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Elevated plasma dimethylglycine is a risk marker of mortality in patients with coronary heart disease

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Abstract

Aim: To investigate whether plasma dimethylglycine was associated with and improved risk prediction of mortality among patients with coronary heart disease (CHD).

Methods: By Cox modelling, we explored the association between plasma dimethylglycine and mortality in two independent cohorts of patients with suspected stable angina pectoris (SAP) ($n = 4156$) and acute myocardial infarction (AMI) ($n = 3733$). We also assessed any improvement in risk prediction by adding plasma dimethylglycine to established CHD risk factors.

Results: Median follow-up time was 4.7 and 7.0 years among patients with SAP and AMI, respectively. Across both cohorts, elevated plasma dimethylglycine levels were linearly associated with increased risk of all-cause mortality (age and gender adjusted hazard ratios (95% confidence interval, CI) were 1.72 (1.21–2.46) and 1.76 (1.42–2.18) when comparing the fourth versus the first plasma dimethylglycine quartile in patients with SAP and AMI, respectively). There was a particularly strong risk association between plasma dimethylglycine and cardiovascular, as compared with non-cardiovascular, mortality (age and gender adjusted hazard ratios (95% CI) 1.94 (1.21–3.11) and 1.43 (0.83–2.47) among patients with SAP and 1.97 (1.50–2.59) and 1.44 (1.02–2.04) among patients with AMI, respectively). The relationship between dimethylglycine and all-cause and cardiovascular mortality was only slightly attenuated in analyses adjusted for established CHD risk factors. Plasma dimethylglycine also improved risk prediction for all-cause and cardiovascular mortality, and especially among patients with AMI.

Conclusions: Elevated plasma dimethylglycine was associated with and improved risk prediction of mortality in patients with suspected or verified CHD. This relationship was stronger for death from cardiovascular, as compared with non-cardiovascular, causes.

Keywords

Angina pectoris, acute myocardial infarction, coronary heart disease, dimethylglycine, mortality

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Background

The one-carbon metabolite dimethylglycine (DMG) is a component of the choline oxidation pathway, and is formed from betaine during the remethylation of homocysteine to methionine, catalysed by the enzyme betaine-homocysteine methyl transferase (BHMT). BHMT induction is associated with increased hepatic production of apolipoprotein (apo) B,¹ and BHMT activity also determines hepatic levels of the ubiquitous methyl donor *S*-adenosylmethionine (SAM).² Both apo B and SAM are crucial components for the synthesis and assembly of very low density lipoproteins,³ the remnants of which are causally related to atherosclerosis. Further, the one-carbon metabolism and the availability of SAM may affect epigenetic regulation,⁴ which has been associated with cardiovascular disease (CVD),⁵ a major cause of death in most European countries.⁶

Elevated plasma DMG levels are related to increased risk of acute myocardial infarction (AMI) in patients with either suspected or established coronary heart disease (CHD).^{7,8} The relationship between circulating DMG and mortality risk has not been studied in larger cohorts or over longer periods of time, although a small study with short follow-up time suggested a link between plasma DMG and all-cause mortality.⁸

We investigated whether plasma DMG was associated with and improved risk prediction of all-cause, cardiovascular and non-cardiovascular mortality in 7889 participants of two large, independent cohorts of patients with suspected stable angina pectoris (SAP) or AMI, of whom the majority were enrolled in two, large B-vitamin intervention trials. The results are presented according to the STrengthening the Reporting of OBServational studies in Epidemiology: Molecular Epidemiology (STROBE-ME) guidelines.⁹

Materials and methods

Study population

The current study population consisted of patients from two separate cohorts: one included 4164 patients, undergoing elective coronary angiography due to suspected SAP during the time period from 2000 to 2004, and has been characterized elsewhere.⁷ Of these patients, 2568 (61.8%) were enrolled in the Western Norway B-vitamin Intervention Trial (WENBIT),¹⁰ a secondary prevention study with B-vitamins among patients with CHD. The other cohort included 3749 participants hospitalized with an AMI and enrolled in the Norwegian Vitamin Trial (NORVIT). This was a multicentre study recruiting patients between 1998 and 2002, and had identical study treatment design to the WENBIT.

Patients with missing data on plasma DMG at baseline were excluded, leaving 4156 and 3733 patients with

SAP and AMI for the final analyses, respectively (Supplemental Figure 1 online).

All patients provided written, informed consent. The study was approved by the regional ethics committee and was carried out according to the Declaration of Helsinki.

