FULL-LENGTH ORIGINAL RESEARCH

Impaired bone and muscle development in young people treated with antiepileptic drugs

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Summary

Objective: **Antiepileptic drugs (AEDs) are associated with reduced bone density, balance impairment, and increased fracture risk in adults. However, pediatric data are limited. Therefore, we aimed to examine bone, muscle, and balance outcomes in young patients taking AEDs.**

Methods: **We undertook a case–control study utilizing an AED exposure–discordant matched-pair approach. Subjects were aged 5–18 years with at least 12 months of AED exposure. Pairs were twins, nontwin siblings and first cousins, sex- and age-matched (to within 2 years), allowing for greater power than with unrelated control subjects. Dual energy x-ray absorptiometry (DXA), peripheral quantitative computed tomography (pQCT), and muscle force/balance were tested, with questionnaires were administered for bone health and epilepsy details.**

Results: **Twenty-three pairs were recruited, (median age 12.9 years [subjects] and 13.5 years [controls])—7 twin, 14 sibling, and 2 cousin pairs. Those taking AEDs had an increased prevalence of fractures (15 fractures in 8 subjects, compared with 4 fractures in 3 controls, $p < 0.01$). Trabecular volumetric bone mineral density (vBMD) measured by pQCT at the 4% site (tibia) was reduced by 14% ($p = 0.03$) in subjects. Subjects exerted a decreased maximum force compared to body weight (F_{max} total/g) at the tibia. There were no differences seen in either bone mineral parameters measured by DXA or balance measures.**

Significance: **Young people taking AEDs reported more fractures and had reductions in tibial vBMD and lower limb muscle force compared to their matched controls. These findings suggest that further exploration of bone health issues of young patients on AED therapy is required. Longitudinal studies are required to confirm these changes in the muscle–bone unit and to further explore the clinical outcomes.**

KEY WORDS: **Fractures, Epilepsy, Bone density.**

Antiepileptic drug (AED) therapy remains the mainstay of treatment for most people with epilepsy. There is increasing awareness of the adverse effects of AED use, including potential negative effects on bone health.[1] This adverse outcome is of concern as this group already has an increased fracture risk due to factors such as other medications that

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Key Points

- Young people taking AEDs for >1 year have an increased risk of fracture
- Young people on AEDs show reduced trabecular bone mineral density, which correlates with clinically observed fractures
- Young people on AEDs show reduced muscle force which may in part explain the reduced bone density

may induce unsteadiness, neurodevelopmental disorders (e.g., cerebral palsy), and seizure-related falls.[2] Previous adult studies show increased rates of fractures in epileptic populations[3,4] and it has been estimated that each year of AED use amounts to about a 4–6% increased odds ratio (OR) of fracture in patients with epilepsy.[5]

Studies in adults have provided strong evidence for an association of AED therapy with bone disease, clearly showing lower bone mass in patients prescribed AEDs compared with those not taking these agents.[6–8] Balance impairment is seen as well as lower bone mass, with poorer performance on static and dynamic clinical balance tests.[9,10] It is important that multiple AED use and duration of therapy are independent predictors of increased sway and fracture risk in the epileptic population.[5] This suggests that it is not only the presence of AED therapy but also the manner of therapy that influences outcome severity. The major relevant finding for the pediatric population from the adult research is that individuals who commenced AED therapy up to 18 years of age had poorer bone health outcomes. They were found to have decreased bone mass[6,11,12] and increased fracture risk[5] compared with those who commenced from 18 years and older. The effect of AED exposure during this critical period of skeletal development is especially important, because a failure to appropriately accrue bone in childhood may lead to reduced bone mass and increased fracture risk in adults.

