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Dietary Choline Intake Is Directly Associated with Bone Mineral Density in the Hordaland Health Study[1,2]

Jannike Øyen,[3,6,9]* Clara Gram Gjesdal,[3,7] Therese Karlsson,[7] Gard FT Svingen,[4] Grethe S Tell,[6] Elin Strand,[7] Christian A Drevon,[10] Kathrine J Vinknes,[10] Klaus Meyer,[11] Per Magne Ueland,[7,5] and Ottar Nygård[4,8]

[3]Departments of Rheumatology and [4]Heart Disease and [5]Laboratory of Clinical Biochemistry, Haukeland University Hospital, Bergen, Norway; [6]Department of Global Public Health and Primary Care, [7]Department of Clinical Science, and [8]KG Jebsen Centre for Diabetes Research, Department of Clinical Science, University of Bergen, Bergen, Norway; [9]National Institute of Nutrition and Seafood Research, Bergen, Norway; [10]Department of Nutrition, Institute of Basic Medical Sciences, Faculty of Medicine, University of Oslo, Oslo, Norway; and [11]BEVITAL AS, Bergen, Norway

Abstract

Background: Choline is an important nutrient either obtained from a variety of foods or synthesized endogenously, and it is the precursor of betaine. We previously reported positive associations between plasma free choline and bone mineral density (BMD). Animal studies suggest an impact of dietary choline on bone metabolism, but the role of dietary intake of choline and betaine for human bone health is unknown.

Objectives: The main aims were to examine the associations of dietary choline, choline species, and betaine with BMD and to study the relations between dietary and plasma free choline and betaine.

Methods: Study subjects were participants in the Hordaland Health Study, including 2649 women and 1983 men (aged 46–49 or 71–74 y). BMD was measured by dual-energy X-ray absorptiometry, and dietary intake was obtained by using a validated 169-item food-frequency questionnaire. Risk associations were assessed by logistic regression and correlations by ρ (Spearman's bivariate rank order correlation).

Results: Subjects in the lowest compared with the highest tertile of dietary total choline, free choline, glycerophosphocholine, phosphocholine, phosphatidylcholine, and sphingomyelin had a higher risk of low–femoral neck BMD, defined as the lowest BMD quintile. Particularly strong associations were found among middle-aged men for intake of free choline (OR: 1.83; 95% CI: 1.24, 2.69; $P = 0.002$) and glycerophosphocholine (OR: 2.13; 95% CI: 1.43, 3.16; $P < 0.001$) and among elderly women for total choline (OR: 1.96; 95% CI: 1.33, 2.88; $P = 0.001$) and phosphatidylcholine (OR: 1.94; 95% CI: 1.33, 2.84: $P = 0.001$) intake. No significant associations were observed between dietary betaine and BMD. Dietary total choline, free choline, glycerophosphocholine, phosphatidylcholine, and sphingomyelin correlated weakly with plasma free choline (ρ: 0.07, 0.05, 0.07, 0.07, and 0.05, respectively; $P < 0.01$). Dietary betaine correlated with plasma betaine (ρ: 0.23; $P < 0.001$).

Conclusion: Dietary choline was positively associated with BMD in middle-aged and elderly participants. *J Nutr* 2017;147:572–8.

Keywords: bone mineral density, community-dwelling participants, dietary betaine, dietary choline, glycerophosphocholine, phosphatidylcholine, phosphocholine, plasma betaine, plasma choline, sphingomyelin

Introduction

Choline is an important nutrient obtained from a variety of foods (1). In European populations the main sources of dietary choline are meat, milk, grain, egg, and fish (2). According to North American health authorities, an adequate intake of choline is 425 and 550 mg/d for women and men, respectively (3). However, studies from the United States (4, 5) and Europe (2) suggest that intake of choline may be inadequate.

The total choline content in foods includes free choline in addition to choline species, such as glycerophosphocholine, phosphocholine, phosphatidylcholine, and sphingomyelin (6). Choline may also be formed by de novo biosynthesis via methylation of phosphatidylethanolamine, catalyzed by

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*To whom correspondence should be addressed. E-mail: jannike.oyen@nifes.no.

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phosphatidylethanolamine *N*-methyltransferase (7). Choline is required for the synthesis of acetylcholine and phospholipids, which are necessary components of lipoproteins and cellular membranes (1, 7). In the mitochondria, choline is oxidized to betaine, which serves as an intracellular osmolyte in addition to being a methyl donor in the remethylation of homocysteine catalyzed by betaine-homocysteine methyltransferase (BHMT)[12] (1, 7). Conversion of choline to betaine is the only endogenous pathway of betaine synthesis, although betaine may also be obtained from the diet. Foods with the highest content of betaine are whole grain, wheat bran, wheat germ, spinach, and shrimp (6, 8).

