Monotherapy with Levetiracetam versus Older AEDs: A Randomized Comparative Trial of Effects on Bone Health

Tahir Hakami[1,2,7], Terence J O'Brien[1,2], Sandra J Petty[2], Mary Sakellarides[2], Jemma Christie[2],[*]Susan Kantor[4], Marian Todaro[1,2], Alexandra Gorelik[5], Markus J Seibel[6], Raju Yerra[1], John D Wark[2,3]

[1] Department of Neurology, the Royal Melbourne Hospital. [2] Department of Medicine, the University of Melbourne. [3] Bone & Mineral Medicine, the Royal Melbourne Hospital. [4] Bone Densitometry Unit, the Royal Melbourne Hospital. [5] The Melbourne EpiCentre, the Royal Melbourne Hospital, Melbourne, Australia. [6] Bone Research Program, ANZAC Research Institute, The University of Sydney, Sydney, Australia [7] Faculty of Medicine, Jazan University, Saudi Arabia

Correspondence John D. Wark MB.BS, Ph.D., FRACP, Department of Medicine, The Royal Melbourne Hospital, The University of Melbourne, 4th Floor Clinical Sciences Building Royal Parade, The Royal Melbourne Hospital, Parkville 3050, Victoria, AUSTRALIA, Telephone: +61 3 9342 7109, Facsimile: +61 3 9348 2254, Mobile: +61 419314625, Email: jdwark@unimelb.edu.au.

Word count: 3, 838 words

Abstract: 250 words

Tables/Figures: 5

References: 30

Acknowledgements: The authors acknowledge Annie Roten and Tess Bright (Department of Neurology, The Royal Melbourne Hospital, Victoria, Australia) and Lauren Day (The University of Melbourne, The Department of Medicine, The Royal Melbourne Hospital) for their assistance with data collection and Dr. Ashwini Kale (The Bone Densitometry Unit, The Royal Melbourne Hospital) for her assistance with DXA and pQCT scanning.

[*] Sue Kantor has passed away in December 2012.

Funding: This was an investigator-initiated study partly funded by UCB Pharma. UCB Pharma had no role in the study design, data collection, analysis, interpretation of data, nor in the preparation of this article. All authors had full access to the data. The corresponding author had full access to the data and had final responsibility for the decision to submit for publication.

ABSTRACT

Background: Long-term anti-epileptic drug (AED) therapy is associated with increased fracture risk. This study tested whether substituting the newer AED levetiracetam has less adverse effects on bone than older AEDs.

Methods: An open-label randomized comparative trial. Participants had "failed" initial monotherapy for partial epilepsy and were randomized to substitution monotherapy with levetiracetam or an older AED (carbamazepine or valproate sodium). Bone health assessments, performed at 3 and 15 months, included areal bone mineral density (aBMD) and content at lumbar spine (LS), total hip (TH), forearm (FA), and femoral neck (FN), radial and tibial peripheral quantitative computed tomography and serum bone turnover markers. Main outcomes were changes by treatment group in aBMD at LS, TH, and FA, radial and tibial trabecular BMD and cortical thickness.

Results: 70/84 patients completed assessments (40 in levetiracetam- and 30 in older AED group). Within-group analyses showed decreases in both groups in LS (-9.0%; $p<0.001$ in levetiracetam vs. -9.8%; $p<0.001$ in older AED group), FA (-1.46%; $p<0.001$ vs. -0.96%; $p<0.001$ respectively) and radial trabecular BMD (-1.46%; $p=0.048$ and -2.31%; $p=0.013$ respectively). C-terminal telopeptides of type I collagen (βCTX; bone resorption marker) decreased in both groups (-16.1%; $p=0.021$ vs. -15.2%; $p=0.028$ respectively) whereas procollagen I N-terminal peptide (PINP; bone formation marker) decreased in older AED group (-27.3%; $p=0.008$). The treatment groups did not differ in any of these measures.

Conclusions: Use of both levetiracetam and older AEDs was associated with bone loss over 1 year at clinically-relevant fracture sites and a reduction in bone turnover.

Trial Registration anzctr.org.au Identifier: ACTRN12606000102572

KEYWORDS: Epilepsy; Antiepileptic drugs; Bone health.

INTRODUCTION

Chronic antiepileptic drug (AED) therapy is associated with bone disease and increased fracture risk [1]. Several studies have revealed this association but the mechanisms remain controversial [2-8]. Decreased bone mineral density (BMD), the most significant predictor of fracture risk, has been observed in patients using "older generation" AEDs, particularly inducers of cytochrome p450 (CYP450) enzymes; carbamazepine, phenytoin, phenobarbital and primidone [9]. The impact of the older AED valproate, a CYP450 inhibitor, on bone is still controversial, but a number of studies also show an association of treatment with this drug with decreased BMD [4-6, 10]. It is still unclear whether the "newer generation" AEDs that have been introduced into practice over the last two decades, including gabapentin, lamotrigine, topiramate, tiagabine, oxcarbazepine, levetiracetam, and zonisamide, adversely affect bone health.

The American Academy of Neurology (AAN) subcommittee report observed that newer AEDs were "equivalent" in controlling seizures, but might be better tolerated (i.e. have less adverse effects) than older AEDs [11]. Of particular relevance, the newer AEDs do not enhance or inhibit liver enzymes. Evidence from uncontrolled trials has raised the possibility that lamotrigine has limited negative effects on bone [5, 6, 12, 13]. In contrast, some studies report low bone density in people on long-term treatment with oxcarbazepine [14], gabapentin [3], and topiramate [15]. Levetiracetam is a "new generation" AED that is structurally and mechanistically distinct to other AEDs. It has a favorable efficacy, tolerability, and pharmacokinetic profile [16, 17]. It therefore potentially has less adverse effects on bone but data are limited. A prospective cohort study reported no significant bone loss in drug-naïve epilepsy patients treated with levetiracetam [18]. A cross-sectional study, in contrast, reported lower BMD in patients taking levetiracetam compared to those taking topiramate, lamotrigine, carbamazepine, or valproate monotherapy [19].

