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Vitamin D supplementation and bone markers in ambulatory children on long-term valproic acid therapy. A prospective interventional study

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ABSTRACT

Purpose: Our aim was to investigate any adverse effects of long-term valproic acid (VPA) therapy on bone biochemical markers in ambulatory children and adolescents with epilepsy, and the possible benefits of vitamin D supplementation on the same markers.
Methods: In this single center, the prospective interventional study levels of 25-hydroxyvitamin D (25OHD) and the bone turnover indices of Crosslaps (CTX), total alkaline phosphatase (tALP), osteoprotegerin (OPG), and the receptor activator for nuclear factor kB (RANK) ligand (sRANKL) were assessed before and after one year of vitamin D intake (400 IU/d) and were compared with those of clinically healthy controls. Fifty-four ambulatory children with mean (±standard deviation [SD]) age 9.0 ± 4.5 yrs on VPA (200–1200 mg/d) long-term monotherapy (mean: 3.2 ± 2.6 yrs) were studied, before and after a year's vitamin D intake (400 IU/d).
Results: Nearly half of the cases were vitamin D insufficient/deficient with mean levels 23.1 ± 12.8 vs 31.8 ± 16.2 ng/mL of controls ($p = 0.004$) and after the year of vitamin D intake increased to 43.2 ± 21.7 ng/mL ($p < 0.0001$). In parallel, serum CTX and tALP had a decreasing trend approaching control levels but OPG and sRANKL did not change and were not different from controls. However, after vitamin D intake, a positive correlation was seen between 25OHD and OPG but not before.
Conclusions: The findings imply a higher bone turnover in the young patients on long-term VPA therapy that decreased after vitamin D intake.

1. Introduction

The incidence of epilepsy in childhood and adolescence is quite high and concerns 2.5–7% of the ages up to 18 years, who are in need of treatment. For this, there are several antiepileptic drugs (AEDs) like the classic ones (valproic acid [VPA], carbamazepine, phenobarbital, and phenytoin) and those of a newer generation (lamotrigine, oxcarbamazepine, topiramate, gabapentin, levetiracetam).

However, since the 70s, the long-term antiepileptic therapy started to be associated with bone and vitamin D disorders. The association between epilepsy, AEDs, bone metabolism, and vitamin D status has been recognized for more than 30 years [1].

Although there are some conflicting reports regarding bone mineral density among children with varying epilepsy syndromes treated with AEDs, many studies report clinically significant reductions in bone mineral density [2–4]. This is so even in the case of monotherapy with VPA which is a widely used drug in this age group and very effective for a broad range of seizure types [3–6]. Also, even though reports agree in that vitamin D insufficiency/deficiency is quite high in this population [7,8], those implicating vitamin D deficiency to bone complications are controversial [8–10]. In addition, information about the possible effects of vitamin D replenishment in these patients is scarce.

Vitamin D insufficiency is quite prevalent even among ambulatory children whose epilepsy is well-controlled on monotherapy. Although VPA is not an enzymatic inducer and should not interfere with vitamin D metabolism, most studies with this drug have shown decreased 25OHD levels [7,10–12].

Childhood is a critical time for bone mineralization, since the peak bone mass is acquired up to the end of adolescence [13]. It is also known the significant role that vitamin D plays in the skeleton's growth, as well as its positive correlations between vitamin D and Bone Mineral Density (BMD) across a wide range of vitamin D levels reported in the general population in the National Health and Nutrition Examination Survey (NHANES) study [14].

The abnormalities in bone metabolism induced by VPA are still conflicting and difficult to evaluate because of widely varying study designs [15,16]. Although it is not an enzyme inhibitor drug and should not

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affect the cytochrome P450 enzymes involved in the vitamin D metabolism, it is believed to affect calcium (Ca) balance, by reducing its intestinal absorption and renal loss together with loss of phosphate (Pi). It also seems to have a direct effect on bone turnover by inhibiting osteoblasts and stimulating osteoclasts [17].