Studies of health-related effects of dietary choline in community-dwelling participants have shown that low and high intakes may be associated with adverse health effects. High choline intake has been related to increased risk of colorectal adenomas (9). High intake of choline and betaine have also been related to a favorable body composition in humans (10). Low choline intake has been associated with hepatic fat accumulation, liver and muscle damage (11), and impaired cognitive function (12). In animal studies, diets low in choline may adversely affect bone health (13, 14). Our group previously reported that low concentrations of plasma free choline (15) and dimethylglycine (16), the immediate betaine catabolite, were associated with low bone mineral density (BMD) in humans, whereas no relations were observed with plasma betaine (15). However, it is unclear whether intake of choline and betaine is reflected in their systemic concentrations and if such intakes influence bone health in humans (17–20).

The aims of this study were to examine associations of dietary choline and choline species as well as betaine with BMD and to examine correlations between intake of total choline and betaine with plasma concentrations of free choline and betaine in a cross-sectional study of community-dwelling middle-aged and older adults.

Methods

Study population. Participants in this cross-sectional study were from the Hordaland Health Study (HUSK) in Western Norway and examined in 1998–2000. The HUSK was a collaboration between the University of Bergen, the Norwegian Institute of Public Health, and local health services. The 9187 invited participants were born in 1925–1927 (older cohort) or 1950–1951 (middle-aged cohort) and had previously participated in the Hordaland Homocysteine Study in 1992–1993 (21).

A total of 7074 participants (77% of those invited) met for examinations and completed self-administered questionnaires about health status, lifestyle factors, and use of medications. Of the 7074 participants attending the health examinations, we obtained femoral neck BMD measurements from 5378 subjects, and of these, 4762 completed an FFQ. The FFQ was handed out on the day of the health examination, filled out at home, and returned by mail to the HUSK project center. Participants with a very low [<3000 kJ or 717 kcal for women (n = 57); <3300 kJ or 788 kcal for men (n = 13)] or a very high [>15,000 kJ or 3583 kcal for women (n = 21); >17,500 kJ or 4180 kcal for men (n = 39)] estimated daily energy intake were excluded. This left 4632 subjects, of whom 65.5% attended the health examination (1600 women and 1024 men aged 46–49 y, and 1049 women and 959 men aged 71–74 y).

[12] Abbreviations used: BHMT, betaine-homocysteine methyltransferase; BMD, bone mineral density; CRP, C-reactive protein; hs-CRP, high-sensitive C-reactive protein; HUSK, Hordaland Health Study; ρ, Spearman's bivariate rank order correlation.

The study was approved by the Regional Committee for Medical and Health Research Ethics. Each participant signed an informed consent form.

BMD measurements. BMD was measured by DXA on a stationary fan beam densitometer (Expert-XL; Lunar Company Inc.) operated by 4 skilled technicians (22). The left hip was scanned except when there was a history 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.

Assessment of dietary intake. Information on dietary intake was obtained by using a validated 169-item FFQ (23), which assessed habitual diet during the past year and included frequency alternatives, the number of portions, and portion sizes. The FFQ was previously validated against multiple, weighed dietary records for 14 d in elderly men and women and against serum carotenoids and FA composition in adipose tissue and serum (23–25). The frequency of consumption was specified per day, week, or month. Information on the use of supplements was obtained from the FFQ and included in the calculations. A database and a software system (KBS software, version 3.2; University of Oslo) developed at the Department of Nutrition, University of Oslo, were used to calculate nutrient intake. Choline and betaine intakes were estimated according to the USDA choline database (26). Total choline was calculated as the sum of free choline and choline from glycerophosphocholine, phosphocholine, phosphatidylcholine, and sphingomyelin.

Analytic procedures. Nonfasting blood samples were collected in evacuated tubes containing EDTA, chilled, and centrifuged (1000 × *g*; 10 min; 4°C) within 1–3 h. EDTA-plasma was stored at −80°C. Plasma free choline, betaine, and cotinine were measured by LC-MS/MS 6–8 y after collection without any thaw-freeze cycles (27, 28). CVs were 3.8–7.6% for plasma free choline and 5–11.7% for plasma betaine (27). Within-day and between-day CVs were determined on sample replicates on controls (29). Plasma cotinine concentrations ≥85 nmol/L were used to identify current smokers (30). High-sensitive C-reactive protein (hs-CRP) was determined by a novel immunoassay based on matrix-assisted laser desorption and ionization time-of-flight mass spectrometry (31). The analyses were performed at the BEVITAL laboratory, Bergen, Norway.

Other measurements. Detailed information about measurements of weight, height, BMI (in kg/m^2), physical activity, and hormone replacement therapy has been presented previously (15).

Statistical analysis. Categorical variables are expressed as numbers (percentages) and continuous variables as medians (IQRs). All dietary nutrients were energy-adjusted by using the residual method (32). Trends across tertiles of dietary total choline and betaine were tested by logistic and linear regression for categorical and continuous variables, respectively. The analyses were adjusted for sex and age group. Sex was adjusted for age group. One-factor ANOVA with Bonferroni's correction was used to compare choline intake between sex and age group. We explored potential nonlinear relations between dietary intake of total choline and BMD in all participants in generalized additive models adjusting for sex and age group.

