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Plasma methylmalonic acid predicts risk of acute myocardial infarction and mortality in patients with coronary heart disease: A prospective 2-cohort study

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Abstract. Dhar I, Lysne V, Ulvik A, Svingen GFT, Pedersen ER, Bjørnestad EØ, et al. Plasma methylmalonic acid predicts risk of acute myocardial infarction and mortality in patients with coronary heart disease: A prospective 2-cohort study. *J Intern Med.* 2023;**293**:508–519.

Background. Elevated plasma methylmalonic acid (MMA) is reported in patients with established coronary heart disease (CHD) and is considered a marker of vitamin B12 deficiency. Moreover, MMA-dependent reactions have been linked to alterations in mitochondrial energy metabolism and oxidative stress, key features in the pathophysiology of cardiovascular diseases (CVDs).

Objectives. We examined whether plasma MMA prospectively predicted the long-term risk of acute myocardial infarction (AMI) and mortality.

Methods and results. Using Cox modeling, we estimated hazard ratios (HRs) for endpoints according to per 1-SD increment of log-transformed plasma MMA in two independent populations: the Western Norway Coronary Angiography Cohort (WECAC) (patients evaluated for CHD; n = 4137) and the Norwegian Vitamin Trial (NORVIT) (patients hospitalized with AMI; n = 3525). In WECAC and NORVIT, 12.8% and 18.0% experienced an AMI, whereas 21.8% and 19.9% died, of whom 45.5% and 60.3% from CVD-related causes during follow-up (range 3–11 years), respectively. In WECAC, age- and gender-adjusted HRs (95% confidence interval) were 1.18 (1.09–1.28), 1.25 (1.18–1.33), and 1.28 (1.17–1.40) for future AMI, total mortality, and CVD mortality, respectively. Corresponding risk estimates were 1.19 (1.10–1.28), 1.22 (1.14–1.31), and 1.30 (1.19–1.42) in NORVIT. These estimates were only slightly attenuated after multivariable adjustments. Across both cohorts, the MMA-risk association was stronger in older adults, women, and non-smokers.

Conclusions. Elevated MMA was associated with an increased risk of AMI and mortality in patients with suspected or verified CHD.

Keywords: biological markers, coronary heart disease, energy metabolism, methylmalonic acid

Introduction

Atherosclerosis is a multifactorial progressive disease characterized by the accumulation of lipids and fibrous elements in the arterial walls, thus potentially obliterating arterial flow and hampering tissue perfusion, leading to cardiovascular disease (CVD) [1]. Although atherosclerotic CVD-related mortality trends have decreased during the last decades, atherosclerotic CVD remains a major

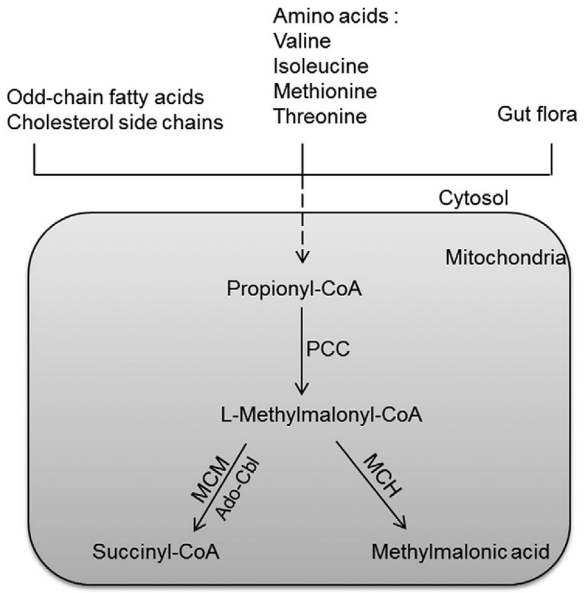

Fig. 1 *Schematic presentation of methylmalonic acid metabolism. Ado-cbl, adenosyl-cobalamin; MCH, methylmalonyl-CoA hydrolase; MCM, methylmalonyl-CoA mutase; PCC, propionyl-CoA carboxylase.*

determinant of global fatality burden [2]. Thus, the identification of new risk markers may improve cardiovascular risk assessment and enhance our understanding of underlying mechanisms.

