Arteriosclerosis, Thrombosis, and Vascular Biology

JOURNAL OF THE AMERICAN HEART ASSOCIATION

American Heart Association®

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Arterioscler Thromb Vasc Biol. 2013;33:2041-2048; originally published online May 30, 2013;
doi: 10.1161/ATVBAHA.113.301714
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Print ISSN: 1079-5642. Online ISSN: 1524-4636

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Objective**—**Dimethylglycine is linked to lipid metabolism, and increased plasma levels may be associated with adverse prognosis in patients with coronary artery disease. We evaluated the relationship between plasma dimethylglycine and risk of incident acute myocardial infarction in a large prospective cohort of patients with stable angina pectoris, of whom approximately two thirds were participants in a B-vitamin intervention trial. Model discrimination and reclassification when adding plasma dimethylglycine to established risk factors were obtained. We also explored temporal changes and the test–retest reliability of plasma dimethylglycine.

Approach and Results**—**Four thousand one hundred fifty patients (72% men; median age 62 years) were included. Plasma dimethylglycine was associated with several traditional coronary artery disease risk factors. During a median follow-up of 4.6 years, 343 (8.3%) patients experienced an acute myocardial infarction. The hazard ratio (95% confidence interval) for acute myocardial infarction was 1.95 (1.42–2.68; P<0.001) when comparing plasma dimethylglycine quartile 4 to 1 in a Cox regression model adjusted for age, sex, and fasting status. Adjusting for traditional coronary artery disease risk factors only slightly modified the estimates, which were particularly strong among nonsmokers and among patients with serum triglyceride or apolipoprotein B100 levels ≤median (P for interaction=0.004, 0.004, and 0.03, respectively). Plasma dimethylglycine improved discrimination and reclassification and had high test–retest reliability.

Conclusions**—**Plasma dimethylglycine is independently related to incident acute myocardial infarction and enhances risk prediction in patients with stable angina pectoris. Our results motivate further studies on the relationship between 1-carbon metabolism and atherothrombosis. A potential interplay with lipid and energy metabolism merits particular attention. **(*Arterioscler Thromb Vasc Biol*. 2013;33:2041-2048.)**

Key Words: angina pectoris ■ biological markers ■ lipids ■ acute myocardial infarction ■ smoking

An increased risk of ischemic heart disease has been observed in patients with elevated blood choline,[1] plasma total homocysteine (tHcy),[2] and betaine[3] levels, although homocysteine-lowering B-vitamin treatment did not reduce risk of future cardiovascular disease (CVD) events in secondary prevention trials.[4] The tertiary amine dimethylglycine (DMG) is produced from betaine during the remethylation of homocysteine to methionine, catalyzed by betaine-homocysteine methyltransferase (BHMT; enzyme commission 2.1.1.5), an enzyme mainly confined to the liver and kidney.[5] DMG is metabolized to sarcosine in the mitochondria,[6] providing 1-carbon units for the formation of 5,10-methylenetetrahydrofolate.[7] A smaller proportion of DMG is excreted unmetabolized in the urine.[6] Blood levels of DMG relate to BHMT activity,[8,9] but the association between circulating DMG and intracellular BHMT activity is complex, and DMG provides negative feedback on BHMT at physiological concentrations.[8] Lipid-lowering therapy with fibrates is associated with elevated tHcy levels[10] and reduced DMG catabolism,[11] thus linking both homocysteine and DMG with peroxisome proliferator-activated receptor α activation.

BHMT induction has been related to enhanced hepatic apolipoprotein B (apoB) transcription and very low-density lipoprotein excretion,[12] and betaine supplementation in humans has been associated with increased levels of low-density lipoprotein cholesterol in serum.[13] The G allele of the single nucleotide polymorphism BHMT 742 G>A (rs3733890) is related to both higher plasma DMG levels[14] and more extensive coronary artery disease (CAD) in the elderly.[15] Notably, in a recent, small study of patients

Received on: January 14, 2013; final version accepted on: May 16, 2013.

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The online-only Data Supplement is available with this article at http://atvb.ahajournals.org/lookup/suppl/doi:10.1161/ATVBAHA.113.301714/-/DC1.

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with established CAD, plasma DMG was associated with increased risk of all-cause death, acute myocardial infarction (AMI), and hospitalization for heart failure.[3] This suggests that the flux through BHMT or other determinants of plasma DMG may be related to the development of atherosclerotic CAD.

We explored the associations between baseline characteristics and plasma DMG and investigated the relationship between plasma DMG and the risk of subsequent AMI in a large cohort of patients undergoing coronary angiography for stable angina pectoris. We also evaluated the improvement in model discrimination and reclassification of patients at risk when adding plasma DMG to a model containing traditional CAD risk factors. In addition, we studied temporal changes and the test–retest variability of plasma DMG. The findings were reported according to the STrengthening the Reporting of OBservational studies in Epidemiology-Molecular Epidemiology (STROBE-ME) statement.[16]

Materials and Methods

Materials and Methods are available in the online-only Supplement.

Results

Characteristics of the Study Population According to Quartiles of Plasma DMG

Baseline characteristics of the study population are given in Table 1. The cohort consisted of 72.0% men, and the median (5th–95th percentile) age was 62 (44–78) years. Thirty-one percent of the participants were current smokers, 11.8% were diagnosed with diabetes mellitus, 46.7% had hypertension, and 40.3% had a history of previous myocardial infarction. Baseline revascularization with either percutaneous coronary intervention or coronary artery bypass grafting was performed in 2177 (52.4%) patients.

Median (5th–95th percentile) plasma DMG was 4.1 (2.6–7.3) µmol/L. Plasma DMG levels were higher in men (4.2 [2.7–7.5] µmol) than in women (3.8 [2.4–6.9] µmol/L; $P<0.001$) and higher in nonfasting (4.3 [2.6–7.4] µmol/L) compared to fasting patients (3.8 [2.4–7.0] µmol/L; $P<0.001$).

There was a positive linear relationship between incremental DMG quartiles and age and C-reactive protein, whereas a negative association was observed with estimated glomerular filtration rate (eGFR). Subjects in higher DMG quartiles more

Table 1. Baseline Characteristics of the Total Study Cohort According to Quartiles of Plasma Dimethylglycine

		Quartiles of Plasma DMG						
	n*	All	First	Second	Third	Fourth	P_{trend}†	P_{trend}§
Plasma DMG, µmol/L	4150	4.1 (2.6–7.3)	2.9 (2.2–3.3)	3.8 (3.4–4.1)	4.6 (4.2–5.0)	6.0 (5.2–10.3)	…	…
Male sex, n (%)	4150	2987 (72.0)	647 (62.3)	752 (72.6)	789 (75.8)	799 (77.3)	<0.001	…
Age, y	4150	62 (44–78)	59 (42–76)	62.0 (45–77)	62.0 (45–78)	65 (45–80)	0.001	…
Current smoking, n (%)	4150	1311 (31.6)	317 (30.5)	285 (27.5)	333 (32.0)	376 (36.4)	0.001	<0.001
Diabetes mellitus, n (%)	4150	491 (11.8)	135 (13.0)	109 (10.5)	115 (11.0)	132 (12.8)	0.97	0.29
BMI, kg/m²	4147	26.3 (21.1–33.7)	26.5 (20.7–33.7)	26.3 (21.3–33.6)	26.4 (21.5–34.3)	26.1 (21.0–33.6)	0.04	0.68
Plasma glucose, mmol/L	4147	5.6 (4.4–11.2)	5.6 (4.3–11.5)	5.6 (4.4–10.5)	5.7 (4.4–11.0)	5.7 (4.4–11.8)	0.004	0.29
Hypertension, n (%)	4150	1939 (46.7)	442 (42.5)	448 (43.2)	497 (47.7)	552 (53.4)	<0.001	<0.001
Extent of CAD, n (%)	4150						<0.001	0.06
No stenotic vessels		1044 (25.2)	321 (30.9)	263 (25.4)	248 (23.8)	212 (20.5)		
1-vessel disease		963 (23.2)	233 (22.4)	252 (24.3)	265 (25.5)	213 (20.6)		
2-vessel disease		925 (22.3)	224 (21.6)	241 (23.3)	228 (21.9)	232 (22.4)		
3-vessel disease		1218 (29.3)	261 (25.1)	280 (27.0)	300 (28.8)	377 (36.5)		
LVEF, %	4150	65 (40–80)	68 (45, 80)	68 (43, 80)	65 (40, 80)	65 (36, 80)	<0.001	1.000
Previous MI, n (%)	4150	1674 (40.3)	349 (33.6)	397 (38.3)	412 (39.6)	516 (49.9)	<0.001	<0.001
Previous CBV, n (%)	4150	288 (6.9)	49 (4.7)	59 (5.7)	67 (6.4)	113 (10.9)	<0.001	<0.001
Previous PAD, n (%)	4150	374 (9.0)	69 (6.6)	64 (6.2)	106 (10.2)	135 (13.1)	<0.001	<0.001
Previous CABG, n (%)	4150	478 (11.5)	110 (10.6)	110 (10.6)	116 (11.1)	142 (13.7)	0.03	1.00
Previous PCI, n (%)	4150	796 (19.2)	194 (18.7)	183 (17.7)	192 (18.4)	227 (22.0)	0.05	0.08
Serum CRP, mg/L	4150	1.8 (0.4–12.6)	1.6 (0.3–10.9)	1.6 (0.3–10.6)	1.8 (0.4–11.5)	2.2 (0.4–19.1)	<0.001	<0.001
eGFR, mL/min per 1.73m²	4150	91 (57–111)	96 (70–115)	92 (63–110)	89 (58–109)	84 (41–109)	<0.001	<0.001
Plasma levels of 1-carbon metabolites								
Choline, µmol/L	4150	9.7 (6.4–14.7)	8.5 (5.7–12.3)	9.5 (6.5–13.7)	10.1 (7.0–14.5)	11.2 (7.5–16.8)	<0.001	<0.001
Betaine, µmol/L	4150	39.1 (23.2–63.7)	32.6 (19.3–51.5)	38.4 (24.0–59.0)	41.5 (26.1–63.5)	44.9 (27.1–72.6)	<0.001	<0.001
tHcy, µmol/L	4150	10.4 (6.7–18.5)	9.6 (6.3–15.8)	10.1 (6.8–15.6)	10.8 (6.9–17.5)	11.7 (7.3–22.7)	<0.001	<0.001
Methionine, µmol/L	4150	26.6 (18.0–42.0)	24.5 (17.4–39.1)	26.2 (18.0–41.1)	27.0 (18.5–41.1)	28.5 (18.7–46.1)	<0.001	<0.001
Sarcosine, µmol/L	1727	6.8 (5.3–8.9)	6.5 (5.1–8.5)	6.7 (5.2–8.3)	6.9 (5.7–8.8)	7.0 (5.6–9.8)	<0.001	<0.001

(*Continued*)

Table 1. Continued

		Quartiles of Plasma DMG						
	n*	All	First	Second	Third	Fourth	P_{trend}†	P_{trend}§
Markers of B-vitamin status								
Plasma riboflavin, nmol/L	4125	11.2 (4.4–48.7)	11.9 (4.6–46.2)	11.3 (4.5–45.8)	11.3 (4.4–47.3)	11.6 (4.2–59.1)	0.02	0.12
Serum folate, nmol/L	4148	10.1 (4.9–35.0)	10.3 (5.0–38.4)	10.3 (5.2–34.1)	10.1 (4.9–31.6)	9.7 (4.6–38.5)	0.10	0.07
Plasma PLP, nmol/L	4125	41.3 (18.7–124.2)	43.0 (19.1–121.5)	41.5 (20.2–108.8)	40.9 (18.3–128.4)	39.7 (17.2–133.7)	0.43	0.17
Serum cobalamin, pmol/L	3658	362 (177–706)	360 (172–689)	364 (182–678)	365 (185–698)	362 (175–800)	0.63	0.36
Plasma MMA, μmol/L	4150	0.16 (0.10–0.32)	0.15 (0.10–0.26)	0.16 (0.10–0.29)	0.17 (0.11–0.32)	0.18 (0.11–0.39)	<0.001	<0.001
Serum lipids and apolipoproteins								
Total cholesterol, mmol/L	4148	4.9 (3.5–7.1)	5.0 (3.6–7.3)	4.9 (3.5–7.2)	4.9 (3.5–7.1)	4.8 (3.3–6.9)	<0.001	0.02
LDL-C, mmol/L	4147	2.9 (1.7–5.0)	3.0 (1.8–5.0)	2.9 (1.7–5.1)	2.9 (1.7–5.0)	2.8 (1.6–4.7)	0.004	0.10
HDL-C, mmol/L	4149	1.2 (0.8–2.0)	1.3 (0.8–2.1)	1.2 (0.8–2.0)	1.2 (0.8–2.0)	1.2 (0.8–1.9)	<0.001	<0.001
Triglycerides, mmol/L	4146	1.5 (0.7–3.7)	1.5 (0.7–4.0)	1.5 (0.7–3.7)	1.5 (0.7–3.5)	1.5 (0.7–3.6)	0.70	0.66
ApoB100, g/L	4150	0.87 (0.57–1.36)	0.87 (0.57–1.37)	0.86 (0.58–1.37)	0.87 (0.58–1.36)	0.86 (0.56–1.30)	0.41	0.87
ApoA1, g/L	4150	1.30 (0.92–1.80)	1.32 (0.91–1.86)	1.30 (0.92–1.80)	1.30 (0.94–1.78)	1.27 (0.92–1.76)	<0.001	0.02
BHMT 742 G>A	2424							
GG		1272 (52.5)	276 (45.6)	307 (50.5)	344 (56.7)	345 (57.1)	<0.001	<0.001
GA		992 (40.9)	271 (44.8)	268 (44.1)	226 (37.2)	227 (37.6)		
AA		160 (6.6)	58 (9.6)	33 (5.4)	37 (6.1)	32 (5.3)		
Medications, n (%)	4150							
β-blocker		3005 (72.4)	700 (67.4)	722 (69.7)	771 (74.1)	812 (78.5)	<0.001	<0.001
ACEI and ARB		1322 (31.9)	283 (27.2)	286 (27.6)	349 (33.5)	404 (39.1)	<0.001	<0.001
Statin		3323 (80.1)	797 (76.7)	843 (81.4)	855 (82.2)	828 (80.1)	0.05	0.65
Aspirin		3389 (81.7)	833 (80.2)	859 (82.9)	844 (81.1)	853 (81.5)	0.20	0.34

Continuous variables are reported as median (5th–95th percentiles), and categorical variables are reported as counts (%). ACEI indicates angiotensin-converting enzyme inhibitor; apoA1, apolipoprotein A1; apoB, apolipoprotein B; ARB, angiotensin receptor blocker; BHMT, betaine-homocysteine methyltransferase; BMI, body mass index; CABG, coronary artery bypass grafting; CAD, coronary artery disease; CBV, cerebrovascular disease; CRP, C-reactive protein; DMG, dimethylglycine; eGFR, estimated glomerular filtration rate; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; LVEF, left ventricular ejection fraction; MI, myocardial infarction; MMA, methylmalonic acid; PAD, peripheral artery disease; PCI, percutaneous coronary intervention; PLP, pyridoxal phosphate; and tHcy, total homocysteine.

*Patients with valid measurements.

†Unadjusted.

‡Adjusted for age and sex.

often were smokers, had hypertension and established CVD, whereas plasma DMG was not associated with left ventricular ejection fraction and was only borderline significantly related to the number of stenosed coronary arteries at angiography. As expected, DMG was strongly related to other 1-carbon metabolites (plasma choline, betaine, tHcy, methionine, and sarcosine), but except for a positive association with plasma methylmalonic acid, there was no relationship between DMG and various markers of B-vitamin status in age- and sex-adjusted analyses. We further observed an inverse trend between plasma DMG quartiles and total cholesterol, high-density lipoprotein cholesterol, and apolipoprotein A1 levels, whereas there were minor or no associations with other lipid parameters. Patients with higher DMG more often used β-blockers and angiotensin-converting enzyme inhibitors and angiotensin receptor blockers. We did not observe any association between plasma DMG and plasma glucose, body mass index, or diabetes mellitus.

When comparing fasting with nonfasting patients (Tables I and II in the online-only Data Supplement), we observed similar associations with DMG for most variables (P for interaction ≥0.05), except for left ventricular ejection fraction and previous myocardial infarction (P for interaction=0.01 for both).

The minor A allele frequency of the BHMT 742 G>A polymorphism was 0.27, and the genotypes were in Hardy–Weinberg equilibrium[17] for all patients investigated, as well as for cases and controls separately (P≥0.06). Plasma DMG was inversely related to the minor allele, but we did not find any association between the polymorphism and the extent of CAD (P for trend=0.43) in age- and sex-adjusted analysis.

