Seizure: European Journal of Epilepsy 69 (2019) 186–192

Contents lists available at ScienceDirect

Seizure: European Journal of Epilepsy

journal homepage: www.elsevier.com/locate/seizure

Vitamin D abnormalities and bone turn over analysis in children with epilepsy in the Western Cape of South Africa.

Edward Kija[a,g], Barry E. Gidal[b], Alexander Shapson-Coe[c], Shihaam Cader[d], George van der Watt[e], Steve Delport[f], Jo M Wilmshurst[a,*]

[a] Department of Paediatric Neurology, Red Cross War Memorial Children's Hospital, Neuroscience Institute, University of Cape Town, South Africa
[b] University of Wisconsin School of Pharmacy, Madison, WI, USA
[c] School of Clinical Medicine, University of Cambridge, Cambridge, UK
[d] Department of Dietetics: Red Cross War Memorial Children's Hospital, Cape Town, South Africa
[e] Department of Chemical Pathology: Red Cross War Memorial Children's Hospital, Cape Town, South Africa
[f] Department of Endocrinology: Red Cross War Memorial Children's Hospital, Cape Town, South Africa
[g] Department of Paediatrics and Child Health, Muhimbili University of Health and Allied Sciences, Dar Es Salaam, Tanzania

ARTICLE INFO

Keywords:
Bone mineral metabolism
Vitamin D
Children
Africa
Antiseizure medications
Epilepsy

ABSTRACT

Purpose: The effects of antiseizure medications (ASMs) on bone metabolism is inconsistent. Most studies are in high income settings and none from sub-Saharan Africa.

Methods: A hospital based cross-sectional study in a paediatric epilepsy service with a comparison group assessed vitamin D metabolism.

Results: Seventy-five children with epilepsy and 75 comparison group were recruited. Median age for children with epilepsy was 9 years (range 1–17 years) and controls 3 years (range 1–12 years). Vitamin D deficiency occurred in 11(16.2%) children with epilepsy versus 6 (8.8%) control group ($p = 0.29$). Vitamin D insufficiency occurred in 30 (44.1%) children with epilepsy compared to 27(39.7%) control group. Children on ASMs had lower mean vitamin D levels than the control group ($p = 0.02$). Children on enzyme-inducing ASMs had lower mean vitamin D levels ($p = 0.08$), vitaminD_2 ($p = 0.0018$), vitaminD_3 ($p = 0.004$), serum phosphate levels ($p = 0.000$), and higher mean parathyroid hormone levels ($p = 0.03$) compared to controls. There was no difference in dietary intake and ancestry, although the dietary content of both groups was low in vitamin D products.

Conclusions: Low vitamin D levels were common in children from both groups, but statistically lower for the children on ASMs. Children on enzyme-inducing ASMs need screening for vitamin D deficiency. The literature supports extending this for all children on ASMs. This is the first study to report that children on enzyme-inducing ASMs have lower levels of Vitamin D_2 and D_3 levels, probably as result of increased destruction of vitamin D. Improved vitamin D intake for children in vulnerable settings is important.

1. Introduction

An estimated 50 million people worldwide have epilepsy, 85% of whom reside in the low and low-middle income countries (LMICs) [1]. In sub-Saharan Africa only 36% of children with epilepsy have access to antiseizure medications (ASMs) and 95% of this group are managed with phenobarbital [2]. One of the chronic side effects of ASMs are abnormalities in bone metabolism [3,4].

Vitamin D is a prohormone derived in the skin from 7-dehydrocholesterol to form Vitamin D_3 and from a plant derivative ergosterol to form Vitamin [5]. The Vitamin D undergoes 25-hydroxylation in the liver to form 25-hydroxyvitamin D_3 and subsequently 1α hydroxylation in the kidneys to form 1α, 25 dihydroxy-vitamin D_3. The degradation of Vitamin D_3 in the kidneys occurs through an enzyme CYP24 to form the metabolites 24, 25 dihydroxy-vitamin D_3 and 1α, 24, 25 trihydroxy-vitamin D_3 [6,7]. In vitro studies support that CYP3A4 catalyzes the hydroxylation of 125 dihydroxy vitamin D_3 into inactive metabolites in the liver and small intestines, where CYP24A1 is nearly absent, which results in decreased intestinal calcium uptake. Notably, CYP3A4 also contributes to the catabolism of 25 hydroxy vitamin D_3 by catalyzing its hydroxylation into 425 dihydroxy vitamin D_3 [8].

ASMs induce hepatic cytochrome P450 enzymes, especially

* Corresponding author.
E-mail addresses: edwardkija@yahoo.co.uk (E. Kija), jo.wilmshurst@uct.ac.za (J.M. Wilmshurst).

https://doi.org/10.1016/j.seizure.2019.04.020
Received 23 December 2018; Received in revised form 25 April 2019; Accepted 28 April 2019

Table 1
Summary of studies showing inconsistent findings on Vitamin D level and ASM by year of publication and Class of study.

