Epilepsy and bone health

University of Oslo 2011, Faculty of Medicine

Department of Neurology, Rikshospitalet

By

Stud. Med. Senaria Matapour and

Stud. Med. Amandeep Kaur Malhi

Supervisor: Prof. Dr. Med. Erik Taubøll

Contents

Preface

The theme of this special assignment was chosen by inspiration from our supervisor Prof. Dr. Med. Erik Taubøll's work in the field at The Department of Neurology at Rikshospitalet University hospital. We would like to express our gratitude to Prof. Taubøll for his guidance and patience through the process.

We are also very grateful for all the help and support from Dr. Med. Erik Sætre, Department of Neurology, Ullevål University hospital.

This assignment would not have taken its final shape without the outstanding help in statistics from Navdeep Singh, M. Scient, and Actuary, former student at Institute of Mathematics, Department of Mathematics and Natural Sciences at University of Oslo. And thanks to Navdeep Singh Malhi, student at Institute of Mathematics, Department of Mathematics and Natural Sciences at University of Oslo, for support in statistical interpretation.

Finally, we would like to thank our nearest family for always being the most important support in our lives. And especially David Amar Si Saïd Norberg and Dr. Jai Paul Singh Dhillon.

Senaria Matapour and Amandeep Kaur Malhi, Oslo 2011

Abstract

Purpose: Initially make a literary overview of epilepsy and bone health, bone structure and physiology, hormonal regulation of bone metabolism, osteoporosis and enzyme inducing and non-enzyme inducing AEDs.
The clinical study part assesses the comparative effects of the non-enzyme inducing drug lamotrigine (LTG) and enzyme inducing drug carbamazepine (CBZ) on bone health.

Methods: In the clinical study patients aged 65 years or older, who had experienced at least two unprovoked partial and/or generalized tonic clonic seizures, were randomized to receive LTG (n = 93) or CBZ (n = 92) according to a multicenter double-blind, parallel group design. Trial duration was 40 weeks and included a 4-week dose escalation followed by a maintenance phase during which dosages could be adjusted according to response. Initial, maintenance and maximum dosages were 25 mg, 100 mg, and 500 mg per day for LTG, and 100 mg, 400 mg, and 2,000 mg per day for CBZ, respectively. Patient data used in the study is from 64 patients in the LTG group and 44 in the CBZ group. This selection is based on the availability of the desired bone health measuring parameters in each patient, which are estrogen, progesterone, osteocalcin, PTH, testosterone, β-CrossLaps, Ca^{2+}, total protein, alkaline phosphatase and TSH.

Results: There was a significant difference in calcium levels-where patients in the CBZ group had lower values than patients in the LTG group. Bone metabolism parameters were investigated, such as β-CrossLaps, ALP and osteocalcin, but no significant differences were found between the 2 groups. Further, there were not significant alterations in hormonal levels, except that progesterone in the CBZ group was significantly lower than the LTG group.

Conclusions: Calcium levels were significantly lower in elderly epilepsy patients taking CBZ compared to LTG. This is consistent with earlier observations that an enzyme inducing antiepileptic drug like CBZ is more likely to affect bone health than a non-enzyme inducing drug like LTG. However, the difference in calcium levels could not be explained by differences in bone metabolism or endocrine effects. The minor difference in progesterone is not considered to be of biological importance.

Epilepsy

Epilepsy is a common chronic neurological disease in the developed world, where approximately 5 to 10 individuals per 1000 are affected [1, 2]. The disease is characterized by seizures. More specifically, epilepsy is the recurrent appearance of epileptic seizures caused by continuous intracranial pathological conditions. A seizure is a momentary disturbance of brain function which manifests itself as a clinical phenomenon (seizures) and characteristic changes in EEG. A majority of epilepsy cases has an idiopathic etiology (40%), while vascular lesions (15%), head injuries (5 %), alcohol and drug abuse (6 %), neoplasms (6%), infections (2%), central nervous system developmental disturbances and genetic predisposition count for the rest [3].

The seizures can be partial or generalized, by means of affecting a localized part of the brain or the brain as a whole, respectively. The partial seizures can be categorized as simple, where consciousness is totally intact, and complex, where consciousness is reduced. Partial seizures can develop into generalized. Generalized seizures are absences, tonic clonic seizures (GTK), tonic seizures, myoclonic seizures or atonic seizures. Beside these two major groups there is also a category of other types that cannot be classified strictly into either one of them.

The diagnosis is based on a thorough anamnesis, clinical examination, and examination of anatomical structures by MRI and CT, functional examination by EEG, SPECT and PET. Other, less used, diagnostic procedures are tests of cerebral spinal fluid and neuropsychological examination. Epilepsy is treated not only with drugs, but also by focusing on the avoidance of factors that can trigger seizures, by providing medical assistance in a seizure situation, by psychological supportive therapy and surgically [3]. Medical treatment is however the cornerstone of treatment, which will be the main focus of this assignment.

Patients with epilepsy experience several other consequences than only the seizures themselves; Prejudices, social stigma, social maladaptation, isolation, restrictions in education transportation and vocation, increased fall and accident tendency and decreased bone density due to use of antiepileptic drugs (AEDs) are only some of them [4]. Reduced bone density as a side effect of AEDs is not necessarily common knowledge among medical doctors. In a survey of 624 neurologists only 28 % were aware of the bone density decreasing effects of AEDs [5]. The use of AEDs is not only limited to epilepsy treatment. AEDs are also commonly used in treatment of patients with neuropathic pain, migraine and psychiatric

disorders; hence the necessity of more knowledge about AED induced reduction in bone density is important [6].

An increased fracture risk has been reported in patients with epilepsy, in particular linked to tonic clonic seizures. The risk is increased furthermore with the use of antiepileptic, drugs that increase bone turnover and occasionally cause a mineralization defect, leading to decreased bone strength with increased fracture risk [7] [8].

The association between AEDs and decreased bone density has been known since the first study in the 1960s [9]. After this there has been produced a growing number of literary works underlining this association. The initial studies were done on institutionalized patients. These patients might have had limited mobility and little sun exposure. However more recent studies suggest that decreased bone density is observed in children and adults with epilepsy, whether institutionalized or ambulatory.

Bone structure and metabolism, and hormonal regulation

Bone tissue is a dynamic and metabolically very active tissue. It continuously undergoes resorption and formation (ossification). It provides mechanical protection for internal organs, anchorage for muscles, contains the blood cell producing bone marrow, provides with storage of important minerals and iron, allows humans to direct motion and facilitates the locomotion process. Besides these mechanical functions bone provides a protective housing for mineral ions (Ca^{2+}, Mg^{2+}, PO_4^{3-}) [10].

Macroscopical and biochemical structure. Macroscopically bone tissue has two main structural elements: Substantia spongiosa, the spongy or trabecular bone mass, constituting the inner part, and substantia compacta, a dense bone mass, constituting the outer part. The outermost membrane like layer covering the substantia compacta is called periost. As bone matures it becomes more and more dense. The general features of both compact and trabecular bone are similar. Both are solid mineralized matrices with small canals called canaliculi, spaces called lacunaes and bone cells. Biochemically bone is a composite material consisting of mineral, collagen, water, noncollagenous proteins, lipids, vascular elements and cells. The different bones in the body are composed to resist the load imposed by functional activities [11].

Biochemical markers of resorption and formation. The matrix of bone changes with growth and development. Vitamine D, calcium and phosphorus are essential contributes in the process of resorption and formation [12]. In an anabolic condition there is an increase in the levels of bone specific alkaline phosphatase, osteocalcin, type I procollagen C-terminal peptides and type I procollagen N-terminal peptides [13, 14]. A catabolic condition is characterized by an increase in degradation products, that is carboxy-terminal telopeptide type I collagen, tartrate-resistant acid phosphatase, bone specific hydroxyproline, pyrodinoline crosslinks and deoxypyridinoline crosslinks [15].

Cell types. As any connective tissue bone tissue consists of cells and extracellular matrix. There are mainly five types of cells in bone tissue; Stem cell like osteoprogenitor cells, osteoblasts, osteoclasts, osteocytes and periost cells that makes the outer covering layer.

The osteoprogenitor cells differentiate from the primitive mesenchymal cells. These cells are found near the ossification centers, that is where bone formation starts in a specific bone, in fetuses and in the deep layers of periost after birth and the rest of human life. During bone

formation osteoprogenitor cells undergo division and develop into osteoblasts. This activity is at its highest in fetal life and during the growth period. Peak bone mass is reached at age 30. Amongst the hormones influencing early osteogenesis the most important is parathyroid hormone (PTH), which stimulates osteogrogenitor population.

Osteoblasts are the bone forming cells and gate keepers of hormonal activity. They synthetize and secrete organic bone matrix. Osteoblasts are stimulated by interleukin-1, -6 and -11. The production of these interleukins is stimulated by circulating hormones like parathyroid hormone and 1,25-dihydroxycholecalciferol (active vitamin D). Besides their responsiveness to endocrine factors, osteoblasts are also responsible for the production of paracrine and autocrine factors for the recruitement of osteoprogenitors, the growth of preosteoblasts and the regulation of osteoclastic resorption of the mineralized bone matrix.

Osteocytes are the actual bone cells, by representing the most abundant cell in the skeleton. They originate from osteoblasts. They are important in communicating the bone tissue's condition to the surface, although osteoblasts receive and convey the majority of local and systemic signals. As mineralization of the matrix envelopes the osteoblast, morphologic changes are induced in the cell when the osteoblast progresses to the fully developed osteocyte.

Osteoclasts have a bone destructing function. Their function is directly stimulated by the same factors as osteoblasts, that is interleukin-1, -6 and -11 and indirectly by parathyroid hormone. [16] [17].

Minerals of bone metabolism. As mentioned earlier, bone provides housing for mineral ions like Ca^{2+}, Mg^{2+} and PO_4^{3-}. 99 % of the calcium in a human body is stored in bone tissue, making it the biggest reserve of calcium. It is found in three fractions, namely the ionized fraction (Ca^{2+}) (45-50%), the complexed fraction (10-15 %) and the protein bound fraction (40 %). The sum of these three is the total serum calcium, which is fairly constant in a healthy person [18]. Approximately 1 % of the calcium reserve works as a buffer to the calcium levels in the blood. The buffer regulation system aims to keep the calcium levels within a narrow area, because fluctuation beyond this may result in fatal consequences. However, the buffer system is capable of adjusting just small changes. Larger changes are controlled by the hormone system, by affecting the bone turnover with parathyroid hormone, calcitonin and the active form of vitamin D [19].

Phosphate is present in blood chiefly as inorganic and organic phosphates, nearly all of the latter residing in the erythrocytes. Determination is often referred to as inorganic phosphorus rather than inorganic phosphate, since the phosphate determined is usually reported in terms of phosphorus. Only inorganic phosphate is measured in routine clinical settings. In normal healthy subjects the total phosphate concentration in plasma is 3,9 mmol/L, of which only 0,8-1,4 mmol/L is inorganic phosphate, remaining being phospholipids and other organic compounds. 10 % of plasma inorganic phosphate is protein bound, 6 % is complexed with calcium or magnesium and 84 % is present in the form of ions $H_2PO_4^-$ and HPO_4^{2-}. The relative proportion depends on the pH. The ratio varies between 1:1 in acidosis, 1:4 at pH 7,4 and 1:9 in alkalosis. The extracellular phosphorus pool and therefore the serum phosphate levels are influenced by phosphate intake, intestinal absorption, exchange with the bone reservoir and renal excretion [20].

Magnesium is the most prevalent divalent cation and the second most prevalent cation in the body. The total amount of magnesium in human body is approximately 25 g, of which 50-60 % is in bone and the remaining 40-50 % in soft tissues. One third of magnesium in the skeleton resides on the bone surface, thereby being exchangeable. It serves as a reservoir for maintaining a normal extracellular magnesium concentration. Extracellular magnesium counts for only 1 % of the total magnesium amount. Magnesium in plasma is found in three fractions: Ionized (Mg^{2+}, 55 %), protein bound (30 %) and complexed with phosphate, citrate and other anions (15 %) [21].