Methylmalonic acid (MMA) is a dicarboxylic acid produced via the enzyme methylmalonyl-CoA hydrolase from L-methylmalonyl-CoA, which is formed from propionyl-CoA, a catabolite of several amino acids and odd-chain fatty acids and cholesterol [3] (Fig. 1). Alternatively, L-methylmalonyl-CoA can be converted to succinyl-CoA in a reaction catalyzed by the vitamin B12–dependent enzyme, methylmalonyl-CoA mutase [3]. Accordingly, increased systemic MMA is considered a marker of vitamin B12 deficiency [3, 4]. Increased MMA has also been linked to mitochondrial dysfunction and oxidative stress [5–7], both processes playing key roles in atherogenesis [1, 8]. Furthermore, an activation of peroxisome proliferator–activated receptor (PPAR) α, a major regulator of lipid, glucose, and amino acid energy metabolism [9], has been shown to increase plasma MMA in rodents [10]. MMA is excreted by the kidneys, and MMA elevation is observed in patients with reduced glomerular filtration rate (GFR) [11].

Taken together, these observations suggest that MMA availability may be cross-linked to both mitochondrial energy metabolism, impaired renal function, and the risk of atherosclerotic CVD. However, the few population studies that have investigated the relationship of circulating MMA with CVD showed inconsistent results [7, 12–14]. A case-control study reported significantly higher plasma MMA among patients with myocardial infarction as compared with healthy controls, despite no difference in vitamin B12 concentrations [12]. Also, increased MMA was found in patients with renal failure, the latter predominantly among those with a history of cardiovascular complications [13]. Moreover, recent studies have demonstrated a positive relationship between MMA and increased mortality in the general population [7, 14]. On the other hand, circulating MMA was not associated with the risk of future cardiovascular events in a small prospective study among patients surviving acute myocardial infarction (AMI) [15].

We investigated the association of plasma MMA with the long-term risk of AMI and mortality using data from two large cohorts of patients with either suspected or verified coronary heart disease (CHD).

Materials and methods

Study cohorts

The current study used data from two large clinical cohorts. The Western Norway Coronary Angiography Cohort (WECAC) consisted of 4166 patients who underwent elective coronary angiography for suspected stable angina pectoris between 2000 and 2004 and has been described in detail elsewhere [16]. Approximately 2/3 of these patients participated in the Western Norway B-Vitamin Intervention Trial (WENBIT, NCT00354081), which evaluated the effects of oral B-vitamin treatment on future clinical cardiovascular outcomes and mortality. The Norwegian Vitamin Trial (NORVIT, NCT00266487) enrolled 3749 participants who were hospitalized for AMI between 1998 and 2002 [17]. WENBIT and NORVIT had identical study intervention protocols and patients in both trials received treatment with folic acid (FA) plus vitamin B12 and/or vitamin B6, or placebo. In the current study, we included only baseline measurements, and patients with any missing data on plasma MMA and covariates incorporated in the risk models as well as those with a high plasma MMA value of ≥ 1 μmol/L were excluded, yielding 4137 patients in WECAC and 3525 patients in

NORVIT eligible for the final analyses. The study was carried out according to the Declaration of Helsinki and was approved by the Regional Medical and Health Ethics Committee, the Norwegian Medicines Agency, and the Norwegian Data Inspectorate. All study subjects provided written informed consent.

Baseline data and laboratory analyses

Data on medical history, including CVD risk factors and medications, were collected from self-administered questionnaires and validated against hospital records when available. Smoking status and diabetes mellitus were defined or classified as previously described [16].

Serum low-density lipoprotein cholesterol was calculated on the basis of the Friedewald formula. The estimated GFR (eGFR) rate was obtained using the Chronic Kidney Disease Epidemiology Collaboration formula [18]. Serum concentrations of apolipoprotein (apo) A1 and apo B 100 were measured on Roche Hitachi 917 and 912 systems (Roche Diagnostics), respectively, using reagent kits of type Tina-quant (Roche Diagnostics, GmbH, Mannheim, Germany). Plasma vitamin A (Vit-A) (as all-trans retinol), cobalamin [19], and acylcarnitines [20] were determined as previously described. Plasma concentrations of MMA were measured by gas chromatography–tandem mass spectrometry (GC–MS/MS) [21], whereas plasma neopterin was quantified with liquid chromatography/MS [22] at Bevital AS laboratory, Bergen, Norway (www.bevital.no) (see Supporting Information for description). The intraclass correlation coefficient of plasma MMA is 0.82 (95% confidence interval [CI]: 0.80–0.84) [19].

Follow-up and clinical endpoints

The primary endpoints were AMI, total mortality, and CVD mortality, whereas the secondary outcome of interest was non-CVD death. AMI was classified according to the International Statistical Classification of Disease (ICD) 10th Revision (coded I21, I22, I46.1, R96, and R98), and CVD mortality included the cause of death coded I00–I99 or R96 according to the ICD-10 system. WECAC participants were followed up from baseline until 2009 for the AMI endpoint or 2012 for death, whereas patients in NORVIT were followed throughout 2004 (for AMI events) or 2007 (for death). Data on AMI were collected from the CVD in Norway project (www.cvdnor.no) [23], which includes all CVD discharge diagnoses from any public hospital in Norway during 1994–2009. Information on fatal events was obtained from the Norwegian Cause of Death Registry (https://www.fhi.no/en/hn/health-registries/cause-of-death-registry/). In both registries, endpoint data were combined with each study participant's unique 11-digit personal identification number.

