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ORIGINAL ARTICLE

Plasma dimethylglycine, nicotine exposure and risk of low bone mineral density and hip fracture: the Hordaland Health Study

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Abstract
Summary In the large community-based Hordaland Health Study, low plasma dimethylglycine was associated with low bone mineral density in both middle-aged and elderly subjects and to an increased risk of subsequent hip fracture among the elderly. These associations seemed to be particularly strong among subjects exposed to nicotine.
Introduction Dimethylglycine (DMG) is a product of the choline oxidation pathway and formed from betaine during the folate-independent remethylation of homocysteine (Hcy) to methionine. Elevated plasma DMG levels are associated with atherosclerotic cardiovascular disease and inflammation, which in turn are related to osteoporosis. High plasma total Hcy and low plasma choline are associated with low bone mineral density (BMD) and hip fractures, but the role of plasma DMG in bone health is unknown.
Methods We studied the associations of plasma DMG with BMD among 5315 participants (46–49 and 71–74 years old) and with hip fracture among 3310 participants (71–74 years old) enrolled in the Hordaland Health Study.
Results In age and sex-adjusted logistic regression models, subjects in the lowest versus highest DMG tertile were more likely to have low BMD (odds ratio [OR] 1.68, 95 % confidence interval [CI] 1.43–1.99). The association was stronger in participants exposed compared to those unexposed to nicotine (OR 2.31, 95 % CI 1.73–3.07 and OR 1.43, 95 % CI 1.16–1.75, respectively, p interaction=0.008). In the older cohort, Cox regression analyses adjusted for sex showed that low plasma DMG was associated with an increased risk of hip fracture (hazard ratio [HR] 1.70, 95 % CI 1.28–2.26). A trend toward an even higher risk was found among women exposed to nicotine (HR 3.41, 95 % CI 1.40–8.28).
Conclusion Low plasma DMG was associated with low BMD and increased risk of hip fractures. A potential effect modification by nicotine exposure merits particular attention.

Keywords Bone mineral density · Dimethylglycine · Hip fracture · Nicotine exposure · One-carbon metabolism · Smoking

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Introduction

Dimethylglycine (DMG) is a metabolite of the choline oxidation pathway and is produced from betaine during the

remethylation of homocysteine (Hcy) to methionine, catalyzed by betaine-homocysteine methyl transferase (BHMT) [1, 2]. DMG is demethylated in the mitochondria, leading to the subsequent formation of sarcosine and glycine [3]. This process yields formate to be used in the one-carbon metabolism [4] (Fig. 1), as well as for the synthesis of the universal methyl donor *S*-adenosylmethionine [4]. Thus, DMG metabolism is linked to nucleotide synthesis and may also affect epigenetic regulation [5].

We previously observed that high plasma levels of total Hcy [6, 7] and low levels of choline, but not betaine [8], were associated with low bone mineral density (BMD) and subsequent increased risk of hip fracture in a community-based study. Plasma DMG levels have been associated with increased serum levels of C-reactive protein (CRP) [9] and impaired renal function [10], as well as with increased risk of acute myocardial infarction (AMI) [9]. Notably, both inflammation [11, 12], renal failure [13], and cardiovascular disease (CVD) [14, 15] are related to osteoporosis. However, the role of plasma DMG in relation to bone health has not previously been reported.

The adverse effects of smoking on bone health are well known [16, 17], and in our previous report from the Hordaland Health Study (HUSK), plasma choline levels were found to be lower among smokers than among nonsmokers [18]. We have also shown that among patients with suspected stable angina pectoris, plasma choline is lower [19], whereas plasma DMG is higher in smokers [9]. Notably, the relation of plasma choline status to BMD and hip fracture was strongest in subjects exposed to nicotine [8], and the relationship between plasma choline [19] and DMG [9] with risk of incident AMI was confined to nonsmokers. These observations suggest that smoking may modify disease risk associated with components of the choline oxidation pathway.

The aim of this community-based study was to examine relations of plasma DMG with BMD and subsequent risk of hip fracture, and the possible effect modification by nicotine exposure.

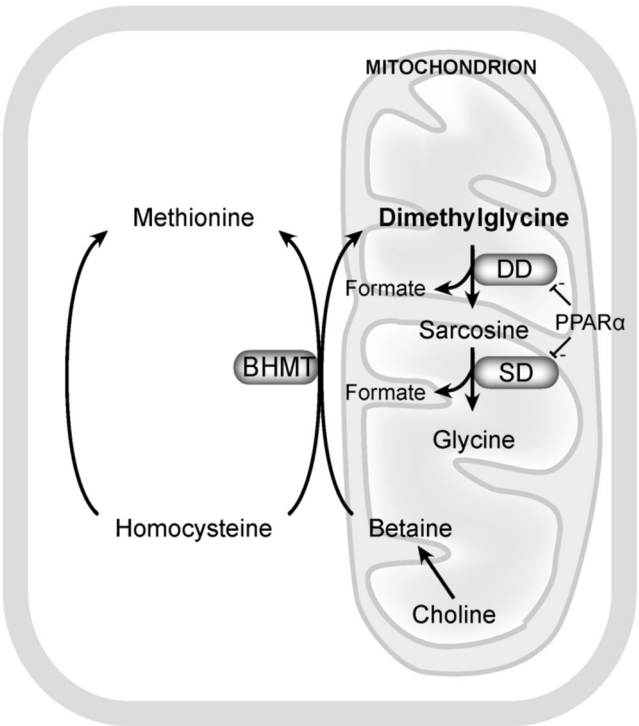

Fig. 1 The relationship between the choline oxidation pathway and BHMT-mediated homocysteine remethylation. DMG is formed during this reaction, and PPARα activation inhibits DMG catabolism (several factors could influence BHMT activity, such as dietary fat, insulin, and redox status). *BHMT* betaine-homocysteine methyltransferase, *DD* dimethylglycine dehydrogenase, *DMG* dimethylglycine, *PPARα* peroxisome proliferator-activated receptor α, *SD* sarcosine dehydrogenase