ORs for being in the lowest quintile of femoral neck BMD according to sex- and age-specific tertiles of dietary choline and betaine were estimated in logistic regression analyses. We explored potential interactions between sex and dietary choline intake in relation to BMD in the 2 age groups by introducing a product term between tertiles of the dietary choline variables (continuous) and sex (dichotomous) in the binary logistic regression analyses. The logistic regression models were adjusted for age group and sex as well as potential confounding factors, including BMI, nicotine exposure, physical activity, hs-CRP, and dietary intakes of vitamin D and calcium. Further adjustment for dietary protein intake as well as estrogen supplementation (in women) did not alter the risk estimates and were left out of the multivariate model (data not shown). Unadjusted analyses were also performed, and the results were similar but somewhat weaker than in the adjusted analyses; thus, we present results from the adjusted models.

The correlations between dietary and plasma concentrations of choline and betaine were assessed by using Spearman's bivariate rank order correlation (ρ).

Two-tailed P values <0.05 were considered statistically significant. The analyses were performed by using SPSS for windows (IBM SPSS Statistics 22) and software package mgcv for R, version 3.1.2 (The R Foundation for Statistical Computing).

Results

Study population. Characteristics of participants according to tertiles of energy-adjusted dietary intake of total choline and betaine are presented in **Table 1**.

Median energy-adjusted total choline intakes in middle-aged women and men were 255 mg/d (IQR: 63 mg/d) and 259 mg/d (IQR: 74 mg/d), respectively, and in elderly women and men 265 mg/d (IQR: 61 mg/d) and 258 mg/d (IQR: 61 mg/d), respectively. Age- and sex-specific analyses showed that elderly women had higher intakes than middle-aged women (P = 0.001) and middle-aged men (P = 0.015). Dietary intake of total choline was positively correlated with intakes of meat, fish, eggs, milk, fiber, calcium, and protein but negatively associated with the intakes of carbohydrates and fat (Table 1). Dietary choline was also positively correlated with nicotine exposure and BMI and inversely associated with hs-CRP.

Dietary betaine was positively associated with dietary fiber and carbohydrates as well as plasma betaine but negatively associated with intakes of meat, fish, eggs, milk, vitamin D, calcium, and total fat as well as with any nicotine exposure and BMI (Table 1).

Three hundred forty-two (21.4%) middle-aged and 128 (12.2%) elderly women reported current use of hormone-replacement therapy (P < 0.001), and there were no significant differences between use and nonuse across tertiles of choline or betaine intake.

TABLE 1 Characteristics of the participants (women and men aged 46–49 and 71–74 y old) in total and by tertiles of energy-adjusted intake of total choline and betaine[1]