Predictors of Subsequent AMI

Median (5th–95th percentile) follow-up time was 4.6 (1.6–6.8) years, constituting a total of 18 848 patient-years. Three hundred forty-three (8.3%) patients experienced an AMI, of which 103 (30.0%) were fatal. The incidence rate for AMI was thus 1.8 events per 100 patient-years. Figure 1 depicts a Kaplan–Meier plot of event-free survival time in quartiles of DMG, showing reduced survival with increasing DMG quartiles (P<0.001).

Using Cox regression analyses we found an approximately linear trend between plasma DMG and subsequent AMI (Table 2 and Figure 2). In an unadjusted Cox regression

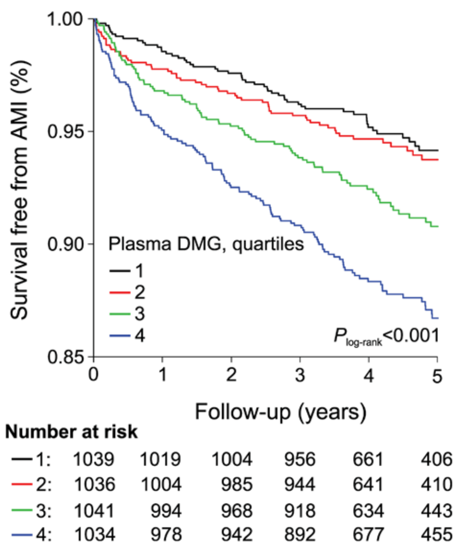


Figure 1. Kaplan–Meier event-free survival curves for patients with plasma dimethylglycine in quartiles 1 to 4. The *x* axis is trimmed at 5 years. AMI indicates acute myocardial infarction; and DMG, dimethylglycine.

model and a model adjusted for fasting status only, the hazard ratio (HR) (95% confidence interval [CI]) for AMI was 2.43 (1.78–3.31; *P*<0.001) and 2.46 (1.80–3.37; *P*<0.001), respectively, when comparing the highest versus the lowest quartiles of DMG. Corresponding HRs (95% CI) for AMI were 1.95 (1.42–2.68; *P*<0.001) and 1.82 (1.32–2.51; *P*<0.001) in models 1 and 2, respectively, and there was a trend toward a stronger association between DMG and fatal versus nonfatal AMI (*P* for interaction=0.06; Table III in the online-only Data Supplement).

Among 1-carbon metabolites in the choline pathway and markers of B-vitamin status, only plasma tHcy was related to the outcome in a similar way as plasma DMG (HR [95% CI] for AMI in the fourth versus the first tHcy quartile, 1.77 [1.28–2.45; *P*=0.001] in Cox model 2; Table IV in the online-only Data Supplement). Furthermore, the relationship between DMG and AMI was only marginally affected by separately adjusting for these parameters in the Cox model 2 (Table V in the online-only Data Supplement), whereas including eGFR in model 2 somewhat weakened the relationship (HR [95% CI] for the fourth versus the first DMG quartile, 1.56 [1.11–2.19; *P*=0.01]).

As depicted in Figure 3 and Table VI in the online-only Data Supplement, the risk of subsequent AMI when comparing the highest versus the lowest plasma DMG quartile in Cox model 2 was stronger in nonsmokers (HR [95% CI], 2.53 [1.64–3.91; *P*<0.001]) and in patients with serum triglycerides (TG) or apoB100 ≤median (HR [95% CI], 3.04 [1.73–4.34; *P*<0.001] and 2.15 [1.32–3.49; *P*=0.002], respectively), whereas there was no association between DMG and incident AMI among smokers or among those with serum TG or apoB100 levels above the median (*P* for interaction=0.004, 0.004, and 0.03, respectively). Accordingly, in patients with DMG levels above median, neither smoking nor high serum TG or apoB100 levels were statistically significantly associated with incident AMI (Table VII in the online-only Data Supplement). Because statin therapy influences circulating TG and apoB100 levels, we excluded subjects who either altered their statin doses or started statin therapy at baseline (654 patients, 42 AMI events) and obtained similar results (data not shown). There were borderline statistically significant stronger associations between plasma DMG and AMI in patients with eGFR ≤median (*P* for interaction=0.06) and age >median (*P* for interaction=0.07). We did not find any effect modification by any of the other subgroup parameters (*P* for interaction ≥0.16; Table VI in the online-only Data Supplement).

When further exploring these subgroups according to fasting status, we found some variations in the relationship between plasma DMG and incident AMI (Table VIII in the online-only Data Supplement). Notably, in patients with fasting TG levels ≤median, we observed a >7-fold increased risk of AMI in the upper versus the lower DMG quartile. A particularly high risk of incident AMI was also noticed in fasting patients with serum apoB100 ≤median. However, fasting status did not add any statistically significant effect modification to any of the subgroups or when examining the total population (*P* for interaction ≥0.05). We found no association between the BHMT 742 G>A polymorphism and the risk of incident AMI in case–control analyses (Tables IX and X in the online-only Data Supplement).

Table 2. Hazard Ratios for Incident Acute Myocardial Infarction According to Plasma Dimethylglycine

	Unadjusted		Model 1*		Model 2†	
	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value
Plasma DMG						
Quartiles						
First	Reference		Reference		Reference	
Second	1.08 (0.75–1.55)	0.68	0.98 (0.68–1.40)	0.90	1.02 (0.71–1.46)	0.92
Third	1.57 (1.13–2.20)	0.01	1.36 (0.97–1.90)	0.08	1.34 (0.95–1.88)	0.09
Fourth	2.43 (1.78–3.31)	<0.001	1.95 (1.42–2.68)	<0.001	1.82 (1.32–2.51)	<0.001
Trend	1.38 (1.25–1.53)	<0.001	1.29 (1.17–1.43)	<0.001	1.25 (1.13–1.38)	<0.001
Per 1 SD‡	1.40 (1.28–1.52)	<0.001	1.31 (1.20–1.44)	<0.001	1.27 (1.15–1.40)	<0.001

CI indicates confidence interval; DMG, dimethylglycine; and HR, hazard ratio.

*Model 1 adjusted for age, sex, and fasting status.

†Model 2 adjusted for age, sex, fasting status, serum apolipoprotein A1 and apoB100, diabetes mellitus, smoking, and hypertension.

‡Log transformed.

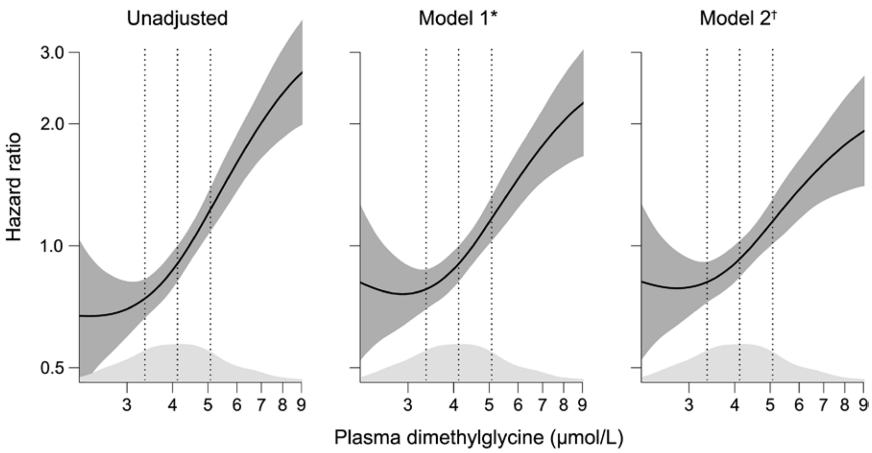


Figure 2. The dose–response relationship between log-transformed plasma dimethylglycine levels and the hazard ratio of incident acute myocardial infarction. Data and the smoothed splines are fitted by various generalized additive Cox models, using 4 *df*. Shaded areas around the curves depict 95% confidence intervals. The *x* axis is trimmed, excluding the lower and upper 2.5 percentiles. Kernel density plots are superimposed along the *x* axis, with vertical dotted lines depicting (from the left) the 25th, 50th, and 75th percentiles of the population. *Adjusted for age, sex, and fasting status. †Adjusted for age, sex, fasting status, smoking, diabetes mellitus, hypertension, and serum apolipoprotein B100 and A1.

Discrimination and Reclassification of AMI by Plasma DMG

When added to the Cox regression model 2, plasma DMG increased the *C* statistic (95% CI) by 0.012 (0.001–0.022). Even larger increments were observed in subgroups of nonsmokers and those with serum TG and apoB100 ≤median, although the latter was only borderline statistically significant (Table 3). The addition of plasma DMG to a logistic regression model containing the same variables as the Cox regression model 2 also improved reclassification (net reclassification improvement >0 [95% CI]: 0.246 [0.113–0.380], 0.346 [0.171–0.521], 0.308 [0.108–0.509], and 0.250 [0.058–0.441] in the total population, nonsmokers, and patients with TG or apoB100 ≤median, respectively).

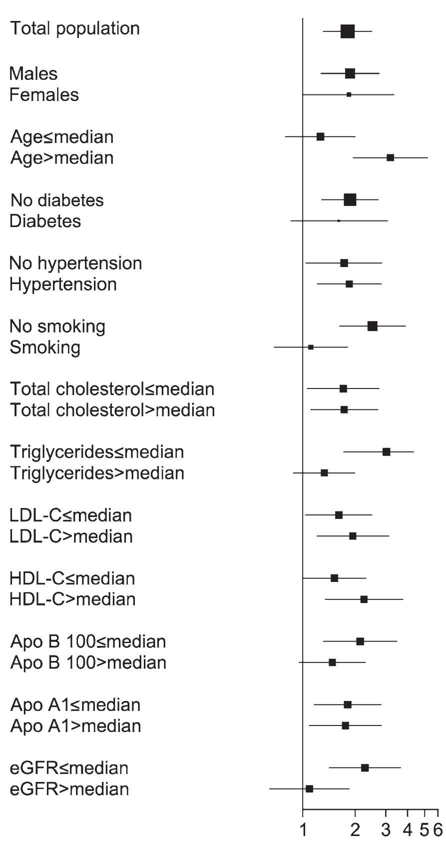


Figure 3. Forest plot depicting the hazard ratios for incident acute myocardial infarction in the fourth vs the first quartile of plasma dimethylglycine in the total population and in subgroups of traditional risk factors for coronary artery disease. Box areas illustrate the sample sizes, and horizontal lines depict 95% confidence intervals (CIs). ApoB indicates apolipoprotein B; ApoA1, apolipoprotein A1; eGFR, estimated glomerular filtration rate; HDL-C, high-density lipoprotein cholesterol; and LDL-C, low-density lipoprotein cholesterol.

Changes in Plasma DMG Over Time and Test–Retest Reliability

Mean (SEM) plasma DMG levels rose from 4.29 (0.03) μmol/L at baseline to 4.45 (0.03) μmol/L at the end of study in the 2565 patients enrolled in Western Norway B-vitamin Intervention Trial (WENBIT; *P*<0.001). The median (5th–95th percentile) follow-up time was 3.3 (1.8–5.0) years. However, a temporal increase in DMG levels from baseline to the end of study was only seen in patients randomized to receive vitamin B6 (*P*<0.001), whereas a borderline statistically significant increase was observed in those allocated to receive placebo (*P*=0.07). There were no time-dependent changes in plasma DMG among patients treated with folic acid, vitamin B12, and vitamin B6 (*P*=0.94) or in those allocated to receive folic acid and vitamin B12 (*P*=0.22). No interaction with fasting status was observed (*P*≥0.08). The coefficients of reliability for plasma DMG and log-transformed plasma DMG throughout these repeated measurements were 0.93 and 0.73, respectively.

Discussion

This large, prospective cohort study of patients undergoing elective coronary angiography for stable angina pectoris showed that patients with elevated plasma DMG levels were more likely to experience an AMI, even after extensive adjustment for traditional cardiovascular risk factors. The association was stronger than for most other metabolites related to the choline oxidation pathway and was most pronounced in nonsmokers and in subjects with low serum apoB100 or TG levels, in whom a particularly strong relationship was suggested among the minority with fasting blood samples at baseline. Plasma DMG also significantly improved discrimination and reclassification of patients at risk and showed high test–retest reliability over >3 years.

Table 3. Model Discrimination and Reclassification

	C Statistic (95% CI)			NRI >0 (95% CI)			
	Model Without DMG	Model With DMG	*P* Value	Total	*P* Value	Events	Nonevents
Total population	0.690 (0.660 to 0.719)	0.701 (0.672 to 0.730)	0.04	0.246 (0.113 to 0.380)	<0.001	0.189 (0.059 to 0.320)	0.057 (0.026 to 0.088)
Smoking status							
Nonsmokers	0.700 (0.662 to 0.739)	0.724 (0.687 to 0.762)	0.01	0.346 (0.171 to 0.521)	<0.001	0.227 (0.057 to 0.398)	0.119 (0.081 to 0.156)
Smokers	0.645 (0.598 to 0.692)	0.647 (0.599 to 0.694)	0.59	0.010 (−0.199 to 0.219)	0.93	0.053 (−0.148 to 0.254)	−0.043 (−0.099 to 0.013)
Serum triglycerides*							
≤Median	0.696 (0.654 to 0.738)	0.727 (0.685 to 0.769)	0.01	0.308 (0.108 to 0.509)	0.003	0.200 (0.004 to 0.396)	0.108 (0.065 to 0.152)
>Median	0.690 (0.650 to 0.730)	0.693 (0.653 to 0.732)	0.41	0.142 (−0.038 to 0.322)	0.12	0.102 (−0.072 to 0.276)	0.040 (−0.005 to 0.084)
Serum apoB100							
≤Median	0.713 (0.672 to 0.755)	0.732 (0.693 to 0.772)	0.06	0.250 (0.058 to 0.441)	0.01	0.153 (−0.033 to 0.339)	0.096 (0.052 to 0.141)
>Median	0.672 (0.631 to 0.713)	0.676 (0.635 to 0.717)	0.29	0.149 (−0.038 to 0.337)	0.12	0.103 (−0.079 to 0.285)	0.046 (0.002 to 0.090)

ApoB indicates apolipoprotein B; CI; confidence interval; DMG; dimethylglycine; and NRI >0, continuous net reclassification improvement.
*Excluding 4 subjects with missing serum triglyceride values, of whom no one experienced an incident acute myocardial infarction.

DMG and CAD

Data on the relationship between DMG and CAD are scarce. Most importantly, our results extend the findings from an investigation of 531 patients with recent acute coronary syndrome, reporting a positive relationship between plasma DMG at baseline and the risk of incident AMI during ≈2.5 years of follow-up.[3] Increased urinary DMG levels have been observed in patients with premature vascular disease,[18] and serum levels of sarcosine, the immediate catabolic product of DMG, has been associated with restenosis after percutaneous coronary intervention.[19]

DMG and Other Baseline Characteristics

In the present investigation, DMG levels were higher among patients with established CVD, and we observed a borderline significant relationship between DMG and the extent of CAD, as evaluated by coronary angiography. In line with findings in the general population,[14] we found the BHMT 742 G>A G allele to be associated with higher plasma DMG levels. This allele has been related to more extensive CAD in elderly subjects.[15] However, in agreement with a recent meta-analysis,[20] we did not observe any overall association between the BHMT 742 G>A genotype and extent of CAD in the present study.

As in previous reports[3,14,21] plasma DMG levels in the current study were higher in men than in women. DMG was also higher among patients with established CAD risk factors, such as older age, hypertension, and smoking. We found a strong inverse association between plasma DMG levels and eGFR, in agreement with earlier studies among patients with renal failure.[8,22] B vitamins are important as cofactors in 1-carbon metabolism, and folate deficiency has been related to increased BHMT flux.[21] DMG tended to be weakly, inversely related to serum folate at baseline in nonfasting subjects, and folic acid supplementation (in WENBIT) seemed to prevent a time-dependent 4% to 10% increase in plasma DMG during follow-up. There was a positive association between baseline plasma DMG and methylmalonic acid, although no relationship was observed between DMG and cobalamin levels.

Possible Mechanisms

Experimental studies have demonstrated that dietary betaine increases the expression of both BHMT and apoB, suggesting a link at the level of gene transcription.[12] We found no or only weak, negative associations between plasma DMG quartiles and serum total cholesterol, TG, low-density lipoprotein cholesterol, and apoB100; however, putative associations may be masked by statin therapy. The relationship between DMG and AMI was most pronounced among patients with low TG or apoB100 levels, and there was a tendency toward an even stronger association among patients in the fasting state. Others have demonstrated that TG lowering by the peroxisome proliferator-activated receptor α agonist WY14 643 reduces transcription of the enzymes involved in DMG and sarcosine catabolism.[11] Of note, peroxisome proliferator-activated receptor α influences the handling of energy substrates derived from lipids,[23] amino acids,[24] and carbohydrates.[25] Hence, high plasma DMG levels may be related to the regulation of lipid and energy metabolism.