Participants and methods

Study population

The current study includes participants of the HUSK in Western Norway, and the baseline examinations were conducted during 1998 to 2000. The 9187 invited participants were born 1925 to 1927 (older cohort) and 1950 to 1951 (middle-aged cohort) [20]. A total of 7074 participants (77 %) met for examinations and completed self-administered questionnaires about health status, lifestyle factors, and use of medications.

BMD was measured at baseline in 5408 persons (76.4 %) at Haukeland University Hospital, Bergen, Norway [6]. Of these, 30 scans were invalid or rejected due to hip malformations or bilateral hip prostheses. Plasma DMG and cotinine measurements were missing in 63 participants, leaving 5315 participants eligible for the BMD subpopulation of HUSK (Fig. 2).

All 7074 participants in HUSK were followed until they experienced their first hip fracture or were censored at death or on December 31, 2009. Only 13 participants in the middle-aged cohort suffered a hip fracture during the follow-up period. Thus, hip fracture analyses were carried out only in the older cohort (n=3341) of whom 31 participants were excluded due to missing DMG and cotinine measurements. This left a population of 1855 women and 1455 men eligible for the hip fracture analyses (Fig. 2).

Baseline data collection

BMD

BMD was measured by dual X-ray absorptiometry (DXA) on a stationary fan beam densitometer (Expert-XL; Lunar Company Inc., Madison, Wis), operated by four skilled technicians [6]. The left hip was scanned except when there was a history

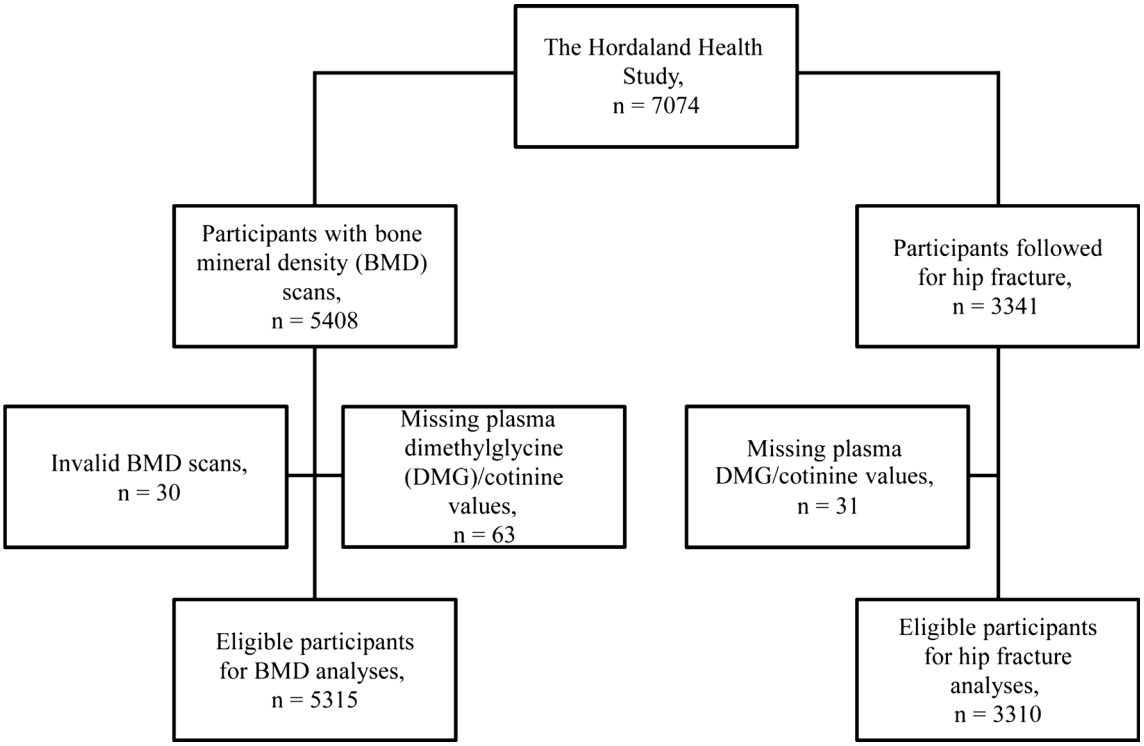

Fig. 2 Flow chart showing the selection of participants eligible for the study

of hip fracture or insertion of a hip joint prosthesis. Femoral neck BMD was used in the analyses, and having low femoral neck BMD was defined as being in the lowest quintile in each age and sex group. Further descriptions have been presented previously [6].

