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Plasma Choline, Nicotine Exposure, and Risk of Low Bone Mineral Density and Hip Fracture: The Hordaland Health Study

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ABSTRACT

Choline, obtained from diet and formed by biosynthesis, is the immediate precursor of betaine. Animal studies suggest an impact of choline on bone metabolism. We examined the associations of plasma choline and betaine with bone mineral density (BMD), the risk of hip fractures, and possible effect-modification by nicotine exposure. The Hordaland Health Study (1998 to 2000) included 7074 women and men (ages 46 to 49 or 71 to 74 years). In 5315, BMD was measured. The oldest ($n = 3311$) were followed for hip fractures through 2009. Risk associations were studied by logistic and Cox regression by comparing the lowest and middle tertiles with the highest, as well as trends across tertiles of plasma choline and betaine. In analyses adjusted for sex and age, participants in the lowest (odds ratio [OR] = 2.00, 95% confidence interval [CI] 1.69–2.37) and middle (OR = 1.39, CI 1.17–1.66) tertiles of plasma choline had an increased risk of low BMD (lowest quintile) (p trend < 0.001). Separate analyses for sex and age groups revealed the strongest relations in elderly women (lowest tertile: OR = 2.84, CI 1.95–4.14; middle tertile: OR = 1.80, CI 1.22–2.67, p trend < 0.001), and highest OR among those in the lowest tertile who were exposed to nicotine (OR = 4.56, CI 1.87–11.11). Low plasma choline was also associated with an increased risk of hip fracture in elderly women and men (lowest tertile: hazard ratio [HR] = 1.45, CI 1.08–1.94; middle tertile: HR = 1.13, CI 0.83–1.54, p trend = 0.012). In elderly women, the HR for hip fracture was 1.90 (CI 1.32–2.73) and 1.36 (CI 0.92–1.99) (p trend < 0.001) for lowest and middle tertiles of choline, and the highest HR was found among women in the lowest tertile exposed to nicotine (HR = 2.68, CI 1.16–6.19). Plasma betaine was not related to BMD or hip fracture. Low plasma choline was associated with low BMD in both sexes and increased the risk of hip fracture in elderly women. These results should motivate further studies on choline, nicotine exposure, and bone metabolism. © 2014 American Society for Bone and Mineral Research.

KEY WORDS: BONE MINERAL DENSITY; HIP FRACTURE; PLASMA BETAINE; PLASMA CHOLINE; PLASMA COTININE; NICOTINE EXPOSURE

Introduction

Associations of plasma choline and betaine with bone health in humans have not been reported. A role of choline in bone metabolism is suggested by the findings of reduced bone formation[1,2] and increased bone resorption[2] in rats fed a choline-deficient diet. Choline and betaine supplementation in humans has been found to lower plasma homocysteine levels,[3,4] and high homocysteine has been associated with low bone mineral density (BMD) and increased risk of subsequent hip fracture.[5,6]

Choline is an essential nutrient obtained from a variety of foods such as eggs, salmon, beef, pork, liver, soybean, and wheat germ,[7] and is also formed by de novo biosynthesis by the methylation of phosphatidylethanolamine.[8] Choline is important for synthesis of acetylcholine and transport of lipoproteins, as well as for the formation of phospholipids and blood and membrane lipids.[8,9] Phosphatidylcholine (PC) is a phospholipid that incorporates choline as a head group and is the most abundant choline form, accounting for 95% of the total choline in mammalian tissue.[10] In the mitochondria, choline is oxidized to betaine, which serves as an osmolyte and a methyl donor in the betaine-homocysteine methyltransferase reaction.[8,9] Plasma PC concentrations of polyunsaturated fatty acids have been positively associated with BMD in men and with a decreased risk of hip fracture in both genders.[11] Further, fatty acids may be related to skeletal health via the production of eicosanoids and

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cytokines in bone modeling and remodeling,[12] through peroxisome proliferator-activated receptors (PPARs) activation,[13] by the production of lipid mediators reducing inflammation,[14] and by improved osteoblast function[15] and calcium transport.[16]

The association of smoking with low BMD and increased fracture risk is well established.[17] The underlying mechanisms for these associations are not fully explored, but smoking has adverse effects on skeletal remodeling and bone cells[18] and negative influence on sex hormones among both women and men.[19–21] In addition, increased fat oxidation,[22] reduced levels of antioxidant vitamins, and increased oxidative stress and inflammation[23] are observed in smokers. Further, plasma choline has been shown to be lower in smokers than in nonsmokers,[24] and smoking is known to alter the structure and cause fragmentation of phospholipids,[25] thereby decreasing the content of overall availability of PC. Given that smoking interferes with choline metabolism and availability and itself is a major risk factor for osteoporosis, there may be an effect modification by smoking on choline and the risk of osteoporosis.

We hypothesized that low levels of plasma choline are associated with low BMD and increased risk of hip fracture. We also included plasma betaine because choline is the only precursor of betaine and betaine gives a clue as to whether the effects of choline are linked to one-carbon metabolism or to phospholipids. Thus, the aim of this community-based study was to examine associations of plasma choline and betaine with BMD and subsequent risk of hip fractures, as well as to evaluate a potential effect modification by nicotine exposure (plasma cotinine) in these associations.

Materials and Methods

Study population

The study subjects were participants in the Hordaland Health Study (HUSK) in Western Norway, where the baseline examination was conducted from 1998 to 2000. HUSK was conducted in collaboration between the University of Bergen, the Norwegian Institute of Public Health, and local health services. A total of 9187 subjects born in 1925 to 1927 and 1950 to 1951 who had previously participated in the Hordaland Homocysteine Study in 1992 to 1993[26] were invited, and 7074 (77.0%) participated.