The effect modification by smoking is not readily explained; however, the current and previous studies have shown that levels of various 1-carbon metabolites are associated with smoking.[26–28] Furthermore, the observation that plasma DMG did not predict risk of AMI in several groups of patients with a high burden of other risk factors may suggest common mechanistic pathways or masking of the DMG–AMI association in such patients.

Adding eGFR to the Cox regression model 2 somewhat attenuated the association between plasma DMG levels and future AMI, and we found a borderline statistically significant effect modification by eGFR. Declined renal function is considered a major CAD risk factor[29]; hence, our findings indicate that the enhanced risk of AMI by high plasma DMG levels could partly be mediated through similar mechanisms as in subjects with renal impairment.

An increased risk of ischemic heart disease has been associated with elevated blood levels of choline,[1] betaine,[3] and tHcy.[2] Choline is the precursor of betaine and thus interrelated with homocysteine in the production of DMG. Notably, 2 recent studies have proposed a plausible mechanism as to how dietary choline and phosphatidylcholine may promote atherosclerosis[30] and augment the risk of cardiovascular events and mortality via gut microbial–dependent formation of trimethylamine *N*-oxide.[31] We did not assess dietary data in the current study nor did we measure circulating trimethylamine *N*-oxide levels. However, prospective studies in the general population have not found statistically significant associations between choline and betaine intake and the risk of CVD.[1] Accordingly, in our study, no relationship between either plasma choline or betaine levels and the risk of AMI was observed in multivariate analyses, also including DMG. Furthermore, the association of AMI risk with plasma DMG was only slightly attenuated by adjustment for tHcy, making homocysteine an unlikely confounder. Thus, our findings extend the current knowledge of the association between 1-carbon metabolites and CVD.

High plasma DMG levels may reflect altered flux through BHMT, influencing liver *S*-adenosylmethionine levels[32] and thereby the availability of methyl groups for transmethylation reactions, including synthesis of phosphatidylcholine, the major phospholipid in very low-density lipoprotein particles. Thus, increased DMG may be linked to changes in lipoprotein assembly. However, the BHMT 742 G>A polymorphism was not associated with the extent of CAD nor was it related to the risk of incident AMI, despite plasma DMG levels being inversely associated with the minor allele. Importantly, if the A allele were to provide protection from suffering an AMI, the issue of statistical power must be considered. The various genetic models would require a maximum event rate of ≈4% in groups with the minor allele, compared with the 8.3% event rate in the total population, using α- and β-levels set to 0.05 and 0.80, respectively. Furthermore, as CAD is considered being of multifactorial etiology, an ≈50% reduction in event rate is not likely caused by 1 single genetic determinant.[33] The BHMT pathway is the only source of DMG. Thus, large Mendelian randomization studies[34] of BHMT genotypes could shed more light on whether DMG is causally related to AMI risk. However, genes are often multifunctional, hence their pleiotropic effects are part of the inherent weaknesses of such studies.[35]

Strengths and Limitations

The major strengths of this study are its prospective design and large study sample and detailed clinical characterization of the population. Furthermore, we have recently shown that plasma DMG is stable during both short- and long-term storage[36] and now report a high test–retest reliability of plasma DMG, which allow 1-exposure assessment of DMG status. We cannot rule out underreporting or misclassification of clinical end points, which would weaken rather than strengthen the observed associations. Furthermore, residual confounding might influence the assessment of risk predictors in observational cohort studies, which do not allow inference on causality or on flux through metabolic pathways. We mainly studied white, elderly men with stable coronary heart disease, and our results may not apply to women and subjects in other age and ethnic groups or to subjects with clinical features other than stable angina pectoris.

Conclusion

In conclusion, we found that plasma DMG levels are strongly and independently associated with risk of future AMI in patients with stable angina pectoris and adds improvement in risk prediction and discrimination, particularly in subgroups at presumably lower risk. The current findings motivate further studies to elucidate possible mechanisms of 1-carbon metabolism in atherothrombosis. Such research should also consider the potential interaction with energy and lipid metabolism.

Acknowledgments

We express our gratitude to the recruiting physicians, nurses, laboratory personnel, and other coworkers at Haukeland University Hospital, Bergen, Stavanger University Hospital, Stavanger, Bevital A/S, Bergen, and the Lipid research group at the Institute of Medicine, University of Bergen, Bergen, Norway. All authors take responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation.

Sources of Funding

This work has been performed in cooperation with the Department of Heart Disease, Haukeland University Hospital, Bergen, Norway, the Western Norway Regional Health Authority, and the Foundation to Promote Research Into Functional Vitamin B12 Deficiency, Bergen, Norway.

Disclosures

None.

References

1. Ueland PM. Choline and betaine in health and disease. *J Inherit Metab Dis*. 2011;34:3–15.
2. Clarke R, Collins R, Lewington S, et al. Homocysteine Studies C. Homocysteine and risk of ischemic heart disease and stroke: a meta-analysis. *JAMA*. 2002;288:2015–2022.
3. Lever M, George PM, Elmslie JL, Atkinson W, Slow S, Molyneux SL, Troughton RW, Richards AM, Frampton CM, Chambers ST. Betaine and secondary events in an acute coronary syndrome cohort. *PLoS One*. 2012;7:e37883.
4. Clarke R, Halsey J, Lewington S, et al; B-Vitamin Treatment Trialists' Collaboration. Effects of lowering homocysteine levels with B vitamins on cardiovascular disease, cancer, and cause-specific mortality: meta-analysis of 8 randomized trials involving 37 485 individuals. *Arch Intern Med*. 2010;170:1622–1631.
5. Pajares MA, Pérez-Sala D. Betaine homocysteine *S*-methyltransferase: just a regulator of homocysteine metabolism? *Cell Mol Life Sci*. 2006;63:2792–2803.
6. Lever M, George PM, Dellow WJ, Scott RS, Chambers ST. Homocysteine, glycine betaine, and N,N-dimethylglycine in patients attending a lipid clinic. *Metabolism*. 2005;54:1–14.
7. Tibbetts AS, Appling DR. Compartmentalization of mammalian folate-mediated one-carbon metabolism. *Annu Rev Nutr*. 2010;30:57–81.
8. Allen RH, Stabler SP, Lindenbaum J. Serum betaine, *N,N*-dimethylglycine and *N*-methylglycine levels in patients with cobalamin and folate deficiency and related inborn errors of metabolism. *Metabolism*. 1993;42:1448–1460.
9. Schwab U, Alfthan G, Aro A, Uusitupa M. Long-term effect of betaine on risk factors associated with the metabolic syndrome in healthy subjects. *Eur J Clin Nutr*. 2011;65:70–76.

10. Ntaios G, Savopoulos C, Chatzopoulos S, Mikhailidis D, Hatzitolios A. Iatrogenic hyperhomocysteinemia in patients with metabolic syndrome: a systematic review and metaanalysis. *Atherosclerosis*. 2011;214:11–19.
11. Sheikh K, Camejo G, Lanne B, Halvarsson T, Landergren MR, Oakes ND. 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.
12. Sparks JD, Collins HL, Chirieac DV, Cianci J, Jokinen J, Sowden MP, Galloway CA, Sparks CE. 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.
13. Olthof MR, van Vliet T, Verhoef P, Zock PL, Katan MB. Effect of homocysteine-lowering nutrients on blood lipids: results from four randomised, placebo-controlled studies in healthy humans. *PLoS Med*. 2005;2:e135.
14. Fredriksen A, Meyer K, Ueland PM, Vollset SE, Grotmol T, Schneede J. Large-scale population-based metabolic phenotyping of thirteen genetic polymorphisms related to one-carbon metabolism. *Hum Mutat*. 2007;28:856–865.
15. Weisberg IS, Park E, Ballman KV, Berger P, Nunn M, Suh DS, Breksa AP 3rd, Garrow TA, Rozen R. Investigations of a common genetic variant in betaine-homocysteine methyltransferase (BHMT) in coronary artery disease. *Atherosclerosis*. 2003;167:205–214.
16. Gallo V, Egger M, McCormack V, Farmer PB, Ioannidis JP, Kirsch-Volders M, Matullo G, Phillips DH, Schoket B, Stromberg U, Vermeulen R, Wild C, Porta M, Vineis P; STROBE Statement. STrengthening the Reporting of OBservational studies in Epidemiology–Molecular Epidemiology (STROBE-ME): an extension of the STROBE Statement. *PLoS Med*. 2011;8:e1001117.
17. Graffelman J, Camarena JM. Graphical tests for Hardy-Weinberg equilibrium based on the ternary plot. *Hum Hered*. 2008;65:77–84.
18. Lundberg P, Dudman NP, Kuchel PW, Wilcken DE. 1H NMR determination of urinary betaine in patients with premature vascular disease and mild homocysteinemia. *Clin Chem*. 1995;41:275–283.
19. Hasokawa M, Shinohara M, Tsugawa H, Bamba T, Fukusaki E, Nishiumi S, Nishimura K, Yoshida M, Ishida T, Hirata K. Identification of biomarkers of stent restenosis with serum metabolomic profiling using gas chromatography/mass spectrometry. *Circ J*. 2012;76:1864–1873.
20. Singh PR, Lele SS. Folate gene polymorphisms MTR A2756G, MTRR A66G, and BHMT G742A and risk for coronary artery disease: a meta-analysis. *Genet Test Mol Biomarkers*. 2012;16:471–475.
21. Holm PI, Ueland PM, Vollset SE, Midttun Ø, Blom HJ, Keijzer MB, den Heijer M. Betaine and folate status as cooperative determinants of plasma homocysteine in humans. *Arterioscler Thromb Vasc Biol*. 2005;25:379–385.
22. McGregor DO, Dellow WJ, Lever M, George PM, Robson RA, Chambers ST. Dimethylglycine accumulates in uremia and predicts elevated plasma homocysteine concentrations. *Kidney Int*. 2001;59:2267–2272.
23. Berger J, Moller DE. The mechanisms of action of PPARs. *Annu Rev Med*. 2002;53:409–435.
24. Kersten S, Mandard S, Escher P, Gonzalez FJ, Tafuri S, Desvergne B, Wahli W. The peroxisome proliferator-activated receptor alpha regulates amino acid metabolism. *FASEB J*. 2001;15:1971–1978.
25. Ribet C, Montastier E, Valle C, Bezaire V, Mazzucotelli A, Mairal A, Viguerie N, Langin D. Peroxisome proliferator-activated receptor-alpha control of lipid and glucose metabolism in human white adipocytes. *Endocrinology*. 2010;151:123–133.
26. Mannino DM, Mulinare J, Ford ES, Schwartz J. Tobacco smoke exposure and decreased serum and red blood cell folate levels: data from the Third National Health and Nutrition Examination Survey. *Nicotine Tob Res*. 2003;5:357–362.
27. Konstantinova SV, Tell GS, Vollset SE, Nygård O, Bleie Ø, Ueland PM. Divergent associations of plasma choline and betaine with components of metabolic syndrome in middle age and elderly men and women. *J Nutr*. 2008;138:914–920.
28. Ulvik A, Ebbing M, Hustad S, Midttun Ø, Nygård O, Vollset SE, Bønaa KH, Nordrehaug JE, Nilsen DW, Schirmer H, Ueland PM. Long- and short-term effects of tobacco smoking on circulating concentrations of B vitamins. *Clin Chem*. 2010;56:755–763.
29. Schiffrin EL, Lipman ML, Mann JF. Chronic kidney disease: effects on the cardiovascular system. *Circulation*. 2007;116:85–97.
30. Wang Z, Klipfell E, Bennett BJ, et al. Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. *Nature*. 2011;472:57–63.
31. Tang WH, Wang Z, Levison BS, Koeth RA, Britt EB, Fu X, Wu Y, Hazen SL. Intestinal microbial metabolism of phosphatidylcholine and cardiovascular risk. *N Engl J Med*. 2013;368:1575–1584.
32. Strakova J, Gupta S, Kruger WD, Dilger RN, Tryon K, Li L, Garrow TA. Inhibition of betaine-homocysteine *S*-methyltransferase in rats causes hyperhomocysteinemia and reduces liver cystathionine β-synthase activity and methylation capacity. *Nutr Res*. 2011;31:563–571.
33. Roberts R, Stewart AF. Genes and coronary artery disease: where are we? *J Am Coll Cardiol*. 2012;60:1715–1721.
34. Pierce BL, Ahsan H, Vanderweele TJ. Power and instrument strength requirements for Mendelian randomization studies using multiple genetic variants. *Int J Epidemiol*. 2011;40:740–752.
35. Smith GD, Ebrahim S. 'Mendelian randomization': can genetic epidemiology contribute to understanding environmental determinants of disease? *Int J Epidemiol*. 2003;32:1–22.
36. Hustad S, Eussen S, Midttun O, Ulvik A, van de Kant PM, Morkrid L, Gislefoss R, Ueland PM. Kinetic modeling of storage effects on biomarkers related to B vitamin status and one-carbon metabolism. *Clin Chem*. 2012;58:402–410.

Significance

The production of the 1-carbon metabolite dimethylglycine may be connected to cardiovascular disease through its association with lipid metabolism.

In this large, prospective cohort study of patients with stable angina pectoris, we found that plasma dimethylglycine levels were associated with several traditional risk factors of coronary artery disease. Plasma dimethylglycine also showed an independent, strong dose–response relationship with the risk of incident acute myocardial infarction during follow-up for >4 years, and the prediction was more pronounced in several subgroups at presumably lower risk. Interaction analyses suggested a potential connection between 1-carbon and lipid and energy metabolism. Furthermore, plasma dimethylglycine improved risk assessment when added to traditional coronary artery disease risk factors and showed a high degree of test–retest reliability.

Our findings extend the current knowledge of the relationship between 1-carbon metabolism and coronary artery disease and could prove important in risk assessment and understanding atherothrombosis.

Materials and Methods

Study Population

The Bergen Coronary Angiography Cohort (BECAC) consists of 4241 adult, mainly (>99%) white patients who underwent coronary angiography at the Department of Heart Disease, Haukeland University Hospital, Bergen, Norway, between January 2000 and April 2004. More than 95% of patients referred to our department for an elective examination during this period were included in BECAC. The primary aim of BECAC was to study various prognostic markers of cardiovascular end-points and cause-specific mortality in patients with suspected heart diseases. Furthermore, BECAC constituted the source population of patients randomized from our hospital in the Western Norway B vitamin Intervention Trial (WENBIT; ClinicalTrials.gov number NCT00354081), to investigate the effect of B vitamin supplementation on mortality and cardiovascular events.[1] Only patients admitted due to suspected SAP (n=3413) were selected for the current study, of whom 1822 (53.4%) were enrolled in WENBIT. We additionally included 751 WENBIT participants with stable angina pectoris (SAP) and angiographically verified coronary artery disease (CAD), recruited from Stavanger University Hospital, Stavanger, Norway. Patients with any missing baseline covariate incorporated in the risk models (n=13) and one patient with an extremely high plasma dimethylglycine (DMG) level of 257.0 µmol/L were excluded, leaving a total of 4150 patients eligible for the final analyses (Supplemental Figure 1). The study population was followed for the occurrence of acute myocardial infarction (AMI) until December 31st 2006. The study was carried out according to the Declaration of Helsinki and approved by The Regional Committee for Medical and Health Research Ethics and the Norwegian Data Inspectorate. All participants provided written informed consent

Baseline Characteristics

Information on patients' lifestyle and medical history was obtained from self-administered questionnaires and verified by comparing to hospital records. Hypertension and diabetes mellitus were defined according to preexisting diagnoses, and diabetes included both type 1 (n=37) and 2 (n=454). Smoking status was based on self-reported smoking habits and plasma cotinine measurements. Because self-reported smoking generally underestimates the true exposure,[2] patients initially classified as non-smokers, but with serum cotinine≥85 nmol/L, were classified as smokers.[3] Blood sampling, blood pressure measurement and assessment of anthropometric data were performed by trained nurses. Left ventricular ejection fraction (LVEF) was obtained either by echocardiography or by ventriculography performed during cardiac catheterization.

Coronary Angiography

Cardiac catheterization was performed by trained cardiologists and coronary stenoses were confirmed in orthogonal views. A significant coronary artery stenosis was defined by luminal narrowing ≥50% of any epicardial coronary artery [i.e. the right coronary artery (RCA), the left descending artery or the left circumflex artery] or any of their main branches. The extent of CAD was scored by aggregating the number of significantly stenotic arteries to a maximum of three.

