Homocysteine and bone loss in epilepsy

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Summary   Epidemiological studies reveal fracture incidence in epilepsy is twice that of the normal population. Much interest has been focused on Vitamin D, however, considering mixed results on non-enzyme inducing anti-epileptic drugs (AEDs) and bone mineral density (BMD) additional metabolic effects may be to blame. AEDs increase serum homocysteine (s-Hcy) by lowering blood folate levels. An association between elevated homocysteine, BMD and increased fracture incidence has been found in non-epilepsy populations. Additionally, folate and Vitamin B12 levels are independently related to bone mineral density in various non-epilepsy populations. This study supports previous research, which found elevated s-Hcy in subjects taking AEDs and that bone loss is related to the use of enzyme-inducing AEDs and changes in alkaline phosphatase. By one-way ANOVA, subjects on phenytoin monotherapy had significantly higher levels of s-Hcy than those on other AEDs ($F = 5.89$, $p = .016$). Regression analyses revealed homocysteine, fracture history, length of years on AEDs, ethnicity were predictors of spine T scores. Weight and BMI were predictors of both BMD and DEXA T scores. Use of enzyme-inducing AEDs was a negative predictor of spine BMD and T scores, while phenytoin monotherapy was a positive predictor of spine BMD. Lamotrigine was found to be a negative predictor of spine T score. Ambulatory status, menopause and alcohol consumption were predictors of BMD but not T scores. In this study, persons with epilepsy who take nutritional supplementation have 25% lower s-Hcy levels than those who do not. Supplementation continues to be important in preventative epilepsy care.
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Introduction

Osteoporosis is a common bone disease affecting up to 40% of women and 12% of men.[1] An estimated 1.5 million people suffer a bone-disease related fracture annually in the U.S.[2] Hip fractures account for most of the morbidity, mortality and costs of the disease.[3] Costs for the treatment of incident

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osteoporotic fractures are estimated to be $34 billion in 2004, U.S. dollars.[4] Up to 20—30% of postmenopausal women and 50% of men have a secondary cause (i.e. drug therapies, endocrine disorders, eating disorders and immobilization) of osteoporosis.[5] Bone loss can occur after as little as two years of antiepileptic drug (AED) exposure[6,7] and epidemiological studies have found the incidence of fracture in epilepsy to be twice that of the normal population.[8,9] While osteoporosis is generally associated with advancing age it also impacts individuals who are unable to reach optimal bone density during childhood and adolescence,[10] so persons with childhood onset epilepsy may be especially at risk. Whether these changes are primary due to AED treatment or lack of physical activity or a combination of the two is not presently known.[7]

In a study of older women, it was found that those who were continuous users of enzyme-inducing AEDs (phenytoin, phenobarbital, carbamazepine and primidone) had almost double the amount of bone loss at the calcaneus and the total hip on DEXA compared to nonusers.[11] Younger men (age 25—44) on AEDs (phenytoin, carbamazepine, phenobarbital, valproic acid and gabapentin) have been found to have a 1.8% annual loss of bone mineral density (BMD), yielding a 2.5 fold increased prevalence of bone loss at the hip when compared to the healthy U.S. male population.[12] Pack et al. found that persons with epilepsy, taking enzyme-inducing AEDs, are prone to significant loss of bone mass (based on current World Health Organization guidelines); with only 42% having normal bone density compared to 84% expected in the normal population.[13] Bone loss in epilepsy has been traditionally thought to be as a result of Vitamin D deficiency, secondary to enzyme-inducing AED use.[13] Results of non-enzyme inducing antiepileptic drugs such as valproic acid and lamotrigine on bone have in the past been mixed.[14—16] It follows that mechanisms other than Vitamin D may also be involved in the pathogenesis of osteoporosis by AEDs.

Homocysteine, a key intermediate in methionine metabolism, is created as a byproduct of methyl transfer reactions important for the synthesis of nucleic acids, methylated protein, neurotransmitters and phospholipids.[17] Anti-epileptic drugs (AEDs) have been shown to increase serum homocysteine (s-Hcy) by lowering blood folate levels.[18—23] The proposed mechanisms for the folic acid depletion with AEDs include (1) interference with the intestinal absorption of folic acid by increasing the pH (2) interference with folate transport into tissues, and (3) Hepatic microsomal induction and increased folate catabolism.[24—26] Valproate and carbamazepine significantly increase homocysteine levels compared to controls.[20,27] Yoo et al. found a 25% increase in homocysteine in patients taking phenytoin and carbamazepine (but not valproic acid) compared to controls.[28]

In one study of bone density, for each standard deviation increase in the homocysteine level, age and sex-adjusted risk of fracture increased by 30%.[29] This level of increased risk is similar to that previously observed in the risk of cardiovascular disease and dementia according to homocysteine level.[29] Homocysteine levels in the highest quartile double the risk of fracture.[30] Goldbahar et al. found hip and spine bone mineral density was significantly correlated with plasma homocysteine in postmenopausal women.[31] Homocysteine also appears to predict fracture rates in patients with Parkinson's disease.[32] Low folate[33] and Vitamin B12 levels[34] as well as elevated homocysteine[35] are associated with reduced bone mineral density in post menopausal women. In addition, low serum B12 has been associated with a reduction in bone mineral density among adolescents fed a macrobiotic diet.[36]

