–1.76)	0.001	1.18 (1.09–1.27)
Multivariate						
Model 2[c]	1	0.98 (0.77–1.26)	1.08 (0.85–1.37)	1.35 (1.07–1.69)	0.004	1.16 (1.07–1.25)
Model 3[d]	1	0.95 (0.74–1.21)	1.05 (0.82–1.33)	1.31 (1.04–1.64)	0.008	1.15 (1.06–1.24)

CI indicates confidence interval; HR, hazard ratio; SD, standard deviation; NORVIT, Norwegian Vitamin Trial; WECAC, Western Norway Coronary Angiography Cohort.
[a] Log-transformed.
[b] Adjusted for age and gender.
[c] Adjusted for age, gender, body mass index, hypertension, diabetes, and smoking.
[d] Adjusted for co-variates of model 2 and for previous cardiovascular diseases, total cholesterol, HDL-C, triglycerides, and CRP (WECAC) and for previous cardiovascular diseases and total cholesterol (NORVIT).

3.2. Plasma cystathionine and risk of AMI

The median (IQR) follow-up time was 4.8 years (WECAC-patients), and 3.2 years (NORVIT- patients). The number of patients experiencing an AMI during follow-up were 264 (8.7%, WECAC) and 683 (18.2%, NORVIT). Fig. 2 depicts crude Kaplan–Meier curves for event-free survival, showing reduced overall survival across increasing quartiles of baseline cystathionine in both cohorts ($P < 0.001$ by log-rank test).

Among WECAC patients, those in the highest plasma cystathionine quartile compared to the lowest had an increased risk of incident AMI with HR (95% CI) of 2.56 (1.78–3.69) in an unadjusted model. Corresponding HRs (95% CI) for AMI was 2.08 (1.43–3.03) and 1.92 (1.31–2.79) in model 1, and model 2, respectively (Table 1). Further adjustment for potential cofounders (multivariate model 3) only slightly attenuated the risk estimates (Table 1).

Since Hcy is a well-known CVD risk predictor [1,2], and strongly associated with cystathionine, we also evaluated the influence of adjusting the Hcy-AMI event association for cystathionine and vice versa. The risk estimates of cystathionine were numerically stronger compared to that of tHcy in all the analyses and did not appreciably change after adjusting for tHcy in the multivariate model 3 (HR: 1.78; 95% CI, 1.20–2.66). In contrast, the association between tHcy and risk of AMI was no longer statistically significant after additional adjustment for plasma cystathionine in model 2 (1.22; 95% CI, 0.84–1.76) (Supplemental Table 3).

Plasma Met was not associated with AMI risk (HR: 1.04; 95% CI, 0.74–1.47; $P = 0.82$, for quartile 4 vs 1) and adjustment for Met did not influence the cystathionine-AMI associations (data not shown). However, controlling for eGFR or serum cTnT in the multivariate model 3 attenuated the risk estimates considerably between cystathionine and