Blood Collection and Biochemical Analyses

For patients undergoing coronary angiography at Haukeland University Hospital, venous blood samples were drawn at baseline, usually 1-3 days before the

procedure and for patients undergoing coronary angiography at Stavanger University Hospital, samples were drawn immediately after the procedure. Blood sampling was carried out before noon in most patients and plasma DMG levels were inversely associated with time elapsed since last meal. However, this relationship was not observed more than 8 hours after a meal; hence, those patients delivering blood samples 8 hours or longer since last meal were defined as fasting (n=1104 [26.6%]). Among the 2565 WENBIT participants, additional blood samples were drawn at follow-up visits after 1-3 months, at 1 year and at the end of the B vitamin intervention study, although all participants did not attend all four study visits.
Routine laboratory analyses were performed at hospital laboratories of Haukeland University Hospital, Bergen, or Stavanger University Hospital, Stavanger, Norway. Estimated glomerular filtration rate (eGFR) was obtained using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula.[4] For study-specific analyses, serum and plasma were immediately prepared and stored in 2 mL Vacutainer tubes (Becton, Dickinson and Company, United States) at –80^{o}C until thawed and analyzed by laboratory staff blinded to the clinical outcomes of the patients. Plasma total homocysteine (Hcy) and methylmalonic acid (MMA) were measured using the gas chromatography coupled with mass spectrometry procedure,[5] whereas plasma DMG, betaine, methionine, sarcosine, riboflavin and pyridoxal phosphate (PLP) and serum cotinine were analyzed using liquid chromatography-tandem mass spectrometry[6] at Bevital AS, Bergen, Norway (http://www.bevital.no). Serum folate[7] and cobalamin[8] levels were measured by microbiological assays. The within-day Coefficient of Variance (CV) of the DMG assay was <7.2%. Serum C-reactive protein (CRP) was measured using an ultrasensitive immunoassay, with a detection limit of 0.17 mg/L, applying the Behring nephelometer II system (CV 8.1-11.4%; N Latex CRP mono, Behring Diagnostics, Marburg, Germany). Serum levels of apolipoprotein (apo) A1 and apo B 100 were measured on the Hitachi 917 and 912 systems (Roche Diagnostics, GmbH, Mannheim, Germany), respectively. We also investigated the single nucleotide polymorphism (SNP) BHMT 742G>A (rs3733890) in blood samples from 2424 WENBIT participants, using matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry.[9] The genotyping was performed at Bevital AS (www.bevital.no), Bergen, Norway,

Clinical End Points

We obtained information on events from the Cause of Death Registry at Statistics Norway (http://www.ssb.no) and the Western Norway Cardiovascular Registry.[10] The latter contains all CVD discharge diagnoses from the patient administrative systems at Western Norway public hospitals. Medical records were used for verifying the registry data. The revised European criteria published in 2000[11] were applied to classify AMI, including both fatal and non-fatal events, and the study end point was assigned by the WENBIT study end point committee.

Statistical Analyses

Continuous variables are reported as median (5th, 95th percentiles). Patient baseline characteristics across plasma DMG quartiles were assessed, and trends tested by logistic regression for dichotomous variables and by linear median regression[12] for continuous and ordinal data. Mann-Whitney U-test was applied when exploring differences between continuous variables in independent groups.

Survival was studied using Kaplan-Meier plots and the difference in survival across quartiles of DMG was assessed by the log-rank test. Univariate and multivariate Cox regression analysis was used to obtain hazard ratios (HRs) and 95% confidence intervals (CI) of incident AMI for plasma DMG levels. The results were reported according to the 4th vs. the 1st plasma DMG quartile, trend across quartiles and per 1 standard deviation (SD) increment in log transformed plasma DMG. Model 1 included age, gender and fasting status (dichotomous) and model 2 additionally included serum apo A1 and apo B 100, diabetes (dichotomous), smoking status (dichotomous) and hypertension (dichotomous). The assumption of proportional hazards was assessed by inspecting survival plots and calculating scaled Schoenfeld residuals. We explored potential non-linear relationships between logarithmically transformed plasma DMG levels and HR of AMI, deriving the estimates from Cox proportional hazard models with penalized smoothing splines (four degrees of freedom).

B vitamins are important as cofactors in one-carbon metabolism and circulating B vitamin levels have been associated with CAD[13] and risk of coronary events.[14] Thus, we also investigated the potential association between incident AMI and blood levels of several metabolites in the choline pathway, as well as markers of B vitamin status. To assess their impact on the relationship between AMI and DMG, these variables were separately included in Cox model 2, as was eGFR, because of the increased plasma DMG levels observed in patients with renal impairment.[15]

Effect modification was investigated according to strata of traditional CAD risk factors, by adding interaction product terms to Cox model 2. Subgroups were created by using existing categorical variables or splitting continuous variables at the median value. A potential interaction according to treatment with folic acid and vitamin B12 was also investigated among WENBIT participants. Since some subgroup variables were likely to be affected by time elapsed since the last meal, we also studied any additional effect modification by fasting status.

Among 2424 of the WENBIT participants we further assessed the relationship between the BHMT 742 G>A polymorphism and extent of CAD, and the association with AMI in genetic case-control models.[16]

Model discrimination was explored by calculating the *C* statistic from Cox model 2 with and without plasma DMG quartiles added as a continuous variable. A logistic regression model containing the same variables as model 2, but with censored follow-up beyond 1000 days, was used to investigate reclassification. Because there are no established categories of risk in patients with established CAD, the continuous net reclassification improvement [NRI(>0)][17] was obtained.

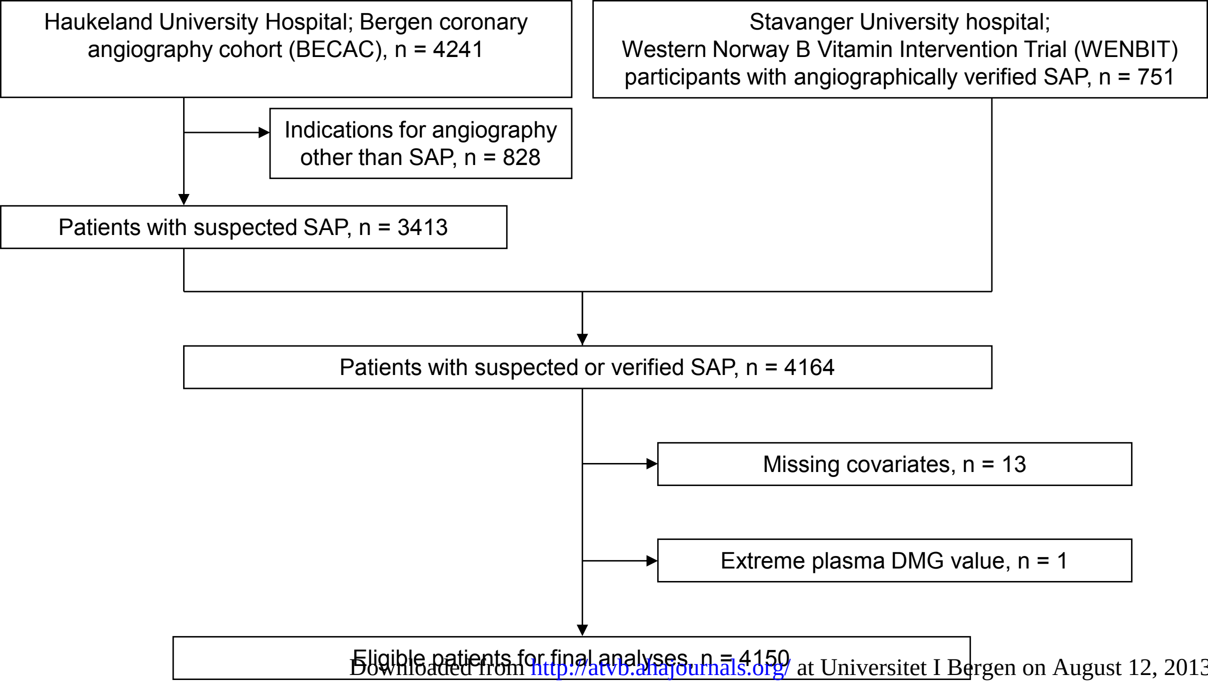
We investigated changes in mean plasma DMG levels, also according to treatment allocation,[1] from baseline to the end of study in WENBIT participants. The Coefficient of Reliability (CoR) of plasma DMG was calculated in 644 of these patients randomized to placebo. The CoR was obtained by calculating the between-person variance as a proportion of the total variance across four visits; CoR≥0.75 suggests excellent reproducibility.[18] Using mixed linear modelling when assessing these longitudinal data allowed us to also include patients who did not attend all four study visits.

The two-sided significance level was set to 0.05 in all statistical models. The computer software packages PASW Statistics 18, Release Version 18.0.1 (SPSS, Inc., 2009, Chicago, IL, www.spss.com), SPSS Sample Power Version 2.0 (SPSS, Inc., 2000, Chicago, IL, www.spss.com) and R version 2.15.0 (The R Foundation for Statistical Computing, Vienna, Austria)[19] were used for statistical analyses.

References

1. Ebbing M, Bleie O, Ueland PM, Nordrehaug JE, Nilsen DW, Vollset SE, Refsum H, Pedersen EK, Nygard O. Mortality and cardiovascular events in patients treated with homocysteine-lowering B vitamins after coronary angiography: A randomized controlled trial. *JAMA: The journal of the American Medical Association*. 2008;300:795-804
2. Gorber SC, Schofield-Hurwitz S, Hardt J, Levasseur G, Tremblay M. The accuracy of self-reported smoking: A systematic review of the relationship between self-reported and cotinine-assessed smoking status. *Nicotine & tobacco research : Official journal of the Society for Research on Nicotine and Tobacco*. 2009;11:12-24
3. SRNT SoBV. Biochemical verification of tobacco use and cessation. *Nicotine & Tobacco Research*. 2002;4:149-159
4. Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF 3rd, Feldman HI, Kusek JW, Eggers P, Van Lente F, Greene T, Coresh J, CKD EPI. A new equation to estimate glomerular filtration rate. *Annals of internal medicine*. 2009;150:604-612
5. Windelberg A, Arseth O, Kvalheim G, Ueland PM. Automated assay for the determination of methylmalonic acid, total homocysteine, and related amino acids in human serum or plasma by means of methylchloroformate derivatization and gas chromatography-mass spectrometry. *Clinical chemistry*. 2005;51:2103-2109
6. Holm PI, Ueland PM, Kvalheim G, Lien EA. Determination of choline, betaine, and dimethylglycine in plasma by a high-throughput method based on normal-phase chromatography-tandem mass spectrometry. *Clinical chemistry*. 2003;49:286-294
7. Molloy AM, Scott JM. Microbiological assay for serum, plasma, and red cell folate using cryopreserved, microtiter plate method. *Methods in enzymology*. 1997;281:43-53
8. Kelleher BP, Broin SD. Microbiological assay for vitamin B12 performed in 96-well microtitre plates. *Journal of clinical pathology*. 1991;44:592-595
9. Meyer K, Fredriksen A, Ueland PM. MALDI-TOF MS genotyping of polymorphisms related to 1-carbon metabolism using common and mass-modified terminators. *Clinical chemistry*. 2009;55:139-149
10. Oyen N, Nygard O, Igland J, Tell GS, Nordrehaug JE, Irgens LM, Cooper JG, Langorgen J, Vollset SE. [Hospital admission rates for cardiovascular diseases in Western Norway, 1992-2001]. *Tidsskrift for den Norske laegeforening: Tidsskrift for praktisk medicin, ny raekke*. 2008;128:17-23
11. Myocardial infarction redefined--a consensus document of the joint European Society of Cardiology/American College of Cardiology Committee for the redefinition of myocardial infarction. *Eur Heart J*. 2000;21:1502-1513
12. Koenker R. *Quantile Regression*. New York, USA: Cambridge University Press; 2011:295-316
13. Verhoef P, Kok FJ, Kruyssen DA, Schouten EG, Witteman JC, Grobbee DE, Ueland PM, Refsum H. Plasma total homocysteine, B vitamins, and risk of coronary atherosclerosis. *Arterioscler Thromb Vasc Biol*. 1997;17:989-995
14. Vanuzzo D, Pilotto L, Lombardi R, Lazzerini G, Carluccio M, Diviacco S, Quadrifoglio F, Danek G, Gregori D, Fioretti P, Cattaneo M, De Caterina R.

Both vitamin B6 and total homocysteine plasma levels predict long-term atherothrombotic events in healthy subjects. *Eur Heart J*. 2007;28:484-491
15. McGregor DO, Dellow WJ, Lever M, George PM, Robson RA, Chambers ST. Dimethylglycine accumulates in uremia and predicts elevated plasma homocysteine concentrations. *Kidney international*. 2001;59:2267-2272
16. Clarke GM, Anderson CA, Pettersson FH, Cardon LR, Morris AP, Zondervan KT. Basic statistical analysis in genetic case-control studies. *Nature protocols*. 2011;6:121-133
17. Pencina MJ, D'Agostino RB Sr, Steyerberg EW. Extensions of net reclassification improvement calculations to measure usefulness of new biomarkers. *Statistics in medicine*. 2011;30:11-21
18. Rosner B. *Fundamentals of biostatistics*. Boston, USA: Brooks/Cole; 2011:568-571
19. R Core Team. R: A language and environment for statistical computing. Vienna, Austria. 2012



Supplemental Figure I. Flowchart depicting the selection of patients eligible for the study.

Supplemental Table I. Baseline Characteristics of Fasting Patients and According to Quartiles of Plasma Dimethylglycine

	N*	All	Quartiles of plasma DMG				P_{trend}†	P_{trend}§
			1st	2nd	3rd	4th		
Plasma DMG µmol/L	1104	3.8 (2.4, 7.0)	2.9 (2.2, 3.3)	3.8 (3.4, 4.1)	4.5 (4.2, 5.0)	6.1 (5.2, 10.2)	-	-
Male gender, n (%)	1104	869 (78.7)	256 (72.3)	307 (81.8)	194 (81.2)	168 (82.4)	0.004	0.001
Age, years	1104	61 (44, 77)	60 (52, 66)	61 (54, 67)	63 (55, 71)	63 (56, 72)	0.01	0.001
Current smoking, n (%)	1104	401 (36.3)	121 (34.2)	104 (33.9)	83 (34.7)	93 (45.6)	0.02	<0.001
Diabetes, n (%)	1104	129 (11.7)	40 (11.3)	38 (12.4)	31 (13.0)	20 (9.8)	0.78	0.51
BMI, kg/m²	1104	26.7 (21.5, 33.9)	26.7 (21.2, 33.3)	26.5 (21.6, 34.1)	27.0 (22.1, 34.8)	26.6 (21.1, 33.8)	0.59	0.29
Plasma glucose, mmol/L	1104	5.5 (4.6, 9.3)	5.5 (4.0, 10.1)	5.5 (4.6, 9.6)	5.6 (4.5, 9.1)	5.6 (4.6, 8.1)	0.19	0.20
Hypertension, n (%)	1104	498 (45.1)	144 (40.7)	125 (40.7)	116 (48.5)	113 (55.4)	<0.001	0.004
Extent of CAD, n (%)	1104						1.00	0.84
No stenotic vessels		225 (20.4)	78 (22.0)	61 (19.9)	48 (20.1)	38 (18.6)		
1-vessel disease		281 (25.5)	87 (24.6)	82 (26.7)	65 (27.2)	47 (23.0)		
2-vessel disease		273 (24.7)	86 (24.3)	77 (25.1)	65 (27.2)	45 (22.1)		
3-vessel disease		325 (29.4)	103 (29.1)	87 (28.3)	61 (25.5)	74 (36.3)		
LVEF, %			65 (44, 80)	65 (40, 80)	65 (37, 80)	60 (38, 80)	0.04	<0.001
Previous MI, n (%)	1104	462 (41.8)	141 (39.8)	130 (42.3)	90 (37.7)	101 (49.5)	0.12	0.13
Previous CBV, n (%)	1104	75 (6.8)	19 (5.4)	21 (6.8)	15 (6.3)	20 (9.8)	0.08	0.51
Previous PAD, n (%)	1104	89 (8.1)	28 (7.9)	14 (4.6)	20 (8.4)	27 (13.2)	0.03	0.13
Previous CABG, n (%)	1104	141 (12.8)	40 (11.3)	39 (12.7)	36 (15.1)	26 (12.7)	0.39	0.62
Previous PCI, n (%)	1104	221 (20.0)	70 (19.8)	61 (19.9)	43 (18.0)	47 (23.0)	0.57	0.26
Serum CRP, mg/L	1104	1.8 (0.3, 12.2)	1.8 (0.3, 12.1)	1.6 (0.3, 9.2)	1.7 (0.3, 12.0)	2.4 (0.5, 18.6)	0.06	0.03
eGFR, mL/min/1.73m²	1104	94 (58, 114)	97 (72, 116)	94 (66, 113)	90 (51, 113)	86 (42, 113)	<0.001	<0.001
Plasma levels of one-carbon metabolites								
Choline, µmol/L	1104	8.9 (5.9, 13.7)	8.1 (5.5, 11.3)	8.6 (6.0, 13.4)	9.4 (6.3, 14.1)	10.5 (7.2, 16.4)	<0.001	<0.001
Betaine, µmol/L	1104	35.9 (21.6, 59.6)	32.0 (19.3, 49.6)	36.7 (22.8, 57.1)	36.7 (23.0, 59.9)	41.7 (26.7, 70.5)	<0.001	<0.001
tHcy, µmol/L	1104	10.4 (6.8, 17.8)	9.6 (6.4, 15.8)	9.8 (6.9, 16.0)	11.0 (7.0, 20.5)	12.2 (7.3, 20.2)	<0.001	<0.001
Methionine, µmol/L	1104	24.3 (17.6, 33.2)	23.0 (17.1, 32.2)	24.7 (17.3, 33.4)	25.1 (17.7, 34.1)	25.4 (18.1, 36.6)	<0.001	<0.001
Sarcosine, µmol/L	281	6.6 (5.2, 9.0)	6.6 (5.0, 8.8)	6.5 (4.5, 8.0)	6.6 (5.6, 9.5)	7.1 (5.8, 11.5)	0.09	0.01
Markers of B-vitamin status								
Plasma riboflavin, nmol/L	1082	11.0 (4.5, 43.9)	10.4 (4.6, 45.3)	11.5 (4.5, 41.9)	11.2 (4.5, 38.2)	11.4 (4.1, 48.9)	0.17	0.34
Serum folate, nmol/L	1103	9.6 (4.6, 30.5)	9.9 (4.6, 30.3)	9.7 (5.1, 33.7)	9.2 (4.3, 25.1)	9.0 (4.4, 37.7)	0.07	0.06
Plasma PLP, nmol/L	1082	37.3 (17.2, 101.6)	39.6 (16.9, 111.0)	36.7 (17.4, 94.4)	37.8 (17.8, 95.1)	34.8 (15.7, 103.8)	0.01	0.17
Serum cobalamin, pmol/L	1104	328 (154, 665)	327 (157, 589)	331 (146, 612)	322 (153, 702)	320 (154, 769)	0.56	0.83