Baseline data and biochemical analyses

Earlier reports have described the collection of baseline data and biochemical analyses, including the routine laboratory measurements carried out at each participating hospital.^{7,11} Among patients with SAP, processed blood samples were immediately stored, whereas such samples among NORVIT participants were sent by mail within 48 hours to the Bevital AS core laboratory, Bergen, Norway (www.bevital.no), and frozen at -80°C until undergoing study-specific analyses as previously described. We obtained data on serum cardiac troponin T concentrations from 4070 patients with SAP, using a high sensitive cardiac troponin T (hs-cTnT) assay on Modular E170 from Roche Diagnostics, with a lower detection limit of 3 ng/l. Among 888 (21.4%) fasting, non-diabetic patients with SAP we also calculated the computer based homeostatic model assessment (HOMA2)¹² of insulin resistance (IR), β -cell function and insulin sensitivity, based upon serum insulin, which was measured together with serum C-peptide in citrate-based samples by a solid phase, two-site chemiluminescent immunometric assay (Immulite 2000) from Siemens Healthcare Diagnostics.

Follow-up and clinical endpoints

Study participants were followed from enrolment and until death or until 1 January 2008, as part of the extended follow-up of the combined WENBIT and NORVIT cohort (www.clinicaltrials.gov; NCT00671346), with the addition of patients with SAP who were not enrolled in the WENBIT (Regional Ethics Approval number 2010/1880). We obtained information on fatal events from the Cause of Death Registry at Statistics Norway (<http://www.ssb.no/en>). The primary endpoint was all-cause mortality, whereas deaths from cardiovascular or non-cardiovascular causes were regarded as secondary outcomes. Similar to what was previously described for the WENBIT participants,¹⁰ patients enrolled in the NORVIT were scheduled for follow-up visits including blood sampling two months after randomization and at the end of the study intervention period.¹¹

Statistical analyses

We used the computer software packages PASW Statistics 21 (SPSS IBM, NY, USA) and R version

3.0.0 (The R Foundation for Statistical Computing, Vienna, Austria) for statistical analyses.

Continuous variables are reported as median (5th–95th percentile) and categorical variables as counts (%). Patient baseline characteristics across plasma DMG quartiles were assessed by binary and ordinal logistic regression for nominal and ordinal data, respectively, whereas median linear regression¹³ was applied for continuous data.

We used Cox regression models to obtain hazard ratios (HRs) of all endpoints, according to quartiles of plasma DMG and as per one standard deviation (SD) increment of logarithmically transformed plasma DMG. The multivariate Cox model included the continuous variables age, and serum total cholesterol, and the categorical variables sex, current smoking, diabetes, and hypertension. For the primary survival analyses, missing data were accounted for by using multiple imputation among two patients with SAP and 170 patients with AMI. The imputation models included the same covariates as the multivariate Cox model. To test the assumption of proportional hazards, we inspected survival plots and calculated scaled Schoenfeld residuals. When analysing secondary endpoints, we also estimated subdistribution hazards of competing events.

Survival analyses were performed also according to subgroups of established CHD risk factors in both cohorts, and according to study treatment with either folic acid or vitamin B6 among WENBIT and NORVIT participants. We previously reported the association between plasma DMG and AMI to be stronger among patients with SAP having low levels of serum triglycerides or apo B100.⁷ Hence, we searched for similar effect modifications regarding mortality in patients with SAP, among whom we also stratified analyses according to fasting status and the number of coronary arteries with significant stenosis at angiography. Among patients with AMI, we did not have information on either of these latter variables.

We used case-control analyses as described by Clarke et al.¹⁴ to test whether the BHMT 742 G > A single nucleotide polymorphism (SNP) (rs3733890) was related to all endpoints among 2427 patients with SAP and 3433 patients with AMI, due to its established relationship with plasma DMG levels.^{7,15}

We explored model discrimination by calculating the C statistic according to the multivariate Cox models with and without DMG. Any added improvement in reclassification by DMG was investigated by calculating the continuous net reclassification improvement (NRI > 0) and the integrated discrimination improvement (IDI),¹⁶ using logistic regression models containing the same variables as the Cox models. For the

reclassification analyses, follow-up was censored beyond 1000 days among patients with SAP and 2000 days among those with AMI, roughly corresponding to the minimum follow-up time in either cohort.

Similar to what was previously studied among patients with SAP,⁷ we investigated temporal changes in plasma DMG concentrations throughout follow-up among patients with AMI, according to study treatment allocation. Among those receiving B-vitamin placebo, we applied linear mixed modelling to assess the coefficient of reliability (CoR) for plasma DMG, to explore its within-subject reproducibility.

The two-sided significance level was set to 0.05 in all statistical models.

Results

Baseline characteristics of the study populations

Supplemental Tables 1 and 2 show the baseline characteristics of the two cohorts. Among patients with AMI, the median (5th–95th percentile) timespan from the index AMI to study specific blood sampling was 4 (2–7) d. Median plasma DMG was slightly higher among patients with SAP than among those with AMI ($p=0.02$). As depicted in Supplemental Figure 2, there was a positive association between the timespan from the index AMI to study specific blood sampling (median (5th–95th percentile) 4 (2–7) d) and plasma DMG concentrations ($\beta=0.10$ in a linear median regression model adjusted for age and gender; $p<0.001$). As compared with the patients with SAP, those with AMI were older and had lower estimated glomerular filtration rate (eGFR), but less often pre-existing CVD, hypertension (all $p<0.001$) or diabetes ($p=0.003$).