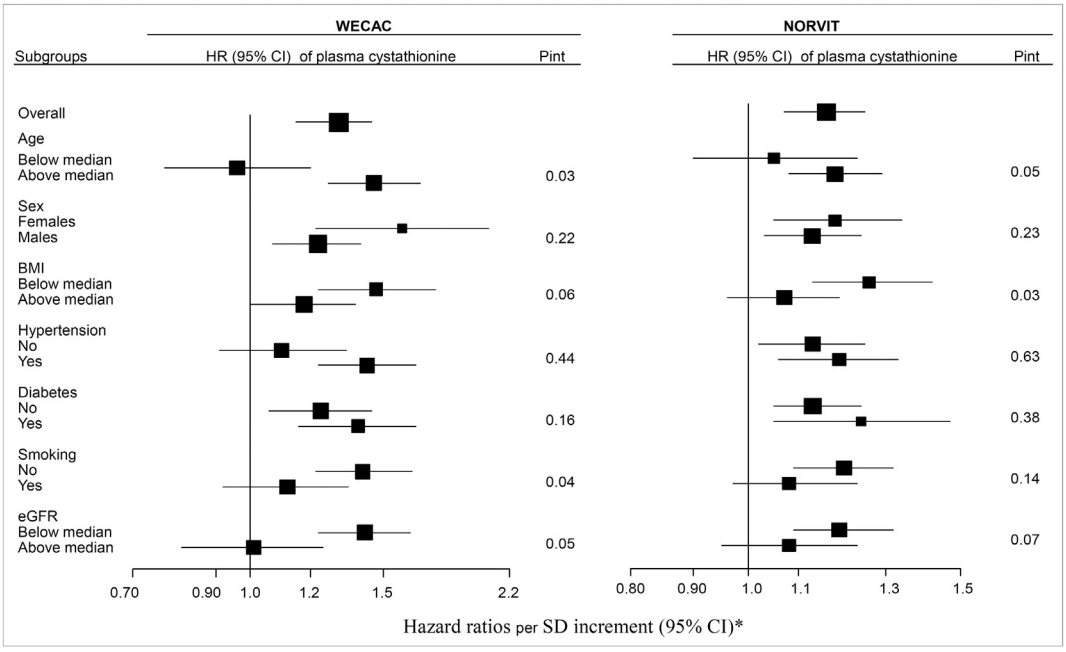

Fig. 3. Forest plot depicting associations between plasma cystathionine and acute myocardial infarction according to subgroups of traditional risk factors for coronary heart disease. The filled squares illustrate the sample sizes, and horizontal lines represent the 95% confidence intervals. BMI indicates body mass index; eGFR, estimated glomerular filtration rate. *Adjusted for age, gender, body mass index, hypertension, diabetes, and smoking.

incident AMI (HR [95% CI] for quartile 4 vs 1, 1.54 [1.04–2.31; $P = 0.04$] and 1.49 [1.01–2.18; $P = 0.05$], respectively).

Among patients in the NORVIT, we also observed increased risk for future AMI with higher plasma cystathionine, although the estimates were numerically somewhat weaker in crude, and all the adjusted analyses (Table 1).

Also, controlling for Hcy (Supplemental Table 3), or eGFR or Met in the multivariate model 3 (HR [95% CI] for quartile 4 vs 1, 1.27 [1.01–1.61; P = 0.04] and 1.30 [1.03–1.65; $P = 0.03$], respectively) slightly attenuated risk associations, and as for the patients in the WECAC, plasma cystathionine was a stronger predictor of future AMI than tHcy (Supplemental Table 3).

3.3. Urine cystathionine and risk of AMI

Among WECAC patients, we observed no association between urinary cystathionine levels and AMI risk in the extended multivariate adjusted model (HR: 1.31; 95% CI, 0.78–1.63; $P = 0.57$, in the fourth vs. first quartile).

3.4. Subgroup analysis

The cystathionine-AMI estimates for both study cohorts according to several traditional CHD risk factors and B-vitamin status are demonstrated in Fig. 3 and Supplemental Fig. 2, respectively.

Among patients in the WECAC, we observed a stronger association between plasma cystathionine and incident AMI in patients with higher median age, and in non-smokers, whereas there was no association among those with lower median age, or in smokers (P for interaction = 0.03, and 0.04, respectively). Additionally, we observed trends towards stronger risk estimates for plasma cystathionine among patients with low as compared to high BMI and eGFR (P for interaction 0.06 and 0.05, respectively) (Fig. 3). There was also a stronger association with risk among patients with plasma PLP below as compared to above median value (P for interaction = 0.02) (Supplemental Fig. 2).

Among NORVIT patients, each of the subgroup analyses confirmed the general pattern observed in the WECAC cohort. Yet again, the association between cystathionine and future AMI was significantly or marginally modified when participants were stratified by age, BMI, or eGFR, (P for interaction = 0.05, 0.03, and 0.07, respectively) (Fig. 3). However, the cystathionine-AMI relationship was not modified by smoking (Fig. 3) or plasma PLP status (Supplemental Fig. 2) (P for interaction >0.14, both).

For both cohorts, the results from subgroup analyses were essentially similar after extended multivariate adjustment (model 3; data not shown).

3.5. Sensitivity analyses

After the exclusion of patients treated with B vitamins, we obtained numerically stronger associations with risk in the remaining 1128 WECAC and 943 NORVIT patients (Supplemental Table 4), although the interactions with treatment were not statistically significant (P for interaction≥0.15) (Supplemental Table 5).

3.6. Relationship between plasma cystathionine, GSH, and H_2S

We next examined the relationship between plasma cystathionine, GSH and H_2S levels in a subset of 80 WECAC patients (Supplemental Table 6). Plasma GSH levels were lower in high than in the low cystathionine group ($P < 0.001$). On the other hand, increased concentrations of plasma lanthionine were observed in the high cystathionine group ($P < 0.001$), whereas there was no significant difference in homolanthionine levels between the two groups ($P > 0.05$).

4. Discussion

4.1. Principal findings

Using data from two independent cohort studies, the WECAC and the NORVIT, we demonstrated that higher plasma cystathionine was associated with an increased risk of incident AMI, independent of baseline traditional CHD risk factors and potential confounders. Across both cohorts, the positive association between plasma cystathionine concentration and AMI risk was most pronounced among older subjects, and those with low BMI or PLP status. Finally, we documented a negative association between plasma cystathionine and total GSH levels versus positive association with concentration of circulating lanthionine in a subset of WECAC patients.