Plasma MMA, µmol/L	1103	0.15 (0.10, 0.30)	0.14 (0.10, 0.25)	0.15 (0.10, 0.31)	0.16 (0.11, 0.31)	0.17 (0.11, 0.37)	<0.001	<0.001
Serum lipids and apolipoproteins								
Total cholesterol, mmol/L	1104	5.0 (3.5, 7.1)	5.1 (3.6, 7.1)	4.9 (3.5, 7.2)	4.9 (3.6, 7.0)	4.7 (3.3, 7.3)	0.01	0.003
LDL-C, mmol/L	1103	2.9 (1.7, 5.0)	3.0 (1.7, 5.0)	2.9 (1.7, 5.0)	2.9 (1.7, 5.1)	2.7 (1.6, 5.1)	0.01	0.04
HDL-C, mmol/L	1103	1.2 (0.8, 2.0)	1.3 (0.8, 2.1)	1.2 (0.8, 1.9)	1.2 (0.8, 2.1)	1.2 (0.8, 1.9)	0.003	0.05
Triglycerides, mmol/L	1103	1.4 (0.6, 3.6)	1.5 (0.6, 4.1)	1.4 (0.6, 3.5)	1.5 (0.7, 3.3)	1.4 (0.6, 3.4)	0.18	1.00
Apo B 100, g/L	1104	0.84 (0.55, 1.32)	0.86 (0.55, 1.33)	0.82 (0.55, 1.34)	0.85 (0.54, 1.35)	0.81 (0.51, 1.30)	0.24	0.49
Apo A1, g/L	1104	1.24 (0.85, 1.76)	1.25 (0.85, 1.81)	1.23 (0.83, 1.69)	1.24 (0.86, 1.78)	1.21 (0.85, 1.73)	0.27	0.21
BHMT 742 G>A	853						0.001	<0.001
GG		462 (54.2)	124 (45.1)	128 (51.8)	118 (64.5)	92 (62.2)		
GA		340 (39.9)	125 (45.5)	109 (44.1)	56 (30.6)	50 (33.8)		
AA		51 (6.0)	26 (9.5)	10 (4.0)	9 (4.9)	6 (4.1)		
Medications, n (%)								
Beta blocker	1104	807 (73.1)	253 (71.5)	219 (71.3)	175 (73.2)	160 (78.4)	0.09	0.24
ACEI and/or ARB	1104	362 (32.8)	97 (27.4)	90 (29.3)	86 (36.0)	89 (43.6)	<0.001	<0.001
Statin	1104	916 (83.0)	291 (82.2)	259 (84.4)	202 (84.5)	164 (80.4)	0.77	0.63
Aspirin	1104	917 (83.1)	300 (84.7)	261 (85.0)	190 (79.59	166 (81.4)	0.12	0.07

Continuous variables are reported as median (5th, 95th percentiles) and categorical variables are reported as counts (%).

ACEI indicates angiotensin converting enzyme inhibitor; apo, apolipoprotein; ARB, angiotensin receptor blocker; BHMT, betaine-homocysteine methyltransferase; BMI, body mass index; CABG, coronary artery bypass grafting; CAD, coronary artery disease; CBV, cerebrovascular disease; CRP, C-reactive protein; DMG, dimethylglycine; eGFR, estimated glomerular filtration rate; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; LVEF, left ventricular ejection fraction; MI, myocardial infarction; MMA, methylmalonic acid; PAD, peripheral artery disease; PCI, percutaneous coronary intervention; PLP, pyridoxal phosphate; tHcy, total homocysteine.

*Patients with valid measurements.

†Unadjusted.

§Adjusted for age and gender.

Supplemental Table II. Baseline Characteristics of Non-Fasting Patients and According to Quartiles of Plasma Dimethylglycine

	N*	All	Quartiles of plasma DMG					
			1st	2nd	3rd	4th	P_{trend}†	P_{trend}§
Plasma DMG µmol/L	3046	4.3 (3.4, 5.2)	2.9 (2.2, 3.3)	3.8 (3.4, 4.1)	4.6 (4.2, 5.0)	6.0 (5.1, 10.3)	-	-
Male gender, n (%)	3046	2118 (69.5)	391 (57.1)	501 (68.7)	595 (74.2)	631 (76.0)	<0.001	<0.001
Age, years	3046	62 (44, 78)	58 (42, 76)	62 (45, 77)	62 (45, 78)	65 (45, 80)	<0.001	<0.001
Current smoking, n (%)	3046	910 (29.9)	196 (28.6)	181 (24.8)	250 (31.2)	283 (34.1)	0.002	<0.001
Diabetes, n (%)	3046	362 (11.9)	95 (13.9)	71 (9.7)	84 (10.5)	112 (13.5)	0.93	0.38
BMI, kg/m^2	3043	26.2 (21.0, 33.7)	26.5 (20.5, 33.9)	26.2 (21.2, 33.3)	26.3 (21.2, 34.2)	26.0 (21.0, 33.5)	0.04	0.47
Plasma glucose, mmol/L	3044	5.7 (4.3, 11.9)	5.7 (4.2, 12.8)	5.6 (4.3, 11.2)	5.7 (4.3, 11.7)	5.8 (4.3, 12.3)	0.03	0.58
Hypertension, n (%)	3046	1441 (47.3)	298 (43.5)	323 (44.3)	381 (47.5)	439 (52.9)	<0.001	0.01
Extent of CAD, n (%)	3046						<0.001	0.04
No stenotic vessels		819 (26.9)	243 (35.5)	202 (27.7)	200 (24.9)	174 (21.0)		
1-vessel disease		682 (22.4)	146 (21.3)	170 (23.3)	200 (24.9)	166 (20.0)		
2-vessel disease		652 (21.4)	138 (20.1)	164 (22.5)	163 (20.3)	187 (22.5)		
3-vessel disease		893 (29.3)	158 (23.1)	193 (26.5)	239 (29.8)	303 (36.5)		
LVEF, %	3046	67 (41, 80)	70 (45, 80)	70 (46, 80)	65 (40, 80)	65 (35, 80)	<0.001	1.00
Previous MI, n (%)	3046	1212 (39.8)	208 (30.4)	267 (36.6)	322 (40.1)	415 (50.0)	<0.001	<0.001
Previous CBV, n (%)	3046	213 (7.0)	30 (4.4)	38 (5.2)	52 (6.5)	93 (11.2)	<0.001	<0.001
Previous PAD, n (%)	3046	285 (9.4)	41 (6.0)	50 (6.9)	86 (10.7)	108 (13.0)	<0.001	<0.001
Previous CABG, n (%)	3046	337 (11.1)	70 (10.2)	71 (9.7)	80 (10.0)	116 (14.0)	0.02	0.63
Previous PCI, n (%)	3046	575 (18.9)	124 (18.1)	122 (16.7)	149 (18.6)	180 (21.7)	0.04	0.19
Serum CRP, mg/L	3046	1.8 (0.4, 12.7)	1.4 (0.4, 10.5)	1.6 (0.4, 10.9)	1.9 (0.4, 11.0)	2.1 (0.4, 19.1)	<0.001	<0.001
eGFR, mL/min/1.73m^2	3046	90 (57, 110)	95 (87, 102)	91 (81, 98)	88 (76, 98)	67 (83, 94)	<0.001	<0.001
Plasma levels of one-carbon metabolites								
Choline, µmol/L	3046	10.0 (6.7, 15.1)	8.6 (6.0, 12.6)	9.7 (7.0, 13.7)	10.3 (7.1, 14.7)	11.4 (7.6, 17.1)	<0.001	<0.001
Betaine, µmol/L	3046	40.5 (24.1, 64.9)	33.2 (19.2, 52.4)	39.4 (24.8, 60.2)	42.9 (27.1, 64.3)	45.6 (27.2, 73.2)	<0.001	<0.001
tHcy, µmol/L	3046	10.4 (6.7, 18.7)	9.6 (6.2, 15.9)	10.1 (6.8, 15.6)	10.8 (6.9, 17.2)	11.5 (7.2, 23.2)	<0.001	<0.001
Methionine, µmol/L	3046	27.8 (18.4, 43.6)	25.9 (17.4, 40.4)	27.2 (18.4, 42.6)	28.0 (18.8, 42.9)	29.7 (18.9, 47.4)	<0.001	<0.001
Sarcosine, µmol/L	1446	6.8 (5.4, 8.9)	6.5 (5.1, 8.5)	6.7 (5.3, 8.4)	6.9 (5.7, 8.7)	7.0 (5.5, 9.7)	<0.001	<0.001
Markers of B-vitamin status								
Plasma riboflavin, nmol/L	3045	11.4 (4.3, 52.2)	11.2 (4.5, 49.5)	11.2 (4.4, 48.2)	11.3 (4.2, 51.8)	11.6 (4.3, 61.6)	0.32	0.77
Serum folate, nmol/L	3046	10.3 (4.9, 37.6)	10.7 (5.3, 42.2)	10.6 (5.2, 34.3)	10.3 (5.1, 34.4)	9.8 (4.6, 38.8)	0.003	0.005
Plasma PLP, nmol/L	3043	43.0 (19.3, 135.6)	44.2 (19.4, 137.6)	43.6 (21.9, 115.8)	41.7 (18.5, 138.5)	41.5 (18.0, 142.6)	0.02	0.08
Serum cobalamin, pmol/L	2595	375 (189, 728)	375 (181. 763)	379 (194, 699)	377 (197, 699)	369 (183, 814)	0.48	0.40

Plasma MMA, µmol/L	3046	0.17 (0.11, 0.32)	0.15 (0.10, 0.26)	0.16 (0.11, 0.29)	0.17 (0.11, 0.32)	0.18 (0.12, 0.40)	<0.001	<0.001
Serum lipids and apolipoproteins								
Total cholesterol, mmol/L	3045	4.9 (3.5, 7.1)	5.0 (3.6, 7.3)	4.9 (3.6, 7.3)	4.9 (3.5, 7.1)	4.8 (3.3, 6.9)	0.01	0.14
LDL-C, mmol/L	3044	3.0 (1.8, 5.0)	3.0 (1.8, 5.1)	3.0 (1.8, 5.2)	2.9 (1.8, 5.0)	2.9 (1.6, 4.7)	0.04	0.20
HDL-C, mmol/L	3046	1.2 (1.0, 1.5)	1.3 (0.8, 2.1)	1.3 (0.8, 2.0)	1.2 (0.8, 2.0)	1.2 (0.8, 1.9)	<0.001	<0.001
Triglycerides, mmol/L	3043	1.5 (0.7, 3.7)	1.5 (0.7, 3.9)	1.5 (0.8, 3.8)	1.5 (0.7, 3.6)	1.5 (0.7, 3.6)	1.00	0.70
Apo B 100, g/L	3046	0.87 (0.58, 1.37)	0.89 (0.58, 1.38)	0.87 (0.59, 1.38)	0.87 (0.59, 1.37)	0.87 (0.56, 1.30)	0.30	0.91
Apo A1, g/L	3046	1.31 (0.97, 1.80)	1.35 (0.98, 1.87)	1.32 (0.98, 1.81)	1.32 (0.97, 1.79)	1.28 (0.95, 1.77)	<0.001	<0.001
BHMT 742 G>A	1571						<0.001	<0.001
GG		810 (51.6)	186 (46.0)	208 (54.0)	222 (54.0)	194 (56.4)		
GA		652 (41.5)	181 (44.8)	177 (43.0)	163 (39.7)	131 (38.1)		
AA		109 (6.9)	37 (9.2)	27 (6.6)	26 (6.3)	19 (5.5)		
Medications, n (%)								
Beta blocker	3046	2198 (72.2)	447 (65.3)	503 (69.0)	596 (74.3)	652 (78.6)	<0.001	<0.001
ACEI and/or ARB	3046	960 (31.5)	186 (27.2)	196 (26.9)	263 (32.8)	315 (38.0)	<0.001	<0.001
Statin	3046	2407 (79.0)	506 (73.9)	584 (80.1)	653 (81.4)	664 (80.0)	0.01	0.30
Aspirin	3046	2472 (81.2)	533 (77.8)	598 (82.0)	654 (81.5)	687 (82.8)	0.03	0.63

Continuous variables are reported as median (5th, 95th percentiles) and categorical variables are reported as counts (%).

ACEI indicates angiotensin converting enzyme inhibitor; apo, apolipoprotein; ARB, angiotensin receptor blocker; BHMT, betaine-homocysteine methyltransferase; BMI, body mass index; CABG, coronary artery bypass grafting; CAD, coronary artery disease; CBV, cerebrovascular disease; CRP, C-reactive protein; DMG, dimethylglycine; eGFR, estimated glomerular filtration rate; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; LVEF, left ventricular ejection fraction; MI, myocardial infarction; MMA, methylmalonic acid; PAD, peripheral artery disease; PCI, percutaneous coronary intervention; PLP, pyridoxal phosphate; tHcy, total homocysteine.

*Patients with valid measurements.

†Unadjusted.

§Adjusted for age and gender.