Statistical analyses

Continuous variables are presented as medians (25th–75th percentile) and categorical variables as percentages (%). Associations of plasma MMA with relevant baseline continuous variables were estimated using the unadjusted Spearman rank correlations, and *t*-tests were performed to compare MMA concentrations across levels of categorical variables.

Cox proportional hazard regression analyses were used to obtain hazard ratios (HRs) (95% CIs) per 1-SD increment of log-transformed plasma MMA (corresponding to a 46% and 41% increment in NORVIT and WECAC, respectively). The basic model (Model 1) was adjusted for age and sex. The multivariable model (Model 2) additionally included established risk factors common to both cohorts: hypertension (yes/no), current smoking (yes/no), diabetes mellitus (yes/no), body mass index (BMI), and serum total cholesterol. Because circulating MMA levels are elevated in patients with renal impairment [11, 13], we additionally included eGFR in the extended model (Model 3). Additional adjustments for WENBIT or NORVIT B-vitamin treatment or medications prescribed at discharge after angiography had negligible impact on the risk associations and, hence, were not included in the final models (data not shown). To analyze potential nonlinear risk associations between plasma MMA and endpoints, MMA was modeled as a penalized spline to Model 1, and the partial effect of MMA was visualized.

In both cohorts, we performed subgroup analyses according to predefined CHD risk factors and serum cobalamin. Circulating MMA may be lowered by B-vitamin treatment [24], hence, we also examined whether the risk associations of MMA were modified according to the FA/B12 or B6 study treatment allocation among WENBIT and NORVIT patients. Importantly, in addition to increasing MMA [10], PPARα agonism is also suggested to enhance the generation of oxysterols

[25], which may regulate the biosynthesis of the active form of Vit-A, i.e retinoic acid (RA) [26]. Hence, we also evaluated the post hoc exploration of the potential effect modifications between circulating MMA and primary outcomes by serum Vit-A (available in the WECAC only). Tests for effect modifications by potential categorical and continuous moderators were performed by entering interaction terms to Model 1. Further, the predicted HRs (95% CI) per 1-SD increments in log-transformed MMA were then plotted as a function of the continuous moderators and presented visually.

Sensitivity toward unobserved confounding was evaluated by applying *E*-values formula to the estimates obtained from the Cox model 2, as recently recommended for observational studies [27]. In both cohorts, we additionally explored model discrimination by calculating areas under receiver operator characteristics curves (ROC-AUC) and the integrated discrimination improvement (IDI) according to the Cox models 2 and 3 with and without MMA. Further, the continuous net reclassification improvement (NRI > 0) [28] was calculated to evaluate any improvement in reclassification by MMA for predicting an adverse event. For these analyses, the follow-up time was censored beyond 3 years for AMI and 5 years for mortality in both patient populations, roughly corresponding to the identical and minimum follow-up for endpoints in either cohort.

Computations were carried out using SPSS for Windows (version 27; SPSS IBM, NY, USA) and R (R Development Core Team, Vienna, Austria, version 4.0.3) and the packages from the *tidyverse* for data handling, *survival* for survival analyses and *ggplot2, ggridges*, and *patchwork* for visualization.

Results

Baseline characteristics

Baseline characteristics of the two study cohorts are presented in Table 1. In the WECAC population, median (25th–75th percentile) age was 62 (55–70) years, 46.7% had hypertension, 38.6% had diabetes mellitus, and 31.7% were smokers. In NORVIT, 48.5% were smokers, and 28.5% and 9.6% had hypertension and diabetes mellitus, respectively. Median MMA (25th–75th percentile) concentrations were 0.16 (0.13–0.20) in WECAC and 0.17 (0.14–0.22) in NORVIT.

Associations of plasma MMA with selected baseline variables are given in Fig. 2. Across both cohorts, plasma MMA was positively correlated with age and plasma neopterin, and inversely with BMI, eGFR, and serum cobalamin. Moreover, patients with higher MMA were more likely to be women, non-smokers, having hypertension, but were less frequently prescribed with statins.

Further in WECAC, plasma MMA was related to higher levels of Vit-A as well as to serum acylcarnitines, including acetylcarnitine, octanoylcarnitine, and propionylcarnitine.