Country (year)	Study Class	AED Studied	Study Design	Sample size	Main objective	Key Findings
Inaloo et al Southern Iran [26] (2018)	II	CBZ, VPA	Cross sectional	90 (plus 90 controls)	Effect on bone mineral density in Vit D supplemented ambulant children	CBZ, VPA associated with lower BMD. Risk of low BMD was also related to epilepsy as an independent variable.
Sreedharan et al India [23] (2018)	II	CBZ, VPA	Cross sectional	56 (plus 109 controls)	Effect of ASMs on Vit D level of ambulant children compared to controls	Risk of Vit D deficiency in children with monotherapy CBZ and VPA.
Dura-Travea et al Spain [24] (2018)	II	VPA, LEV	Cross sectional	90 (plus 244 controls)	Effect of ASDs on Vit D level of children compared to controls	Risk of Vit D deficiency in children with monotherapy LEV and VPA.
Fong et al Malaysia [27] (2018)	III	All ASMs – CBZ, PB, PHY, OXC, TOP, VPA, CB, LM, GABA, LEV, ZON and VB	Cross sectional	87	Determinants of low BM in ambulant children	Risk factors > 2 ASDs, underweight for age, small frame size. Study didn't state which ASDs.
Chadhuri et al India [25] (2017)	II	CBZ, CB, CLON, LM, PB, VPA, TOP.	Cross sectional	100 (plus 50 controls)	Association of vit D levels in children on monotherapy	Risk greatest with use of VPA and CBZ
Yildz et al Turkey [22] (2017)	III	VPA, CBZ, LEV, PB	Retrospective longitudinal	172	Compared baseline Vit D levels to those after longterm ASDs	Found chronic ASD use associated with increased reduction in Vit D. Did not find any difference across monotherapy with different ASDs studied.
Hasaneen et al Egypt [21] (2017)	II	"All ASMs"	Prospective cross sectional study	70 (plus 82 controls)	Assessed effects on Vit D after 6-24 months and > 24 months on ASDs	Found multiple ASDs for longer duration more associated with lower Vit D. Found same effect on Vit D from all ASDs ("new and old")
Serin et al Turkey [18] (2015)	II	LEV, CBZ, VPA	Cross sectional	59 (plus 13 controls)	Assessed BMD effects on ambulant children after 2 years of ASDs	Found no "considerable bone loss" with any of the ASDs in contrast to other studies. Further LEV was not protective of bone loss.
Yaghini et al Iran [20] (2015)	II	CBZ, PB, PRIM, VPA	Cross sectional	120	Assessed BMD in ambulant children	Found equal effects on BMD from all ASDs, whether enzyme inducers or not.
Nettekoven et al (2008) [42] Germany	III	CBZ,VPA,LVT,LMT,TMP,OXZ	Cross sectional	38 Children Mean 8.4 ± 1.7	Effect of ASDs on Vit D and markers of bone turnover	ASD was associated with low Vit D
Fuleihan et al (2008) [28] Lebanon	II	PHT, PT, CBZ, VPA, LMT, CNZ	Longitudinal	106 Adults (18-60) 88 Children (10-17)	Predictors of BMD	Low Vit D levels, BMD was decreased in adults
Bergqvist et al (2007) [43] USA	II	Newer AED and KD	Longitudinal study	45 Children Mean 5.1 ± 2.7	Vit D status in children with intractable epilepsy on ASD and KD	Newer ASD and KD associated with low Vit D
Kim SH et al (2007) [47] South Korea	II	CBZ, VPA, LMT	Longitudinal study	33 Adults (18-50)	Effect of ASD monotherapy on bone biochemical markers and BMD	CBZ associated with a decrease in BMD and Vit D
Babayigit et al (2006) [48] Turkey	II	CBZ, VPA, OXZ	Longitudinal study	68 children	Effect of ASD on biochemical markers of bone metabolism and BMD	Normal Vit D levels but decreased BMD
Tekgul et al (2006) [49] Turkey	II	CBZ, VPA, PHT	Longitudinal study	30 Children	Effect of ASD monotherapy on bone mineral status	ASD monotherapy was associated with low Vit D
Kumandas S et al (2006) [50] Israel	III	CBZ, VPA	Cross sectional Retrospective	33 Children	Effect of ASD on BMD	Vit D and BMD lower in the ASD group compared to healthy children
Nicolaidou et al (2006) [51] Greece	II	CBZ, VPA	Longitudinal study	51 children	Effect of ASD on Biochemical markers of bone metabolism	ASDs associated with low Vit D
Pack et al (2005) [52] USA	II	CBZ, LMT, PT, VPA	Longitudinal study	93 premenopausal women	Effects of ASD monotherapy on bone mineral status and BMD	Lower Vit D and BMD associated with PT
Vestergaard P et al (2004) [32] Denmark	III	PHT,CBZ,OXZ, CNZ, LMT,VPA,TG,PT,PMD,TMP,VG	Case Control	124655 fracture cases 373962 controls	Fracture risk associated with ASD	Limited increase fracture risk with ASD

(*continued on next page*)

Table 1 (*continued*)

Country (year)	Study Class	AED Studied	Study Design	Sample size	Main objective	Key Findings
Farhat G et al (2002) [3] Lebanon	III	PHT, CBZ, PT, VPA, LMT,CNZ, GBT,ETX,TMP	Cross sectional	42 Adults and 29 children	Effect ASD on Vit D levels and bone density Effect of single and multiple drugs	Low Vit D levels in over 50% of patients. Generalized seizures and polypharmacy were predictors of BMD
Tsukahara et al (2002) [14] Japan	II	CBZ, VPA, PHT, ETX, CNZ	Longitudinal study	18 children (5-16)	Effect of ASD on bone mineral status and BMD	Normal Vit D levels but decreased BMD
Guo et al (2001) [19] Canada	II	LMT, VPA	Longitudinal study	53 Children (3-17)	Long term effects of VPA and LMT on bone metabolism	Normal Vit D levels
Erbayat E et al (2000) [12] Turkey	III	CBZ, VPA	Cross Sectional	36 Children	Effect of ASD monotherapy on bone biochemical markers and BMD	ASD had no effect on bone biochemical markers or Vit D
Stephen et al (1999) [53] Scotland	III	PHT, CBZ, PT, VPA, LMT, GBT, TMP, VG	Case Control	Adults	Effect of ASD on bone mineral status and BMD	ASD was associated with decreased BMD but normal Vit D
Weinstein RS et al (1984) [54] USA	III	PHT, CBZ, PT, VPA, CNZ	Cross sectional	120 Adults	Effect of ASD on ionized Ca and Vit D	Low ionized Ca in patients on ASD but normal Vit D level

PHT: Phenobarbital CBZ: Carbamazepine PT: Phenytoin VPA: Valproic acid LMT: Lamotrigine CNZ: Clonazepam. GBT: Gabapentin ETX: Ethosuximide TMP: Topiramate OXZ: Oxcarbazepine PMD: Primidone TG: Tiagabine. LVT: Levetiracetam VG: Vigabatrin AED: Antiepileptic Drug KD: Ketogenic Diet.