In both cohorts, patients with higher plasma DMG levels were more likely to be current smokers, older and men with pre-existing CVD, and using β -blockers, angiotensin converting enzyme inhibitors (ACEIs) and/or angiotensin receptor blockers (ARBs) than those with lower plasma DMG levels. Patients with higher DMG also had higher serum creatinine and lower eGFR levels, and we observed an inverse relationship between DMG and serum total cholesterol. Among patients with SAP there was a strong association between plasma DMG and serum hs-cTnT, whereas left ventricular ejection fraction (LVEF) levels were not statistically significantly related to DMG levels once adjusted for age and gender. DMG was inversely related to insulin sensitivity, as shown by a positive association with serum C-peptide and HOMA2 β -cell function and IR, among 888 fasting, non-diabetic patients with SAP.

Plasma DMG and mortality risk

The median (5th–95th percentile) follow-up time was 4.7 (2.8–6.8) and 7.0 (1.1–8.8) years, constituting 19,556 and 24,578 patient-years among patients with SAP and AMI, respectively. Among patients with SAP, 308 (7.4%) died (mortality rate 15.7/1000 patient-years), of whom 168 from cardiovascular causes. Among patients with AMI 772 (20.7%) patients died (mortality rate 31.4/1000 patient-years), and 470 deaths had CVD as underlying death cause. Kaplan–Meier curves (Supplemental Figure 3) show an increased risk of all-cause mortality across plasma DMG quartiles in both populations. There were linear relationships between plasma DMG concentrations and all-cause mortality in both cohorts (Figure 1). In age and gender adjusted Cox models the HRs (95% confidence interval (CI)) were 1.72 (1.21–2.46; $p=0.003$) and 1.76 (1.42–2.18; $p<0.001$) for patients with SAP and AMI, respectively, when comparing the fourth to the first quartile of plasma DMG. These estimates were only slightly attenuated in the multivariate models (Table 1) and consistent also when excluding patients who originally had missing data (Supplemental Tables 3 and 4). The associations were similar across subgroups (Supplemental Figure 4), and no statistically significant interactions were seen according to WENBIT and NORVIT study treatment allocation. However, a trend towards a stronger relationship between DMG and mortality was observed among patients with lower serum apo B100 and triglyceride levels (Supplemental Table 5).

The age and gender adjusted HRs (95% CI) for cardiovascular mortality were 1.94 (1.21–3.11); $p=0.006$ and 1.97 (1.50–2.59); $p<0.001$ for the fourth versus the first quartiles of plasma DMG among patients with SAP and AMI, respectively. Again, there was a tendency towards a stronger relationship with plasma DMG among patients with serum apo B100 or triglycerides below the median value (Supplemental Table 6). For non-cardiovascular death, the age and gender adjusted HRs (95% CI) were 1.43 (0.83–2.47; $p=0.20$) and 1.44 (1.02–2.04; $p=0.04$) for the fourth versus the first quartiles of plasma DMG among the SAP and AMI patients, respectively. When performing competing risk analyses according to non-cardiovascular and cardiovascular death, we found similar estimates as in the original analyses (Supplemental Table 7).

Adjusting for fasting status, body mass index, baseline coronary revascularization, medications at discharge (including statins, aspirin, β -blockers and ACEIs and/or ARBs), HOMA2-IR or the time span from the index AMI to blood sampling did not materially influence the association between plasma DMG and either endpoint (data not shown).

Homocysteine and betaine are the immediate metabolic precursors of methionine and DMG, and elevated plasma tHcy has been associated with increased risk of mortality among patients with CHD.¹⁷ Elevated DMG levels have been found among patients with chronic renal disease,¹⁸ hence we also included plasma tHcy and betaine, eGFR and serum creatinine separately from the multivariate Cox models. Among patients with SAP, including plasma tHcy or serum creatinine slightly weakened the relationship between DMG and the endpoints, whereas adjusting for eGFR attenuated the associations to a greater extent. Adjusting for serum hs-cTnT or plasma betaine left the risk estimates essentially unaltered (Supplemental Table 8). Among patients with AMI, including either plasma tHcy and betaine, serum creatinine or eGFR did not influence the risk estimates (Supplemental Table 9).

When including LVEF in the multivariate model among the patients with SAP, the HRs (95% CIs) between DMG and all-cause and CVD death were 1.49 (1.04–2.13); $p=0.03$ and 1.56 (0.97–2.52); $p=0.07$, respectively, comparing the fourth versus the first quartiles. Among patients with AMI we did not have information on left ventricular systolic function or several other important parameters associated with a high risk of short-term mortality,¹⁹ although the NORVIT did not include patients with short life expectancy.¹¹ Thus, we attempted to correct the relationship between plasma DMG and adverse prognosis for such potential confounders by excluding all AMI patients with less than one year of follow-up ($n=184$ (4.9%)), yielding similar results as the original analyses (data not shown).