Herein we report a 12-month randomized comparative study (RCT) aimed at testing the hypothesis that patients randomized to treatment with levetiracetam will show, on serial assessments performed 12 months apart, less changes in: 1) areal bone mineral density (aBMD) at clinically-relevant sites; 2) bone volumetric density and structure; 3) serum markers of bone turnover; compared to those randomized to treatment with an older AED (carbamazepine or sodium valproate). The null hypothesis was that there is no difference between the interventions.

METHODS

Patients

This was a pragmatic, single-centre, prospective, open-label study with blinded endpoint ascertainment. Eligible patients were recruited from the KONQUEST study (*Keppra versus Older AEDs evaluating Neuropsychiatric, Neurocognitive and QUality of life outcomes in treatment of Epilepsy as Substitution monoTherapy*) [20]. Exclusion criteria were a history of bone disease; a treatment history of glucocorticoids, chemotherapy or other medications which could affect bone density; a history of tumor or other medical morbidity known to affect bone health; pregnancy, breast feeding or planned pregnancy in the next year.

Randomization

Enrolled patients had "failed" initial monotherapy for partial epilepsy with an "older" AED (carbamazepine, valproate, or phenytoin) and were randomized to substitution monotherapy treatment with levetiracetam or another older AED, i.e. controlled-release carbamazepine (Tegretol® CR) or enteric-coated sodium valproate (Epilim®). If the initial AED treatment had been carbamazepine or phenytoin the patient was randomized to levetiracetam or valproate, and if the initial AED treatment was valproate the patient was randomized to levetiracetam or carbamazepine. Details of randomization are described in Hakami et al [20]. The primary endpoints of the KONQUEST study were the proportions of patients who showed improvement in depression symptoms and quality of life at three months following randomization [20]. Hence a balanced randomization schedule, based upon baseline Hospital Anxiety and Depression Scale (HADS) [21] depression score permuted blocks, was used to ensure that the two treatment groups were equivalent with regards to the numbers of patients reporting depressive symptomatology (HADS depression score >7) at baseline. Permuted blocks ensured a balance after every 4th treatment allocation, within each level of baseline scores (Figure 1 in Hakami et al. 2012 [20]. The bone health measures were not considered in the randomization, which was conducted by a research scientist who had no contact with study patients. Physicians screening and enrolling patients, and scientists performing and analyzing the bone health measures, were blinded to the patient's treatment allocation.

Treatment

During the initial four-week titration period following randomization, the initial AED was weaned and the study drug increased in two weekly step-ups to a target dose of 1000 mg per day for levetiracetam, 1000 mg per day

for valproate, and 400 mg per day for carbamazepine. After this time, dose adjustments by the treating neurologist were allowed if the patient had further seizures or if there were issues with tolerability. If seizures were unable to be controlled with monotherapy with the study drug, another AED could be added. If intolerable drug side effects persisted, the patient could be withdrawn from the study medication and treated with a different AED. Patients continued to be followed and received all scheduled assessments for the 12-month post-randomization period irrespective of treatment changes.

Outcome assessments

The main bone outcomes were changes over 12 months by treatment group in: (1) aBMD at lumbar spine (LS), total hip (TH), and forearm (FA); (2) trabecular BMD and cortical thickness of the non-dominant radius and tibia; and (3) serum levels of bone turnover markers. Exploratory outcomes were changes in FN aBMD, total body bone mineral content (TB BMC) on DXA and strength-strain index (SSIp) on pQCT scanning. Assessments were performed at 3 months following randomization (to allow time for dose titration of the study drug and weaning of the previous AED to be completed) and repeated 12 months later (i.e. 15 months post randomization).

The assessments included:

1. Dual energy x-ray absorptiometry (DXA) using Hologic QDR® 4500A densitometer (Hologic Inc., Bedford MA, 01730 USA) for aBMD and body composition [22]. The coefficient of variation for aBMD using the Hologic spine phantom was 0.36 – 0.37% throughout the duration of the study and there was no significant drift in mean values.
2. Peripheral quantitative computed tomography (pQCT) for assessment of trabecular and cortical bone volumetric density and geometry at non-dominant tibia and radius using Stratec 3000 XCT version 5.50 (Stratec Medizintechnik GmbH, Durlacher Str. 35 75172 Pforzheim, Germany). Details of pQCT assessment are included in supplementary information;
3. Serum levels of C-terminal telopeptides of type I collagen (β-CTX), a marker of bone resorption; procollagen I N-terminal peptide (PINP), a marker of bone formation, 25-hydroxyvitamin D (25OHD); 1,25-dihydroxyvitamin-D (1, 25$(OH)_2$D), intact parathyroid hormone (iPTH) and calcium.
4. Questionnaires for epilepsy history, exercise, smoking, alcohol intake, medical and medication histories, fracture history, calcium intake, and physical activity [23]. Lifetime smoking was expressed

as total pack years (average cigarettes/day X years/20). Alcohol intake was determined based upon the average number of standard drinks per week in the 12 months prior to the bone densitometry scan. Usual physical activity was determined by a questionnaire at the interview by surveying participants' regular hours of weight-bearing exercise (< 1 hour per week, 2-3 hours per week, 4-7 hours per week, > 7 hour per week) and the average hours were calculated during the last 12 months.

