Plasma Total Homocysteine Level and Bone Mineral Density

The Hordaland Homocysteine Study

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Background: Plasma total homocysteine (tHcy) has been associated with hip fracture but not directly with bone mineral density (BMD). We examined the association of hip BMD with levels of plasma tHcy, folate, and vitamin B_{12} and the methylenetetrahydrofolate reductase (*MTHFR*) 677C→T and 1298A→C polymorphisms.

Methods: Bone mineral density was measured between 1997 and 2000 in 2268 men and 3070 women, aged 47 to 50 and 71 to 75 years, from the Hordaland Homocysteine Study cohort. Low BMD was defined as BMD in the lowest quintile for each sex and age group. Linear, logistic, and generalized additive regression models were used.

Results: Plasma levels of tHcy were inversely related to BMD among middle-aged and elderly women ($P<.001$) but not among men. The multiple adjusted odds ratio for low BMD among subjects with high (≥15 µmol/L [≥2.02 mg/L]) compared with low (<9 µmol/L [<1.22 mg/L]) tHcy level was 1.96 (95% confidence interval, 1.40-2.75) for women and was not significant for men. Additional adjustments for plasma folate level or intake of calcium and vitamin D did not substantially alter the results. Plasma folate level was associated with BMD in women only. We observed no association between BMD and vitamin B_{12} level or the *MTHFR* polymorphisms.

Conclusions: Elevated tHcy and low folate levels were associated with reduced BMD in women but not in men. These findings suggest that tHcy may be a potential modifiable risk factor for osteoporosis in women.

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ELEVATED PLASMA CONCENTRATION of total homocysteine (tHcy) and deficiency of vitamins related to its metabolism, such as vitamin B_{12} and folate, have been associated with various disorders, including cardiovascular disease[1,2] and Alzheimer disease.[3] Likewise, the common 677C→T polymorphism in the methylenetetrahydrofolate reductase (*MTHFR*) gene has been studied extensively in relation to these diseases because the homozygous TT genotype is associated with reduced enzyme activity and higher levels of tHcy compared with the more common CC genotype.[4]

The hypothesis that tHcy may be a risk factor for fracture was suggested by studies of patients with homocystinuria, characterized by very high plasma levels of homocysteine. Among several clinical manifestations, these patients also have high incidence of premature osteoporosis and fractures.[5] Investigations have been further motivated by studies showing that homocysteine inhibits the collagen cross-linking[6] and impairs bone mineralization.[7]

Recently, a positive relationship between tHcy level and osteoporotic fractures has been demonstrated in large epidemiological studies of elderly men and women.[8,9] However, in the Dutch study in which measurements of bone mineral density (BMD) were performed, BMD was not related to tHcy level.[9] A smaller study concluded that BMD was related to serum folate but not to tHcy level.[10] Combined treatment with folate and vitamin B_{12} has been effective in reducing the risk of hip fracture following stroke.[11]

Previous studies have demonstrated an association between *MTHFR* 677C→T polymorphism and BMD,[12-15] which could support the role of homocysteine or folate in bone metabolism. However, results of studies on this polymorphism and the risk of fracture are conflicting. Two studies have reported increased risk of fracture associated with the TT genotype,[13,16] whereas another study found a greater risk of fracture associated with the wild-type C allele.[17] We could not identify any publications on the relation between the *MTHFR* 1298A→C polymorphism and BMD.

A possible relation between levels of tHcy and BMD and fractures has large health implications because hyperhomocysteinemia responds to intake of B vitamins[18] and because of the high incidence of osteoporotic fractures.[19] The aim of the present study was to examine whether key components of the 1-carbon metabolism, including tHcy, vitamin B_{12}, folate, and *MTHFR* 677C→T or 1298A→C polymorphisms, are associated with BMD in a large population-based cohort.

METHODS

STUDY POPULATION

The second round of the Hordaland Homocysteine Study was conducted as part of the Hordaland Health Study (HUSK) from 1997 to 2000 as a collaboration between the National Health Screening Service, The University of Bergen, and local health services. Of the total sample of 9187 men and women born between 1925 and 1927 and 1950 and 1951 who were invited into the main study, 7074 (77.0%) participated. Of these, 5408 (76.4%) participated in the BMD substudy. The Regional Committee for Medical Research Ethics approved the study protocol, and all participants signed an informed consent form.