Of the 7074 participants, 5408 (76.4%) persons met for densitometry measurements at Haukeland University Hospital in Bergen.[5] Of these, 30 subjects were excluded because of invalid BMD scans or bilateral hip prostheses. Further, plasma choline and cotinine measurements were missing in 63 participants. Thus, 5315 participants (1866 women and 1228 men aged 46 to 49 years and 1204 women and 1017 men aged 71 to 74 years) comprise the BMD subpopulation of HUSK.

All 7074 participants in HUSK were followed until the first hip fracture, and observations were censored at death or on December 31, 2009. Because only 13 participants in the middle-aged cohort suffered a hip fracture during follow-up, only the older cohort, 1868 women and 1473 men aged 71 to 74 years at baseline, is included in the hip fracture analyses. Plasma choline and cotinine measurements were missing in 30 subjects. Thus, a cohort of 1856 women and 1455 men were included in the hip fracture analyses. During the follow-up period, 337 women and 474 men died without having suffered a hip fracture.

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

Baseline data collection

Bone densitometry

BMD was measured by dual-energy X-ray absorptiometry (DXA) on a stationary fan beam densitometer (Expert-XL; Lunar Company Inc., Madison, WI, USA). All examinations were conducted by one of four trained technicians. The left hip was scanned unless there was a history of hip prosthesis or fracture. Femoral neck BMD was used in the analyses. Daily scanning of the manufacturer-supplied spine phantom presented no instrumentation drift and a coefficient of variation of less than 0.9% during the entire study period.

Analytic procedures

Blood samples were collected into evacuated tubes containing EDTA, chilled, and centrifuged within 1 to 3 hours. EDTA-plasma was stored at −80°C. Plasma choline, betaine, creatinine, homocysteine, and cotinine concentrations were measured by liquid chromatography-tandem mass spectrometry.[27,28] Plasma cotinine was categorized as <85 and ≥ 85 nmol/L to define participants with no versus any nicotine exposure, respectively.[29] Cotinine is the main metabolite of nicotine and a sensitive marker of recent active and passive tobacco exposure with a half-life of 11 to 37 hours.[30] Plasma folate was measured by a *Lactobacillus casei* microbiological assay.[31] Levels of high sensitive C-reactive protein (CRP) were determined by a novel immunoassay based on matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). All analyses were done at the laboratory Bevital A/S, Bergen, Norway (www.bevital.no).[27]

Other measures

Height and weight were measured with light clothing, and body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters. Self-administered questionnaires included information on health factors such as physical activity, smoking, hormone-replacement therapy, and time (hours) since last meal. Physical activity was categorized as no or light regular activity (<1 hour/week), regular (1 to 2 hours/week), or hard regular activity (≥ 3 hours/week). Smoking was categorized as current, former, and never smoking, and former smoking was included in some of the analyses. Use of estrogen supplements was categorized as current or no use.

Follow-up data collection

Hip fractures

Information on hip fracture was attained from computerized records containing discharge diagnoses for all hospitalizations occurring between the baseline examination in HUSK through December 31, 2009, at the six hospitals in Hordaland County. Hip fracture was defined as the first fracture of the proximal femur occurring during the observation period. Only hip fractures confirmed by a concurrent code of an adequate surgical procedure were included in order to validate the fracture registration; all hospital discharges with an identified hip fracture diagnosis were searched for adequate surgical treatment. Further descriptions of the classification codes were previously described.[6] Information on time of death was obtained from the Norwegian Population Register.

Statistical analyses

Categorical variables are expressed as numbers and percentages and continuous variables as means with standard deviations, and median with interquartile range. Independent sample *t* tests were used for continuous variables and Fisher's exact tests for categorical variables for comparisons between participants with no versus any nicotine exposure.

Lowest quintiles of BMD (g/cm^2) and tertiles of plasma choline (μmol/L) were established for each sex and age group. Odds ratios (ORs) for being in the lowest quintile of femoral neck BMD according to sex- and age-specific tertiles of plasma choline were estimated in unadjusted and adjusted logistic regression analyses. The sex- and age-specific tertiles of plasma choline were also used in analyses including the whole population. To explore the effect of smoking, similar analyses stratified on nicotine exposure and self-reported former smoking was performed. Adjustment variables included sex, age group, BMI, and nicotine exposure in analyses for the whole cohort, and BMI and nicotine exposure in sex- and age group-stratified analyses. In models stratified on nicotine exposure, we adjusted for BMI, sex, and age group when investigating all participants combined, and in analyses additionally stratified on sex and age group, we adjusted for BMI. In addition, we adjusted for plasma folate, creatinine, homocysteine, CRP, time since last meal, physical activity, and use of estrogen supplementation (women only). Similar analyses as described above were conducted for plasma betaine.

Further, we used multiple linear regression analyses with BMD as dependent variable and plasma choline as independent variable with and without adjustment. However, the results were similar to those found using logistic regression analyses; thus, only data from the logistic regression models are presented.

Cox proportional hazards regression models were used to estimate associations of plasma choline and betaine with subsequent hip fractures in the oldest subjects. These analyses were performed without and with adjustments (same variables as for the BMD analyses except from age group), and also stratified according to nicotine exposure and self-reported former smoking. Similar analyses as described above were performed for plasma betaine.

Further, we constructed Kaplan-Meier disease-free survival curves for hip fractures according to tertiles of plasma choline.

Two-tailed p values < 0.05 were considered statistically significant. The analyses were done using SPSS for Windows (IBM SPSS Statistics 19, Chicago, IL, USA).

Results

Study population

Characteristics of the HUSK BMD subpopulation, stratified by sex, age group, and nicotine exposure, are presented in Table 1. Plasma choline and femoral neck BMD were lower among participants with any versus no nicotine exposure within each sex and age group. BMD was higher and choline lower in the youngest compared with the oldest groups. In the older cohort in both genders, plasma betaine was lower among nicotine-exposed versus unexposed participants. Plasma folate and creatinine were lower among those exposed to nicotine in all groups, except for plasma folate in elderly women and plasma creatinine in elderly men. Plasma CRP was higher among participants exposed to nicotine in all groups, apart from in elderly women. In the majority of sex and age groups, participants exposed to nicotine had lower BMI and were less physically active than unexposed participants.