Blood samples

Nonfasting blood samples were collected and stored in EDTA-containing tubes, cooled for 15–30 min, and then centrifuged and stored at −80 °C [21]. Plasma choline, betaine, DMG, cotinine, and serum creatinine concentrations were measured by liquid chromatography-tandem mass spectrometry 6–8 years after collection without any thaw-freeze cycles [22, 23]. Plasma total Hcy was measured by high-performance liquid chromatography [24], whereas serum folate was measured by a *Lactobacillus casei* microbiological assay [25]. Previous studies have shown these biomarkers to be relatively stable during storage under such conditions [26]. Coefficients of variations (CVs) were 3.8–7.6 % for plasma choline, 5–11.7 % for plasma betaine, 2.2–5.8 % for plasma DMG, 5.5–9.5 % for serum creatinine [27], and 2.3–6.2 % for plasma cotinine [22]. Levels of high sensitive C-reactive protein (hs-CRP) were determined by an immunoassay based on matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. CV for hs-CRP was 2.4–7.0 % [28]. Plasma cotinine levels of ≥85 nmol/L were used to identify participants exposed to nicotine [29]. Estimated glomerular filtration rate (eGFR) was obtained using the Modification of Diet in Renal Disease (MDRD) formula [30]. All biochemical analyses were done at Bevital A/S, Bergen, Norway (www.bevital.no) [22].

Additional measures

Height and weight were measured in light clothing, and body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters. Information of health factors including hormone replacement therapy (current or no use), time since last meal (hours), and physical activity were obtained from self-administered questionnaires. Physical activity was classified as no or light regular activity (<1 h/week), and regular (1 to 2 h/week) or hard regular activity (≥3 h/week).

Clinical endpoints

Computerized records comprising discharge diagnoses for all hospitalizations in Hordaland County were searched for hip fractures occurring between the HUSK baseline examinations through December 31, 2009. A hip fracture was defined as the first fracture of the proximal femur occurring during the follow-up period. Only hip fractures confirmed by a concurrent code of an adequate surgical procedure were included [7]. Information on time of death was obtained from the Norwegian Population Register.

Statistical analyses

Categorical variables are summarized as numbers (percentage) and continuous variables as median (interquartile range [IQR]). Logistic regression was used for categorical variables and linear median regression for continuous variables to test for trend across tertiles of plasma DMG.

The relationship between DMG and BMD was explored by linear median and logistic regression. To graphically express the linear relationship between BMD and DMG, we used a generalized additive linear model (GAM). Odds ratios (ORs) for low femoral neck BMD according to age group and sex-specific tertiles of plasma DMG (μmol/L) were estimated by logistic regression analyses. Low BMD was defined as the lowest quintile in each age and sex group. This was done to define estimates for the same number of individuals with lowest BMD. We adjusted for potential confounders: BMI, nicotine exposure, plasma choline, betaine, and total Hcy, serum folate and hs-CRP, eGFR, time since last meal, physical activity, and use of estrogen supplementation (in women).

Kaplan-Meier curves for cumulative incidence of hip fracture according to tertiles of plasma DMG were constructed in the older cohort. The associations between plasma DMG and subsequent hip fracture were obtained by Cox proportional hazards regression, using crude and adjusted models, also stratified according to nicotine exposure. Formal statistical testing of an effect modification by nicotine exposure was carried out by introducing a product term between plasma DMG tertiles as a continuous variable and nicotine exposure status (dichotomous) in the sex-adjusted binary logistic and Cox regression models.

Two-tailed p values <0.05 were considered statistically significant. The analyses were performed using the Statistical Package for the Social Sciences (SPSS) for windows (IBM SPSS Statistics 22, Chicago, IL, USA, www.spss.com) and R version 3.0.0 (The R Foundation for Statistical Computing, Vienna, Austria) [31].

Ethics

The study was approved by the Regional Committee for Medical and Health Research Ethics review for Western Norway, Bergen, Norway. Each participant provided written informed consent.

Funding

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Results

Study population

Characteristics of the HUSK BMD subpopulation, stratified according to age group and tertiles of plasma DMG, are presented in Table 1. Characteristics of participants in each sex and age group according to nicotine exposure have been presented previously [8], and most importantly, participants exposed to nicotine had lower plasma choline, femoral neck BMD, BMI, and higher CRP than unexposed participants. In both age groups, plasma DMG was positively related to BMD, BMI, plasma choline, betaine, and serum creatinine after adjustment for sex (Table 1). In the oldest cohort, the prevalence of diabetes mellitus was highest among those in the highest DMG tertiles (Table 1). Current use of estrogen among women did not differ across the tertiles of plasma DMG.

Baseline characteristics of the HUSK participants included in the hip fracture analyses were similar to the HUSK BMD subpopulation (Supplemental Table S1).

Plasma DMG and femoral neck BMD

We observed a positive linear relationship between plasma DMG and BMD (Beta=0.07, $p<0.001$) (Fig. 3). This association was essentially identical when adjusting for age group and gender (Table 2). BMD was positively associated with plasma DMG in most linear regression models, but not among middle-aged women and men, as well as elderly men who were unexposed to nicotine (Table 2). Further, a higher proportion of subjects in the lowest tertiles of plasma DMG had low femoral neck BMD compared to those in the highest tertiles of plasma DMG (Table 3 and Fig. 4), irrespective of sex and age groups (Supplemental Table S2). However, we found a stronger association between DMG and BMD among participants who were exposed, as compared to those who were unexposed to nicotine (Table 3 and Supplemental Table S2) (p interaction=0.008). In separate analyses according to nicotine exposure in the two age groups, the trend was similar, but a statistically significant interaction was found only among the middle-aged cohort (p interaction=0.017).

Additional adjustments for plasma choline, betaine, and total Hcy, serum folate and hs-CRP, eGFR, time since last meal, physical activity, and use of estrogen supplementation (in women) did weaken some estimates, most of which remained significant (Supplemental Table S2). These variables were also included separately, but the results remained significant in all models (data not shown).