BONE MINERAL DENSITY

Bone mineral density of the hip was obtained by a stationary, dual x-ray densitometer (EXPERT-XL; Lunar Company Inc, Madison, Wis). The scanner was calibrated daily against a standard calibration block. These measurements showed no drift, and the coefficient of variation for measuring total hip BMD was 1.2%.[20] Of the 5408 individuals who came for densitometry, 70 persons were excluded either because of nonwhite descent, incorrectly performed measurements, bilateral hip prosthesis, or severe deformities of the hips.

OTHER 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. Self-administered questionnaires provided information regarding lifestyle and medical history, including data on menopause, physical activity, smoking, consumption of coffee, dietary habits, current medication use, and current and past use of postmenopausal estrogen therapy. Leisure time physical activity was categorized in 3 groups (ie, no strenuous activity [low], strenuous activity 1 to 2 h/wk [moderate], or ≥3 h/wk [heavy]). Smoking habits were categorized as current, ex-smokers, and never smokers. Intake of calcium and vitamin D were assessed by a quantitative food frequency questionnaire based on usual dietary habits during the last year.[21] Total intake comprised daily dietary intake and supplements on a continuous scale. Coffee consumption was measured as the number of cups per day. Estrogen use was categorized as current or no use.

BIOCHEMICAL AND GENETIC MEASUREMENTS

Nonfasting plasma samples were collected in tubes containing EDTA for tHcy, folate, and vitamin B_{12} analyses. Plasma tHcy levels were analyzed by fully automated high-performance liquid chromatography with fluorescence detection. Intra-assay coefficient of variation was lower than 4%.[22] Plasma folate level was determined by a *Lactobacillus casei* microbiological assay[23] and plasma vitamin B_{12} concentrations by a *Lactobacillus leichmannii* microbiological assay.[24] Both the folate and vitamin B_{12} assays were adapted to a microtiter plate format and carried out by a robotic workstation (Micro-laboratory ATplus2; Hamilton Bonaduz AG, Bonaluz, Switzerland). Serum creatinine level was measured colorimetrically using a standard alkaline picrate method. The *MTHFR* 677C→T and 1298A→C genotyping were performed by a real-time polymerase chain reaction.[25]

STATISTICAL ANALYSIS

Linear regression analyses were used to evaluate the association between blood parameters and BMD. Generalized additive regression models (GAMs), as implemented in S-PLUS 6.2 for Windows (Insightful Corporation, Seattle, Wash) were used to generate graphic representations of the dose-response relations between levels of tHcy, folate, and vitamin B_{12} and BMD, adjusted for covariates.[26] A variable was included as a covariate to control for confounding if it affected the β-coefficient in the tHcy-BMD linear regression by 5% or more in any of the age- and sex-specific strata (change-in-estimate criterion for confounder selection).[27] By this procedure our final model included the following covariates: BMI, smoking status, consumption of coffee, plasma creatinine level, level of physical activity, and use of postmenopausal estrogen (women only). Height, weight, age, intake of calcium and vitamin D, and for women, previous use of estrogen and years since menopause were considered but not included. Plasma tHcy level was divided into 4 categories: less than 9.0 µmol/L (reference), 9.0-11.9 µmol/L, 12.0-14.9 µmol/L, and 15 µmol/L or greater (<1.22 mg/L [reference], 1.22-1.61 mg/L, 1.62-2.01 mg/L, and ≥2.02 mg/L).[2,28] Folate and vitamin B_{12} were also divided into 4 categories: plasma folate, less than 3.80 nmol/L, 3.80-4.99 nmol/L, 5.00-8.49 nmol/L, and 8.50 nmol/L or greater (reference) (<1.68 ng/mL, 1.68-2.20 ng/mL, 2.21-3.74 ng/mL, and ≥3.75 ng/mL [reference]); plasma vitamin B_{12}, less than 230.0 pmol/L, 230.0-279.9 pmol/L, 280.0-414.9 pmol/L, and ≥415.0 pmol/L (reference) (<311.65 pg/mL, 311.65-379.39 pg/mL, 379.40-562.32 pg/mL, and ≥562.33 pg/mL [reference]).[28] Low BMD was defined as BMD in the lowest quintile for each sex and age group. The BMD cutoff for men was 0.918 g/cm^2 for the youngest and 0.829 g/cm^2 for oldest age group. The corresponding cutoff values for women were 0.874 g/cm^2 and 0.693 g/cm^2. Logistic regression analyses were used to estimate odds ratios (ORs) for low BMD comparing each category of plasma tHcy, folate, or vitamin B_{12} level with the reference category. Linear representation of indicator variables was used to test for trend.

