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Effects of Etelcalcetide on Bone Microstructure in the Adenine-Induced Chronic Kidney Disease Rat Model

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Abstract

Objective: Chronic kidney disease (CKD) with secondary hyperparathyroidism (SHPT) increases the risk of fragility fractures with deterioration of cortical and trabecular bone microstructure. Etelcalcetide (EC), which is used to treat SHPT, reduces parathyroid hormone (PTH) levels in the blood. However, the details of the effects of EC on the microstructure of cortical and trabecular bone remain unclear. This study investigated whether EC improved the cortical and trabecular bone microstructure in CKD model rats. **Methods:** Eight-week-old, male Wistar rats were fed with a 0.75% adenine diet for 4 weeks to establish the CDK model rats. At 20 weeks of age, the rats were divided into two groups (n = 9 - 11 in each group): CKD group (vehicle administration) and EC group (0.6 mg/kg, daily). EC was injected for 4 weeks starting at 20 weeks of age. After treatment, the biochemical tests, measurement of bone mineral density and bone strength, and evaluation of cortical and trabecular bone microstructure were performed. **Results:** Compared with the CKD group, the EC group showed significantly lower serum blood urea nitrogen, calcium, and inorganic phosphorus levels ($p < 0.05$ to $p < 0.01$), significantly higher cortical area, cortical volume, and cortical thickness ($p <$ 0.05 to $p < 0.01$), and significantly lower cortical porosity ($p < 0.01$). In addition, the CKD group showed decreased bone strength in cortical bone, loss of bone mass in trabecular bone, and deterioration of bone structure, whereas these changes were suppressed in the EC group. **Conclusions:** EC significantly improved cortical microstructure and cortical porosity, suppressing deterioration of cortical bone strength and loss of trabecular bone in the adenine-induced CKD model rats.

Keywords

Chronic Kidney Disease, Secondary Hyperparathyroidism, Osteoporosis, Etelcalcetide

1. Introduction

The number of patients with chronic kidney disease (CKD) is increasing due to the growing elderly population [1] [2]. In addition to age-related osteoporosis, the risk of fragility fractures in elderly CKD patients is known to be increased by abnormalities in bone metabolism [3]. Fractures in CKD patients not only impair activities of daily living (ADL) and quality of life (QOL), but they also affect life expectancy, with mortality reported to increase immediately after fracture onset and to be 3.8 - 11.6 times higher than in non-fracture patients [4]. One of the causes of bone fragility in CKD patients is a CKD-mineral bone disorder (CKD-MBD).

CKD-MBD is an abnormality of bone metabolism caused by impaired renal function, resulting in decreased bone mass and prolonged vascular calcification [5]. In early renal hypofunction, secondary hyperparathyroidism (SHPT) develops due to increased phosphorus excretion by fibroblast growth factor 23 and impaired vitamin D activation [6] [7]. In the stage of renal dysfunction such as stage 4 - 5 CKD, SHPT is further promoted by increased serum phosphorus levels and decreased serum calcium (Ca) levels to maintain the balance between Ca and phosphorus. In addition, SHPT causes osteitis fibrosa, and vitamin D deficiency results in osteomalacia in bone lesions of CKD patients and a mixture of both as an aplastic osteopathy [5]. The continuous secretion of parathyroid hormone (PTH) by SHPT stimulates bone metabolism with increased bone resorption and formation, decreased bone mass, and increased fibrous component. [8]. Especially in cortical bone, increased bone resorption causes deterioration of bone structure, such as thinning of cortical bone and increased cortical porosity, leading to increased fracture risk in CKD [5] [9].

Therefore, it is important to treat SHPT in CKD patients. Recently, a peptide agonist of the calcium-sensing receptor (CaSR), which is a calcimimetic, has been used to treat SHPT in hemodialysis patients [10] [11]. One of the calcimimetics, etelcalcetide (EC), acts on the CaSR and suppresses PTH synthesis, which is expected to control SHPT in CKD patients. The calcimimetics have been reported to correct the CKD-MBD in patients treated with hemodialysis [12] [13]. Although EC is expected to improve bone mineral density (BMD) and bone strength by improving SHPT and CKD-MBD, the details of the effects and influences of EC on both cortical and trabecular bone microstructure are still largely unknown. However, it is difficult to investigate the effects of EC on cortical and trabecular bone microstructure in CKD patients. Therefore, the purpose of the present study was to evaluate the effects of EC on both cortical and trabecular bone microstructure and bone strength in an adenine-induced, advanced-stage CKD rat model.