A homocysteine-associated disturbance in collagen cross-linking has been suggested as a potential mechanism[37,38] and considering the high prevalence of osteoporosis in homocystinuria (a rare autosomal recessive disorder), abnormal homocysteine metabolism may contribute to the development of osteoporosis.[39] Additionally, folate may preserve nitric oxide synthase activity in bone cells leading to a stimulation of osteoblast activity while inhibiting bone catabolism.[40] Supplementation with folic acid and Vitamin B12 has been found to reduce hip fracture in stroke patients by 20%. This is significant since both the supplemented group and the control group had the same number of falls during the follow up period.[41] Furthermore, the administration of high doses of homocysteine in animal models produces convulsive seizures.[42—44] Therefore, elevated homocysteine may represent a mechanism by which the seizure threshold is reduced in some susceptible patients. This mechanism might be of practical relevance, as folate is easily supplemented.[21]

It has been suggested that persons with epilepsy may have a higher folate requirement to maintain a normal homocysteine level.[28] Also little is known about homocysteine in African Americans and Latinos with epilepsy. The Center for Disease Control's National Health and Nutrition Examination Survey (NHANES) recently looked at post-folate fortification of the U.S. food supply and found a significant reduction of s-Hcy levels in all ethnic groups.[45] However, drug induced elevations of homocysteine may not respond fully to fortification and supplementation may be necessary. Homocysteine reduction by vitamin supplementation (folic acid, B6 and

B12) could prove to be as simple and elegant a measure in the reduction of osteoporosis in the epileptic population as calcium and Vitamin D are in the elderly. The goal of this study was to see if there is a relationship between bone loss, homocysteine, demographics (age, gender and ethnicity) or metabolic factors in epilepsy.

Methods

We screened 805 patient charts seen at the Temple University School of Medicine Department of Neurology outpatient epilepsy clinics over the past 2 years. Data on 165 patients, age 18 years or older (range 19—78), included: age, gender, weight, BMI, ambulatory status, years on AEDs, current/previous AEDs, serum levels of homocysteine (s-Hcy), folic acid (s-FA), B12 (s-B12), calcium, alkaline phosphatase, creatinine, blood urea nitrogen (BUN), supplementation patterns and dual-energy x-ray absorptiometry (DEXA) screening results. Data collection took place over a 1 year period. Inclusion criteria included patients age 18 or older, with a diagnosis of epilepsy on AEDs over 2 years. No exclusion criteria were used. Instead documentation of any secondary causes of osteoporosis, based on a paper by Fitzpatrick[5] was collected to observe "what their impact might be." The Temple University Institutional Review Board approved this study and since data collection focused on existing records, written informed consent from each participant was not necessary. All data was gathered, analyzed and stored in a way to protect the patients' identity and medical information in compliance with HIPAA and Temple University IRB guidelines.

Certain variables were recoded for analysis. This included separating serum homocysteine into quartiles as well as performing a log transformation since values are not normally distributed. Age and years on AEDs were recoded generally into quartiles in order to make comparisons by more equal group sizes. BMI was calculated and recoded based on the National Institutes of Health website guidelines.[46] As a retrospective study serum homocysteine levels were not collected as fasting samples. Previous work has found that non-fasting samples are actually preferable[47] and the variation between fasting and non-fasting samples is not enough to recommend strict fasting blood draws.[48] Bone mineral density was measured by DEXA with the Hologic QDR 1000.

Data analysis plan

The analytic plan of this study followed a specific progression. Bone mineral density measured in g/cm^2 and the lowest T score from each site (excluding

Table 1 Demographics of participants

Variables	n (%)
Female	94 (57)
Ethnicity	
African American	75 (46)
Latino	27 (16)
Caucasian	60 (36)
Asian/other	3 (2)
On medical assistance	89 (54)
Body mass index	
Underweight	6 (4)
Normal	53 (33)
Overweight	39 (24)
Obesity	62 (39)
Ambulatory status	
Non ambulant	8 (6)
Ambulant with restrictions	21 (17)
Ambulant without restrictions	61 (48)
Ambulant and exercises	36 (29)
Present smoker	27 (17)
Present drinker	32 (20)
Presence of other secondary risk factors	30 (18)
History of fracture[a]	36 (43)
Taking an osteoporosis medication	19 (16)
Presently taking calcium	52 (32)
Presently taking a multivitamin	76 (46)
Bone density DEXA results	
Normal	38 (28)
Osteopenia	59 (43)
Osteoporosis	40 (29)
No DEXA available	28 (17)
AED use	
Presently on a enzyme-inducing AED	78 (47)
Presently on monotherapy	
PHT	17 (10)
CBZ	20 (12)
VPA	25 (15)
LTG	16 (10)
Presently on multiple AEDs	
Two	33 (35)
Three	13 (13)
Four	1 (2)
Seizure types listed in subject's medical record	
Complex partial	107 (65)
Simple partial	27 (16)
Tonic clonic	38 (23)
Secondary generalized	33 (20)
Absence	9 (5)
Myoclonic	5 (3)
Other (includes not categorized)	27 (16)

[a] Fracture information was available on 83 of 165 subjects.

Ward's triangle) were the primary dependent variables. First, descriptive statistics were used to summarize and describe the data, see Table 1. A one-way ANOVA analysis of demographics, clinical measures and DEXA results based on homocysteine quartiles was performed, see Table 2. One-way ANOVA analysis was also used to determine if there were differences in the independent variables: age, gender, ethnicity, BMI, ambulatory status and length on time on AEDs, see Table 3. Another one—way ANOVA was used to examine various AED monotherapies and the effects of nutritional supplementation on DEXA results, see Table 4. Next, we performed a bivariate (Pearson correlations) analysis to examine relationships between the dependent and independent variables as well as to identify redundancy among independent variables, see Table 5. Correlations provided insight into the variables which would likely hold up in the modeling process.

