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# The effect of levetiracetam on rat bone mass, structure and metabolism

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**Summary**
*Objective:* To determine the effect of levetiracetam (LEV) Lon bone mineral density (BMD), mineral content (BMC), bone markers, body composition and bone mechanical strength in the orchidectomised (ORX) rat model.
*Method:* 16 orchidectomised Wistar rats were divided into control and test groups, 8 rats in each group. The control rats received standard laboratory diet (SLD) while rats in the test group were fed with SLD enriched with LEV for 12 weeks. BMD was measured by dual energy X-ray absorptiometry at the whole body, lumbar spine and femur. Bone marker concentrations were examined of osteoprotegerin (OPG) and insulin-like growth factor 1 (IGF-1) in serum, and amino-terminal propeptide of procollagen type I (PINP), carboxy-terminal cross-linking telopeptide of type I collagen (CTX-I), bone alkaline phosphatase (ALPL), and bone morphogenetic protein 2 (BMP-2) in bone homogenate. The femurs were used for biomechanical testing.
*Results:* Compared to the control group we found lower fat mass, lower BMD in the area of the left femur, lower BMC in both femurs, a reduced concentration of OPG, and an increased concentration of CTX-I of borderline statistical significance ($p = 0.0661$). Biomechanical parameters did not differ between groups.
*Conclusions:* Significant loss of BMD or BMC was seen at the left and right femur area in the LEV group. Administration of LEV in the ORX-rat model significantly decreased levels of OPG (marker of bone formation) in serum and increased levels of CTX-I (marker of bone resorption) in bone homogenate, but results in this study did not reveal any change in biomechanical bone strength. Administration of LEV in the ORX-rat model may reduce adipose tissue. Further studies in animals and humans will be needed to confirm these findings.


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## Introduction

Epilepsy is a condition affecting 0.5—1% of the population in developed countries. The disease is often chronic and lifelong treatment may be required. Antiepileptic drugs (AEDs) can adversely affect bone health. A number of theories have been proposed to explain why AEDs affect bone, but none explains all the reported effects. Cytochrome P450 enzyme-inducing AEDs are most commonly associated with negative impact on bone, but studies also suggest an effect of valproate (Pack, 2008). There is limited evidence that also use of newer AEDs may have adverse effects on bone mineral density (BMD) and metabolism (Heo et al., 2011; Ali et al., 2011; Takahashi et al., 2003; Babacan et al., 2012; Cansu et al., 2008; Mintzer et al., 1996; Ensrud et al., 2008).

Levetiracetam (LEV), (*S*)-α-ethyl-2-oxo-1-pyrrolidine acetamide, an analog of piracetam, is a relatively new broad-spectrum AED with a favorable tolerability and efficacy profile and a low potential for drug interactions. LEV is used in treating partial, generalized and myoclonic seizures. Despite wide therapeutic use of LEV, to our knowledge there is only one animal study, which reports changes in biomechanical strength properties of femoral bones from rats, along with documentation of changes in BMD and biochemical markers of bone turnover. This study demonstrates a biphasic dose-dependent effect of LEV on biomechanical bone strength, which may be related to microstructural changes in bone matrix (Nissen-Meyer et al., 2007).

There is only limited and conflicting data concerning the effect of LEV on bone health in humans (Beniczky et al., 2012; Koo et al., 2013).

We report here our findings in ORX-rats fed on LEV-enriched diet for 12 weeks concerning the impact of LEV on bone mineral density (BMD), content (BMC), bone metabolism markers, and biomechanical properties.

## Methods

### Animals

All animals received humane care in accordance with the guidelines set by the institutional Animal Use and Care Committee of Charles University, Prague, Faculty of Medicine in Hradec Kralove, Czech Republic. The protocol of the experiment was approved by the same committee. The experiments used eight-week-old male albino Wistar rats (Biotest s.r.o., Konarovice, Czech Republic). The animals were hosted in groups of 4 in plastic cages. During the experimental period the animals were maintained at controlled conventional conditions (12 h light and 12 h dark, temperature 22 ± 2 °C, air humidity 30—70%). Tap water and standard laboratory diet (SLD, VELAS, a.s., Lysa nad Labem, Czech Republic) or SLD enriched with LEV were given *ad libitum*. The weights of the rats were monitored once a week.

### Experiment

Rats weighing (270 ± 7 g) at the beginning of the experiment were divided into two groups of 8 animals:

1. CON-R: orchidectomised control fed with SLD
2. LEV-R: orchidectomised rat fed with SLD enriched with LEV (101 mg/25 g of the diet; Levetiracetam, UCB Pharma)

At the beginning of the experiment the rats (CON-R and LEV-R) underwent bilateral orchidectomy under ether anesthesia. On the second day after operation the LEV-R began to receive SLD enriched with LEV and the CON-R only SLD, both diets *ad libitum*. After 12 weeks, the animals were sacrificed by blood withdrawal from the abdominal aorta under ether anesthesia, and the obtained serum was aliquoted and stored at −80 °C for ensuing biochemical analyses. After sacrifice of the rats, both tibiae and femurs were dissected free of soft tissue, wrapped in gauze moistened with saline and frozen to −80 °C till required for analysis.

### Analysis of serum and bone homogenates

Blood serum levels of osteoprotegerin (OPG) and insulin-like growth factor 1 (IGF-1) were determined using the ELISA (Enzyme-Linked Immunosorbent Assay) method. Blood serum levels also of levetiracetam were determined in the middle and at the end of the experiment. Concentrations of levetiracetam in the samples were determined by the modified high-performance liquid chromatography method with UV photodiode-array detection (Lancelin et al., 2007). Levetiracetam and internal standard UCB 17025 were extracted after alkalinization of the sample (0.05 mL) into dichloromethane. Organic solvent was evaporated and the residue was dissolved and injected for HPLC analysis. Compounds were separated on a Zorbax SB-C8 column (Agilent Technologies, USA) at flow rate 1.1 mL/min. The mobile phase was composed of 10% acetonitrile, 7% methanol and 83% of a 20 mM phosphate buffer pH 6.7 with 0.1% triethylamine. UV detection was performed at a wavelength of 200 nm.

Bone homogenate was prepared from the tibiae. After animal sacrifice, both tibiae were carefully excised; after removal of all the surrounding skin, muscle and other soft tissue, they were stored at −80 °C until required. The proximal part of the bone (0.1 g) was disrupted and homogenized in 1.5 ml of phosphate buffer (PBS, PAA Laboratories GmbH, Pasching, Austria) with MagNA Lyser instrument (Roche Applied Science, Germany) at 6500 rpm for 20 s, and cooled on the MagNA Lyser Cooling Block. This procedure was repeated three times. The raw tissue homogenate was centrifuged at 10,000 g at 4 °C for 10 min, and the resulting supernatant was collected and stored at −80 °C.

Levels of the markers carboxy-terminal cross-linking telopeptide of type I collagen (CTX-I), amino-terminal propeptide of procollagen type I (PINP), bone alkaline phosphatase (ALPL) and bone morphogenetic protein 2 (BMP-2) were determined in this bone homogenate, also using the ELISA method.

Bone-marker levels were determined using kits from the firm Uscn Life Science Inc., Wuhan, China (PINP, Procollagen I N-Terminal Propeptide, pg/mL; OPG, Osteoprotegerin, pg/mL; IGF-1 Insulin Like Growth Factor 1, pg/mL; CTX-I, Cross Linked C-Telopeptide Of Type I Collagen; pg/mL; ALPL,