4.2. Cystathionine and CVD in other epidemiological studies

A small study among 14 healthy subjects suggested an inverse relationship between cystathionine concentration and vascular function following oral Met challenge [14]. Additionally, elevated cystathionine levels in plasma were shown in patients with CAD (1), and vascular disease [13]. Compared to these studies, median plasma cystathionine levels were higher in our study. However, this may be attributable to use of different method for measuring plasma cystathionine levels, and higher age of study participants in the present study [19].

4.3. Plasma cystathionine and unfavorable CVD risk profile

Our findings of a generally adverse CVD risk profile among those with higher plasma cystathionine are in line with other studies reporting positive associations between plasma cystathionine with age [19], and BMI [13]. Similar to our findings, plasma cystathionine has been inversely associated with renal function (eGFR) [11], and positively related with serum creatinine [19]. Further, we confirmed a strong positive association between baseline plasma cystathionine and tHcy as shown in earlier studies [11,12]. Extending these results, we also found a strong positive association of baseline plasma cystathionine with serum cTnT levels, as well as with more extensive CAD at angiography, potentially mirroring the inverse relationship with LVEF. These observations further supports overall hypothesis that higher level of plasma cystathionine is associated with increased risk of cardiovascular disease and AMI. However, somewhat unexpectedly we also observed negative associations between plasma cystathionine and smoking. Although reverse causation cannot be ruled out, this finding could possibly also be explained by the inverse correlation between body weight and smoking habits, as reported in previous cross-sectional studies [20].

Interestingly, despite of higher plasma cystathionine levels in NORVIT compared to WECAC, we observed somewhat weaker risk estimates for AMI in NORVIT. This may suggest that plasma cystathionine either may be linked to underlying mechanisms predisposing to the acute coronary event of NORVIT participants, or influenced by the myocardial infarction and associated inflammatory processes per se, which may have masked risk association during long-term follow up. Thus, in patients with acute coronary syndrome, plasma cystathionine may not be a good predictor of AMI outcomes. However, as far as we are aware, the large-scale analysis of long-term prospective relationship between plasma cystathionine and clinical cardiovascular events has not been evaluated previously among patients or in the general population; hence the present investigation extends prior knowledge on atherosclerotic CVD according to cystathionine status.

4.4. Cystathionine, age and BMI

Our study shows that the relationship between plasma cystathionine and AMI was most pronounced among older subjects, and among

patients with low BMI. Older age is associated with reduced levels of folate and vitamin B12, and enzyme activity involved in cystathionine metabolism [19]. Furthermore, age-dependent deterioration of kidney function may also influence cystathionine levels [19]. The mechanism behind the effect modification by BMI is uncertain, however, studies show that body mass negatively influences the hepatic CBS enzyme activity and transcription [21], and thus may attenuate the cystathionine production.

4.5. Cystathionine and kidney function

Inclusion of eGFR in the extended multivariate model attenuated the estimates somewhat among WECAC patients, and in both cohorts we found an evidence of effect modification by eGFR on the cystathionine-AMI relationship. Renal dysfunction imposes an increased risk of atherosclerotic CVD [22], and has consistently been associated with increased cystathionine levels [11,19]. Our findings thus may indicate that elevated plasma cystathionine contributes to the development of vascular complications in patients with renal impairment. Nevertheless, additional studies are required to validate and explain the effect modification from renal function observed in our study, not least because the patients currently studied in general had normal or only mildly impaired renal function.

4.6. Cystathionine, tHcy and AMI

Elevated concentration of tHcy has been associated with increased risk of CVD in several studies [1–3], including our present study. High tHcy is thought to promote atherosclerosis by several mechanisms, such as inducing endothelial dysfunction, oxidative stress, and increased synthesis of collagen and deterioration of arterial wall elasticity [1,14]. However, in both study cohorts, the association between plasma cystathionine and AMI was not influenced by adjusting for tHcy. On the other hand, the attenuation of tHcy-AMI relationship after adjustment for plasma cystathionine, and the findings that cystathionine has a stronger risk association with AMI, suggest that elevated levels of cystathionine in blood may mediate some of the adverse cardiovascular effects of elevated tHcy.

4.7. Cystathionine and methionine

Met is another important precursor of cystathionine. Excess Met intake in humans has been associated with up-regulation of CBS activity and downregulation of the remethylation pathway, and thereby may influence plasma cystathionine concentration [5,7]. We did not evaluate Met intake in the current study; however, in a prospective Nurses' Health Study, the dietary intake of Met was not associated with the risk of CHD [23]. Accordingly, in the current study, plasma Met was not associated with CVD risk, making Met less likely a confounder.

