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Plasma methionine and risk of acute myocardial infarction: Effect modification by established risk factors

Indu Dhar [a, b, *], Vegard Lysne [a], Reinhard Seifert [c], Gard F.T. Svingen [c], Per M. Ueland [a, d], Ottar K. Nygård [a, b, c]

[a] Department of Clinical Science, University of Bergen, 5021, Bergen, Norway
[b] KG Jebsen Center for Diabetes Research, University of Bergen, 5009, Bergen, Norway
[c] Department of Heart Disease, Haukeland University Hospital, 5021, Bergen, Norway
[d] Bevital AS, 5021, Bergen, Norway

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ABSTRACT

Background and aims: Methionine (Met) is an essential amino acid involved in methylation reactions and lipid metabolism. A Met-deficient diet may cause hepatic lipid accumulation, which is considered an independent risk factor for atherosclerosis. However, the prospective relationship between circulating Met and incident acute myocardial infarction (AMI) is unknown.

Methods: We studied the associations of plasma Met and incident AMI in 4156 patients (77% men; median age 62 years) with stable angina pectoris, among whom the majority received lipid lowering therapy with statins. Risk associations were estimated using Cox-regression analyses.

Results: Plasma Met was negatively related to age, serum levels of total cholesterol, low-density lipoprotein cholesterol (LDL-C) and apolipoprotein (apo) B at baseline (all $p \leq 0.05$). During a median follow-up of 7.5 years, 534 (12.8%) patients experienced an AMI. There was no overall association between plasma Met and incident AMI; however, plasma Met was inversely associated with risk among patients with high as compared to low levels of serum LDL-C or apo B 100 (multivariate adjusted HRs per SD [95% CI] 0.84 [0.73–0.96] and 0.83[0.73–0.95], respectively; p-interaction ≤ 0.02). Trends towards an inverse risk relationship were also observed among those younger than 62 years and patients without diabetes or hypertension.

Conclusions: Low plasma Met was associated with increased risk of AMI in patients with high circulating levels of atherogenic lipids, but also in subgroups with presumably lower cardiovascular risk. The determinants of Met status and their relation with residual cardiovascular risk in patients with coronary heart disease should be further investigated.

1. Introduction

Methionine (Met) is an essential sulfur-based amino acid naturally present in the diet, or regenerated from homocysteine by the enzymes Met synthase (MS) or betaine-homocysteine methyltransferase (BHMT), using tetrahydrofolate (THF) and betaine as the methyl donors, respectively (Fig. 1) [1]. Met is in turn metabolized to the global methyl donor S-adenosylmethionine by the enzyme methionine adenosyltransferase [1]. Following the removal of the methyl group and catalyzed by glycine N-methyltransferase (GNMT), the product S-adenosylhomocysteine is hydrolyzed to homocysteine, a risk factor for coronary artery disease (CAD) [2].

Met is involved in numerous metabolic processes, such as, protein synthesis, polyamine metabolism, glutathione synthesis and methylation reactions including DNA [1]. Animal studies also suggest that Met availability regulates the flux through GNMT [3], which has been shown to affect the composition of atherosclerotic lesions, and the regulation of inflammatory responses [4] and hepatic cholesterol metabolism [5]. Indeed, Met deficiency has been directly associated with hepatic lipid accumulation through blocking hepatic very-low-density lipoprotein (VLDL) secretion and inhibiting mitochondrial fatty acid β-oxidation [6,7]. Notably,

* Corresponding author. Department of Clinical Science, University of Bergen, Laboratory Building, 9th floor, Haukeland University Hospital, Jonas Lies vei 87, Bergen, N-5021, Norway.
E-mail address: Indu.Dhar@uib.no (I. Dhar).