2. Methods

2.1. Animal Model and Experimental Design

Eight-week-old, male Wistar rats (n = 9 - 11) (Charles River Laboratories Inc., Tokyo, Japan) were housed in a controlled environment (temperature 23˚C ± 2˚C, humidity 40% ± 20%) with a 12-hour light-dark cycle with free access to water and rat food. The details were described in our previous study [14]. Rats were treated with a 0.75% adenine diet (Oriental Yeast Co., Ltd., Tokyo, Japan) for 4 weeks until 12 weeks of age and then fed a standard rodent chow (CE-7; Clea Japan, Tokyo, Japan) diet to generate CKD model rats (CKD group). The 4-week adenine diet treatment was selected based on previous studies [14] [15], which reported that a 4-week adenine diet increased serum creatinine, phosphorus, and intact-PTH levels at 20 weeks of age and induced non-progressive, irreversible renal failure. In addition, Ferrari *et al.* demonstrated that the adenine-induced CKD model rats had a higher bone metabolism, as well as osteoclast area, than the 5/6 nephrectomized CKD model rats [16]. Based on these results, the adenine-induced CKD model rats at 20 weeks of age used in this study are considered to be appropriate for evaluating the effects of EC on cortical and trabecular bone microstructure in CKD conditions with SHPT.

At 20 weeks of age, the rats were divided into two groups: CKD group (vehicle administration) and EC group (0.6 mg/kg subcutaneous injection of EC daily). Vehicle or EC was injected into the CKD group or EC group, respectively, for 4 weeks starting at 20 weeks of age. EC was dissolved in vehicle (1 mass/volume percent (m/v%) glycine, 1 m/v% d (+)-trehalose dihydrate, 0.27 m/v% disodium succinate hexahydrate, 2 m/v% d-mannitol, 0.9 m/v% benzyl alcohol, pH 4.5), and administered subcutaneously daily for 4 weeks at a dose of 0.6 mg/kg body weight according to a previously reported protocol [17]. The non-CKD control (Cont group) was also included without the adenine diet and treated with vehicle for 4 weeks starting at 20 weeks of age. Body weight (BW) was measured at the beginning (20 weeks of age) and at the end of the experiment. The protocols for all animal experiments were approved in advance by the Animal Experimentation Committee of our institute (approval number a-1-0271), and all subsequent animal experiments adhered to the Guidelines for Animal Experimentation of our institution.

2.2. Tissue Preparation

After sacrifice, the right femur was fixed in 10% neutral-buffered formalin (Wako Chemical Industries, Osaka, Japan) until preparation for measurement of BMD and micro-computed tomography (CT). The left femur was dissected free of soft tissue, wrapped in gauze moistened with saline, and frozen at −80˚C until biomechanical testing.

2.3. Serum Biochemistry Test

At 24 weeks of age, blood samples were collected from the vena cava of rats 24 - 36 hours after EC administration. Blood samples were centrifuged at 3000 rpm for 30 minutes at 4˚C to separate the serum, which was then stored in disposable aliquots at −80˚C until analysis. Blood urea nitrogen (BUN), creatinine (CRE), calcium (Ca), and inorganic phosphorus (IP) levels were measured using a Hitachi Automatic Biochemical Analyzer 7180 (Hitachi Ltd., Tokyo, Japan).

2.4. Cortical and Trabecular Bone Microstructure Analysis

The excised right femur was secured in a specimen holder. Micro-CT was performed using the Cosmo Scan GX II (Rigaku Corporation, Tokyo, Japan) according to the manufacturer's instructions with an isotropic voxel size of 36 μm, energy of 90 kVp, and current of 88 μA. The acquired images were rendered using TRI/3D BON software (Ratoc System Engineering Co., Ltd., Tokyo, Japan). Osteoporosis assessment was performed based on total area (Tt.Ar, mm^2), cortical area (Co.Ar, mm^2), cortical volume (Ct.V, mm^3), cortical area/total area (Ct.Ar/Tt.Ar, %), cortical thickness (Ct.Th, mm), and cortical porosity (Co.Po, %) at the mid femur and bone volume/tissue volume (BV/TV, %), trabecular thickness (Tb.Th, mm), trabecular number (Tb.N, 1/mm), trabecular separation (Tb.Sp, mm), structure model index (SMI), and connectivity density (Conn.D) at the distal femur. The measurement area of the middle femur was a 1000-μm-high region centered on the midpoint of the entire femur. The measurement area of the distal femur was 2000 μm cranially from 500 μm from the reference line connecting the two ends of the growth plate of the sagittal image.

