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Predictors of bone density in ambulatory patients on antiepileptic drugs

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ABSTRACT

Background and aim: Antiepileptic drugs are associated with bone loss and fractures. Data in children is scarce and the impact of new therapies and of low vitamin D is not clear. This study assessed predictors of bone mineral density (BMD) in 225 ambulatory patients with epilepsy.

Methods: BMD and detailed clinical information were obtained from 137 adults mean age of 31 years, on therapy for a mean of 11.7 years, and 88 children mean age of 13 years, on therapy for an average of 4.7 years.

Results: Hypovitaminosis D was common in epileptic patients. BMD was reduced in adults but not children with epilepsy, by 0.3–0.6 SD depending on the skeletal site measured, compared to controls. Duration of treatment, but not vitamin D levels, was negatively correlated with BMD at the hip in adults. Bone density was reduced with the use of both enzyme and non-enzyme-inducing drugs, with both mono- and polytherapy, and was most severely reduced at the spine and hip with the use of enzyme-inducing drugs. In the multivariate analyses, polytherapy in children and duration of therapy and enzyme-inducing drugs in adults were independent predictors of BMD.

Conclusion: Antiepileptic drug therapy is associated with low bone density at clinically relevant skeletal sites, projecting into a possible doubling of fracture risk. Age, therapy duration, polypharmacy and the use of enzyme-inducing drugs were risk factors. Newer drugs may be associated with deleterious effects on bone. Skeletal monitoring with varying intervals, depending on the individual risk profile, is indicated.

Introduction

Each year 200,000 new cases of epilepsy, of which 45,000 children under 15 years, are diagnosed in the United States [1]. By age 20 years, 1% of the population would develop epilepsy and by age 75 years the proportion increases to 3% [1]. Antiepileptic drug (AED) associated osteopathy includes decreased bone mineral density (BMD), increased fracture risk, and overt osteomalacia, most of the data available is from adults [2–4]. Bone changes occur not only in institutionalized patients, but also in ambulatory subjects [5,6].

The AEDs most consistently associated with altered bone metabolism are the enzyme-inducing drugs [6]. The effect of valproic acid on bone has received conflicting results [7,8], whereas that of the newer AEDs is unclear due to limited data. Enzyme-inducing AEDs induce the hepatic cytochrome P450 enzyme system, lower vitamin D levels and decrease bone mineral density (BMD) [6]. Non-enzyme-inducing AEDs do not affect vitamin D metabolism [9]. Other suggested mechanisms involve a direct action on bone cells, impaired calcium absorption, hypogonadism, and calcitonin deficiency [3,10,11]. Although hypovitaminosis D was present in >50% of ambulatory patients on AEDs, BMD was low irrespective of vitamin D levels [5].

The objectives of this study were to elucidate predictors of BMD such as type and mode of antiepileptic drug therapy, treatment duration, and low vitamin D levels, in a large cumulative database of ambulatory children and adults with epilepsy. This would allow the development of paradigms for skeletal monitoring, depending on the patient's individual risk profile based on the identified predictors.

Materials and methods

Study subjects

194 subjects, 106 adults (age 18–60 years) and 88 children and adolescents (10–17 years) with epilepsy presenting to neurologists at the American University of Beirut Medical Center between 2001 and 2003 were recruited. Most were enrolled in a vitamin D supplementation trial, and data gathered at baseline was used for the current analyses [12]. Data from 31 epileptic subjects evaluated in the same time period and reported previously was also included [5]. Therefore, information for 137 adults and 88 children and adolescents is presented. Inclusion criteria were age 10 years or older and

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diagnosis of epilepsy, on chronic anticonvulsant therapy for at least 6 months. Exclusion criteria were hepatic or renal disorders, hypothyroidism, malabsorption, and history of intake of medications that can affect bone turnover other than anticonvulsants.

Controls were age, gender, and ethnic-match healthy subjects, for the adults they were participants in study characterizing peak bone mass in the Lebanese [13], and for the children participants in a vitamin D trial [14].

The study was approved by the Institutional Review Board, and informed consent was obtained from the subjects and/or their legal guardian.

Data collection

A questionnaire was administered to assess type and duration of AEDs, and lifestyle habits. Cumulative duration of type of AED use was considered as the total sum of the duration of the intake of individual drugs within each type (if a subject took phenytoin for 2 years and phenobarbital for 3 years, the cumulative duration would be 5 years, regardless of possible overlap between the two drugs). Antiepileptic drugs taken by the patients were classified as enzyme-inducing AEDs (EIAEDs) or non-enzyme-inducing AEDs (non-EIAEDs). The EIAEDs were phenytoin (*Dilantin*, *Epanutin*), phenobarbital (*Gardenal*, *Luminal sodium*), carbamazepine (*Tegretol*), and primidone (*Mysoline*). The non-EIAEDs were valproic acid (*Depakene*, *Depakote*), lamotrigine (*Lamictal*), clonazepam (*Rivotril*, *Klonopi*), gabapentin (*Neurontin*), topiramate (*Topamax*), ethosuximide (*Zarontin*) and vigabatrin (*Sabril*). Patients on multiple medications were classified in the EIAED group if one of the medications they were taking at the time of the study was an enzyme inducer. Dietary calcium intake was assessed using a food frequency questionnaire validated in Lebanese youth [15], and compared to recommended standards [16]. Weight, height, Body Mass Index (BMI) and 25(OH) vitamin D levels were measured.