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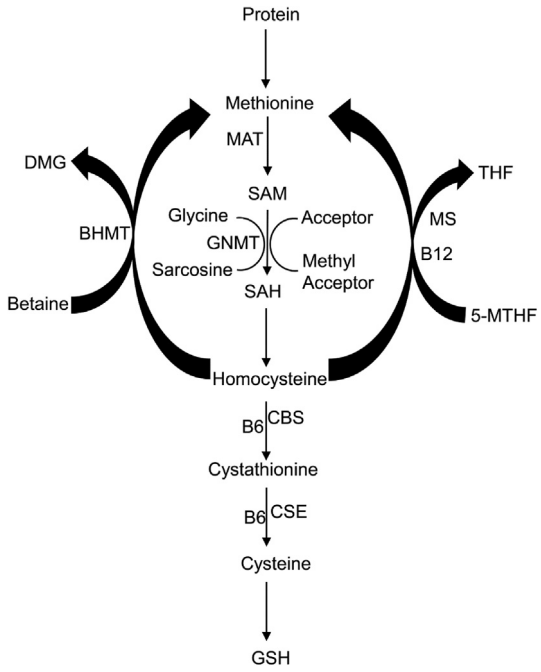

Fig. 1. Methionine metabolism.
MAT, methionine S-adenosyltransferase; SAM, S−adenosylmethionine; SAH, S-adenosylhomocysteine; CBS, cystathionine beta-synthase; BHMT, betaine-homocysteine methyltransferase; MS, methionine synthase; 5-MTHF, 5-methylenetetrahydrofolate; THF, tetrahydrofolate; GSH, glutathione.

hepatic lipid accumulation is consistently related to increased risk of atherosclerosis in several studies [8–10]. Additionally, Met deficiency can induce site-specific hypomethylation [11], which has recently been linked to increased expression of proprotein convertase subtilisin/kexin type 9 (PCSK9), a serine protease involved in the degradation of both hepatic and extrahepatic LDL receptors (LDL-Rs), thereby increasing circulating LDL cholesterol concentrations [12]. Moreover, Met deficiency increases the susceptibility of lipoproteins to oxidation [13], which may further promote oxidized low-density lipoprotein (oxLDL)-induced foam cell formation and progression of atheromatous plaques [4,13].

Taken together, these observations suggest that the availability of the sulfur amino acid, Met, can affect lipid metabolism and thus may be related to the development of atherosclerotic CAD. However, few studies have evaluated the association between circulating Met and cardiovascular disease (CVD), and findings have been inconsistent [2,14,15]. One study found lower concentrations of Met in cases than in controls [2], while another study did not observe such an association [14]. On the other hand, lower Met levels have been associated with increased risk of venous thrombosis [15], which in turn is related to atherosclerosis of the arteries [16]. Nevertheless, the direct relationship between plasma Met and acute myocardial infarction (AMI) is not known.

We investigated the relation of plasma Met with long-term risk of incident AMI in a large prospective cohort of patients with suspected stable angina pectoris (SAP), additionally exploring potential effect modifications by baseline risk factors for CAD.

2. Patients and methods

2.1. Study cohort 14, 15

A total of 4164 adult patients, undergoing elective coronary angiography due to suspected SAP between 1999 and 2004 at Haukeland (n = 3413) and Stavanger (n = 751) University Hospitals in Western Norway were enrolled in this study. The study protocol has been described in detail elsewhere [17]. Of these, 2573 (61.8%) were included in the Western Norway B-vitamin Intervention Trial (WENBIT, NCT00354081), to investigate the effect of vitamin B treatment on all-cause mortality and cardiovascular outcomes [18]. Patients with missing plasma Met data at baseline were excluded from the study, leaving a total of 4156 patients with SAP for the final analyses. The study protocol was approved by the regional ethics committee and the Norwegian Data Inspectorate, and was carried out according to the Declaration of Helsinki. All participants provided informed consent.