2.5. BMD Measurement

BMDs of the femur and lumbar vertebrae (L2-4) were measured using dual-energy X-ray absorptiometry (Horizon® DXA System; Hologic, Bedford, MA, USA). Each region was scanned in "small animal" mode, with the "regional high resolution" scan option. Femoral BMD was measured at the proximal, middle, and distal thirds of the femur.

2.6. Biomechanical Testing

Mechanical testing of the left femoral shaft was performed at room temperature using a material testing machine (MZ500S; Maruto, Tokyo, Japan). The mid-diaphysis of the femur was stabilized by placing it on two supports of the test apparatus placed 18-mm apart. The load of a three-point bending test was applied in the anteroposterior direction midway between the two supports. Load-displacement curves were recorded at a crosshead speed of 5 mm/min. The maximum load (N), stiffness (N/mm), and breaking energy (N mm) were calculated using software for measuring bone strength (CTR win. Version 1.05; System Supply, Nagano, Japan), as described previously [18]. Following the three-point bending test, the distal part of the femur was evaluated using a compression test, as previously described [19]. Load-displacement curves were recorded, and the maximum load (N), stiffness (N/mm), and breaking energy (N mm) were calculated using the same software.

2.7. Statistical Analysis

All data are expressed as mean ± standard deviation (SD) values. A Kolmogorov-Smirnov test showed that all data were normally distributed. Differences among the Cont, CKD, and EC groups were evaluated by one-way analysis of variance (ANOVA) and analyzed by Tukey's multiple comparison test as a post hoc test. All statistical analyses were performed using EZR [20], which is a modified version of R commander designed to add statistical functions frequently used in biostatistics. Values of $p < 0.05$ were considered significant.

3. Results

3.1. Body Weight

To evaluate the health status of the animals, body weight and its changes during the experiment were measured. Although rats in the CKD and EC groups had significantly lower body weights than rats in the Cont group at the baseline and endpoints ($p < 0.01$) (**Figure 1(a)**), there were no significant differences between the CKD and EC groups (**Figure 1(a)**). The body weight change (%) from baseline to endpoint was significantly higher in the CKD group than in the Cont ($p <$ 0.05) and EC ($p < 0.01$) groups (**Figure 1(b)**).

3.2. Serum Biochemistry

In addition, blood biochemical tests were performed to evaluate the effects of 4 weeks of treatment with EC. Serum BUN ($p < 0.01$) (**Figure 2(a)**), CRE ($p <$ 0.01) (**Figure 2(b)**), and IP ($p < 0.05$) (**Figure 2(d)**) were significantly higher in

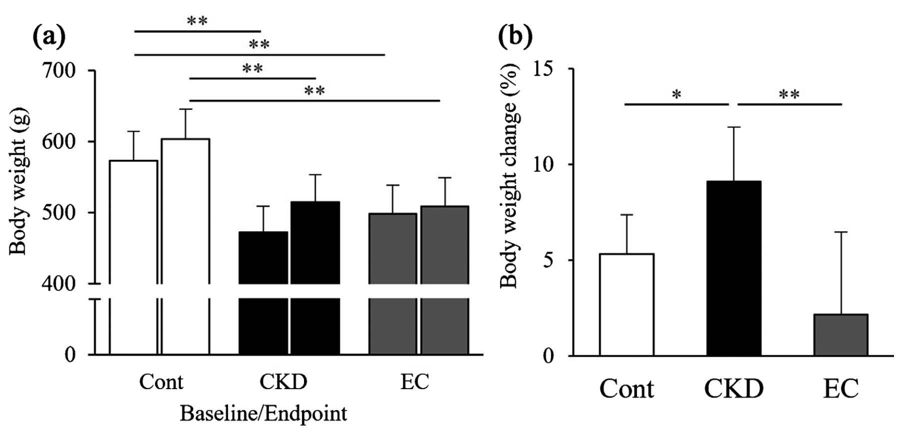

Figure 1. (a) Body weight in each group at baseline and at the endpoint; (b) Percentage change in body weight in each group from baseline. Data are the mean ± SD values (n = 9 - 11 in each group). *$p < 0.05$, **$p < 0.01$ (Tukey's multiple comparison test). Cont: non-CKD control rats, CKD: CKD rats administered vehicle, EC: CKD rats administered etelcalcetide.