Evidence has shown decreased bone mass,[12–15] decreased linear growth, and reduced bone formation in children with epilepsy on AED therapy.[16,17] The results display patterns similar to those of adult studies, where increasing duration and polytherapy are also associated with worse outcomes. However, these studies are based largely on dual energy x-ray densitometry (DXA) scans, with little exploration of skeletal geometry and the problem of confounding by bone size. Therefore, further study is required in this vulnerable population given the high skeletal turnover in childhood and ongoing bone mass accrual. Peripheral quantitative computed tomography (pQCT) overcomes a number of the technical limitations of DXA, and there are data linking pQCT results to long-term risk of fracture.[18]

Therefore, the aims of this study were to examine the within-pair differences in fracture prevalence, and bone measures (by DXA and pQCT), muscle, and balance parameters in age- and sex-matched twin, sibling, and first cousin pairs who were discordant for exposure to AED therapy.

Methods

Participants were recruited from the Australian Twin Registry or Neurology clinics of The Royal Children's Hospital (RCH). Expression of interest (EOI) forms were provided to potential participants before each clinic, and responders were screened for eligibility. Inclusion criteria were age between 5 and 18 years and having a same-sex twin, or a sibling or first cousin within 2 years of their age. All subjects and controls were ambulant (Gross Motor Function Classification System [GMFCS]). Exclusion criteria were the presence of any neurodevelopmental disorders such as cerebral palsy, known primary bone disorders, immobility, or the use of medications known to cause significant skeletal effects. Pairs were discordant for AED exposure, with subjects requiring a minimum of 12 months of therapy, with no use ever for matched controls.

All participants (or their parents) were asked to complete a questionnaire including medical history (and epilepsy history, where applicable), fractures, falls and injuries, and vitamin D or calcium supplements.

All participants underwent a cross-sectional appraisal of their bone health during a single study visit at The Royal Melbourne Hospital, which involved the following assessments:

Serum 25 hydroxyvitamin D (25 OHD) concentrations were measured through direct competitive chemiluminescent immunoassay (CLIA) using a LIAISON Analyser (DiaSorin, Saluggia, Italy) (coefficient of variation [CV] over study period 2.5–2.8%).

Bone age x-ray was performed of the left hand/wrist, read by radiologists and also investigator PS, and compared to a standardized atlas adjusted for sex using the method outlined by Greulich and Pyle.[19] This provided a tool to account for maturational differences.

DXA was performed using a Hologic QDR-4500A densitometer (Hologic, Bedford, MA, U.S.A.). Areal bone mineral density (BMD), bone mineral content (BMC), and soft tissue composition were assessed. Sites scanned were the right total hip (TH), lumbar spine (LS), and the whole body excluding the head ($WB_{less\ head}$). Z-scores were calculated using age- and sex-specific normative data provided by Hologic, based on the Bone Mineral Density in Childhood study data.[20]

pQCT scans generate a cross-sectional image that can be used to calculate volumetric bone mineral density, avoiding the confounding that DXA experiences with short or tall stature.[21] It also examines skeletal geometry, and then combines these measures to generate an estimation of bending strength (the stress-strain index), which infers the risk of fracture for that bone. Scans were completed using a Stratec

XCT 3000 scanner, software version 5.50 (Stratec Medizintechnik GmbH, Pforzheim, Germany) at sites corresponding to 4% (distal end) and 66% of the tibia (effectively mid-shaft) from the distal reference line to assess both trabecular and cortical bone compartments as well as muscle cross-sectional area. Setting a threshold of 710 mg/cm^2 and 181 mg/cm^2 allowed for isolation of the bone and muscle cross-sectional area (CSA), respectively. The contour and peel mode were both set at 1 for analysis of each section as per manufacturer's recommendation.

Muscular force, power, and coordination were assessed by a Leonardo Mechanograph ground reaction force plate (GRFP) (Novotec Medical GmbH, Pforzheim, Germany). Parameters assessed included the single two-legged jump test (s2LJ), which assesses peak power and the multiple one-legged hop test (m1LH), which assesses peak force. Each measure was recorded as best of three attempts, and the parameters were adjusted for bone age, height, and weight. Multiple balance tests (Romberg, semi-tandem, tandem and one-legged balance tests) were applied using the Leonardo Mechanograph GRFP according to the manufacturer's instructions. Balance tests included assessed ellipse area (cm^2) about the center of pressure indicating sway, and relative path length (mm/s) indicating movement velocity.