Supplemental Table III. Hazard Ratios for Incident Fatal and Non-Fatal Acute Myocardial Infarction According to Plasma Dimethylglycine

		Fatal acute myocardial infarction (103 events)					
		Unadjusted		Model 1*		Model 2†	
		HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value
Plasma DMG							
N	4150						
Number of events	103						
Quartiles							
1st		Reference		Reference		Reference	
2nd		1.15 (0.56, 2.36)	0.70	1.00 (0.49, 2.05)	0.99	1.06 (0.52, 2.18)	0.88
3rd		1.84 (0.96, 3.52)	0.07	1.49 (0.77, 2.87)	0.24	1.43 (0.74, 2.77)	0.29
4th		3.30 (1.81, 5.99)	<0.001	2.39 (1.29, 2.87)	0.01	2.18 (1.17, 4.04)	0.01
Trend		1.55 (1.29, 1.87)	<0.001	1.40 (1.16, 1.70)	0.001	1.34 (1.10, 1.62)	0.003
Per 1 SD§		1.55 (1.34, 1.78)	<0.001	1.44 (1.23, 1.68)	<0.001	1.38 (1.17, 1.63)	<0.001
		Non-fatal acute myocardial infarction (240 events)					
		Unadjusted		Model 1*		Model 2†	
		HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value
Plasma DMG							
N	4150						
Number of events	240						
Quartiles							
1st		Reference		Reference		Reference	
2nd		1.05 (0.69, 1.60)	0.82	0.97 (0.64, 1.48)	0.89	1.00 (0.66, 1.53)	1.00
3rd		1.47 (1.00, 2.17)	0.05	1.30 (0.88, 1.93)	0.19	1.29 (0.87, 1.91)	0.21
4th		2.09 (1.45, 3.01)	<0.001	1.75 (1.20, 2.55)	0.004	1.65 (1.13, 2.41)	0.01
Trend		1.31 (1.16, 1.47)	<0.001	1.24 (1.10, 1.40)	0.001	1.20 (1.07, 1.36)	0.003
Per 1 SD§		1.32 (1.18, 1.47)	<0.001	1.25 (1.11, 1.40)	<0.001	1.21 (1.08, 1.37)	0.001

CI indicates confidence interval; HR, hazard ratio; DMG, dimethylglycine; SD, standard deviation.

*Model 1 adjusted for age, gender and fasting status.

†Model 2 adjusted for age, gender, fasting status, serum apolipoprotein A1 and apolipoprotein B 100, diabetes, smoking and hypertension.

§Log transformed.

Supplemental Table IV. Hazard Ratios for Incident Acute Myocardial Infarction According to Plasma Levels of One-Carbon Metabolites and Markers of B-vitamin Status

		Unadjusted		Model 1*		Model 2†		Model 2† + quartiles plasma dimethylglycine	
		HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value
Plasma choline									
N	4150								
Number of events	343								
Quartiles									
1st		Reference		Reference		Reference		Reference	
2nd		1.03 (0.73, 1.45)	0.86	0.94 (0.67, 1.33)	0.74	0.95 (0.69, 1.38)	0.90	0.92 (0.65, 1.30)	0.64
3rd		1.25 (0.90, 1.74)	0.18	1.05 (0.75, 1.47)	0.77	1.13 (0.81, 1.58)	0.49	1.00 (0.71, 1.41)	1.00
4th		1.82 (1.34, 2.47)	<0.001	1.35 (0.98, 1.87)	0.07	1.37 (0.81, 1.58)	0.06	1.12 (0.80, 1.57)	0.52
Trend		1.24 (1.13, 1.37)	<0.001	1.12 (1.01, 1.25)	0.03	1.13 (1.02, 1.25)	0.03	1.05 (0.95, 1.17)	0.35
Per 1 SD§		1.36 (1.22, 1.51)	<0.001	1.22 (1.09, 1.37)	0.001	1.20 (1.07, 1.33)	0.001	1.11 (0.99, 1.25)	0.08
Plasma betaine									
N	4150								
Number of events	343								
Quartiles									
1st		Reference		Reference		Reference		Reference	
2nd		0.70 (0.50, 0.97)	0.03	0.64 (0.46, 0.90)	0.01	0.69 (0.50, 0.97)	0.03	0.66 (0.47, 0.92)	0.01
3rd		1.19 (0.89, 1.59)	0.23	1.04 (0.78, 1.41)	0.78	1.20 (0.89, 1.63)	0.24	1.06 (0.78, 1.45)	0.70
4th		1.13 (0.84, 1.51)	0.42	0.91 (0.67, 1.24)	0.55	1.09 (0.79, 1.49)	0.60	0.90 (0.65, 1.25)	0.54
Trend		1.09 (0.99, 1.20)	0.07	1.02 (0.92, 1.13)	0.70	1.08 (0.98, 1.20)	0.13	1.02 (0.91, 1.13)	0.77
Per 1 SD§		1.13 (1.01, 1.25)	0.03	1.05 (0.93, 1.17)	0.44	1.12 (1.00, 1.25)	0.06	1.03 (0.92, 1.17)	0.59
Plasma tHcy									
N	4150								
Number of events	343								
Quartiles									
1st		Reference		Reference		Reference		Reference	
2nd		1.40 (1.00, 1.98)	0.05	1.23 (0.87, 1.74)	0.25	1.23 (0.87, 1.73)	0.25	1.21 (0.85, 1.70)	0.29
3rd		1.16 (0.81, 1.66)	0.42	0.91 (0.63, 1.31)	0.61	0.85 (0.59, 1.23)	0.39	0.81 (0.56, 1.17)	0.26
4th		2.81 (2.06, 3.82)	<0.001	1.99 (1.44, 2.75)	<0.001	1.77 (1.28, 2.45)	0.001	1.59 (1.14, 2.21)	0.006
Trend		1.39 (1.26, 1.54)	<0.001	1.25 (1.12, 1.38)	<0.001	1.19 (1.07, 1.32)	0.001	1.15 (1.03, 1.27)	0.01

Per 1 SD§		1.37 (1.26, 1.50)	<0.001	1.27 (1.15, 1.40)	<0.001	1.21 (1.10, 1.34)	<0.001	1.16 (1.05, 1.29)	0.004
Plasma methionine									
N	4150								
Number of events	343								
Quartiles									
1st		Reference		Reference		Reference		Reference	
2nd		0.95 (0.71, 1.27)	0.72	0.96 (0.72, 1.29)	0.80	0.98 (0.73, 1.31)	0.88	0.97 (0.72, 1.30)	0.82
3rd		0.75 (0.55, 1.02)	0.07	0.74 (0.54, 1.01)	0.06	0.75 (0.55, 1.03)	0.07	0.69 (0.50, 0.95)	0.02
4th		1.05 (0.79, 1.40)	0.73	1.06 (0.78, 1.43)	0.71	1.06 (0.78, 1.43)	0.70	0.95 (0.70, 1.29)	0.76
Trend		1.00 (0.91, 1.09)	0.92	0.99 (0.90, 1.10)	0.87	0.99 (0.90, 1.10)	0.87	0.95 (0.86, 1.06)	0.36
Per 1 SD		1.00 (0.90, 1.11)	0.96	0.99 (0.89, 1.11)	0.89	0.99 (0.89, 1.11)	0.86	0.95 (0.85, 1.06)	0.33
Plasma sarcosine									
N	2423								
Number of events	160								
Quartiles									
1st		Reference		Reference		Reference		Reference	
2nd		0.72 (0.46, 1.14)	0.16	0.75 (0.47, 1.18)	0.21	0.76 (0.48, 1.21)	0.25	0.72 (0.46, 1.15)	0.17
3rd		1.00 (0.66, 1.53)	0.99	1.01 (0.66, 1.54)	0.96	1.03 (0.67, 1.58)	0.89	0.94 (0.61, 1.45)	0.78
4th		1.02 (0.66, 1.56)	0.94	1.02 (0.66, 1.56)	0.94	1.08 (0.70, 1.66)	0.74	0.94 (0.60, 1.46)	0.77
Trend		1.04 (0.90, 1.19)	0.61	1.03 (0.90, 1.19)	0.65	1.05 (0.91, 1.21)	0.49	1.00 (0.87, 1.16)	0.95
Per 1 SD§		1.09 (0.93, 1.37)	0.30	1.08 (0.93, 1.27)	0.33	1.11 (0.94, 1.30)	0.22	1.05 (0.89, 1.24)	0.54
Plasma riboflavin									
N	4125								
Number of events	339								
Quartiles									
1st		Reference		Reference		Reference		Reference	
2nd		0.99 (0.72, 1.37)	0.97	0.96 (0.70, 1.32)	0.81	1.00 (0.73, 1.38)	0.98	1.01 (0.73, 1.38)	0.97
3rd		1.12 (0.82, 1.53)	0.47	1.04 (0.77, 1.42)	0.78	1.06 (0.77, 1.44)	0.73	1.07 (0.78, 1.46)	0.68
4th		1.35 (1.00, 1.82)	0.05	1.20 (0.89, 1.32)	0.24	1.32 (0.97, 1.78)	0.07	1.31 (0.96, 1.77)	0.09
Trend		1.11 (1.01, 1.22)	0.03	1.07 (0.97, 1.18)	0.93	1.10 (0.99, 1.21)	0.07	1.09 (0.99, 1.21)	0.07
Per 1 SD§		1.14 (1.03, 1.26)	0.01	1.09 (0.98, 1.20)	0.11	1.13 (1.02, 1.25)	0.02	1.12 (1.01, 1.24)	0.03

Serum folate									
N	4148								
Number of events	341								
Quartiles									
1st		Reference		Reference		Reference		Reference	
2nd		1.11 (0.83, 1.48)	0.50	1.14 (0.76, 1.39)	0.39	1.21 (0.91, 1.62)	0.20	1.25 (0.93, 1.67)	0.14
3rd		0.80 (0.58, 1.09)	0.16	0.81 (0.59, 1.11)	0.20	0.89 (0.65, 1.22)	0.46	0.91 (0.66, 1.25)	0.57
4th		1.02 (0.76, 1.37)	0.90	1.03 (0.76, 1.39)	0.86	1.16 (0.85, 1.57)	0.35	1.21 (0.89, 1.64)	0.23
Trend		0.97 (0.89, 1.07)	0.59	0.98 (0.89, 1.07)	0.61	1.01 (0.92, 1.12)	0.79	1.03 (0.93, 1.13)	0.60
Per 1 SD§		1.02 (0.91, 1.13)	0.79	1.01 (0.91, 1.12)	0.89	1.05 (0.95, 1.16)	0.37	1.06 (0.96, 1.18)	0.26
Serum PLP									
N	4125								
Number of events	339								
Quartiles									
1st		Reference		Reference		Reference		Reference	
2nd		0.80 (0.60, 1.06)	0.12	0.84 (0.63, 1.11)	0.21	0.90 (0.68, 1.20)	0.48	0.94 (0.71, 1.25)	0.66
3rd		0.64 (0.48, 0.87)	0.004	0.68 (0.50, 0.92)	0.01	0.81 (0.59, 1.10)	0.18	0.85 (0.63, 1.16)	0.31
4th		0.68 (0.50, 0.91)	0.01	0.73 (0.54, 0.99)	0.04	0.89 (0.66, 1.22)	0.47	0.91 (0.67, 1.24)	0.56
Trend		0.86 (0.79, 0.95)	0.003	0.89 (0.81, 0.98)	0.02	0.95 (0.86, 1.05)	0.35	0.96 (0.87, 1.06)	0.44
Per 1 SD§		0.86 (0.77, 0.96)	0.01	0.89 (0.79, 0.99)	0.04	0.97 (0.86, 1.08)	0.55	0.97 (0.87, 1.08)	0.59
Serum cobalamin									
N	3658								
Number of events	285								
Quartiles									
1st		Reference		Reference		Reference		Reference	
2nd		0.79 (0.57, 1.09)	0.15	0.84 (0.60, 1.16)	0.29	0.84 (0.60, 1.16)	0.28	0.85 (0.61, 1.18)	0.32
3rd		0.88 (0.64, 1.20)	0.41	0.98 (0.71, 1.34)	0.89	0.94 (0.69, 1.30)	0.72	0.95 (0.69, 1.31)	0.76
4th		0.76 (0.55, 1.05)	0.10	0.84 (0.60, 1.18)	0.32	0.86 (0.61, 1.20)	0.36	0.84 (0.60, 1.18)	0.31
Trend		0.93 (0.84, 1.03)	0.16	0.96 (0.87, 1.07)	0.50	0.96 (0.87, 1.07)	0.50	0.96 (0.86, 1.07)	0.45
Per 1 SD§		0.93 (0.82, 1.04)	0.21	0.96 (0.85, 1.08)	0.48	0.96 (0.86, 1.09)	0.54	0.96 (0.85, 1.08)	0.48
Plasma MMA									

N	4150								
Number of events	343								
Quartiles									
1st		Reference		Reference		Reference		Reference	
2nd		0.60 (0.42, 0.85)	0.004	0.52 (0.36, 0.74)	<0.001	0.52 (0.36, 0.74)	<0.001	0.48 (0.33, 0.69)	<0.001
3rd		1.14 (0.84, 1.53)	0.41	0.91 (0.67, 1.24)	0.91	0.94 (0.69, 1.28)	0.94	0.84 (0.61, 1.15)	0.27
4th		1.61 (1.22, 1.53)	0.001	1.13 (0.84, 1.53)	0.43	1.07 (0.79, 1.45)	0.66	0.92 (0.67, 1.25)	0.58
Trend		1.25 (1.13, 1.37)	<0.001	1.11 (1.01, 1.23)	0.04	1.10 (0.99, 1.21)	0.08	1.05 (0.94, 1.16)	0.41
Per 1 SD§		1.27 (1.18, 1.37)	<0.001	1.18 (1.08, 1.28)	<0.001	1.15 (1.05, 1.25)	0.002	1.11 (1.01, 1.21)	0.03

CI indicates confidence interval; DMG, dimethylglycine; HR, hazard ratio; MMA, methylmalonic acid; PLP, pyridoxal phosphate; SD, standard deviation; tHcy, total homocysteine.|

*Model 1 adjusted for age, gender and fasting status.

†Model 2 adjusted for age, gender, fasting status, serum apolipoprotein A1 and apo B 100, diabetes, smoking and hypertension.

§Log transformed.

Supplemental Table V. Hazard Ratios for Incident Acute Myocardial Infarction According to Plasma Dimethylglycine, When Separately Adjusted for One-Carbon Metabolites in The Choline Pathway and Markers of B-vitamin Status

		Model 2* + quartiles plasma choline			Model 2* + quartiles plasma betaine	
		HR (95% CI)	*P* Value		HR (95% CI)	*P* Value
Plasma DMG						
N	4150			4150		
Number of events	343			343		
Quartiles						
1st		Reference			Reference	
2nd		1.00 (0.69, 1.44)	0.98		1.01 (0.70, 1.45)	0.98
3rd		1.30 (0.92, 1.83)	0.14		1.32 (0.93, 1.86)	0.12
4th		1.73 (1.23, 2.42)	0.002		1.78 (1.27, 2.49)	0.001
Trend		1.23 (1.10, 1.37)	<0.001		1.24 (1.12, 1.38)	<0.001
Per 1 SD†		1.25 (1.13, 1.39)	<0.001		1.27 (1.15, 1.40)	<0.001

		Model 2* + quartiles plasma tHcy			Model 2* + quartiles methionine	
		HR (95% CI)	*P* Value		HR (95% CI)	*P* Value
Plasma DMG						
N	4150			4150		
Number of events	343			343		
Quartiles						
1st		Reference			Reference	
2nd		1.00 (0.70, 1.44)	1.00		1.02 (0.71, 1.47)	0.90
3rd		1.28 (0.91, 1.79)	0.16		1.36 (0.97, 1.91)	0.08
4th		1.69 (1.22, 2.34)	0.002		1.86 (1.34, 2.57)	<0.001
Trend		1.22 (1.10, 1.35)	<0.001		1.26 (1.13, 1.40)	<0.001
Per 1 SD†		1.24 (1.13, 1.37)	<0.001		1.28 (1.16, 1.41)	<0.001

		Model 2* + quartiles plasma sarcosine			Model 2* + quartiles plasma riboflavin	
		HR (95% CI)	*P* Value		HR (95% CI)	*P* Value
Plasma DMG						

N	2423			4125		
Number of events	160			339		
Quartiles						
1st		Reference			Reference	
2nd		1.05 (0.64, 1.71)	0.86		0.97 (0.68, 1.41)	0.89
3rd		1.30 (0.81, 2.09)	0.28		1.30 (0.93, 1.83)	0.13
4th		1.80 (1.13, 2.87)	0.01		1.77 (1.29, 2.45)	<0.001
Trend		1.23 (1.06, 1,43)	0.007		1.24 (1.12, 1.38)	<0.001
Per 1 SD†		1.29 (1.11, 1.51)	<0.001		1.26 (1.14, 1.39)	<0.001

		Model 2* + quartiles plasma folate			Model 2* + quartiles plasma PLP	
		HR (95% CI)	*P* Value		HR (95% CI)	*P* Value
Plasma DMG						
N	4148			4125		
Number of events	341			339		
Quartiles						
1st		Reference			Reference	
2nd		1.03 (0.72, 1.49)	0.86		0.98 (0.68, 1.41)	0.90
3rd		1.36 (0.97, 1.91)	0.07		1.30 (0.93, 1.83)	0.12
4th		1.85 (1.33, 2.55)	<0.001		1.77 (1.28, 2.45)	0.001
Trend		1.25 (1.13, 1.39)	<0.001		1.24 (1.12, 1.38)	<0.001
Per 1 SD†		1.27 (1.16, 1.40)	<0.001		1.27 (1.15, 1.39)	<0.001

		Model 2* + quartiles plasma cobalamin			Model 2* + quartiles plasma MMA	
		HR (95% CI)	*P* Value		HR (95% CI)	*P* Value
Plasma DMG						
N	3658			4150		
Number of events	285			343		
Quartiles						
1st		Reference			Reference	
2nd		1.02 (0.70, 1.49)	0.91		1.01 (0.70, 1.46)	0.95
3rd		1.33 (0.94, 1.90)	0.11		1.32 (0.94, 1.85)	0.11
4th		1.71 (1.21, 2.41)	0.002		1.77 (1.27, 2.46)	0.001

Trend	1.22 (1.09, 1.36)	0.001	1.24 (1.11, 1.37)	<0.001
Per 1 SD†	1.23 (1.11, 1.37)	<0.001	1.26 (1.14, 1.39)	<0.001

CI indicates confidence interval; DMG, dimethylglycine; HR, hazard ratio; MMA, methylmalonic acid; PLP, pyridoxal phosphate; SD, standard deviation; tHcy, total homocysteine.