Follow-up and events

Median (25th–75th percentile) length of follow-up time was 7.5 (6.3–8.7) (AMI) and 10.3 (9.2–11.6) years (death) in WECAC and 3.1 (2.3–3.5) (AMI) and 6.9 (6.0–7.9) (death) in NORVIT. In WECAC, 529 (12.8%) participants experienced an AMI, whereas 900 (21.8%) subjects died whereof 410 (45.5%) from CVDs. In NORVIT, 636 (18.0%) suffered an AMI, and 700 (19.9%) subjects died, of whom 422 (60.3%) due to cardiovascular causes.

Plasma MMA and risk of AMI and mortality

In both cohorts, plasma MMA concentrations were associated with an increased risk of AMI, total, and CVD-death in age- and sex-adjusted analyses (WECAC: HR [95% CI] per 1-SD: 1.18 [1.09–1.28], 1.25 [1.18–1.33], and 1.28 [1.17–1.40], respectively; NORVIT: HR [95% CI] per 1-SD: 1.19 [1.10–1.28], 1.22 [1.14–1.31], and 1.30 [1.19–1.42], respectively) (Table 2). The risk relationships remained also after multivariable adjustments and were slightly attenuated after additional adjusting for eGFR (Table 2). Accordingly, we found an approximately linear positive relationship between plasma MMA as a continuous variable and study outcomes in both study populations (Fig. 3). In WECAC, we also observed a positive association between MMA and non-CVD death in age- and sex-adjusted analyses (HR: 1.22 [1.13–1.33]), which persisted after multivariable adjustments. The risk relationship between MMA and non-CVD death was less pronounced in NORVIT after basic adjustments (Table 2).

Vitamin B12 is an important cofactor in MMA metabolism [3, 4], but additional adjustment for serum cobalamin did not materially influence the

Table 1. *Baseline characteristics of patients in two Norwegian clinical populations*

	WECAC $n = 4137$	NORVIT $n = 3525$
MMA (μmol/L)	0.16 (0.13–0.20)	0.17 (0.14–0.22)
Age (year)	62 (55–70)	63 (54–72)
Male sex (%)	72.0	74.1
BMI (kg/m^2)	26 (24–28)	26 (24–28)
Hypertension	46.7	28.5
Diabetes mellitus	38.6	9.6
Current smoking	31.7	48.5
eGFR (ml/min per 1.73 m^2)	91 (78–99)	77 (65–89)
Serum vitamin A (μmol/L)	2.82 (2.5–3.3)	NA
Serum neopterin (nmol/L)	8.2 (6.7–10.3)	8.7 (6.8–11.7)
Plasma methionine (μmol/L)	26.6 (22.4–32.0)	29.6 (25.1–34.2)
LVEF (%)	65 (60–70)	NA
B-vitamin status		
Serum folate (nmol/L)	10.1 (7.4–14.8)	7.99 (5.9–11.2)
Plasma PLP (nmol/L)	41.2 (29.5–59.8)	28.5 (20.4–40.3)
Serum cobalamin (pmol/L)	363 (275–467)	360 (284–455)
Serum lipid parameters		
Total cholesterol (mmol/L)	4.90 (4.3–5.7)	5.7 (4.9–6.5)
LDL-C (mmol/L)	2.90 (2.4–3.7)	NA
ApoB 100 (g/L)	0.87 (0.73–1.04)	NA
HDL-C (mmol/L)	1.20 (1.0–1.5)	NA
Apo A1 (g/L)	1.30 (1.1–1.5)	NA
Serum acylcarnitines (μmol/L)		
Acetylcarnitine	5.93 (4.8–7.5)	NA
Octanoylcarnitine	0.14 (0.1–0.2)	NA
Propionylcarnitine	0.43 (0.34–0.54)	NA
Extent of CAD, (%)		
No significant stenosis	25.1	NA
1-vessel disease	23.3	NA
2-vessel disease	22.3	NA
3-vessel disease	29.3	NA
Medications at discharge (%)		
Aspirin	81.6	89.0
Statins	80.2	82.1
β-Blocker	72.5	91.5
Future AMI		
Follow-up time (year)	7.5 (6.3–8.7)	3.1 (2.3–3.5)
Events, (%)	12.8	18.0
Incidence rate/1000 person-years	17.8	67.1
Total death		
Follow-up time (year)	10.3 (9.2–11.6)	6.9 (6.0–7.9)
Events (%)	21.8	19.9
Incidence rate/1000 person-years	22.1	30.8

(*Continued*)

Table 1. *(Continued)*

	WECAC $n = 4137$	NORVIT $n = 3525$
CVD death		
Follow-up time (year)	10.3 (9.2–11.6)	6.9 (6.0–7.9)
Events (%)	9.9	12.0
Incidence rate/1000 person-years	10.1	18.6

Note: Continuous variables are presented as medians (25th–75th percentile), and categorical variables are reported as %. Abbreviations: AMI, acute myocardial infarction; apoA1, apolipoprotein A1; apoB, apolipoprotein B; BMI, body mass index; CAD, coronary artery disease; CVD, cardiovascular disease; eGFR, estimated glomerular filtration rate; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; LVEF, left ventricular ejection fraction; MMA, methylmalonic acid; NORVIT, Norwegian Vitamin Trial; PLP, pyridoxal-5′-phosphate; WECAC, Western Norway Coronary Angiography Cohort.