Analysis of covariance was used to compare adjusted mean BMD among *MTHFR* 677C→T and 1298A→C genotypes. To examine the potential effect of plasma folate level on the association between genotype and BMD, participants were dichotomized with plasma folate values of 5.0 nmol/L (2.2 ng/mL) as the cutoff value. We used the software Haplin (http://www.uib.no/smis/gjessing/genetics/software/haplin/; Gjessing HK and Lie RT, 2005) for haplotype analyses. Because the genotype data are unphased, individuals heterozygous in both markers have ambiguous haplotypes. In Haplin, this is accounted for by using the EM algorithm to reconstruct the haplotypes. Confidence intervals are computed using jackknife resampling. There are 4 possible haplotypes at the locus, A-C, C-T, C-C, and C-T. We used the A-C haplotype as reference and assumed a multiplicative model for the odds of having low BMD.[29] This means that an individual with a combination of the A-C and C-C haplotypes has an odds equal to

$$\text{Odds}_{\text{ref}} \times \text{OR}_{\text{a-c}} \times \text{OR}_{\text{c-c}} = \text{Odds}_{\text{ref}} \times \text{OR}_{\text{c-c}},$$

where odds_{ref} is the odds of low BMD for individuals homozygous in the A-C haplotype, and $\text{OR}_{\text{c-c}}$ denotes the OR associ-

Table 1. Characteristics of the Study Population, the Hordaland Homocysteine Study*

Characteristic	Women, Age, y		Men, Age, y	
	47-50 (n = 1856)	71-75 (n = 1205)	47-50 (n = 1238)	71-75 (n = 1030)
BMD, g/cm^2	0.98 ± 0.13	0.80 ± 0.13	1.03 ± 0.14	0.96 ± 0.15
Plasma tHcy, µmol/L	9.2 ± 3.2	11.7 ± 5.7	10.9 ± 3.5	12.9 ± 4.1
Plasma folate, nmol/L	8.5 ± 6.2	9.4 ± 8.3	7.2 ± 3.8	7.5 ± 5.3
Plasma vitamin B_{12}, pmol/L	380.8 ± 145.7	412.8 ± 328.5	369.8 ± 142.9	379.5 ± 294.8
BMI	24.8 ± 4.0	26.2 ± 4.2	26.1 ± 3.3	26.0 ± 3.2
Coffee consumption, No. of cups/d	3.8 ± 2.5	3.0 ± 2.2	4.6 ± 3.0	3.3 ± 2.0
Smoking status				
Never	749 (40.2)	728 (60.4)	429 (34.7)	254 (24.7)
Former	489 (26.2)	324 (26.9)	417 (33.7)	622 (60.4)
Current	626 (33.6)	153 (12.7)	392 (31.7)	154 (15.0)
Physical exercise				
No or little activity	674 (36.5)	473 (43.1)	462 (37.6)	291 (29.2)
Moderate activity	966 (52.3)	558 (50.8)	568 (46.3)	546 (54.8)
Heavy activity	208 (11.3)	67 (6.1)	198 (16.1)	159 (16.0)
Postmenopausal estrogen				
Current user	318 (17.1)	57 (4.7)	NA	NA
Nonuser	1547 (82.9)	1148 (95.3)	NA	NA

Abbreviations: BMD, bone mineral density of total hip; BMI, body mass index (calculated as weight in kilograms divided by the square of height in meters); NA, not applicable; tHcy, total homocysteine.

Conventional unit conversion factors: To convert tHcy to milligrams per liter, divide by 7.397; folate to nanograms per milliliter, divide by 2.266; and vitamin B_{12} to picograms per milliliter, divide by 0.738.