Hormonal regulation. Several hormones are involved in the regulation of bone metabolism; Growth hormone, parathyroid hormone, calcitonin, calcitriol (vitamin D), thyroidea stimulating hormone (TSH), leptin, serotonin and estrogens.

Growth hormone, also called somatotropine, drives the growth of bone until adult size is reached, and is the main regulator of height. It is a product of the anterior pituitary gland and it increases the rate of mitosis in osteoblasts, drives the skeletal cells towards a committed phenotype, increases protein synthesis and inhibits apoptosis. Growth hormone stimulates bone tissue to secrete insulin like growth factor 1 (IGF-1), which in turn stimulates bone itself to growth in a paracrine and autocrine fashion [22].

Parathyroid hormone (PTH) is one of the hormones that play a part in maintaining the strength and health of the bone matrix by functioning to control the level of blood calcium**.** In fact, calcium is needed for a number of metabolic processes other than for bone formation,

including blood clot formation, nerve impulse conduction, and muscle cell contraction. When a low blood calcium condition exists, the parathyroid glands respond by releasing PTH**.** This hormone stimulates osteoclasts to break down bone tissue, and as a result, calcium salts are released into the blood. PTH keeps calcium in a narrow physiological range by direct actions on bone and kidney tissue and an indirect action via 1,25-dihydroxy vitamin D on the intestinal tract. PTH release is regulated by serum calcium concentrations. Hypocalcemia stimulates release of PTH from the parathyroid gland. By reabsorption of calcium in the distal convoluted tubule of the kidney or by osteoclast mediated bone resorption, serum calcium concentrations increase. A rise in extracellular calcium inhibits secretion of PTH [23].

Calcitonin reduces the blood calcium concentration by opposing the effects of TSH. It does so by inhibiting osteoclast function and thereby resorption of bone tissue, and by inhibiting calcium absorption in the intestine. Calcitonin is secreted by a positive feedback by high blood calcium levels, and vice versa with low levels. Other effects are in preventing postprandial hypercalcemia resulting from absorption of Ca^{2+}. In its skeleton preserving actions, calcitonin protects against calcium loss from skeleton during periods of calcium mobilization, such as pregnancy and, especially, lactation. It also inhibits reabsorption of phosphate in the kidney tubules and increases tubular reabsorption of Ca^{2+}, leading to decreased excretion in the urine. Other effects are in preventing postprandial hypercalcemia resulting from absorption of Ca^{2+} [24].

Vitamin D also plays an essential role in the regulation of calcium by promoting calcium absorption in the intestines, promoting bone resorption by increasing osteoclast number, maintaining optimal calcium and phosphate levels for bone formation, and allowing proper functioning of parathyroid hormone to maintain serum calcium levels. It promotes calcification of bone tissue by increasing the concentration of calcium and phosphorus in the extracellular fluid. It's synthesized under UV light exposure in skin. Once formed, vitamin D3 is metabolized sequentially in the liver and kidney to 1,25-dihydroxyvitamin D [25].

Hypothyroidism causes impaired bone formation and growth retardation whereas thyrotoxicosis results in accelerated growth, advanced bone age and decreased bone mass. The thyroid gland detects low levels of calcium and stimulates secretion of calcitonin, which is an indirect control mechanism. TSH directly stimulates bone growth by recruiting and enhancing activity of osteoblasts [26].

Amongst other hormones that in a minor extent contribute to the regulation of bone metabolism is leptin, a hormone derived from fat tissue. Leptin can affect bone metabolism via direct signaling from the brain and that although leptin acts to reduce cancellous bone, it conversely increases cortical bone.

Mechanisms of bone loss

Etiology of bone deprivation.

Primary osteoporosis, non-modifiable factors. The dynamics of bone tissue might switch over to a catabolic state due to several different factors. Amongst these there are some which are not subjects to modification; I. e. age. Increasing age is associated with bone loss in both genders, but distinctly in a larger extent in women. Menopause in women causes a decrease in estrogen levels which in turn leads to bone deprivation. 5 % of the bone mass is deprivated yearly 5-10 years after onset of menopause. Decrease in testosterone levels in men has a similar, but milder effect. Bone density is also linked to ethnicity, where European and Asian descent predisposes for osteoporosis. A small stature and low BMI, and paradoxically also a height over average is a risk factor. And last, but not least, hereditary factors also play an essential role; probably 80 % of variability in bone density might be explained by genetics [27]; There is postulated an association between the LRP5 gene and osteoporosis and increased fracture risk; There is however need for more research on this. Peak bone mass, which is reached at an age of 20-30 years in a human body is amongst other factors influenced by heredity. After the age of 40 years bone mass is gradually reduced because of a change in the remodeling process which favours resorption. Bone loss is calculated to be approximately 1 % per year in both genders.

The osteoporotic changes are most striking in the trabecular bone, which is the most active part of bone tissue. The trabecles are perforated, hence causing irreversible bone loss. Trabecular bone is found in vertebras and long tubal bones, which are common sites of osteoporotic fractures. The non-modifiable factors might be categorized as primary osteoporosis, whilst so called secondary osteoporosis is related to underlying diseases and medical treatment, which will be discussed later.

Modifiable factors are many, illustrating the complexity of bone loss, and nonetheless the opportunity to provide and encourage prophylactic treatment. Inadequate diet leads to malnutrition and insufficient supplement of vitamins, especially vitamin D, and minerals, calcium, magnesium and phosphorus, zinc, boron, iron, fluoride, copper, vitamin A, K, E and C, are shown to be protective against premature bone loss. Vitamin D is not commonly found in food. The major natural sources of vitamin D are fatty fish and fatty fish oils including cod liver oil. Certain foods are fortified with vitamin D, including cereals, bread products, and

milk. Older persons and persons with eating disorders are especially prone to suffer from lack of essential vitamins and minerals. Dairy products are the most important source of dietary calcium.

Season variations can affect the production of vitamin D3 in the skin. Use of sunscreen creams and ultraviolet radiation substantially reduces the cutaneous production of vitamin D3.

Excess alcohol consumption increases risk of bone loss, and so does tobacco smoking by inhibiting osteoblast activity. Smoking has amongst many other hazardous effects on health the ability to break down exogenous estrogen, lead to lower body weight and induce an earlier onset of menopause – all contributing to reduce bone density.

Secondary osteoporosis. There are also a number of medical conditions and medical treatments that might decrease bone density, causing so called secondary osteoporosis. Gastrointestinal diseases causing malnutrition, i.e. coeliac disease, Morbus Crohn, may lead to inadequate absorption of important vitamins and minerals. Endocrine disturbances like hyperthyroidism, reduced function of the gonads, primary hyperparathyroidism and Cushing's disease are also worth mentioning. Bone marrow diseases like myeloma and cancer metastases into bone marrow may disturb normal bone metabolism. Others are kidney diseases, connective tissue diseases, i.e. Bechterew's disease, Rheumatoid arthritis, psychiatric diseases, i.e. Anorexia nervosa or other eating disorder, increased homocystein and medicines; corticosteroids used for longer than 3 months [28]. Some drugs have osteopenic qualities; Some of these are aluminum containing antacids, several AEDs, aromatase inhibitors, some cytostatics, cyclosporine A, glucocorticoids, gonadotropin releasing hormone, heparin, lithium, medroxyprogesterone acetate, PPIs, SSRIs, tamoxifen, thiazides and excess thyroid hormones [29].

Osteomalacia occurs due to inadequate mineralization of bone. Osteomalacia increases fracture risk. Bone mineralization begins at birth and plateaus in the third decade of life with subsequent gradual bone loss as a natural process of aging. Many of the effects of osteomalacia overlap with the more common osteoporosis, but the two diseases are significantly different. There are two main causes of osteomalacia:

1. Insufficient calcium absorption from the intestine because of lack of dietary calcium or a deficiency of or resistance to the action of vitamin D.

2. Phosphate deficiency caused by increased renal losses. The causes of adult osteomalacia are many; insufficient supply of vitaimin D or phosphorus, renal tubular acidosis, malnutrition during pregnancy, malabsorption syndrome, hypophosphatemia, chronic renal failure, tumor induced osteomalacia, coeliac disease and long term anticonvulsant therapy.

The mechanisms underlying the bone-depleting effects of AEDs are however only partly known. Yet, increased fracture rates have been reported in persons with epilepsy receiving AEDs [30].

Pathophysiology of osteoporosis. Around the age of 30-35, trabecular bone loss begins. Women may lose as much as 50 %, while men lose about 30 % [31]. Osteoporosis develop through three main mechanisms; inadequate *peak bone mass* (the skeleton develops insufficient mass and strength during growth), *excessive bone resorption* and *inadequate formation* of new bone during remodeling. An interplay of these three mechanisms underlies the development of fragile bone tissue. Hormonal factors strongly determine the rate of bone resorption. For instance, the lack of estrogen as a result of menopause increases bone resorption as well as decreasing the deposition of new bone that normally takes place in weight bearing bones. The α-form of the estrogen receptor appears to be the most important in regulating bone turnover [32].

Osteoporosis

Osteoporosis ("porous bones", from Greek) is a disease of bones that leads to an increased risk of fractures. WHO defines it as a bone mineral density that is 2.5 standard deviations or more below the mean peak bone mass (average of young, healthy adults) as measured by DEXA; Dual energy absorptiometry, which is two x-ray beams with different energy levels aimed at the patients skeleton. Dual-energy X-ray absorptiometry is the most widely used and most thoroughly studied bone density measurement technology.

Osteoporosis risks can be reduced with lifestyle changes and sometimes medication. Lifestyle changes include diet and exercise, and preventing falls. Medication includes calcium, vitamin D supplements, bisphosphonates, calcitonin, HRT and selective estrogen modulators (SERMs) [33].

Chemical biomarkers of osteoporosis. Measuring chemical biomarkers is a simple and useful way to detect bone loss. The enzyme cathepsin K breaks down type-I collagen protein, an important constituent in bones. Prepared antibodies can recognize the fragment, as a way to diagnose osteoporosis [34]. Increased urinary excretion of C-telopeptides, a type-I collagen breakdown product, is also a biomarker for osteoporosis [35].

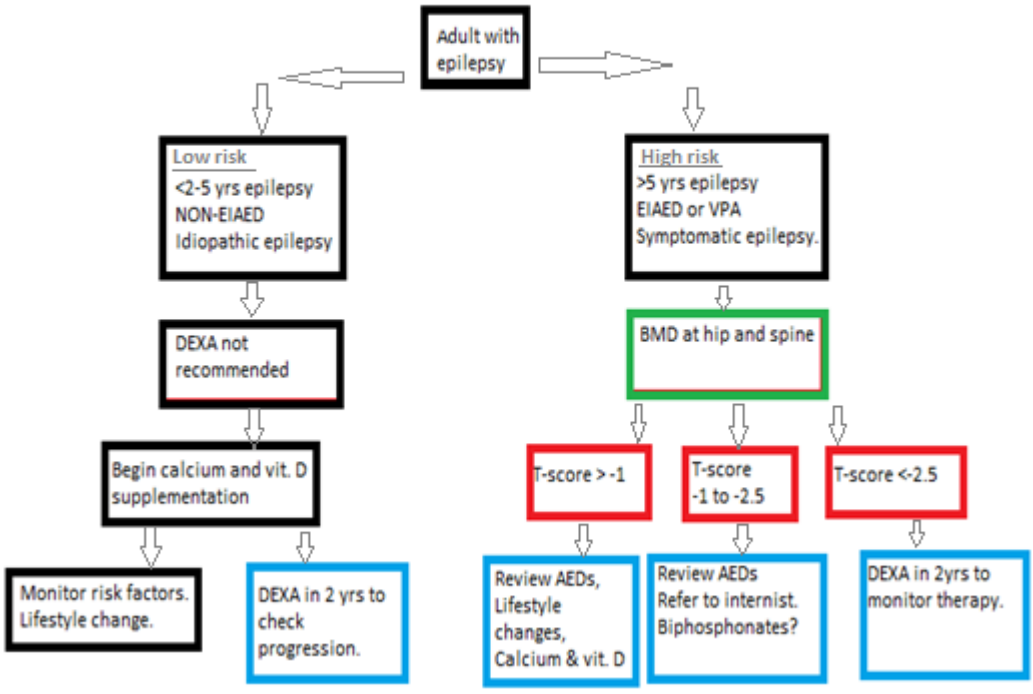
Clinical decision rule. Clinical decision rules is another diagnostic tool that has the ability to predict the risk of osteoporotic fractures. The ”Q Fracture score” was developed in 2009 and is based on age, BMI, smoking status, alcohol use, rheumatoid arthritis, cardiovascular disease, type 2 diabetes, asthma, use of tricyclic antidepressants or corticosteroids, liver disease, and a history of falls in men. In women hormone replacement therapy, parental history of osteoporosis, gastrointestinal malabsorption, and menopausal symptoms are also taken into account [36].