Finally, multivariate regression analysis was performed, see Table 6. Multivariate regression allows for the examination of multiple independent variables on each dependent variable. The regression analysis was performed using a backward serial process to eliminate non-significant variables until only significant ones remained in the model, see Table 6. Demographic and clinical variables have been found to be significant predictors of osteoporosis. Hence, independent variables included in the regression analysis included demographics, medical management factors and clinical markers. The goal was to determine the degree to which components from these areas explain bone mineral density and DEXA T scores for the spine and hip. SPSS version 13.0 was used for all of the statistical analyses.

Results

This was an adult epilepsy population: mean age: 45 years (S.D. = 13.6, range 18—81), there were 71 males and 94 females. Seventy-five were African American, 27 Latino, 60 Caucasian and 3 Asian/Other, average length of AED exposure: 25 years (S.D. = 14.6, range 1—64). Complete demographics are displayed in Table 1. DEXA results were found for 130 patients and homocysteine levels for 158

Table 2 Clinical characteristics of subjects in each quartile of homocysteine — mean (standard deviation)

	Quartile 1 (n = 41)	Quartile 2 (n = 40)	Quartile 3 (n = 39)	Quartile 4 (n = 38)	P value[*]
Age	37.1 (11.5)	45.2 (12.9)	48.2 (12.9)	49.6 (14.1)	.000
BMI	29.7 (7.8)	28.9 (6.9)	29.6 (6.4)	27.3 (6.2)	.382
Years on AEDs	20.2 (11.5)	28.2 (15.4)	22.2 (15.6)	26.5 (14.2)	.048
Serum Hcy	5.9 (1.1)	8.2 (0.6)	10.7 (1.1)	18.3 (7.4)	.000
Range	2.7—7.2	7.1—9.0	9.1—12.3	12.4—50.0	NA
Serum folic acid	17.9 (6.8)	17.9 (6.7)	15.3 (7.2)	12.8 (7.3)	.005
Serum B12	624.7 (378.2)	655.7 (415.7)	558.7 (282.4)	493.2 (252.2)	.216
Serum calcium	9.2 (0.4)	9.2 (0.4)	9.3 (0.4)	9.3 (0.5)	.704
Alkaline phosphatase	97.4 (36.9)	94.4 (36.7)	110.2 (57.4)	120.2 (119.2)	.355
BUN	11.4 (3.9)	14.6 (3.8)	14.8 (4.5)	15.0 (8.3)	.009
Serum creatinine	0.8 (0.2)	0.9 (0.2)	1.2 (1.3)	1.1 (0.3)	.080
Spine mineral density (g/cm^2)	.93 (0.28)	1.02 (0.25)	1.01 (0.28)	.98 (0.20)	.514
Spine T score	−1.33 (1.34)	−1.25 (1.57)	−1.12 (1.52)	−1.56 (1.78)	.724
Hip mineral density (g/cm^2)	.81 (.21)	.79 (.22)	.83 (.26)	.77 (.17)	.727
Hip T Score	−0.81 (1.28)	−1.47 (1.22)	−0.94 (1.62)	−1.50 (0.94)	.081
Other osteoporosis risk factors					
Yes	7	7	6	7	
No	34	33	33	31	.988[**]
Fracture history					
Yes	8	11	14	13	.144[**]
No	12	9	8	4	

NA, not applicable. Other osteoporosis risk factors include; corticosteroid use, thyroid hormone use, eating disorders, cancer treatment.
[*] Analysis of variance.
[**] Chi square.

Table 3 Demographics and bone marker dependent variables — mean (standard deviation)