Height was measured using a wall-mounted stadiometer (Holtain, Limited, Pembrokeshire, United Kingdom), and weight was measured using a mechanical beam scale (Colonial Weighing Australia, Victoria, Australia). Body mass index (BMI) was calculated.

Statistical analyses

As this was an exploratory study, there were no existing literature upon which to base a sample size estimation. The data analyses were carried out using the IBM SPSS Statistics for Windows version 20 (IBM Corp, Armonk, NY, U.S.A.). Analysis was undertaken by the same investigators who had performed the investigations. Age-matched Z-scores were produced to standardize patient's height, weight, and BMI. Continuous data were presented as mean ± standard deviation (SD) if normally distributed data or median (interquartile range, IQR) if skewed, and n (%) for categorical data. The differences in fracture prevalence and pubertal staging were analyzed by a McNemar test. All continuous variables of interest (e.g., bone mineral parameters) were tested for normality using Shapiro Wilk's test prior to data analysis. Normally distributed data were analyzed by a paired *t*-test, and skewed data were analyzed by a Wilcoxon signed-rank test to assess within-pair differences. Some comparisons were performed both unadjusted and after adjustment for relevant covariates. The BMD of the LS, TH, and $WB_{less\ head}$ results were corrected for bone age and height for both subjects and controls, whereas s2LJ and m1LH parameters were corrected for bone age, height, and weight.

Standard protocol approvals, registrations, and patient consents

This study was approved by the Human Research Ethics Committee (HREC) of the Royal Melbourne Hospital and Royal Children's Hospital, as well as by the Australian Twin Registry. Written informed consent was obtained from all subjects and controls or from their guardians as appropriate.

Results

There were 23 subjects using AEDs, with a median (IQR) AED therapy duration of 4.0 (IQR 2–9) years. The majority of AEDs used were non–enzyme-inducing agents (61%) (Table 1). Thirteen subjects were prescribed 2 AEDs and 3 subjects were on 3 agents. The groups had similar age distributions with mean (SD) of 12.8 ± 2.9 years for subjects and 12.9 ± 3.3 for controls, p = 0.722. There were no significant within-pair differences in height (p = 0.79), weight (0.82), or BMI (0.98). Mean height Z-score was −0.01 SD ± 0.99. Both weight and BMI had a mean Z-score of 0 ± 1 SD. There was no difference in BMI between those on valproate compared with those on other AEDs (p = 0.82). It is notable that the groups were well matched for level of skeletal maturation, with the bone age comparable between groups with a median 13 years (IQR 11–15) in subjects and median 13 years (IQR 8–18) for controls. The groups were also well matched for height (subjects' median height 157.1 cm, IQR 142.6–166.6, compared with 155.2 cm, IQR 148.2–168.4 for controls, p = 0.94). The serum 25-OHD levels tended to be higher in subjects compared to controls (58.7 ± 24.8 nmol/L compared to 50.68 ± 14.4 nmol/L), but the difference was not significant (p = 0.20). No participants reported taking vitamin D or calcium supplements.

There was a lifetime history of 15 fractures in 8 subjects (3 having 1 fracture, 3 having 2 fractures, and 2 having 3 fractures), compared to 4 fractures in 3 controls (Fig. 1;

Table 1. AEDs used by participant

AED	Number of subjects
Valproate	13
Topiramate	6
Lamotrigine	6
Levetiracetam	4
Clobazam	4
Lacosamide	2
Carbamazepine	2
Clonazepam	2
Oxcarbazepine	2
Pregabalin	1
Sulthiame	1
Ethosuximide	1

Note that 16 of 23 patients were on combination therapy.