The BHMT 742 G > A SNP and its relationship with plasma DMG and endpoints

For patients with SAP, we observed no departure from Hardy–Weinberg equilibrium regarding the BHMT 742 G > A SNP ($p=0.09$), whereas among patients with AMI, such a deviation was present ($p=0.001$). Those with higher DMG levels more often carried the G allele in both cohorts. However, we did not find any relationship between the BHMT 742 G > A genotypes and either outcome in case–control analyses (Supplemental Tables 10–15).

Discrimination and risk reclassification

For both cohorts, adding DMG to the multivariate Cox models made modest increments in the C statistics for all-cause and cardiovascular mortality, but not for non-cardiovascular mortality (Supplemental Table 16). However, the improvement was statistically significant only among patients with AMI. Accordingly, when

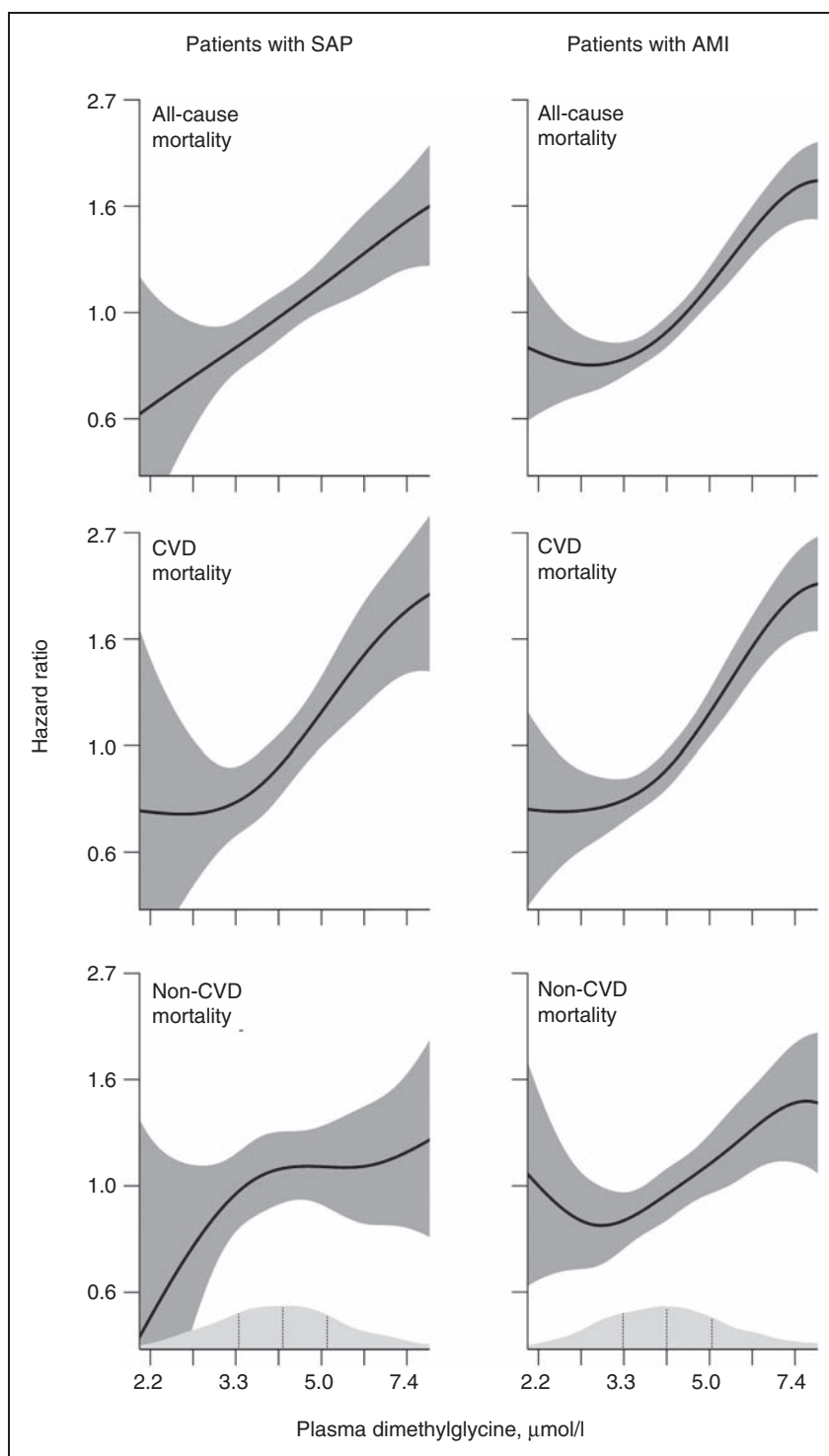


Figure 1. The age and gender adjusted association between plasma dimethylglycine and all-cause, cardiovascular and non-cardiovascular mortality among patients with suspected stable angina pectoris and acute myocardial infarction, respectively. The shaded areas surrounding the solid splines depict 95% confidence intervals. Kernel density plots are superimposed along the X-axis of the lower panels. The vertical, dotted lines depict the 25th, 50th and 75th percentiles, respectively. AMI: acute myocardial infarction; CVD: cardiovascular disease; SAP: stable angina pectoris