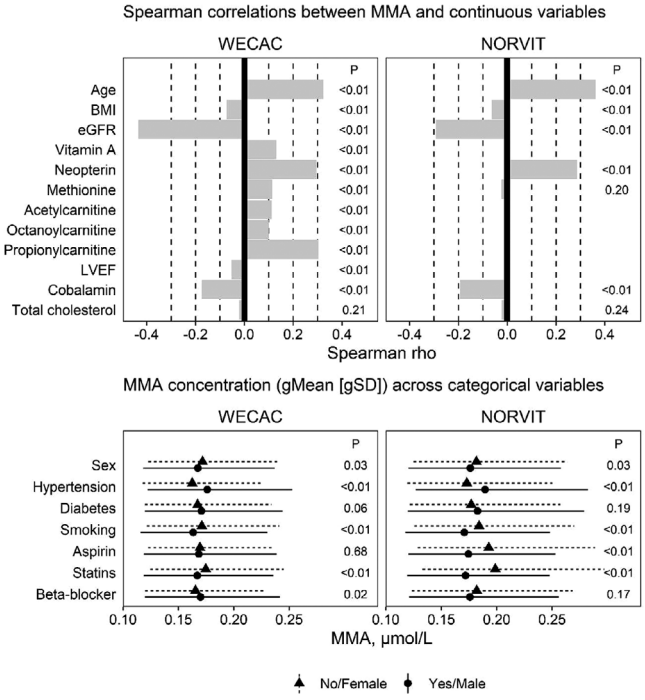

Fig. 2 *Association between plasma methylmalonic acid and selected baseline variables in two Norwegian clinical populations. BMI, body mass index; eGFR, estimated glomerular filtration rate; LVEF, left ventricular ejection fraction; NORVIT, Norwegian Vitamin Trial; Vit-A, vitamin A measured as retinol; WECAC, Western Norway Coronary Angiography Cohort.*

relationship between MMA and outcomes (data not shown), nor was cobalamin related to any outcome per se (Table S1).

Subgroup analyses

Across both cohorts, plasma MMA showed generally stronger risk associations in patients with higher age (Fig. 4), in women and in nonsmokers (Fig. S1). Further, MMA-risk associations were more pronounced in those who had higher serum Vit-A levels (available in WECAC only) (Fig. 4).

In either cohort, no effect modifications were seen according to WENBIT or NORVIT study intervention (Table S2) or serum cobalamin levels (Fig. 4)

Sensitivity analyses and model discrimination, and reclassification

In WECAC, an application of sensitivity analysis to the multivariable model provided HRs (lower CIs) of 1.45 (1.27), 1.56 (1.42), and 1.59 (1.40) for AMI, total, and CVD mortality, respectively. Corresponding *E*-values were 1.49 (1.32), 1.51 (1.36), and 1.64 (1.45) in NORVIT (Table S3).

Table 2. *The association between plasma methylmalonic acid and acute myocardial infarction (AMI) events and mortality in two Norwegian clinical populations*

	Unadjusted		Model 1[a]		Model 2[b]		Model 3[c]	
	HR (95% CI)	*p*-Value	HR (95% CI)	*p*-Value	HR (95% CI)	*p*-Value	HR (95% CI)	*p*-Value
WECAC								
AMI	1.29 (1.19–1.39)	<0.001	1.18 (1.09–1.28)	<0.001	1.16 (1.07–1.26)	<0.001	1.12 (1.03–1.23)	0.012
Total death	1.49 (1.41–1.58)	<0.001	1.25 (1.18–1.33)	<0.001	1.22 (1.14–1.29)	<0.001	1.15 (1.08–1.23)	<0.001
CVD death	1.55 (1.43–1.68)	<0.001	1.28 (1.17–1.40)	<0.001	1.24 (1.13–1.35)	<0.001	1.17 (1.06–1.29)	0.002
Non-CVD death	1.45 (1.34–1.56)	<0.001	1.22 (1.13–1.33)	<0.001	1.20 (1.10–1.30)	<0.001	1.14 (1.04–1.25)	0.006
NORVIT								
AMI	1.35 (1.26–1.45)	<0.001	1.19 (1.10–1.28)	<0.001	1.18 (1.09–1.27)	<0.001	1.17 (1.08–1.26)	<0.001
Total death	1.58 (1.49–1.69)	<0.001	1.22 (1.14–1.31)	<0.001	1.19 (1.11–1.27)	<0.001	1.16 (1.08–1.25)	<0.001
CVD death	1.68 (1.55–1.82)	<0.001	1.30 (1.19–1.42)	<0.001	1.27 (1.16–1.38)	<0.001	1.22 (1.11–1.34)	<0.001
Non-CVD death	1.43 (1.29–1.59)	<0.001	1.10 (0.98–1.23)	0.119	1.07 (0.95–1.20)	0.271	1.07 (0.95–1.20)	0.283