Therefore, it is of importance to continue searching further for possible factors/mechanisms involved in the process of VPA treatment of these young patients with the old (alkaline phosphatase, Crosslaps [CTX]) and some new bone biochemical markers like osteoprotegerin (OPG) and the receptor activator for nuclear factor kB (RANK) ligand (sRANKL). This may help in minimizing the adverse effects of the antiepileptic therapy on their bone mass.

Osteoprotegerin is a glycoprotein soluble member of the tumor necrosis factor (TNF) receptor superfamily and is produced by many different tissues and cell types including osteoblasts. It functions as a decoy receptor for the receptor activator for nuclear factor kB (RANK) ligand (RANKL), a member of the TNF superfamily that is expressed on the surfaces of osteoblast lineage cells and is an obligate requirement for osteoclast formation and function. When OPG binds to RANKL, it neutralizes its proresorptive activity by preventing it binding to its physiologic effect or receptor, RANK, thus inhibiting osteoclastogenesis [18]. Osteoprotegerin may also promote bone regeneration and enhance new bone formation [19].

Studies to date for possible effects of vitamin D supplementation on bone metabolism in children on antiepileptic therapy are very limited, inconsistent, and only one recent in adults/adolescents [20]. Even though most recommend the prescription of vitamin D supplementation, there is still a debate regarding the optimal dose and its exact impact on bone mineralization [21].

Therapies that modulate bone turnover in other disorders have been reported to influence OPG and RANKL production [22,23]. Investigation of the possible beneficial effects of vitamin D supplementation in the bone metabolism of these children is of great importance for two reasons. First, because they constitute a large part of the general youth population and second, due to the fact that the maximum bone density is achieved up to the end of adolescence.

The aim of the present study was to study the effects of long-term monotherapy with VPA on some new biochemical bone markers as well as old ones in children and adolescents, and also to investigate any possible beneficial effects of vitamin D supplementation on the same markers. For this goal, we determined serum markers of bone turnover including proteins of the RANK/RANKL/OPG system, total alkaline phosphatase (tALP), and CTX in children up to 18 yrs of age before and one year after vitamin D intake (400 IU/d) who were on long-term monotherapy with VPA.

2. Methods

2.1. Study design-subjects

This was a prospective interventional study. Patients were recruited from the Epilepsy Pediatrics Clinic of the University Hospital of Ioannina. Fifty-four ambulatory children up to 18 yrs of age (mean: 9.0 ± 4.5 yrs; median: 9.0) monitored for epilepsy who were treated with VPA for over 12 months (mean: 3.2 ± 2.6 yrs; median: 2.5) with dosages (200–1200 mg/d; median: 700) were included in the study. All children were seizure-free. None of them had seizures that had been treated with other AEDs before. All the diagnoses were made by one neurologist (I.N.) on the basis of clinical history and seizure semiology, electroencephalography (EEG), cerebral computerized tomography (CT) scan, or magnetic resonance imaging (MRI). No restrictions were based on the etiology or localization of the seizures. Exclusion criteria were medical treatment with substances that have a known impact on bone metabolism (e.g., corticosteroids, bis-phosphonates, calcitonin), other risk factors for secondary osteoporosis such as endocrine diseases (e.g., diabetes mellitus), inflammatory diseases (e.g., rheumatoid arthritis), malignancies, malabsorption (e.g., Crohn disease), or kidney diseases. A group of 55 children of comparable age (8.3 ± 4.3; median: 8.0) who attended the pediatric clinics for programmed follow-up either for radiological investigation (urinary tract ultrasound or cystourethrography) because of urinary infections or for investigations for nocturnal enuresis, headaches, episodes of loss of consciousness, chronic abdominal pain, and paleness were used as controls. None of them was on any medication at that time. Both patients and controls came from the same area of Epirus.