*Model 2 adjusted for age, gender, fasting status, serum apolipoprotein A1 and apo B 100, diabetes, smoking and hypertension.

†Log transformed.

Supplemental Table VI. Hazard Ratios for Incident Acute Myocardial Infarction According to Plasma Dimethylglycine in Subgroups of Traditional CAD Risk Factors (Whole Population) and Folic Acid Treatment (WENBIT Participants only)

	Gender				Age			
	Males		Females		≤median		>median	
N	2987		1163		2174		1976	
Number of events	266		77		132		211	
	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value
Plasma DMG								
Quartiles								
1st	Reference		Reference		Reference		Reference	
2nd	1.15 (0.75, 1.76)	0.53	0.70 (0.33, 1.50)	0.36	0.65 (0.38, 1.11)	0.11	1.72 (1.00, 2.94)	0.05
3rd	1.41 (0.94, 2.11)	0.09	1.14 (0.60, 2.16)	0.70	1.06 (0.66, 1.69)	0.81	2.08 (1.24, 3.48)	0.01
4th	1.86 (1.13, 2.74)	0.002	1.76 (0.97, 3.20)	0.06	1.27 (0.80, 2.01)	0.31	3.20 (1.96, 5.24)	<0.001
Trend	1.24 (1.10, 1.40)	<0.001	1.25 (1.02, 1.53)	0.04	1.12 (0.96, 1.31)	0.15	1.43 (1.25, 1.64)	<0.001
Per 1 SD*	1.28 (1.14, 143)	<0.001	1.23 (1.03, 1.47)	0.02	1.10 (0.93, 1.30)	0.27	1.45 (1.30, 1.62)	<0.001

	Smoking				Diabetes			
	No		Yes		No		Yes	
N	2839		1311		3659		491	
Number of events	202		141		272		71	
	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value
Plasma DMG								
Quartiles								
1st	Reference		Reference		Reference		Reference	
2nd	1.10 (0.67, 1.81)	0.71	0.95 (0.55, 1.62)	0.84	1.13 (0.75, 1.70)	0.57	0.68 (0.30, 1.55)	0.36
3rd	1.57 (0.99, 2.49)	0.06	1.09 (0.66, 1.79)	0.75	1.39 (0.94, 2.05)	0.10	1.22 (0.61, 2.45)	0.58
4th	2.53 (1.64, 3.91)	<0.001	1.12 (0.69, 1.82)	0.65	1.88 (1.29, 2.73)	0.001	1.62 (0.86, 3.08)	0.14
Trend	1.41 (1.23, 1.61)	<0.001	1.05 (0.90, 1.23)	0.54	1.25 (1.11, 1.40)	<0.001	1.23 (0.99, 1.53)	0.06
Per 1 SD*	1.38 (1.23, 1.56)	<0.001	1.11 (0.95, 1.29)	0.19	1.28 (1.15, 1.42)	<0.001	1.23 (0.98, 1.54)	0.07

	Hypertension				Previous myocardial infarction			
	No		Yes		No		Yes	
N	2211		1939		2475		1674	
Number of events	148		195		117		226	

	HR (95% CI)	P Value	HR (95% CI)	P Value	HR (95% CI)	P Value	HR (95% CI)	P Value
Plasma DMG Quartiles								
1st	Reference		Reference		Reference		Reference	
2nd	0.99 (0.57, 1.70)	0.96	1.03 (0.63, 1.69)	0.90	0.83 (0.46, 1.48)	0.52	1.10 (0.69, 1.77)	0.69
3rd	1.42 (0.86, 2.36)	0.17	1.25 (0.79, 1.97)	0.35	1.17 (0.69, 1.97)	0.56	1.44 (0.92, 2.26)	0.11
4th	1.74 (1.05, 2.86)	0.03	1.86 (1.22, 2.84)	0.004	1.36 (0.80, 2.30)	0.26	1.90 (1.25, 2.88)	0.003
Trend	1.24 (1.06, 1.45)	0.009	1.26 (1.10, 1.44)	0.001	1.14 (0.96, 1.35)	0.14	1.26 (1.11, 1.43)	<0.001
Per 1 SD*	1.21 (1.03, 1.42)	0.02	1.31 (1.16, 1.48)	<0.001	1.17 (0.99, 1.40)	0.07	1.28 (1.14, 1.44)	<0.001

	Estimated glomerular filtration rate				Folic acid treatment†			
	≤median		>median		No		Yes	
N	2170		1980		1287		1278	
Number of events	228		115		96		115	
	HR (95% CI)	P Value	HR (95% CI)	P Value	HR (95% CI)	P Value	HR (95% CI)	P Value
Plasma DMG Quartiles								
1st	Reference		Reference		Reference		Reference	
2nd	1.21 (0.71, 2.07)	0.49	0.87 (0.52, 1.45)	0.60	1.20 (0.67, 2.16)	0.55	0.95 (0.53, 1.73)	0.88
3rd	1.54 (0.94, 2.52)	0.09	1.15 (0.70, 1.90)	0.59	1.29 (0.72, 2.28)	0.39	1.43 (0.81, 2.53)	0.22
4th	2.29 (1.43, 3.66)	0.001	1.10 (0.65, 1.86)	0.74	1.35 (0.75, 2.45)	0.32	2.02 (1.17, 3.50)	0.01
Trend	1.34 (1.17, 1.54)	<0.001	1.05 (0.89, 1.24)	0.55	1.10 (0.92, 1.33)	0.30	1.31 (1.10, 1.57)	0.003
Per 1 SD*	1.33 (1.20, 1.49)	<0.001	1.02 (0.84, 1.24)	0.84	1.07 (0.88, 1.31)	0.49	1.48 (1.23, 1.78)	<0.001

	Serum total cholesterol				Serum low density lipoprotein cholesterol			
	≤median		>median		≤median		>median	
N	2111		2037		2092		2055	
Number of events	169		174		174		169	
	HR (95% CI)	P Value	HR (95% CI)	P Value	HR (95% CI)	P Value	HR (95% CI)	P Value
Plasma DMG Quartiles								
1st	Reference		Reference		Reference		Reference	
2nd	0.82 (0.47, 1.42)	0.47	1.21 (0.74, 1.95)	0.45	0.74 (0.44, 1.26)	0.26	1.31 (0.79, 2.18)	0.29

3rd	1.14 (0.68, 1.90)	0.62	1.54 (0.98, 2.43)	0.06	1.07 (0.66, 1.74)	0.78	1.64 (1.02, 2.64)	0.04
4th	1.72 (1.07, 2.76)	0.03	1.74 (1.12, 2.72)	0.02	1.62 (1.04, 2.51)	0.03	1.95 (1.22, 3.13)	0.01
Trend	1.27 (1.09, 1.48)	0.002	1.21 (1.05, 1.39)	0.01	1.25 (1.08, 1.44)	0.003	1.24 (1.08, 1.44)	0.003
Per 1 SD*	1.29 (1.13, 1.47)	<0.001	1.23 (1.06, 1.41)	0.01	1.27 (1.11, 1.44)	<0.001	1.24 (1.08, 1.43)	0.003

	Serum high density lipoprotein cholesterol				Serum triglycerides			
	≤median		>median		≤median		>median	
N	2133		2015		2160		1986	
Number of events	204		139		157		186	
	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value
Plasma DMG								
Quartiles								
1st	Reference		Reference		Reference		Reference	
2nd	0.98 (0.62, 1.56)	0.95	1.04 (0.58, 1.88)	0.89	1.20 (0.63, 2.28)	0.58	0.99 (0.64, 1.55)	0.97
3rd	1.02 (0.65, 1.60)	0.94	1.87 (1.11, 3.15)	0.02	2.19 (1.22, 3.91)	0.01	1.01 (0.66, 1.56)	0.96
4th	1.53 (1.01, 2.32)	0.04	2.26 (1.35, 3.78)	0.002	3.04 (1.73, 5.34)	<0.001	1.34 (0.89, 2.00)	0.16
Trend	1.17 (1.03, 1.34)	0.02	1.36 (1.16, 1.60)	<0.001	1.50 (1.27, 1.77)	<0.001	1.10 (0.97, 1.26)	0.15
Per 1 SD*	1.27 (1.11, 1.45)	0.001	1.27 (1.11, 1.47)	0.001	1.36 (1.20, 1.55)	<0.001	1.20 (1.04, 1.38)	0.03

	Serum apolipoprotein B 100				Serum apolipoprotein A1			
	≤median		>median		≤median		>median	
N	2146		2004		2122		2027	
Number of events	163		180		200		143	
	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value
Plasma DMG								
Quartiles								
1st	Reference		Reference		Reference		Reference	
2nd	0.92 (0.52, 1.64)	0.73	1.09 (0.68, 1.74)	0.73	1.00 (0.61, 1.65)	0.99	1.02 (0.60, 1.74)	0.94
3rd	1.38 (0.82, 2.33)	0.23	1.33 (0.85, 2.06)	0.21	1.43 (0.90, 2.28)	0.13	1.21 (0.74, 1.99)	0.45
4th	2.15 (1.32, 3.49)	0.002	1.49 (0.96, 2.30)	0.08	1.82 (1.17, 2.83)	0.01	1.77 (1.10, 2.84)	0.02
Trend	1.36 (1.16, 1.59)	<0.001	1.15 (1.00, 1.32)	0.05	1.26 (1.10, 1.44)	0.001	1.22 (1.05, 1.43)	0.01
Per 1 SD*	1.32 (1.16, 1.51)	<0.001	1.19 (1.03, 1.36)	0.02	1.27 (1.12, 1.44)	<0.001	1.25 (1.08, 1.45)	0.003

CAD indicates coronary artery disease, CI; confidence interval; DMG, dimethylglycine; HR, hazard ratio; SD, standard deviation, WENBIT; Western Norway B-Vitamin Intervention Trial.

Models are adjusted for age, gender, serum apolipoprotein AI and apolipoprotein B 100, diabetes mellitus, smoking and hypertension.
*Log transformed
†Participants in the Western Norway B-Vitamin Intervention Trial only.

Supplemental Table VII. Unadjusted Associations Between Traditional CAD Risk Factors and Incident Acute Myocardial infarction, According to Plasma Dimethylglycine Levels

	Total population HR (95% CI)	Plasma DMG ≤median HR (95% CI)	Plasma DMG >median HR (95% CI)	*P* for interaction
Male gender	1.35 (1.04, 1.74)	1.46 (0.96, 2.20)	1.14 (0.83, 1.57)	0.35
Age, years†	1.60 (1.42, 1.79)	1.24 (1.03, 1.50)	1.73 (1.49, 2.00)	0.01
Smoking	1.54 (1.24, 1.91)	2.05 (1.43, 2.93)	1.23 (0.94, 1.62)	0.03
Diabetes	2.09 (1.61, 2.71)	2.05 (1.32, 3.18)	2.13 (1.54, 2.95)	0.87
Hypertension	1.52 (1.23, 1.89)	1.61 (1.13, 2.30)	1.38 (1.05, 1.80)	0.49
Previous MI	2.89 (2.31, 3.61)	2.67 (1.85 3.84)	2.82 (2.12, 3.75)	0.83
Serum lipids and lipoproteins*				
Triglycerides, mmol/L†	1.13 (1.01, 1.25)	1.26 (1.06, 1.49)	1.05 (0.92, 1.21)	0.11
Apolipoprotein B 100, g/dL	1.13 (1.02, 1.25)	1.23 (1.04, 1.44)	1.10 (0.96, 1.25)	0.30
Apolipoprotein A1, g/dL†	0.85 (0.77, 0.95)	0.90 (0.75, 1.07)	0.84 (0.74, 0.96)	0.58
eGFR, mL/min/1.73m^2*†	0.76 (0.71, 0.80)	0.72 (0.57, 0.91)	0.79 (0.74, 0.84)	0.51

CAD indicates coronary artery disease; CI, confidence interval; DMG, dimethylglycine; eGFR, estimated glomerular filtration rate; HR, hazard ratio; MI, myocardial infarction.

*Per 1 standard deviation.

†Log transformed.

Supplemental Table VIII. Hazard Ratios for Incident Acute Myocardial Infarction According to Plasma Dimethylglycine in Subgroups of Traditional CAD Risk Factors (Whole Population) and Folic Acid Treatment (WENBIT Participants only), Stratified for Fasting Status

	Male gender				Female gender			
	Non-fasting		Fasting		Non-fasting		Fasting	
N	2118		869		928		235	
Number of events	196		70		59		18	
	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value
Plasma DMG								
Quartiles								
1st	Reference		Reference		Reference		Reference	
2nd	0.95 (0.56, 1.61)	0.84	1.54 (0.75, 3.16)	0.24	0.51 (0.21, 1.25)	0.14	1.73 (0.38, 7.89)	0.48
3rd	1.43 (0.89, 2.30)	0.14	1.05 (0.47, 2.37)	0.90	0.82 (0.38, 1.75)	0.60	2.34 (0.65, 8.43)	0.19
4th	1.61 (1.02, 2.54)	0.04	2.49 (1.24, 4.98)	0.01	1.55 (0.80, 2.98)	0.19	2.48 (0.60, 10.23)	0.21
Trend	1.32 (1.05, 1.39)	0.007	1.30 (1.04, 1.62)	0.02	1.21 (0.96, 1.53)	0.11	1.37 (0.89, 2.10)	0.15
Per 1 SD*	1.25 (1.10, 1.43)	0.001	1.33 (1.06, 1.65)	0.01	1.25 (1.01, 1.54)	0.04	1.31 (0.90, 1.92)	0.16

	Age≤median				Age>median			
	Non-fasting		Fasting		Non-fasting		Fasting	
N	1565		609		1481		495	
Number of events	101		31		154		57	
	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value
Plasma DMG								
Quartiles								
1st	Reference		Reference		Reference		Reference	
2nd	0.57 (0.30, 1.09)	0.09	0.85 (0.32, 2.28)	0.75	1.27 (0.65, 2.45)	0.48	2.87 (1.11, 7.37)	0.03
3rd	1.12 (0.66, 1.90)	0.66	0.82 (0.28, 2.42)	0.72	1.78 (0.97, 3.29)	0.06	2.54 (0.97, 6.66)	0.06
4th	1.21 (0.71, 2.04)	0.49	1.21 (0.48, 3.08)	0.69	2.54 (1.42, 4.53)	0.002	5.04 (2.02, 12.61)	0.001
Trend	1.12 (0.94, 1.34)	0.20	1.05 (0.77, 1.44)	0.75	1.39 (1.18, 1.63)	<0.001	1.54 (1.20, 1.99)	0.001
Per 1 SD*	1.08 (0.89, 1.31)	0.46	1.08 (0.77, 1.52)	0.65	1.46 (1.28, 1.67)	<0.001	1.45 (1.17, 1.80)	0.001

	Smoking		Non-smoking	
	Non-fasting	Fasting	Non-fasting	Fasting
N	910	401	2136	703
Number of events	95	46	160	42

	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value
Plasma DMG Quartiles								
1st	Reference		Reference		Reference		Reference	
2nd	0.69 (0.34, 1.41)	0.31	1.48 (0.64, 3.46)	0.36	0.89 (0.50, 1.58)	0.68	1.95 (0.71, 5.37)	0.20
3rd	1.11 (0.61, 2.01)	0.74	0.93 (0.36, 2.41)	0.88	1.36 (0.81, 2.28)	0.25	2.13 (0.78, 5.84)	0.14
4th	0.79 (0.43, 1.44)	0.45	1.95 (0.87, 4.38)	0.10	2.25 (1.39, 3.65)	0.001	3.37 (1.27, 8.96)	0.02
Trend	0.97 (0.80, 1.17)	0.75	1.20 (0.92, 1.56)	0.18	1.39 (1.19, 163)	<0.001	1.43 (1.07, 1.91)	0.02
Per 1 SD*	1.08 (0.89, 1.31)	0.45	1.14 (0.89, 1.47)	0.29	1.35 (1.18, 1.55)	<0.001	1.58 (1.17, 2.15)	0.003