Serum samples were analyzed across two laboratories due to limited local technician availability: 1) the ANZAC Research Institute, Sydney, Australia: samples for 32 patients, 15 randomized to levetiracetam and 17 to older AEDs; 2) The Melbourne Health Shared Pathology Service (MHSPS): samples for 48 patients, 26 randomized to levetiracetam and 22 to older AEDs). Samples from each treatment arm and from each patient were measured in the same assay run; assay details are included in supplementary information.

Statistical analysis

The sample size calculation was based upon the depression scores primary endpoint of the KONQUEST study, as described in Hakami et al. 2012 [20]. The study was not specifically powered for bone health measures, which were secondary endpoints. Analyses were undertaken on per protocol basis. Patients who did not attend for their 12-month follow-up bone health assessments were excluded from the analysis. The analysis comprised adjusted comparison between the treatment groups for 12-month percentage change in aBMD at LS, TH, and FA; trabecular BMD and cortical thickness of the non-dominant radius and tibia; and serum levels of bone turnover markers. The changes in LS, TH, FA, and FN aBMD in the combined treatment groups were also compared to those in a healthy untreated reference group. The DXA and pQCT measures were adjusted for age, baseline height and weight, and time interval. Paired t-tests and independent t-tests were utilised to assess mean within-group absolute change and between-group differences, respectively. Categorical variables were compared using either Chi-square (χ^2)/Fisher's exact test for repeated measures. As the two laboratories used different assay methodology and laboratory references (Table e-1), agreements between the results measuring paired samples in the two laboratories were assessed using: Pearson's correlation and Bland Altman analysis (see supplemental materials).

Mixed-effects Restricted Maximum Likelihood (REML) regression analysis was fitted to examine the effect of specific factors and covariates on the outcomes of interest. Factors examined included treatment groups, sex and

menopausal status, and alcohol consumption. Covariates included age, height, weight change, follow-up interval, calcium intake, and life-time smoking. REML analysis was utilized due to its advantages over standard linear modelling (where all observations are assumed to be independent) and accounts for both the within-group and between-group variation in the dataset.

Results were presented as mean (SD)/ or median (IQR) and percentage of change. Level of significance was set at < 0.05. However, due to the number of comparisons between main outcomes, level of significance for Univariate analysis was adjusted using the Bonferroni adjustment and set at 0.01. All analyses were performed using the statistical package Stata SE/10·1 for Windows (StataCorp, TX, USA).

Ethics approval and registration

The study protocol was approved by the Melbourne Health Human Research Ethics Committee. All participants provided written informed consent. The study was registered as an International Standard Randomized Controlled Trial with the Australian New Zealand Clinical Trial Registry (ACTRN12606000102572).

RESULTS

Patient recruitment and follow-up

Over 23 months (February 2006 – December 2008), 84 patients were enrolled (51 (60.7%) males: mean (SD) age 43.5 (17.8) years) and 33 (39.3%) females: mean age 41.2 (17.1) years). Forty-five patients (53.6%) were randomized into the levetiracetam treatment group and 39 patients (46.4%) to the older AED treatment group (24 valproate and 15 carbamazepine). The treatment groups were similar in demographic and clinical characteristics (Table 1).

Figure 1 is a flow chart for enrolled patients. Thirty-one of 45 patients randomized to levetiracetam (69%) and 20 of 39 patients randomized to older AEDs (51%) completed the study on assigned monotherapy**.** In total, 14/84 (16·7%) of patients withdrew or died during the study, and this did not significantly differ between the treatment groups (five randomized to levetiracetam, three to carbamazepine and six to valproate sodium). Withdrawals from the study were due to:

- Non-compliance with the study procedures - 10 patients (two levetiracetam, two carbamazepine, and six valproate);

- Medication side effects – one patient (levetiracetam, balance problem and poor seizure control);
- Conversion to high grade brain tumor - one patient (levetiracetam);
- Death – two patients (one patient taking carbamazepine died of sudden unexpected death in epilepsy (SUDEP), one patient taking levetiracetam died of carcinoma of the stomach).

Main bone outcomes

On DXA scanning, there was no difference between the treatment groups in the percentage change in aBMD from baseline to 12 months at LS (-9.0% in the levetiracetam group vs. -9.8% in the older AED group; p=0.53, independent t-test), FA (-1.46% vs. -0.96% respectively; p=0.14, independent t-test), or TH (-0.21% vs. -0 .84% respectively; p=0.11, independent t-test). On pQCT, there was no significant difference between the treatment groups in the percentage change in trabecular BMD and cortical thickness at both non-dominant radius and tibia sites (Table 4).

Within-group analysis, however, showed a decrease in both groups in LS aBMD (-9.0%; $p< 0.001$ in the levetiracetam group and -9.8%; $p< 0.001$ in the older AED group, paired t-test) and FA (-1.46%; $p< 0.001$ in the levetiracetam group and -0.96%; $p< 0.001$ in the older AED group, paired t-test) (Table 2). TH aBMD decreased in the older AED group (-0 .84%; $p< 0.001$, paired t-test) but not in the levetiracetam group (-0.21%; $p= 0.56$, paired t-test). Both groups also showed a significant decrease in serum βCTX levels, a marker of bone resorption (-16.1%; $p= 0.021$ in the levetiracetam group and -15.2%; $p= 0.028$ in the older AED group, paired t-test). Serum P1NP concentrations, marker of bone formation, significantly decreased in the older AED group (-27.3%; $p= 0.008$, paired t-test) and showed a trend towards decrease in the levetiracetam group (-20.9%; $p=$ 0.14, paired t-test) (Table 3).