Plasma DMG and the risk of hip fracture

During a median (IQR) follow-up time of 10.8 (1.8)years, a hip fracture occurred in 277 (8.4 %) of the older subjects, and

Table 1 Characteristics of subjects included in the analyses of femoral neck bone mineral density (BMD) according to age group and plasma dimethylglycine (DMG), in the Hordaland Health Study

	Middle-aged cohort (46–49 years)					Older cohort (71–74 years)				
	N	All	Tertiles of plasma DMG			*N*	All	Tertiles of plasma DMG		
			1st	2nd	3rd			1st	2nd	3rd
Age, years, mean (SD)	3094	48.6 (0.8)	48.2 (0.9)	48.7 (0.8)	48.8 (0.7)	2221	73.0 (0.9)	72.6 (1.0)	73.1 (0.9)	73.4 (0.8)
Plasma DMG, μmol/L	3094	4.4 (1.5)	3.4 (0.6)	4.4 (0.6)	5.6 (1.1)	2221	4.4 (1.5)	3.5 (0.7)	4.4 (0.5)	5.8 (1.3)
Male sex, *n* (%)	3094	1228 (39.7)	409 (39.7)	411 (39.7)	408 (39.6)	2221	1017 (45.8)	342 (45.7)	336 (45.8)	339 (45.8)
Any nicotine exposure, *n* (%)[a]	3094	1128 (36.5)	368 (35.7)	381 (36.8)	379 (36.8)	2221	371 (16.7)	136 (18.2)	118 (16.1)	117 (15.8)
No regular physical activity, *n* (%)	3062	1138 (37.2)	362 (35.7)	401 (39.1)	375 (36.7)	2077	755 (36.4)	257 (36.6)	240 (35.4)	258 (37.0)
Diabetes mellitus, *n* (%)	3067	24 (0.8)	6 (0.6)	9 (0.9)	9 (0.9)	2183	143 (6.6)	40 (5.4)	31 (4.3)	72 (9.9)[b]
Current use of corticosteroids, *n* (%)	3094	18 (0.6)	6 (0.6)	6 (0.6)	6 (0.6)	2221	43 (1.9)	11 (1.5)	12 (1.6)	20 (2.7)
eGFR, mL/min/1.73 m^2	3087	80.9 (16.1)	82.0 (16.1)	80.2 (16.4)	80.6 (16.6)	2214	70.6 (16.5)	72.2 (15.9)	70.6 (16.9)	69.1 (16.6)
BMD, g/cm^2	3094	0.961 (0.168)	0.956 (0.176)	0.961 (0.168)	0.968 (0.168)[c]	2221	0.809 (0.189)	0.797 (0.191)	0.815 (0.197)	0.815 (0.183)[c]
BMI, kg/cm^2	3094	24.9 (4.7)	24.7 (4.4)	24.9 (4.7)	25.2 (4.9)[c]	2221	25.9 (4.8)	25.9 (4.7)	25.7 (4.7)	26.1 (4.8)[d]
Plasma										
Choline, μmol/L	3094	9.2 (2.7)	8.5 (2.3)	9.2 (2.5)	10 (2.7)[b]	2221	10.1 (3.0)	9.4 (2.7)	10.2 (2.7)	10.9 (3.5)[b]
Betaine, μmol/L	3094	36.5 (15.3)	34.2 (14.1)	36.6 (15.4)	39.4 (16.3)[b]	2221	39.6 (15.0)	36.2 (14.2)	40.2 (14.5)	41.7 (16.3)[b]
tHcy, μmol/L	3094	9.7 (3.4)	9.7 (3.5)	9.8 (3.5)	9.7 (3.4)	2221	11.9 (4.4)	11.8 (4.3)	11.9 (4.4)	12.2 (4.4)
Serum										
Folate, nmol/L	3090	6.7 (3.9)	6.6 (3.5)	6.7 (4.0)	6.8 (4.1)[d]	2210	6.7 (4.0)	6.6 (4.2)	6.6 (4.3)	6.9 (3.7)
Creatinine, μmol/L	3094	77.2 (15.9)	76.4 (15.5)	77.7 (15.9)	77.5 (16.0)[d]	2221	82.4 (19.9)	80.1 (18.3)	82.6 (18.1)	84.6 (23.0)[b]
hs-CRP, mg/L	2860	1.11 (2.14)	1.07 (2.03)	1.10 (2.15)	1.20 (2.34)	2128	2.09 (3.22)	2.15 (3.39)	2.05 (3.15)	2.09 (3.19)

Data are presented as median with interquartile range in parenthesis, when not otherwise indicated

BMI body mass index, *hs-CRP* high sensitive C-reactive protein, *eGFR* estimated glomerular filtration rate, *Hcy* homocysteine