Two hundred fifty (13.4%) of the middle-aged women and 239 (19.5%) of middle-aged men were former smokers. The corresponding numbers for elderly women and men were 229 (19%) and 525 (51.6%), respectively.

During a median follow-up time of 10.8 years for elderly women and men, hip fracture was recorded among 191 women (144 in nicotine unexposed) and 86 men (62 in nicotine unexposed) (Supplemental Table S1). Similar characteristics as for the HUSK BMD subpopulation were found for the HUSK participants included in the hip fracture analyses (Supplemental Table S1).

Plasma choline and bone mineral density

The risk of having low BMD, defined as being in the lowest quintile of BMD, was analyzed in a logistic regression model. For the whole population combined, participants in the lowest (OR = 2.00, 95% confidence interval [CI] 1.69–2.37) and middle (OR = 1.39, CI 1.17–1.66) tertiles of plasma choline compared with the highest had an increased risk of low BMD after adjustment for sex and age group (p trend < 0.001). This difference between tertiles was still significant after additional adjustment for BMI and nicotine exposure. Similar results were found in analyses stratified on sex and age group (Table 2).

Further, in models stratified by nicotine exposure for the whole cohort, participants who were exposed to nicotine and were in the lowest (OR = 2.55, CI 1.89–3.44) and middle tertiles (OR = 1.66, CI 1.21–2.81) of plasma choline compared with the highest had an increased risk of low BMD in analyses adjusted for sex and age group (p trend < 0.001). However, a significant association was also found among participants who were unexposed to nicotine (lowest tertile: OR = 1.63, CI 1.32–2.01; middle tertile: OR = 1.26, CI 1.02–1.56, p trend < 0.001). The results were similar after further adjustment for BMI. In analyses stratified on sex, age group, and nicotine exposure, an increased risk of low BMD was found in all groups of participants exposed to nicotine as well as among unexposed middle-aged and elderly women (Table 2).

In analyses for all participants combined, additional stratification on self-reported former smoking showed significant increased risk of low BMD among former smokers with low plasma choline after adjustment for sex and age group (lowest tertile: OR = 1.82, CI 1.27–2.60; middle tertile: OR = 1.40, CI 0.98–2.01, p trend = 0.001). In analyses further stratified on sex and age group, similar findings were observed for middle-aged and elderly women; however, the results were not significant for men (data not shown).

For all regression models, further adjustment for plasma folate, creatinine, homocysteine, CRP, time since last meal, physical activity, and use of estrogen supplement (women only) did not materially change the results.

No statistically significant associations between plasma betaine and BMD were found in any of the groups (data not shown).

Plasma choline and hip fracture

In Cox proportional hazard regression models for elderly women and men combined, the hazard ratios (HR) for subsequent hip fracture were, respectively, 1.45 (95% CI 1.08–1.94) and 1.13

Table 1. Characteristics of Participants of the Hordaland Health Study Included in the BMD Analyses by Age Group, Sex, and Nicotine Exposure[a]

	Middle-aged cohort (46 to 49 years)						Older cohort (71 to 74 years)					
	Women			Men			Women			Men		
	All	No nicotine exposure	Any nicotine exposure	All	No nicotine exposure	Any nicotine exposure	All	No nicotine exposure	Any nicotine exposure	All	No nicotine exposure	Any nicotine exposure
No. of participants	1866	1183	683	1228	783	445	1204	1016	188	1017	834	183
Femoral neck BMD (g/cm^2), mean (SD)	0.96 (0.13)	0.97 (0.13)	0.94[b] (0.12)	0.99 (0.13)	1.00 (0.14)	0.96[b] (0.13)	0.76 (0.12)	0.77 (0.11)	0.73[b] (0.13)	0.90 (0.14)	0.91 (0.14)	0.86[b] (0.13)
BMI (kg/m^2), mean (SD)	24.8 (4.0)	25.1 (4.1)	24.3[b] (3.7)	26.2 (3.3)	26.6 (3.3)	25.9[d] (3.3)	26.2 (4.2)	26.6 (4.1)	24.3[b] (4.1)	26.0 (3.1)	26.1 (3.0)	25.1[b] (3.6)
No regular physical activity, *n* (%)	674 (36.5)	394 (33.7)	280[c] (41.4)	464 (38.2)	269 (34.6)	195[c] (44.4)	470 (43.0)	388 (41.9)	82 (48.8)	285 (29.0)	221 (27.4)	64[d] (36.4)
Use of estrogen supplements, *n* (%)	346 (24.8)	197 (22.4)	149[c] (29.0)	–	–	–	135 (15.0)	111 (14.6)	24 (16.9)	–	–	–
Plasma choline (μmol/L), median (IQR)	8.9 (2.5)	8.9 (2.5)	8.8[d] (2.4)	9.8 (2.8)	9.9 (2.8)	9.4[b] (2.7)	9.6 (2.8)	9.7 (2.8)	9.3[d] (2.5)	10.8 (3.0)	11.0 (3.1)	10.1[c] (3.2)
Plasma betaine (μmol/L), median (IQR)	32.3 (13.1)	32.7 (13.4)	31.5 (12.4)	42.7 (14.5)	42.6 (14.0)	42.8 (15.2)	35.8 (13.3)	36.3 (13.3)	34.2[d] (14.3)	44.0 (16.2)	44.4 (15.6)	41.0[c] (15.6)
Plasma folate (nmol/L), median (IQR)	6.9 (4.2)	7.1 (4.1)	6.6[b] (4.3)	6.4 (3.5)	6.6 (3.8)	6.1[c] (3.1)	7.0 (4.7)	7.2 (4.8)	5.9 (4.3)	6.3 (3.5)	6.5 (3.7)	5.8[d] (2.9)
Plasma creatinine (μmol/L), median (IQR)	72.2 (11.4)	72.7 (10.9)	71.1[b] (12.3)	86.3 (13.9)	88.0 (12.8)	84.0[b] (13.7)	75.4 (14.0)	75.7 (13.8)	74.3[d] (15.0)	91.1 (17.3)	91.5 (17.1)	89.2 (18.1)
Plasma CRP, (mg/L), median (IQR)	0.94 (2.03)	0.82 (1.71)	1.23[d] (2.39)	1.11 (2.13)	0.86 (1.54)	1.72[b] (2.82)	2.08 (3.23)	2.01 (3.25)	2.31 (3.13)	2.10 (3.17)	2.01 (3.04)	2.79[c] (5.17)