CPY3A4, leading to the increased catabolism of vitamin D, causing reduction of calcium absorption from the intestine, hyperparathyroidism and increased calcium demineralization from the bones [9–13]. Despite the plausible logic behind enzyme induction and ASMs, the data is inconsistent with several studies failing to demonstrate low levels of calcium and phosphate with enzyme-inducing ASMs [14,15].

Conflicting study methodologies have challenged comparison across studies such that meta-analyses conclude that ASMs may be associated with decreased bone mineral density (BMD) in children with epilepsy [16,17]. But studies have also reported no ASM effect on BMD or vitamin D levels [12,18,19], and others found no variance in the effect of enzyme-inducing ASMs compared to non-inducing ASMs on BMD [20–22]. Some specifically highlight the increased impact from monotherapy with sodium valproate, carbamazepine and levetiracetam [23–26]. Other studies conclude that the key risks are polypharmacy and chronic ASM use [21,22,27]. Table 1 summaries some of the key cohort studies illustrating the variation in findings.

Compared to healthy controls, abnormalities in bone metabolism and an increased risk of fractures are more common in children with epilepsy who are treated with ASMs [28–31]. The fracture risk is higher for hepatic enzyme-inducing ASMs such as carbamazepine, phenobarbital and phenytoin than the non-inducers [28]. Sodium valproate, carbamazepine, phenobarbital and clonazepam also have a dose response relationship to the risk of fractures [32]. Further the dysregulation on bone metabolism reported with sodium valproate and or oxcarbazepine chronic therapy may affect physical growth [33].

This study was conducted to determine the impact of ASMs on vitamin D and secondary markers of bone turn over in children with epilepsy who attend an epilepsy service in sub-Saharan Africa.

2. Methodology

The study was undertaken at the epilepsy service at the Red Cross War Memorial Children's Hospital (RCWMCH) in Cape Town, South Africa. This is a University affiliated tertiary referral center, which is the largest children's hospital in sub-Saharan Africa. Children with epilepsy attend for a wide spectrum of health needs from those who require primary care through to those with complex quaternary needs. The data was collected over a 10-month consecutive period.

Inclusion criteria for the study were children with epilepsy attending the epilepsy service at RCWMCH who were on single or multiple ASDs for at least 3 months duration.

The comparison group were children who came for non-urgent surgical day outpatient procedures at RCWMCH. These children had routine bloods and intravenous line insertion for their surgical procedures.

Exclusion criteria were children at risk of conditions which affect bone metabolism, e.g. non-ambulatory (inappropriate for developmental age), renal diseases, hyperparathyroidism, gastrointestinal disorders, diabetes mellitus, cerebral palsy, hemiplegia and liver insufficiency. Also children on oral corticosteroids as well as those on calcium or vitamin D supplementation. Further recruited children whose Vitamin D samples proved inadequate for analysis were excluded.

All children who met the inclusion criteria were consecutively enrolled throughout the year to include the winter and summer seasons until the sample size was reached.

The sample size calculated was 63 (supplemental file Figure 1). The sample size was increased by 10% to make allowance for incomplete data, non-responses and other factors that may decrease the yield of responses. Thus, the minimum sample size was 70 for the cases. The control group had 70 patients to make up a ratio of 1:1 for cases and controls.

Data collected included demographic details, dietary intake (as recorded by registered dietician), average sunlight exposure hours, seizure onset and the types of seizures, ASMs (inclusive of initiation age,

dosage, duration, levels and so on). Blood samples were undertaken for 25-hydroxycholecalciferol, serum calcium, phosphate and parathyroid hormone.

Vitamin D insufficiency was noted at 20–29 ng/ml and Vitamin D deficiency at < 20 ng/ml [34].

Dietary assessment of the daily intake of the common dietary sources of vitamin D was undertaken by a registered dietician (SC). The food sources were assessed for frequency and average amounts of intake of common food groups such as fish, beef, eggs, dairy products (fresh milk, yoghurt and cheese) and cod liver oil. The average intake was viewed as adequate if it met the recommended minimal daily requirements of at least 400IU [35]. The 24 h food recall method was used as well to generate more information in-line with standard data capturing procedures [36].

Data entry, cleaning and analysis were done using SPSS (Statistical Package for Social Science) software version 18.

The ethical clearance to conduct the study was obtained from the University of Cape Town Faculty of Health Sciences Human Research Ethics Committee (HREC REF: 560/2014) and University of Wisconsin Institutional Review Board.

3. Results

During the study period 150 children met the inclusion criteria and were recruited. Seventy-six children were of mixed ancestry, 60 indigenous African and 14 European ancestries. Seventy-five children with epilepsy and 75 controls were recruited. The median age for the children with epilepsy was 9 years of age (range 1–17 years) and controls 3 years of age (range 1–12 years). The male to female ratio for the children with epilepsy was 1.3:1 and controls 4:1. The majority of the controls were boys because the common cause of admission for the day surgical ward is circumcision.