Note: All values are HRs per 1-SD increment of the log-transformed methylmalonic acid.
Abbreviations: CI, confidence interval; CVD, cardiovascular disease; HR, hazard ratio; NORVIT, Norwegian Vitamin Trial; SD, standard deviation; WECAC, Western Norway Coronary Angiography Cohort.
[a]Adjusted for age and sex.
[b]Adjusted for age, sex, body mass index, hypertension, diabetes, smoking, total cholesterol.
[c]Model 2 additionally adjusted for eGFR.

Across both cohorts, adding plasma MMA to multivariable model 2 provided significant improvement in patient IDI statistics for predicting 3-year AMI, 5-year total and CVD mortality, and in ROC-AUC for 5-year CVD mortality. These estimates remained statistically significant only in NORVIT after including eGFR to the model 2. When including MMA to multivariate models, we did not observe reproducible improvements in reclassification across the cohorts (Table S4).

Discussion

In two large prospective cohorts of patients with suspected or verified CHD, elevated plasma MMA predicted increased long-term risk of AMI, total, and CVD mortality. The risk associations were independent of traditional cardiovascular risk factors, including indicators of renal function, as well as serum cobalamin. Furthermore, the risk associations were most pronounced in older adults, women, and nonsmokers, as well as among patients with higher Vit-A concentrations.

Strengths and limitations

The strengths of the study include its large sample size, detailed baseline clinical characteristics, rigorous endpoint evaluation, and external validation, facilitated by accurate MMA quantification using the GC–MS/MS method. MMA has been reported to have excellent within-person reproducibility [19], which allows the assessment of MMA status by a single measurement and reduces the risk of regression–dilution bias [29]. In addition, the estimated *E*-values in the current study indicate that the observed associations are less likely to be explained by unmeasured confounders [27].

Our study also has several limitations. First, the majority of patients received B-vitamin treatment, which may limit the generalizability of our findings. However, within-trial study treatment with B-vitamins did not affect the risk estimates, nor did we find any interactions by such treatment allocation on the MMA-related risk associations, although a lack of conclusive results may also relate to the loss of power due to small subgroups. Second, we mainly studied Caucasian elderly subjects, of which the majority were males with either suspected or verified CHD. Hence, our results may not necessarily extrapolate to populations with other demographic characteristics. Third, we did not evaluate the polymorphism in 3-hydroxybutyryl-CoA hydrolase, which catalyzes the cobalamin-independent degradation of valine and represents an independent determinant of

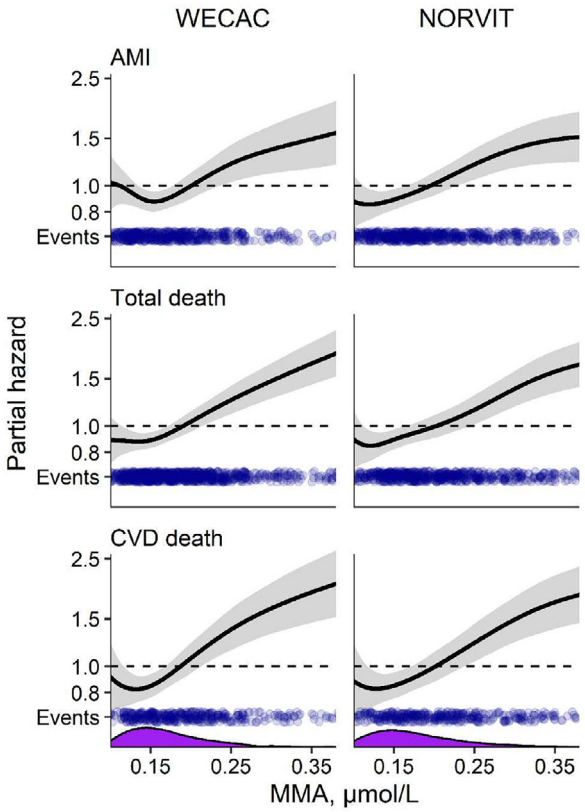

Fig. 3 *Age- and sex-adjusted risk relationships between plasma methylmalonic acid and acute myocardial infarction and mortality in two Norwegian clinical populations. The shaded areas around the splines depict the 95% confidence interval. A Kernel density plot is superimposed along the x-axis. AMI, acute myocardial infarction; CVD, cardiovascular disease; MMA, methylmalonic acid; NORVIT, Norwegian Vitamin Trial; WECAC, the Western Norway Coronary Angiography Cohort.*