*Values are given as mean ± SD for continuous variables and number (percentage) for categorical variables. Total numbers may vary between different variables according to different numbers of missing data.

ated with the C-C haplotype. Except for generalized additive regression and halotype analyses, all statistical analyses were carried out using the SPSS for Windows (version 11.0; SPSS, Chicago, Ill).

RESULTS

Plasma tHcy level was higher among men than among women and higher among the oldest than among the middle-aged group (**Table 1**). Bone mineral density was symmetrically distributed in all groups, and the difference in BMD between age groups was most pronounced among women. All analyses were performed for both femoral neck and total hip, with similar results. We therefore report results for total hip only.

The relationship between the level of tHcy and BMD by GAM provided dose-response curves adjusted for smoking status, BMI, plasma creatinine level, coffee consumption, physical activity, and use of postmenopausal estrogen therapy (**Figure 1**). Plasma levels of tHcy were inversely related to BMD among women, while no relation to BMD was seen among men. A corresponding multiple linear regression analysis adjusting for the same parameters showed that the linear relationships were highly significant ($P<.001$) for both middle-aged and elderly women, with regression coefficients $\beta=0.004$ and $\beta=0.003$, respectively. Adding level of plasma folate or vitamin B_{12}, years since menopause, and intake of vitamin D and calcium in the model essentially did not change the results. Including age to the final model did not change the estimates, and age was not a predictor of BMD within the different groups.

The GAM plots demonstrated a positive relation between plasma folate level and BMD among women, but the curve for middle-aged women leveled off at 10 nmol/L (4.4 ng/mL) (**Figure 2**). The relationship between plasma folate level and BMD was significant by multiple linear regression analysis (adjusting for smoking status, BMI, plasma creatinine level, coffee consumption, level of physical activity, and use of postmenopausal estrogen therapy) among elderly ($\beta=0.001$; $P=.003$) but not among middle-aged women. Vitamin B_{12} level was not significantly related to BMD in multiple linear regression analyses, and there was no apparent threshold effect. Thus, we do not present the GAM curve for this relation.

High levels of tHcy or low levels of folate were significantly associated with low BMD among women but not among men (**Table 2**). The unadjusted OR for having low BMD among women in the highest level of tHcy (≥15 µmol/L [≥2.02 mg/L]) compared with the lowest level (<9 µmol/L [<1.22 mg/L]) was 1.96 ($P<.001$). Adjustment for smoking status, BMI, plasma creatinine level, coffee consumption, level of physical activity, and use of postmenopausal estrogen therapy strengthened the relationship. Further inclusion of years since menopause and dietary intake of vitamin D and calcium did not change the risk estimates. Among women, the adjusted OR for having low BMD in the lowest (<3.80 nmol/L [<1.7 ng/mL]) compared with the highest (≥8.50 nmol/L [≥3.8 ng/mL]) folate level was 1.55 ($P=.02$).

The genotype frequencies (for 677C→T: CC, 49.2%; CT, 42.3%; and TT, 8.5%; and for 1298A→T: AA, 45%; AC, 44.2%; and CC, 10.8%) were similar to those found in other studies of whites,[30] and the observed genotype distribution in the study population is in Hardy-Weinberg equilibrium.