Dual energy X-ray absorptiometry. DEXA is the gold standard for diagnosis of osteoporosis. Osteoporosis is diagnosed when the bone mineral density is less than or equal to 2.5 standard deviations below that of a young adult reference population. This is translated as a T-score. The World Health Organization has established the following diagnostic guidelines [37]:

• T-score -1.0 or greater is normal value
• T-score between -1.0 and -2.5 is low bone mass (or osteopenia)
• T-score -2.5 or below is osteoporosis

Importance of screening. Population surveys indicate that patients with epilepsy have 2-6 times the fracture rate of the general population [38]. Postmenopausal women with epilepsy where osteopenia may occur as a result of physical impairment, incoordination or gait instability are at a particularly increased risk for fractures [39].
There is insufficient evidence to make recommendations about the optimal intervals for repeated screening and the appropriate age to stop screening. Clinical prediction rules are available to guide selection of women ages 60–64 for screening.
No firm recommendations for measuring bone mass in patients with epilepsy exist. It is unclear if children with epilepsy should routinely be screened. A suggested algorithm for screening adults with epilepsy is presented in Fig. 1. [40].

Patient with BMD T-score of −1 or higher do not require repeated measurements unless there have been changes in the risk factors. Patients with an osteopenic BMD should usually have a BMD re-measured in a 2 years' time.



Prophylaxis. Patients with risk of developing osteoporosis are encouraged to quit smoking and aim towards moderation of alcohol intake. Achieving a higher peak bone mass through exercise and proper nutrition during adolescence is important for the prevention of osteoporosis [41]. Exercise and adequate nutrition throughout one's life delays bone degeneration. Supplement with vitamin B and calcium is also an essential part of prophylaxis.

Medication. Bisphosphonate can be used prophylactic in high risk patients and in treating manifest osteoporosis. Other medicines prescribed for prevention of osteoporosis are selective estrogen receptor modulators (SERMs). Estrogen replacement therapy remains a good treatment for prevention of osteoporosis but, at this time, is not recommended unless there are other indications for its use as well, for example menopausal symptoms. There is uncertainty and controversy about whether estrogen should be recommended in women in the first decade after the menopause [42].

Treatment. The medications can be classified as anti-resorptive or bone anabolic. Antiresorptive agents inhibit bone resorption, while bone anabolic agents build bone. Lifestyle changes are an important aspect of treatment. A major problem is gaining long-term adherence to therapy [43].

Antiresorptive agents

• *Bisphosphonates:* In confirmed osteoporosis, bisphosphonate drugs are the first-line treatment in women. Oral bisphosphonates are relatively poorly absorbed, and must therefore be taken on an empty stomach, with no food or drink to follow for the next 30 minutes. They are associated with esophagitis and are therefore sometimes poorly tolerated.

• *Estrogen analogs, Raloxifene, Calcitonin:* Calcitonin works by directly inhibiting osteoclast activity via the calcitonin receptor. Calcitonin receptors have been identified on the surface of osteoclasts.

Bone anabolic agents

• *Teriparatide***:** It acts like parathyroid hormone and stimulates osteoblasts. It is used mostly for patients with established osteoporosis, with particularly low BMD, and who have several risk factors for fracture or cannot tolerate the oral bisphosphonates.

• *Calcium salts***.**

• *Sodium fluoride* [44].

Epilepsy and bone health

There are approximately 20 AEDs available today, each having their own characteristic effects and side-effects. There are several potential explanations for the bone depleting properties of AEDs, which have been known for almost five decades. For example, postmenopausal women with epilepsy and postmenopausal women taking AEDs have higher hip fracture rates [45] [46]. Another study showed increased overall fracture rates in ambulatory epileptic patients, whose median age was 43, when compared with a randomly selected control population [47]. Those who are especially vulnerable to AED-associated bone disease are epileptic patients who also have non-AED related risk factors.

Non AED related risk factors are what we can call confounding factors when studying bone health in an epileptic population. These are factors which can contribute to bone depletion without any AED treatment. For example a study from 2007 analyzed the diet of children with severe epilepsy by measuring the most important vitamins and minerals, and this study concluded that they had a suboptimal diet [48]. In addition reduced sunlight exposure, which especially patients in Scandinavia are prone to, has an immense impact on their vitamin D levels. There is also suggested that having epilepsy enhances sedentary lifestyles, for instance because of low self-esteem and consequently isolation, overprotective relationships, anxiety and depression [49]. Some people with epilepsy embrace the myth that physical exercise may provoke seizures, when in reality less than 10 % of the epileptic population experiences exercise induced seizures [50]. Adequate physical activity is a cornerstone in developing and maintaining normal bone density. Also high caffeine intake, smoking and high alcohol consumption are risk factors, which may be more prevalent in people leading a sedentary lifestyle [51].

AED induced bone loss has been explained through several theories in medical science literature, and most of the theories are trying to explain the AEDs induced bone loss through alteration of the activity of bodily processes. Antiepileptic drugs (AEDs), especially those that affect the liver enzymes, e.g., phenytoin, carbamazepine, phenobarbital and valproate are associated with increased fracture rate and low bone mineral density [52]. Up until today, no prospective, controlled studies on bone health in the epilepsy population are performed. All published studies have limitations, where detailed information on epilepsy type, AED exposure time, and previous treatment is lacking. Thus, the results should be interpreted with

caution. In patients treated with polytherapy, the effect of a particular drug on bone health is impossible to evaluate. Further, in some studies, confounding factors have not been taken into consideration. However, it is a fact that evidence of impaired bone health in patients with epilepsy is accumulating.

AEDs associated bone disorders are result of a multifactorial causation that is most likely enhanced by non-AEDs related risk factors. Regarding AEDs' role in bone health depletion, large RCTs are needed [53].

AEDs induced mechanisms causing reduced bone density.
Enzyme inducing (EIAEDs) and non-enzyme inducing AEDs (non-EIAEDs)

Since the 1960s until the recent years a growing number of scientifically based publications have been trying to claim an association between AEDs and reduced bone health. Generally the results are assuming an alteration of bone metabolism towards a catabolic state and consequently decreased bone mineral density. Several mechanisms have been suggested to explain the negative impact AEDs might have on bone health and to prevent further progress of the condition after disease manifestation.
Hence, the serious effects of AEDs on bone health there is not sufficient awareness of this problem amongst general practitioners and specialists. And the mechanisms behind it are more intricate than previously assumed.
Based on their qualities AEDs can be categorized into CYP3A4 enzyme-inducing antiepileptic drugs (EIAEDs) like carbamazepine, phenytoin, and oxcarbazepine or as non-EIAEDs like valproic acid, levetiracetam, and lamotrigine

Postulated mechanisms. Hepatic induction of the cytochrome P450 enzyme system, which in turn increases catabolism of vitamin D is the main theory reported. Enzyme inducing AEDs (EIAEDs) induce the metabolism of vitamin D to inactive metabolites. Vitamin D is inert and must be activated to exert its biological effect. Vitamin D compounds are primarily catabolized by oxidation, and each oxidative step leads to progressive loss of biological activity. These steps involve many enzymes, including many CYP enzymes. CYP-enzyme inducing AEDs are described in both animal and human studies and the result of their effect is that they cause increased conversion of vitamin D to polar inactive metabolites in the liver microsomes, reducing bioavailable vitamin D [54].

However this cannot explain why also Valproate (VPA) is found to reduce bone health, and that there is evidence of bone turnover independent of low vitamin D levels [55]. In one study men and women with epilepsy who were receiving AEDs had accelerated bone turnover with elevated markers of bone formation and bone resorption. However, only the women showed biochemical signs of vitamin D deficiency [55]. In another study 12 male epileptic patients were evaluated before treatment with carbamazepine and again 1 year later. Markers of bone turnover were increased, although serum levels of calcium, phosphate, magnesium, PTH and vitamin D metabolites were normal. These studies show that mechanisms other than vitamin

D deficiency can explain the accelerated bone turnover seen in some patients receiving AEDs [56].

Reduced bioavailability of vitamin D, leads to decreased absorption of calcium in the gut, and in turn hypocalcaemia and therefore increased circulating PTH. PTH extracts more calcium from the bones [57]. Furthermore, there is evidence of inhibition of the cellular response to PTH, where fetal rats treated with phenytoin or phenobarbital demonstrated an impaired response to PTH [58]. Inhibition of the bone resorptive response to PTH could lead to hypocalcaemia, which is a frequent finding in epileptic patients [59].

Calcitonin deficiency has also been presented as a possible mechanism, which also can accelerate bone turnover by inhibiting osteoclast mediated bone resorption. These results were seen both in vivo and in vitro [60].

A study has shown that rats treated with PTH have reduced absorption of calcium in the gut, which leads to hypocalcaemia and consequently hyperparathyroidism. No such changes were seen in rats treated with Phenobarbital (PB) [61]. One study in rats looked at the intestinal calcium transport effects phenytoin and phenobarbital may have. Rats treated with phenytoin had markedly decreased calcium absorption, whereas rats treated with phenobarbital showed calcium absorption similar to controls. These findings suggest that in those patients treated with phenytoin, impaired calcium absorption may play a role [62].

Another theory focuses on hyperparathyroidism, which activates bone resorption. The hyperparathyroidism in these patients were independent of their vitamin D status [63].

Epilepsy as a disease itself is also a contributor in altering concentrations of reproductive hormones, which can result in reproductive disturbances. Epileptic seizures are associated with increased secretion of pituitary hormones, including an increase in prolactin and LH pulse frequency, making conditions as PCO and hypogonadism more prevalent in the epileptic population. CBZ also inhibited testosterone formation at clinically relevant doses in an in vitro Leydig cell model. Replacement of VPA with LTG gave normalization of endocrine function within the one-year follow-up in women with a previously diagnosed reproductive endocrine disorder possibly related to VPA medication. This result shows that LTG is not responsible for changes in endocrine and metabolic functions in women with epilepsy [64].

For many years, the main theory was that enzyme-inducing AEDs (e.g., phenobarbital, phenytoin, and carbamazepine) caused an accelerated vitamin D hydroxylation to inactive forms, resulting in hypocalcaemia, secondary hyperparathyroidism, increased bone turnover, and higher rates of bone loss. However, later studies have shown that AEDs may cause bone loss in the absence of vitamin D deficiency [65].