BMD = bone mineral density; BMI = body mass index; IQR = interquartile range; CRP = C-reactive protein.
Total numbers may vary between variables because of varying numbers of missing data.
[a]No nicotine exposure = plasma cotinine <85 nmol/L; any nicotine exposure = plasma cotinine ≥85 nmol/L.
[b]$p < 0.001$,
[c]$p < 0.01$,
[d]$p < 0.05$ for comparisons between participants with no and any nicotine exposure in the various age groups and gender.

Table 2. Odds Ratios (OR) for Low Femoral Neck BMD[a] According to Tertiles[b] of Plasma Choline by Baseline Age Group, Sex, and Nicotine Exposure[c] in the Hordaland Health Study

		Model 1[d]		Model 2[e]		No nicotine exposure[f]		Any nicotine exposure[f]	
Tertiles of plasma choline (μmol/L)	*n*	OR (95% CI)	*p* trend	OR (95% CI)	*p* trend	OR (95% CI)	*p* trend	OR (95% CI)	*p* trend
Middle-aged cohort (46 to 49 years)									
Women	1866		<0.001		0.005		0.041		0.041
≤8.13	625	1.79 (1.34–2.37)		1.52 (1.13–2.04)		1.50 (1.01–2.22)		1.59 (1.02–2.49)	
8.14–9.74	621	1.36 (1.02–1.83)		1.22 (0.90–1.65)		1.15 (0.77–1.73)		1.30 (0.81–2.07)	
>9.74	620	1 (ref.)		1 (ref.)		1 (ref.)		1 (ref.)	
Men	1228		0.001		0.009		0.633		0.001
≤8.87	409	1.83 (1.29–2.61)		1.63 (1.13–2.35)		1.13 (0.70–1.82)		2.92 (1.57–5.41)	
8.88–10.60	412	1.29 (0.89–1.86)		1.23 (0.84–1.80)		0.89 (0.55–1.43)		2.22 (1.16–4.24)	
>10.60	407	1 (ref.)		1 (ref.)		1 (ref.)		1 (ref.)	
Older cohort (71 to 74 years)									
Women	1204		<0.001		<0.001		<0.001		0.001
≤8.78	401	2.84 (1.95–4.14)		2.74 (1.83–4.09)		2.34 (1.49–3.66)		4.56 (1.87–11.11)	
8.79–10.50	405	1.80 (1.22–2.67)		1.83 (1.21–2.76)		1.70 (1.08–2.69)		2.35 (0.92–5.98)	
>10.50	398	1 (ref.)		1 (ref.)		1 (ref.)		1 (ref.)	
Men	1017		0.001		0.009		0.222		0.004
≤9.80	339	1.87 (1.28–2.73)		1.69 (1.14–2.51)		1.33 (0.84–2.11)		3.15 (1.34–7.41)	
9.81–11.70	343	1.22 (0.82–1.82)		1.21 (0.80–1.83)		1.25 (0.79–1.96)		1.11 (0.42–2.93)	
>11.70	335	1 (ref.)		1 (ref.)		1 (ref.)		1 (ref.)	

BMD = bone mineral density; CI = confidence interval.
[a]Lowest quintile in each sex and age group.
[b]Age- and sex-specific tertiles of plasma choline.
[c]No nicotine exposure = plasma cotinine <85 nmol/L; any nicotine exposure = plasma cotinine ≥85 nmol/L.
[d]Unadjusted.
[e]Adjusted for body mass index (BMI, kg/m^2) and nicotine exposure.
[f]Adjusted for BMI.

Table 3. Risk (Hazard Ratio) of Hip Fracture During Follow-Up[a] According to Tertiles[b] of Plasma Choline by Sex and Nicotine Exposure[c] in Participants Aged 71 to 74 Years at Inclusion in the Hordaland Health Study

Tertiles of plasma choline (μmol/L)	All[d]			No nicotine exposure[e]			Any nicotine exposure[e]		
	n/events	HR (95% CI)	*p* trend	Events	HR (95% CI)	*p* trend	Events	HR (95% CI)	*p* trend
Women	1856/191		0.003			0.040			0.020
≤8.85	619/83	1.72 (1.19–2.49)		58	1.54 (1.02–2.33)		25	2.57 (1.11–5.97)	
8.86–10.50	620/64	1.37 (0.93–2.01)		49	1.25 (0.82–1.92)		15	1.69 (0.69–4.14)	
>10.50	617/44	1 (ref.)		37	1 (ref.)		7	1 (ref.)	
Men	1455/86		0.328			0.633			0.290
≤9.84	487/27	0.77 (0.46–1.30)		20	0.87 (0.48–1.59)		7	0.59 (0.21–1.64)	
9.85–11.80	482/28	0.81 (0.48–1.35)		19	0.72 (0.39–1.32)		9	1.19 (0.46–3.10)	
>11.80	486/31	1 (ref.)		23	1 (ref.)		8	1 (ref.)	