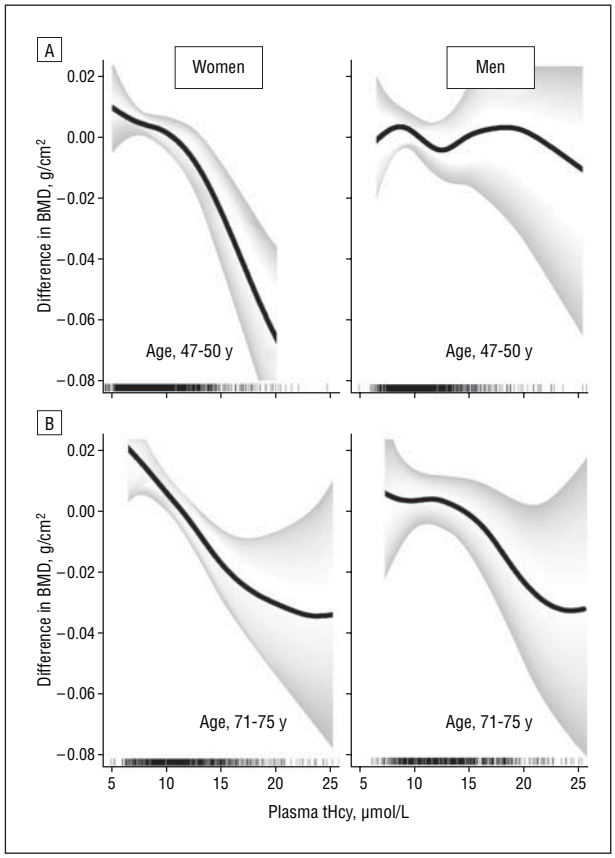

Figure 1. Dose-response relation between plasma total homocysteine (tHcy) level and bone mineral density (BMD) obtained by generalized additive regression adjusted for body mass index, smoking status, creatinine level, consumption of coffee, level of physical activity, and, for women, use of postmenopausal estrogen therapy. Shaded areas represent 95% confidence intervals. The model generates a reference scale, where zero corresponds to the BMD value associated with the mean tHcy concentration for all subjects in the age- and sex-specified strata. Ranges from 1% to 99% percentiles of exposure variables are included. The rug plot along the bottom of each graph depicts each observation. To convert tHcy to milligrams per liter, divide by 7.397.

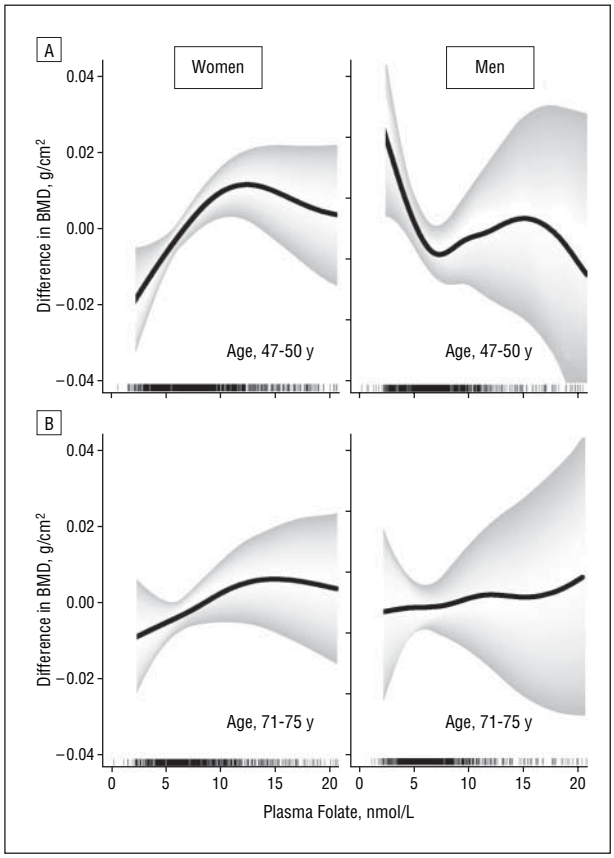

Figure 2. Dose-response relation between plasma folate level and bone mineral density (BMD) obtained by generalized additive regression adjusted for body mass index, smoking status, creatinine level, consumption of coffee, level of physical activity, and, for women, use of postmenopausal estrogen therapy. Shaded areas represent 95% confidence intervals. The model generates a reference scale, where zero corresponds to the BMD value associated with the mean folate concentration for all subjects in the age- and sex-specified strata. Ranges from 1% to 99% percentiles of exposure variables are included. The rug plot along the bottom of each graph depicts each observation. To convert folate to nanograms per milliliter, divide by 2.266.

Table 3 gives the adjusted mean BMD for the different 677C→T genotypes. The heterozygous CT group had lower BMD compared with the CC group. This was also seen among all women combined and among women with folate levels of 5.0 nmol/L or greater (≥2.2 ng/mL). However the TT genotype was not associated with lower BMD compared with the other genotypes. Nor was there any effect modification by low serum folate level among subjects with TT genotype. There was no significant association between the *MTHFR* 1298A→C polymorphism and BMD (data not shown). The estimated haplotype frequencies (95% confidence interval) were as follows: A-C, 37.7% (36.7%-38.8%); C-C, 33.0% (32.0%-34.0%); and A-T, 29.3% (28.3%-30.3%). The C-T haplotype was very rare (estimated frequency 0.15%). Consequently, OR estimates for this haplotype are unimportant and imprecise and not presented. There was no association between haplotypes and risk of low BMD (**Table 4**).