Exploratory outcomes

There was a difference between the treatment groups in the change in FN aBMD (-0.47% in the levetiracetam group vs. -1.45% in the older AED group; $p= 0.005$, independent t-test) and the whole body BMC (-0.16% vs. 0.60% respectively; $p= 0.012$, independent t-test) (Table 2). Males and females in either group were not different in the changes in LS, FA, TH, or FN aBMD ($p \geq 0{\cdot}05$). On pQCT scanning, patients randomized to an older AED had significant decreases in all measures of trabecular bone as well as cortical BMD and strength-strain index (SSIp) at the non-dominant radius (Table 4). Compared to those randomized to an older AED,

patients randomized to levetiracetam had higher cortical BMD, but lower total bone area$_{Cortical}$ and periosteal circumference at the non-dominant radius (Table 4). Patients randomized to an older AED had higher SSIp and endosteal circumference at the non-dominant tibia (supplemental material – table e-3).

REML analysis for factors and covariates affecting bone density

Mixed-effects restricted maximum likelihood (REML) regression analysis was fitted to examine effects of some factors and covariates on areal bone mineral density (aBMD) at the lumbar spine, forearm, total hip and femoral neck (details of REML analysis of the lumbar spine aBMD are described in the supplemental material (table e-4). The analysis showed that 88% of variance was attributed to between-patient differences. Post-menopausal female status was a borderline significant predictor for the change in the lumbar spine aBMD ($p= 0.047$, $\chi 2$ test). The REML analysis for aBMD change at the other sites (forearm, total hip and femoral neck) also showed that 88% of the unexplained residual variation existed at the between-patient level. Weight change showed a trend towards significance at three sites: the lumbar spine ($p= 0.071$, $\chi 2$ test), forearm ($p= 0.055$, $\chi 2$ test) and femoral neck ($p= 0.044$, $\chi 2$ test).

Comparison of serial changes with a healthy, untreated reference group

It was not ethically feasible to include an untreated control group in the trial; hence a reference group was included to test for instrument stability. Data for the reference group were obtained from J.D Wark research group's database at the University of Melbourne, Australia. All participants were scanned on the same dual energy x-ray absorptiometry (DXA) and were scanned contemporaneously with the clinical trial participants. Patients in the treatment groups who completed the 15-month assessment [total = 70, mean (SD) age: 43·7 (17·9) years] were compared with healthy reference subjects [n = 71: mean age 41·7 (15·1) years] for the changes in aBMD on DXA scanning.

Patients in the treatment group were predominantly male (61·4% vs. 25·3%; $p<0.001$, $\chi 2$ test) and had shorter time to follow-up scan (median (IQR): 14·8 (13·0-18·7) vs. 26·9 (24·8-32·6), $p< 0·001$, Man-Whitney test) compared to healthy subjects. The changes in adjusted aBMD were different between the treatment group and reference group at the LS (-9·4% vs. -0·9% respectively; $p< 0·001$, independent t-test), FA (-1·24% vs. -0·40% respectively; $p< 0·001$, independent t-test) and FN (-0·87% vs. -0·02% respectively; $p< 0·001$, independent t-

test). The groups did not differ in the TH aBMD change (-0·45 vs. -0·41% respectively; p= 0·852, independent t-test).

DISCUSSION

To our knowledge, this is the first RCT to compare the effects of different AEDs on longitudinal measures of bone health. Concern about possible adverse effects of chronic AED treatment, particularly with the older AEDs, has become increasingly prominent amongst clinicians and epilepsy sufferers and their families. Over the last two decades, several newer AEDs have been approved for clinical use, with hope that these may have less adverse effects. Levetiracetam is a newer generation AED with a unique mechanism of action, binding to the SV2A receptor on neuronal synaptic vesicles where it is believed to inhibit vesicular exocytosis and thereby reduce synaptic excitability. The drug does not enhance liver enzymes or result in other metabolic changes [16, 17]. Although efficacy and tolerability of levetiracetam have been evaluated in several controlled trials [24-29], there has been no previous randomized controlled trial examining effects of levetiracetam on bone health.

In this study, patients with epilepsy were randomized to monotherapy with one of two older AEDs (carbamazepine or valproate) or to treatment with the newer AED levetiracetam. AED treatments were compared extensively with serial assessments performed 12 months apart, including historical bone health risk factors, areal bone mineral measures (with DXA), bone volumetric density and structure (with pQCT), and biochemical markers of bone turnover. Carbamazepine and valproate were analyzed as a single group despite their different mechanisms of action, as previous studies suggested that both have bone effects. A number of studies report that carbamazepine, valproate, and lamotrigine monotherapy in premenopausal women with epilepsy do not differ in their adverse effects on 25-OHD, PTH, markers of bone turnover or bone mineral density [5, 6].

We found that patients randomized to both the older AEDs and to levetiracetam showed changes in bone density and structure on serial DXA and pQCT studies. In both treatment groups, there were significant decreases over 12 months in aBMD at the lumbar spine, forearm, and femoral neck, common sites of fracture in patients with osteoporosis. In both treatment groups there were decreases in trabecular BMD, total trabecular bone area, total cortical bone area, and polar strength strain index at the non-dominant radius. The latter is an index of long bone bending strength. These findings suggest that chronic treatment with both the older AEDs and the newer

generation AED, levetiracetam, is associated with adverse effects on bone health. Similarly, a retrospective study found that in 17 subjects treated with levetiracetam, 70% had low BMD [19]. In contrast, levetiracetam monotherapy in 61 patients with recent-onset epilepsy was associated with an increase in BMD at the lumbar spine with no change in biochemical bone markers [18]. The latter study included individuals at age range of 13–55 years some of whom were continuing to increase BMD and therefore the findings are likely related to the age as opposed to the direct effect of levetiracetam [30] but this is unlikely to be the sole explanation for the difference in findings.