Events = hip fractures; HR = hazard ratio; CI = confidence interval.
[a]Median 10.8 years follow-up time.
[b]Age- and sex-specific tertiles of plasma choline.
[c]No nicotine exposure = plasma cotinine <85 nmol/L; any nicotine exposure = plasma cotinine ≥85 nmol/L.
[d]Adjusted for body mass index (BMI, kg/m^2) and nicotine exposure.
[e]Adjusted for BMI.

(0.83–1.54) (*p* trend = 0.012) for those in the lowest and middle tertiles of plasma choline compared with the highest, after adjustment for sex. The result was similar after further adjusting for BMI and nicotine exposure. In corresponding analyses stratified on sex, elderly females with low plasma choline had an increased risk of subsequent hip fractures, in both unadjusted (lowest tertile: HR = 1.90, CI 1.32–2.73; middle tertile: HR = 1.36, CI 0.92–1.99, *p* trend = 0.001) and adjusted models (Table 3). No significant differences in risk of hip fracture according to plasma choline tertiles were observed among elderly men (Table 3).

Disease-free survival curves for hip fractures according to tertiles of plasma choline for elderly females are shown in Fig. 1, with the highest incidence of hip fracture in the lowest tertile, and a significant trend across tertiles.

Further, in models stratified on nicotine exposure for elderly women and men combined, no significant increased risk of hip fracture was observed (data not shown). However, when we stratified on nicotine exposure and sex, both elderly women who were exposed (lowest tertile: HR = 2.68, CI 1.16–6.19; middle tertile: HR = 1.56, CI 0.64–3.83, *p* trend = 0.012) and unexposed to nicotine (lowest tertile: HR = 1.66, CI 1.10–2.50; middle tertile: HR = 1.28, CI 0.84–1.97, *p* trend = 0.015) had an increased hip fracture risk in unadjusted and adjusted analyses (Table 3). For elderly men, no significant association between plasma choline and risk of hip fracture was found (Table 3).

In analyses additionally stratified on former smoking, no significant increased risk of hip fracture was found in former smokers according to plasma choline in any of the groups (data not shown).

Further adjustments for plasma folate, creatinine, homocysteine, CRP, time since last meal, physical activity, and use of estrogen supplements (in women) did not materially change the results.

The results presented above were similar when plasma choline was used as a continuous variable in Cox proportional hazard regression analyses.

There were no significant associations between plasma betaine and risk of hip fracture (data not shown).

Discussion

In this large community-based study, we have shown that low plasma choline is associated with low BMD in both women and men and increased risk of hip fracture in older women. The strongest association was seen among older female participants. No significant relations were found between plasma betaine and BMD or risk of hip fracture.

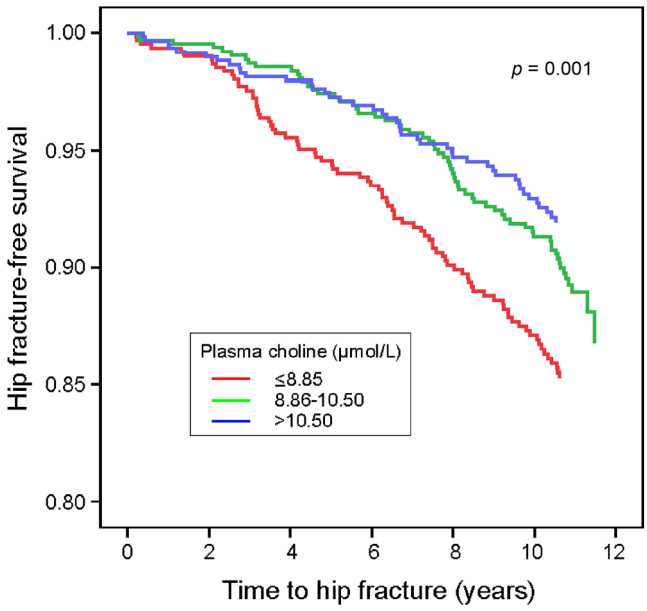

Fig. 1. Kaplan-Meier disease-free survival curves for hip fractures in 1856 women (ages 71 to 74 years at inclusion), according to tertiles of plasma choline. The *p* value for trend is across tertiles. Results are from the Hordaland Health Study.

Strengths of this study include the community-based cohort design, the large number of participants, and more than 10 years follow-up for hip fractures. All BMD measurements were performed on the same DXA machine. To estimate nicotine exposure, we used plasma cotinine, which has been reported to correlate better with various effects of smoking than self-reported smoking.[32] However, blood samples and other measures were collected only at baseline. Thus, we have no information on potential changes in plasma choline, betaine, cotinine, or BMI during follow-up.

Studies of health-related effects of choline in community-dwelling participants are few, and we are not aware of published studies on plasma choline in relation to human bone health. In rats, a choline-deficient diet has been shown to alter mandibular bone remodeling by a reduction in osteogenesis.[1] In another study on rats, a diet low in methionine and choline led to fat accumulation in the liver, decreased bone formation, and increased bone resorption, resulting in lower cancellous and cortical bone mass compared with controls.[2] In healthy humans with diets low in choline, fatty liver and liver or muscle damage have been observed.[33,34]

The required daily intake of choline is somewhat lower for women (425 mg/d) than for men (550 mg/d).[35] This could partly be explained by increased endogenous de novo synthesis of PC catalyzed by the estrogen-dependent phosphatidylethanolamine N-methyltransferase (PEMT).[36,37] The 2005 National Health and Nutrition Examination Survey indicates that only 2% of postmenopausal women consumed the recommended intake of choline.[38] Thus, estrogen deficiency in postmenopausal women may promote osteoporosis as well as low choline.