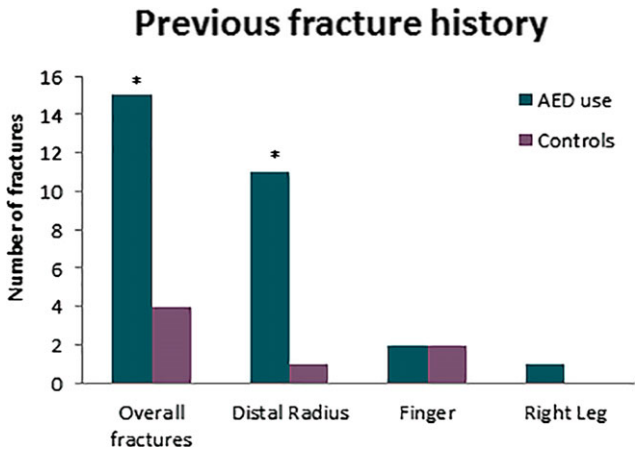

Figure 1.
Frequency of fractures overall, at the right or left distal radius, finger, and right leg in the antiepileptic drug (AED) users compared to the non-users. Sites shown are distal radius (right or left), finger, and right leg (NB this does not cover all fracture sites). The fractures at the distal radius ($p = 0.002$) and the overall fracture prevalence showed a statistically significant difference between groups and are indicated with (*).
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$p < 0.01$). Therefore, a history of fracture was noted in 35% of subjects compared with 17% of controls. All of these fractures were sustained after AED treatment commenced. Twelve of the fractures among subjects occurred due to trauma from falling off playground equipment or playing sports, with only three sustained during an epileptic seizure. Mean age at first fracture was comparable between groups (8.1 ± 3.4 years vs. 8.3 ± 2.8 years).

The most frequent fracture site was the distal radius. There were 11 such fractures in subjects compared to only one distal radius fracture in controls (Fig. 1; $p < 0.01$). A family history of osteoporosis was reported by 6 of the 23 families, 4 of whom reported a history of fracture in a grandmother. There were insufficient numbers to perform further sub-analyses of factors driving fractures such as specific AED use.

Both crude and adjusted BMD (aBMD) measurements were consistently around 5% lower in subjects than controls, but the difference did not reach statistical significance at any site (LS, TH, and $WB_{less\ head}$) (Table 2).

pQCT scanning demonstrated a clinically significant 14.4% lower trabecular volumetric BMD (vBMD) in subjects ($p = 0.03$) (Fig. 2A) and a 7.6% deficit approaching statistical significance in total vBMD ($p = 0.07$) at the 4% site of the tibia (Table 3). There were no significant within-pair differences in geometrical parameters, polar stress-strain index (SSI), and muscle CSA at the 66% site of the tibia. After adjustments for bone age and height, previously significant findings remained thus, while the between-pair differences in total CSA, trabecular area, and polar SSI between subjects and controls increased, but did not reach statistical significance, with adjusted CSA of 998 mm^2 (±139) in subjects as opposed to 1,040 mm^2 (±139) in controls ($p = 0.3$), and adjusted SSI of 1,622 mm^3 (±352) in subjects compared with 1,738 mm^3 (±352) in controls ($p = 0.27$).

Muscle function testing revealed a 17.5% deficit in maximum force (F_{max}), from the m1LH test on the weight-bearing leg in subjects, with a mean force exertion of 1.04 kN compared to 1.26 kN in nonusers ($p < 0.01$). Subjects also showed a 15.3% deficit in maximum force relative to body weight, exerting a mean force of 2.21 (*Fg) compared to 2.61 (*Fg) ($p < 0.01$) (Fig. 2B). There were no significant within-pair differences between users and nonusers in Romberg, semi-tandem, tandem, and one-legged balance tests (data not shown). There were also no between-group differences in physical activity levels.

When plotting Fmax against trabecular density (Fig. 3), there was no clear relationship for subjects and controls, but a trend toward higher Fmax leading to improved trabecular density in both groups.