Evaluations were performed during the follow-up visits, and samples were taken at the same time with the routine samples for drug monitoring and other tests just before the start of vitamin D (400 IU/d) and 12 months after. Body weight and height were recorded at each visit, and body mass index (BMI) was calculated as BMI = mass [kg] / (height [m])^2. Blood samples collected to determine serum levels of Ca, Pi, tALP, OPG, sRANKL, and CTX were centrifuged, and serum was stored at −20 to −30 °C until further processing.

2.2. Standard protocol approvals, registrations, and patient consents

The study protocol was approved by the Institutional Review Board of the University Hospital of Ioannina. Informed consent was obtained by the parents according to the local regulations.

2.3. Biochemical analysis

Serum analysis of sRANKL and OPG was performed according to the protocols using commercially available kits from BioVendor (Heidelberg, Germany) with sandwich enzyme immunoassays. Detection limit for sRANKL was 0.4 pmol/L; intra-assay coefficient of variation (CV) was 11% at the level of 370 pmol/L; and inter-assay CV was 12% at the level of 310 pmol/L. Osteoprotegerin detection limit was 0.03 pmol/L, intra-assay CV 3.8%, and inter-assay CV was 7% at the level of 4.8 pmol/L. Serum CTX was determined according to the protocol of commercially available Enzyme-Linked Immunosorbent Assay (ELISA) kit from Immunodiagnostic Systems (IDS) (Frankfurt, Germany). Detection limit was 0.02 ng/mL, intra-assay CV was 1.8%, and inter-assay CV was 2.5% at the level of 2.0 ng/mL. Serum Ca, P, and ALP were analyzed on an Automatic Biochemical Analyzer (AXSYM, Abbott).

2.4. Statistical analysis

The results are shown as mean ± standard deviation (SD) and median, since the values did not show normal distribution. The distribution of data was checked using the Kolmogorov–Smirnov test. The Mann–Whitney nonparametric test was used as appropriate for identifying the differences between groups. Bivariate correlations were analyzed using the Pearson's correlation coefficients (r) to identify associations among the parameters, like duration of treatment, VPA dosage, 25OHD levels, and the other biochemical indices of bone turnover. A value of $p < 0.05$ was considered statistically significant.

3. Results

As shown in Table 1, the patient's characteristics were comparable to those of controls, and their BMI was in the normal range. Table 2 shows the results of the biochemical parameters studied that are expressed as means ± SDs and median. Serum basal 25OHD levels were significantly lower than of controls (23.1 ± 12.8 vs 31.8 ± 16.2 ng/mL; $p = 0.004$), and at individual level, nearly half of the patients (38%) had levels < 20 ng/mL, nearly twice the number of controls (20%).

However, their serum Pi levels did not differ from the controls. Despite the lower than controls mean serum Ca levels (9.9 ± 0.5 vs 10.2 ± 0.4 mg/dl; $p = 0.027$) (Table 2), none of the patients had values below 8.5 mg/dl. Serum CTX levels were found significantly higher than controls (1.38 ± 0.55 vs 1.15 ± 0.53 ng/mL; $p = 0.044$) while

Table 1
Characteristics of patients on valproic acid and controls as means (±SD) and median values.

	Patients	Controls	p
N	54	55	
Age (yrs)	9.0 ± 4.5	8.9 ± 3.9	NS
(Median)	(9.0)	(8.0)	
Gender (M/F)	30/24	25/30	
Years of treatment (yrs)	3.18 ± 2.6	–	
(Median)	(2.25)	–	
Valproic dose (mg)			
(Mean ± SD)	682 ± 287	–	
(Median)	(700)	–	
Body weight (kg)	36.8 ± 21.0	36.0 ± 18.5	NS
(Median)	(28.8)	(33.0)	
BMI (kg/m^2)	20.1 ± 5.0	18.9 ± 5.0	NS
(Median)	(18.1)	(17.8)	

NS: Not significant.

serum ALP, OPG, and sRANKL levels were not different from controls (208 ± 43 vs 187 ± 70 IU/L, 3.50 ± 2.54 vs 2.54 ± 1.23 pmol/L, and 411 ± 298 vs 386 ± 201 pmol/L, respectively).