COMMENT

In our large population-based study on the associations between homocysteine-related factors and total hip BMD, the plasma tHcy level was significantly related to BMD in middle-aged and older women but not in men. A positive relation between plasma folate level and BMD was observed among women, although weaker than the relation between tHcy level and BMD. Bone mineral density was not related to vitamin B_{12} level or the *MTHFR* 677C→T or 1298A→C polymorphisms.

Whereas several studies indicate a relation between plasma tHcy levels and risk of fracture,[8,9] data are sparse on the relation between plasma tHcy level and BMD. Two previous studies reported that there was no relationship between tHcy levels and BMD,[9,10] whereas in our study, tHcy level was significantly related to BMD among women but not among men. The difference in BMD by each standard deviation of tHcy among middle-aged women was 0.012 g/cm^2 and among elderly women 0.018 g/cm^2. The mechanism linking tHcy level to BMD is equivocal. Studies of patients with homocystinuria indicate that an elevated homocysteine level impairs collagen cross-linking[6,31] and bone mineralization.[7] However, plasma tHcy levels are influenced by dietary intake of folate, cobalamin, riboflavin, and vitamin B_6,[32] and high levels of tHcy may reflect low intake of fruits and vegetables[33] or

Table 2. Odds Ratio for Low Bone Mineral Density (Lowest Quintile for Age Group and Sex) at Different Plasma Levels of tHcy, Folate, and Vitamin B_{12}

	Women					Men				
		Adjusted for Age		Multiple Adjusted*			Adjusted for Age		Multiple Adjusted*	
Plasma Level	No. (%)	OR (95% CI)	*P* Value for Trend	OR (95% CI)	*P* Value for Trend	No. (%)	OR (95% CI)	*P* Value for Trend	OR (95% CI)	*P* Value for Trend
tHcy, µmol/L										
<9.0	243 (17.9)	1.00 (Reference)	<.001	1.00 (Reference)	<.001	92 (20.0)	1.00 (Reference)	.43	1.00 (Reference)	.72
9.0-11.9	208 (19.6)	1.15 (0.93-1.43)		1.14 (0.90-1.44)		180 (18.8)	0.93 (0.70-1.24)		1.01 (0.74-1.37)	
12.0-14.9	90 (21.3)	1.31 (0.99-1.74)		1.30 (0.95-1.79)		115 (21.3)	1.09 (0.79-1.50)		1.12 (0.79-1.60)	
≥15	64 (28.7)	1.96 (1.40-2.75)		2.19 (1.48-3.25)		64 (21.1)	1.08 (0.74-1.57)		1.02 (0.66-1.56)	
Folate, nmol/L										
<3.8	64 (25.0)	1.61 (1.16-2.23)	.004	1.55 (1.07-2.23)	.02	43 (17.7)	0.94 (0.64-1.40)	.68	0.81 (0.53-1.24)	.26
3.8-4.9	82 (20.8)	1.26 (0.94-1.69)		1.18 (0.86-1.63)		71 (18.9)	1.02 (0.73-1.44)		0.96 (0.67-1.38)	
5.0-8.4	283 (20.2)	1.22 (0.99-1.51)		1.24 (0.99-1.56)		234 (21.3)	1.19 (0.91-1.54)		1.15 (0.87-1.53)	
≥8.5	173 (17.2)	1.00 (Reference)		1.00 (Reference)		99 (18.6)	1.00 (Reference)		1.00 (Reference)	
Vitamin B_{12}, pmol/L										
<230.0	62 (20.8)	1.05 (0.76-1.44)	.75	0.97 (0.68-1.37)	.61	58 (25.4)	1.46 (1.02-2.10)	.03	1.22 (0.82-1.81)	.25
230.0-279.9	69 (17.3)	0.83 (0.62-1.13)		0.87 (0.63-1.21)		73 (21.9)	1.19 (0.86-1.66)		1.14 (0.80-1.62)	
280.0-414.9	268 (20.1)	1.00 (0.82-1.23)		1.02 (0.82-1.27)		203 (18.8)	0.99 (0.77-1.27)		0.97 (0.74-1.28)	
>415.0	206 (20.0)	1.00 (Reference)		1.00 (Reference)		117 (19.0)	1.00 (Reference)		1.00 (Reference)	

Abbreviations: CI, confidence interval; OR, odds ratio; tHcy, total homocysteine.
Conventional unit conversion factors: See Table 1.
*Adjusted for age, body mass index, smoking status, plasma creatinine concentration, coffee consumption, level of physical activity, and current use of postmenopausal estrogen (for women only).