	n	Spine (g/cm^2)	Spine T-score	Hip (g/cm^2)	Hip T-score	Serum Hcy	Serum folate	Vitamin B12	Calcium	Alkaline phosphatase
Age										
Under 35	43	0.96 (.25)	−1.13 (1.17)	0.82 (.20)	−1.06 (1.00)	9.20 (5.00)	15.5 (7.4)	578.97 (348.9)	9.4 (0.4)	92.5 (35.0)
36−45	32	1.04 (.22)	−1.23 (1.87)	0.78 (.16)	−1.04 (1.13)	9.31 (3.99)	17.8 (7.2)	643.61 (384.4)	9.1 (0.3)	121.0 (128.4)
46−54	52	0.98 (.24)	−1.15 (1.32)	0.83 (.23)	−1.07 (1.49)	11.87 (7.84)	14.6 (7.2)	534.34 (297.7)	9.3 (0.4)	103.2 (47.6)
Over 55	35	0.96 (.28)	−1.94 (1.77)	0.74 (.23)	−1.69 (1.33)	11.82 (4.16)	17.2 (6.9)	597.10 (341.1)	9.2 (0.5)	113.2 (50.2)
Gender										
Male	68	1.03 (.26)	−1.23 (1.67)	0.84 (.20)	−1.37 (0.98)	11.57 (5.48)	16.6 (7.1)	583.95 (306.4)	9.3 (0.4)	97.8 (39.0)
Female	89	0.96 (.24)	−1.42 (1.44)	0.77 (.22)	−1.07 (1.49)	9.95 (6.14)	15.6 (7.3)	578.98 (357.4)	9.2 (0.4)	112.1 (85.5)
Ethnicity										
African American	74	1.02 (.26)	−1.50 (1.65)	0.80 (.22)	−1.39 (0.88)	10.87 (6.56)	16.1 (7.8)	612.24 (318.6)	9.3 (0.5)	124.4 (93.4)[*]
Latino	27	1.04 (.27)	−0.99 (1.32)	0.83 (.22)	−0.98 (1.38)	12.07 (7.38)	13.6 (6.6)	568.52 (389.4)	9.3 (0.4)	90.5 (30.1)[*]
Caucasian	57	0.91 (.21)	−1.29 (1.50)	0.78 (.20)	−1.05 (1.65)	9.75 (3.94)	17.0 (6.6)	551.69 (342.2)	9.3 (0.4)	91.0 (38.9)[*]
BMI										
Underweight	6	0.69 (.38)	−2.97 (1.53)[**]	0.77 (.06)	−1.89 (0.93)[***]	9.46 (6.42)	17.6 (7.2)	667.20 (449.3)	9.5 (0.2)	200.2 (288.7)[**]
Normal weight	52	0.95 (.26)	−1.67 (1.38)[**]	0.69 (.19)	−1.96 (1.14)[***]	10.52 (5.38)	16.2 (7.0)	571.66 (245.4)	9.3 (0.5)	100.9 (33.6)[**]
Overweight	38	0.96 (.19)	−1.53 (1.38)[**]	0.79 (.17)	−1.24 (0.93)[***]	12.29 (8.24)	14.8 (7.6)	532.09 (333.0)	9.2 (0.5)	101.3 (55.1)[**]
Obesity	61	1.06 (.23)	−0.80 (1.59)[**]	0.89 (.24)	-0.53 (1.32)[***]	9.80 (4.09)	16.3 (7.3)	583.70 (350.9)	9.3 (0.4)	103.9 (48.3)[**]
Ambulatory status										
Non-ambulatory	6	0.90 (.21)	−2.75 (1.32)	0.68 (.16)[*]	−2.22 (.78)[**]	11.24 (6.09)	18.44 (8.4)	1065.29 (733.9)[**]	9.13 (.57)	202.9 (242.7)[***]
Ambulant w/ restrictions	17	0.85 (.31)	−1.53 (1.62)	0.67 (.16)[*]	−1.97 (1.53)[**]	10.51 (3.88)	18.61 (6.7)	675.16 (444.4)[**]	9.21 (.62)	103.1 (52.3)[***]
Ambulant w/o restrictions	49	1.00 (.21)	−1.33 (1.18)	0.82 (.23)[*]	−1.00 (1.10)[**]	10.16 (4.76)	16.00 (7.1)	542.22 (212.2)[**]	9.25 (.37)	103.0 (39.9)[***]
Ambulant and exercises	27	0.97 (.21)	−1.10 (1.47)	0.86 (.23)[*]	−0.84 (1.49)[**]	10.08 (5.64)	15.55 (7.0)	538.30 (338.1)[**]	9.40 (.41)	80.3 (20.8)[***]
Length on AEDs										
<12 years	40	0.92 (.19)	−1.57 (1.23)	0.82 (.26)	−1.06 (1.50)	10.50 (5.59)	18.2 (6.3)[*]	566.45 (282.5)	9.3 (0.5)	106.1 (49.2)
12 to 24 years	40	1.03 (.35)	−1.11 (1.86)	0.82 (.22)	−0.97 (1.30)	9.95 (4.18)	14.2 (7.6)[*]	548.31 (238.2)	9.3 (0.4)	101.8 (33.6)
25 to 35 years	36	1.03 (.19)	−1.13 (1.56)	0.80 (.16)	−1.15 (1.01)	11.16 (8.33)	14.3 (7.4)[*]	568.09 (391.1)	9.2 (0.5)	117.1 (122.0)
>36 years	38	0.96 (.20)	−1.53 (1.47)	0.76 (.21)	−1.60 (1.28)	11.11 (5.18)	17.2 (6.9)[*]	639.00 (411.2)	9.3 (0.3)	99.9 (44.1)

[*] $p < .05$.
[**] $p < .01$.
[***] $p < .001$.

Table 4 AEDs, nutritional supplements and bone markers — mean (standard deviation)