2.2. Baseline data

Clinical information about patient's lifestyle, medical history, CVD risk factors and medications were obtained from self-administered questionnaires/through interviews; and was checked against hospital records. Smoking status was based on self-reported smoking habits and serum cotinine levels ≥85 nmol/L at baseline [17]. Diabetes mellitus was defined as previously diagnosed, or having a baseline plasma fasting glucose concentration >7 mmol/l, non-fasting glucose concentration >11.1 mmol/l or HbA1c >6.5%, according to the American Diabetes Association (ADA) guidelines [19]. Hypertension was defined according to pre-existing diagnoses. The angiographic extent of CAD was scored as nonsignificant stenosis or as single, double or triple vessel disease (0–3) according to the number of coronary arteries with a significant (>50%) narrowing of the lumen.

2.3. Follow-up and study end points

Patients were followed-up from enrollment until suffering from an AMI or throughout 2009. Information on study outcomes was obtained from the Western Norway Cardiovascular Registry, reporting on all patients being discharged with a CVD diagnosis from any 42 Norwegian public hospitals during 1994–2009 [20]. The primary endpoint was total AMI (fatal and nonfatal), which was classified according to the 2000 revised definition of AMI criteria [21].

2.4. Biochemical analyses

Details on the collection, storage and biochemical analysis of plasma samples have been described previously [17]. Routine biochemical analyses were performed at the local laboratories in each recruiting hospital, whereas study-specific analyses were carried out by Bevital AS, Bergen, Norway (http://www.bevital.no). Plasma concentrations of Met and total homocysteine (tHcy) were measured by gas chromatography–tandem mass spectrometry. Serum apolipoprotein (apo) A1 and apo B 100 were analyzed using Hitachi 917 and 912 systems, respectively, from Roche Diagnostics.

2.5. Statistical analysis

Baseline variables are reported as median (interquartile range (IQR)) or counts (percentages) as appropriate. Patient baseline characteristics across quartiles of plasma Met were compared, by median linear or logistic regression for continuous and categorical variables, respectively.

The association between plasma Met and risk of AMI was estimated using Cox regression models. The hazard ratios (HRs) and 95% confidence intervals (CI) were reported according to quartiles of plasma Met, and per 1 standard deviation (SD) increment in log transformed plasma Met. The simple model included age

(continuous), gender (male/female) and fasting status (yes/no), and the multivariate model additionally included current smoking (yes/no), hypertension (yes/no), diabetes mellitus (yes/no), physical activity (≥2 days/week), and estimated glomerular filtration rate (eGFR), body mass index (BMI), and serum apo A1 and apo B (all continuous). Further adjustments for statin treatment or C-reactive protein (CRP) did not appreciably alter the risk estimates and were excluded in the final model (data not shown).The assumption of proportionality was tested by inspecting log-log plots and calculating scaled Schoenfeld residuals.

To investigate potential effect modifications, survival analyses were performed according to subgroups of baseline serum lipid parameters (including LDL cholesterol, apoB, high-density lipoprotein-cholesterol and apoA1), and according to traditional risk factors for coronary heart disease (CHD) such as age, gender, BMI, diabetes, hypertension smoking and eGFR. Continuous variables were dichotomized according to their median value, and interactions were tested by adding interaction products terms to the respective Cox models.

The computer software packages PASW Statistics 21 (SPSS IBM, NY, USA) and R (R Development Core Team, version 3.2.1) were used to perform statistical analyses. In all statistical models, p values were two-sided and $p < 0.05$ was considered statistically significant.

3. Results

3.1. Baseline characteristics

Baseline characteristics of the study patients (n = 4156) according to quartiles of plasma Met are presented in Table 1. The overall median (IQR) age was 62 years, 77% were men, 32% were current smokers, 48% were diagnosed with hypertension, 33% had diabetes mellitus and 44% had a history of AMI. There was no association between Met quartiles and several traditional CHD risk factors, including previous CHD, hypertension, diabetes and smoking, whereas a significant inverse relationship was observed with eGFR and CRP. Moreover, plasma Met was positively associated with apoA1, but inversely with high-density lipoprotein cholesterol (HDL-C), total cholesterol and LDL-C. A trend towards an inverse relationship was also seen with apo B. Notably, the lipid associations were also present after adjusting for statin treatment ($p < 0.001$). Plasma Met was not related to plasma tHcy but showed a strong positive association with plasma pyridoxal 5′-phosphate (PLP). Furthermore, patients with higher Met less likely used aspirin and diuretics, but more frequently took metformin.