The overall magnitude of effect, and the nature and pathogenesis of bone disease affecting patients taking AEDs remains to be determined. Based on the relatively small amount of longitudinal data available, chronic AED use may result in approximately 1.5-2.0% annual bone loss at clinically-relevant fracture sites [2]. However, this study showed a decrease in BMD over a 12-month interval (-9·4% in the lumbar spine, -1.24% in forearm, and -0·87% in femoral neck), which was greater than those in healthy reference subjects of similar age and not taking any medications (-0·9% in the lumbar spine, -0.4% in forearm, and -0·02% in femoral neck). This bone loss is potentially of clinical significance and warrants further study to determine whether this rate of loss is sustained longer-term.

We also found that patients randomized to older AED treatment had a decrease in serum levels of βCTX and PINP, indicating reduced overall bone turnover. Patients randomized to levetiracetam treatment had a decrease in serum levels of βCTX, indicative of reduced bone resorption. The mean 25-OHD level was in the low sufficient/ replete range (50 – 80 nmol/L) in both treatment groups over the study period, without significant change over time. The mechanism underlying bone deficits in patients taking AEDs remains not understood. One plausible hypothesis is that, given that all AEDs ultimately act to decrease neuronal excitability, they also have analogous effects on bone cells, with adverse consequences on bone health. This hypothesis would be consistent with the finding in this study that AED treatments reduced not only bone formation but also bone resorption. Another hypothesis is that the mechanism of bone loss could independently (apart of the potential effect of AEDs) be related to epilepsy itself, which require further investigation.

Unlike other studies in the literature that reported levetiracetam in patients with new onset epilepsy, our study included a clinically-important, but understudied group of patients in whom initial treatment with an “older”

AED had "failed" due to either inadequate seizure control or intolerable AED side effects. Therefore it cannot be excluded that the bone loss was a result of poor seizure control and/or delayed adverse effect of pre-randomization drug(s). It should also be acknowledged that, because carbamazepine and valproate have different effects on cytochrome P450 enzymes, the two drugs may have variable effects on bone and therefore the combined analysis would make the data difficult to interpret. However, final sample size of subjects in subgroups who continued to receive the AED to which they were randomized and were taking that AED in monotherapy was very small (8 for carbamazepine and 12 for valproate). The subjects in the two subgroups were also heterogeneous, including men and women (pre- and post-menopausal) over a wide age range. Therefore, it may not be valid to analyze data separately for carbamazepine and valproate groups and to draw clinically-relevant conclusions.

While this unique study has a number of strengths, including its RCT design, it also has limitations. First, the number of participants studied was relatively small, and not primarily powered to detect differences in bone health measures between treatment groups. The study was also limited by the relatively short-term follow-up period and the clinically-mandated changes in AED treatment regimes in some patients in the interval. Analysis of the serum bone turnover markers in two different laboratories is a limitation, which we took careful steps to minimize. Another potential limitation is that the treatments were open-labeled, which means that there was greater potential for information bias arising from patient, doctor and investigator preconceptions. However, the outcome assessors were blinded to the treatment assignment.

In conclusion, this RCT, in which patients were randomized to monotherapy treatment with either the newer AED levetiracetam or one of the older AEDs, carbamazepine or valproate, demonstrated significant bone loss at clinically-relevant sites over twelve months, accompanied by changes in the serum levels of bone turnover markers. Although this study was not sufficiently powered to detect significant differences between the treatment groups, the results highlight the need for further research characterising the mechanisms underlying the adverse effects of AEDs on bone health, for further longitudinal comparative studies of different specific treatment options, and for patients and clinicians to be informed of the potential implications of AED treatment on bone health and fracture risk, and the need to monitor for this adverse effect.

Disclosure of Conflicts of Interest: Dr. O'Brien reports grants from UCB Pharma, during the conduct of the study; grants from NHMRC, grants from RMH Neuroscience Foundation, grants from Eisai, outside the

submitted work. Dr Petty has given an invited lecture on an unrelated topic at a symposium, which was sponsored by UCB Pharma in 2015; honorarium was paid to an employer and no personal funding was received. Dr Seibel reports grants from the Australian NHMRC and speaker fees from Amgen, Eli Lilly and Sanofi outside the submitted work. Dr. Wark reports grants from UCB PHARMA, during the conduct of the study; grants from Australian NHMRC, outside the submitted work. Others have nothing to disclose.