	n	Spine (g/cm^2)	Spine T-Score	Hip (g/cm^2)	Hip T-Score	Serum Hcy	Serum folate	Serum B12	Calcium	Alkaline phosphate
On PHT monotherapy										
No	142	0.98 (.24)	−1.28 (1.57)	0.81 (.22)	−1.17 (1.35)	10.25 (4.80)[*]	16.4 (7.1)	593.1 (348.5)	9.3 (0.4)[*]	101.2 (69.5)[*]
Yes	16	1.05 (.32)	−1.81 (1.22)	0.73 (.15)	−1.48 (0.70)	14.16 (11.54)[*]	13.1 (7.4)	472.2 (180.9)	9.0 (0.4)[*]	146.8 (61.7)[*]
On CBZ monotherapy										
No	142	0.99 (.24)	−1.26 (1.62)	0.79 (.21)	−1.19 (1.39)	10.84 (6.02)	15.9 (7.2)	580.9 (337.0)	9.3 (0.5)	107.7 (74.7)
Yes	17	0.97 (.31)	−1.57 (1.48)	0.88 (.20)	−0.83 (0.72)	8.96 (4.61)	17.1 (7.1)	602.1 (408.4)	9.3 (0.3)	97.7 (24.6)
On VPA monotherapy										
No	133	0.98 (.25)	−1.41 (1.61)	0.79 (.22)	−1.23 (1.33)	10.55 (5.92)	15.9 (7.2)	549.4 (307.2)[**]	9.2 (0.4)[**]	110.6 (72.6)[*]
Yes	24	1.07 (.18)	−0.86 (1.07)	0.83 (.20)	−0.98 (0.93)	11.16 (5.89)	17.0 (7.4)	758.0 (438.9)[**]	9.5 (0.4)[**]	79.5 (44.5)[*]
On LTG monotherapy										
No	143	0.99 (.26)	−1.47 (1.74)	0.80 (.21)	−1.25 (1.18)	10.88 (6.10)	15.9 (7.3)	582.2 (349.3)	9.2 (0.4)[*]	107.3 (73.1)
Yes	15	0.94 (.17)	−1.32 (1.52)	0.78 (.27)	−0.87 (1.96)	8.43 (2.64)	17.0 (6.6)	568.5 (186.4)	9.5 (0.4)[*]	95.6 (28.3)
Presently taking a multivitamin										
No	88	1.00 (.22)	−1.37 (1.51)	0.83 (.22)	−1.09 (1.04)	12.03 (7.15)[**]	13.9 (7.4)[***]	503.6 (308.2)[**]	9.3 (0.5)	113.2 (89.2)
Yes	76	0.96 (.27)	−1.31 (1.59)	0.76 (.20)	−1.32 (1.53)	9.11 (3.57)[**]	18.4 (6.3)[***]	670.9 (351.3)[**]	9.3 (0.4)	98.0 (37.8)
Presently taking calcium										
No	112	1.00 (.25)	−1.27 (1.62)	0.82 (.22)	−1.15 (1.08)	10.53 (4.91)	15.6 (7.4)	574.4 (331.3)	9.3 (0.4)	107.6 (79.4)
Yes	52	0.95 (.25)	−1.47 (1.37)	0.76 (.21)	−1.29 (1.65)	10.92 (7.61)	16.7 (6.8)	594.5 (354.5)	9.2 (0.6)	102.9 (45.8)

Alkaline phosphate — serum alkaline phosphatase.
[*] $p < .05$.
[**] $p < .01$.
[***] $p < .001$.

Table 5 Pearson correlation matrix of demographics/laboratory variables

Variables	Spine (g/cm^2)	Spine T score	Hip (g/cm^2)	Hip T score
Age	−0.04	−0.16	−0.13	−0.19[*]
Gender	−0.14	−0.06	−0.17	0.12
Ethnicity	−0.18[*]	0.07	−0.03	0.12
Body weight	0.31[***]	0.37[***]	0.36[***]	0.47[***]
Body mass index	0.25[**]	0.29[***]	0.30[***]	0.45[***]
Ambulatory status	0.13	0.24[*]	0.28[**]	0.31[**]
Years on AEDs	0.04	−0.02	−0.10	−0.17
Presently on enzyme-inducing AEDs	−0.03	−0.15	−0.05	−0.16
Presently taking a multivitamin	−0.08	0.02	−0.16	−0.09
Presently taking calcium	−0.11	−0.06	−0.13	−0.05
Fracture history	0.04	−0.13	−0.01	−0.08
Present smoker	0.24[**]	0.11	0.07	0.04
Present drinker	0.02	0.11	0.28[**]	0.18[*]
Menopause status	−0.22	−0.17	−0.28[**]	−0.12
Presence of other risk factors	−0.10	−0.07	−0.09	−0.07
s-Hcy	0.09	−0.02	−0.08	−0.10
log-Hcy	0.06	−0.05	−0.06	−0.11
s-FA	−0.09	−0.09	0.01	−0.03
s-B12	−0.13	−0.16	−0.01	−0.13
Serum calcium	0.08	0.09	0.16	0.07
Serum alkaline phosphatase	−0.09	−0.26[**]	−0.06	−0.06
BUN	−0.08	−0.05	0.02	−0.04
Serum creatinine	0.12	0.04	0.10	−0.03

[*] $p \leq .05$.
[**] $p \leq .01$.
[***] $p \leq .001$.

patients. Of the 130 subjects who obtained a DEXA scan of the spine 40 had osteopenia (men = 14, women = 26) and 30 had osteoporosis (men = 14, women = 16) and 60 were normal (men = 28, women = 32). For those who had a DEXA of the hip 64 had osteopenia (men = 33, women = 31), 13 had osteoporosis (men = 6, women = 7) and 52 were normal (men = 17, women = 35). We were able to determine from extensive historical chart review that 47 subjects had a history of fracture (African American = 18, Latino = 3 and Caucasian = 26) and 36 did not (African American = 17, Latino = 7 and Caucasian = 12).

Clinical characteristics by quartile of homocysteine

Clinical characteristics were separated by quartiles of homocysteine. Age was significantly higher in the ascending quartiles (F = 7.46, p = .000) by one-way ANOVA, see Table 2. Years on AEDs were higher in quartiles 2 and 4 compared with 1 and 4 but this was not significant. Serum folate levels were significantly lower in each successive quartile (F = 4.42, p = .005). BUN levels also rose with each increment the in homocysteine quartile (F = 4.01, p = .009). There were no significant differences in the presence of other osteoporosis risk factors in each quartile (Chi square = .132, p = .988). No significant increase in fracture history was noted in successive quartiles (Chi square = 5.41, p = .144).