We have also today well established knowledge that AEDs may give rise to endocrine disturbances, which may have negative impact on bone health. The theory suggests that AEDs increase the sex steroid metabolism, leading to decreased levels of bioavailable endogenous estradiol and testosterone, which results in increased rates of bone loss. "Non-epileptic rats fed with LEV, PHT and VPA for 3 months were studied. PHT and VPA reduced bone mineral density (BMD) and content (BMC) in one or more bone compartments. VPA induced increased bone turnover, whereas modest changes were observed for PHT. LEV did not affect bone mass density or content in these animals. Surprisingly low dose LEV was associated with reduced biomechanical strength and reduced levels of the bone formation marker osteocalcin". The conclusion made from this research was that it demonstrates affection of bone health through bone quality, not only BMD- which is the most widely used method today. By using rats in these experiments, one will eliminate variations caused by lifestyle, genes, individual disease characteristics and compliance issues, but unfortunately less useful when drawing conclusions on humans [66].

Which AEDs are bone hostile? The AEDs that are mostly associated with decreased bone health are Primidone (PRM), Carbamazepine (CBZ), Phenytoin (PHT) and Phenobarbital (PB), where the common denominator is that they are inducers of cytochrome P450 enzyme system [67] [68] [69]. One study shows that lamotrigine (LTG) gives no significant change in BMD and bone metabolism [70], whereas another study [71] using computer-linked DXA showed that Valproate (VPA), which is a non-inducer, gave a 14% reduction of BMD compare with healthy controls. We have less information on new AEDs. Preliminary data indicate that LTG does not cause bone loss, while drugs which inhibit carbonic anhydrase (topiramate, zonisamide, acetazolamide) may theoretically affect bone health by causing a metabolic acidocis.

A closer look at the two AEDs chosen in our clinical study:

Carbamazepine (CBZ) is an anticonvulsant drug used in the treatment of epilepsy, prophylactically in bipolar disorder, trigeminal neuralgia, alcohol abstinence, centrally contingent diabetes insipidus, symptomatic relief of neurological pain in diabetes mellitus and acute manic episodes. CBZ has enzyme inducing qualities, which is correlated with altered bone, calcium and vitamin D metabolism [72].

The main mechanism of action is carried out by blocking voltage-gated sodium channels and consequently inhibiting the influx of Na^+ into the nerve cells, making them less excitable. Carbamazepine has also been shown to potentiate GABA receptors made up of alpha1, beta2, gamma2 subunits. When taken in a single oral dose, peak plasma concentrations of unchanged carbamazepine are reached within 4 to 24 hours. The elimination half-life of unchanged carbamazepine in the plasma averages approximately 36 hours following a single oral dose, whereas after repeated administration, which leads to autoinduction of hepatic enzymes, it averages only 16 to 24 hours, depending on the duration of the medication.

Like phenytoin and phenobarbital, CBZ is an inducer of the cytochrome P450 enzyme system; however studies find conflicting results when evaluating its effect on bone and mineral metabolism and BMD. Some of the studies do report disturbances in bone and mineral metabolism, while others do not find any abnormalities. A study carried out by Tjellesen et al. found that in 30 patients receiving CBZ there were decreased levels of calcium concentrations and elevated total alkaline phosphatases [73]. In a study of 21 outpatients treated with CBZ, 3 had hypocalcemia, 1 had hypophosphatemia and 4 had elevated alkaline phosphatases [74]. In contrast to Tjellesen et al.'s studies another study showed 25(OH) D levels significantly lower than in controls. There was found evidence of increased bone turnover with elevated markers of formation and resorption after 1 year [75] and 2 years of medical treatment [76]. In a study of 21 healthy adults without epilepsy, treated with CBZ for 10 weeks did not find significant elevations of markers of bone formation and resorption [77].

Lamotrigine (LTG) is one of the newer drugs available at the market and is used alone in mono- or in combination with other drugs in polytherapy in the treatment of epilepsy, partial or generalized seizures, tonic clonic seizures or seizures related to Lennox-Gastaut syndrome. Monotherapeutical treatment is common in absence seizures. In bipolar disorder it is used

prophylactically to avoid the occurrence of depressive episodes. It's not used in treatment of mania or depression. This illustrates that the drug has a wide spectrum of action. In placebo-controlled clinical studies, lamotrigine has been shown to be effective in reducing seizure frequency and the number of days with seizures when added to existing antiepileptic drug therapy in adult patients with partial seizures, with or without generalized tonic clonic seizures, which are not satisfactorily controlled.

LTG is a non-enzyme inducing anticonvulsant. It function of action is carried out by inhibiting the voltage-gated sodium channels in a similar fashion as CBZ and by affecting the release of glutamate and aspartate. Lamotrigine acts presynaptically on voltage-gated sodium channels to decrease glutamate release. Lamotrigine is rapidly and completely absorbed following oral administration, reaching peak plasma concentrations 1.4 to 4.8 hours post-dosing. The plasma half-life averages 33 hours after single doses. Concomitant Antiepileptic Drugs: In patients with epilepsy, concomitant administration of lamotrigine with enzyme-inducing AEDs (phenytoin, carbamazepine, primidone or phenobarbital) decreases the mean lamotrigine t1/2 to 13 hours. Concomitant administration of lamotrigine with valproic acid significantly increases t1/2 and decreases the clearance of lamotrigine, whereas concomitant administration of lamotrigine with valproic acid plus enzyme-inducing AEDs can prolong t1/2 up to approximately 27 hours. Acetaminophen was shown to slightly decrease the t1/2 and increase the clearance of lamotrigine. Current studies suggest that lamotrigine has limited (if any) effect on bone health, but again, data are inconsistent.

Despite conflicting data an overall assessment might justify the conclusion that EIAEDs are most consistently associated with skeleton abnormalities.

Summary

Epilepsy is the recurrent appearance of epileptic seizures caused by continuous intracranial pathological conditions. The diagnosis is based upon a thorough anamnesis, clinical examination, and examination of anatomical structures by MRI and CT, functional examination by EEG, SPECT and PET. Epilepsy is treated not only with drugs, but also by focusing on factors that can trigger seizures, how to provide medical assistance in a seizure situation, by psychological supportive therapy and surgically. Medical treatment, antiepileptic drugs (AEDs), is the cornerstone of treatment.

Reduced bone density as a side effect of AEDs is not necessarily common knowledge among general practitioners. In a survey of 624 neurologists only 28 % were aware of the bone density decreasing effects of AEDs. The use of AEDs is not only limited to epilepsy treatment. AEDs are also commonly used in treatment of patients with neuropathic pain, migraine and psychiatric disorders. The association between AEDs and decreased bone density has been known since the first study in the 1960s. After this there has been produced a growing number of literary works underlining this association.

Peak bone mass is reached at age 30. Several hormones are involved in the regulation of bone metabolism; Growth hormone, parathyroid hormone, calcitonin, calcitriol (vitamin D), thyroidea stimulating hormone (TSH), leptin, serotonin and estrogens. Growth hormone, also called somatotropine drives the growth of bone until adult size is reached, and is the main regulator of height. Parathyroid hormone (PTH) is one of the hormones that play a part in maintaining the strength and health of the bone matrix by functioning to control the level of blood calcium. The skeleton is the largest reservoir (99%) for calcium in the body. Calcium can be stored in or removed from the skeleton to regulate serum calcium levels. When calcium homeostasis is in balance, bone resorption and formation are equivalent. If not, PTH concentration in serum will rise, and if this state is prolonged, it will lead to increased osteoclast activity, thus increasing resorption of bone. The role of the thyroid hormone named calcitonin, that increases bone deposition, is less clear. Regions rich in trabecular bone such as the hip and spine more susceptible to fractures. Prior to osteoporotic fracture, there are manifest reduced bone density (BMD), and also "microcracks", which are replaced by weaker bone.

Osteomalacia is due to inadequate mineralization of the bone. Osteomalacia increases fracture risk. Many of the effects of osteomalacia overlap with the more common osteoporosis, but the two diseases are significantly different. The causes of adult osteomalacia are many; insufficient nutrition of vitaimin D or phosphorus, renal tubular acidosis, malnutrition during pregnancy, malabsorption syndrome, hypophosphatemia, chronic renal failure, tumor-induced osteomalacia, coeliac disease and long term anticonvulsant therapy.

Osteoporosis: ("porous bones", from Greek) is a disease of bones that leads to an increased risk of fracture. WHO defines it as a bone mineral density that is 2.5 standard deviations or more below the mean peak bone mass (average of young, healthy adults) as measured by DXA; Dual energy absorptiometry. Dual-energy X-ray absorptiometry is the most widely used and most thoroughly studied bone density measurement technology. No firm recommendations for measuring bone mass in patients with epilepsy exist. Osteoporosis develop through three main mechanisms; inadequate *peak bone mass* (the skeleton develops insufficient mass and strength during growth), *excessive bone resorption* and *inadequate formation* of new bone during remodeling. An interplay of these three mechanisms underlies the development of fragile bone tissue. Hormonal factors strongly determine the extent of bone resorption, although reduced bone health is a multifactorial condition, including inadequate sunlight exposure, poor diet, institutionalization/ inadequate daily exercise. The health effects from osteoporosis are enormous and the prevalence and health care costs are high.

Osteoporosis risks can be reduced with lifestyle changes and sometimes medication. Bisphosphonate can be used prophylactic in high risk patients and in treating manifest osteoporosis. The medical treatment of manifest osteoporosis can be classified as anti-resorptive or bone anabolic. Antiresorptive agents inhibit bone resorption, while bone anabolic agents build bone. Standard guidelines are needed for prevention, screening and treatment of BMD loss in epilepsy. A study by Jain et al. found out that more than 90% of patients wanted more information about epilepsy, and 75% reported they were not given enough information about AED side effects, which includes reduced bone health. A study of Elliott et al. shows that if the physician addresses quality-of-life issues (worrying about seizure, possible side effects of AEDs that are prescribed, and overall well-being) it may improve the outcomes related to bone loss.

For many years, the main theory behind AEDs bone depleting sideeffect was that enzyme-inducing AEDs (e.g., phenobarbital, phenytoin, carbamazepine) caused an accelerated vitamin D hydroxylation to inactive forms, resulting in hypocalcaemia, secondary hyperparathyroidism, increased bone turnover, and higher rates of bone loss. However, later studies have shown that AEDs may cause bone loss in the absence of vitamin D deficiency. We have also today well established knowledge that AEDs may give rise to endocrine disturbances, which may have negative impact on bone health. The disease epilepsy itself is also a contributor in altering concentrations of reproductive hormones, which can result in reproductive disturbances.

The AEDs that are mostly associated with decreased bone health are Primidone (PRM), Carbamazepine (CBZ), Phenytoin (PHT) and Phenobarbital (PB), where the common denominator is that they are inducers of cytochrome P450 enzyme system. One study shows that lamotrigine (LTG) gives no significant change in BMD and bone metabolism, whereas another study using computer-linked DXA showed that Valproate (VPA), which is a non-inducer, gave a 14% reduction of BMD compare with healthy controls. There exists less information on new AEDs. Preliminary data indicate that LTG does not cause bone loss, while drugs which inhibit carbonic anhydrase (topiramate, zonisamide, acetazolamide) can in theory reduce bone health by causing a metabolic acidocis.

Since the 1960s until the recent years a growing number of scientifically based publications have been trying to claim an association between AEDs and reduced bone health. Generally the results are assuming an alteration of bone metabolism towards a catabolic state and consequently decreased bone mineral density. Based on their qualities AEDs can be categorized into CYP3A4 enzyme-inducing antiepileptic drugs (EIAEDs) like carbamazepine, phenytoin, and oxcarbazepine or as non-EIAEDs like valproic acid, levetiracetam, and lamotrigine. Hepatic induction of the cytochrome P450 enzyme system, which in turn increases catabolism of vitamin D is the main theory reported. IEAEDs cause several different alterations in metabolism of vitamins and minerals related to bone physiology. Carbamazepine and lamotrigine are the two drugs studied in the clinical study part. CBZ has enzyme inducing qualities, which are correlated with altered bone, calcium and vitamin D metabolism. LTG is a non-enzyme inducing AED, which is generally not related to altered bone metabolism, but current data is conflicting.

CLINICAL STUDY

SUMMARY

Purpose: To assess the comparative effects of the non-enzyme inducing drug lamotrigine (LTG) and enzyme inducing drug carbamazepine (CBZ) on bone health.