Of the total, 68 cases and 68 controls had adequate samples for the Vitamin D studies. The mean vitamin D values for children with epilepsy was lower than the control group (27.4 ng/ml versus 30.7 ng/ml), this was statistically significant. However, when the number of children from the groups with deficiency or insufficiency was assessed, the proportion of children with epilepsy on ASMs with vitamin D levels less than 20 ng/ml, i.e., vitamin D deficiency, was 16.2% (n = 11) compared to 8.8% (n = 6) in the control group, this trend was not statistically significant (p = 0.29). Similarly vitamin D insufficiency was identified in a further 44% (n = 30) of the children on ASMs and 40% (n = 27) of the control group. This equated to 60.2% of the children with epilepsy having vitamin D deficiency or insufficiency, compared to 48.5% of the control group, this was also not statistically significant. As such vitamin D deficiency or insufficiency was found in a high proportion of the population from both groups, but children with epilepsy were statistically more likely have lower vitamin D levels, although not necessary in the deficiency or insufficiency range.

The mean Vitamin D, 24,25$(OH)_2D_3$, serum calcium, and serum phosphate were all statistically found to be significantly lower in children with epilepsy compared to the control group (Table 2). One clinically asymptomatic five year old boy in the control group was hypocalcaemic, with a raised serum phosphate and normal vitamin D levels. He was referred to endocrinology for further assessment based on these findings.

The vitamin D2 and D3 were significantly lower in patients with epilepsy who were on cytochrome P450 inducers (carbamazepine and phenobarbital) compared to those on cytochrome P450 non-inducers and controls (Table 3).

Twenty-four children were prescribed enzyme-inducing ASMs (carbamazepine and phenobarbital) and 44 were prescribed non-enzyme inducers (Table 4). Fifty-two children were on one to two ASMs and 16 were on three or more ASMs. The type of ASMs, number of ASMs, seizure semiology (focal or generalized) and whether a child had a known epilepsy syndrome or not, were not associated with vitamin D deficiency. When the data was analysed for those on monotherapy (n = 26) compared to two or more ASMs there was no statistical difference found. Median duration on ASMs was 63 months (range 12–176 months).

There was no difference in the dietary intake between children with epilepsy on ASMs and controls. The 24 h recall suggested moderate intake of vitamin D foods such as one to two cups of fresh milk daily, followed by eggs up to three times a week. However other foods such as fish and beef were generally only eaten once a week and no children had cod liver oil.

Among children with epilepsy, the mean vitamin D level was 25.9 ng/ml ± 7.8 and 29.1 ng/ml ± 8.6 (p value = 0.14) in children with African (n = 60) and mixed ancestry (n = 76) respectively. Further the ancestry of the children was not associated with any statistical difference in vitamin D levels across the control and the epilepsy group.

4. Discussion

Overall vitamin D deficiency was evident in more children with epilepsy (16%) than without (8.8%), but this was not statistically significant. Vitamin D insufficiency was present in similar proportions of the children with epilepsy compared to the control group. Children with epilepsy on enzyme-inducing ASMs had lower mean vitamin D levels, lower mean serum phosphate and a higher mean parathyroid hormone levels compared to the control group.

This study established that vitamin D deficiency or insufficiency is common in children with epilepsy on ASMs in this region of sub-Saharan Africa, occurring in 60.3% of the study group. Similar results were found in other studies undertaken in Australia, USA, Denmark, Spain, Turkey, Israel, Iran, Egypt, and Korea where the prevalence of vitamin D deficiency or insufficiency in children with epilepsy ranged between 50–66% [20–24,26,32,34,37]. As demonstrated since vitamin D deficiency is common in children with epilepsy, where there is capacity, all patients on ASMs should be investigated for vitamin D deficiency and supplemented accordingly. In a resource limited setting this may be challenging to implement and clinicians may need to resort to promotion of a healthy diet and access to adequate daylight time. The health economics of routine vitamin D supplementation of children on long term ASMs versus serial screening of vitamin D levels and even DEXA scans is yet to be delineated. Development of a clinical practice guideline would be a useful aid to assist motivating for local resources to enable appropriate screening and management of children with epilepsy on ASM.

Vitamin deficiency or insufficiency was also common in the control group affecting 48.5%. Key vitamin D promoting foods were considered lacking for the children in both groups, namely fish, beef and cod liver oil. This may have compounded the worryingly low levels identified in many of the children and highlights the need to include details of dietary intake in the clinical assessment of children based in resource limited settings. Whilst children from both groups were at risk of low vitamin D levels, those in the epilepsy group had statistically significant lower levels.

In this study children with epilepsy on ASMs had significantly lower levels of serum calcium and phosphate levels compared to the control group. Similar findings were seen in a case control study in Nigeria which found children with epilepsy had lower serum calcium levels compared to the control group but there was no difference in serum phosphate between the two groups [38]. A study in Turkey also found children on carbamazepine and sodium valproate had lower serum calcium levels compared to the control group [12]. Other reports have shown that hypocalcaemia and hypophosphataemia are associated with the use of ASMs [11,12,39]. ASDs, particularly cytochrome P450 enzyme inducers, cause reduction of vitamin D leading to impaired bone metabolism and low serum calcium and phosphate.

This study showed that children with epilepsy on cytochrome P450

Table 2
Biochemical parameters of children with epilepsy compared to the control group.