Assays

Serum 25-OH vitamin D was measured by a competitive protein-binding assay with inter- and intra-assay CV <13% for the value of 47 ng/ml (Diasorin Incstar, Diasorin, Saluggia, Italy). Vitamin D deficiency was defined as a 25-OHD <10 ng/ ml (26 nmol/L), and insufficiency as a level between 10 and 20 ng/ml (26–39 nmol/L) [17]. Data was also analyzed using the recently suggested desirable level of 30 ng/ml for children and adults [18].

Dual-Energy X-ray Absorptiometry

BMD of the lumbar spine (L1-L4), the hip and 1/3 radius for adults and lumbar spine and total body for children and adolescents, were performed at 0 and 12 months using Dual-Energy X-ray Absorptiometry (DXA). The first thirty one adults had their BMD measured on a Lunar DPX-L densitometer (Lunar Madison Wisconsin, USA), the remaining 106 adults and all pediatric subjects had their measurements on a Hologic 4500A densitometer (Hologic Waltham, MA, USA). A cross-calibration formula based on 72 adult subjects measured on both machines, at the time of the transition, allowed the expression of BMD in terms of Hologic units. The formulas derived were very similar to those published [19].

Lumbar spine (Hologic)=0.835×lumbar spine (Lunar)+0.04 (R^2=0.95).
Total hip BMD (Hologic)=0.968×total hip (Lunar)−0.031 (R^2=0.82).
One-third radius BMD (Hologic)=0.856×1/3 radius (Lunar)+0.106 (R^2=0.78).

In-vivo quality control was derived from same day duplicate measurements performed on patients during the study period. The CV % mean (SD) for the spine and total hip and forearm was <1% (±0.8%) based on 124 duplicates and 0.9±0.7% at the total body based on 30 duplicates.

T-score calculation

For adults, two *T*-scores were calculated. One using mean and SD for peak bone mass from the NHANES densitometer western database for the hip and the densitometer software for the spine and forearm, as recommended by the International Osteoporosis Foundation. The other *T*-score was derived using mean and SD for peak bone mass of an identical ethnic group derived from a population based Lebanese reference sample [13]. *T*-scores of epileptic adult subjects were compared with zero to determine whether BMD in this population differed significantly from that of the relevant representative reference group.

Statistical analysis

Results were expressed as mean±SD. All analyses were implemented separately for adult and children–adolescents patients (<18 years) using SPSS version 10 software (SPSS, Chicago, Illinois). Comparisons of continuous variables between two subgroups of subjects were performed using a two-tailed *t*-test, and between three subgroups by analysis of variance (ANOVA). All analyses were conducted for the overall study group and also by gender. The association between the outcome variable (BMD/BMC) and the correlates was examined by bivariate analysis (Pearson's correlation) and then further evaluated through adjusted stepwise multiple regression analyses using SAS (SAS version 9.1, Carey, North Carolina, USA). The correlates of interest were age, weight, duration of AED therapy, type (EIAEDs vs non-EIAEDs) and mode (single vs multiple) of therapy. Significance was at a $p<0.05$, *p* values were not adjusted for multiple comparisons.

Results

Adult subjects

Clinical characteristics of the study group

The mean age was 31±11.2 years, median 28 years [range: 17–69 years].

The etiologies of epilepsy were: idiopathic (39%), cryptogenic/congenital (36%), trauma (16%), infection (6%), neoplasm (1.5%), and cerebrovascular (1.5%). The mean duration of intake of AEDs was 11.4±10.4 years: 72% of adults were taking enzyme-inducing AEDs, and 48% of them were on multiple drug therapy. Mean calcium intake was 551±406 mg/day. There were no gender differences in duration of AEDs, type or mode of therapy used (data not shown).

Prevalence of vitamin D insufficiency and deficiency

The mean value for serum 25(OH)D levels was 14.9±9.4 ng/ml (38.7±24.4 nmol/L). This level was significantly lower than that of age–gender and ethnic-matched controls (Table 1). Prevalence of hypovitaminosis D (25(OH)D levels <20 ng/ml) was significantly higher in patients compared to controls, the proportions were 74% versus 53%, respectively, $p<0.01$. Similarly, using a cut-off of <30 ng/ml, the proportions were 86% in patients compared to 71% in controls ($p=0.001$).

Bone mineral density

In epileptic adults, bone density at the spine and hip but not forearm, and *T*-scores at the three sites, were significantly lower than that of age, gender and ethnically matched controls (Table 1). Subgroup analyses by gender revealed a consistently lower BMD at all skeletal sites that reached significance at the total hip and forearm in males and females (Appendices A-1 and A-2) compared to controls. Similarly, bone density *T*-scores were significantly reduced compared to peak bone mass as provided by the densitometer database or the Lebanese database at the spine, total hip and 1/3 radius in the overall group (Table 1). It was similarly reduced by subgroup analyses in both genders with the exception of the spine in women (Appendices A-1 and A-2).