Discussion

The current study used an AED-discordant, same-sex, related-pair design to demonstrate new, clinically relevant information relating fracture prevalence, bone health, and muscle strength in 5- to 18-year-old epilepsy patients taking AEDs. Young people taking AEDs had a significantly higher prevalence of fractures at the distal radius, a

Table 2. Crude and adjusted (for bone age and height) dual energy x-ray absorptiometry (DXA) parameters of subjects versus controls

	Crude			Adjusted		
	Subjects	Control	p-Value	Subjects	Control	p-Value
LS BMD (g/cm^2)	0.78 ± 0.20	0.82 ± 0.19	0.53	0.79 ± 0.11	0.83 ± 0.11	0.16
TH BMD (g/cm^2)	0.80 ± 0.18	0.84 ± 0.15	0.41	0.81 ± 0.12	0.85 ± 0.12	0.19
$WB_{less\ head}$ BMC (g)	1095.44 ± 493.79	1138.40 ± 488.79	0.77	1082.61 ± 188.70	1151.23 ± 188.70	0.23

No significant within-pair differences were seen (paired *t*-test).
Parameters include the bone mineral density (BMD) of the lumbar spine (LS), total hip (TH), and bone mineral content (BMC) of the whole body excluding the head ($WB_{less\ head}$). Results are displayed as mean ± standard deviation. p-Values compare within pair differences between users and nonusers.

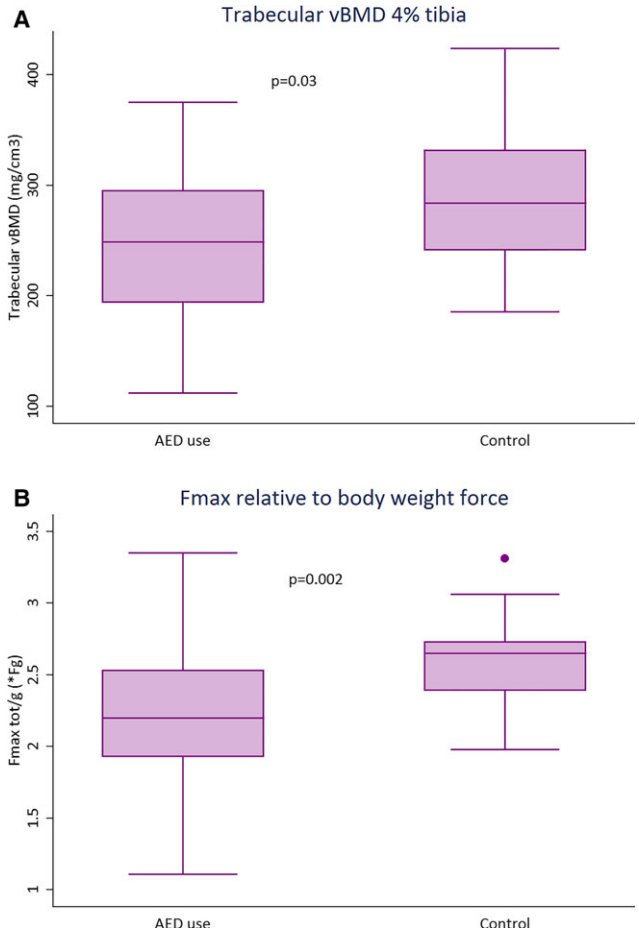

Figure 2.

(**A**) Trabecular volumetric bone mineral density (vBMD), as measured by peripheral quantitative computerized tomography (pQCT), adjusted for bone age and height at the 4% site of the tibia, comparing antiepileptic drug (AED) users against controls. The shaded box indicates the interquartile range (IQR), and the horizontal black line indicates the median trabecular vBMD for each group. (**B**) Maximum force (F_{max}) relative to body weight force adjusted for bone age, height, and weight in AED users compared to controls. The shaded box indicates the IQR and the horizontal black line indicates the median F_{max} for each group. The p-value was calculated by paired *t*-test.

clinically significant reduction in trabecular vBMD at the 4% site of the tibia, and lower maximum force exerted on the lower limb. Cases and controls were well matched for height, age, and level of skeletal maturation.