			Tertiles of dietary total choline intake				Tertiles of dietary betaine intake			
	N	All (*n* = 4632)	First (*n* = 1542)	Second (*n* = 1546)	Third (*n* = 1544)	*P*-trend[2]	First (*n* = 1542)	Second (*n* = 1546)	Third (*n* = 1544)	*P*-trend[2]
Women	2649	2649 (57.2)[3]	882 (57.2)	884 (57.2)	883 (57.2)	1.00	882 (57.2)	884 (57.2)	883 (57.2)	1.00
Nicotine exposure[4]	4580	1243 (27.1)	368 (24.2)	388 (25.4)	487 (31.8)	<0.001	480 (31.1)	403 (26.1)	360 (23.6)	<0.001
No physical activity	4505	1618 (34.9)	556 (37.3)	558 (36.9)	504 (33.6)	0.035	571 (37.0)	569 (36.8)	478 (31.8)	<0.001
Femoral neck BMD, g/cm^2	4632	0.908 [0.211][5]	0.896 [0.208]	0.913 [0.211]	0.918 [0.211]	<0.001	0.907 [0.120]	0.907 [0.215]	0.913 [0.216]	0.40
Total fat mass, kg	4632	22.1 [11.4]	20.9 [11.4]	21.9 [11.1]	23.4 [11.9]	<0.001	22.9 [11.7]	22.1 [11.3]	21.1 [11.4]	<0.001
Total lean mass, kg	4632	44.2 [17.8]	44.1 [18.0]	44.5 [18.1]	44.1 [17.6]	<0.001	44.1 [17.9]	44.3 [18.1]	44.2 [17.5]	0.93
BMI, kg/m^2	4632	25.3 [4.7]	24.8 [4.6]	25.2 [4.7]	26.0 [4.9]	<0.001	27.7 [4.9]	25.3 [4.7]	24.9 [4.6]	<0.001
Plasma										
Choline, μmol/L	4612	9.6 [2.9]	9.4 [2.8]	9.5 [2.8]	9.7 [2.9]	0.033	9.4 [2.8]	9.6 [2.8]	9.7 [2.9]	0.17
Betaine, μmol/L	4612	38.0 [15.2]	38.2 [15.0]	38.3 [15.0]	37.3 [15.5]	0.85	36.1 [13.8]	38.3 [15.5]	39.7 [16.1]	<0.001
Serum										
hs-CRP, mg/L	4612	1.40 [2.75]	1.59 [2.79]	1.47 [2.79]	1.43 [2.84]	0.008	1.39 [2.78]	1.55 [2.86]	1.53 [2.81]	0.90
Daily dietary intake										
Total choline, mg	4632	259 [66]	216 [31]	259 [20]	311 [45]	<0.001	262 [69]	258 [63]	257 [66]	0.14
Free choline, mg	4632	70.7 [16.8]	62.8 [12.0]	70.9 [12.2]	80.5 [17.9]	<0.001	70.6 [18.0]	70.5 [15.6]	70.9 [16.7]	0.08
Glycerophosphocholine, mg	4632	59.7 [26.8]	47.9 [18.7]	60.7 [20.0]	75.4 [29.8]	<0.001	59.1 [28.4]	60.4 [26.0]	59.4 [26.5]	0.42
Phosphocholine, mg	4632	10.2 [5.5]	7.7 [3.7]	10.4 [3.9]	13.7 [6.2]	<0.001	10.7 [5.8]	9.9 [5.3]	10.1 [5.6]	<0.001
Phosphatidylcholine, mg	4632	114.6 [40.0]	93.0 [24.5]	115.9 [24.8]	141.9 [39.0]	<0.001	117 [38]	113 [39]	114 [41]	0.002
Sphingomyelin, mg	4632	8.9 [3.6]	7.0 [2.6]	8.9 [2.3]	11.1 [3.4]	<0.001	8.9 [3.8]	8.8 [3.6]	8.8 [3.4]	0.005
Betaine, mg	4632	134 [39]	136 [37]	135 [38]	132 [42]	0.006	108 [21]	134 [12]	166 [30]	<0.001
Meat, g	4632	102 [55]	95 [52]	104 [53]	105 [60]	<0.001	113 [59]	102 [52]	90 [48]	<0.001
Fish, g	4632	75.7 [52.6]	64.7 [49.1]	76.6 [46.3]	86.2 [60.0]	<0.001	78.4 [56.1]	76.6 [52.0]	71.4 [51.2]	<0.001
Eggs, g	4632	15.4 [11.8]	9.0 [6.8]	16.2 [9.7]	20.0 [14.1]	<0.001	16.3 [14.2]	15.5 [11.7]	13.7 [12.0]	<0.001
Milk, g	4632	255 [244]	171 [163]	270 [238]	365 [313]	<0.001	266 [257]	259 [250]	242 [229]	0.002
Vitamin D, μg	4632	7.0 [9.0]	19.7 [6.9]	20.1 [6.2]	21.0 [7.5]	<0.001	7.5 [9.0]	6.9 [8.0]	6.7 [8.0]	<0.001
Calcium, mg	4632	753 [410]	683 [256]	778 [250]	874 [317]	<0.001	790 [393]	728 [399]	744 [444]	<0.001
Energy, kJ	4632	8046 [3383]	8187 [3582]	7752 [3215]	8213 [3300]	0.41	8352 [3292]	7754 [3170]	7957 [3643]	<0.001
Fiber, E%	4632	2.3 [0.8]	2.2 [0.7]	2.3 [0.7]	2.4 [0.9]	<0.001	2.1 [0.7]	2.3 [0.7]	2.5 [0.8]	<0.001
Protein, E%	4632	16.0 [3.0]	14.7 [2.5]	16.1 [2.6]	17.2 [2.9]	<0.001	16.0 [3.1]	16.0 [3.1]	16.0 [3.0]	0.32
Carbohydrate, E%	4632	50.9 [7.8]	51.9 [8.0]	51.0 [7.9]	49.7 [7.6]	<0.001	48.7 [7.5]	50.6 [7.6]	52.8 [7.2]	<0.001
Total fat, E%	4632	31.2 [7.0]	31.9 [7.2]	31.0 [6.9]	30.9 [6.6]	<0.001	33.2 [6.6]	31.3 [6.8]	29.3 [6.4]	<0.001
SFA, E%	4632	12.2 [3.2]	12.5 [3.4]	12.1 [3.1]	11.9 [3.0]	<0.001	13.0 [3.0]	12.1 [3.0]	11.1 [3.0]	<0.001
MUFA, E%	4632	9.9 [2.4]	9.9 [2.5]	9.9 [2.4]	9.9 [2.2]	0.54	10.6 [2.2]	9.9 [2.3]	9.2 [2.3]	<0.001
PUFA, E%	4632	6.4 [2.4]	6.5 [2.7]	6.3 [2.4]	6.3 [2.2]	<0.001	6.5 [2.6]	6.3 [2.4]	6.2 [2.3]	<0.001