[a] Any nicotine exposure = plasma cotinine ≥85 nmol/L

[b] p for trend across tertiles <0.001

[c] p for trend across tertiles <0.01

[d] p for trend across tertiles <0.05. All analyses are adjusted for sex

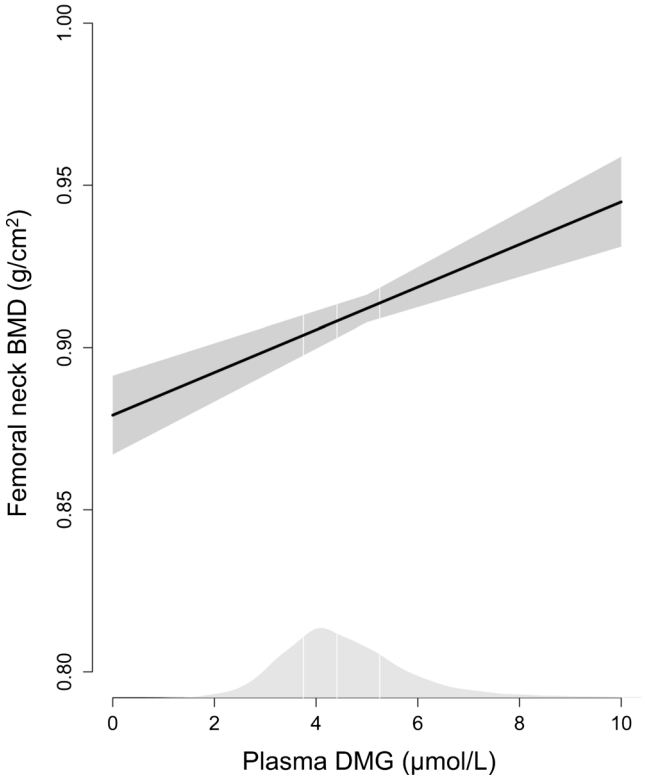

Fig. 3 Spline curve showing the association between plasma dimethylglycine (DMG) and femoral neck bone mineral density (BMD) in 3094 women and men aged 46–49 years and 2221 women and men aged 71–74 years at inclusion

the highest incidence was seen among subjects in the lowest DMG tertile (Fig. 5, Supplemental Table S1). Accordingly, sex-adjusted Cox regression analyses estimated an increased risk of hip fracture (hazard ratio [HR] 1.70, 95 % confidence interval [CI] 1.28–2.26) among those in the lowest compared to the highest tertile of plasma DMG (Table 4). The relationship was consistent across subgroups (p for interaction >0.115); however, we observed a trend toward an even higher risk among women exposed to nicotine (HR 3.41, 95 % CI 1.40–8.28) (Table 4). The results were similar after additional adjustment for BMI and nicotine exposure (Table 4), and still significant, but somewhat weaker when including plasma choline, betaine, and total Hcy, serum folate and hs-CRP, eGFR, time since last meal, physical activity, and use of estrogen supplementation (in women) in the analyses (data not shown). We also adjusted for BMD among 2221 older participants with valid measurements, rendering the association between DMG and hip fracture essentially unaltered (HR 1.45, 95 % CI 0.95–2.21, p=0.085).

Table 2 Associations between femoral neck bone mineral density and plasma dimethylglycine in multiple regression analyses according to nicotine exposure in the whole cohort, and separately in age groups, in the Hordaland Health Study

	N	Model 1[a]		Model 2[a]		Model 3[a]	
		Beta[b]	p value	Beta[b]	p value	Beta[b]	p value
All participants	5315	0.06	<0.001	0.05	<0.001	0.06	<0.001
46–49 years							
All	3094	0.08	<0.001	0.05	0.002	0.07	<0.001
Women	1866	0.09	<0.001	0.06	0.005	0.08	<0.001
Men	1228	0.05	0.067	0.04	0.101	0.05	0.099
71–74 years							
All	2221	0.07	<0.000	0.06	0.001	0.07	<0.001
Women	1204	0.11	<0.001	0.09	0.002	0.10	0.002
Men	1017	0.06	0.081	0.06	0.065	0.07	0.032
No nicotine exposure							
All	3816	0.04	0.010	0.03	0.042	0.03	0.032
46–49 years							
All	1966	0.04	0.097	0.04	0.097	0.03	0.179
Women	1183	0.06	0.060	0.03	0.319	0.05	0.097
Men	783	0.01	0.733	0.01	0.722	0.06	0.869
71–74 years							
All	1850	0.05	0.028	0.05	0.030	0.05	0.032
Women	1016	0.09	0.004	0.06	0.037	0.10	0.014
Men	834	0.03	0.381	0.04	0.285	0.03	0.460
Any nicotine exposure							
All	1499	0.14	<0.001	0.12	<0.001	0.013	<0.001
46–49 years							
All	1128	0.15	<0.001	0.14	<0.001	0.14	<0.001
Women	683	0.15	<0.001	0.12	0.001	0.15	<0.001
Men	445	0.14	0.003	0.11	0.018	0.13	0.008
71–74 years							
All	371	0.20	<0.001	0.19	<0.001	0.20	<0.001
Women	188	0.22	0.002	0.21	0.002	0.24	0.006
Men	183	0.22	0.002	0.21	0.003	0.24	0.002

No nicotine exposure = plasma cotinine <85 nmol/L; any nicotine exposure = plasma cotinine ≥85 nmol/L

[a] Model 1: adjusted for age and sex; model 2: adjusted for age, sex, BMI, and nicotine exposure; model 3: adjusted for age, sex, BMI, nicotine exposure, plasma choline, betaine, and total homocysteine, serum folate, and high sensitive C-reactive protein, estimated glomerular filtration rate, time since last meal, physical activity, and use of estrogen supplementation (in women). Age group-stratified models are not adjusted for age, and nicotine exposure-stratified models are not adjusted for nicotine exposure

[b] Beta = estimated standardized regression coefficient

Discussion

In this large community-based study, low plasma DMG was associated with low BMD in both middle-aged and elderly subjects and with an increased risk of subsequent hip fracture among the elderly.