	Diabetes				No diabetes			
	Non-fasting		Fasting		Non-fasting		Fasting	
N	362		129		2684		975	
Number of events	54		17		201		71	
	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value
Plasma DMG Quartiles								
1st	Reference		Reference		Reference		Reference	
2nd	0.64 (0.26, 1.60)	0.34	0.94 (0.13, 6.95)	0.96	0.89 (0.53, 1.49)	0.65	1.81 (0.92, 3.59)	0.09
3rd	0.89 (0.40, 1.99)	0.78	2.88 (0.56, 14.74)	0.20	1.41 (0.89, 2.23)	0.15	1.09 (0.51, 2.35)	0.83
4th	1.29 (0.64, 2.58)	0.48	3.18 (0.62, 16.15)	0.16	1.69 (1.08, 2.64)	0.02	2.20 (1.12, 4.32)	0.02
Trend	1.12 (0.89, 1.43)	0.34	1.56 (0.95, 2.56)	0.08	1.25 (1.09, 1.43)	0.002	1.22 (0.98, 1.51)	0.07
Per 1 SD*	1.16 (0.91, 1.50)	0.24	1.78 (0.97, 3.25)	0.06	1.28 (1.13, 1.45)	<0.001	1.24 (1.01,1 52)	0.04

	Hypertension				No hypertension			
	Non-fasting		Fasting		Non-fasting		Fasting	
N	1441		498		1605		606	
Number of events	141		54		114		34	
	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value
Plasma DMG Quartiles								
1st	Reference		Reference		Reference		Reference	
2nd	0.86 (0.47, 1.57)	0.62	1.32 (0.56, 3.12)	0.52	0.76 (0.39, 1.47)	0.41	1.86 (0.68, 5.09)	0.23

3rd	1.12 (0.65, 1.93)	0.69	1.35 (0.58, 3.12)	0.49	1.39 (0.79, 2.45)	0.26	1.35 (0.43, 4.24)	0.61
4th	1.61 (0.97, 2.65)	0.06	2.28 (1.04, 4.98)	0.04	1.50 (0.85, 2.64)	0.16	3.02 (1.08, 8.43)	0.04
Trend	1.22 (1.04, 1.43)	0.02	1.30 (1.01, 1.67)	0.04	1.21 (1.01, 1.45)	0.04	1.35 (0.98, 1.86)	0.06
Per 1 SD*	1.29 (1.12, 1.48)	<0.001	1.34 (1.05, 1.71)	0.02	1.20 (0.99, 1.46)	0.06	1.22 (0.91, 1.63)	0.18

	Previous myocardial infarction				No previous myocardial infarction			
	Non-fasting		Fasting		Non-fasting		Fasting	
N	1212		462		1833		642	
Number of events	165		61		90		27	
	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value
Plasma DMG								
Quartiles								
1st	Reference		Reference		Reference		Reference	
2nd	0.77 (0.43, 1.38)	0.38	1.93 (0.85, 4.38)	0.11	0.76 (0.38, 1.50)	0.43	1.13 (0.37, 3.44)	0.83
3rd	1.19 (0.71, 2.00)	0.52	1.76 (0.74, 4.21)	0.20	1.16 (0.64, 2.10)	0.63	1.04 (0.35, 3.05)	0.95
4th	1.46 (0.90, 2.37)	0.13	2.91 (0.34, 6.35)	0.007	1.23 (0.67, 2.25)	0.51	1.74 (0.59, 5.14)	0.32
Trend	1.21 (1.04, 1.40)	0.02	1.36 (1.08, 1.71)	0.01	1.11 (0.91, 1.36)	0.28	1.17 (0.82, 1.67)	0.40
Per 1 SD*	1.24 (1.07, 1.42)	0.003	1.29 (1.04, 1.61)	0.02	1.16 (0.95, 1.42)	0.14	1.20 (0.83, 1.74)	0.34

	Estimated glomerular filtration rate≤median				Estimated glomerular filtration rate>median			
	Non-fasting		Fasting		Non-fasting		Fasting	
N	1694		476		1352		628	
Number of events	170		58		85		30	
	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value
Plasma DMG								
Quartiles								
1st	Reference		Reference		Reference		Reference	
2nd	0.96 (0.49, 1.86)	0.90	2.00 (0.79, 5.07)	0.15	0.76 (0.41, 1.40)	0.37	1.28 (0.50, 3.26)	0.61
3rd	1.53 (0.85, 2.76)	0.16	1.46 (0.58, 3.64)	0.42	1.14 (0.65, 2.00)	0.65	1.29 (0.43, 3.88)	0.66
4th	2.11 (1.20, 3.71)	0.009	2.79 (1.17, 6.64)	0.02	0.92 (0.50, 1.69)	0.78	1.41 (0.51, 3.89)	0.51
Trend	1.36 (1.15, 1.60)	<0.001	1.31 (1.02, 1.69)	0.04	1.01 (0.83, 1.23)	0.90	1.11 (0.81, 1.53)	0.51
Per 1 SD*	1.37 (1.21, 1.55)	<0.001	1.27 (1.02, 1.59)	0.04	0.94 (0.75, 1.19)	0.62	1.16 (0.81, 1.67)	0.41

	Folic acid therapy†				No folic acid therapy†			

	Non-fasting		Fasting		Non-fasting		Fasting	
N	814		464		851		436	
Number of events	79		36		68		28	
	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value
Plasma DMG								
Quartiles								
1st	Reference		Reference		Reference		Reference	
2nd	0.77 (0.38, 1.56)	0.47	1.51 (0.48, 4.73)	0.48	0.96 (0.44, 2.07)	0.91	1.70 (0.65. 4.44)	0.28
3rd	1.32 (0.69, 2.52)	0.40	1.61 (0.48, 5.35)	0.44	1.33 (0.68, 2.62)	0.41	1.11 (0.37, 3.35)	0.85
4th	1.44 (0.74, 2.80)	0.28	3.99 (1.43, 11.18)	0.008	1.46 (0.73, 2.91)	0.28	0.97 (0.28, 3.38)	0.96
Trend	1.19 (0.96, 1.48)	0.11	1.60 (1.16, 2.20)	0.004	1.16 (0.93, 1.44)	0.19	0.97 (0.68, 1.38)	0.97
Per 1 SD*	1.35 (1.07, 1.70)	0.01	1.83 (1.34, 2.50)	<0.001	1.11 (0.88, 1.41)	0.39	0.95 (0.64, 1.41)	0.81

	Serum total cholesterol≤median				Serum total cholesterol>median			
	Non-fasting		Fasting		Non-fasting		Fasting	
N	1564		547		1481		556	
Number of events	131		38		124		50	
	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value
Plasma DMG, quartil								
Quartiles								
1st	Reference		Reference		Reference		Reference	
2nd	0.70 (0.36, 1.34)	0.28	1.09 (0.37, 3.19)	0.88	0.89 (0.49, 1.64)	0.72	1.88 (0.84, 4.23)	0.13
3rd	1.07 (0.60, 1.91)	0.82	1.17 (0.39, 3.51)	0.78	1.44 (0.85, 2.45)	0.18	1.36 (0.57, 3.26)	0.49
4th	1.41 (0.82, 2.43)	0.22	3.12 (1.20, 8.10)	0.02	1.62 (0.96, 2.73)	0.07	2.15 (0.92, 5.01)	0.08
Trend	1.21 (1.01, 1.43)	0.04	1.52 (1.11, 2.08)	0.01	1.22 (1.03, 1.44)	0.02	1.21 (0.93, 1.58)	0.15
Per 1 SD*	1.26 (1.08, 1.48)	0.004	1.41 (1.09, 1.82)	0.009	1.23 (1.04, 1.45)	0.01	1.25 (0.94, 1.66)	0.12

	Serum low density lipoprotein cholesterol≤median				Serum low density lipoprotein cholesterol>median			
	Non-fasting		Fasting		Non-fasting		Fasting	
N	1519		582		1525		521	
Number of events	131		43		124		45	
	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value
Plasma DMG								
Quartiles								

1st	Reference		Reference		Reference		Reference	
2nd	0.65 (0.34, 1.23)	0.19	0.90 (0.35, 2.31)	0.83	0.96 (0.52, 1.77)	0.88	2.89 (1.15, 7.31)	0.03
3rd	1.05 (0.60, 1.84)	0.87	1.08 (0.41, 2.82)	0.88	1.48 (0.86, 2.56)	0.16	1.48 (0.56, 3.92)	0.44
4th	1.47 (0.87, 2.48)	0.15	2.03 (0.89, 4.63)	0.10	1.60 (0.93, 2.74)	0.09	3.61 (1.40, 9.36)	0.008
Trend	1.23 (1.04, 1.46)	0.02	1.30 (0.98, 1.72)	0.07	1.21 (1.02, 1.43)	0.03	1.34 (1.01, 1.78)	0.04
Per 1 SD*	1.28 (1.09, 1.49)	0.002	1.28 (0.98, 1.66)	0.07	1.22 (1.03, 1.44)	0.02	1.45 (1.08, 1.95)	0.02

	Serum high density lipoprotein cholesterol≤median				Serum high density lipoprotein cholesterol>median			
	Non-fasting		Fasting		Non-fasting		Fasting	
N	1600		558		1444		545	
Number of events	163		45		92		43	
	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value
Plasma DMG Quartiles								
1st	Reference		Reference		Reference		Reference	
2nd	0.80 (0.47, 1.36)	0.41	1.67 (0.63, 4.39)	0.30	0.78 (0.35, 1.74)	0.54	1.85 (0.77, 4.46)	0.17
3rd	0.97 (0.59, 1.58)	0.89	1.08 (0.37, 3.16)	0.89	1.86 (0.96, 3.60)	0.06	1.70 (0.70, 4.14)	0.24
4th	1.29 (0.81, 2.05)	0.28	2.85 (1.17, 6.91)	0.02	2.13 (1.11, 4.07)	0.02	1.85 (0.74, 4.60)	0.19
Trend	1.13 (0.97, 1.31)	0.11	1.38 (1.04, 1.82)	0.03	1.38 (1.13, 1.69)	0.002	1.19 (0.91, 1.57)	0.21
Per 1 SD*	1.25 (1.07, 1.45)	0.004	1.45 (1.06, 1.98)	0.02	1.28 (1.07, 1.53)	0.006	1.21 (0.94, 1.55)	0.15

	Serum triglycerides≤median				Serum triglycerides>median			
	Non-fasting		Fasting		Non-fasting		Fasting	
N	1566		594		1477		509	
Number of events	113		44		142		44	
	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value
Plasma DMG Quartiles								
1st	Reference		Reference		Reference		Reference	
2nd	0.63 (0.28, 1.42)	0.27	4.14 (1.16, 14.77)	0.03	0.92 (0.54, 1.57)	0.76	1.13 (0.50, 2.57)	0.77
3rd	1.70 (0.89, 3.26)	0.11	3.56 (0.97, 13.09)	0.06	1.01 (0.61, 1.67)	0.97	0.93 (0.39, 2.24)	0.87
4th	2.29 (1.22, 4.29)	0.01	7.26 (2.12, 24.92)	0.002	1.19 (0.74, 1.92)	0.47	1.43 (0.62, 3.30)	0.40
Trend	1.48 (1.21, 1.80)	<0.001	1.63 (1.22, 2.19)	0.001	1.07 (0.92, 1.25)	0.37	1.10 (0.83, 1.44)	0.52

Per 1 SD*	1.36 (1.17, 1.58)	<0.001	1.46 (1.14, 1.88)	0.003	1.18 (1.00, 1.39)	0.06	1.18 (0.89, 1.58)	0.25

	Serum apolipoprotein B 100≤median				Serum apolipoprotein B 100>median			
	Non-fasting		Fasting		Non-fasting		Fasting	
N	1479		593		1567		511	
Number of events	115		39		140		49	
	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value
Plasma DMG								
Quartiles								
1st	Reference		Reference		Reference		Reference	
2nd	0.57 (0.29, 1.12)	0.10	5.18 (1.13, 23.73)	0.03	1.07 (0.59, 1.94)	0.82	1.07 (0.49, 2.30)	0.87
3rd	0.97 (0.54, 1.74)	0.93	4.90 (1.03, 23.37)	0.05	1.55 (0.91, 2.64)	0.11	0.79 (0.35, 1.78)	0.57
4th	1.28 (0.75, 2.19)	0.37	12.61 (2.88, 55.20)	0.001	1.75 (1.04, 2.94)	0.04	1.11 (0.49, 2.49)	0.80
Trend	1.18 (0.99, 1.41)	0.07	1.92 (1.38, 2.66)	<0.001	1.23 (1.04, 1.44)	0.01	1.00 (0.77, 1.30)	0.98
Per 1 SD*	1.22 (1.03, 1.44)	0.02	1.58 (1.25, 2.00)	<0.001	1.25 (1.08, 1.46)	0.003	1.02 (0.76, 1.37)	0.91

	Serum apolipoprotein A1≤median				Serum apolipoprotein A1>median			
	Non-fasting		Fasting		Non-fasting		Fasting	
N	1459		663		1586		441	
Number of events	153		47		102		41	
	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value
Plasma DMG								
Quartiles								
1st	Reference		Reference		Reference		Reference	
2nd	0.70 (0.39, 1.6	0.23	2.48 (0.87, 7.07)	0.09	0.95 (0.48, 1.90)	0.89	1.12 (0.48, 2.63)	0.29
3rd	1.19(0.72, 1.98)	0.50	2.14 (0.72, 6.37	0.17	1.31 (0.71, 2.41)	0.39	0.86 (0.34, 2.15)	0.75
4th	1.36 (0.83, 2.22)	0.22	4.52 (1.67, 12.28)	0.003	1.90 (1.06, 3.39)	0.03	1.28 (0.53, 3.10)	0.58
Trend	1.18 (1.01, 1.38)	0.04	1.53 (1.16, 2.03)	0.003	1.28 (1.06, 1.54)	0.01	1.05 (0.79, 1.39)	0.75
Per 1 SD*	1.23 (1.06, 1.43)	0.006	1.44 (1.12, 1.84)	0.004	1.30 (1.09, 1.54)	0.003	1.10 (0.81, 1.48)	0.54

CAD indicates coronary artery disease, CI; confidence interval; DMG, dimethylglycine; HR, hazard ratio; SD, standard deviation, WENBIT; Western Norway B-Vitamin Intervention Trial.

Models are adjusted for age, gender, serum apolipoprotein AI and apolipoprotein B 100, diabetes mellitus, smoking and hypertension.

*Log transformed

†Participants in the Western Norway B-Vitamin Intervention Trial only.

Supplemental Table IX. Contigency Tables for Case-Control Analyses According to The BHMT 742 G>A Single Nucleotide Polymorphism, by Different Genetic Models

Full genotype table				
	GG	GA	AA	*P* Value*
Controls, n=2218	1161	912	145	0.76
Cases, n=206	111	80	15	

Dominant model			
	GG	GA+AA	*P* Value
Controls, n=2218	1161	1057	0.72
Cases, n=206	111	95	

Recessive model			
	GG+GA	AA	*P* Value
Controls, n=2218	2073	145	0.66
Cases, n=206	191	15	

Multiplicative model			
	G	A	*P* Value
Controls, number of alleles	3234	1202	0.91
Cases, number of alleles	302	110	

Additive model				
	GG	GA	AA	P_{trend}†
Controls, n=2218	1161	912	145	≥0.52
Cases, n=206	111	80	15	

BHMT indicates betaine-homocysteine methyl transferase.
*Fischer's exact test.
†Cochrane-Armitage test for various trends.

Supplemental Table X. Case-Control Analyses According to The BHMT 742 G>A Single Nucleotide Polymorphism, by Different Genetic Models

	Unadjusted Odds ratio (95% CI)	*P* Value
Dominant model		
GG	Reference	
GA+AA	0.94 (0.71, 1.25)	0.67
Recessive model		
GG+GA	Reference	
AA	1.12 (0.65, 1.95)	0.68
Multiplicative model		
G	Reference	
A	0.98 (0.78, 1.23)	0.86
Additive model		
GG	Reference	
GA	0.92 (0.68, 1.24)	0.57
AA	1.08 (0.61, 1.91)	0.79
Trend	0.98 (0.78, 1.24)	0.86

BHMT indicates betaine-homocysteine methyl transferase; CI, confidence interval.