Table 1
Baseline characteristics according to the quartiles of plasma methionine.

	n[a]	Total cohort	Quartiles of plasma methionine				p_{trend}
			Q1 (≤23.5)	Q2 (23.5–27.6)	Q3 (27.6–33.3)	Q4 (>33.3)	
			(n = 1054)	(n = 1025)	(n = 1044)	(n = 1033)	
Age, y	4156	62 (15)	63 (15)	62 (14)	62 (15)	61 (16)	0.009
Male sex, n (%)	4156	2334 (77)	658 (62.4)	739 (72.1)	786 (75.3)	807 (78.1)	<0.001
Fasting, n (%)	3982	1135 (28.5)	414 (40.7)	358 (36.7)	282 (28.3)	81 (8.1)	<0.001
BMI, kg/m^2	4153	26 (4)	26 (5)	26 (4)	26 (5)	26 (5)	0.16
Physical activity, 2 times/week	3884	2155 (55.5)	461 (46.2)	520 (54)	568 (58.9)	606 (63.2)	<0.001
CHD risk factors, n%							
Diabetes mellitus	4118	978 (32.5)	414 (39.8)	389 (38.4)	374 (36.0)	388 (37.7)	0.19
Hypertension	4156	1442 (47.5)	511 (48.5)	465 (45.4)	471 (45.1)	496 (48.0)	0.79
Current smoking	4128	978 (32.3)	343 (32.9)	317 (31.2)	335 (32.2)	319 (31.0)	0.46
Cardiovascular history, n (%)							
Prior MI	4156	1332 (44)	441 (41.8)	412 (40.2)	413 (39.6)	412 (39.9)	0.34
Prior PAD	4156	288 (9.5)	89 (8.4)	96 (9.4)	98 (9.4)	94 (9.1)	0.61
Angiographic evidence of CAD, n (%)	4156						0.05
Nonsignificant stenosis	1044		234 (22.2)	258 (25.2)	268 (25.7)	284 (27.5)	
1-vessel disease	965		260 (24.7)	233 (22.7)	234 (22.4)	238 (23.0)	
2-vessel disease	926		234 (22.2)	247 (24.1)	239 (22.9)	206 (19.9)	
3-vessel disease	1221		326 (30.9)	287(28.0)	303 (29.0)	305 (29.5)	
eGFR, mL/min per 1.73 m^2	4155	91 (21)	93 (21)	91 (21)	90 (20)	89 (22)	<0.001
CRP, mg/L	4154	1.78 (2.80)	2.05 (3.70)	1.77 (2.66)	1.70 (2.55)	1.65 (2.43)	<0.001
Serum lipids,							
Total cholesterol, mmol/L	4154	4.90 (1.40)	5.0 (1.42)	4.90 (1.48)	4.88 (1.50)	4.80 (1.40)	<0.001
LDL cholesterol, mmol/L	4152	2.90 (1.30)	2.97 (1.39)	2.90 (1.30)	3.0 (1.30)	2.90 (1.30)	0.01
ApoB, g/L	4155	0.87 (0.31)	0.88 (0.33)	0.87 (0.31)	0.87 (0.31)	0.85 (0.30)	0.05
HDL cholesterol, mmol/L	4155	1.20(0.50)	1.29 (0.50)	1.20 (0.50)	1.20 (0.45)	1.20 (0.50)	0.04
ApoA1, g/L	4154	1.30 (0.35)	1.27 (0.37)	1.30 (0.35)	1.29 (0.32)	1.31 (0.35)	0.001
Plasma markers of B-vitamin status and homocysteine							
Folate, nmol/L	4154	10.1(7.4)	9.71 (7.29)	10.1 (7.49)	10.1 (7.53)	10.6 (7.48)	0.25
Cobalamin (B12), pmol/L	3664	362 (193)	347 (180)	357 (188)	368 (198)	377 (206)	0.28
PLP, nmol/L	4132	41.3 (30.3)	35.2 (25.6)	40.9 (31.1)	42.9 (30.6)	47.2 (31.3)	<0.001
tHcy, μmol/L	4156	10.4 (3.90)	10.3 (4.08)	10.5 (4.01)	10.4 (3.76)	10.5 (3.74)	0.64
Medications, n (%)							
Statins	4156	3333 (80.2)	851 (80.7)	834 (81.4)	832 (79.7)	816 (79.0)	0.21
Aspirin	4156	3393 (81.6)	886 (84.1)	844 (82.3)	837 (80.2)	826 (80.0)	0.007
Metformin	4156	192 (4.6)	47 (4.5)	46 (4.5)	37 (3.5)	62 (6.0)	0.065
β-Blockers	4156	3013 (72.5)	764 (72.5)	741 (72.3)	770 (73.8)	738 (71.4)	0.79
Diuretics	4156	714 (17.2)	212 (20.1)	172 (16.8)	163 (15.6)	167 (16.2)	0.012
ACEI and ARB	4156	1326 (31.9)	345 (32.7)	329 (32.1)	328 (31.4)	324 (31.7)	0.45