Table 3. Adjusted Mean BMD According to the *MTHFR* 677C→T Genotype, the Hordaland Homocysteine Study

	Men*		Women*		All†	
Folate Level	BMD (95% CI)	*P* Value, Test for Trend/ Test for Homogeneity	BMD (95% CI)	*P* Value, Test for Trend/ Test for Homogeneity	BMD (95% CI)	*P* Value, Test for Trend/ Test for Homogeneity
All folate levels						
CC	0.917 (0.911-0.923)	.13/.004	1.002 (0.994-1.011)	.24/.48	0.954 (0.949-0.959)	.03/.004
CT	0.902 (0.895-0.909)		0.996 (0.987-1.005)		0.941 (0.935-0.947)	
TT	0.919 (0.904-0.935)		0.993 (0.972-1.013)		0.951 (0.939-0.964)	
Folate <5.0 nmol/L						
CC	0.911 (0.895-0.926)	.99/.09	1.010 (0.994-1.026)	.53/.21	0.958 (0.947-0.969)	.79/.04
CT	0.894 (0.879-0.908)		0.990 (0.973-1.007)		0.942 (0.931-0.953)	
TT	0.925 (0.897-0.953)		1.012 (0.981-1.044)		0.968 (0.947-0.989)	
Folate ≥5.0 nmol/L						
CC	0.919 (0.912-0.926)	.11/.03	1.000 (0.990-1.010)	.25/.28	0.953 (0.947-0.959)	.02/.03
CT	0.905 (0.897-0.912)		0.999 (0.988-1.010)		0.941 (0.935-0.948)	
TT	0.918 (0.900-0.936)		0.977 (0.951-1.004)		0.943 (0.927-0.958)	

Abbreviations: BMD, bone mineral density; CI, confidence interval.
Conventional unit conversion factor: To convert folate to nanograms per milliliter, divide by 2.266.
*Adjusted for age.
†Adjusted for age and sex.

impaired intestinal absorption, which may also lead to deficiencies of nutrients necessary for bone mineralization, such as vitamin D and calcium. Adjusting for intake of vitamin D and calcium did not change the results of our study.

Estrogen deficiency is associated with a moderate increase in plasma tHcy levels,[34] and serum levels of estradiol are associated with BMD in elderly women.[35] We have not measured serum estradiol levels and cannot rule out that endogenous estrogen levels confound the observed relationship between levels of tHcy and BMD. Renal function may affect the tHcy-BMD relationship because a decline in glomerular filtration rate increases tHcy levels[36] and renal failure could be detrimental to bone.[37] There is, however, no evidence that the age-related decline in glomerular filtration rate is independently associated with BMD, in fact, only patients with end-stage renal disease have been shown to have a decreased BMD.[38,39] Only 5

participants in our study had severe kidney dysfunction defined as an estimated glomerular filtration rate lower than 30 mL/min per 1.73 m^2 (using the simplified Modification of Diet in Renal Disease Study equation). Adding creatinine level or glomerular filtration rate from the simplified Modification of Diet in Renal Disease Study equation to the statistical models actually strengthened the relationship between the level of tHcy and BMD. Thus, renal impairment is an unlikely explanation for the observed association between the level of tHcy and BMD. Elevated plasma tHcy levels are associated with established risk factors for osteoporosis, including old age, smoking, high coffee consumption, and lack of exercise.[32,40] The association between plasma tHcy levels and BMD was slightly attenuated after adjusting for smoking, coffee consumption, and level of exercise. However, these factors could not explain the relation of BMD to levels of tHcy or folate observed in this study.