	Patients with Epilepsy n = 75	Control Group n = 75	Patient Mean – Control Mean (95% CI)	p-value	Normal ranges
Vitamin D deficiency (%)	16% n = 11	8.8% n = 6			**NA**
Vitamin D (ng/ml)	**N = 68**	**N = 68**			
Mean ± SD	27.56 ± 8.6	30.30 ± 7.4	−2.74	**0.024**	> 30 ng/ml
(95% CI)	(25.48, 29.64)	(28.51, 32.09)	(-5.46, -0.02)	**0.049	
24,25(OH)$_2$D$_3$ (ng/ml)*	**N = 68**	**N = 68**			
Mean ± SD	2.14 ± 0.9	2.58 ± 1.1	−0.44	**0.014**	–
(95% CI)	(1.92, 2.36)	(2.31, 2.85)	(-0.78, -0.1)	**0.012	
25(OH)D$_2$ (ng/ml)*	**N = 68**	**N = 68**			
Mean ± SD	0.39 ± 0.3	0.4 ± 0.1	−0.01	0.78	–
(95% CI)	(0.32, 0.46)	(0.38, 0.42)	(-0.09, 0.07)	**0.79	
25(OH)D$_3$/24,25(OH)2D3*	**N = 68**	**N = 68**			
Mean ± SD	14.17 ± 4.3	13.15 ± 3.9	1.02	0.152	–
(95% CI)	(13.13, 15.21)	(12.21, 14.09)	(-0.37, 2.41)	**0.149	
25(OH)D$_3$/D$_3$*	**N = 68**	**N = 68**			
Mean ± SD	32.71 ± 2.4	34.26 ± 2.5	−1.55	0.715	–
(95% CI)	(32.13, 33.29)	(33.65, 34.87)	(-2.38, -0.72)	**0.00033	
Serum Calcium	**N = 73**	**N = 69**			2.23 – 2.58
Mean ± SD	2.38 ± 0.09	2.34 ± 0.12	0.04	**0.026**	mmol/L
(95% CI)	(2.36, 2.4)	(2.31, 2.37)	(0.0046, 0.075)	**0.027	
Calcium Corrected	**N = 71**	**N = 68**			2.2-2.6
Mean ± SD	2.34 ± 0.08	2.32 ± 0.12	0.02	0.553	mmol/L
(95% CI)	(2.32, 2.36)	(2.29, 2.35)	(-0.01, 0.05)	**0.252	
Serum Phosphate	**N = 72**	**N = 68**			0.81 – 1.49
Mean ± SD	1.39 ± 0.2	1.76 ± 0.7	−0.37	**0.000**	mmol/L
(95% CI)	(1.34, 1.44)	(1.59, 1.93)	(-0.55, -0.19)	****0.000071**	
Parathyroid Hormone	**N = 68**	**N = 52**			1.6 – 6.9
Mean ± SD	3 ± 1.51	2.7 ± 0.97	0.3	0.187	pmol/L
(95% CI)	(2.63, 3.37)	(2.43, 2.97)	(-0.15, 0.75)	**0.189	

enzyme-inducing ASMs had higher mean parathyroid hormone levels compared to those on cytochrome P450 enzyme non-inducing drugs. Children with epilepsy on enzyme-inducing ASDs had also higher parathyroid hormone levels compared to the control group. Similar findings were shown in cross sectional studies in Malaysia and Australia where children with epilepsy on ASMs had elevated parathyroid hormone levels [34,40]. In our study, serum vitamin D levels were significantly lower in children on cytochrome P450 enzyme-inducing ASMs compared to the control group. Serum vitamin D levels were also lower in children with cytochrome P450 enzyme-inducing ASMs compared to those on cytochrome P450 enzyme non-inducing drugs, however the difference did not reach statistical significance.

The mean 25(OH)D_2 and mean 24,25(OH)$_2$D$_3$ were significantly lower in children with epilepsy on enzyme-inducing ASMs compared to those on enzyme non-inducing drugs and the control group. The 25(OH)D_2 is derived from hydroxylation of ergosterol and 24,25(OH)$_2$D$_3$ a metabolite of Vitamin D is produced from hydroxylation of 25(OH)D_3 [6,41]. Enzyme-inducing ASMs cause increased

Table 4
Clinical Parameters and Vitamin D levels in children with epilepsy.

	Vitamin D Level		
	Less than 20 ng/ml Number %	Greater than 20 ng/ml Number %	P value
Enzyme Inducers			
Yes (N = 24)	6 54.5	18 31.6	0.144
No (N = 44)	5 45.6	39 68.4	
No of ASMs used			
1-2 (N = 52)	9 81.8	43 75.4	0.648
> 3 (N = 16)	2 18.2	14 14.6	
Seizure Semiology			
Focal (N = 39)	7 63.6	32 56.1	0.645
Generalized (N = 29)	4 36.4	25 23.9	
Epilepsy Syndrome			
Yes (N = 18)	3 27.3	15 26.3	1.0
No (N = 50)	8 72.7	42 73.7	

Table 3
Comparison Vitamin D, D2, D3 and Parathyroid Hormone in children with Epilepsy on cytochrome P450 inducers, inhibitors and controls.