Continuous variables are presented as medians (interquartile range), and categorical variables are reported as counts (%).
BMI indicates body mass index; CAD, coronary artery disease; CHD, coronary heart disease; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; MI, myocardial infarction; PAD, peripheral artery disease; PLP, pyridoxal phosphate; tHcy, total homocysteine.
[a] Patients with valid measurements.

3.2. Plasma Met and incident AMI

A total of 534 (12.8%) patients experienced an AMI during the median (IQR) follow-up of 7.5 (2.4) years. Risk association between plasma Met and AMI are presented in Supplemental Table 1 and illustrated in Supplemental Fig. 1. There were no significant overall associations between plasma Met and subsequent AMI. The multivariate adjustments did not appreciably affect the risk estimates (Supplemental Table 1).

3.3. Subgroup analyses

Fig. 2 depicts age, gender and fasting status adjusted risk estimates between plasma Met and future AMI according to several established risk factors for coronary artery disease. Among patients with high (above median) versus low serum LDL-C or apoB levels, plasma Met showed an inverse association with incident AMI (HR per SD [95% CI] was 0.85 [0.74–0.97; $p = 0.02$] and 0.84 [0.74–0.96; $p = 0.01$], respectively; p for interaction$\leq$ 0.02). The risk estimates did not appreciably change after multivariate adjustment (Fig. 3). The Met-AMI association was not modified by serum HDL-C or ApoA1 (p for interaction >0.15).

We also observed an inverse association between plasma Met and incident AMI in patients aged 65 years or below (younger), and those without diabetes or hypertension, whereas there was no association among those aged above 65 years (elderly), and in patients with diabetes or hypertension (Figs. 2 and 3) (p for interaction = 0.07, 0.04 and 0.06, respectively in age, gender and fasting status adjusted model).

Approximately 2/3 of these patients enrolled in the WENBIT received treatment with either folic acid and/or vitamin B6 throughout 2006. We did not find any effect modification by any of the intervention treatments on the risk estimates of plasma Met (Supplemental Table 2).

4. Discussion

4.1. Principal findings

In this large prospective cohort study among 4156 patients undergoing elective coronary angiography for suspected SAP, we observed no overall association between plasma Met and incident AMI followed for an average of >7 years, mirroring the lack of any overall relationship between plasma Met and previous CVD also at baseline. However, we found that plasma concentration of Met was inversely associated with risk among patients with high LDL-cholesterol or apoB levels, as well as among younger patients and those without pre-exiting diabetes or hypertension.