After the one-year vitamin D supplementation, the patient's 25OHD levels increased significantly from 23.1 ± 12.8 to 43.2 ± 21.7 ng/mL ($p < 0.0001$) and became higher also from controls ($p = 0.004$) (Table 2). Still, 4% continued to have lower than 20 ng/mL 25OHD levels. Serum Ca and Pi did not show any changes while serum CTX levels decreased approaching those of controls, and similarly, the serum tALP had a decreasing trend ($p = 0.065$) (Table 2). Serum OPG and sRANKL did not change and again did not differ from controls.

Spearman correlation analysis of the patient's results showed no correlations between years of VPA treatment and the parameters examined. A VPA dose dependence of CTX was found before vitamin D intake ($r = 0.40$, $p = 0.01$) which was sustained even afterwards ($+0.47$, $p = 0.002$) (Table 3). At the same time, there was a negative dose effect on OPG levels ($r = -0.44$, $p = 0.04$) but again it continued to hold after the vitamin D supplementation ($r = -0.47$, $p = 0.002$). However, after vitamin D supplementation, a strong positive correlation was found between serum 25OHD and OPG ($r = 0.37$, $p = 0.013$) that was not seen before (Table 3). Also, after vitamin D, there were negative correlations between CTX and sRANKL ($r = -0.33$, $p = 0.03$) and between CTX and OPG (-0.31, $p = 0.04$). At both times, an age dependence of CTX (before: $r = +0.45$, $p = 0.003$; after: $r = +0.46$, $p = 0.002$) and a negative one of OPG (before: $r = -0.46$, $p = 0.002$; after $r = -0.45$, $p = 0.002$) were observed but not of 25OHD (Table 3).

Table 2
The mean values (M ± SD) and median of the serum biochemical parameters studied in the patients on valproic acid treatment before and after one-year vitamin D supplementation (400 IU/d) and of controls.

Parameter	Patients		Controls
	Before vitamin D	After vitamin D	
25OHD (ng/mL)	23.1 ± 12.8*,a	43.2 ± 21.7*,b	31.8 ± 16.2
	(Median: 21.0)	(Median: 38.8)	(Median: 29.4)
Ca (mg/dl)	9.9 ± 0.5ǂ	9.9 ± 0.4***	10.2 ± 0.4
	(Median: 10.0)	(Median: 9.9)	(Median: 10.3)
Pi (mg/dl)	5.3 ± 0.7	4.6 ± 0.8	4.7 ± 0.8
	(Median: 5.4)	(Median: 4.6)	(Median: 4.9)
tALP (IU/L)	208 ± 43	181 ± 62	187 ± 70
	(Median: 211)	(Median: 174)	(Median: 194)
CTX (ng/mL)	1.38 ± 0.55**	1.29 ± 0.54	1.15 ± 0.53
	(Median: 1.42)	(Median: 1.30)	(Median: 1.12)
OPG (pmol/L)	3.50 ± 2.54	3.11 ± 2.42	2.54 ± 1.23
	(Median: 2.77)	(Median: 2.57)	(Median: 2.63)
sRANKL (pmol/L)	411 ± 298	500 ± 420	386 ± 201
	(Median: 343)	(Median: 372)	(Median: 370)

a vs b: $p < 0.0001$.
* $p = 0.004$.
** $p = 0.044$.
*** $p = 0.0002$.
ǂ $p = 0.027$ (from controls).

Table 3
Spearman correlations in the patient group before and after vitamin D supplementation.