Correlation between 25(OH)D levels, BMD and duration of AED therapy

Vitamin D levels did not correlate with BMD at any measured site, either in the overall study group, or in gender subgroups (Appendix

Table 1
Clinical characteristics and bone density data in epileptic adults and controls

	Epileptic adults	Control adults	*p* value
	N=137	*N*=212	
Age, years	31±11.2	29.6±3.8	0.16
BMI, kg/m^2	25.8±4.4	25.2±4.6	0.21
25-OHD, ng/ml	14.9±9.4	19.9±9.5	0.000
Total calcium intake, mg/day	541±407	328±245	0.000
Lumbar spine BMD, g/cm^2	0.98±0.12	1.01±0.10	0.04
Lumbar spine *T*-score (densitometer database)	−0.91±1.13**	−0.65±0.94**	0.03
Lumbar spine *T*-score (Lebanese database)	−0.25±1.13**	0***	0.03
Hip BMD, g/cm^2	0.90±0.13	0.94±0.13	0.005
Hip *T*-score (NHANES database)	−0.87±1.01**	−0.46±0.96**	0.000
Hip *T*-score (Lebanese database)	−0.46±1.08**	0***	0.000
1/3 radius, g/cm^2	0.70±0.06	0.71±0.06	0.08
1/3 radius *T*-score (densitometer database)	−0.77±1.06**	−0.17±0.86**	0.000
1/3 radius *T*-score (Lebanese database)	−0.59±1.2**	0***	0.000

SI conversion factor: To convert 25(OH)D to nmol/L, multiply by 2.6.

** Significantly different from peak bone mass (ie from zero) by one sided *t*-test at $p<0.05$.

*** Control adults constitute the Lebanese reference group for peak bone mass determination (Ref. [13]) and in whom the *T*-score is by definition 0, using a Lebanese reference.

A-3). 25(OH)D levels did not correlate with duration of intake of AED therapy (data not shown), nor with cumulative duration of intake of AED (enzyme-inducing and non-enzyme-inducing) evaluated as a class or as individual drugs, with the exception of carbamazepine (Appendix A-4).

Duration of AED therapy inversely correlated with BMD at the total hip ($r=-0.21$, $p<0.05$) and trochanter ($r=-0.26$, $p<0.01$) in the overall adult group, and in women with comparable correlation coefficients (Appendix A-3). This negative correlation was present only in the subgroup of adults who started AED therapy before age 18 ($N=80$ subjects), and was present at the total hip ($R=-0.274$, $p=0.016$), femoral neck ($R=-0.237$, $p=0.037$) and trochanter ($R=-0.386$, $p<0.0001$).

Cumulative duration of intake of enzyme-inducing AEDs inversely correlated with total hip ($r=-0.24$, $p<0.05$) and trochanter ($r=-0.30$, $p<0.05$) BMD in the overall adult group (Appendix A-4), and in adult women at the trochanter only ($r=-35$, $p<0.01$) (Appendix A-5). The correlation coefficients were highest at the three hip sites with phenytoin use in the overall study group ($r=-0.32$ to -0.48, $p<0.05$, Appendix A-4) and in adult women ($r=-0.59$ to -0.71, $p<0.001$, Appendix A-5). Cumulative duration of intake of carbamazepine also negatively correlated with trochanter BMD in the overall adult group ($r=-0.21$, $p<0.05$) (Appendix A-4), and in adult women ($r=-0.29$, $p<0.05$, Appendix A-5).

In the overall study group, negative correlation coefficients were noted between cumulative duration of intake of some non-EIAEDs, and bone density at the hip, that only achieved significance for valproate ($r=-0.27$, $p<0.05$, Appendix A-4).

Impact of type of antiepileptic drug (enzyme-inducing versus non-enzyme-inducing) and mode of therapy (single versus multiple) on bone density

Compared to controls and to patients on non-EIAEDs, patients on EIAEDs had the lowest BMD and lowest *T*-scores at the lumbar spine and at the hip (Table 2), but they were also older and were on therapy longer. Males on EIAEDs had lower femoral neck and trochanter BMD as compared to those on non-EIAEDs, but these subjects were also older (Appendix A-6).

Compared to controls, patients on polytherapy had lower BMD and *T*-scores at the hip and lower *T*-scores at the forearm (Table 3). Compared to controls, patients on single therapy also had lower BMD and *T*-scores at the hip and at the forearm (Table 3).

Table 2
Clinical characteristics and bone mineral density (BMD) in adult patients on enzyme and non-enzyme-inducing AEDs and controls

Variables	Control	Non-enzyme inducers	Enzyme inducers	*p* value
	N=212	*N*=39	*N*=98	ANOVA*
Age, years	29.6±3.8[a]	25.9±8.4[a]	33.1±11.5[a]	0.000
BMI, kg/m^2	25.2±4.6	24.9±3.1	26.1±4.7	0.18
25(OH)D, ng/ml	19.9±9.5	15.5±9.3	14.7±9.6	0.000
Duration of therapy, years	NA	9.0±7.4	12.4±11.2	0.04
Lumbar spine BMD, g/cm^2	1.006±0.10[a]	1.015±0.11[b]	0.966±0.12[a,b]	0.008
T-score (Lebanese data)	0.005±0.97[a]	0.07±1.0[b]	−0.39±1.1[a,b]	0.005
Hip BMD, g/cm^2	0.936±0.13[a]	0.924±0.14	0.885±0.13[a]	0.006
T-score (Lebanese data)	0.004±1.0[a]	−0.16±1.2[b]	−0.58±1.02[a,b]	0.000
1/3 radius, g/cm^2	0.715±0.05[a]	0.693±0.06[a]	0.708±0.06	0.109
T-score (Lebanese data)	0.001±1.0[a,b]	−0.58±1.3[b]	−0.59±1.1[a]	0.000

SI conversion factor: To convert 25(OH)D to nmol/L, multiply by 2.6.
[a,b]Values that share the same superscript are statistically different between the 3 subgroups, by LSD, post-Hoc, at $p<0.05$.
* *p* value between the 3 subgroups by ANOVA.