Methods: Patients aged 65 years or older, who had experienced at least two unprovoked partial and/or generalized tonic-clonic seizures, were randomized to receive LTG (n = 93) or CBZ (n = 92) according to a multicenter double-blind, parallel group design. Trial duration was 40 weeks and included a 4-week dose escalation followed by a maintenance phase during which dosages could be adjusted according to response. Initial maintenance and maximum dosages were 25 mg, 100 mg, and 500 mg per day for LTG, and 100 mg, 400 mg, and 2,000 mg per day for CBZ, respectively. In the LTG group, 68 patients (73%) completed the 40-week study period compared with 61 (67%) in the CBZ group, a non-significant difference. Patient data used in the current study is from 64 patients in the LTG group and 44 in the CBZ group. This selection is based on the availability of the desired bone health measuring parameters in each patient, which are estrogen, progesterone, osteocalcin, PTH, testosterone, β-CrossLaps, Ca^{2+}, total protein, alkaline phosphatase and TSH.

Results: There was a significant difference in calcium levels, where patients in the CBZ group had lower values than patients in the LTG group. Bone metabolism parameters were investigated, such as β-CrossLaps, ALP and osteocalcin, but no significant differences were found. Further there were no significant alterations in hormonal levels, except that progesterone in the CBZ group was significantly lower than in the LTG group.

Conclusion: Calcium levels were significantly lower in elderly epilepsy patients taking CBZ compared to LTG. This is consistent with earlier observations that an enzyme inducing antiepileptic drug like CBZ is more likely to affect bone health than a non-enzyme inducing drug like LTG. However, the difference in calcium levels could not be explained by differences in bone metabolism or endocrine effects. The minor difference in progesterone is not considered to be of biological importance.

Key Words: Epilepsy—Elderly—Carbamazepine—Lamotrigine — Monotherapy—Randomized controlled trial – Bone health.

INTRODUCTION

The incidence of epilepsy increases with increasing age, due to several different factors. The prevalence of epilepsy is also developing in an increasing fashion in industrialized countries. The cornerstone of treatment is antiepileptic medication, which has since the early research in the1960s been related to decreased bone density. An elderly population group is especially prone to the additive bone deprivating effects of aging, hormone changes, decreased mobility, reduced sun exposure, polytherapy, poor diet and AED use. In the northern part of the world the sun exposure is limited.

The aim of the study has been analyzing quantitative changes in bone health measuring parameters, for CBZ and LTG, respectively in an RCT. By observing the effects of these two drugs the aim will also point towards distinguishing the non-enzyme inducing qualities of LTG and enzyme inducing qualities' of CBZ effect on the mentioned parameters.

METHODS

Eligibility criteria

Patients were enrolled in the study according to the following inclusion criteria:
(i) age ≥65 years, (ii) newly diagnosed epilepsy, with a history of two or more recurrent unprovoked seizures either partial (with or without secondary generalization) or primarily generalized tonic-clonic, and at least one of the seizures during the previous 6 months; (iii) clinical indication to initiate AED treatment; (iv) life expectancy >1 year and (v) willingness to provide written free informed consent. Main exclusion criteria were (i) a history of absence, tonic, atonic or myoclonic seizures; (ii) >2-week intake of any AED in the previous 6 months, or any previous intake of CBZ or LTG; iii) treatment with any AED for five elimination half-lives in the period immediately preceding study entry; (iv) severe psychiatric disease or severe intellectual impairment; (v) acute or chronic hepatic failure; (vi) significant unpaced AV defect; (vii) alcohol or substance abuse; and (viii) clinically significant abnormalities in blood chemistry tests. The study protocol was approved by the Ethics Committees of the participating centers.

Study design

The study was conducted according to a randomized double-blind, double-dummy parallel-group design. A total of 29 centers from four countries (Finland, France, Italy, and Norway)

contributed to enrolment of patients. Patients fulfilling the eligibility criteria were randomized to receive LTG (Lamictal, 25 and 100 mg chewable/dispersible tablets, GSK, Brentford U.K.) or sustained release CBZ (Tegretol, 100 and 200 mg divisible tablets, Novartis, Camberley U.K.) together with double-dummy placebo of the alternative treatment. A subset of CBZ 100 mg tablets were split into one half to meet dosing requirement during escalation. In order to maintain the blind, active and placebo tablets were encapsulated and packaged in a double-blind, double-dummy presentation.

The 40-week treatment period included an initial 4-week dose escalation phase and a 36-week maintenance phase. In the escalation phase, LTG and CBZ were administered at a dosage of 25 mg and 100 mg respectively at night for the first 2 weeks, and increased to 25 mg b.i.d. and 100 mg b.i.d., respectively, during weeks 3 and 4. If intolerable adverse events occurred during the first 4 weeks, the patient was required to exit the study. On week 5, LTG was increased to 50 mg b.i.d. and CBZ to 200 mg b.i.d. If seizures occurred during the maintenance phase, increases in dosages were allowed no more rapidly than 50 mg/week for LTG or 200 mg/week for CBZ, up to a maximum of 250 mg b.i.d. for LTG or 1,000 mg b.i.d. for CBZ. Conversely if the subjects developed intolerable adverse effects, dosage could be reduced as considered indicated by the investigator, down to a minimum of 75 mg/day LTGor 300 mg/day CBZ. Plasma drug concentrations were not made available to the investigator; however, undetectable (<0.4 μg/mL LTG or < 0.1 μg/mL CBZ) or high levels (>20 μg/mL LTG or >12 μg/mL CBZ) were notified, without disclosing the nature of the treatment, and it was at the investigator's discretion to determine whether a treatment change was needed within the permitted dose range. In all cases, the blind was preserved by having patients simultaneously increase or decrease active tablets and matching placebo. Subjects not tolerating the minimum allowed maintenance dose were required to exit the trial. If subjects who had dose reductions subsequently experienced seizures, the dose could be increased again at the investigator's discretion. Subjects experiencing unacceptable seizure control and/or intolerable adverse events that could not be managed by dose adjustment, and subjects unwilling to remain in the trial for any reason, had to be withdrawn. Unless dictated otherwise by safety considerations, patients withdrawn from treatment underwent a 4-week down taper phase. For subjects who completed the study, the code was broken and the allocated treatment could be continued on an open label basis as clinically indicated.

The primary endpoint was retention on the allocated treatment. Other endpoints included seizure-freedom rates for the last 36 and 20 weeks, time to first seizure, adverse event (AE)

reports, and tolerability as quantified by the Liverpool Adverse Event Profile (AEP) score (Baker et al., 1997) in the 19-item version utilized by Gilliam et al. (2004).
Subjects were seen in the clinic at screening, at randomization (week 0) and on treatment weeks 2, 4, 12, 20, 28, 36, and 40. Safety assessments included blood pressure, pulse rate, and body weight (at screening and weeks 2, 4, 12, 20, 28, 36, 40, or last visit), recording of treatment-emergent AEs by unstructured interview and medical examination as required (all visits), detailed neurological and physical examination and ECG (at screening and week 40 or last visit), as well as standard hematology and biochemistry tests (at screening and weeks 4, 12, 20, 28, 40, or last visit). Samples for plasma drug concentration measurements were also obtained at study visits, but results were not made available to the treating physician. Details of study medications, including pill counts as a check for compliance, and any associated treatment were recorded at each visit.
Patients were instructed to contact the clinic in the event of seizure recurrence or AEs potentially requiring dose adjustment, and additional study visits were allowed as indicated. Demographic and clinical data were collected in especially designed Case Report Forms (CRFs). Seizures were recorded by subjects in seizure diaries, and entered into the CRF at each study visit. The AEP questionnaires were completed at screening and at visits on treatment weeks 12, 28, and 40 (or last visit).

Bone health parameters

In the present study, concerning bone health, we have investigated the following parameters:
1) General: Calcium, alkaline phosphatase, total protein.
2) Bone metabolism: osteocalcin and β-CrossLaps.
3) Endocrinology: PTH, estrogen, progesterone, testosterone, TSH.

STATISTICS

The statistical program used for analysis and data presentation is R project for statistical computing.

The main analyses made are T-tests comparing the effects of CBZ and LTG on the bone health of the whole group, T-tests comparing the data of women in the two groups and T-tests comparing the data of men in the two groups.

Normal distribution of the parameter values in men and women, respectively, in both groups are selectively presented in QQ-plots.

RESULTS

Study subjects ranged in age from 65 to 91 years, and most had symptomatic epilepsy, except a modest predominance of males over females in the CBZ cohort; the two groups were well balanced in terms of demographic and clinical characteristics. The number of patients completing the 40-week treatment was 68 (73%) in the LTG group and 61 (67%) in the CBZ group. No patient was excluded from the analysis based on results of plasma drug concentration measurements.

Reference values

Biochemical markers of bone metabolism in patients being treated with CBZ and LTG, respectively, compared with reference values of healthy adults. Source: www.prosedyrer.no, Brukerhåndbok i medisinsk biokjemi (User manual in Medical Biochemistry).

As shown in table 1, age and genders are determinators for different reference values. For estrogen we calculated the average and used it as reference value in the study analysis.

Table 1

Reference values

Estrogen		**Testosterone**	
Follicular phase	80-790 pmol/L		
Mid phase	700-2100 pmol/L	Men:	
Luteal phase	80-850 pmol/L	16–19 years	8,4 - 29,2 nmol/L
Postmenopause	6-100 pmol/L	20–50 years	9,1 - 55,2 nmol/L
Men	20-130 pmol/L	>50 years	6,3 - 26,3 nmol/L *
Average	13-115 pmol/L *		
Progesterone		**Crosslaps**	< 0,76 µg/ml *
Women			
		Calcium	
Follicular phase	0,6 - 3,6 nmol/L	Women and men	2,15 - 2,51 mmol/L *
Mid luteal phase	19,0 - 76,0 nmol/L		
Men:	0,6 - 2,5 nmol/L *		
		Total protein	
Osteocalcin		Women and men	Plasma: 64 - 79 g/L *
Women and men			
11-15 years	1,7 - 13,4 nmol/L	**ALP**	
16-20 years	1,7 - 8,4 nmol/L	Women and men	
	>20 år: 0,6 - 3,4 nmol/L*	0 - 17 years	35 - 400 U/L
		> 17 years	35 - 105 U/L *
PTH			
16-50 years	1,0 - 5,7 pmol/L	**TSH**	
50-65 years	1,2 - 7,5 pmol/L	Women and men	0,5 - 4,4 mIE/L *
>65 years	1,5 - 9,1 pmol/L *	16-20 years	

*Values used in our statistical analysis.

T-tests

T-tests are performed on the data for the whole group, for women and for men, respectively, to assess whether there is any statistically significant difference between them.
A P-value below 0,05 qualifies to rejection of the null hypothesis.

Table 2

T-test for LTG and CBZ all patients

Parameter	P-value	CI 95%		SD CBZ	SD LTG
E2	0.508	- 3.04	6.10	14.3	27.4
Progesterone	1.344e-5	0.08	0.20	0.21	0.20
Osteocalcin	0.850	- 4.81	3.97	10.4	23.2
PTH	0.447	-12.5	5.54	23.7	41.5
Testosterone	0.153	- 1.52	0.24	2.39	2.17
β -CrossLaps	0.374	- 0.03	0.09	0.18	0.27
Calcium	0.013	0.01	0.08	0.08	2.32
Total protein	0.581	- 1.15	2.04	4.08	69.5
ALP	0.460	-17.3	7.88	30.5	82.4
TSH	0.784	- 0.58	0.44	1.18	1.85

As shown in table 2, the P-value for calcium reveals a statistically significant difference between the two groups. The confidence interval of calcium confirms this finding. However, when one looks at women and men separately (table 3 and 4), the P-values shows no significance. A possible explanation might be that by an increase in studied population subsequently increases the possibility to reveal a statistically significant difference.