A direct effect of folate status on bone tissue has been hypothesized,[10] and a positive association between folate levels and lumbar spine BMD has previously been reported in postmenopausal women.[10,14] We observed that plasma folate levels were positively related to BMD in elderly women only, and this relationship was weaker than that observed between levels of tHcy and BMD. Whether the observed association between levels of folate and BMD is mediated through homocysteine or by a direct effect on bone by folate itself cannot be determined from the present study.

Women with pernicious anemia have lower BMD[41] and increased risk of fracture[42] compared with healthy women. In the present study, we found no association between plasma levels of vitamin B_{12} and BMD. In contrast, in the Framingham Offspring Study cohort there was an association between BMD and vitamin B_{12} status.[43] This may be related to different vitamin B_{12} assay or different vitamin B_{12} status or folate status in the United States and the Norwegian study.

Whereas some studies have demonstrated a relationship between BMD and the *MTHFR* 677C→T polymorphism,[12,13] this association has been shown by other investigators only under conditions of impaired folate status[14] or low dietary intake of riboflavin.[15] We and others[17] found no association between *MTHFR* and BMD. This may seem as a paradox because the TT genotype predisposes for hyperhomocysteinemia in the general population.[4] One likely explanation is that our study may not have sufficient statistical power to detect a small risk enhancement associated with the mean tHcy level increment of 2.8 µmol/L (0.38 mg/L) found in subjects with the TT compared with the CT-CC genotypes. Furthermore, results from previous studies[15,44] suggest that riboflavin may play a key role in preventing low BMD in the TT genotype. Thus, different intake of riboflavin may to some extent explain why TT genotype is associated with low BMD in some populations and not in others. Another possibility is that the observed association between levels of plasma tHcy and BMD is mediated by folate.

A strength of our study is the large number of community-dwelling participants. Only 2 narrow age groups were studied, and a large number of subjects were included in each age group, eliminating the confounding effect of age. The study also incorporated the main components of the 1-carbon metabolism, including homocysteine, folate and vitamin B_{12}, the 677C→T and 1298A→C polymorphism of the *MTHFR* gene, and dietary intake.

Table 4. Odds Ratio for Low Bone Mineral Density (Lowest Quintile for Age-Group and Sex) at Different Haplotypes of *MTHFR*, the Hordaland Homocysteine Study

	Women		Men	
Haplotype	OR (95% CI)	*P* Value	OR (95% CI)	*P* Value
All folate levels				
1298A-677C	1.00 (Reference)		1.00 (Reference)	
1298C-677C	0.97 (0.83-1.13)	.72	1.00 (0.84-1.19)	>.99
1298A-677T	1.10 (0.94-1.28)	.23	1.01 (0.84-1.21)	.89
Folate <5.0 nmol/L				
1298A-677C	1.00 (Reference)		1.00 (Reference)	
1298C-677C	1.07 (0.76-1.48)	.70	0.88 (0.61-1.25)	.46
1298A-677T	1.00 (0.73-1.38)	>.99	0.95 (0.67-1.35)	.78
Folate ≥5.0 nmol/L				
1298A-677C	1.00 (Reference)		1.00 (Reference)	
1298C-677C	0.93 (0.78-1.11)	.41	1.03 (0.84-1.26)	.76
1298A-677T	1.12 (0.94-1.33)	.21	1.04 (0.84-1.29)	.70

Abbreviations: CI, confidence interval; OR, odds ratio.
Conventional unit conversion factor: To convert folate to nanograms per milliliter, divide by 2.266.

The cross-sectional nature of the study, however, precludes any inferences about causality. Furthermore, because only whites were included, study results cannot be generalized to other ethnic groups. Participants were recruited from the community at large, with no exclusion by diseases that may influence bone health. This makes the results more generalizable but may weaken the observed associations. It should also be noted, for comparison with other studies, that the plasma samples were nonfasting.

In conclusion, our study suggests that plasma tHcy level is an independent risk factor for low BMD among women but not among men. It remains unclear whether the increased risk is mediated directly by homocysteine levels or whether elevated plasma tHcy level is simply a reflection of an unhealthy lifestyle. If the modest associations observed are causal, the public health implications may be significant. Use of folate and other B vitamins for reducing plasma tHcy levels is efficient, safe, and inexpensive. Randomized trials are needed before any formal recommendations can be made concerning B vitamin supplementation for prevention of bone loss.

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