Table 3
Clinical characteristics and bone mineral density (BMD) in adult patients on single and multiple therapy compared to controls

Variables	Control	Single therapy	Multiple therapy	*p* value*
	N=212	*N*=71	*N*=66	
Age, years	29.6±3.8	30.9±12	31.1±10.3	0.24
BMI, kg/m^2	25.2±4.6	25.6±4.3	25.9±4.4	0.438
25(OH)D, ng/ml	19.9±9.5	14.9±8.2	14.9±10.6	0.000
Duration of therapy, years	NA	7.9±7.6	15.2±11.7	0.000
Lumbar spine BMD, g/cm^2	1.006±0.10	0.98±0.12	0.980±0.12	0.11
T-score (Lebanese data)	0.005±0.97	−0.25±1.2	−0.25±1.1	0.08
Hip BMD, g/cm^2	0.936±0.13[a]	0.905±0.14	0.887±0.12[a]	0.01
T-score (Lebanese data)	0.004±1.0[a,b]	−0.36±1.2[a]	−0.56±0.96[b]	0.000
1/3 radius, g/cm^2	0.715±0.05[a]	0.693±0.07[a]	0.71±0.06	0.04
T-score (Lebanese data)	0.001±1.0[a,b]	−0.71±1.1[a]	−0.46±1.3[b]	0.000

SI conversion factor: To convert 25(OH)D to nmol/L, multiply by 2.6.
[a,b]Values that share the same superscript are statistically different between the 3 subgroups, by LSD, post-Hoc, at $p<0.05$.
* *p* value between the 3 subgroups by ANOVA.

There were no significant differences in BMD at any skeletal sites between subjects on single vs multiple therapy in the overall group (Table 3), nor by subgroup analyses within genders except at the forearm (Appendix A-7).

Predictors of bone density parameters and 25(OH)D on multivariate analyses

Because subjects who were on enzyme-inducing drugs and on multiple therapy tended to be older and on longer duration of therapy, and of the high correlation between use of enzyme-inducing drugs and multiple therapy, linear regression analyses were implemented with BMD as the outcome and age, weight, duration of therapy, type and mode of therapy as the predictors. The use of enzyme-inducing AEDs was a negative predictor of BMD in the adult overall group at the LS, total hip, FN and at the trochanter after adjusting for gender, age, weight, duration of therapy, and number of AEDs, partial R^2 varying between 2.9 and 3.6% (Table 4). Duration of therapy was a significant predictor of trochanteric BMD after adjustment for the same covariates with an R^2 of 5.8%, $p=0.007$ (Table 4).

Pediatric subjects

Clinical characteristics of the study group

The mean age was 13±2 years, and the mean duration of AEDs used at the time of study was 4.7±4 years. The etiology of epilepsy were: idiopathic (52%), cryptogenic/congenital (38%), trauma (7%), and cerebrovascular (3%). 55% of those were taking enzyme-inducing AEDs, and 33% were on multiple AED therapy. There were no gender

Table 4
Partial R^2 (%)* and *p* value for the relative impact of the various predictors on bone density at the lumbar spine (LS), total hip (Thip), femoral neck (FN), trochanter (Tro) and forearm (FA) in adults in the adjusted analyses

Predictors	LS		Thip		FN		Tro		FA	
	R^2	*p*	R^2	*p*	R^2	*p*	R^2	*p*	R^2	*P*
Age	–	–	–	–	8.9%	0.005	–	–	–	–
Weight	3.7%	0.051	2.7%	<0.001	21%	<0.001	16%	<0.001	26%	0.04
AED type	3.6%	0.052	3.6%	0.025	2.9%	0.039	3.6%	0.03	–	–
A E D number	–	–	–	–	–	–	–	–	–	–
A E D duration	2.22%	0.12	2.14%	0.077	–	–	5.8%	0.007	–	–

* Partial R^2 adjusted in a stepwise linear regression model entering age, weight, gender, antiepileptic drug (AED) type, AED number and AED duration of treatment.

Table 5
Baseline characteristics of epileptic children and controls

	Epileptic children	Control children	*p* value
	N=88	*N*=111	
Age, years	13.0±2.0	13.3±2.0	0.45
Weight, kg	51.3±15.8	51.2±15.3	0.94
Height, cm	153.4±12.1	155.2±11.7	0.28
25(OH)D, ng/ml	18.5±8.1	15.3±6.8	0.004
Calcium, mg/day	567±341	736±341	0.001
Lumbar spine BMD, g/cm^2	0.77±0.17	0.76±0.15	0.69
Subtotal body BMD, g/cm^2	0.84±0.12	0.85±0.11	0.39
Subtotal body BMC, g	1209±433	1231±400	0.72

SI conversion factor: To convert 25(OH)D to nmol/L, multiply by 2.6.

differences in duration, type or mode of therapy. Mean calcium intake was 567±341 mg/day, lower than that of healthy age, ethnic and gender-matched controls (Table 5).