Dependent variables: Bone density and DEXA T scores

Analysis by one-way ANOVA indicated no significant differences in spine mineral density, spine DEXA T-scores, hip mineral density and hip DEXA T-scores based on age, gender or ethnicity, see Table 3. Mean s-Hcy levels in mmol/L (S.D.) were 10.9 (6.6) for African Americans, 12.1 (7.4) Latinos, 9.8 (3.9) Caucasians and 9.8 (4.8) for Asian/Other. One-way ANOVA revealed no significant differences between s-Hcy (F = 1.00, p = .39), s-FA (F = 1.25, p = .29), s-B12 (F = .33, p = .80) and ethnicity, see Table 3. There was a trend towards significance for homocysteine and age (F = 2.64, p = .051) that became

Table 6 Multivariate regression analysis summary

	Measure	Spine mineral density[a]		Spine T score[b]		Hip mineral density[c]		Hip T score[d]	
		B	Beta	B	Beta	B	Beta	B	Beta
Gender	(Male = 0, female = 1)	—		−1.321	−.427[****]	—		—	
Weight	Scale	.002	.307[***]	—		—		.014	.447[****]
BMI (category)	Ordinal	—		.547	.340[***]	.066	.314[****]	—	
Ethnicity	Ordinal	—		.855	.534[****]	—		—	
Taking calcium supplements	(No = 0, yes = 1)	—		−.567	−.182[*]	—		—	
Menopause	(No = 0, yes = 1)	—		—		−.251	−.399[****]	—	
Present drinker	(No = 0, yes = 1)	—		—		.118	.222[**]	—	
Ambulatory status	Ordinal	—		—		.060	.241[**]	.391	.239[**]
Other risk factors	(No = 0, yes = 1)	—		—		—		−.633	−.190[**]
History of fracture	(No = 0, yes = 1)	—		−.608	−.197[*]	—		—	
Years on AEDs	Scale	—		−.041	−.393[****]	—		—	
On enzyme-inducing AED	(No = 0, yes = 1)	−.050	−.173[*]	−1.196	−.390[***]	—		—	
On PHT monotherapy	(No = 0, yes = 1)	.235	.305[***]	—		—		—	
On LTG monotherapy	(No = 0, yes = 1)	—		−1.067	−.239[**]	−.131	−.208[**]	—	
Blood urea nitrogen	Scale	—		−.057	−.202[*]	—		—	
Serum alkaline phosphatase	Scale	—		−.005	−.321[**]	—		—	
Log serum homocysteine	Scale	—		−3.492	−.801[***]	—		—	
Homocysteine quartile	Ordinal	—		1.141	.769[***]	—		—	

Spine and hip mineral density are measured in g/cm^2; BMI category coded a underweight = 0, normal weight = 1, overweight = 2, obesity = 3; ethnicity was coded as African American = 0, Latino = 1, Caucasian = 2, Asian/other = 3; ambulatory status was coded as non-ambulant = 1, ambulant w/restrictions = 2, ambulant w/o restrictions = 3, ambulant and exercises = 4; serum homocysteine was recoded in the following quartiles: quartile 1 = 2.7—7.2, quartile 2 = 7.3—9.0, quartile 3 = 9.1—12.3, quartile 4 = 12.4—50.0.

[*] $p \leq .10$.
[**] $p \leq .05$.
[***] $p \leq .01$.
[****] $p \leq .001$.
[a] $R^2 = 0.12$, $F = 2.99$, $n = 99$, $p = .007$.
[b] $R^2 = 0.50$, $F = 6.30$, $n = 58$, $p = .000$.
[c] $R^2 = 0.48$, $F = 6.71$, $n = 83$, $p = .000$.
[d] $R^2 = 0.36$, $F = 6.08$, $n = 93$, $p = .000$.

significant when log transformed ($F = 4.15$, $p = .007$).

Analysis of BMI, by one-way ANOVA, found significant differences in spine T-score ($F = 5.15$, $p = .002$), hip T-score ($F = 11.71$, $p = .000$) and serum alkaline phosphatase ($F = 3.94$, $p = .010$). Ambulatory status was significant for hip mineral density ($F = 2.82$, $p = .044$), hip t-score ($F = 4.17$, $p = .008$), Vitamin B12 level ($F = 5.34$, $p = .002$) and serum alkaline phosphatase ($F = 6.76$, $p = .000$). Length on time on AEDs was only significant for serum folate ($F = 3.20$, $p = .025$).

AED monotherapy and supplementation results

By one-way ANOVA, subjects on phenytoin monotherapy had significantly higher levels of s-Hcy than those on other AEDs ($F = 5.89$, $p = .016$), see Table 4. Persons on valproic acid monotherapy had significantly higher levels of Vitamin B12 ($F = 7.13$, $p = .008$), serum calcium ($F = 7.83$, $p = .006$) and lower serum alkaline phosphatase ($F = 4.02$, $p = .047$). Subjects taking a multivitamin had significantly lower serum homocysteine ($F = 10.09$, $p = .002$) as well as higher serum folic acid ($F = 15.61$, $p = .000$) and serum Vitamin B12 levels ($F = 8.89$, $p = .003$). Only seven subjects had s-FA below the laboratory normal range of 5.4 ng/ml. Multivitamin and calcium supplementation had no significant effect on bone markers.

Bivariate analysis

Pearson bivariate correlation analysis on all variables was performed. Spine and hip mineral density and DEXA T scores were not significantly correlated with s-Hcy. Serum alkaline phosphatase was inversely correlated with spine T score ($r = -.26$, $p = .004$). Surprisingly, length of years on AEDs was not correlated with spine T score ($r = -.015$, $p = .865$) or hip T score ($r = -0.16$, $p = .057$). As expected, body weight and BMI were significantly correlated with bone mineral density and DEXA T scores. Ambulatory status was significantly correlated with spine T score ($r = .24$, $p = .019$), hip mineral density ($r = .28$, $p = .008$) and hip T score ($r = .31$, $p = .002$), see Table 5.