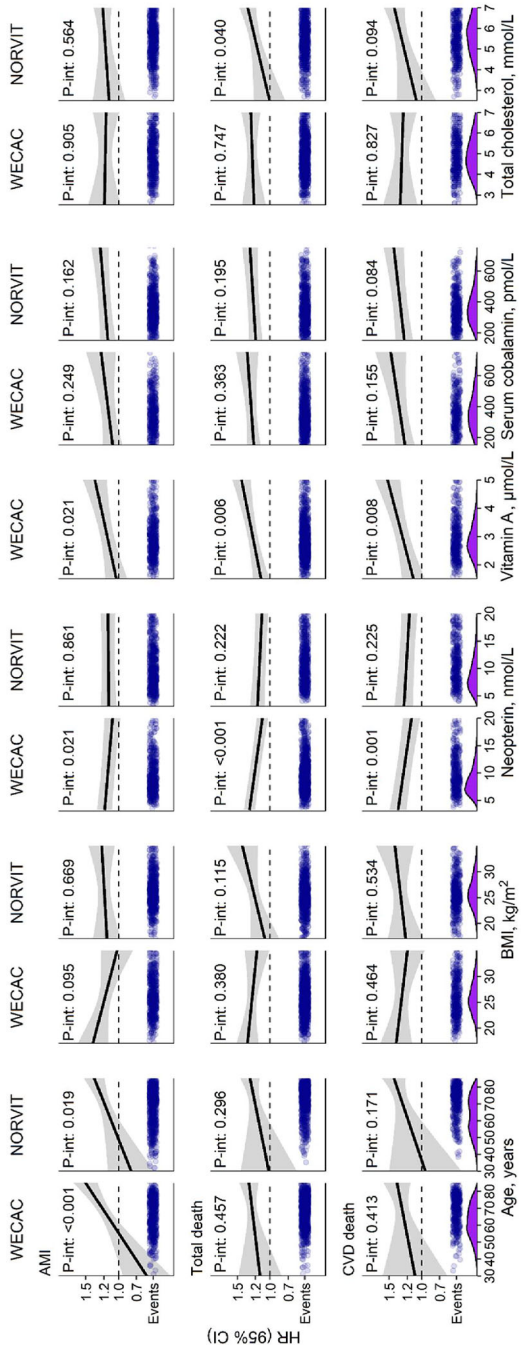

Fig. 4 *Age- and sex-adjusted association between plasma methylmalonic acid (log transformed) per SD and acute myocardial infarction and mortality according to continuous variables. The shaded areas around the splines depict the 95% confidence interval. A Kernel density plot is superimposed along the x-axis. AMI, acute myocardial infarction; BMI, body mass index; CI, confidence interval; HR, hazard ratio; NORVIT, Norwegian Vitamin Trial; WECAC, Western Norway Coronary Angiography Cohort.*

MMA, accounting for 9.9% of the variation in this trait [30]. Fourth, due to the lack of data on succinyl-CoA and L-methylmalonyl-CoA, we could not evaluate the influence of these substances on MMA-associated risk. Fifth, plasma MMA improved the IDI statistics for all endpoints predominantly in the NORVIT population (patients surviving AMI). Further, no reproducible improvements in the patient reclassification were observed across the cohorts. These results suggest a limited prognostic value of MMA beyond traditional risk factors but may indicate a potential role of MMA to improve long-term risk prediction, particularly in patients with acute coronary syndrome. Finally, the obser-

vational nature of this study does not allow for causal inferences.

Plasma MMA and CVD in previous studies

Existing data regarding MMA and CVD risk are sparse. Higher plasma MMA concentrations have been observed in patients with CVD compared to healthy controls [12]. Recent studies in general population reported an association between MMA and mortality risk [7, 14], whereas a study among patients with AMI observed no association between MMA and CHD risk [15]. To our knowledge, the present study is the first large-scale prospective investigation of the long-term relation between plasma MMA and clinical cardiovascular outcomes and mortality among patients with either suspected or established CHD.