	Before vitamin D supplementation		After vitamin D supplementation	
	r	p	r	p
Age vs 25OHD		NS		NS
Age vs CTX	+0.45	0.003	+0.46	0.002
Age vs OPG	−0.46	0.002	−0.45	0.002
VPA dose vs CTX	+0.40	0.01	+0.47	0.002
VPA dose vs OPG	−0.44	0.04	−0.47	0.002
25OHD vs OPG		NS	+0.37	0.013
CTX vs sRANKL		NS	−0.33	0.03
CTX vs OPG		NS	−0.31	0.04

4. Discussion

The adverse effects of antiepileptic therapy on bone metabolism of children and adolescents are of great interest since that is the age when the rate of bone mineral accrual reaches its maximum [13]. Hence, from the infant age up to the adulthood, the higher bone density achieved the better prevention of skeletal problems in later life. It has also been reported that increasing duration of AED treatment in ambulatory children may decrease bone mineral density [16] and elevate the risk of osteopenia [4,24]. This dependence was not seen in our patients, since we could not detect a correlation between years of treatment and either CTX, ALP, OPG, or sRANKL levels.

Vitamin D deficiency has also been implicated in those patients since it is found to occur in a higher incidence than in the general population and to deteriorate further with the duration of therapy according to some reports [12,25]. Although the vitamin D levels were significantly lower in our patients, no relation with the years of treatment was found, which may be because of the relatively short time. But, there was a dose effect on CTX and a negative one on OPG and continued to hold even after the vitamin D supplementation.

Despite reports that monotherapy with nonenzyme inducing AEDs (non-EIAEDs) like VPA [26,27] should not have a detrimental effect on vitamin D metabolism, still, there are others which associate them with poor bone health [7,28]. We observed low 25OHD levels (<20 ng/mL) in nearly half of our patients (38%), which was nearly twice of the controls (20%) and similar to other reports with VPA monotherapy [7,8]. However, after the year's vitamin D intake, there was a significant decrease to 4%. The controversy is whether the increased vitamin D turnover is due to VPA treatment itself or not, since it is believed not to induce but rather inhibit cytochrome p450 enzymes [29]. However, Cerveny et al. [30] and Vrzal et al. [31] from in vitro experiments in human hepatocytes and human embryonic kidney cells reported that VPA in therapeutic concentrations increased basal and vitamin D-induced expression of CYP24A and CYP3A4 through direct activation of the pregnane X receptor (PXR) pathway leading to catabolism of vitamin D and thus lowering its activity.

Another theory proposed by El-Hajj Fuleihan et al. [32] suggests that the low 25(OH)D levels may reflect impact of disease per se rather than of the drug on vitamin D levels and that agrees with our results since we did not see any correlation of 25(OH)D with the years of treatment. Another intriguing possibility could be that certain drugs may affect vitamin D levels independent of the cytochrome P450 system. Also, the way of life of these patients might be an additional detrimental factor even though all patients were ambulatory.

A theory that even the non-EIAEDs may contribute to bone loss by inhibiting intestinal absorption of Ca again has yet to be clarified since a lot of studies including ours do not find low serum Ca or Pi nor high Parathyroid Hormone (PTH) levels [16,27].

However, despite the above theories, the question of whether this insufficiency/deficiency may have an adverse effect on bone health is still under debate, and more so, since there is not enough information as to whether vitamin D supplementation would be of benefit. In our study, the tALP levels did show a trend to decrease after the vitamin D intake becoming similar to controls. Babayigit et al. [4] found higher tALP levels despite normal 25OHD, Ca, Pi, and PTH levels, and Voudris et al. [33] reported only the bone isoezyme higher but not the tALP. There are also others who have not found differences in biochemical markers despite the lower BMDs [3,34].

In addition, we observed increased CTX levels at the start of the study which after the year's vitamin D intake approached the control levels. Other studies all with young patients on VPA monotherapy give conflicting results. Rieger-Wettengl et al. [35] have reported elevated urinary levels of deoxy-pyridinoline, a marker of bone resorption, in agreement with our findings with another resorption marker (CTX). Tsukahara et al. [2] reported reduced concentrations of both formation and resorption markers like serum osteocalcin (OC), carboxy-terminal propeptide of type I procollagen (PICP), and carboxy-terminal telopeptide of type I collagen (ICTP) while Verrotti et al. [15] reported the same markers increased and Öner et al. [3] only the OC higher.