Low plasma choline levels may reflect low dietary intake of choline or perhaps be a marker of a generally poor diet or represent changes in energy metabolism. In a previous study of the same study population,[39] egg consumption was the only dietary item that was significantly positively related to plasma choline. We have also shown that high levels of plasma choline and low levels of plasma betaine were associated with components of the metabolic syndrome,[24] which also may indicate insulin resistance[40,41] and mitochondrial dysfunction.[42] Our present results propose the opposite with regard to osteoporosis. However, further studies are needed to determine what the most important determinants of plasma choline are.

PC availability may increase the proliferation of osteoblast progenitors.[43] In addition, PC is a ligand for PPARs alpha (α) and gamma (γ),[44,45] which play central roles in the lipid and carbohydrate metabolism.[46] Rat studies have demonstrated that PPARα activation seems to play a protective role in the regulation of bone metabolism,[47] whereas PPARγ gene polymorphisms have been associated with bone loss and osteoporosis.[47,48] Stimulation of PPARγ may also cause mesenchymal stem cells to differentiate into adipocytes instead of osteoblasts.[49,50] Hence, activation of PPARs may represent a link between low choline and low BMD.

We have previously reported that high levels of homocysteine are associated with low BMD and increased risk of hip fracture in the current study population.[5,6] Supplementation with choline has been shown to lower plasma homocysteine levels,[4] and there is an inverse relation between plasma levels of homocysteine and choline.[24] Thus, there may be a link between the effects of choline and homocysteine on bone health; however, future studies are needed to investigate if these markers are causal mediators of pathogenic effects on bone metabolism.

Low intakes of choline and betaine have been related to increased concentrations of the inflammatory markers CRP, interleukin-6, and tumor necrosis factor-α (TNF-α),[51] which are also associated with osteoporosis.[52,53] In addition, chronic inflammatory diseases increase the risk of osteoporosis.[54] Dietary choline has also been shown to inhibit macrophages, possibly by an increased rate of PC hydrolysis.[55] Further, inflammatory reactions can be diminished by intakes of choline[56] and betaine[57] by increasing S-adenosylmethionine and decreasing S-adenosylhomocysteine. The former has been found to downregulate TNF-α,[58] whereas the latter may exert a stimulatory effect on release of inflammatory cytokines.[57] Although our results were not affected by adjusting for CRP, we cannot rule out that low-grade inflammation is a mediator of an adverse effect from low choline on bone health.

We found strong associations between low plasma choline and BMD in nicotine-exposed participants, and these effects were independent of BMI. Smoking is known to be associated with oxidative stress and inflammation,[23] altered structure and fragmentation of phospholipids and low availability of PC,[25] increased fat oxidation,[22] low BMI,[59,60] and low levels of estrogens.[20] The relations of nicotine exposure with inflammation (CRP) and BMI were confirmed in the present study, and low BMI and estrogen deficiency are known risk factors of osteoporosis.[17]

In conclusion, we found that low plasma choline was associated with low BMD in both women and men and with increased risk of hip fracture in elderly women. Additional studies are needed to understand the biologic role of choline in bone health. These results should motivate further studies on choline, nicotine exposure, and bone metabolism, with the prospect to create the grounds for future intervention studies.

Disclosures

All authors state that they have no conflicts of interest.

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References

1. Gorustovich AA, Esposito MA, Guglielmotti MB, Giglio MJ. Mandibular bone remodeling under a choline-deficient diet: a histomorphometric study in rats. J Periodontol. 2003;74(6):831–7.
2. Iwamoto J, Seki A, Sato Y, Matsumoto H, Takeda T, Yeh JK. Effect of vitamin K2 on cortical and cancellous bone mass and hepatic lipids in

rats with combined methionine-choline deficiency. Bone. 2011;48(5):1015–21.