	Cytochrome P450 Inducers n = 14	Cytochrome P450 Non-inducers n = 48	P value	Cytochrome P450 Inducers n = 14	Controls n = 75	P value	Normal range
Mean Vitamin D	24.67 ± 11.4	27.96 ± 7.7	0.23	24.67 ± 11.4	30.72 ± 7.4	**0.08**	**> 30 ng/ml**
Mean Vitamin D2*	0.25 ± 0.07	0.44 ± 0.37	**0.000004**	0.25 ± 0.07	0.4 ± 0.17	**0.0018**	–
Mean Vitamin D3*	1.61 ± 1.06	2.26 ± 0.86	**0.028**	1.61 ± 1.06	2.58 ± 0.86	**0.004**	–
Corrected Serum Calcium	2.29 ± 0.06	2.36 ± 0.08	**0.009**	2.29 ± 0.06	2.33 ± 0.12	0.15	2.2-2.6 mmol/L
Serum Phosphate	1.41 ± 0.23	1.38 ± 0.25	0.7	1.41 ± 0.23	1.77 ± 0.7	**0.0015**	**0.81-1.49** mmol/L
Parathyroid Hormone	4.47 ± 2.33	2.64 ± 0.99	**0.02**	4.47 ± 2.33	2.7 ± 0.97	**0.03**	**1.6-6.9** pmol/L

destruction of both forms of vitamin D, thus leading to little formation of $25(OH)D_2$ and $24,25(OH)_2D_3$. This is the first study to report that children on enzyme-inducing ASMs have lower levels of $25(OH)D_2$ and $24,25(OH)_2D_3$ levels probably as result of increased destruction of vitamin D.

The median duration of ASMs was five and a half years so the group had established time period of epilepsy and prolonged time period of prescribed ASMs. However, this study did not show difference in the number of ASMs used between children with vitamin D deficiency and those without, compared to other studies which have shown polytherapy to be associated with lower vitamin D levels [19,21,22,27,28,40,42,43]. Our cohort was recruited from a tertiary center which manages a greater proportion of children with severe forms of epilepsy requiring multiple ASMs. As such the number of children on monotherapy was less than the number on multiple ASMs which may have underpowered this analysis.

The type of seizures, either focal or generalized were not associated with the vitamin D level in this study. Similar findings were reported in cross sectional studies of children with epilepsy conducted in Australia and Malaysia [34,40]. The data from this study further supports that seizure type is not associated with the vitamin D level.

This study also found that vitamin D levels were not associated with whether a child had an epilepsy syndrome. Similar results were demonstrated by a hospital based cross sectional study conducted in Australia [34]. This further emphasizes that vitamin D level is not affected by the type of epilepsy or its aetiology, but rather by the choice of ASMs a patient receives.

Similar to the studies conducted in Australia and Malaysia, this study showed no difference in the dietary intake between children with epilepsy with vitamin D deficiency and those without [34,40]. The primary source of vitamin D is sunshine and dietary intake contributes less than 10% of the requirement [44]. This study further reiterates that the key lifestyle determinant of the vitamin D level is sun exposure and not dietary intake. Sufficient Vitamin D can be produced in fair skin individuals following 10 to 15 min of sun exposure between 10am and 3 pm in summer, spring and autumn. Longer duration of sun exposure will be required for individuals of African descent [45].

Studies from South East Asia have shown that ethnicity does affect the vitamin D levels. A hospital based cross sectional study in Malaysia showed children of Indian ethnicity had lower vitamin D levels compared to the other ethnic groups [40]. A multinational study conducted in Indonesia, Thailand, Vietnam and Malaysia also showed children of Indian ethnicity had lower vitamin D levels compared to the other ethnic groups [46]. In contrary, this study showed no difference in the mean vitamin D levels between the children of African and mixed ancestry. Thus ethnicity may not be a contributing factor to the Vitamin D level in children in this sub-Saharan African region.

Literature on the vitamin D metabolism and ASMs in individuals with epilepsy has revealed inconsistent results (Table 1). Most of these studies were undertaken in Europe and North America in patients who were well controlled on monotherapy.

This is the first study to assess vitamin D levels in children with epilepsy in a sub-Saharan African setting. It is the first study to assess not only parameters of bone metabolism but also other vitamin D metabolites and dietary intake. Previous studies assessed vitamin D levels and other parameters of bone metabolism without including the vitamin D metabolites [34,40,42].

Limitations of this study included that the control group was significantly younger which may have affected the results. However, the effect should have been small since age does not affect bone metabolism before puberty. Further the limited access to children on monotherapy limited delineating the effects of individual ASMs on vitamin D levels.

In conclusion many of the children from both the control and the study group had low vitamin D levels, but this was statistically more marked for the children with epilepsy on ASM. Children with epilepsy on enzyme-inducing ASM have lower mean of vitamin D levels compared to those on enzyme non-inducing drugs. Our data supported that children on enzyme-inducing ASM should be investigated and managed for vitamin D deficiency (using 25(OH) vitamin D). According to the literature there may be a case for extending this to all children on ASMs. Further, holistic care should be implemented for children in resource limited settings where dietary intake is potentially insufficient and correction of this could protect vulnerable children from exacerbation of inadequate vitamin D levels.

COI and Funding Declaration

The authors declare no conflict of interests and this study was partly funded by a Global Burden of Disease award from International Child Neurology Association (ICNA).

Author Contributions

Edward Kija, Jo Wilmshurst, George Van der Watt, Shihaam Cader and Steve Delport were involved in conceiving and designing of the study. Edward Kija and Shihaam Cader collated the data. Barry Gidal and Marc Drezner facilitated the analysis of Vitamin D levels. Edward Kija and Alex Shapson-Coe conducted the data analysis. Edward Kija wrote the first draft and Jo Wilmshurst, and Barry Gidal reviewed and contributed to the manuscript.

Acknowledgements

This research formed the thesis component towards the Mphil degree in paediatric neurology successfully completed by Edward Kija. The children and their parents/guardians who were involved into this study. The Department of Neurology and Department of Paediatric Surgery. The African Paediatric Fellowship Programme for supporting Edward Kija's training in South Africa. Also Dr Marc Drezner of School of Medicine and Public Health, University of Wisconsin, Madison, WI, USA, who assisted with the analysis of the bone mineral studies.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.seizure.2019.04.020.