Increased bone turnover with predominant bone resorption may become evident, as the increase in age and possibly as the duration of treatment is prolonged. We saw only an age dependence of CTX and a negative one with OPG before but also after vitamin D treatment but not with the years of antiepileptic treatment, which was not very long.

Increased bone turnover leading to decreased bone mass has also been reported in adults on valproate [36]. Even when Ca and Pi levels are kept normal in such patients, there may be a persistent increase in bone turnover. Histologically, biopsies of patients that are treated with either enzyme inducing or enzyme inhibiting AEDs suggest that the bone disease is due mainly to an increased frequency of remodeling activation and bone turnover, rather than a mineralization defect [37]. Hence, it seems that the mechanism of action is probably an effect on osteoblast–osteoclast interaction rather than direct Ca effects. In support of the above theory is that in our patients after the vitamin D intake, CTX decreased to comparable control levels, and a positive correlation was seen between 25OHD levels and OPG concentrations.

We did not observe significant differences in the circulating OPG and sRANKL levels between patients and controls, while Bauer et al. [38] in newly diagnosed adults after 3 months of treatment on VPA saw an increase in OPG but not in the sRANKL. Also, we did not see changes in these two parameters after vitamin D but we saw negative relations between CTX and OPG or CTX and sRANKL, again an indication of a positive effect on bone turnover. Rauchenzauner et al. [26] in children on VPA monotherapy also found normal serum OPG and sRANKL, even though their population differed from ours in that it was not vitamin D insufficient/deficient.

4.1. Strengths

As it is already pointed out, relative studies are scarce with reference to exogenous vitamin D intake. In a study with ambulatory patients aged 10–18 yrs on long-term AEDs therapy, two different doses of vitamin D (400 & 2000 IU) given daily led to increases in bone density after one year irrespective of the dose [21]. In our study, also, the 400 IU/d dosage for one year led to a slight improvement of the biochemical bone markers.

4.2. Limitations

A longer follow-up might be needed to get clearer results, and/or even higher doses of vitamin D might have been necessary, since a dose of 400 IU represents the daily needs.

5. Conclusions

It can be concluded, therefore, that long-term treatment even with VPA induces a state of increased bone turnover in normally active children, and that these changes occasionally could lead to osteopenia. Increased bone turnover with predominant bone resorption in these patients may become evident, as they age and the duration of treatment is prolonged. Even though the effect of VPA cannot be readily explained by vitamin D metabolism alone, it may additionally act by stimulating osteoclast activity causing an imbalance between bone formation and resorption and contributing to bone loss. Vitamin D supplementation then seems to act beneficially, and those subjects should be monitored for their vitamin D status as to be treated accordingly. Still, there is an increased need for more prospective intervention trials and clearer results.

Declaration of Competing Interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Author contributions

Dr. Papassava collected the samples and history data; Dr. Nakou made the diagnosis and did the follow-up of the patients; Dr. Siomou collected samples and history data of the control subjects; Dr. Cholevas and Dr. Challa made the biochemical analysis, analyzed the data, and interpreted the results; and Dr. Tzoufi read, edited, and approved the final manuscript.