Prevalence of vitamin D insufficiency and deficiency

Mean value for serum 25(OH) vitamin D level in the pediatric group on AED were 18.5±8.1 ng/ml (48.1±21.1 nmol/L), higher than gender and ethnic-matched healthy controls (Table 4). Prevalence of hypovitaminosis D (25(OH)D levels <20 ng/ml) was lower in children on AEDs as compared to controls, 57% versus 79%, respectively, $p<0.01$. Using a cut-off of 30 ng/ml, 79 patients (90%) had 25(OH)D levels <30 ng/ml as compared to 106 (95%) controls ($p=0.12$).

Comparison of bone mineral density to local database

In the pediatric group, no difference was found in spine BMD or total body BMD and BMC when compared to an age–gender and ethnic-matched population [14] (Table 5).

Correlation between 25(OH)D levels, duration of AED therapy and BMD

Vitamin D levels did not correlate with BMD or BMC at any measured site in the overall pediatric study group nor by gender (data not shown). Vitamin D levels did not show a consistent trend for correlations with cumulative duration of drug use (data not shown). There were no correlations between duration of AED therapy, duration of intake of any of the enzyme-inducing or non-enzyme-inducing AEDs and BMD or BMC, at any of the measured sites in children and adolescents (data not shown).

Impact of type of antiepileptic drug (enzyme-inducing versus non-enzyme-inducing) and mode of therapy (single versus multiple) on bone density

There were no differences in bone density between patients on enzyme-inducing drugs or non-enzyme-inducing drugs in the overall study group, or by gender (data not shown). ANOVA analyses showed no effect of AED type and mode compared to controls (data not shown).

Correlates of bone density parameters on multivariate analyses

Linear regression analyses adjusting for age, weight, duration of therapy, type and mode of therapy only revealed a negative effect of polypharmacy in the overall group on subtotal BMD, and on the lumbar spine (partial $R^2=1.5$, $p=0.04$), and a similar effect at subtotal BMC (partial $R^2=1.7\%$, $p=0.03$).

Discussion

Ambulatory adults, but not children, on chronic AEDs had low BMD and vitamin D levels compared to age, gender and ethnic-matched controls. Duration of treatment, but not vitamin D levels, negatively correlated with hip bone density, and such associations were only present in adults who started AED therapy before the age of 18 years. BMD was most severely reduced with enzyme-inducing drugs at the spine and hip in adults. Independent predictors of bone mass were duration of treatment and use of enzyme-inducing drugs in adults and polytherapy in children.

Calcium intake was below recommended guidelines both in children and adults [16]. The majority of study subjects in this study, 74% of adult and 57% of pediatric patients had hypovitaminosis D [16], with mean serum 25(OH)D levels that were lower than controls, a finding that was independent of type of therapy [5,10,11,20]. Thus the low 25(OH)D levels may reflect impact of disease per say rather than drug on vitamin D levels, or raise the intriguing possibility that certain drugs may affect vitamin D levels independently of the cytochrome P450 system. In children with epilepsy, although 25(OH)D levels were low, they were higher than controls reflecting higher tendency for vitamin supplementation. The lack of any association between vitamin D levels and BMD in the current study is in concordance with publications demonstrating alterations in bone metabolism independent of vitamin D levels [4,8–10,21], including studies on enzyme-inducing drugs [21]. The positive correlations between vitamin D and BMD across a wide range of vitamin D levels reported in the general population in the NHANES study [22], and the lack of any correlation between vitamin D and BMD in patients with epilepsy in the current study, could be explained by vitamin D-independent pathways such as impaired calcium absorption [23], inhibition of osteoblastic function [24], hyperparathyroidism, calcitonin deficiency [6], and altered sex steroid metabolism [25]. Conversely, the observed increments in BMD in patients with epilepsy given vitamin D [12] can be due to improved calcium absorption due to the high vitamin D levels reached, suppression of hyperparathyroidism, in addition to a beneficial effect of vitamin D on muscle and subsequently on bone [14].

Bone density in adults with epilepsy was lower than that of age, gender and ethnic-matched controls. The onset for the deleterious effect of antiepileptic drugs on bone density is unclear. A recent longitudinal study suggested that bone loss continues even after prolonged therapy [26], a finding consistent with the inverse correlation noted between bone density and duration of therapy we observed. The pattern of the decrements in BMD, being most severe at the forearm points to preferential cortical bone loss [5,10,11,27], and was present in both genders. In the Study of Osteoporotic Fractures, there was substantial bone loss not only at the hip (a cortical site) but also at the calcaneus (a trabecular site) [26]. The magnitude of the decrements in bone density noted in our study of 0.3–0.6 SD, and that of others [26,27], would project into a possible doubling in fracture risk if carried into later life [28]. Antiepileptic drugs are indeed a risk factor for hip fractures [4].