Table 1. The association between plasma dimethylglycine and all-cause, cardiovascular and non-cardiovascular death

	Univariate		Adjusted for age and sex		Multivariate model ^a	
	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value
All-cause death						
Patients with SAP						
Fourth vs. first quartile	2.52 (1.78–3.57)	<0.001	1.72 (1.21–2.46)	0.003	1.63 (1.14–2.33)	0.007
Per 1 SD ^b	1.30 (1.21–1.41)	<0.001	1.19 (1.09–1.31)	<0.001	1.15 (1.05–1.26)	0.003
Patients with AMI						
Fourth vs. first quartile	2.80 (2.27–3.44)	<0.001	1.76 (1.42–2.18)	<0.001	1.68 (1.36–2.09)	<0.001
Per 1 SD ^b	1.49 (1.40–1.58)	<0.001	1.27(1.19–1.35)	<0.001	1.25 (1.17–1.33)	<0.001
Cardiovascular death						
Patients with SAP						
Fourth vs. first quartile	2.96 (1.87–4.70)	<0.001	1.94 (1.21–3.11)	0.006	1.82 (1.13–2.92)	0.01
Per 1 SD ^b	1.36 (1.24–1.50)	<0.001	1.25 (1.12–1.40)	<0.001	1.19 (1.06–1.33)	0.002
Patients with AMI						
Fourth vs. first quartile	3.12 (2.39–4.07)	<0.001	1.97 (1.50–2.59)	<0.001	1.89 (1.43–2.48)	<0.001
Per 1 SD ^b	1.58 (1.47–1.71)	<0.001	1.36 (1.25–1.47)	<0.001	1.34 (1.23–1.45)	<0.001
Non-cardiovascular death						
Patients with SAP						
Fourth vs. first quartile	1.99 (1.17–3.40)	0.01	1.43 (0.83–2.47)	0.20	1.37 (0.79–2.37)	0.26
Per 1 SD ^b	1.21 (1.06–1.39)	0.005	1.11 (0.95–1.29)	0.20	1.08 (0.93–1.26)	0.33
Patients with AMI						
Fourth vs. first quartile	2.31 (1.65–3.24)	<0.001	1.44 (1.02–2.04)	0.04	1.38 (0.97–1.95)	0.07
Per 1 SD ^b	1.33 (1.20–1.48)	<0.001	1.12 (1.00–1.26)	0.05	1.10 (0.98–1.23)	0.11

^aIncluding age, gender, hypertension, diabetes, current smoking and serum total cholesterol; ^bLog transformed; AMI: acute myocardial infarction; CI: confidence interval; DMG: dimethylglycine; HR: hazard ratio; SAP: stable angina pectoris; SD: standard deviation

adding plasma DMG to traditional CHD risk factors, we obtained a more substantial increase in the continuous NRI (NRI > 0) and IDI for all-cause and CVD mortality among patients with AMI as compared with those with SAP.

Within-subject reproducibility and variability of plasma DMG

Among 935 patients randomized to placebo in the NORVIT, the CoR for plasma DMG was 0.72 (0.66 for log-transformed plasma DMG), based on repeated measurements over median (5th–95th percentile) 7.1 (1.0–8.8) years. This estimate compares to a CoR of 0.93 (0.73 for log-transformed plasma DMG) among the patients with SAP, as reported earlier.⁷

We found an increase in estimated median plasma DMG levels from baseline to the final study visit among patients with AMI allocated to placebo or vitamin B6 therapy. DMG levels did not, however, differ over time among the patients receiving folic acid (Supplemental Table 17). Similar observations have previously been made among the patients with SAP.⁷

Discussion

Principal findings

In the current study plasma DMG was associated with long-term mortality, and in particular cardiovascular death, in two independent populations, totaling almost 8000 patients with SAP or AMI. Accordingly, risk prediction was improved by adding plasma DMG to established CHD risk factors, especially among patients with AMI. The high within-subject reproducibility of plasma DMG previously reported among patients with SAP⁷ was validated throughout repeated measurements in patients with AMI.

Baseline characteristics and their relationship to plasma DMG levels

Median plasma DMG levels were marginally higher among patients with SAP than among those with AMI. However, the average plasma DMG concentration increased slightly with time from the index AMI to

baseline study blood sampling among AMI patients in the NORVIT. This may be explained by stress-related increments in plasma cortisol,²⁰ decreased insulin concentrations and impaired insulin sensitivity,²¹ as glucocorticoids induce and insulin inhibit BHMT transcription.²² In addition, increased plasma DMG has been observed among patients with renal impairment.¹⁸ Since eGFR was lower among patients with AMI, renal dysfunction, which is common in patients with acute coronary syndrome,²³ may have contributed to the in-hospital time-dependent increase in plasma DMG levels.