Looking at the bone metabolism parameters ALP, osteocalcin and β-CrossLaps in table 2, no significant difference between the two groups in this matter exists to explain the different calcium levels, not even when separating men and women data.

Table 3

T-test for LTG and CBZ in women

	P-value	CI 95%	SD CBZ	SD LTG
E2	0.248	-3.71 13.95	9.34	18.93
Prog	0.001	0.05 0.19	0.06	0.16
Oste	0.101	-13.62 1.29	10.75	10.94
PTH	0.082	-19.92 1.26	14.89	16.56
Test	0.364	-0.73 0.29	0.84	0.15
β-CLaps	0.556	-0.11 0.06	0.09	0.19
Ca	0.110	-0.02 0.14	0.11	0.09
Tot. p	0.812	-2.64 2.09	2.84	3.97
ALP	0.942	-19.80 21.30	20.07	42.15
TSH	0.479	-1.86 0.92	1.95	1.44

Table 4

T-test for LTG and CBZ in men

	P-value	CI 95%	SD CBZ	SD LTG
E2	0.559	-2.91 5.34	8.47	8.12
Prog	4.416 e-05	0.10 0.27	0.11	0.22
Oste	0.464	-3.42 7.40	11.52	10.14
PTH	0.898	-12.42 14.13	25.38	28.17
Test	0.803	-0.74 0.95	1.57	1.83
β-CLaps	0.115	-0.02 0.14	0.15	0.16
Ca	0.130	-0.01 0.07	0.07	0.08
T. prot	0.254	-0.88 3.28	4.16	3.97
ALP	0.333	-21.48 7.47	34.52	17.03
TSH	0.460	-0.47 0.21	0.64	0.68

Further, an analysis was performed on hormone levels, including progesterone, estrogen (E2), testosterone, PTH and TSH to see if there was any difference here that could explain the significant calcium decrease in the CBZ group. Progesterone had a statistically significant P-value, also when analyzing at men and women separately. However, these progesterone levels are very low, and are biologically of low value.

Other analyses

In the following tables there is a presentation of analyses, that is mean values, standard deviations, minimum and maximum values of the different parameters.

CBZ

Table 6

Calcium, ALP, total protein

		N	Mean	SD	Min.	Max.
Ca	**W**	13	2,31	0,11	2,09	2,42
	M	31	2,32	0,06	2,2	2,41
	All	44	2,31	0,08	2,09	2,42
ALP	**W**	13	77,36	20,06	53	119
	M	31	84,35	34,52	41	210
	All	44	82,38	31,03	41	210
Tot.	**W**	13	69,09	2,84	65	73
p	**M**	31	69,71	4,16	61	77
	All	44	69,53	3,81	61	77

LTG

		N	Mean	SD	Min.	Max.
Ca	**W**	29	2.37	0.09	2.26	2.59
	M	35	2.35	0.08	2.2	2.54
	All	64	2.36	0.08	2.2	2.59
ALP	**W**	29	78.11	42.15	30	268
	M	35	77.35	17.03	39	115
	All	64	77.69	30.49	30	268
Tot.	**W**	29	68.81	3.97	59	75
p	**M**	35	70.91	3.97	63	78
	All	64	69.98	4.07	59	78

CBZ

Table 5

Osteocalcin, PTH, β-CrossLaps

		N	Mean	SD	Min.	Max.
Osteo	**W**	13	28,92	10,75	18	54
	M	31	20,77	11,26	7	58
	All	44	23,18	10,4	7	58
PTH	**W**	13	40,81	14,89	24	76,62
	M	31	43,04	25,37	14,36	116
	All	44	41,47	41,5	14,36	116
β-	**W**	13	0,29	0,08	0,18	0,48
Cros	**M**	31	0,26	0,15	0,06	0,72
	All	44	0,27	0,18	0,06	0,72

LTG

		N	Mean	SD	Min.	Max.
Osteo	**W**	29	22.8	10.9	9	46
	M	35	22.8	10.1	11	54
	All	64	22.8	10.4	9	54
PTH	**W**	29	33.7	16.6	8.46	68.27
	M	35	41.66	28.2	10.2	165.2
	All	64	38.0	23.7	8.46	165.2
β-	**W**	29	0.27	0.19	0.04	0.85
Cros	**M**	35	0.33	0.16	0.13	0.7
	All	64	0.30	0.18	0.04	0.85

CBZ

Table 7

Estrogen, progesterone, testosterone, TSH

		N	Mean	SD	Min.	Max.
E2	**W**	13	21,06	9,34	12,7	43,1
	M	31	30,05	8,47	17,4	61,3
	All	44	27,4	9,57	12,7	61,3
Prog	**W**	13	0,13	0,06	0,05	0,26
	M	31	0,22	0,11	0,05	0,5
	All	44	0,20	0,10	0,05	0,5
Test	**W**	13	0,37	0,84	0,02	3,16
	M	31	3,98	1,57	0,29	7,07
	All	44	2.91	2,16	0,02	7,07
TSH	**W**	13	2,79	1,94	0,27	6,01
	M	31	1,47	0,63	0,6	2,92
	All	44	1,84	1,28	0,27	6,01

LTG

		N	Mean	SD	Min.	Max.
E2	**W**	29	26.19	18.93	11.2	89.1
	M	35	31.26	8.12	14.5	56.6
	All	64	28.93	14.26	11.2	89.1
Prog	**W**	29	0.26	0.16	0.1	0.73
	M	35	0.42	0.22	0.11	1.12
	All	64	0.34	0.21	0.1	1.12
Test	**W**	29	0.15	0.15	0.02	0.81
	M	35	4.09	1.83	0.03	6.84
	All	64	2.28	2.39	0.02	6.84
TSH	**W**	29	2.32	1.44	0.16	6.17
	M	35	1.35	0.68	0.22	2.93
	All	64	1.78	1.18	0.16	6.17

Fig. 1

CBZ women, QQ plots of ost, c.laps, ca, ALP.

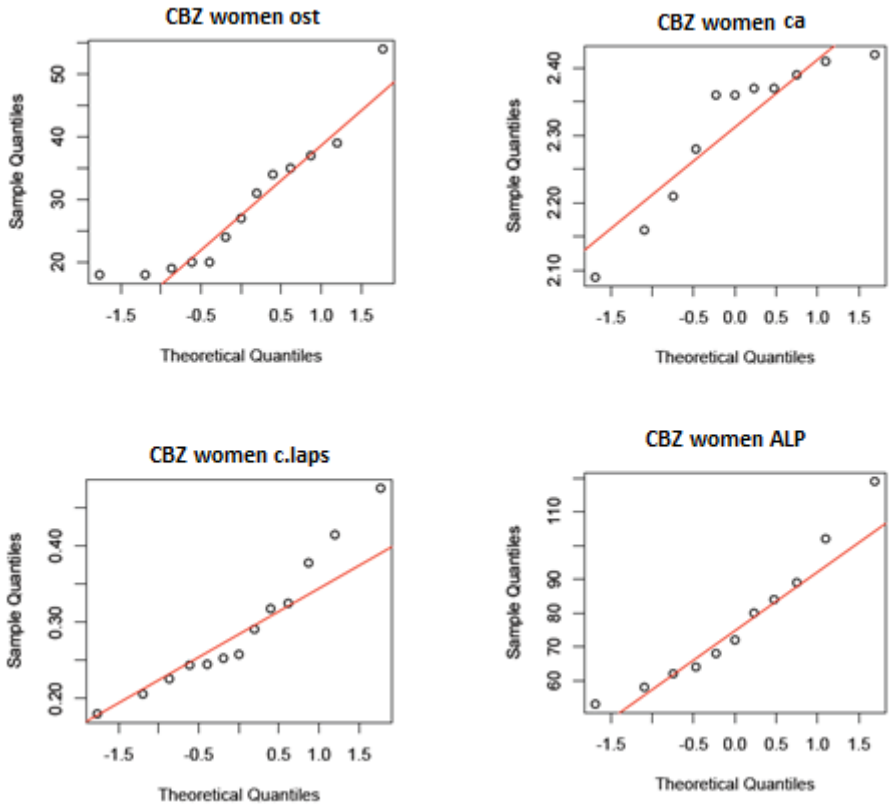


Fig. 2

LTG men, QQ plots of ost, c.laps, ca, ALP

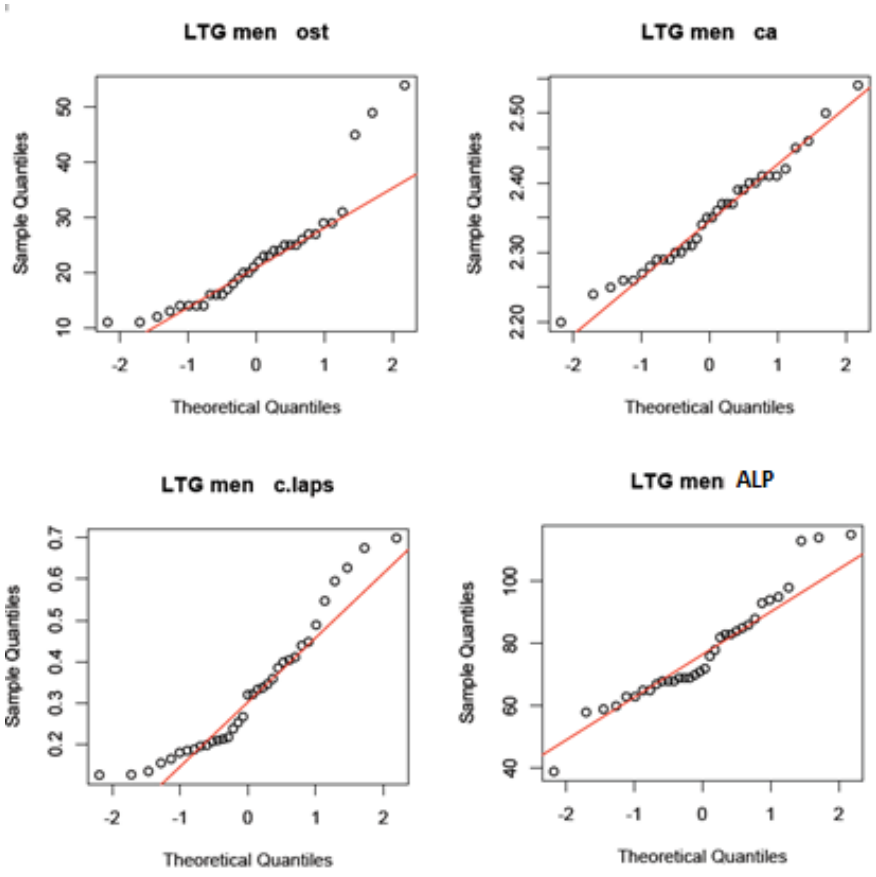
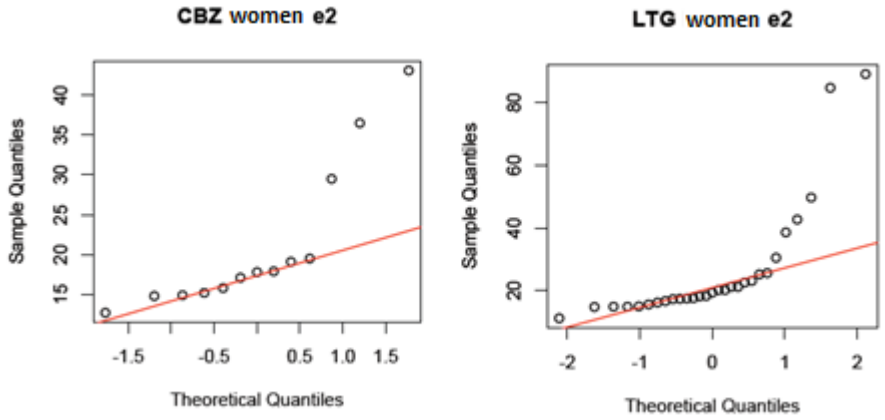


Fig. 3

CBZ and LTG, QQ plots of E2 in women.