The determination of significant predictors of bone loss would be instrumental to derive guidelines for the evaluation and monitoring of patients on antiepileptic therapy. We are unaware of any study evaluating all potential predictors within the same large data set and across age spectrum. The association between duration of therapy and bone density was demonstrated in several [5,7,27] but not all studies [6,9]. The above conflicting reports could be in part explained by the confounding effect of type of therapy with duration of therapy, as well as age of start of therapy. Indeed, in the current study, the correlation coefficients between duration of therapy and bone density were larger and more consistent in the subgroup of patients on enzyme-inducing drugs, both in the overall study group and by gender. Furthermore, these negative associations were present in the subgroup of adults who started AEDs before age 18 years, underscoring the particularly negative impact of such drugs when started at a vulnerable period for mineral accretion (adolescence) and skeletal consolidation (young adulthood). BMD tended to be the lowest both in the overall study group, and by gender, in adult subjects on enzyme-inducing drugs, compared to subjects on non-inducing drugs and controls. These observations are in keeping with the reproducible

deleterious impact of enzyme-inducing drugs on bone metabolism as confirmed in recent studies conducted in ambulatory patients [26,27]. The impact of newer, enzyme-sparing therapies, on bone metabolism is debatable [6,11]. The lower BMD in subjects on non-enzyme-inducing drugs compared to controls and the negative correlation between hip BMD and duration of valproate therapy point to a deleterious effect of newer drugs on bone, and needs to be investigated further. Polytherapy affects bone metabolism in institutionalized subjects, but data in ambulatory subjects is scarce [5]. In most studies, duration of therapy, intake of enzyme-inducing drugs, and polytherapy, were highly correlated variables, thus rendering the evaluation of the independent contribution of each of these predictors difficult in most studies, due to limitations from sample size. Such analyses were possible in the current study and revealed a more deleterious effect of polytherapy compared to monotherapy in children and of enzyme-inducing drugs compared to non-enzyme-inducing drugs in adults. Polytherapy may reflect poorer health status, but that was not assessed in this study.

Bone density was unaffected in children, a finding possibly explained by the relatively shorter treatment duration, and the increasing use of monotherapy and of enzyme-sparing drugs [5,8]. Studies in the pediatric age groups are few, limited by small sample sizes (N=9–53), and have for the most only evaluated the impact of valproate, carbamazepine and in one study lamotrigine [7,8,29,30]. While three smaller studies (N=19–28 depending on the study) [7,29,30] revealed a deleterious effect of valproate on bone density in children, such findings were not confirmed in the largest study using the same drugs (N=53, [8]). In the current study, polypharmacy was the only independent predictor of low bone mass in children. The limited data in children, a critical time for bone mass accretion, renders further studies a necessity in this vulnerable age group.

There are limitations to this study including the recruitment of the study group from a tertiary care center with more severe cases, the lack of fracture data, and its cross-sectional nature. However, longitudinal studies have validated bone loss observed in cross-sectional studies [26]. Recruitment of patients with epilepsy from the population would be impossible due to the lack of a national epilepsy registry. The magnitude of the decrements in vitamin D and BMD, compared to other studies and to controls, and the fact that all patients were ambulatory make the case for a representative study group. Other limitations include the inability to dissect the impact of individual drugs independently on bone density, the impact of previous versus current intake of EIAED, and of AED intake on bone mass in relationship to achievement of skeletal maturation. However, such limitations could only be overcome through a multi-center study design.

The strengths of the study are the inclusion of age, gender and ethnic-match controls, the evaluation of both adults and children within the same center, measurement of BMD with modern densitometry techniques, and the large sample size allowing the dissection of several predictors of bone mass concomitantly within the same dataset.

Bone density is reduced in ambulatory adults on antiepileptic drugs, with a projected doubling of fracture risk. Independent predictors of BMD were polypharmacy in children, duration of treatment and the use of enzyme-inducing drugs in adults, but not vitamin D levels. Mono-therapy and the use of non-enzyme-inducing drugs were also associated with low bone density in adults, albeit to a lesser extent. Careful skeletal assessment and monitoring, with varying intervals depending on the above predictors are recommended.

Disclosure

The authors have reported no conflict of interest.

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Appendix

Appendix A-1

Comparison between epileptic men and the group of reference control men

	Epileptic men	Control men	p value
	N=62	N=62	
Age, years	32±12	28.4±3.8	0.02
Weight, kg	80±15.3	80.4±13.4	0.88
Height, cm	172±6.6	173±5.4	0.25
BMI, kg/m^2	27±4.4	26.8±4.4	0.85
25(OH)D, ng/ml	16.6±9.3	23.7±7.5	0.000
Total calcium intake, mg/day	599±494	321±259	0.000
Lumbar spine BMD, g/cm^2	0.98±0.13	1.02±0.12	0.058
Lumbar spine T-score (densitometer database)	−1.02±1.22*	−0.61±1.11*	0.058
Lumbar spine T-score (Lebanese database)	−0.36±1.10*	0**	0.058
Hip BMD, g/cm^2	0.95±0.12	1.01±0.15	0.015
Hip T-score (densitometer database)	−0.73±0.92*	−0.25±1.15	0.015
Hip T-score (Lebanese database)	−0.41±0.81*	0**	0.015
1/3 radius, g/cm^2	0.74±0.06	0.77±0.04	0.008
1/3 radius T-score (densitometer database)	−1.32±1.11*	−0.78±0.88*	0.008
1/3 radius T-score (Lebanese database)	−0.62±1.37*	0**	0.008

SI conversion factor: To convert 25(OH)D to nmol/L, multiply by 2.6.
*Significantly different from zero by one sided t-test at $p<0.05$.
**Control adults constitute the Lebanese reference group for peak bone mass determination and in whom the T-score is by definition 0, using a Lebanese reference database (Ref. [13]).