Fracture risk is the most clinically relevant marker of bone strength. Our finding of increased distal radial fractures, in particular, corroborates other pediatric studies, which identify the distal radius as the most commonly fractured site in otherwise well children.[22,23] The fracture rate in the control group is consistent with population studies of fracture rates in childhood, which suggest around one in three young people will have a fracture prior to the age of 17 years.[24]

DXA data from this study showed a trend toward reduced BMD and BMC in the subjects, which would be in keeping with the published literature. The relatively small sample size may have prevented finding deficits, which were found on the more sensitive measure of pQCT. As mentioned previously, there is a large volume of published data showing lower Z-scores in pediatric and adult patients on AED therapy. The use of pQCT is important in furthering our understanding of bone health issues in this cohort. Although still the mainstay of clinical bone health assessment in pediatrics, DXA is confounded by variations in stature because it measures areal rather than volumetric bone density, and this limits its diagnostic utility when assessing the growing skeleton.[21] However, pQCT measures volumetric BMD and allows for assessment of skeletal geometry, as well as separation of the cortical and trabecular compartments.

Studies have shown a strong association between trabecular vBMD and risk of distal radius fracture.[18,25] Therefore, our finding of significantly reduced trabecular density at the 4% site ties in with the observation of increased distal radial fractures in the young people taking AEDs.

Previous literature has identified trabecular bone as a site of force dissipation and a marker of bone strength,[26,27] suggesting that bone strength is compromised at this site if the vBMD is reduced. The large surface area provided by the trabecular meshwork may allow for rapid interaction with endocrine and other environmental stimuli. This in turn will be detrimental to bone strength,[28] and hence this site is potentially vulnerable to adverse stimuli such as medications.

Another possible explanation for the observed reduction in vBMD is diminished responsiveness of the bone adaptation process.[29] Muscles exert forces on the bone, and the bone then adapts its structure to increase strength and prevent fracture.[30–33] Therefore, if bone formation does not respond appropriately to the mechanical loading it encounters, this will increase fracture risk. Hence, it is important to consider bone and muscle as one functional unit.[32]

The multiple one-legged hop showed significant differences in maximal force production in the lower limb. This finding suggests that the lower limbs of subjects on AEDs are exposed to lower peak forces. This may indicate a neurologic effect of AEDs, possibly involving muscle function.[34] Given that peak force has been positively associated with total bone area and vBMD of the tibia,[35,36] this loss may have a direct impact on skeletal development and fracture risk.

The nature of the fracture incidents may also suggest a neurologic effect of AEDs on muscle coordination, power, or balance, resulting in a heightened risk of accidental falls. Adult studies have been able to recapitulate balance impairment in clinical testing but this was not seen in the current study sample, possibly related to the relatively short duration of AED exposure in our study. Alternatively, the balance tests may not have been sufficiently challenging, or a

Table 3. Unadjusted peripheral quantitative computed tomography (pQCT) parameters of subjects versus controls

	Subjects	Controls	p-Value
Tibia 4% site			
Trabecular vBMD (mg/cm^3)	244.37 ± 63.37	285.60 ± 59.09	0.03*
Total vBMD (mg/cm^3)	290.32 ± 45.56	314.31 ± 42.14	0.07
Total CSA (mm^2)	1001.78 ± 284.58	1036.83 ± 232.48	0.65
Trabecular area (mm^2)	450.70 ± 128.07	466.49 ± 104.61	0.65
Tibia 66% site			
Total CSA (mm^2)	482.82 ± 160.13	497.47 ± 142.24	0.75
Cortical area (mm^2)	230.82 ± 72.78	240.70 ± 70.83	0.64
Periosteal circumference (mm)	12.24 ± 2.04	12.46 ± 1.79	0.69
Endosteal circumference (mm)	8.76 ± 1.90	8.89 ± 1.69	0.81
Cortical thickness (mm)	3.47 ± 0.83	3.57 ± 0.86	0.69
Cortical vBMD (mg/cm^3)	1066.56 ± 56.33	1054.58 ± 71.32	0.53
Polar SSI (mm^3)	1639.57 ± 788.86	1721.70 ± 694.12	0.71
Muscle area (mm^2)	4563.85 ± 1759.05	4610.0 ± 1085.66	0.92

Parameters at the 4% tibial site include the following: trabecular volumetric bone mineral density (vBMD), total vBMD total cross-sectional area (CSA), and trabecular area. Parameters measured at the 66% site include the following: total CSA, cortical area, periosteal circumference, endosteal circumference, cortical thickness, cortical vBMD, polar stress-strain index (SSI), and muscle area. Results are displayed as mean ± standard deviation. A p-value <0.05 indicates statistical significance and is denoted with (*).