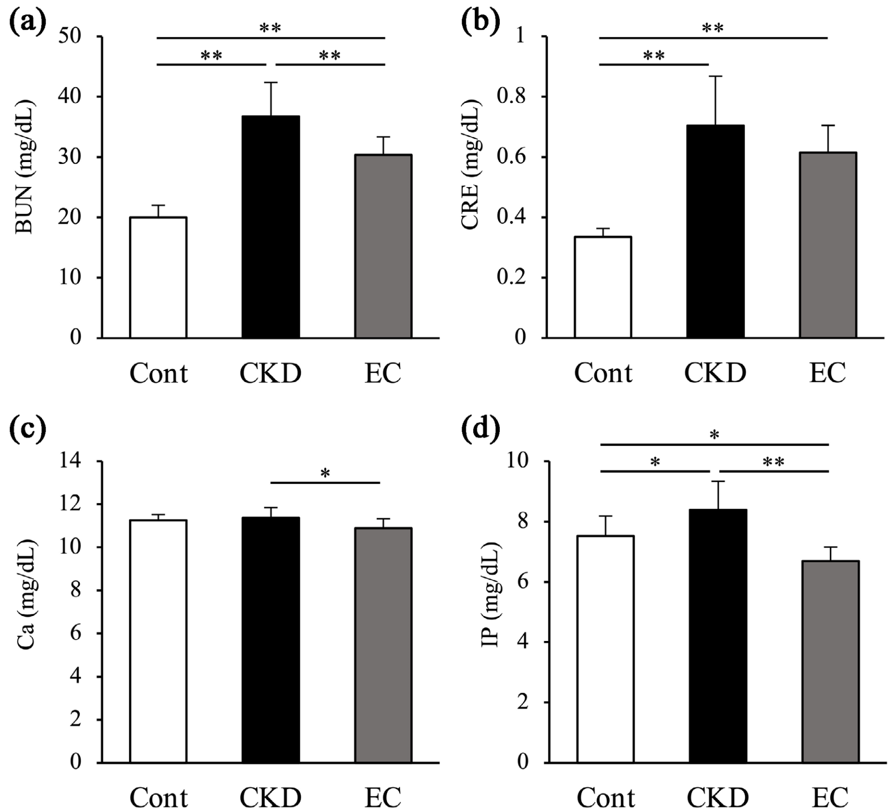

Figure 2. (a) Serum blood urea nitrogen (BUN); (b) Serum creatinine (CRE); (c) Serum calcium (Ca); (d) Serum inorganic phosphorus (IP). Data are the mean ± SD values (n = 9 - 11 in each group). $^*p < 0.05$, $^{**}p < 0.01$ (Tukey's multiple comparison test). Cont: non-CKD control rats, CKD: CKD rats administered vehicle, EC: CKD rats administered etelcalcetide.

the CKD group than in the Cont group. Although the serum BUN level was significantly decreased ($p < 0.01$) in the CKD rats after 4 weeks of EC treatment (**Figure 2(a)**), it was still significantly higher than that in the Cont group ($p < 0.01$). The serum CRE level did not show a significant change with EC treatment compared with the CKD group, and it was significantly higher than that in the Cont group ($p < 0.01$) (**Figure 2(b)**). EC treatment significantly decreased serum Ca levels compared with the CKD group ($p < 0.05$) (**Figure 2(c)**), and serum IP decreased significantly compared with the CKD ($p < 0.01$) and Cont ($p < 0.05$) groups (**Figure 2(d)**).

3.3. Effects of EC on Micro-CT Cortical Bone Parameters

Axial micro-CT images of the femoral diaphysis showed that rats in the CKD group had higher cortical porosity than rats in the Cont group (**Figure 3(a)**). In addition, treatment with EC decreased cortical porosity with an increase in cortical bone thickness in the EC rats (**Figure 3(a)**).