Age was the only demographic variable correlated with s-Hcy ($r = .19$, $p = .02$). For those currently taking enzyme-inducing AEDs (PHT, CBZ, PB, OXC, PRM) there was an inverse correlation with serum calcium ($r = -.29$, $p = .000$). Serum alkaline phosphatase was positively correlated with serum homocysteine ($r = .19$, $p = .020$), PHT monotherapy ($r = .16$, $p = .041$) and negatively correlated with

VPA monotherapy ($r = -.16$, $p = .049$). Serum homocysteine was inversely correlated with s-FA ($r = -.33$, $p = .000$) and s-B12 ($r = -.18$, $p = .035$).

Regression analyses

Regression models were developed to predict bone mineral density and DEXA T scores at the spine and hip using multiple linear regression, see Table 6. The R^2 scores for spine and hip mineral density were .12 and .48. The R^2 values for spine and hip DEXA T scores were .50 and .36. The regression models reveal that different factors predict bone mineral density and DEXA T score. Gender was a negative predictor in females for spine T score and hip mineral density, which follows accepted risk factors for osteoporosis. Weight was a positive predictor for spine mineral density and hip T score, while BMI (by category) was a positive predictor of spine T score and hip mineral density. For spine T score, ethnicity was a positive predictor indicating that African Americans and Latinos have better T scores than Caucasians and Asians.

Use of calcium supplements was a negative predictor of spine T score. Menopausal status was a negative predictor of hip mineral density. Present use of alcohol was a positive predictor of hip mineral density. Ambulatory status was a positive predictor of hip mineral density and hip T score. The presence of other risk factors (corticosteroid and thyroid hormone use, eating disorders and cancer treatment) was only a predictor for hip T score. Number of years on AEDs was a negative predictor of spine T score. Present use of an enzyme-inducing AED was a negative predictor of spine mineral density and spine T score. Present use of PHT monotherapy was found to be a positive predictor of spine mineral density, while LTG monotherapy was a negative predictor of spine T score and hip mineral density. History of fracture, BUN, serum alkaline phosphatase and log converted serum homocysteine were all negative predictors of spine T score. Serum Hcy when separated by quartiles positively predicts spine T score. Age was also not a predictor in any of the models. Also, the use of other AEDs (CBZ or VPA) as monotherapy did not factor into any of the regression models.

Discussion

The medical literature over the past thirty years has established a strong link between antiepileptic medications and metabolic bone loss;[49] however the emphasis has primarily been on determining which AEDs are worse for bone density in various

populations. Prevention has been focused on recommendations for DEXA screening and supplementation with calcium and Vitamin D.[50] This is the first study to look at homocysteine and bone loss in epilepsy. In this study, log transformed values of s-Hcy appear to predict spine T score. A trend towards increased fractures in the higher quartiles appears possible; however this may be due to the statistically significant increase in subject age in successive quartiles. Goldbahar et al., found negative correlations between log homocysteine and bone mineral density in both the spine and hip, but the effect was not predictive in their final regression models.[31] Their results revealed age, menopause, BMI, alkaline phosphatase, creatinine and folate were predictors. Several of these factors also appear in of the predictive models in this study. Higher levels of blood urea nitrogen in the successive quartiles, while still in the normal range, may indicate changes in kidney function as a potential factor in elevated homocysteine for epilepsy. This finding is interesting since BUN made it to the final prediction model for spine T score and homocysteine levels are known to predict renal function loss and cardiovascular events in end-stage renal disease.[51]

A correlation between serum folate and serum homocysteine was evident in the bivariate analysis, which supports the current epilepsy literature.[52] In the present study patients who reported supplementation had significantly lower serum homocysteine supporting the possibility of an increased need for folate in those taking AEDs, despite normal folate levels. Additionally, patients taking multivitamins were compared to those who were not. Since nutritional supplements are recommended as part of clinical care this demonstrates that multivitamins can reduce serum homocysteine levels in persons taking AEDs by approximately 25% (9.11 versus 12.3), supporting previous work by Yoo and Hong, see Table 4.[28]

While an average serum homocysteine level of 10.7 mmol/L in our subjects is considered acceptable by most laboratories there is evidence that homocysteine levels within the range currently considered to be normal, the risk for coronary disease rises, regardless of age and sex, with no threshold effect.[53] A recent analysis of NHANES by the Centers for Disease Control, looking at post folate fortification of foods, found 78% of the US population had homocysteine levels below 9 mmol/ug.[45] In this study, over 50% of subjects had homocysteine levels *above* this level. This is of concern since over half of our patients reported taking a multivitamin. Based on our data, the trend for elevated s-Hcy in the non-Caucasian population may also be related to ethnicity and/or related dietary habits.

There were no significant differences in DEXA T-scores in the univariate dependent variable analysis based on ethnicity or gender, which is contrary to generally accepted risk factors for fracture. However, when the multivariate regression analysis was performed ethnicity was found to be a positive predictor of spine T score, while gender was a negative predictor supporting the generally accepted increased risk of osteoporosis for Caucasians and women.

This study also supports previous research that mechanisms of bone loss in epilepsy appear to be related to the use of enzyme-inducing AEDs and elevated levels of alkaline phosphatase. While changes in calcium have been suggested as mechanisms of AED-induced bone loss[49] it is important to note that serum calcium was normal in our subjects despite reaching statistical significance in some analyses shown in Table 4. Since we did not assess Vitamin D status or parathyroid hormone levels in this study we cannot say this precludes the presence of Vitamin D deficiency or compensatory hyperparathyroidism as a cause of the bone loss, especially since normal calcium levels may be present in these cases.