[1] All dietary nutrients were energy-adjusted. BMD, bone mineral density; E%, percentage of total energy intake; hs-CRP, high-sensitive C-reactive protein.
[2] *P*-trend across tertiles of choline and betaine intakes was calculated by linear regression for continuous variables and logistic regression for dichotomous variables. The analyses were adjusted for sex and age group. Sex was adjusted for age group.
[3] Number; percentage in parentheses (all such values).
[4] Any nicotine exposure = plasma cotinine ≥85 nmol/L.
[5] Median; IQR in brackets (all such values).

Dietary choline and betaine in relation to BMD. The positive relation between intake of total choline and BMD in all subjects combined is shown in **Figure 1** (β: 0.05, $P = 0.001$). Furthermore, participants in the lowest compared with highest tertile of dietary total choline, free choline, glycerophosphocholine, phosphocholine, phosphatidylcholine, and sphingomyelin were more prone to have a low BMD (**Table 2**). In analyses stratified by age group and sex, similar results were found among middle-aged men for whom the (nominal) strongest associations were observed for intakes of free choline (OR: 1.83; 95% CI: 1.24, 2.69; $P = 0.002$), glycerophosphocholine (OR: 2.13; 95% CI: 1.43, 3.16; $P < 0.001$), and phosphocholine (OR: 1.81; 95% CI: 1.23, 2.66; $P = 0.003$). In elderly women similar associations were seen for the intakes of total choline, free choline, phosphatidylcholine, and sphingomyelin (Table 2). Significant interactions between sex and dietary intake of total choline (P-interaction = 0.001), phosphatidylcholine (P-interaction < 0.001), and sphingomyelin (P-interaction = 0.046) were found in relation to risk of low BMD among the elderly and for glycerophosphocholine (P-interaction = 0.046) among the middle-aged age group.

No significant associations were observed between dietary betaine and BMD (Table 2).

Dietary choline and betaine in relation to plasma free choline and betaine. Intakes of total choline, free choline, glycerophosphocholine, phosphocholine, and phosphatidylcholine were weakly positively correlated with plasma free choline in the whole population (ρ: 0.07, 0.05, 0.07, 0.07, and 0.05, respectively; $P < 0.01$). In analyses stratified by sex and age group, dietary total choline correlated weakly with plasma concentration of free choline among middle-aged and elderly men (ρ: 0.08 and 0.06, respectively; $P < 0.05$) but not among women (**Table 3**).

Overall (ρ: 0.23, $P < 0.001$) and in all sex and age groups, dietary betaine was significantly correlated with plasma betaine (Table 3).

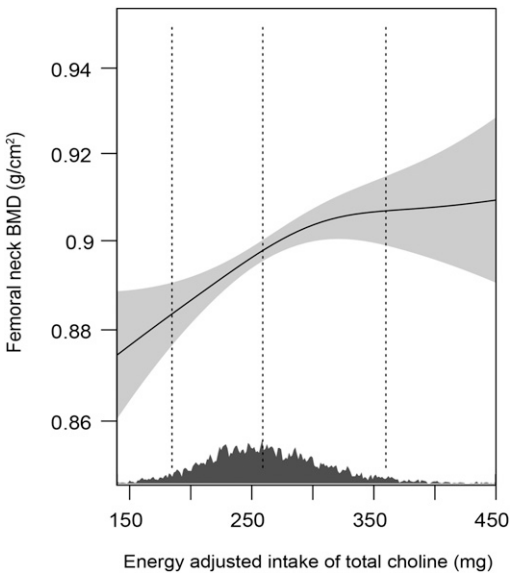

FIGURE 1 Spline curve (generalized additive linear model) showing the association between energy-adjusted intake of total choline and femoral neck BMD in 4632 women and men aged 46–49 and 71–74 y in the Hordaland Health Study. The model is adjusted for sex and age group. The solid line is the dose-response curve; the gray area represents the 95% CIs. BMD, bone mineral density.

Discussion

Intakes of total free choline, glycerophosphocholine, and phosphocholine were positively associated with BMD in middle-aged men, and intakes of total choline, free choline, phosphatidylcholine, and sphingomyelin were positively associated with BMD in elderly women. No significant associations with BMD were observed for dietary betaine. We observed a weak positive correlation between total choline intake and plasma concentration of free choline and a moderate positive correlation between betaine intake and plasma betaine.

To our knowledge, our study is the first to examine the associations of habitual intake of choline and betaine with BMD in humans. Our current findings support our previous observations suggesting an association of low concentrations of plasma free choline (15) and dimethylglycine (16) with low BMD, whereas no significant relations were observed between plasma betaine and BMD (15).