An association between plasma DMG and an adverse cardiovascular risk profile was previously reported among the patients with SAP,⁷ and was now validated among patients with AMI. Accordingly, a strong relationship was observed between plasma DMG and serum hs-cTnT levels among patients with SAP.

The inverse association between baseline serum folate and plasma DMG, previously described among fasting patients with SAP,⁷ was confirmed among patients with AMI.

Plasma DMG and the risk of adverse outcomes

Little is known about the association between DMG and health outcomes in general. Plasma DMG predicted AMI among essentially the same patients constituting the current study population with SAP,⁷ as well as among 534 patients with a recent acute coronary syndrome.⁸ Moreover, in the latter population higher levels of DMG and betaine were associated with all-cause mortality throughout approximately two years of follow-up.⁸

In the current study, the relationship between plasma DMG and all-cause mortality was consistent across both cohorts. However, it tended to be more robust among patients with AMI, possibly due to a higher mortality rate. Among patients with SAP, we previously observed a trend towards a stronger relation between plasma DMG and fatal, as compared with non-fatal, AMI during follow-up. In line with this, our present results suggest that DMG is even more strongly associated with cardiovascular than with non-cardiovascular mortality.

Possible mechanisms

A relationship between plasma DMG and an adverse cardiovascular risk profile seems consistent. However, the association with adverse outcomes does not appear to be mediated by traditional CHD risk factors alone, nor is it explained by increased levels of the metabolic precursors betaine and homocysteine.

The kidneys contain large amounts of betaine and, together with the liver, harbour most of the BHMT in the body.³ Low eGFR, indicating impaired renal function, is considered a major risk factor of both all-cause and cardiovascular mortality,²⁴ and increased plasma DMG levels have been observed among patients with uremia.¹⁸ In the current study populations, the eGFR was somewhat lower among patients with AMI as compared with patients with SAP, but indicated adequate renal function in most patients. Still, the risk estimates were attenuated after adjusting for eGFR and serum creatinine among patients with SAP, but not among those with AMI. Notably, the production of creatinine is dependent on glycine,²⁵ which can be methylated into sarcosine via glycine-*N*-methyltransferase (GNMT). Activation of the peroxisome proliferator receptor (PPAR) α in rats reduces the genetic transcription of enzymes involved in DMG catabolism, but also GNMT, reflected by a rise in urinary DMG and plasma glycine levels.²⁶ PPAR α agonists are also well-known for their ability to elevate circulating creatinine concentrations, independent of a decline in renal function.²⁷ We previously showed that the association between plasma DMG levels and risk of AMI was particularly strong among patients with either low serum apo B100 or triglyceride levels, indicating a possible link between plasma DMG and endogenous PPAR α stimulation.⁷ In the present study we found a similar trend according to both all-cause and cardiovascular mortality among the patients with SAP. Thus, endogenous PPAR α stimulation, rather than declined kidney function alone, might explain the association between creatinine and DMG metabolism.

Adjusting for hs-cTnT among the patients with SAP did not influence the association between plasma DMG and the endpoints, although these biomarkers were strongly related at baseline. While ischaemic myocardial cell death mainly has been explained by necrosis, cardiomyocyte apoptosis was suggested to confer the increased risk of cardiovascular mortality related to higher levels of hs-cTnT among patients with stable CHD.²⁸ Apoptosis of cardiomyocytes seems partly dependent on mitochondrial processes,²⁹ and DMG metabolism inside the mitochondrion may influence the production of nucleotides,³⁰ potentially affecting the cells' regenerative abilities. However, DMG did not relate to LVEF among patients with SAP, once adjusted for age and gender, although the risk estimates were somewhat attenuated when adding LVEF to the Cox models. Although data on left ventricular systolic function and cardiac cell death were lacking among patients with AMI, plasma DMG predicted death also beyond one year post discharge from hospital. This suggests that DMG levels do not solely reflect increased cardiomyocyte death and left ventricular systolic function. More

importantly, knowledge of plasma DMG may add prognostic information beyond that obtained by conventional biomarkers of myocardial damage.

The link between BHMT induction and hepatic apo B and SAM production has previously been discussed more thoroughly,⁷ and could partly account for the association between plasma DMG and adverse prognosis, especially regarding cardiovascular events. However, a somewhat paradoxical inverse association between serum total cholesterol levels and plasma DMG was consistent in both cohorts. This is in line with a trend towards an inverse relationship between DMG and serum apo B and low-density lipoprotein (LDL)-cholesterol, but also apo A1 and high-density lipoprotein (HDL)-cholesterol, among patients with SAP, as reported earlier.⁷ However, the use of statins did not differ according to DMG quartiles, and adjusting the multivariate Cox model for statin treatment did not change the risk estimates, suggesting that mechanisms other than those conferred by statin therapy are responsible for the associations observed between plasma DMG and serum lipid levels.