Illustrating outlayers.

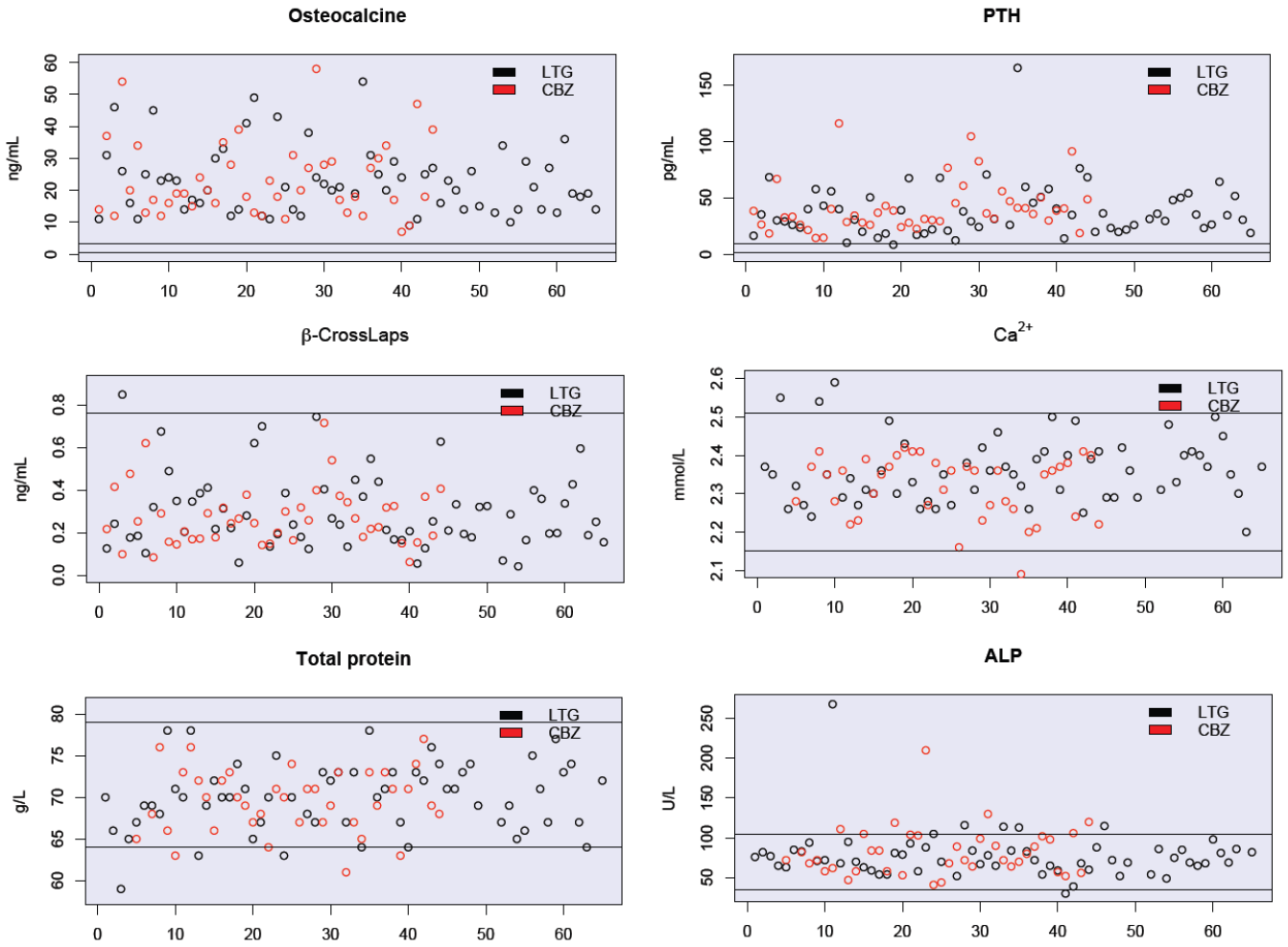


The QQ plots for the measured parameters represent normal distribution.

Figure 3 illustrates some typical outlayer values, whilst the remaining values are normally distributed.

Fig. 4

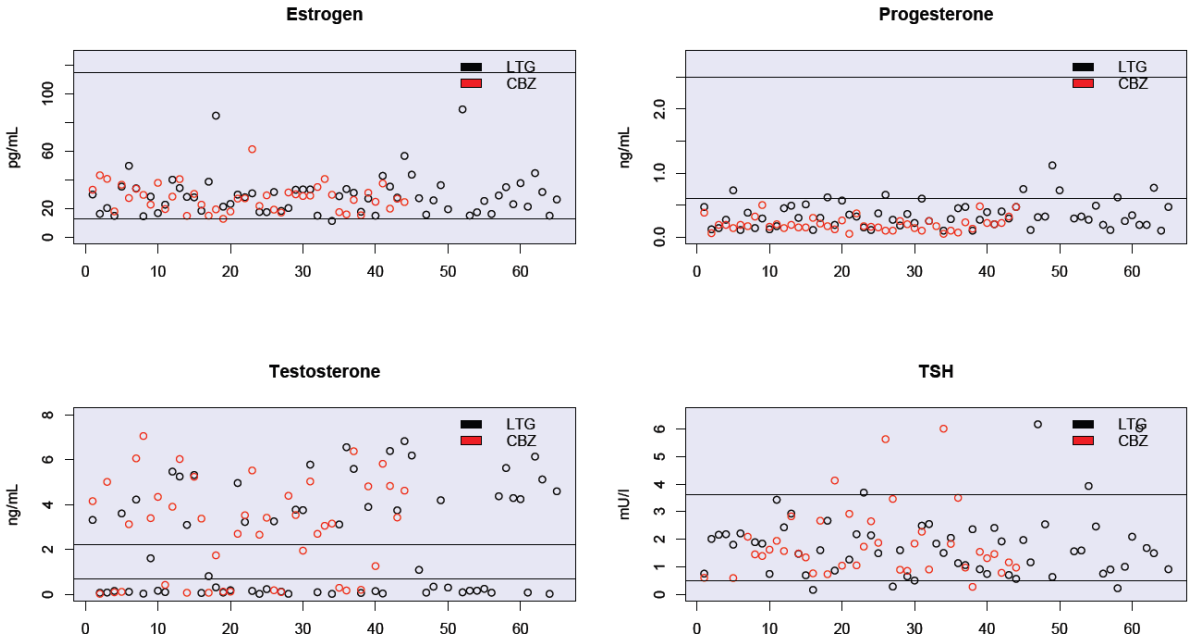
Observed measurements of bone metabolism parameters for all patients



Black lines illustrating upper and lower reference values.

Fig. 5

Observed measurements of hormones for all patients



DISCUSSION

The statistical analysis shows significant differences in calcium and progesterone levels, between the CBZ and LTG group, where patients in the CBZ group had lower values. The importance of this finding of lowered calcium levels, which is directly correlated to bone health, in the CBZ group might strengthen the theory about AEDs bone depleting qualities. In this study the focus has been to measure ten different physiological parameters related to bone health, which are also mentioned in other related literature trying to explain AEDs' effect on bone health. Other factors able to influence calcium levels might be those who haven't been measured in this study, e.g. insufficient diet, comorbidity, polymedication with more. The studied subjects' age (>65 years) might be a contributing factor. However, in spite of lack of studying these factors, the process of randomization from a more or less homogenous population into equal groups makes the groups quiet equal.

The study investigated bone metabolism parameters such as β-CrossLaps, ALP and osteocalcin comparing the two groups, but found no significant changes between them. There was not any significant alteration in hormonal levels that could explain the reduction of

calcium in the CBZ group either, except progesterone in the CBZ group that was significantly lower than the LTG group. This is neither necessarily of biological importance nor practical significance, because the actual progesterone level is quite low originally, that even minor changes can give statistically significant differences between CBZ and LTG.

It is also an important point that it is difficult to compare results of therapeutic drug trials in younger patients with an elderly population. First of all, the etiology of the disease differs across ages, comorbidity and comedication are more prevalent among people >65 years of age, and this makes the two different age groups incomparable. Age can affect pharmacokinetics; especially elderly patients tend to achieve seizure control at lower AED dosages and lower serum AED levels than younger subjects [78].

However the study has several quality strengthening aspects. I. e. participants from different countries, randomization in regard to gender and age.

The study reveals changes in the chosen bone health measuring parameters. A significant change in only one parameter, calcium, doesn't necessarily justify the rejection of theory that AEDs have bone depleting qualities. In future studies, there might be need for following the subjects over a longer period, study a larger population of subjects, have base line values for comparison and a control group and also study other parameters than in the current study, that is phosphate, biologically active vitamin D metabolites, calcitonin, IGF-I and homocysteine.

Reference list

1. Sander JW. *The epidemiology of epilepsy revisited*. Curr Opin Neurol 2003; 16(2): 165-70.
2. Svendsen T, Lossius M, Nakken KO. *Age specific prevalence of epilepsy in Oppland County, Norway*. Acta Neurol Scand 2007; 116(5): 307-11.
3. Taubøll*: Epilepsi – en kort oversikt*. A lecture for medical students, Rikshospitalet University Hospital.
4. Taubøll and Nakken. *Bone loss associated with use of antiepileptic drugs.* Informa healthcare 2010; 1.
5. Valmadrid C, Voorhees C, Litt B, et al. *Practice patterns of neurologists regarding bone and mineral effects of antiepileptic drug therapy*. Arch Neurol. 2001; 58: 1369-74.
6. Landmark CJ. *Antiepileptic drugs in non-epilepsy disorders: Relations between mechanisms of action and clinical efficacy*. CNS drugs 2008; 22 (1): 27-47.
7. Mossekilde L, Hansen HH, Christensen MS. *Fractional intestinal calcium absorption in epileptics on anticonvulsant therapy; short term effects of 1,25-dihydroxycholecalciferol and 25-hydroxycholecalciferol* . Acta Med. Scand 1979; 205: 401-4.
8. Dymling JF, Lindgren L, Walloe A. *Biochemical variables related to calcium metabolism in epileptics*. Acta Med Scand 1979; 205: 405-9.
9. Pack. *The association between antiepileptic drugs and bone disease*. Current review. 2003: 1.
10. Marcus et. al. *Dynamics of bone and cartilage metabolism.* 2nd ed. Great Britain. Academic press publications: 203-204.
11. Geneser. 2006. *Histologi – På molekylærbiologisk grundlag*. 6th ed. Denmark. Publishing house Munksgaard Denmark: 282-283.
12. Fitzpatrick LA. *Pathophysiology of bone loss in patients receiving anticonvulsant therapy.* Epilepsy Behav 2004; 5: S3-15.
13. Valsamis HA, Arora SK, Labban B, et al. *Anti-epileptic drugs and bone metabolism.* Nutr. Metab (Lond) 2006; 3-36
14. Pack AM, Walczak TS, *Bone health in women with epilepsy: Clinical features and potential mechanisms.* Int Rev Neurobiol. 2009; 83: 305-28.