4.2. Strengths and limitations

The major strengths of this study include the large sample size, detailed clinical characterization of the population and its-long term prospective design. Although the possibility of residual confounding cannot be ruled out in observational cohort studies, the adjustment for established CHD risk factors minimizes the risk of

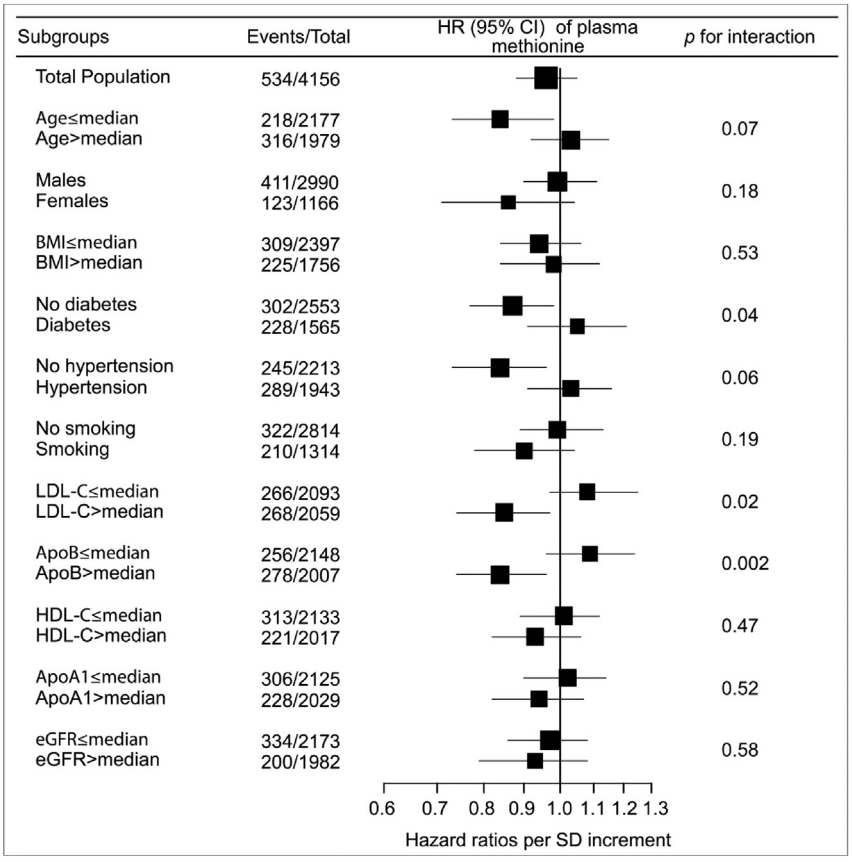

Fig. 2. Risk association between plasma methionine and incident AMI according to subgroups of established risk factors for coronary artery disease.
The black squares represent sample size and horizontal lines represent the 95% CI. AMI indicates acute myocardial infarction; ApoA, apolipoprotein A; ApoB, apolipoprotein B; BMI, body mass index; eGFR, estimated glomerular filtration rate; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; SD, standard deviation. Adjusted for age, gender, and fasting status.