References

[1] Ngugi AK, Bottomley C, Kleinschmidt I, Sander JW, Newton CR. Estimation of the burden of active and life-time epilepsy: a meta-analytic approach. Epilepsia 2010;51:883–90.
[2] Kariuki SM, Matuja W, Akpalu A, et al. Clinical features, proximate causes, and consequences of active convulsive epilepsy in Africa. Epilepsia 2014;55:76–85.
[3] Farhat G, Yamout B, Mikati MA, Demirjian S, Sawaya R, El-Hajj Fuleihan G. Effect of antiepileptic drugs on bone density in ambulatory patients. Neurology 2002;58:1348–53.
[4] Sheth RD, Binkley N, Hermann BP. Progressive bone deficit in epilepsy. Neurology 2008;70:170–6.
[5] DeLuca HF. Overview of general physiologic features and functions of vitamin D. Am J Clin Nutr 2004;80:1689S–96S.
[6] Jones G, Strugnell SA, DeLuca HF. Current understanding of the molecular actions of vitamin D. Physiol Rev 1998;78:1193–231.
[7] Bikle DD. Vitamin D metabolism, mechanism of action, and clinical applications. Chem Biol 2014;21:319–29.
[8] Prytuła A, Cransberg K, Raes A. CYP3A4 is a crosslink between vitamin D and calcineurin inhibitors in solid organ transplant recipients: implications for bone health. Pharmacogenomics J 2017;17:481–7.
[9] Zhou C, Assem M, Tay JC, et al. Steroid and xenobiotic receptor and vitamin D receptor crosstalk mediates CYP24 expression and drug-induced osteomalacia. J Clin Invest 2006;116:1703–12.
[10] Shellhaas RA, Barks AK, Joshi SM. Prevalence and risk factors for vitamin D insufficiency among children with epilepsy. Pediatr Neurol 2010;42:422–6.
[11] Ali II, Schuh L, Barkley GL, Gates JR. Antiepileptic drugs and reduced bone mineral density. Epilepsy Behav 2004;5:296–300.
[12] Erbayat Altay E, Serdaroğlu A, Tümer L, Gücüyener K, Hasanoğlu A. Evaluation of bone mineral metabolism in children receiving carbamazepine and valproic acid. J Pediatr Endocrinol Metab 2000;13:933–9.