15. Dreznet MK. *Treatment of anticonvulsant drug-induced bone disease.* Epilepsy Behav. 2004; 5: S41-7.
16. Geneser. 2006. *Histologi – På molekylærbiologisk grundlag.* 6th ed. Denmark. Publishing house Munksgaard Denmark: 287-292.
17. Marcus et. al. *Dynamics of bone and cartilage metabolism.* 2nd ed. Great Britain. Academic press publications: 227-238.
18. Marcus et. al. *Dynamics of bone and cartilage metabolism.* 2nd ed. Great Britain. Academic press publications: 487.
19. Geneser. 2006. *Histologi – På molekylærbiologisk grundlag*. 6th ed. Denmark. Publishing house Munksgaard Denmark: 303-304.
20. Marcus et. al. *Dynamics of bone and cartilage metabolism*. 2nd ed. Great Britain. Academic press publications: 495.
21. Marcus et. al. *Dynamics of bone and cartilage metabolism*. 2nd ed. Great Britain. Academic press publications: 499.
22. Marcus et. al. *Dynamics of bone and cartilage metabolism*. 2nd ed. Great Britain. Academic press publications: 99-100.
23. Marcus et. al. *Dynamics of bone and cartilage metabolism*. 2nd ed. Great Britain. Academic press publications: 273.
24. Geneser. 2006. *Histologi – På molekylærbiologisk grundlag*. 6th ed. Denmark. Publishing house Munksgaard Denmark: 615.
25. Geneser. 2006. *Histologi – På molekylærbiologisk grundlag*. 6th ed. Denmark. Publishing house Munksgaard Denmark: 304-305.
26. Geneser. 2006. *Histologi – På molekylærbiologisk grundlag.* 6th ed. Denmark. Publishing house Munksgaard Denmark: 612.
27. Pack AM, Morrell MJ. *Epilepsy and bone health in adults.* Epilepsy Behav 2004; S41-7.
28. Dr. Jan Falch, Norsk Elektronisk legehåndbok, 2011; *Osteoporose.* https://vpn2.uio.no/+CSCO+dh756767633A2F2F79727472756E61716F62786E2E6162++/endokrinologi/tilstander-og-sykdommer/beinvev-og-vitamin-d/osteoporose-1184.html
29. Erik Taubøll, Karl O. Nakken. *Bone loss associated with use of antiepileptic drugs.* Expert opinion 2010.
30. Pack AM. *The association between Antiepileptic drugs and Bone disease*, Vol 3 2003; 91-95.

31. Alldredge BK, Koda-Kimble MA, Young LY, Kradjan WA, Guglielmo BJ. *Applied therapeutics: the clinical use of drugs.* Philadelphia: Wolters Kluwer Health/Lippincott Williams & Wilkins. 2009; pp. 101–3.
32. Raisz L. *Pathogenesis of osteoporosis: Concepts, conflicts, and prospects*. J Clin Invest 2005; 115 (12): 3318–25.
33. Raisz L. *Pathogenesis of osteoporosis: Concepts, conflicts, and prospects.* J Clin Invest 2005; 115 (12): 3318–25.
34. Pack AM. *The association between Antiepileptic drugs and Bone disease*, Vol 3 2003; 91-95.
35. Meunier, Pierre (1998). *Osteoporosis: Diagnosis and Management.* London: Taylor and Francis. ISBN 1-85317-412-2.
36. Brian K Alldredge; Koda-Kimble, Mary Anne; Young, Lloyd Y.; Wayne A Kradjan; B. Joseph Guglielmo (2009). *Applied therapeutics: The clinical use of drugs.* Philadelphia: Wolters Kluwer Health/Lippincott Williams & Wilkins. pp. 101–3. ISBN 0-7817-6555-2.
37. Souverein et al., 2005. *Incidence of fractures among epilepsy patients.* Blackwell Publishing.
38. Harden. 2003; *Contribution of current and past traumatic events to pseudoseizures and epilepsy*. Pubmed Central.
39. Sheth et al. 2007; *Screening for bone health in epilepsy*. Blackwell Publishing.
40. Meng-Yi Weng MD and Nancy E. Lane MD. *Medication-induced Osteoporosis*. Current Osteoporosis Reports**.** 2007, 5:139–145.
41. Riggs, B.L.; Melton, Lj 3.r.d. (2005). *The worldwide problem of osteoporosis: insights afforded by epidemiology.* Bone 17 (5 Suppl): 505S–511.
42. Elliott JO, Jacobsen MP, Seals BF. *Self-efficacy, knowledge, health beliefs, quality of life, and stigma in relation to osteoprotective behaviors in epilepsy*. Epilepsy & Behavior 2006; 9: 478–491.
43. Brian K Alldredge; Koda-Kimble, Mary Anne; Young, Lloyd Y.; Wayne A Kradjan; B. Joseph Guglielmo (2009). *Applied therapeutics: the clinical use of drugs.* Philadelphia: Wolters Kluwer Health/Lippincott Williams & Wilkins. pp. 101–3. ISBN 0-7817-6555-2.
44. Meunier, Pierre (1998). *Osteoporosis: Diagnosis and Management*. London: Taylor and Francis. ISBN 1-85317-412-2.

45. Jaglal SB, Kreiger N, Darlington GA. *Lifetime occupational physical activity and risk of hip fracture in women*. Ann Epidemiol 1995; 5 (4): 321-4.
46. Cummings SR, Nevitt MC, Browner WS, et al. *Risk factors for hip fracture in white women. Study of osteoporotic fracture research group*. N Engl J Med 1995,332 (12):767-73.
47. Vestergaard P, Tigaran S, Reinmark L, et al. *Fracture risk is increased in epilepsy.* Acta Neurol Scand 1999; 99: 269-75.
48. Volpe SI, Schall JI, Gallagher PR, et al. *Nutrient intake of children with intractable epilepsy compared with healthy children*. J Am Diet Assoc 2007; 107(6):1014-18.
49. Nakken KO, Taubøll E. *Bone loss associated with use of antiepileptic drugs*. Expert Opin. Drug Saf. 2010; 9 (4): 561-571.
50. Nakken KO. *Physical exercise in outpatients with epilepsy*. Epilepsia 1999; 40 (5): 643-51.
51. Svalheim S, Røste L, Nakken KO, Taubøll E. *Bone health in adult with epilepsy*. Acta Neurol Scand: 2011; 124 (Suppl. 191):89-95.
52. Svalheim S, Røste L, Nakken KO, Taubøll E. *Bone health in adult with epilepsy.* Acta Neurol Scand: 2011; 124 (Suppl. 191):89-95.
53. Fitzpatrick LA. *Pathophysiology of bone loss in patients receiving anticonvulsant therapy.* Epilepsy Behav 2004; 5: S3-15.
54. Svalheim S, Taubøll E, Luef G, Lossius A, Rauchenzauner M, Sandvand F, Bertelsen M, Mørkrid L, Gjerstad L. *Differential effects of levetiracetam, carbamazepine, and lamotrigine on reproductive endocrine function in adults*. Epilepsy & Behavior 16 (2009) 281-287.
55. Valimaki MJ, Tiihonen M, Laitinen K, Tahtela R, Karkkainen M, Lamberg-Allardt C, Makela P, Tunninen R. *Bone mineral density measured by dual-energy X-ray absorptiometry and novel markers of bone formation and resorption in patients on anti-epileptic drugs*. J Bone Miner Res 1994;9:631–637.
56. Verrotti A, Greco R, Morgese G, et al. *Increased bone turnover in epileptic patients treated with carbamazepine*. Ann Neurol 2000; 47: 385-8.
57. Taubøll E, Isojärvi JIT, Gjerstad L. *Gender-specific side-effects of antiepileptic drugs.* 2009 Elsevier Ltd.
58. Gough H, Goggin T, Bissessar A, Baker M, Crowley M, Callaghan N. *A comparative study of the relative influence of different anti-convulsant drugs, UV exposure and diet on vitamin D and calcium.* Q J MED 1986; 59 (230): 569-77.

59. Sheth RD, Wesolowski CA, Jacob JC, Penney S, Hobbs GR, Riggs JE, Bodensteiner JB. *Effect of carbamazapine and valproate on bone mineral density*. J Pediatr 1995; 127:256–262.
60. Valimaki MJ, Tiihonen M, Laitinen K, Tahtela R, Karkkainen M, Lamberg-Allardt C, Makela P, Tunninen R. *Bone mineral density measured by dual-energy X-ray absorptiometry and novel markers of bone formation and resorption in patients on anti-epileptic drugs*. J Bone Miner Res 1994; 9:631–637.
61. Koch HV, Kratf D, von Herrath D; *Influence of diphenylhydantoin and phenobarbital on intestinal calcium transport in the rat. Epilepsia* 1972; 13: 829-41.
62. Sato Y, Kondo I, Ishida S, Motooka H, Takayama K, Tomita Y, Maeda H, Satoh K. *Decreased bone mass and increased bone turnover with valproate therapy in adults with epilepsy.* Neurology 2001;57:445–449
63. Taubøll E, Isojärvi JIT, Gjerstad L. *Gender-specific side-effects of antiepileptic drugs.* 2009 Elsevier Ltd.
64. Nissen-Meyer LSH, Svalheim S, Taubøll E, Gjerstad L, Reinholt FP, Jemtland R. *How can antiepileptic drugs affect bone mass, structure and metabolism?* Lessons from animal studies. 2007 British Epilepsy Association, Published by Elsevier Ltd.
65. Nissen-Meyer LSH, Svalheim S, Taubøll E, Gjerstad L, Reinholt FP, Jemtland R. *How can antiepileptic drugs affect bone mass, structure and metabolism?* Lessons from animal studies. 2007 British Epilepsy Association, Published by Elsevier Ltd.
66. Sheth RD, Wesolowski CA, Jacob JC, Penney S, Hobbs GR, Riggs JE, Bodensteiner JB. *Effect of carbamazapine and valproate on bone mineral density*. J Pediatr 1995; 127:256–262.
67. Gough H, Goggin T, Bissessar A, Baker M, Crowley M, Callaghan N. *A comparative study of the relative influence of different anti-convulsant drugs, UV exposure and diet on vitamin D and calcium.*
68. Valimaki MJ, Tiihonen M, Laitinen K, Tahtela R, Karkkainen M, Lamberg-Allardt C, Makela P, Tunninen R. *Bone mineral density measured by dual-energy X-ray absorptiometry and novel markers of bone formation and resorption in patients on anti-epileptic drugs.* J Bone Miner Res 1994; 9:631–637.
69. Stephen LJ, McLellan AR, Harrison JH, Shapiro D, Dominiczak MH, Sills GJ, BrodieMJ. *Bone density and antiepileptic drugs: a case controlled study*. Seizure 1999; 8:339–342.

70. Taubøll E, Isojärvi JIT, Gjerstad L. *Gender-specific side-effects of antiepileptic drugs*. 2009 Elsevier Ltd.
71. Sato Y, Kondo I, Ishida S, Motooka H, Takayama K, Tomita Y, Maeda H, Satoh K. *Decreased bone mass and increased bone turnover with valproate therapy in adults with epilepsy.* Neurology 2001;57:445–449
72. Verrotti A, Greco R, Morgese G, Chiarelli F. *Increased bone turnover in epileptic patients treated with carbamazepine.* Ann Neurol 2000; 47:358-8.
73. Tjellesen L. Christiansen C. *Serum vitamin D metabolites in epileptic patients treated with 2 different anticonvulsant drugs.* Acta Neurol Scand 1982; 66:335-41.
74. Hoikka V, Alhava EM, Karjalainen P, et al. *Carbamazepine and bone mineral metabolism.* Actal Neurol Scand 1984; 70:77-80.
75. Verotti A, Greco R, Latini G, Morgese G, Chiarelli F. *Increased bone turnover in epileptic patients treated with carbamazepine.* Epilepsia 2000; 47:385-8.
76. Verotti A, Greco R, Latini G, Morgese G, Chiarelli F. *Increased bone turnover in prepubertal, pubertal and postpubertal patients receiving carbamazepine.* Epilepsia 2002; 47:358-8.
77. Bramswig S, Zittermann A, Berthold HK. *Carbamazepine does not alter biochemical parameters of bone turnover in healthy male adults.* Calcif Tissue Int 2003; Jul 24 [Epub ahead of print].
78. Erik Saetre, Emilio Perucca, Jouko Isojarvi and Leif Gjerstad. An International Multicenter Randomized Double-Blind Controlled Trial of Lamotrigine and Sustained-Release Carbamazepine in the Treatment of Newly Diagnosed Epilepsy in the Elderly. *Epilepsia*, **48**(7):1292–1302, 2007.