3. Olthof MR, Verhoef P. Effects of betaine intake on plasma homocysteine concentrations and consequences for health. Curr Drug Metab. 2005;6(1):15–22.
4. Olthof MR, Brink EJ, Katan MB, Verhoef P. Choline supplemented as phosphatidylcholine decreases fasting and postmethionine-loading plasma homocysteine concentrations in healthy men. Am J Clin Nutr. 2005;82(1):111–7.
5. Gjesdal CG, Vollset SE, Ueland PM, Refsum H, Drevon CA, Gjessing HK, Tell GS. Plasma total homocysteine level and bone mineral density: the Hordaland Homocysteine Study. Arch Intern Med. 2006;166(1):88–94.
6. Gjesdal CG, Vollset SE, Ueland PM, Refsum H, Meyer HE, Tell GS. Plasma homocysteine, folate, and vitamin B 12 and the risk of hip fracture: the Hordaland Homocysteine Study. J Bone Miner Res. 2007;22(5):747–56.
7. Zeisel SH, Mar MH, Howe JC, Holden JM. Concentrations of choline-containing compounds and betaine in common foods. J Nutr. 2003;133(5):1302–7.
8. Ueland PM. Choline and betaine in health and disease. J Inherit Metab Dis. 2011;34(1):3–15.
9. Zeisel SH. Choline: an essential nutrient for humans. Nutrition. 2000;16(7–8):669–71.
10. Zeisel SH, da Costa KA. Choline: an essential nutrient for public health. Nutr Rev. 2009;67(11):615–23.
11. Farina EK, Kiel DP, Roubenoff R, Schaefer EJ, Cupples LA, Tucker KL. Plasma phosphatidylcholine concentrations of polyunsaturated fatty acids are differentially associated with hip bone mineral density and hip fracture in older adults: the Framingham Osteoporosis Study. J Bone Miner Res. 2012;27(5): 1222–30.
12. Kruger MC, Coetzee M, Haag M, Weiler H. Long-chain polyunsaturated fatty acids: selected mechanisms of action on bone. Prog Lipid Res. 2010;49(4):438–49.
13. Krey G, Braissant O, L'Horset F, Kalkhoven E, Perroud M, Parker MG, Wahli W. Fatty acids, eicosanoids, and hypolipidemic agents identified as ligands of peroxisome proliferator-activated receptors by coactivator-dependent receptor ligand assay. Mol Endocrinol. 1997;11(6):779–91.
14. Herrera BS, Ohira T, Gao L, Omori K, Yang R, Zhu M, Muscara MN, Serhan CN, Van Dyke TE, Gyurko R. An endogenous regulator of inflammation, resolvin E1, modulates osteoclast differentiation and bone resorption. Br J Pharmacol. 2008;155(8): 1214–23.
15. Watkins BA, Li Y, Lippman HE, Feng S. Modulatory effect of omega-3 polyunsaturated fatty acids on osteoblast function and bone metabolism. Prostaglandins Leukot Essent Fatty Acids. 2003;68(6):387–98.
16. Coetzer H, Claassen N, van Papendorp DH, Kruger MC. Calcium transport by isolated brush border and basolateral membrane vesicles: role of essential fatty acid supplementation. Prostaglandins Leukot Essent Fatty Acids. 1994;50(5):257–66.
17. Russell RG, Espina B, Hulley P. Bone biology and the pathogenesis of osteoporosis. Curr Opin Rheumatol. 2006;18(Suppl 1):S3–10.
18. Walker LM, Preston MR, Magnay JL, Thomas PB, El Haj AJ. Nicotinic regulation of c-fos and osteopontin expression in human-derived osteoblast-like cells and human trabecular bone organ culture. Bone. 2001;28(6):603–8.
19. Hannan MT, Felson DT, Dawson-Hughes B, Tucker KL, Cupples LA, Wilson PW, Kiel DP. Risk factors for longitudinal bone loss in elderly men and women: the Framingham Osteoporosis Study. J Bone Miner Res. 2000;15(4):710–20.
20. Michnovicz JJ, Hershcopf RJ, Naganuma H, Bradlow HL, Fishman J. Increased 2-hydroxylation of estradiol as a possible mechanism for the anti-estrogenic effect of cigarette smoking. N Engl J Med. 1986;315(21):1305–9.
21. Tanko LB, Christiansen C. An update on the antiestrogenic effect of smoking: a literature review with implications for researchers and practitioners. Menopause. 2004;11(1):104–9.
22. Jensen EX, Fusch C, Jaeger P, Peheim E, Horber FF. Impact of chronic cigarette smoking on body composition and fuel metabolism. J Clin Endocrinol Metab. 1995;80(7):2181–5.
23. Yanbaeva DG, Dentener MA, Creutzberg EC, Wesseling G, Wouters EF. Systemic effects of smoking. Chest. 2007;131(5):1557–66.
24. Konstantinova SV, Tell GS, Vollset SE, Nygard O, Bleie O, Ueland PM. Divergent associations of plasma choline and betaine with components of metabolic syndrome in middle age and elderly men and women. J Nutr. 2008;138(5):914–20.
25. Frey B, Haupt R, Alms S, Holzmann G, Konig T, Kern H, Kox W, Rustow B, Schlame M. Increase in fragmented phosphatidylcholine in blood plasma by oxidative stress. J Lipid Res. 2000;41(7):1145–53.
26. Nygard O, Vollset SE, Refsum H, Stensvold I, Tverdal A, Nordrehaug JE, Ueland PM, Kvale G. Total plasma homocysteine and cardiovascular risk profile. The Hordaland Homocysteine Study. JAMA. 1995;274(19):1526–33.
27. Midttun O, Hustad S, Ueland PM. Quantitative profiling of biomarkers related to B-vitamin status, tryptophan metabolism and inflammation in human plasma by liquid chromatography/tandem mass spectrometry. Rapid Commun Mass Spectrom. 2009;23(9):1371–9.
28. Holm PI, Ueland PM, Kvalheim G, Lien EA. Determination of choline, betaine, and dimethylglycine in plasma by a high-throughput method based on normal-phase chromatography-tandem mass spectrometry. Clin Chem. 2003;49(2):286–94.
29. Gorber SC, Schofield-Hurwitz S, Hardt J, Levasseur G, Tremblay M. The accuracy of self-reported smoking: a systematic review of the relationship between self-reported and cotinine-assessed smoking status. Nicotine Tob Res. 2009;11(1):12–24.
30. Benowitz NL. Nicotine addiction. Prim Care. 1999;26(3):611–31.
31. O'Broin S, Kelleher B. Microbiological assay on microtitre plates of folate in serum and red cells. J Clin Pathol. 1992;45(4):344–7.
32. Perez-Stable EJ, Benowitz NL, Marin G. Is serum cotinine a better measure of cigarette smoking than self-report? Prev Med. 1995;24(2):171–9.
33. Fischer LM, daCosta KA, Kwock L, Stewart PW, Lu TS, Stabler SP, Allen RH, Zeisel SH. Sex and menopausal status influence human dietary requirements for the nutrient choline. Am J Clin Nutr. 2007;85(5):1275–85.
34. da Costa KA, Badea M, Fischer LM, Zeisel SH. Elevated serum creatine phosphokinase in choline-deficient humans: mechanistic studies in C2C12 mouse myoblasts. Am J Clin Nutr. 2004;80(1):163–70.
35. Institute of Medicine. Dietary reference intakes for folate, thiamin, riboflavin, niacin, vitamin B12, pantothenic acid, biotin, and choline. Washington, DC: National Academy Press; 1998.
36. da Costa KA, Kozyreva OG, Song J, Galanko JA, Fischer LM, Zeisel SH. Common genetic polymorphisms affect the human requirement for the nutrient choline. FASEB J. 2006;20(9):1336–44.
37. Resseguie M, Song J, Niculescu MD, da Costa KA, Randall TA, Zeisel SH. Phosphatidylethanolamine N-methyltransferase (PEMT) gene expression is induced by estrogen in human and mouse primary hepatocytes. FASEB J. 2007;21(10):2622–32.
38. Jensen HH, Batres-Marquez SP, Carriquiry A, Schalinske KL. Choline in the diets of the U.S. population: NHANES, 2003–2004. FASEB J. 21: lb219.
39. Konstantinova SV, Tell GS, Vollset SE, Ulvik A, Drevon CA, Ueland PM. Dietary patterns, food groups, and nutrients as predictors of plasma choline and betaine in middle-aged and elderly men and women. Am J Clin Nutr. 2008;88(6):1663–9.
40. Eckel RH, Grundy SM, Zimmet PZ. The metabolic syndrome. Lancet. 2005;365(9468):1415–28.
41. Raubenheimer PJ, Nyirenda MJ, Walker BR. A choline-deficient diet exacerbates fatty liver but attenuates insulin resistance and glucose intolerance in mice fed a high-fat diet. Diabetes. 2006;55(7): 2015–20.
42. Nisoli E, Clementi E, Carruba MO, Moncada S. Defective mitochondrial biogenesis: a hallmark of the high cardiovascular risk in the metabolic syndrome? Circ Res. 2007;100(6):795–806.
43. Karsenty G, Wagner EF. Reaching a genetic and molecular understanding of skeletal development. Dev Cell. 2002;2(4):389–406.