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References

[1] Offermann G, Pinto V, Kruse R. Antiepileptic drugs and vitamin D supplementation. Epilepsia 1979;20(1):3–15.
[2] Tsukahara H, Kimura K, Todoroki Y, Ohshima Y, Hiraoka M, Shigematsu Y, et al. Bone mineral status in ambulatory pediatric patients on long-term antiepileptic drug therapy. Pediatr Int 2002;44:247–53.
[3] Öner N, Kaya M, Karasalihoğlou S, Karaca H, Celtic S, Tütüncüler F. Bone mineral metabolism changes in epileptic children receiving valproic acid. J Paediatr Child Health 2004;40(8):470–3.
[4] Babayigit A, Dirik E, Bober E, Cakmakci H. Adverse effects of antiepileptic drugs on bone mineral density. Pediatr Neurol 2006;35:177–81.
[5] Espinosa PS, Salazar JC, Yu L, Mendiondo MS, Robertso WC, Baumann RJ. Lack of valproic acid-associated weight gain in prepubertal children. Pediatr Neurol 2008; 39:177–80.
[6] Moosa ANV. Antiepileptic drug treatment of epilepsy in children. Continuum (Minneap Minn) 2019 Apr;25(2):381–407. https://doi.org/10.1212/CON.0000000000000712.
[7] Nicolaidou P, Georgouli H, Kotsalis H, Matsinos Y, Papadopoulou A, Fretzayas A, et al. Effects of anticonvulsant therapy on vitamin D status in children: prospective monitoring study. J Child Neurol 2006;21(3):205–9. https://doi.org/10.2310/7010.2006.00050.
[8] Nettekoven S, Ströhle A, Trunz B, Wolters M, Hoffmann S, Horn R, 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(12):1369–77.
[9] Borusiak P, Langer T, Heruth M, Karenfort M, Bettendorf U, Jenke ACW. Antiepileptic drugs and bone metabolism in children: data from 128 patients. J Child Neurol 2013; 28(2):176–83.
[10] Tosun A, Karaca SE, Unuvar T, Yurekli Y, Yenisey C, Omurlu IK. Bone mineral density and vitamin D status in children with epilepsy, cerebral palsy, and cerebral palsy with epilepsy. Childs Nerv Syst 2017;33:153–8.
[11] Shellhaas RA, Barks AK, Joshi SM. Prevalence and risk factors for vitamin D insufficiency among children with epilepsy. Pediatr Neurol 2010;42:422–6. https://doi.org/10.1016/j.pediatrneurol.2009.12.005.