Possible mechanisms

Plasma MMA and mitochondrial dysfunction. Mitochondrial dysfunction, characterized by reduced electron transport chain activity and ATP generation, likely plays an important role in the initiation and development of atherosclerosis [8]. In this context, a previous study demonstrated that MMA potentially inhibits the mitochondrial use of lactate through the inhibition of lactate dehydrogenase–catalyzed lactate/pyruvate conversion, suggesting an impairment of energy metabolism [31]. In-line with these observations, both in vitro and in vivo studies have shown that MMA may alter mitochondrial energy metabolism [5, 6, 32]. It is, therefore, interesting that in the WECAC population, plasma MMA demonstrated positive associations with several acylcarnitines, which may serve as indicators of mitochondrial dysfunction [16, 33]. However, and importantly, PPARα activation, which influences the handling of energy from lipids, amino acids, and glucose [9], was shown to cause an increase in systemic MMA [10]. Thus, elevated circulating MMA possibly represents a protective feedback response to regulate energy balance.

Plasma MMA, age, sex, vitamin A, and smoking status. In our study, we observed that plasma MMA was more strongly related to outcomes in older adults, women subjects, nonsmokers, and those with higher circulating Vit-A levels. A previous study reported an increase in MMA with aging, due to age-related deterioration of renal function and vitamin B12 status [34]. Thus, increases in both MMA and age may have interactive effects, which may contribute to adverse cardiovascular outcomes. The biological mechanisms behind the sex, Vit-A, and smoking interaction are unclear. However, PPARα expression is reported to be lower in females than in males [35]. Because MMA is upregulated with PPARα activation [10], the sex-specific observation in our study can simply be because women have much lower risk than men; the reference value of risk is lower, making the chance for an increase in risk much higher. Of note, PPARα activation also increases hepatic levels of cholesterol metabolites, namely, oxysterols [25], which in turn are shown to stimulate the production of all-trans RA, an active form of Vit-A [26]. Therefore, given the assumption that increased Vit-A reflects increased intracellular RA synthesis, concurrent with higher MMA may reflect PPARα activation, suggests an interplay between cholesterol, MMA, and Vit-A metabolism. Unfortunately, we were not able to quantify circulating concentrations of RA. Smoking, on the other hand, is suggested to have no influence on circulating MMA [36]. However, we observed higher plasma MMA concentrations as well as MMA-related adverse risk profiles in nonsmokers. To our knowledge, the current study is the first to report that nonsmokers may be more susceptible to cardiovascular impairments as a result of elevated MMA. Further studies are nevertheless needed to confirm our findings and explore possible mechanisms.

Plasma MMA, B12 status, and renal function. Elevated MMA may indicate impaired vitamin B12 status [3, 4], which could explain an inverse correlation between MMA and serum cobalamin at baseline across both cohorts. However, plasma vitamin B12 status, together with age, sex, and renal function, has been reported to account for only less than 17% of the overall variation in MMA concentration [4]. Moreover, a prior study found no significant association between plasma vitamin B12 and CHD risk [12]. Together with our results, this suggests that the risk relationship between MMA and outcome is not explained by vitamin B12 status. Alternatively, high plasma MMA could reflect renal dysfunction [11, 13, 34], which is considered an independent risk factor for CVD [37]. In our study, plasma MMA levels were strongly associated with lower eGFR in both cohorts at baseline; however, adjusting for eGFR slightly attenuated the risk relationships. Further, neither cohort included a majority of patients with severely impaired kidney function; thus, we cannot exclude an influence of renal function on the observed results, although it

is unlikely that the current findings are explained by kidney function alone.

Plasma MMA and non-CVD mortality. Another important finding was the strong positive association between plasma MMA and deaths attributed to non-CVD causes in the WECAC population. We also observed a trend toward increased non-CVD mortality risk in NORVIT, albeit not statistically significant; however, the lack of significant association could be related to a much higher proportion of patients dying from CVD causes. Importantly, a recent study showed that the age-induced accumulation of MMA may favor tumor progression and aggressiveness [38]. Others have linked higher systemic MMA to cognitive decline [39] and osteoporosis [40]. These observations may explain some of the conferring effects of MMA that increase the risk of non-CVD deaths. Nevertheless, more studies are required to investigate MMA in a wider perspective beyond CVDs.

Conclusions

In conclusion, this study of two large, independent cohorts of patients with either suspected or established CHD suggests that elevated plasma MMA is associated with an increased risk of AMI and mortality. The adverse long-term risk among patients with higher MMA might mirror alterations in mitochondrial function and energy homeostasis. The current findings motivate further studies to elucidate possible pro-atherogenic mechanisms related to disturbances of the one-carbon, energy, and lipid metabolisms.

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Conflict of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Disclaimer

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