A recent study found subjects on lamotrigine or valproate monotherapy had less negative effects on bone markers and improved bone density compared to phenytoin and carbamazepine monotherapy.[16] Our data on DEXA spine and hip T scores, while not significant, support a similar pattern. Interestingly, in the multivariate analysis lamotrigine was found to be a negative predictor of spine T score and hip mineral density. While lamotrigine has anti-folate properties it is unlikely this fully explains our results. Since the mean length of AED exposure in this study was 25 years it more likely that previous exposure to other AEDs may be to blame. In reviewing the individuals on LTG monotherapy the majority were Caucasian (9 of 16) and female (10 of 16), which is likely a reflection of current prescribing recommendations. There were no differences based on age, weight, BMI and years on AEDs in the LTG monotherapy group. Also, contrary to published research we found PHT monotherapy to be a positive predictor of spine mineral density. A similar analysis for the LTG group was performed and the majority of individuals taking PHT monotherapy were African American (13 of 17) and male (9 of 17) who are generally less likely to experience bone loss. Again there were no differences based on age, weight, BMI and years on AEDs.

Weight and body mass index appear to be significant predictors of bone mineral density and DEXA T scores in person with epilepsy. However, short of promoting obesity for the prevention of osteoporosis

it makes sense that more beneficial health strategies such as exercise, nutritional supplementation and possibly bisphosphonates be recommended—especially to those who are very thin. Interestingly, present alcohol consumption was found to be a positive predictor of hip mineral density. While alcoholism has a negative effect on bone,[54] moderate intake in the literature appears to be protective.[55,56]

Not surprisingly, additional factors found in the multivariate analysis such as menopause status, other risk factors, use of calcium supplements and history of fracture support their ability to predict DEXA T scores or bone mineral density. Years on AEDs do not consistently predict bone density or DEXA T scores. This supports previous research that AEDs affect bone health rather quickly. Since ambulatory status is a positive predictor of hip mineral density and hip T score it supports the importance of physical activity for reducing fracture risk.[57,58] While bone mineral density is most developed in childhood and adolescence[59] exercise is still vital throughout adulthood especially in persons taking seizure medications.

Limitations

The use of a retrospective, cross-sectional design (without controls) may have prevented us from detecting some effects, especially since almost half of the subjects took multivitamins. This study also included some older individuals with a diagnosis of epilepsy at mid-life who may have bone loss irrespective of AED use. Also, length of time subjects were on each AED was not available from chart review. Any determination of a time-dependent effect of AEDs on bone density would have to be based on strict historical documentation or through a prospective design. The comparisons of different monotherapies provide some additional insight, but again using only length of years on AEDs limits our ability to generalize this study. Additionally, while a majority of the DEXA scans and blood test results were from the same facilities some results came from outside labs whose equipment and methodologies may vary. The majority of DEXA reports provided only T score so no analysis could be performed using Z scores.

An assessment of Vitamin D level would have been useful in reporting our results in comparison with other studies. While a study of Vitamin D would have been beneficial in this population, Vitamin D status had not been obtained on a routine basis over the past few years due to the poor reliability of Vitamin D assays and unreliable normal ranges suggested in the literature.[60,61] Insurance reimbursement for Vitamin D studies was also a concern. This study does not diminish the importance of Vitamin D in persons taking AED but adds support to the literature for other potential markers.

Conclusion

It appears that AEDs negatively affect bone density regardless of race or gender. African Americans and those of Latino background are generally thought to have significantly less risk for fracture. Little in the way of ethnicity data have been presented in the literature on AED induced bone loss. It appears from our data that African Americans with epilepsy have a reduction in their bone density similar to Caucasians. In contrast, Latinos may experience the least bone loss from AEDs. In this study, AED-induced bone loss was similar in both men and women. Therefore, further research and recommendations for prevention need to include men.

Diagnosis by DEXA scan improves the adoption of certain behaviors, increases interest in knowledge of calcium in foods and increases the use of calcium supplements.[62] For persons with childhood exposure to AEDs, greater than 5 years of adult AED use (especially enzyme-inducing AEDs), and for women over age 50 (regardless of length of AED use) a DEXA should be recommended.

Six AEDs (PHT, CBZ, PB, PRM, OXC, LTG) have known anti-folate mechanisms in pregnancy, cardiovascular protection and bone loss. Based on the safety of calcium, Vitamin D and multivitamin supplementation they continue to be important for preventative care in this population. The prospect of long term use of bisphosphonates may not be as benign. Currently, no studies are available to support their use in preventing or treating AED-induced bone loss. Until clinical guidelines can be supported by evidence-based research, clinicians will have to weight the risks and benefits on an individualized basis.

Implications for practice

It is standard practice at the Temple University epilepsy clinic is to address bone health and nutrition with all patients and to communicate treatment issues to primary care physicians. This involves gathering a list of medications and supplements at each visit as part of a standardized visit flow sheet. The importance of taking a standard multivitamin/mineral supplement and calcium with Vitamin D in relation to bone health is discussed with each patient. Risk factors such as smoking, alcohol

consumption, small frame, low body weight and menopausal status are also discussed when clinically appropriate. Prescriptions for the DEXA, lab tests and any specific supplements are provided to patients as necessary. Recommendations for supplements include: calcium 1000 mg daily, a multivitamin that contains 400 units of Vitamin D and folic acid 1 mg (or more) for women of childbearing potential. Exercise is recommended to patients based on their neurologic status. Dietary recommendations include a diet rich in dairy products, fish, beans, leafy greens and nuts/seeds.

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