We did not have data on dietary intake of either choline or betaine, which may affect plasma DMG levels due to substrate availability for the BHMT pathway. However, no relationship has been found between estimated choline and betaine intake and CVD risk in the general population.³ Moreover, a recent report suggested that the association between circulating choline and betaine levels and adverse cardiovascular prognosis may be modified by gut microbial dependent elevations in circulating trimethylamine-*N*-oxide (TMAO) levels.³¹ Unfortunately, we did not have information on plasma TMAO levels, thus we were unable to carry out any interaction analyses with DMG. Explaining the risk associations in the current study by increased DMG production alone may fall short also because B-vitamin intervention in neither the WENBIT nor the NORVIT showed any overall effect on outcomes,^{10,11} despite a likely decreased BHMT flux by folic acid supplementation. Moreover, we did not observe any increased mortality risk according to BHMT G742A genotypes. On the other hand, a low minor allele frequency may cause insufficient power to detect any associations with hard clinical outcomes, and the full pleiotropic effects of genes might not be captured by measuring one single metabolite.

Among patients with SAP we also observed a strong relationship between plasma DMG and insulin resistance, potentially attributable to BHMT induction.²² However, despite low statistical power in this subgroup, adjusting for HOMA2-IR did not seem to attenuate the risk estimates, indicating that the association of elevated DMG levels with adverse prognosis is not mediated solely by impaired insulin sensitivity.

Taken together, whether the risk association between DMG and mortality in the current study is explained by mechanisms involved in increased DMG production, impaired DMG catabolism or excretion, or a combination thereof, is still elusive.

Strengths and limitations

The major strength of this prospective cohort study is the large number of well-characterized patients evaluated for CHD in two separate populations. Detection bias of the endpoints is unlikely, because we used each patient's unique 11 digit personal identification number to link with health registries with almost 100% national coverage. Also, the high within-subject reproducibility of plasma DMG was similar among patients with AMI as previously reported among patients with SAP⁷ and in the general population.³²

In the NORVIT, the study centres sent the blood samples by mail to Bevital AS, thus exposing the samples to room temperature for several days. However, plasma DMG is highly stable even when not stored in a cold environment for up to eight days.³³ By using one core laboratory for investigating samples from all the study centres in both cohorts, we have minimized the potential for analytical bias.

Conclusion

This study of two large, independent cohorts of patients with either presumed or verified CHD suggests plasma DMG to be a potential risk marker of all-cause mortality. The association between DMG and adverse prognosis seems to be driven by cardiovascular, rather than non-cardiovascular, events. Furthermore, plasma DMG improves risk prediction beyond traditional CHD risk factors, and we confirmed a high within-subject reproducibility, allowing one time point assessment of biomarker status. Our results motivate further investigation into the relationship between one-carbon and choline metabolism and CHD.

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Conflict of interest

The authors declare that there is no conflict of interest.