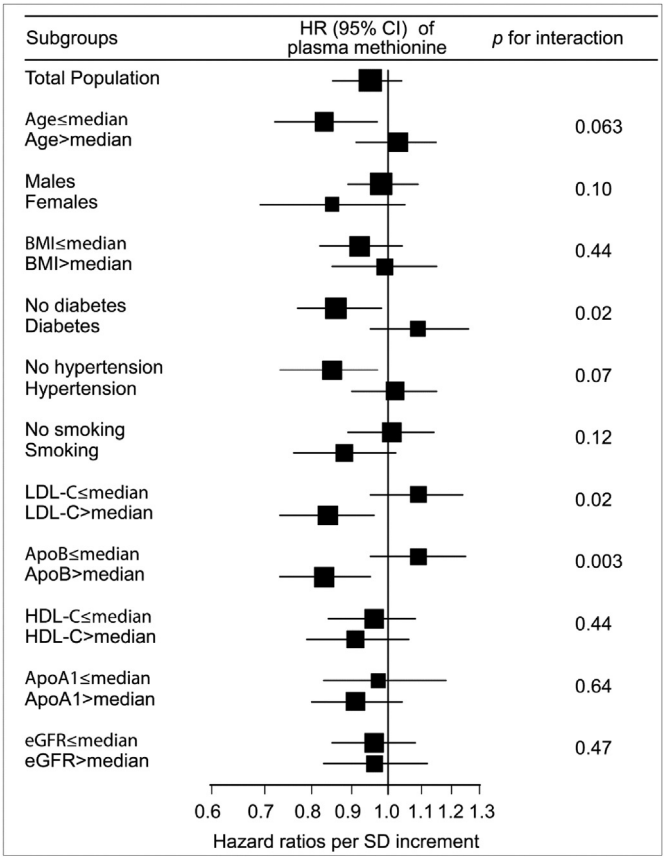

Fig. 3. Forest plot illustrating association between plasma methionine per SD (log transformed) and incident AMI according to traditional risk factors.
The black squares represent sample size and horizontal lines represent the 95% CI. AMI indicates acute myocardial infarction; ApoA, apolipoprotein A; ApoB, apolipoprotein B; BMI, body mass index; eGFR, estimated glomerular filtration rate; HDL-C, high-density lipoprotein cholesterol; HR, hazard ratio; LDL-C, low-density lipoprotein cholesterol; SD, standard deviation. Adjusted for age, gender, fasting status, BMI, diabetes, hypertension, smoking, physical activity, eGFR, serum apolipoprotein A1 and apoB.

residual confounding as an explanation for the observed associations.

There are some additional aspects, which merit consideration. 1) The average follow-up period was quite long (7.5 years), which may have weakened any true associations [22]. 2) Another potential weakness includes the single measurement of Met concentrations at baseline with no data on possible longitudinal variability. Moreover, a prior study from a subsample of the current cohort demonstrated poor within-subject reproducibility for plasma Met (intraclass correlation coefficient: 0.30 [95% CI: 0.26–0.35]) [23], which may have underestimated the true associations, due to regression dilution effect. 3) Serum Met is very oxidation-sensitive and may have degraded over time in the freezers before analysis [24]. However, in the current study, all blood specimen have been stored at a stable −80 °C, with little or no signs of deteriorations during storage. 4) Finally, the majority of blood samples were drawn from non-fasting subjects, which may pose a challenge because of the influence of fasting status on plasma Met concentrations [25]. However, in the current study, adjustment for fasting status did not alter total associations or the interaction effects.

4.3. Met and CVD in other epidemiological studies

Data on the relationship between Met and CVD are limited. A case-control study among 185 patients with recurrent venous thrombosis and 500 healthy subjects showed a positive relationship between low Met concentration and venous thrombosis risk [15]. Another study reported low Met levels in plasma in patients with CVD [2], but others showed no such association [14]. However, as far as we are aware, the present study is the first large-scale analysis of the long-term prospective relation on plasma Met and clinical cardiovascular outcomes among patients with suspected SAP.

4.4. Met, lipid parameters and AMI risk

ApoB is the vital component of the LDL particle and high ApoB is a well-documented risk factor for atherosclerotic CVD [26]. In the current study, we observed an association between low Met and incident AMI among patients with high LDL-C or apoB levels, suggesting that Met availability may affect lipid metabolism. These effects may involve GNMT, since a Met-deficient condition may lead to reduced flux through GNMT [27]. Notably, reduced GNMT flux has been shown to attenuate hepatic lipid uptake, and induce hyperlipidemia [5], in addition to increasing lipid accumulation in macrophages and upregulating inflammatory responses [4], which may promote formation of oxLDL-induced foam cell inside the artery wall [4,13]. Because Met is crucial for methylation reactions [11], it is interesting that a link has been suggested between hypomethylation and increased PCSK9 expression, which can further increase levels of circulating LDL-C via intracellular degradation of LDLR [12]. Thus, future studies should investigate whether Met is primarily exerting its effects by mechanisms regulating GNMT flux or via epigenetic modification of PCSK9 expression.