Appendix A-2

Comparison between epileptic women at baseline and the group of reference control

	Epileptic women	Control women	p value
	N=75	N=150	
Age, years	30.1±10.4	30.1±3.7	0.95
Weight, kg	62.5±11.1	62.0±11.8	0.77
Height, cm	159±5.7	159±5.6	0.61
BMI, kg/m^2	24.8±4.1	24.5±4.5	0.25
25(OH)D, ng/ml	13.4±9.4	18.8±9.8	0.000
Total calcium intake, mg/day	494±313	331±239	0.000
Lumbar spine BMD, g/cm^2	0.98±0.12	1.00±0.09	0.25
Lumbar spine T-score (densitometer database)	−0.82±1.04*	−0.67±0.87*	0.25
Lumbar spine T-score (Lebanese database)	−0.16±1.16	0**	0.25
Hip BMD, g/cm^2	0.85±0.13	0.90±0.10	0.003
Hip T-score (densitometer database)	−0.98±1.07*	−0.55±0.85*	0.003
Hip T-score (Lebanese database)	−0.50±1.26*	0**	0.003
1/3 radius, g/cm^2	0.67±0.05	0.69±0.04	0.000
1/3 radius T-score (densitometer database)	−0.30±0.75*	0.10±0.71	0.000
1/3 radius T-score (Lebanese database)	−0.56±0.14*	0**	0.000

SI conversion factor: To convert 25(OH)D to nmol/L, multiply by 2.6.
*Significantly different from zero by one sided t-test at $p<0.05$.
**Control adults constitute the Lebanese reference group for peak bone mass determination and in whom the T-score is by definition 0, using a Lebanese reference database (Ref. [13]).

Appendix A-3

Correlation coefficients between bone density and duration of AEDs or serum 25(OH)D level in the overall adult group and by gender

	Overall		Males		Females	
Variables	Duration of AED therapy	25(OH)D	Duration of AED therapy	25(OH)D	Duration of AED therapy	25(OH)D
Adults $N=137$						
Lumbar spine BMD	0.05	−0.02	0.07	0.10	0.35	−0.15
Hip BMD	−0.21*	0.01	−0.10	0.06	−0.24*	−0.17
Femoral neck BMD	−0.10	0.05	0.03	0.18	−0.14	−0.16
Trochanter BMD	−0.26**	0.002	−0.16	0.08	−0.27*	−0.17
1/3 radius	0.03	0.07	0.05	−0.19	0.16	0.07

*Values show a significant correlation at $p<0.05$.
**Values show a significant correlation at $p<0.01$.

Appendix A-4

Correlation coefficients between cumulative duration of intake of AEDs (past plus current) with serum 25(OH)D level or bone density in adults

	25 (OH)D	Lumbar spine BMD	Total hip BMD	Femoral neck BMD	Trochanter BMD	1/3 radius BMD
Cumulative duration of intake of all enzyme-inducing drugs, $N=106$	0.09	−0.02	−0.24*	−0.13	−0.30**	0.04
Phenytoin, $N=55$	0.04	−0.11	−0.44**	−0.32*	−0.48**	0.02
Phenobarbital, $N=25$	−0.17	0.33	−0.01	0.19	−0.11	0.20
Carbamazepine, $N=90$	0.22*	−0.004	−0.10	−0.13	−0.21*	0.06
Cumulative duration of intake of all non-enzyme-inducing drugs, N=81	−0.10	0.10	−0.10	0.01	−0.17	0.17
Valproate, $N=63$	−0.15	0.11	−0.18	−0.10	−0.27*	0.20
Lamotrigine, $N=35$	−0.006	0.22	0.06	0.07	−0.004	0.05
Clonazepam, $N=13$	0.05	0.08	0.30	0.48	0.07	0.46
Gabapentin, $N=18$	−0.11	−0.38	−0.20	−0.09	−0.30	0.08
Topamirate, $N=16$	0.18	−0.26	0.45	0.29	0.37	0.18

*Values show significant correlation at $p<0.05$.
**Values show significant correlation at $p<0.01$.

Appendix A-5

Correlation coefficients between duration of intake of AEDs (past plus current) with serum 25(OH)D level or bone densities in adult women

	25 (OH)D	Lumbar spine BMD	Total hip BMD	Femoral neck BMD	Trochanter BMD	1/3 radius BMD
Cumulative duration of intake of all enzyme-inducing drugs, $N=58$	0.22	−0.06	−0.26	−0.21	−0.35**	−0.01
Phenytoin, $N=26$	0.17	−0.28	−0.59**	−0.51**	−0.71**	−0.19
Phenobarbital, $N=15$	−0.22	0.56*	0.10	0.30	−0.19	0.25
Carbamazepine, $N=54$	0.47**	−0.07	−0.15	−0.25	−0.29*	−0.12
Cumulative duration of intake of all non-enzyme-inducing drugs, $N=50$	−0.06	0.26	−0.02	0.11	−0.11	0.24
Valproate, $N=36$	−0.10	0.29	−0.10	0.01	−0.22	0.22
Lamotrigine, $N=23$	0.06	0.35	0.11	0.13	−0.01	0.20
Clonazepam, $N=9$	−0.12	−0.24	0.19	0.47	−0.08	0.20

*Values show a significant correlation at $p<0.05$.
**Values show a significant correlation at $p<0.0$.