REFERENCES

1. van Staa TP, Leufkens HG, Cooper C (2002) Utility of medical and drug history in fracture risk prediction among men and women. Bone 31:508-514

2. Vestergaard P (2005) Epilepsy, osteoporosis and fracture risk - a meta-analysis. Acta Neurol Scand 112:277-286

3. Ensrud KE, Walczak TS, Blackwell TL, Ensrud ER, Barrett-Connor E, Orwoll ES (2008) Antiepileptic drug use and rates of hip bone loss in older men: a prospective study. Neurology 71:723-730

4. Petty SJ, Paton LM, O'Brien TJ, Makovey J, Erbas B, Sambrook P, Berkovic SF, Wark JD (2005) Effect of antiepileptic medication on bone mineral measures. Neurology 65:1358-1365

5. Pack AM, Morrell MJ, Marcus R, Holloway L, Flaster E, Done S, Randall A, Seale C, Shane E (2005) Bone mass and turnover in women with epilepsy on antiepileptic drug monotherapy. Ann Neurol 57:252-257

6. Pack AM, Morrell MJ, Randall A, McMahon DJ, Shane E (2008) Bone health in young women with epilepsy after one year of antiepileptic drug monotherapy. Neurology 70:1586-1593

7. Andress DL, Ozuna J, Tirschwell D, Grande L, Johnson M, Jacobson AF, Spain W (2002) Antiepileptic drug-induced bone loss in young male patients who have seizures. Archives of neurology 59:781-786

8. Souverein P, Webb, DJ, Petri, H, Weil, J, Van Staa, TP, Egberts, T. (2005) Incidence of Fractures among Epilepsy Patients: A Population-based Retrospective Cohort Study in the General Practice Research Database. Epilepsia 46:304-310

9. Pack AM (2011) Treatment of epilepsy to optimize bone health. Current treatment options in neurology 13:346-354

10. Sato Y, Kondo I, Ishida S, Motooka H, Takayama K, Tomita Y, Maeda H, Satoh K (2001) Decreased bone mass and increased bone turnover with valproate therapy in adults with epilepsy. Neurology 57:445-449

11. French JA, Kanner AM, Bautista J, Abou-Khalil B, Browne T, Harden CL, Theodore WH, Bazil C, Stern J, Schachter SC, Bergen D, Hirtz D, Montouris GD, Nespeca M, Gidal B, Marks WJ, Jr., Turk WR, Fischer JH, Bourgeois B, Wilner A, Faught RE, Jr., Sachdeo RC, Beydoun A, Glauser TA (2004) Efficacy and tolerability of the new antiepileptic drugs I: treatment of new onset epilepsy: report of the Therapeutics and Technology Assessment Subcommittee and Quality Standards Subcommittee of the American Academy of Neurology and the American Epilepsy Society. Neurology 62:1252-1260

12. Pack AM, Morrell MJ, McMahon DJ, Shane E (2011) Normal vitamin D and low free estradiol levels in women on enzyme-inducing antiepileptic drugs. Epilepsy & behavior : E&B 21:453-458

13. Kim SH, Lee JW, Choi KG, Chung HW, Lee HW (2007) A 6-month longitudinal study of bone mineral density with antiepileptic drug monotherapy. Epilepsy & behavior : E&B 10:291-295

14. Mintzer S, Boppana P, Toguri J, DeSantis A (2006) Vitamin D levels and bone turnover in epilepsy patients taking carbamazepine or oxcarbazepine. Epilepsia 47:510-515

15. Heo K, Rhee Y, Lee HW, Lee SA, Shin DJ, Kim WJ, Song HK, Song K, Lee BI (2011) The effect of topiramate monotherapy on bone mineral density and markers of bone and mineral metabolism in premenopausal women with epilepsy. Epilepsia 52:1884-1889

16. French J, Edrich P, Cramer JA (2001) A systematic review of the safety profile of levetiracetam: a new antiepileptic drug. Epilepsy research 47:77-90

17. Lyseng-Williamson KA (2011) Levetiracetam: a review of its use in epilepsy. Drugs 71:489-514

18. Koo DL, Joo EY, Kim D, Hong SB (2013) Effects of levetiracetam as a monotherapy on bone mineral density and biochemical markers of bone metabolism in patients with epilepsy. Epilepsy research 104:134-139

19. Beniczky SA, Viken J, Jensen LT, Andersen NB (2012) Bone mineral density in adult patients treated with various antiepileptic drugs. Seizure 21:471-472

20. Hakami T, Todaro M, Petrovski S, Macgregor L, Velakoulis D, Tan M, Matkovic Z, Gorelik A, Liew D, Yerra R, O'Brien TJ (2012) Substitution Monotherapy With Levetiracetam vs Older Antiepileptic Drugs: A Randomized Comparative Trial. Archives of neurology 69:1563-1571

21. Zigmond AS, Snaith RP (1983) The hospital anxiety and depression scale. Acta Psychiatr Scand 67:361-370

22. Briot K, Roux C (2005) What is the role of DXA, QUS and bone markers in fracture prediction, treatment allocation and monitoring? Best Pract Res Clin Rheumatol 19:951-964

23. MacInnis RJ, Cassar C, Nowson CA, Paton LM, Flicker L, Hopper JL, Larkins RG, Wark JD (2003) Determinants of bone density in 30- to 65-year-old women: a co-twin study. J Bone Miner Res 18:1650-1656

24. Zaccara G, Messori A, Cincotta M, Burchini G (2006) Comparison of the efficacy and tolerability of new antiepileptic drugs: what can we learn from long-term studies? Acta Neurol Scand 114:157-168

25. Weintraub D, Buchsbaum R, Resor SR, Jr., Hirsch LJ (2007) Psychiatric and behavioral side effects of the newer antiepileptic drugs in adults with epilepsy. Epilepsy & behavior : E&B 10:105-110

26. Steinhoff BJ, Somerville ER, Van Paesschen W, Ryvlin P, Schelstraete I (2007) The SKATE study: an open-label community-based study of levetiracetam as add-on therapy for adults with uncontrolled partial epilepsy. Epilepsy research 76:6-14

27. Leppik IE, Biton V, Sander JW, Wieser HG (2003) Levetiracetam and partial seizure subtypes: pooled data from three randomized, placebo-controlled trials. Epilepsia 44:1585-1587