44. Chakravarthy MV, Lodhi IJ, Yin L, Malapaka RR, Xu HE, Turk J, Semenkovich CF. Identification of a physiologically relevant endogenous ligand for PPARalpha in liver. Cell. 2009;138(3):476–88.
45. Davies SS, Pontsler AV, Marathe GK, Harrison KA, Murphy RC, Hinshaw JC, Prestwich GD, Hilaire AS, Prescott SM, Zimmerman GA, McIntyre TM. Oxidized alkyl phospholipids are specific, high affinity peroxisome proliferator-activated receptor gamma ligands and agonists. J Biol Chem. 2001;276(19):16015–23.
46. Lalloyer F, Staels B. Fibrates, glitazones, and peroxisome proliferator-activated receptors. Arterioscler Thromb Vasc Biol. 2010;30(5):894–9.
47. Stunes AK, Westbroek I, Gustafsson BI, Fossmark R, Waarsing JH, Eriksen EF, Petzold C, Reseland JE, Syversen U. The peroxisome proliferator-activated receptor (PPAR) alpha agonist fenofibrate maintains bone mass, while the PPAR gamma agonist pioglitazone exaggerates bone loss, in ovariectomized rats. BMC Endocr Disord. 2011;11:11.
48. Tamaki J, Iki M, Morita A, Ikeda Y, Sato Y, Kajita E, Kagamimori S, Kagawa Y, Yoneshima H. Peroxisome proliferator-activated receptor gamma polymorphism is related to peak bone mass: the JPOS study. Osteoporos Int. 2010;21(2):321–9.
49. Ali AA, Weinstein RS, Stewart SA, Parfitt AM, Manolagas SC, Jilka RL. Rosiglitazone causes bone loss in mice by suppressing osteoblast differentiation and bone formation. Endocrinology. 2005;146(3):1226–35.
50. Rzonca SO, Suva LJ, Gaddy D, Montague DC, Lecka-Czernik B. Bone is a target for the antidiabetic compound rosiglitazone. Endocrinology. 2004;145(1):401–6.
51. Detopoulou P, Panagiotakos DB, Antonopoulou S, Pitsavos C, Stefanadis C. Dietary choline and betaine intakes in relation to concentrations of inflammatory markers in healthy adults: the ATTICA study. Am J Clin Nutr. 2008;87(2):424–30.
52. Ershler WB, Keller ET. Age-associated increased interleukin-6 gene expression, late-life diseases, and frailty. Annu Rev Med. 2000;51:245–70.
53. Lacativa PG, Farias ML. Osteoporosis and inflammation. Arq Bras Endocrinol Metabol. 2010;54(2):123–32.
54. Hardy R, Cooper MS. Bone loss in inflammatory disorders. J Endocrinol. 2009;201(3):309–20.
55. Grove RI, Allegretto NJ, Kiener PA, Warr GA. Lipopolysaccharide (LPS) alters phosphatidylcholine metabolism in elicited peritoneal macrophages. J Leukoc Biol. 1990;48(1):38–42.
56. Innis SM, Davidson AG, Melynk S, James SJ. Choline-related supplements improve abnormal plasma methionine-homocysteine metabolites and glutathione status in children with cystic fibrosis. Am J Clin Nutr. 2007;85(3):702–8.
57. Purohit V, Abdelmalek MF, Barve S, Benevenga NJ, Halsted CH, Kaplowitz N, Kharbanda KK, Liu QY, Lu SC, McClain CJ, Swanson C, Zakhari S. Role of S-adenosylmethionine, folate, and betaine in the treatment of alcoholic liver disease: summary of a symposium. Am J Clin Nutr. 2007;86(1):14–24.
58. Chawla RK, Watson WH, Eastin CE, Lee EY, Schmidt J, McClain CJ. S-adenosylmethionine deficiency and TNF-alpha in lipopolysaccharide-induced hepatic injury. Am J Physiol. 1998;275(1 Pt 1):G125–9.
59. Hermann AP, Brot C, Gram J, Kolthoff N, Mosekilde L. Premenopausal smoking and bone density in 2015 perimenopausal women. J Bone Miner Res. 2000;15(4):780–7.
60. Johnell O, Gullberg B, Kanis JA, Allander E, Elffors L, Dequeker J, Dilsen G, Gennari C, Lopes Vaz A, Lyritis G, et al. Risk factors for hip fracture in European women: the MEDOS Study. Mediterranean Osteoporosis Study. J Bone Miner Res. 1995;10(11):1802–15.