We observed weak-to-moderate positive correlations between dietary intake and plasma concentrations of choline and betaine. Plasma free choline seems to be most predictive of BMD because the OR for having low BMD was somewhat stronger for the lowest compared with the highest tertiles of plasma free choline (OR: 2.00; 95% CI: 1.17, 1.66) (15) than for the lowest compared with the highest tertiles of dietary choline (OR: 1.36; 95% CI: 1.14, 1.63) when comparing the whole population. In other studies, the relations between dietary and circulating choline (17, 18) as well as between dietary and systemic betaine concentrations (19) have been divergent. This may be due to differences in doses, study durations, or study cohorts. In addition, de novo choline synthesis may be increased when intake is insufficient (33, 34) thereby weakening potential correlations.

The associations between choline intake and BMD are supported by observations in animal studies because choline-deficient rats had altered mandibular bone remodeling detected as a marked reduction in osteogenesis (13) and reduced bone formation and increased bone resorption, promoting reduced cancellous and cortical bone mass (14). Phosphatidylcholine is a ligand for PPARα and PPARγ (35, 36). Activation of PPARα may have a protective role in regulation of bone metabolism (37), whereas activation of PPARγ may lead mesenchymal stem cells to differentiate into adipocytes and not osteoblasts (38).

Inflammation is associated with low BMD (39). Our findings of an inverse association between choline intake and plasma C-reactive protein (CRP) are in accordance with previous studies in which a low dietary intake of choline was associated with an increased concentration of the inflammatory markers CRP, IL-6, and TNF-α (40), which are also inversely associated with BMD (41, 42). CRP was the only inflammatory marker available in our study, and adjusting for CRP did not materially change the association between dietary choline and BMD, suggesting that the relation is not mediated by inflammatory mechanisms alone.

We have previously reported that elevated plasma homocysteine concentrations were related to low BMD in women in the HUSK (22) in agreement with results from a recent study published by others (43). Choline supplementation (44) and dietary intake (4) may reduce plasma homocysteine concentrations; thus, there may be a connection between effects of choline and homocysteine on BMD in women.

Low choline intake has been associated with metabolic syndrome and nonalcoholic fatty liver disease in animal models (45). However, in the current cohort high plasma concentrations of free choline and low betaine were related to

TABLE 2 ORs for low bone mineral density according to age- and sex-specific tertiles of energy-adjusted intakes of choline and betaine in all participants and by age group and sex[1]