The strengths of this study include its large number of participants, the community-based cohort design, extensive clinical and biochemical information, and the long follow-up

Table 3 Odds ratios (OR) for low femoral neck bone mineral density (lowest quintile in each sex and age group) according to tertiles of plasma dimethylglycine (DMG) and nicotine exposure in the whole cohort, and separately in age groups, in the Hordaland Health Study

Plasma DMG tertiles	*N*	Adjusted for age and sex[a]		Adjusted for age, sex, BMI, and nicotine exposure[a]	
		OR (95 % CI)	*p* value	OR (95 % CI)	*p* value
All participants	5315		<0.001[b]		<0.001[b]
Tertile 1	1778	1.68 (1.43–1.99)	<0.001	1.63 (1.37–1.94)	<0.001
Tertile 2	1767	1.23 (1.03–1.46)	0.021	1.16 (0.97–1.39)	0.107
Tertile 3	1770	1 (ref.)		1 (ref.)	
46–49 years	3094		<0.001[b]		0.001[b]
Tertile 1	1030	1.56 (1.25–1.95)	<0.001	1.50 (1.19–1.89)	0.001
Tertile 2	1034	1.33 (1.06–1.66)	0.014	1.27 (1.00–1.60)	0.047
Tertile 3	1030	1 (ref.)		1 (ref.)	
71–74 years	2221		<0.001[b]		<0.001[b]
Tertile 1	748	1.86 (1.44–2.40)	<0.001	1.81 (1.38–2.36)	<0.001
Tertile 2	733	1.09 (0.83–1.44)	0.523	1.01 (0.76–1.35)	0.943
Tertile 3	740	1 (ref.)		1 (ref.)	
No nicotine exposure					
All participants	3816		0.001[b]		0.003[b]
Tertile 1	1274	1.43 (1.16–1.75)	0.001	1.38 (1.11–1.70)	0.004
Tertile 2	1268	1.08 (0.87–1.34)	0.474	1.05 (0.84–1.31)	0.682
Tertile 3	1274	1 (ref.)		1 (ref.)	
46–49 years	1966		0.151[b]		0.324[b]
Tertile 1	662	1.24 (0.92–1.66)	0.152	1.16 (0.86–1.58)	0.325
Tertile 2	653	1.11 (0.82–1.49)	0.508	1.08 (0.79–1.47)	0.622
Tertile 3	651	1 (ref.)		1 (ref.)	
71–74 years	1850		0.001[b]		<0.001[b]
Tertile 1	612	1.64 (1.22–2.20)	0.001	1.62 (1.20–2.20)	<0.001
Tertile 2	615	1.06 (0.77–1.44)	0.737	(0.73–1.40)	0.493
Tertile 3	623	1 (ref.)		1 (ref.)	
Any nicotine exposure					
All participants	1499		<0.001[b]		<0.001[b]
Tertile 1	504	2.31 (1.73–3.07)	<0.001	2.20 (1.63–2.95)	<0.001
Tertile 2	499	1.55 (1.16–2.08)	0.004	1.42 (1.04–1.93)	0.025
Tertile 3	496	1 (ref.)		1 (ref.)	
46–49 years	1128		<0.001[b]		<0.001[b]
Tertile 1	368	2.16 (1.54–3.04)	<0.001	2.07 (1.46–2.94)	<0.001
Tertile 2	381	1.68 (1.19–2.37)	0.003	1.57 (1.10–2.24)	0.012
Tertile 3	379	1 (ref.)		1 (ref.)	
71–74 years	371		<0.001[b]		0.001[b]
Tertile 1	136	2.70 (1.57–4.62)	<0.001	2.53 (1.44–4.45)	0.001
Tertile 2	118	1.22 (0.69–2.19)	0.493	1.03 (0.56–1.88)	0.930
Tertile 3	117	1 (ref.)		1 (ref.)	

Age and sex-specific tertiles of plasma DMG. No nicotine exposure = plasma cotinine <85 nmol/L; any nicotine exposure = plasma cotinine ≥85 nmol/L

CI confidence interval

[a] Age group-stratified models are not adjusted for age, and nicotine exposure-stratified models are not adjusted for nicotine exposure

[b] *p* value for trend across tertiles

time for hip fractures. Low plasma DMG was associated with both low BMD at baseline and with future risk of fracture, thereby strengthening the relationship between DMG status and bone health. Blood samples and lifestyle variables were collected only at baseline; thus, we have no information on possible changes in plasma DMG or lifestyle variables during

Fig. 4 Forest plot showing the odds ratios for low femoral neck bone mineral density according to tertiles of plasma dimethylglycine by baseline age group and sex. *Box sizes* are proportional to population sizes, and *vertical lines* depict 95 % confidence intervals. The Hordaland Health Study