4.8. Cystathionine and influence of B-vitamin intervention

B-vitamins folic acid, B12 and B6 are the important cofactors, which participate in cystathionine metabolism [18,19]. Elevation of plasma cystathionine levels therefore may result from nutritional or functional insufficiencies of one or several of these co-factors. Interestingly, low B-vitamin status is suggested to be risk marker of CHD [1,6,23]. The risk associations of cystathionine tended towards being stronger among patients treated with placebo in the WENBIT and NORVIT, as well as patients in the WECAC not randomized to WENBIT; however, we did not observe any significant interaction between plasma cystathionine and B-vitamin interventions on AMI occurrence in either cohort. On the other hand, in both study populations, we observed a steep inverse relationship between plasma cystathionine and PLP levels at baseline. Further, the AMI risk association of cystathionine in WECAC was almost entirely restricted to those with low PLP, indicating that the association of elevated cystathionine levels with adverse prognosis is related to altered B6 metabolism rather than low intake of B6.

4.9. Possible mechanisms

We observed a positive association between plasma cystathionine and lanthionine, a sulfur-containing amino acid produced by CBS during H_2S formation [6]. Previous studies have revealed that exposure to prooxidants such as hydrogen peroxide upregulates CBS and is associated with increased cystathionine levels [8]. Others have found that H_2S production is stimulated in response to increased oxidative stress [24]. Although H_2S has been shown to be cardioprotective [24], it remains to be elucidated whether elevated H_2S associated with cystathionine is playing protective or detrimental role. Nonetheless, elevated plasma cystathionine may reflect increased hepatic CBS activity due to increased oxidative stress. This observation was further supported by the strong associations between plasma and urinary cystathionine found in WECAC. However, urine cystathionine was not associated with AMI risk, indicating that the cystathionine-related atherogenesis may not be solely mediated through increased hepatic CBS activity.

Alternatively, high plasma cystathionine levels may reflect impaired CSE, which has been associated with oxidative injury in CSE-deficient (CSE−/−) mice [10]. Notably, the activity of CSE but not CBS is reduced by inadequate B6 status [25]. Thus, high cystathionine may be related to, and be a measure of, reduced intracellular CSE activity, the rate limiting step for synthesis of the vital antioxidant GSH [5,10]. Our observation of low plasma GSH levels in patients with high cystathionine supports this concept. A resultant increased oxidative stress may have participated in processes leading to inflammatory responses, mitochondrial dysfunction, and ultimately cell apoptosis and death [26]. This is further supported by the strong positive relationship between cystathionine and serum cTNT concentrations at baseline, and the attenuation of the risk estimate of cystathionine after adjusting for cTNT in the extended Cox model 3 among WECAC patients.

5. Strengths and limitations

The major strengths of our study include the large sample sizes, detailed clinical baseline characteristics, its prospective design with long-term follow-up and the unbiased event ascertainment procedures. Also, the findings were consistent across two independent study populations.

There are some limitations to our study. 1) Among NORVIT patients, blood samples were exposed to room temperature for 2 days, which could potentially alter plasma cystathionine concentrations; however, plasma cystathionine levels are stable during short-term storage even at room temperature [27]. 2) Unfortunately, analyses on H_2S, GSH and cTNT levels were not replicated in NORVIT because of the lack of data. 3) Concentration of cystathionine in plasma is reported to be higher during non-fasting than fasting conditions [28]. In WECAC, the majority of blood specimen was drawn from non-fasting subjects, and in NORVIT, data on fasting status was not available. However, adjustment for fasting status did not appreciably attenuate the risk associations in WECAC, suggesting that the observed associations are unlikely to be confounded by fasting status. In addition, there was no information available in either cohort on possible diurnal variations in plasma cystathionine. However, within-subject reproducibility of plasma cystathionine over time has been reported to be moderate [29], indicating that the actual risk relationships are even stronger, due to regression-dilution bias [30]. 4) We studied elderly, mostly male patients with CHD, and our findings may thus not be applicable to populations with other characteristics. 5) Finally, as this is an observational study, the potential of residual confounding is an inherent limitation; hence the inference about causality cannot be made.

6. Conclusions

In conclusion, the results of our two large, independent cohorts of patients with either suspected or verified CHD demonstrate that elevated plasma cystathionine levels are strongly associated with risk of future AMI. This association was independent of traditional CHD risk factors and potential confounders. Our findings motivate further studies to explore possible pro-atherogenic mechanisms related to disturbances of the one carbon and transsulfuration pathway.

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Disclosures

None.

Author contributions

OKN designed research; ID, GFTS, ERP, BD, PMU, KHB, JFG and OKN conducted research; ID and OKN interpreted data; ID performed statistical analysis and wrote the manuscript; GFTS, AU, ES, PMU and ON critically revised the manuscript. All authors read and approved the final version of the manuscript.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijcard.2018.04.083.

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