Dietary variables and tertiles	All[2] (n = 4632)			Women,[3] age (y)						Men,[3] age (y)					
				46–49 (n = 1600)			71–74 (n = 1049)			46–49 (n = 1024)			71–74 (n = 959)		
	n	OR (95% CI)	P	n	OR (95% CI)	P	n	OR (95% CI)	P	n	OR (95% CI)	P	n	OR (95% CI)	P
Total choline, mg/d			0.001			0.047			0.001			0.08			0.50
T1: 215.7 (31.3)	1542	1.36 (1.14, 1.63)	0.001	533	1.37 (1.01, 1.87)	0.044	349	1.96 (1.33, 2.88)	0.001	341	1.39 (0.96, 2.03)	0.08	319	0.88 (0.60, 1.29)	0.51
T2: 259.0 (20.3)	1546	1.10 (0.92, 1.33)	0.29	534	1.37 (1.01, 1.87)	0.045	350	1.44 (0.97, 2.15)	0.07	342	0.78 (0.52, 1.16)	0.22	320	0.83 (0.56, 1.22)	0.34
T3: 311.4 (44.7)	1544	1 (ref)		533	1 (ref)		350	1 (ref)		341	1 (ref)		320	1 (ref)	
Free choline, mg/d			<0.001			0.32			0.022			0.002			0.24
T1: 59.7 (8.0)	1547	1.41 (1.18, 1.69)	<0.001	538	1.17 (0.86, 1.60)	0.32	349	1.58 (1.07, 2.32)	0.020	341	1.83 (1.24, 2.69)	0.002	319	1.26 (0.85, 1.86)	0.25
T2: 70.8 (4.8)	1541	1.25 (1.04, 1.50)	0.017	529	1.29 (0.95, 1.18)	0.10	350	1.49 (1.01, 2.19)	0.045	342	1.13 (0.75, 1.69)	0.57	320	1.06 (0.71, 1.57)	0.79
T3: 84.4 (11.8)	1544	1 (ref)		533	1 (ref)		350	1 (ref)		341	1 (ref)		320	1 (ref)	
Glycerophosphocholine, mg/d			0.001			0.38			0.43			<0.001			0.17
T1: 42.7 (11.5)	1542	1.36 (1.14, 1.63)	0.001	533	1.15 (0.85, 1.57)	0.37	349	1.17 (0.80, 1.72)	0.42	341	2.13 (1.43, 3.16)	<0.001	319	1.31 (0.89, 1.93)	0.17
T2: 59.7 (9.4)	1546	1.28 (1.07, 1.54)	0.007	534	1.45 (0.97, 2.19)	0.07	350	1.37 (0.94, 1.99)	0.11	342	1.45 (0.97, 2.19)	0.07	320	1.02 (0.69, 1.53)	0.91
T3: 81.2 (18.1)	1544	1 (ref)		533	1 (ref)		350	1 (ref)		341	1 (ref)		320	1 (ref)	
Phosphocholine, mg/d			0.001			0.16			0.12			0.002			0.39
T1: 6.8 (2.4)	1542	1.36 (1.14, 1.63)	0.001	533	1.25 (0.92, 1.69)	0.16	349	1.34 (0.92, 1.94)	0.12	341	1.81 (1.23, 2.66)	0.003	319	1.18 (0.80, 1.74)	0.39
T2: 10.2 (1.8)	1546	1.12 (0.93, 1.34)	0.24	534	1.20 (0.86, 1.57)	0.24	350	1.03 (0.70, 1.51)	0.89	342	1.19 (0.80, 1.78)	0.39	320	1.02 (0.68, 1.51)	0.93
T3: 14.7 (4.3)	1544	1 (ref)		533	1 (ref)		350	1 (ref)		341	1 (ref)		320	1 (ref)	
Phosphatidylcholine, mg/d			0.016			0.020			<0.001			0.84			0.09
T1: 78.5 (17.2)	1542	1.25 (1.04, 1.49)	0.016	533	1.45 (1.07, 1.98)	0.018	349	1.94 (1.33, 2.84)	0.001	341	1.04 (0.71, 1.51)	0.84	319	0.71 (0.48, 1.06)	0.09
T2: 104.6 (11.9)	1546	1.12 (0.93, 1.34)	0.22	534	1.44 (1.05, 1.96)	0.022	350	1.25 (0.84, 1.86)	0.28	342	0.84 (0.57, 1.24)	0.38	320	0.92 (0.63, 1.35)	0.67
T3: 136.0 (28.0)	1544	1 (ref)		533	1 (ref)		350	1 (ref)		341	1 (ref)		320	1 (ref)	
Sphingomyelin, mg/d			0.005			0.21			0.008			0.10			0.86
T1: 6.4 (1.9)	1542	1.29 (1.08, 1.54)	0.005	533	1.21 (0.90, 1.63)	0.21	349	1.67 (1.14, 2.45)	0.008	341	1.38 (0.94, 2.03)	0.10	319	1.04 (0.70, 1.53)	0.86
T2: 8.9 (1.0)	1542	1.09 (0.91, 1.31)	0.34	534	0.93 (0.68, 1.27)	0.67	350	1.37 (0.93, 2.03)	0.11	342	1.20 (0.81, 1.77)	0.37	316	1.04 (0.70, 1.53)	0.85
T3: 11.7 (2.3)	1548	1 (ref)		533	1 (ref)		350	1 (ref)		341	1 (ref)		324	1 (ref)	
Betaine, mg/d			0.89			0.99			0.91			0.80			0.56
T1: 108.2 (20.5)	1542	1.01 (0.85, 1.21)	0.89	533	1.00 (0.74, 1.35)	0.99	349	1.02 (0.70, 1.49)	0.91	341	0.95 (0.64, 1.40)	0.80	319	1.13 (0.76, 1.67)	0.55
T2: 134.3 (12.0)	1546	1.06 (0.88, 1.26)	0.55	534	0.98 (0.73, 1.34)	0.92	350	1.03 (0.71, 1.50)	0.88	342	1.12 (0.77, 1.64)	0.55	320	1.14 (0.77, 1.69)	0.51
T3: 166.1 (30.4)	1544	1 (ref)		533	1 (ref)		350	1 (ref)		341	1 (ref)		320	1 (ref)	

[1] Tertiles of dietary variables are medians (IQRs). Low bone mineral density is defined as being in the lowest quintile of bone mineral density in each sex and age group. hs-CRP, high-sensitive C-reactive protein; ref, reference; T, tertile.
[2] Adjusted for BMI, plasma cotinine (nicotine exposure), hs-CRP, energy-adjusted dietary vitamin D, calcium, physical activity, age group, and sex.
[3] Adjusted for BMI, plasma cotinine (nicotine exposure), hs-CRP, energy-adjusted dietary vitamin D, calcium, and physical activity.

certain components of the metabolic syndrome (46). The metabolic syndrome (47, 48) and nonalcoholic fatty liver disease (49) have been related to increased risk of low BMD in some studies.