4.5. Plasma status of Met

Low plasma Met concentrations may be due to inadequate intake of proteins or nutritional insufficiency [1,2,15,25]. We did not evaluate protein intake in the current study. Although, we found no significant interaction between B-vitamin treatment and Met on AMI occurrence, we cannot exclude an influence of this treatment on the observed results. It is however, unlikely that a low Met status in the current study reflects suboptimal nutritional status or vitamin-dependent impaired homocysteine remethylation. Alternatively, low plasma Met concentrations may relate to low BHMT activity [1]. Interestingly, low BHMT activity has been shown to impair the transcription of peroxisome proliferator-activated receptor (PPAR) α [28], a nuclear factor involved in lipid-lowering as well as downregulation of GNMT and the enzymes of the transsulfuration pathway [29]. Thus, low Met could also be consequence of increased downstream catabolism due to low endogenous PPARα activity. Accordingly, we observed an inverse association between Met and serum LDL-cholesterol, and apoB levels and also CRP at baseline. Nevertheless, Met metabolism is complex and previous studies have shown that a Met-deficient state increases flux through BHMT [30] and inhibits transsulfuration flux via feedback mechanisms [1]. Thus, it remains an open question whether low systemic Met is secondary to impaired BHMT, increased downstream catabolism or a combination of both.

4.6. Plasma Met, age and incident AMI

Our subgroup analysis also suggests that the inverse risk association between plasma Met and AMI was found exclusively in the younger age group. The mechanisms behind this effect modification are unclear, but there are some plausible interpretations. First, this may be due to differences in baseline risk, as baseline risk determines the magnitude of the association as well as relative effect [31]. Another possibility is the one of selection bias, whereby

older patients in the current population may comprise a selected group who are less prone to develop CVD. Nevertheless, further studies are required to explicate the age-related differences in the association between circulating Met and CHD incidence.

4.7. Plasma Met in relation to diabetes and hypertension

We found significant inverse risk associations between plasma Met and incident AMI in patients without diabetes or hypertension. The lack of association between plasma Met and incident AMI in diabetes or hypertensive subjects might be due to use of medications. However, the effects of treatment therapies are complex due to their potential impact on Met metabolism. In fact, metformin treatment in diabetic patients causes vitamin B-12 malabsorption [32], which may in turn alter Met status. On the other hand, antihypertensive therapy increases the bioavailability of nitric oxide [33,34], which can modulate Met status directly by inactivating MS [35]. Therefore, further investigations are certainly needed to explore the role of circulating Met in high-risk cardiovascular subsets, with a particular focus on diabetes and hypertension.

4.8. Conclusions

In conclusion, the results of our large, prospective cohort of patients with SAP do not support an overall independent association of Met with incident AMI. However, low levels of plasma Met are associated with increased risk of AMI among participants having high circulating LDL-cholesterol or apoB levels, as well as among subjects who are young and those without diabetes mellitus or hypertension (low-risk for CVD). Future studies are warranted to explore these interrelationships further and to evaluate their potential clinical implications. Such research should also consider the potential interaction with the use of lipid-lowering therapy on clinical outcomes.

Conflicts of interest

The authors declared they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

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Author contributions

OKN designed research; ID, VL, SR, GFTS, PMU, and OKN conducted research; ID and OKN interpreted data; ID performed statistical analysis and drafted the manuscript; ID, GFTS, PMU, and ON critically revised the manuscript. All authors read and approved the final version of the manuscript.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.atherosclerosis.2018.03.038.

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