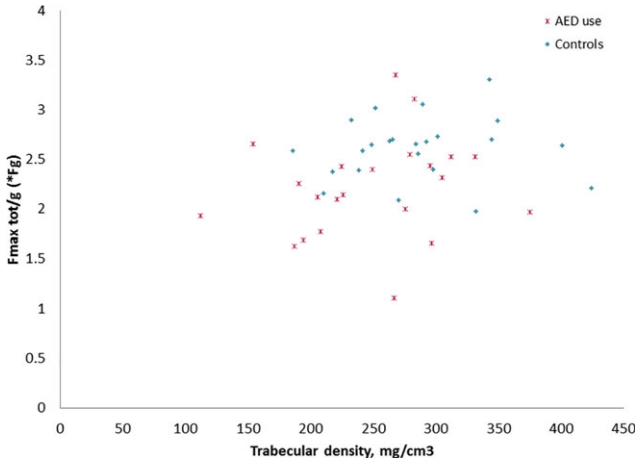

Figure 3.
Trabecular density plotted against F_{max} for subjects taking AEDs and controls.
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larger sample size may be needed to identify any deficits in balance with AED use (or subgroup such as AED type, duration, or polytherapy) in this population.

In our study, vitamin D status was not reduced in users compared with nonusers. Therefore, unlike the classical hypothesis that AEDs exerted their effects on bone via impaired vitamin D metabolism, our data suggest an effect of AEDs on bone and the muscle–bone unit independent of changes in vitamin D metabolism.

The possibility of a direct effect of AEDs on reducing bone formation remains. Animal model work demonstrates a direct effect of carbamazepine and phenytoin on voltage-gated sodium channels within osteoblasts,[37] which may explain the observed reduction in bone density. However, data for other ion channels, other AEDs, and secondary pathways are still required. Valproate has also been shown to increase bone resorption (based on bone turnover marker results), without impacting vitamin D metabolite levels.[38] However, AED-independent mechanisms related specifically to the epilepsy state are also possible, with recent evidence that with a specific genetic murine model of epilepsy (the Pten knockout mouse), reduced bone mass accrual was seen in the absence of AED use.[39] However further study is needed due to the number of genetic mutations associated with epilepsy/and epilepsy syndromes, with the relationship of these then in turn needing to be linked to bone phenotypes. Overall, although these results are intriguing, the underlying mechanism for low bone mass in this population remains unclear.

The study design has offered several advantages. We matched pairs for sex and as well as possible for age. Furthermore, recruitment of twins, siblings, and cousins allowed for at least partial control for genetic and environmental influences, and therefore more powerful results despite the relatively small number of subjects. The use of pQCT imaging is novel in this group, allowing adjustment for bone size and shape in a growing population. In addition, Leonardo mechanography provided insights into the functional muscle–bone relationship in adolescents and increased the clinical relevance of the results. Unlike many other such studies, we adjusted for bone age and development. Despite utilizing matched pairs to control within-pair differences, the study design presents some limitations. The group was heterogeneous, especially with respect to type of AED used, type of epilepsy, pubertal stage, and duration of treatment past 12 months. We were unable to assess the differential effects of individual or enzyme-inducing AEDs and polytherapy on bone parameters due to the relatively small sample size. In addition, the use of sibling pairs

resulted in an inherent age-matching limitation within such pairs.

In summary, this study suggests that children who are prescribed AEDs have an increased occurrence of fracture, decreased bone strength, and lower limb muscle function compared to their controls. These results need to be validated in a larger, longitudinal study investigating the association between AED exposure and adverse outcomes in the developing skeleton over time.

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Disclosure of Conflict of Interest

Mackay MT reports funding from Pfizer for an unrelated project concerning epilepsy and bone health. Petty SJ and Wark JD report funding from Novartis and UCB Pharma for unrelated projects concerning epilepsy and bone health. The remaining authors report no conflicts of interest to disclose. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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