TABLE 3 Correlations between plasma concentration of free choline and energy-adjusted dietary total choline, free choline, glycerophosphocholine, phosphocholine, phosphatidylcholine, and sphingomyelin, as well as plasma concentration of betaine and dietary betaine[1]

	All (n = 4632)	Women, age (y)		Men, age (y)	
		46–49 (n = 1600)	71–74 (n = 1049)	46–49 (n = 1024)	71–74 (n = 959)
Total choline	0.07***	−0.01	0.05	0.06*	0.08*
Free choline	0.05**	−0.03	0.01	0.05	0.03
Glycerophosphocholine	0.07***	−0.02	−0.00	0.04	−0.01
Phosphocholine	0.02	−0.02	−0.00	0.02	0.01
Phosphatidylcholine	0.07***	0.01	0.08*	0.06	0.13***
Sphingomyelin	0.05**	−0.01	0.03	0.02	0.07*
Betaine	0.23***	0.06*	0.07*	0.19***	0.20***

[1] Data are Spearman's bivariate rank order correlation values. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

In analyses stratified by sex and age group, we found the highest OR between choline intake and BMD among middle-aged men and elderly women. A weak significant association was also found in middle-aged women for total choline and phosphatidylcholine, whereas no significant associations were observed in elderly men. In our earlier observations, the strongest positive association between plasma concentration of free choline and BMD was seen among elderly women (15). The median daily intake of choline was higher among elderly women than middle-aged men and women. These sex and age differences may be partly explained by transcriptional regulation of human BHMT by estrogen and androgen (50). Estrogen induces expression of the phosphatidylethanolamine *N*-methyltransferase gene in premenopausal women, thereby stimulating endogenous choline production (51). In a clinical study of choline deficiency sequelae, premenopausal women had a higher endogenous production of choline than postmenopausal women did (11). These findings may explain our results of a weak, significant association between choline intake and BMD among the women in their 40s, considering that the average age at menopause is ~50 y. Unfortunately, we did not have information about menstrual status, although adjusting for use of estrogen supplements did not alter the results. We previously have shown that low plasma dimethylglycine also was associated with low

BMD (16), and although neither dietary nor systemic betaine (15) has been related to BMD, our results may suggest that a low choline intake may reduce flux via BHMT, thereby leading to low dimethylglycine. Because BHMT flux is associated with transcription of apoB, the main protein of VLDL (52), future studies should evaluate if the association between low choline and low BMD may be mediated by impairment of lipid metabolism.

Among middle-aged men, significant associations with BMD were observed for glycerophosphocholine and phosphocholine but not phosphatidylcholine, whereas the opposite was observed in elderly women. This may be due to differences in intakes, bioavailability (6), or metabolism of choline species, factors that may cause residual confounding. Further studies should explore the effects of different dietary choline species and choline intake from different foods on BMD.

Strengths of our study include the large number of participants recruited from the community and not selected because of specific conditions or diseases, which decreases the risk of reverse causation. We had extensive clinical and biochemical information, and we observed that choline intake was positively correlated with BMI, fat mass, smoking, physical activity, and intakes of vitamin D, calcium, and proteins. A high intake of betaine had the opposite relation regarding BMI, fat mass, smoking, and vitamin D. A low BMI (53), fat mass (54), lack of physical activity (55), smoking (56), and low intakes of vitamin D, calcium (57), and protein (58) may be related to low BMD and thus confound our findings between choline intake and BMD. Adjusting for these factors strengthened the associations somewhat, suggesting that additional mechanisms may explain the association between choline intake and BMD.

The current FFQ has not been validated for choline or betaine intake but has been evaluated against a 14-d weighed dietary record, FAs in adipose tissue, and serum carotenoids (23–25). Our dietary choline and betaine data were calculated according to a US database (26) because no Norwegian data exist, thus the amount of choline and betaine in some foods may not accurately reflect the content in Norwegian foods. However, the main sources of dietary choline according to the US database are meat, fish, eggs, and milk (4), which are common foods in the Norwegian diet (59). Notably, intakes of these foods were also positively associated with total choline intake in this study population. The main sources of dietary choline are expected to be very similar in Norway and the United States.

Intakes of choline and betaine in our population were similar to those reported in Norwegian patients with suspected stable coronary heart disease (60). Moreover, the validity of using an FFQ for the calculation of choline and betaine intakes has been reported by others (4). In agreement with other studies among European (2) and US populations (4, 5), our participants reported choline intakes below current recommendations. Thus, the FFQ used in the current study seemed to provide plausible data on choline and betaine intakes, although misclassification cannot be disregarded.

In conclusion, intake of choline was positively associated with BMD and with plasma free choline among middle-aged and elderly community-dwelling participants. These results should encourage further studies on dietary choline and bone health to elucidate any clinical applicability for dietary intervention.

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JØ, CGG, TK, GFTS, GST, ES, PMU, and ON designed the research (project conception, development of the overall research plan, and study oversight); CGG, GST, and ON conducted the research (hands-on conduct of the experiments and data collection); KM and PMU were responsible for the biochemical analysis; CAD and KJV were responsible for the assessment of dietary intake; JØ performed the statistical analysis and wrote the first draft of the manuscript. All authors critically revised the manuscript and read and approved the final manuscript.

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