28. Kuba R, Novotna I, Brazdil M, Kocvarova J, Tyrlikova I, Mastik J, Rektor I Long-term levetiracetam treatment in patients with epilepsy: 3-year follow up. Acta Neurol Scand 121:83-88

29. Bootsma HP, Ricker L, Diepman L, Gehring J, Hulsman J, Lambrechts D, Leenen L, Majoie M, Schellekens A, de Krom M, Aldenkamp AP (2008) Long-term effects of levetiracetam and topiramate in clinical practice: A head-to-head comparison. Seizure 17:19-26

30. Pack A (2013) Levetiracetam treatment does not result in broken bones. Epilepsy Curr 13:83-84

436 **Table 1 Baseline demographic and clinical characteristics**
437

Characteristic	Levetiracetam (n= 45)	Older AEDs (n= 39)	p –value
Mean age (SD), years	40.3 (17.1)	45.3 (17.8)	0.19
Sex, No. (%)			
Male	24 (53)	27 (69)	0.14
Female premenopausal	13 (29)	8 (21)	
Female postmenopausal	8 (18)	4 (10)	
Weight, mean (SD), kg	74.7 (12.7)	76.8 (17.7)	0.22
Height, mean (SD), metre	1.70 (0.10)	1.72 (0.09)	0.27
Age of seizure onset, median (IQR), y	30 (19-42)	29 (23-48)	0.17
Pre-randomization AED, No. (%)			
Carbamazepine	19 (42.2)	21 (53.8)	0.42
Phenytoin sodium	9 (20)	8 (20.5)	
Valproate sodium	16 (35.6)	9 (23.1)	
Duration of treatment before randomization, median (IQR), months	12 (5-75)	7 (4-30)	0.11
Seizure frequency before randomization, median (IQR), No./y	1.0 (1-2)	3.0 (0.4-24.0)	0.16
Life-time smoking (average cigarettes/day X years/20), median (IQR)	7.9 (2.9-21.5)	12.0 (5.9-31.5)	0.31
Calcium intake in the last three months, mean (SD), mg /day	1276 (814.30)	1337 (613.00)	0.72
Physical activity, No. (%)			
0-1 hr. per week	31 (68.9)	22 (56.4)	0.82
2-7 hrs. per week	7 (15.5)	8 (20.5)	
> 7 hrs. per week	2 (4.4)	3 (7.7)	
Alcohol intake, No. (%)			
Never or occasional	20 (44.4)	12 (30.8)	0.22
1-20 units per week	21 (46.6)	20 (51.3)	
> 20 units per week	0	6 (15.4)	

438 ABBREVIATIONS: AED: antiepileptic drug; SD: standard deviation; IQR: interquartile range

Table 2 DXA bone mineral measures

DXA measure	Within levetiracetam group change				Within older AED group change				Between group difference, p-value
	Baseline mean (SD)	Mean change (SD)	% Change	p-value	Baseline mean (SD)	Mean change (SD)	% Change	p-value	
Lumbar spine aBMD (g/cm^2)	1.027 (0.07)	-0.094 (0.04)	- 9.0	< 0.001***	1.030 (0.07)	-0.10 (0.03)	- 9.8	< 0.001***	0.53
Total hip aBMD (g/cm^2)	0.960 (0.08)	-0.002 (0.02)	- 0.21	0.56	0.962 (0.06)	-0.008 (0.01)	- 0.84	< 0.001***	0.11
Femoral neck aBMD (g/cm^2)	0.848 (0.08)	-0.004 (0.012)	- 0.47	0.026*	0.840 (0.08)	-0.011 (0.01)	- 1.45	< 0.001***	0.005**
Forearm aBMD (g/cm^2)	0.597 (0.044)	-0.009 (0.015)	- 1.46	< 0.001***	0.603(0.05)	-0.006 (0.004)	- 0.96	< 0.001***	0.14
TB BMC (g/cm)	2344.5 (289.7)	-3.72 (28.86)	- 0.16	0.420	2404 (349.9)	13.86 (27.15)	+ 0.60	0.454	0.012*

The comparison of within-group mean change and percentage change and between-group difference over a 12 month period in the changes in DXA bone mineral measures. Significant within groups change or between groups difference is indicated by * for $P < 0.05$, ** for $P < 0.01$, and *** for $P < 0.001$.

ABBREVIATIONS: aBMD: areal bone mineral density; TB BMC: total body bone mineral content; g/cm^2: grams per square centimeter; g/cm: grams per centimeter; SD: standard deviation; -: decrease; +: increase.

Table 3 Serum markers of bone turnover, vitamin D, intact parathyroid hormone and calcium.

Blood test	Within levetiracetam group change				Within older AED group change				Between groups difference, p-value
	Baseline mean (SD)	Mean change (SD)	% Change	p-value	Baseline mean (SD)	Mean change (SD)	% Change	p-value	
βCTX ng/ml	0.39 (0.22)	-0.06 (0.13)	- 16.1	0.021*	0.32 (0.12)	-0.05 (0.13)	- 15.2	0.028*	0.94
PINP µg/l	62.6 (31.1)	-6.8 (22.8)	- 20.9	0.14	51.7 (25.1)	-10.86 (20.0)	- 27.3	0.008**	0.17
25 OHD nmol/l	56.3 (28.5)	2.34 (25.3)	+ 4.15	0.81	52.0 (23.7)	7.0 (25.3)	+ 13.39	0.15	0.33
1,25(OH)2D pmol/l	97.68 (33.1)	-8.7 (36.2)	- 8.87	0.22	75.9 (24.5)	-0.53 (20.7)	- 0.70	0.90	0.32
iPTH pmol/l	5.1 (1.8)	-0.26 (0.21)	- 5.2	0.22	4.6 (2.3)	--0.30 (1.8)	- 6.36	0.58	0.97
Calcium mmol/l	2.37 (0.12)	-0.03 (0.16)	- 1.41	0.25	2.37 (0.13)	-0.03 (0.12)	- 1.17	0.26	0.88

The comparison of within-group mean change and percentage change and between-group difference over a 12 month period in the changes in serum markers of bone turnover, vitamin D, intact parathyroid hormone and calcium. Significant within groups change or between groups difference is indicated by * for $P < 0.05$, ** for $P < 0.01$, and *** for $P < 0.001$.