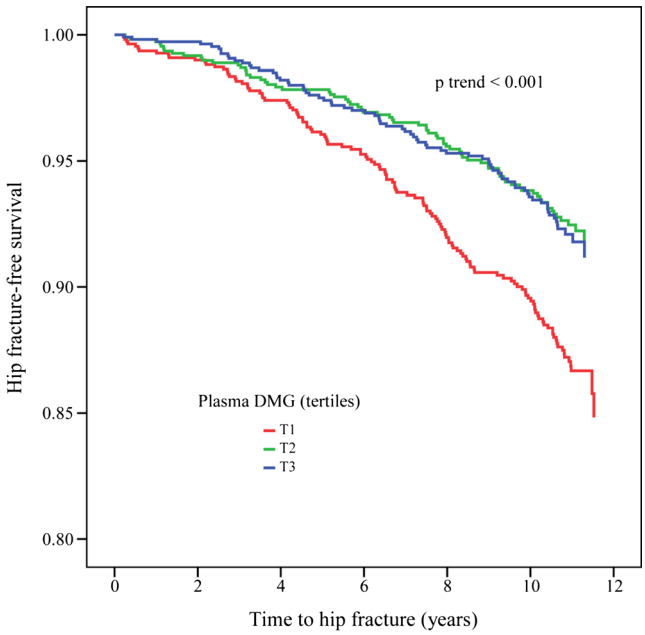

Fig. 5 Kaplan-Meier disease-free survival curves for hip fractures in 1855 women and 1455 men (ages 71 to 74 years at inclusion), according to age and sex-specific tertiles of plasma dimethylglycine (DMG) (μmol/L); women, T1≤3.86 μmol/L, T2 3.87–4.74 μmol/L, and T3>4.74 μmol/L; men, T1≤4.19 μmol/L, T2 4.20–5.23 μmol/L, and T3>5.23 μmol/L. *p* for trend across tertiles. The Hordaland Health Study

follow-up. However, the within-person reproducibility has been shown to be fair to very good, interclass correlation coefficient of 0.55–0.73[26] and 0.93 [9], indicating that baseline DMG values also are representative of levels during follow-up in this cohort not supplemented with folic acid. Moreover, risk estimation based on a single measurement tends to attenuate the true associations, due to regression dilution bias [32].

Systemic DMG concentrations are lower among fasting than nonfasting subjects [9]. The blood samples in the current study were not collected during fasting; however, adjustment for time since last meal did not alter the results. Thus, it is not likely that the current findings are confounded by fasting status. In addition, there are no data on possible diurnal variations in plasma DMG, but as mentioned, the within-person reproducibility has been shown to be fair to very good [9, 26], demonstrating that a single measurement allows assessment of DMG status over time.

Other sources of nicotine than smoking could affect the plasma cotinine levels. Especially, the use of smokeless tobacco-like snuffing is prevalent among residents in the Nordic countries [33]. The study participants did not provide information on smokeless tobacco use; however, during 1998–2000, snuffing was rare among adults and elderly in Norway, and we do not consider this to be of importance in the interpretation of our findings.

Earlier, we showed that high plasma total Hcy [6, 7] and low plasma choline, but not betaine, were associated with low BMD and hip fracture risk in the same cohort [8], and to our knowledge, this is the first study on DMG and bone health. Adjustment for both plasma total Hcy and plasma choline did not alter the results, suggesting the relationship between DMG and bone health to be independent of metabolic precursors. In addition, adjustment for BMD in the hip fracture analyses indicated similar point estimate, although not statistically significant. This was probably due to low sample size (Fig. 1), as 105 hip fracture cases were lost in the BMD-adjusted analyses.

In general, data on circulating levels of DMG in relation to clinical outcomes are sparse. Higher plasma levels of both DMG [9, 34] and choline [35, 36] have been associated with increased risk of atherosclerotic cardiovascular disease (ACVD) and coronary heart disease in particular. Osteoporosis and ACVD are related in epidemiological studies [14] and also share some of the same risk factors [14, 15] including components of the metabolic syndrome [37–39], smoking, and hyperlipidemia [40]. Nevertheless, our results indicate that high levels of plasma choline and DMG actually are associated with higher BMD and a decreased risk of hip fracture. This rather counterintuitive finding might relate to mechanisms involving the choline oxidation pathway (Fig. 1).

We previously suggested that the increased risk of incident AMI [9], and total and CVD death [41] among patients with elevated plasma DMG might be a related to impaired

Table 4 Risk (hazard ratio) of hip fracture during follow-up according to tertiles of plasma dimethylglycine (DMG), sex, and nicotine exposure in participants aged 71 to 74 years at inclusion, in the Hordaland Health Study