[12] Lee Y-J, 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(2):153–9. https://doi.org/10.1016/j.pediatrneurol.2014.10.008.
[13] Baxter-Jones A, Faulkner RA, Forwood MR, Mirwald RL, Bailey DA. Bone mineral accrual from 8 to 30 years of age: an estimation of peak bone mass. J Bone Miner Res 2011;26:1729–39.
[14] Bischoff-Ferrari HA, Dietrich T, Orav EJ, Dawson-Hughes B. Positive association between 25-hydroxy vitamin D levels and bone mineral density: a population based study of younger and older adults. Am J Med 2004;116:634–9.
[15] Verrotti A, Coppola G, Parisi P, Mohna A, Chiarelli F. Bone and calcium metabolism and antiepileptic drugs. Clin Neurol Neurosurg 2010;112:1–10.
[16] Gröber U, Kisters K. Influence of drugs on vitamin D and calcium metabolism. Dermatoendocrinol 2012;4(2):158–66.
[17] Hofbauer LC, Heufelder AE. The role of receptor activator of nuclear factor-κB ligand and osteoprotegerin in the pathogenesis and treatment of metabolic bone diseases. JCME 2000;85(7):2355–63.
[18] Yao Y, Wang G, Wang Z, Zhang H, Liu C. Synergistic enhancement of new bone formation by recombinanat human bone morphogenetic protein-2 and osteoprotegerin in trans-sutural distraction osteogenesis: a pilot study in dogs. J Oral Maxillofac Surg 2011;69:e446–55.
[19] Mikati MA, Dib B, Yamout B, Sawaya R, Rahi AC, el-Hajj Fuleihan G. Two randomized vitamin D trials in ambulatory patients on anticonvulsants: impact on bone. Neurology 2006;67:2005–14.
[20] Valsamis HA, Arora SK, Labban B, McFarlane SI. Antiepileptic drugs and bone metabolism. Nutr Metab 2006;3:36–47. https://doi.org/10.1186/1743-7075-3-36.
[21] Buxton EC, Yao W, Lane NE. Changes in serum receptor activator of nuclear factor-kB ligand, osteoprotegerin, and interleukin-6 levels in patients with glucocorticoid-induced osteoporosis treated with human parathyroid hormone (1–34). J Clin Endocrinol Metab 2004;89:3332–6.
[22] Zheng C-M, Chu P, Wu C-C, Ma W-Y, Hung K-C, Hsu Y-H, et al. Association between increased serum osteoprotegerin levels and improvement in bone mineral density after parathyroidectomy in hemodialysis patients. Tokushima J Exp Med 2012; 226:19–27. https://doi.org/10.1016/j.pediatrneurol.2014.10.008.
[23] 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 (Biomed Biotechnol) 2015;16(7):611–21.
[24] Verrotti A, Agostinelli S, Coppola G, Parisi P, Chiarelli F. A 12-month longitudinal study of calcium metabolism and bone turnover during valproate monotherapy. Eur J Neurol 2010;17:232–7.
[25] Fong CY, Riney CJ. Vitamin D deficiency among children with epilepsy in South Queensland. J Child Neurol 2014;29:368–73.
[26] Rauchenzauner M, Griesmacher A, Tatarczyk T, Haberlandt E, Strasak A, Zimmerhackl LB, et al. Chronic antiepileptic monotherapy, bone metabolism and body composition in non-institutionalized children. Dev Med Child Neurol 2009; 52(3):283–8. https://doi.org/10.1111/j.1469-8749.2009.03402.x.
[27] 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.
[28] Sheth RD, Wesolowski CA, Jacob JC, Penney S, Hobbs GR, Riggs JE, et al. Effect of carbamazepine and valproate on bone mineral density. J Pediatr 1995;127:256–62.
[29] Wen X, Wang JS, Kivistö KT, Neuvonen PJ, Backman JT. In vitro evaluation of valproic acid as an inhibitor of human cytochrome P450 isoforms: preferential inhibition ofcytochrome P450 2C9 (CYP2C9). Br J Clin Pharmacol 2001;52:547–53.
[30] Cerveny L, Svecova L, Anzenbacherova E, Vrzal R, Staud F, Dvorak Z, et al. Valproic acid induces CYP3A4 and MDR1 gene expression by activation of constitutive androstane receptor and pregnane X receptor pathways. Drug Metab Dispos 2007; 35(7):1032–41.
[31] Vrzal R, Doricakova A, Novotna A, Bachleda P, Bitman M, Pavek P, et al. Valproic acid augments vitamin D receptor-mediated induction of CYP24 by vitamin D3: a possible cause of valproic acid-induced osteomalacia? Toxicol Lett 2011;200(3):146–53.
[32] 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(1):149–55.
[33] Voudris K, Moustaki M, Zeis PM, Dimou S, Vagiakou E, Tsagris B, et al. Alkaline phosphatase and its isoenzyme activity for the evaluation of bone metabolism in children receiving anticonvulsant monotherapy. Seizure 2002;11:377–80.
[34] Kumandas S, Koklu E, Gümüs H, Koklu S, Kurtoglu S, Karakukcu M, et al. Effect of carbamezapine and valproic acid on bone mineral density, IGF-I and IGFBP-3. J Pediatr Endocrinol Metab 2006;19(4):529–34.
[35] Rieger-Wettengl G, Tutlewski B, Stabrey A, Rauch F, Herkenrath P, Schauseil-Zipf U, et al. Analysis of the musculoskeletal system in children and adolescents receiving anticonvulsant monotherapy with valproic acid or carbamazepine. Pediatrics 2001;108(6):E107.
[36] Sato Y, Kondo I, Ishida S, Motooka H, Takayama K, Tomita Y, et al. Decreased bone mass and increased bone turnover with valproate therapy in adults with epilepsy. Neurology 2001;57(3):445–9.
[37] Drezner MK. Treatment of anticonvulsant drug-induced bone disease. Epilepsy Behav 2004;5(Suppl. 2):S41–7.
[38] Bauer S, Hofbauer LC, Rauner M, Strzelczyk A, Kellinghaus C, Hallmeyer-Elgner S, et al. Early detection of bone metabolism changes under different antiepileptic drugs(ED-BoM-AED) — a prospective multicenter study. Epilepsy Res 2013;106: 417–22.