ABBREVIATIONS: βCTX: C-terminal telopeptides of type I collagen; PINP: Procollagen 1 N-terminal peptide; 25OHD: 25-hydroxyvitamin-D (25OHD); (1,25(OH)2D): 1,25-dihydroxyvitamin-D; iPTH: Intact parathyroid hormone; ng/ml: nanograms per millilitre; µg/l: micrograms per litre; nmol/l: nanomoles per litre; pmol/l: picomoles per litre; mmol/l: millimoles per litre; SD: standard deviation; -: decrease; +: increase.

Table 4 pQCT measures at the non-dominant radius

pQCT measure	Within levetiracetam group change				Within older AED group change				Between group difference, p-value
	Baseline mean (SD)	Mean change (SD)	% Change	p-value	Baseline mean (SD)	Mean change (SD)	% Change	p-value	
Total bone area $_{Trabecular}$ (mm^2)	349.83 (108.28)	-7.06 (17.6)	- 1.89	0.047*	384.29 (51.05)	-11.30 (15.84)	- 2.79	0.006**	0.41
Trabecular bone area (mm^2)	263.20 (99.10)	-6.87 (39.0)	- 2.43	0.062	293.10 (45.68)	-15.63 (14.82)	- 4.94	< 0.001***	0.09
Trabecular BMC (mg/mm)	57.56 (21.59)	-2.14 (5.53)	- 3.41	0.054	63.83 (9.53)	-4.17 (5.08)	- 6.22	0.002**	0.21
Trabecular BMD (mg/ccm)	218.89 (16.81)	-3.27 (8.17)	- 1.46	0.048*	221.16 (15.33)	-5.03 (7.93)	- 2.31	0.013*	0.47
Total bone area $_{Cortical}$ (mm^2)	113.77 (30.09)	-2.31 (2.50)	- 1.91	< 0.001***	124.37 (21.13)	-0.88 (1.28)	- 0.69	0.007**	0.026*
Cortical bone area (mm^2)	89.46 (22.56)	-0.58 (3.82)	- 0.61	0.44	97.03 (15.57)	-1.09 (3.93)	- 1.10	0.24	0.66
Cortical BMC (mg/mm)	112.29 (17.44)	0.54 (4.68)	+ 0.47	0.55	119.05 (18.94)	-1.12 (5.58)	- 0.94	0.40	0.28
Cortical BMD (mg/ccm)	1220.19 (17.40)	4.79 (14.65)	+ 0.39	0.10	1214.82	-5.77 (11.00)	- 0.48	0.035*	0.011*

Table 4 pQCT measures at the non-dominant radius (continued)

Cortical thickness (mm)	3.25 (0.41)	0.04 (0.20)	+ 1.05	0.36	3.37 (0.24)	-0.04 (0.20)	- 1.28	0.36	0.20
Periosteal circumference (mm)	38.87 (2.9)	-0.4 (0.42)	- 1.0	< 0.001***	39.87 (3.6)	-0.14 (0.26)	- 0.4	0.050	0.041*
Endosteal circumference (mm)	17.65 (1.6)	-0.60 (1.4)	- 3.7	0.040*	18.5 (2.1)	0.10 (0.98)	+ 0.5	0.70	0.094
SSIp	261.89 (112.93)	-6.03 (14.09)	- 2.06	0.032*	298.22 (70.45)	-9.14 (17.08)	- 2.96	0.031*	0.50

The comparison of within-group mean change and percentage change and between-group difference over a 12 month period in the changes in pQCT measures at the non-dominant radius. Significant within groups change or between groups difference is indicated by * for P < 0.05, ** for P < 0.01, and *** for P < 0.001.

ABBREVIATIONS: BMD: bone mineral density; BMC: bone mineral content; mm2: millimetre square; mg/mm: milligram per millimetre, mg/ccm: milligrams per cubic centimetre; SD: standard deviation; -: decrease; +: increase; SSIp: polar strength-strain index.

Figures Legends

Fig 1 Flow diagram of patient enrolment, treatment allocation and follow-up

Author/s:
Hakami, T; O'Brien, TJ; Petty, SJ; Sakellarides, M; Christie, J; Kantor, S; Todaro, M; Gorelik, A; Seibel, MJ; Yerra, R; Wark, JD

Title:
Monotherapy with Levetiracetam Versus Older AEDs: A Randomized Comparative Trial of Effects on Bone Health

Date:
2016-06-01

Citation:
Hakami, T., O'Brien, T. J., Petty, S. J., Sakellarides, M., Christie, J., Kantor, S., Todaro, M., Gorelik, A., Seibel, M. J., Yerra, R. & Wark, J. D. (2016). Monotherapy with Levetiracetam Versus Older AEDs: A Randomized Comparative Trial of Effects on Bone Health. CALCIFIED TISSUE INTERNATIONAL, 98 (6), pp.556-565. https://doi.org/10.1007/s00223-016-0109-7.