Plasma DMG tertiles	n/events	Adjusted for sex[a]		Adjusted for sex, BMI and nicotine exposure[a]	
		HR (95 % CI)	*p* value	HR (95 % CI)	*p* value
All participants	3310/277		<0.001[b]		<0.001[b]
Tertile 1	1109/128	1.70 (1.28-2.26)	<0.001	1.60 (1.21-2.14)	0.001
Tertile 2	1105/74	0.96 (0.70-1.33)	0.822	0.91 (0.67-1.27)	0.107
Tertile 3	1096/75	1 (ref.)		1 (ref.)	
Women	1855/191		0.001[b]		0.004[b]
Tertile 1	620/86	1.81 (1.27-2.58)	0.001	1.65 (1.16-2.36)	0.006
Tertile 2	620/57	1.18 (0.80-1.73)	0.402	1.12 (0.76-1.64)	0.579
Tertile 3	615/48	1 (ref.)		1 (ref.)	
Men	1455/86		0.069[b]		0.071[b]
Tertile 1	489/42	1.49 (0.92-2.41)	0.107	1.48 (0.91-2.39)	0.116
Tertile 2	485/17	0.59 (0.32-1.08)	0.089	0.57 (0.31-1.05)	0.070
Tertile 3	481/27	1 (ref.)		1 (ref.)	
No nicotine exposure					
All participants	2714/206		0.007[b]		0.012[b]
Tertile 1	894/90	1.53 (1.11-2.12)	0.011	1.49 (1.08-2.07)	0.017
Tertile 2	912/56	0.92 (0.64-1.32)	0.651	0.90 (0.63-1.30)	0.588
Tertile 3	908/60	1 (ref.)		1 (ref.)	
Women	1552/144		0.035[b]		0.063[b]
Tertile 1	506/60	1.51 (1.02-2.24)	0.040	1.44 (0.97-2.13)	0.073
Tertile 2	517/42	1.01 (0.66-1.55)	0.955	0.99 (0.64-1.51)	0.952
Tertile 3	529/42	1 (ref.)		1 (ref.)	
Men	1162/62		0.095[b]		0.090[b]
Tertile 1	388/30	1.56 (0.87-2.81)	0.134	1.58 (0.88-2.83)	0.128
Tertile 2	395/14	0.72 (0.36-1.44)	0.347	0.72 (0.36-1.44)	0.346
Tertile 3	379/18	1 (ref.)		1 (ref.)	
Any nicotine exposure					
All participants	596/71		0.005[b]		0.008[b]
Tertile 1	215/38	2.20 (1.21-4.01)	0.010	2.07 (1.13-3.77)	0.018
Tertile 2	193/18	1.12 (0.56-2.22)	0.751	1.03 (0.52-2.05)	0.929
Tertile 3	188/15	1 (ref.)		1 (ref.)	
Women	303/47		0.103[b]		0.009[b]
Tertile 1	114/26	3.41 (1.40-8.28)	0.007	3.02 (1.24-7.37)	0.015
Tertile 2	103/15	2.20 (0.85-5.67)	0.103	1.88 (0.72-4.86)	0.195
Tertile 3	86/6	1 (ref.)		1 (ref.)	
Men	293/24		0.396[b]		0.399[b]
Tertile 1	101/12	1.40 (0.59-3.33)	0.442	1.40 (0.59-3.32)	0.447
Tertile 2	90/3	0.34 (0.09-1.27)	0.110	0.35 (0.09-1.28)	0.111
Tertile 3	102/9	1 (ref.)		1 (ref.)	

Median 10.8 years follow-up time. Age and sex-specific tertiles of plasma DMG. No nicotine exposure = plasma cotinine <85 nmol/L; any nicotine exposure = plasma cotinine ≥85 nmol/L

CI confidence interval, *Events* hip fractures, *HR* hazard ratio

[a] Sex group-stratified models are not adjusted for sex, and nicotine exposure-stratified models are not adjusted for nicotine exposure

[b] *p* value for trend across tertiles

catabolism via increased activity of peroxisome proliferator-activated receptor alpha (PPARα), rather than increased DMG production per se. Notably, increased expression of PPARα agonists in rats has been related to higher BMD and increased

medullary bone area [42, 43]. Moreover, mice models have suggested a link between enhanced genetic PPARα expression and increased hepatic choline levels, potentially related to altered methylation status of the PPARα promoter [44]. Thus, lower plasma choline and DMG levels associated with adverse bone health could be related to decreased PPARα activity and increased downstream catabolism via DMG dehydrogenase. Increased catabolism of DMG may enhance the availability of methyl groups to be used in epigenetic regulation, such as methylation of DNA. Although such modulation is complex and may include both global and focal hypomethylation and hypermethylation, experimental data have linked enhanced DNA methylation to repressed transcription of genes associated with increased bone resorption [45].

Low dietary intake of the DMG precursors choline and betaine has been related to increased concentrations of inflammatory markers, such as CRP, interleukin-6, and tumor necrosis factor-α [46], all of which are also positively associated with osteoporosis risk [12, 47]. Low systemic DMG levels have also been observed among patients with chronic obstructive pulmonary disease, a condition related to low-grade systemic inflammation [48]. In the current study, however, we observed a positive trend between DMG and CRP among the middle-aged cohort, and we previously reported a positive relationship between plasma DMG and serum CRP among patients with suspected coronary heart disease [9]. However, none of these studies, including the current, accounted for dietary intake. Thus, the relationship between DMG and inflammation remains elusive. However, adjusting for CRP did not attenuate the relationship between DMG and BMD or hip fracture risk to any particular degree, suggesting the association not to be mediated by inflammatory mechanisms alone.

Also contrary to what was observed among patients with CHD [9, 41], there was no significant difference in plasma DMG between nicotine-exposed versus nicotine-unexposed participants. However, we found a stronger association between DMG and BMD among participants who were exposed, as compared to those who were unexposed to nicotine. By comparison, the risk association between plasma DMG and incident AMI was confined to non-smokers [9]. The mechanisms behind the current effect modification are unclear, but smokers typically have increased levels of inflammation markers [49], increased fat oxidation [50], lower BMI [51], and lower levels of estrogens [52], all being risk factors of osteoporosis [16], as well. We confirmed the associations of nicotine exposure with inflammation (CRP) and BMI. Furthermore, smokers have altered levels of several B vitamins crucial for reactions in the choline oxidation pathway [53]. Smoking also transforms the structure and causes decomposition of phospholipids [54], leading to reduced availability of phosphatidylcholine, and thus potentially attenuates substrate availability for DMG production.

In conclusion, low levels of plasma DMG were associated with low BMD and increased risk of hip fracture. Low plasma DMG may reflect low flux through BHMT and/or increased catabolism due to low PPARα gene expression. Future studies should examine determinants of plasma choline, betaine, and DMG, as well as primary dietary and lifestyle factors that stimulate the BHMT activity and DMG production and their relation relation to BMD.

Conflicts of interest None.

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