[13] Wang Z, Lin YS, Dickmann LJ, et al. Enhancement of hepatic 4-hydroxylation of 25-hydroxyvitamin D3 through CYP3A4 induction in vitro and in vivo: implications for drug-induced osteomalacia. J Bone Miner Res 2013;28:1101–16.
[14] Tsukahara H, Kimura K, Todoroki Y, et al. Bone mineral status in ambulatory pediatric patients on long-term anti-epileptic drug therapy. Pediatr Int 2002;44:247–53.
[15] Kulak CA, Borba VZ, Bilezikian JP, Silvado CE, Paola L, Boguszewski CL. Bone mineral density and serum levels of 25 OH vitamin D in chronic users of anti-epileptic drugs. Arq Neuropsiquiatr 2004;62:940–8.
[16] Zhang Y, Zheng YX, Zhu JM, Zhang JM, Zheng Z. Effects of antiepileptic drugs on bone mineral density and bone metabolism in children: a meta-analysis. J Zhejiang Univ Sci B 2015;16:611–21.
[17] Vestergaard P. Effects of antiepileptic drugs on bone health and growth potential in children with epilepsy. Paediatr Drugs 2015;17:141–50.
[18] Serin HM, Koç ZP, Temelli B, Esen İ. The bone mineral content alterations in pediatric patients medicated with levetiracetam, valproic acid, and carbamazepine. Epilepsy Behav 2015;51:221–4.
[19] Guo CY, Ronen GM, Atkinson SA. Long-term valproate and lamotrigine treatment may be a marker for reduced growth and bone mass in children with epilepsy. Epilepsia 2001;42:1141–7.
[20] Yaghini O, Tonekaboni SH, Amir Shahkarami SM, Ahmad Abadi F, Shariat F, Abdollah Gorji F. Bone mineral density in ambulatory children with epilepsy. Indian J Pediatr 2015;82:225–9.
[21] Hasaneen B, Elsayed RM, Salem N, et al. Bone mineral status in children with epilepsy: biochemical and radiologic markers. J Pediatr Neurosci 2017;12:138–43.
[22] Yildiz EP, Poyrazoglu Ş, Bektas G, Kardelen AD, Aydinli N. Potential risk factors for vitamin D levels in medium- and long-term use of antiepileptic drugs in childhood. Acta Neurol Belg 2017;117:447–53.
[23] Sreedharan M, Devadathan K, Mohammed Kunju PA, et al. Vitamin d deficiency in Ambulant Children on carbamazepine or sodium valproate monotherapy. Indian Pediatr 2018;55:307–10.
[24] Durá-Travé T, Gallinas-Victoriano F, Malumbres-Chacón M, Moreno-Gónzalez P, Aguilera-Albesa S, Yoldi-Petri ME. Vitamin D deficiency in children with epilepsy taking valproate and levetiracetam as monotherapy. Epilepsy Res 2018;139:80–4.
[25] Chaudhuri JR, Mridula KR, Rathnakishore C, Balaraju B, Bandaru VS. Association of 25-Hydroxyvitamin d deficiency in pediatric epileptic patients. Iran J Child Neurol 2017;11:48–56.
[26] Inaloo S, Paktinat M, Saki F, et al. Bone mineral density loss in ambulatory children with epilepsy in spite of using supplemental vitamin D in Southern Iran: a case-control study. J Bone Miner Metab 2018.
[27] Fong CY, Kong AN, Noordin M, Poh BK, Ong LC, Ng CC. Determinants of low bone mineral density in children with epilepsy. Eur J Paediatr Neurol 2018;22:155–63.
[28] El-Hajj Fuleihan G, Dib L, Yamout B, Sawaya R, Mikati MA. Predictors of bone density in ambulatory patients on antiepileptic drugs. Bone 2008;43:149–55.
[29] Gniatkowska-Nowakowska A. Fractures in epilepsy children. Seizure 2010;19:324–5.
[30] Souverein PC, Webb DJ, Petri H, Weil J, Van Staa TP, Egberts T. Incidence of fractures among epilepsy patients: a population-based retrospective cohort study in the General Practice Research Database. Epilepsia 2005;46:304–10.
[31] Simm PJ, Seah S, Gorelik A, et al. Impaired bone and muscle development in young people treated with antiepileptic drugs. Epilepsia 2017;58:1931–8.
[32] Vestergaard P, Rejnmark L, Mosekilde L. Fracture risk associated with use of anti-epileptic drugs. Epilepsia 2004;45:1330–7.
[33] Lin CM, Fan HC, Chao TY, et al. Potential effects of valproate and oxcarbazepine on growth velocity and bone metabolism in epileptic children- a medical center experience. BMC Pediatr 2016;16:61.
[34] Fong CY, Riney CJ. Vitamin D deficiency among children with epilepsy in South Queensland. J Child Neurol 2014;29:368–73.
[35] Administration. FaD. A Food Labeling Guide. Guidance for Industry. Center for Food Safety and Applied Nutrition 2013.
[36] Bailey RL, Dodd KW, Goldman JA, et al. Estimation of total usual calcium and vitamin D intakes in the United States. J Nutr 2010;140:817–22.
[37] Lee YJ, Park KM, Kim YM, Yeon GM, Nam SO. Longitudinal change of vitamin D status in children with epilepsy on antiepileptic drugs: prevalence and risk factors. Pediatr Neurol 2015;52:153–9.
[38] Oladipo OO, Lesi FE, Ezeaka VC. Plasma magnesium and calcium levels in children with epilepsy in lagos. Niger Postgrad Med J 2007;14:26–9.
[39] Sheth RD. Metabolic concerns associated with antiepileptic medications. Neurology 2004;63:S24–9.
[40] Fong CY, Kong AN, Poh BK, et al. Vitamin D deficiency and its risk factors in Malaysian children with epilepsy. Epilepsia 2016;57:1271–9.
[41] Beckman MJ, Tadikonda P, Werner E, Prahl J, Yamada S, DeLuca HF. Human 25-hydroxyvitamin D3-24-hydroxylase, a multicatalytic enzyme. Biochemistry 1996;35:8465–72.
[42] Nettekoven S, Ströhle A, Trunz B, et al. Effects of antiepileptic drug therapy on vitamin D status and biochemical markers of bone turnover in children with epilepsy. Eur J Pediatr 2008;167:1369–77.
[43] Bergqvist AG, Schall JI, Stallings VA. Vitamin D status in children with intractable epilepsy, and impact of the ketogenic diet. Epilepsia 2007;48:66–71.
[44] Bringhurst RF, Demay MB, Krane SM, Kronenberg HM. Bone and mineral metabolism in health and disease. In: Kasper D, Fauci A, Hauser S, Longo D, Jameson JL, Loscalzo J, editors. Harrison's principles of internal medicine. 19e edNew York: McGraw-Hill Medical Publishing Division; 2001. p. 423–5.
[45] Rovner AJ, O'Brien KO. Hypovitaminosis D among healthy children in the United States: a review of the current evidence. Arch Pediatr Adolesc Med 2008;162:513–9.
[46] Poh BK, Rojroongwasinkul N, Nguyen BK, et al. 25-hydroxy-vitamin D demography and the risk of vitamin D insufficiency in the South East Asian Nutrition Surveys (SEANUTS). Asia Pac J Clin Nutr 2016;25:538–48.
[47] Kim SH, Lee JW, Choi KG, Chung HW, Lee HW. A 6-month longitudinal study of bone mineral density with antiepileptic drug monotherapy. Epilepsy Behav 2007;10:291–5.
[48] Babayigit A, Dirik E, Bober E, Cakmakci H. Adverse effects of antiepileptic drugs on bone mineral density. Pediatr Neurol 2006;35:177–81.
[49] Tekgul H, Serdaroglu G, Huseyinov A, Gökben S. Bone mineral status in pediatric outpatients on antiepileptic drug monotherapy. J Child Neurol 2006;21:411–4.
[50] Kumandas S, Koklu E, Gümüs H, et al. Effect of carbamezapine and valproic acid on bone mineral density, IGF-I and IGFBP-3. J Pediatr Endocrinol Metab 2006;19:529–34.
[51] Nicolaidou P, Georgouli H, Kotsalis H, et al. Effects of anticonvulsant therapy on vitamin D status in children: prospective monitoring study. J Child Neurol 2006;21:205–9.
[52] Pack AM, Morrell MJ, Marcus R, et al. Bone mass and turnover in women with epilepsy on antiepileptic drug monotherapy. Ann Neurol 2005;57:252–7.
[53] Stephen LJ, McLellan AR, Harrison JH, et al. Bone density and antiepileptic drugs: a case-controlled study. Seizure 1999;8:339–42.
[54] Weinstein RS, Bryce GF, Sappington LJ, King DW, Gallagher BB. Decreased serum ionized calcium and normal vitamin D metabolite levels with anticonvulsant drug treatment. J Clin Endocrinol Metab 1984;58:1003–9.