Appendix A-6

Clinical characteristics and bone mineral density (BMD) in adult patients on enzyme and non-enzyme-inducing AEDs, in the overall study group and by gender

	Enzyme inducers			Non-enzyme inducers			p value*
	All	Females	Males	All	Females	Males	
	$N=98$	$N=50$	$N=48$	$N=39$	$N=25$	$N=14$	
Age, years	33.1 ± 11.5	31.7 ± 10.4[a]	34.4 ± 12.5[b]	25.9 ± 8.4	26.8 ± 9.6[a]	24.3 ± 5.4[b]	0.000
Weight, kg	71.6 ± 16.6	63.3 ± 12.4	80.2 ± 16.1	67.6 ± 13.1	61 ± 7.8	79.2 ± 12.6	0.18
Height, cm	165 ± 9.1	158.3 ± 6.1	172 ± 6.1	164 ± 8.3	160 ± 4.8	171.3 ± 8.5	0.57
Duration of therapy, years	12.4 ± 11.2	13.8 ± 11.6	10.8 ± 10.7	9.0 ± 7.4	10.1 ± 8.3	7.1 ± 5.2	0.04
25-OHD, ng/ml	14.7 ± 9.6	13.3 ± 9.7	16.0 ± 9.3	15.5 ± 9.3	13.6 ± 8.4	18.8 ± 9.9	0.68
Total calcium intake, mg/d	532 ± 404	479 ± 287	587 ± 497	603 ± 410	540 ± 360	717 ± 484	0.37
Lumbar spine BMD, g/cm^2	0.97 ± 0.12	0.97 ± 0.12	0.96 ± 0.12	1.01 ± 0.11	1.00 ± 0.09	1.03 ± 0.15	0.03
Hip BMD, g/cm^2	0.88 ± 0.13	0.84 ± 0.12	0.93 ± 0.11	0.92 ± 0.14	0.88 ± 0.13	1.00 ± 0.13	0.12
Femoral neck BMD, g/cm^2	0.77 ± 0.11	0.74 ± 0.11	0.80 ± 0.11[b]	0.83 ± 0.13	0.80 ± 0.12	0.90 ± 0.13[b]	0.008
Trochanter BMD, g/cm^2	0.65 ± 0.11	0.62 ± 0.12	0.68 ± 0.09[b]	0.70 ± 0.12	0.67 ± 0.12	0.75 ± 0.12[b]	0.02
1/3 radius, g/cm^2	0.71 ± 0.07	0.66 ± 0.05	0.75 ± 0.05	0.69 ± 0.06	0.68 ± 0.04	0.72 ± 0.08	0.27

SI conversion factor: To convert 25-OH D to nmol/L, multiply by 2.6.
*p value between overall groups, all enzyme inducers versus all non-enzyme inducers.
[a,b]Values that share the same superscript are statistically different between enzyme-inducers and non-enzyme-inducers subgroups at $p<0.05$.

Appendix A-7

Clinical characteristics and bone mineral density (BMD) in adult patients on single or multiple therapy, in the overall study group and by gender

	Single therapy			Multiple therapy			p value*
	All	Females	Males	All	Females	Males	
	$N=71$	$N=41$	$N=30$	$N=66$	$N=34$	$N=32$	
Age, years	30.9 ± 12	29.2 ± 11.2	33.2 ± 12.7	31.1 ± 10.3	31.1 ± 9.3	31.1 ± 11.5	0.91
Weight, kg	69.4 ± 14.6	63.3 ± 12.6	77.7 ± 12.9	71.6 ± 17	61.6 ± 9.0	82.2 ± 17.1	0.41
Height, cm	164 ± 9.0	158.4 ± 6.3	171.8 ± 5.8	165.4 ± 8.8	159.4 ± 4.9	171.8 ± 7.4	0.38
Duration of therapy, years	7.9 ± 7.6	8.9 ± 8.3[a]	6.5 ± 6.2[b]	15.2 ± 11.7	17 ± 11.7[a]	13.3 ± 11.5[b]	0.000
25-OHD, ng/ml	14.9 ± 8.3	13.7 ± 8.0	16.5 ± 8.3	14.9 ± 10.6	13.1 ± 10.5	16.8 ± 10.5	0.98
Total calcium intake, mg/d	559 ± 304	547 ± 356	575 ± 214	544 ± 491	443 ± 243	651 ± 648	0.83
Lumbar spine BMD, g/cm^2	0.98 ± 0.12	0.98 ± 0.13	0.97 ± 0.10	0.98 ± 0.13	0.99 ± 0.10	0.98 ± 0.15	0.78
Hip BMD, g/cm^2	0.90 ± 0.14	0.86 ± 0.13	0.95 ± 0.11	0.89 ± 0.13	0.84 ± 0.11	0.94 ± 0.13	0.74
Femoral neck BMD, g/cm^2	0.78 ± 0.13	0.76 ± 0.13	0.81 ± 0.12	0.80 ± 0.12	0.76 ± 0.10	0.84 ± 0.12	0.61
Trochanter BMD, g/cm^2	0.67 ± 0.11	0.65 ± 0.13	0.70 ± 0.09	0.65 ± 0.11	0.61 ± 0.1	0.69 ± 0.11	0.26
1/3 radius, g/cm^2	0.69 ± 0.06	0.65 ± 0.04[a]	0.75 ± 0.05	0.71 ± 0.06	0.68 ± 0.04[a]	0.74 ± 0.06	0.048

SI conversion factor: To convert 25-OH D to nmol/L, multiply by 2.6.
*p value between overall groups, all patients on single therapy versus all patients on multiple therapy.
[a,b]Values that share the same superscript are statistically different between single and multiple therapy subgroups at $p<0.05$.

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