References

- Sparks JD, Collins HL, Chirieac DV, et al. Hepatic very-low-density lipoprotein and apolipoprotein B production are increased following in vivo induction of betaine-homocysteine S-methyltransferase. *Biochem J* 2006; 395: 363–371.
- Teng YW, Mehedint MG, Garrow TA, et al. Deletion of betaine-homocysteine S-methyltransferase in mice perturbs choline and 1-carbon metabolism, resulting in fatty liver and hepatocellular carcinomas. *J Biol Chem* 2011; 286: 36258–36267.
- Ueland PM. Choline and betaine in health and disease. *J Inherit Metab Dis* 2011; 34: 3–15.
- Fuso A, Nicolia V, Cavallaro RA, et al. DNA methylase and demethylase activities are modulated by one-carbon metabolism in Alzheimer's disease models. *J Nutr Biochem* 2011; 22: 242–251.
- Duthie SJ. Epigenetic modifications and human pathologies: Cancer and CVD. *Proc Nutr Soc* 2011; 70: 47–56.
- Fifth Joint Task Force of the European Society of Cardiology; European Association of Echocardiography; European Association of Percutaneous Cardiovascular Interventions; European Heart Rhythm Association; Heart Failure Association; European Association for Cardiovascular Prevention & Rehabilitation; European Atherosclerosis Society; International Society of Behavioural Medicine; European Stroke Organisation; European Society of Hypertension; European Association for the Study of Diabetes; European Society of General Practice/Family Medicine; International Diabetes Federation Europe; European Heart Network. European guidelines on cardiovascular disease prevention in clinical practice (version 2012): The Fifth Joint Task Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice (constituted by representatives of nine societies and by invited experts). *Eur J Prev Cardiol* 2012; 19: 585–667.
- Svingen GF, Ueland PM, Pedersen EK, et al. Plasma dimethylglycine and risk of incident acute myocardial infarction in patients with stable angina pectoris. *Arterioscler Thromb Vasc Biol* 2013; 33: 2041–2048.
- Lever M, George PM, Elmslie JL, et al. Betaine and secondary events in an acute coronary syndrome cohort. *PLoS One* 2012; 7: e37883.
- Gallo V, Egger M, McCormack V, et al. Strengthening the Reporting of Observational studies in Epidemiology – Molecular Epidemiology (STROBE-ME): An extension of the STROBE Statement. *PLoS Med* 2011; 8: e1001117.
- Ebbing M, Bleie O, Ueland PM, et al. Mortality and cardiovascular events in patients treated with homocysteine-lowering B vitamins after coronary angiography: A randomized controlled trial. *JAMA* 2008; 300: 795–804.
- Bonaa KH, Njolstad I, Ueland PM, et al. Homocysteine lowering and cardiovascular events after acute myocardial infarction. *N Engl J Med* 2006; 354: 1578–1588.
- Wallace TM, Levy JC and Matthews DR. Use and abuse of HOMA modeling. *Diabetes Care* 2004; 27: 1487–1495.
- Koenker R. *Quantile regression*, 1st ed. New York, USA: Cambridge University Press, 2011, p.366.
- Clarke GM, Anderson CA, Pettersson FH, et al. Basic statistical analysis in genetic case-control studies. *Nat Protoc* 2011; 6: 121–133.
- Fredriksen A, Meyer K, Ueland PM, et al. Large-scale population-based metabolic phenotyping of thirteen genetic polymorphisms related to one-carbon metabolism. *Hum Mutat* 2007; 28: 856–865.
- Pencina MJ, D'Agostino Sr RB and Steyerberg EW. Extensions of net reclassification improvement calculations to measure usefulness of new biomarkers. *Stat Med* 2011; 30: 11–21.
- Nygard O, Nordrehaug JE, Refsum H, et al. Plasma homocysteine levels and mortality in patients with coronary artery disease. *N Engl J Med* 1997; 337: 230–236.
- McGregor DO, Dellow WJ, Lever M, et al. Dimethylglycine accumulates in uremia and predicts elevated plasma homocysteine concentrations. *Kidney Int* 2001; 59: 2267–2272.
- Hamm CW, Bassand JP, Agewall S, et al. ESC Guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation: The Task Force for the management of acute coronary syndromes (ACS) in patients presenting without persistent ST-segment elevation of the European Society of Cardiology (ESC). *Eur Heart J* 2011; 32: 2999–3054.
- Chopra MP, Thadani U, Aber CP, et al. Plasma cortisol, urinary 17-hydroxycorticoids, and urinary vanillyl mandelic acid after acute myocardial infarction. *Br Heart J* 1972; 34: 992–997.
- Shah B, Amoroso NS and Sedlis SP. Hyperglycemia in nondiabetic patients presenting with acute myocardial infarction. *Am J Med Sci* 2012; 343: 321–326.
- Ratnam S, Wijekoon EP, Hall B, et al. Effects of diabetes and insulin on betaine-homocysteine S-methyltransferase expression in rat liver. *Am J Physiol Endocrinol Metab* 2006; 290: E933–E939.
- Marenzi G, Cabiati A, Bertoli SV, et al. Incidence and relevance of acute kidney injury in patients hospitalized with acute coronary syndromes. *Am J Cardiol* 2013; 111: 816–822.
- Chronic Kidney Disease Prognosis Consortium, Matsushita K, van der Velde M, et al. Association of estimated glomerular filtration rate and albuminuria with all-cause and cardiovascular mortality in general population cohorts: A collaborative meta-analysis. *Lancet* 2010; 375: 2073–2081.
- Wyss M and Kaddurah-Daouk R. Creatine and creatinine metabolism. *Physiol Rev* 2000; 80: 1107–213.

26. Sheikh K, Camejo G, Lanne B, et al. Beyond lipids, pharmacological PPARalpha activation has important effects on amino acid metabolism as studied in the rat. *Am J Physiol Endocrinol Metab* 2007; 292: E1157–E1165.
27. Sica DA. Fibrate therapy and renal function. *Curr Atheroscler Rep* 2009; 11: 338–342.
28. Omland T, de Lemos JA, Sabatine MS, et al. A sensitive cardiac troponin T assay in stable coronary artery disease. *N Engl J Med* 2009; 361: 2538–2547.
29. Kubli DA and Gustafsson AB. Mitochondria and mitophagy: The yin and yang of cell death control. *Circ Res* 2012; 111: 1208–1221.
30. Tibbetts AS and Appling DR. Compartmentalization of mammalian folate-mediated one-carbon metabolism. *Annu Rev Nutr* 2010; 30: 57–81.
31. Wang Z, Tang WH, Buffa JA, et al. Prognostic value of choline and betaine depends on intestinal microbiota-generated metabolite trimethylamine-*N*-oxide. *Eur Heart J*. 3 Feb 2014 [Epub ahead of print].
32. Cope EL, Shrubsole MJ, Cohen SS, et al. Intraindividual variation in one-carbon metabolism plasma biomarkers. *Cancer Epidemiol Biomarkers Prev* 2013; 22: 1894–1899.
33. Hustad S, Eussen S, Midttun O, et al. Kinetic modeling of storage effects on biomarkers related to B vitamin status and one-carbon metabolism. *Clin Chem* 2012; 58: 402–410.