Analysis of cortical bone at the femoral diaphysis by micro-CT showed a significant increase in Ct.Po in the CKD group compared with normal rats (Cont group) ($p < 0.01$) (**Figure 3(b)**). There were no significant differences in other

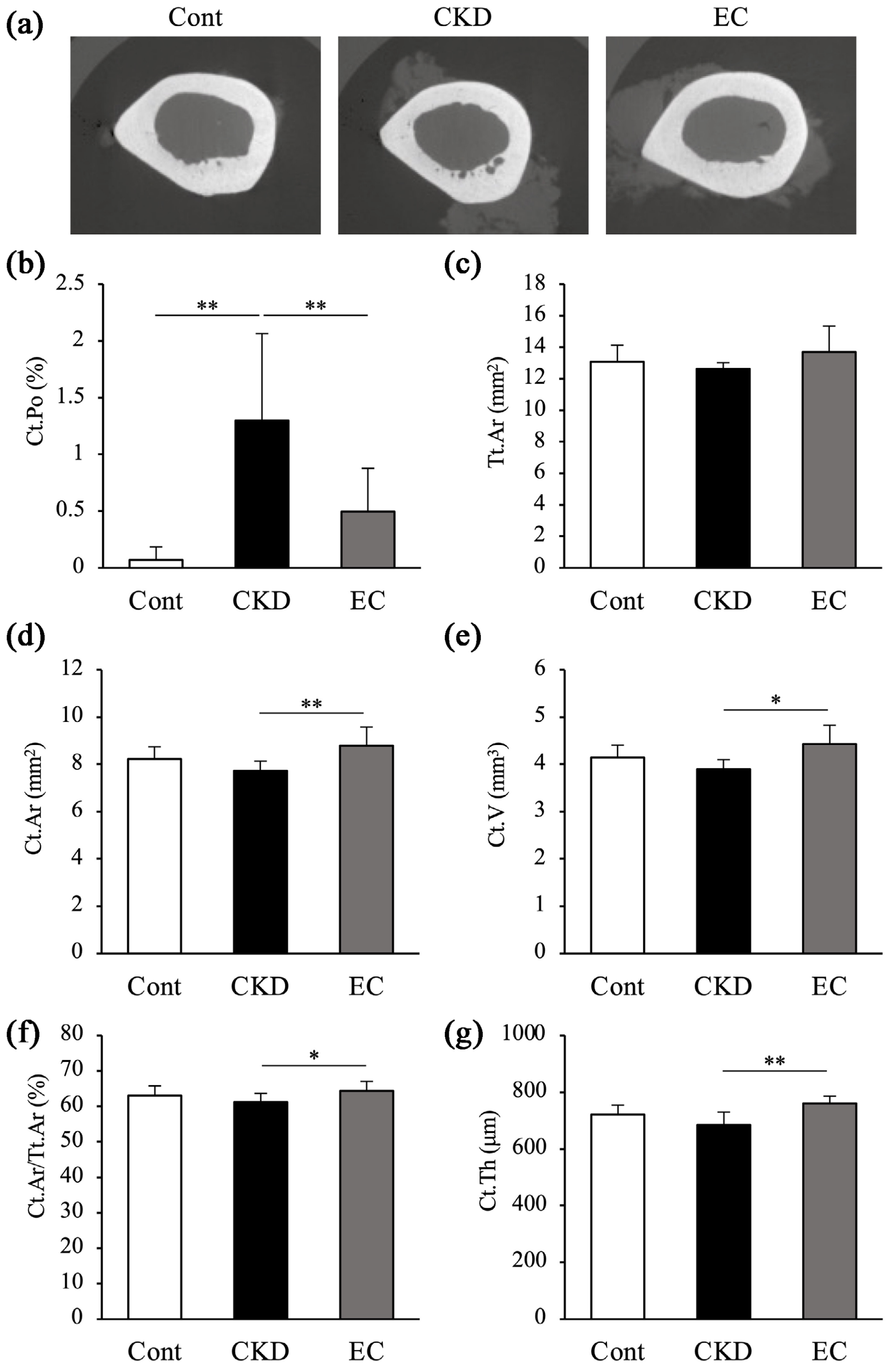

Figure 3. (a) Representative micro-CT images of cortical bone transverse sections from each group; (b) Cortical porosity (Ct.Po); (C) Total area (Tt.Ar); (d) Cortical area (Ct.Ar); (e) Cortical volume (Ct.V); (f) Cortical area/total area (Ct.Ar/Tt.Ar); (g) Cortical thickness (Ct.Th). Date are the mean ± SD values (n = 9 - 11 in each group). **p* < 0.05, ***p* < 0.01 (Tukey's multiple comparison test). Cont: non-CKD control rats, CKD: CKD rats administered vehicle, EC: CKD rats administered etelcalcetide.

cortical bone parameters between the Cont and CKD groups (Figures 3(c)-(g)). EC treatment significantly increased Ct.Ar ($p < 0.01$) (Figure 3(d)), Ct.V ($p < 0.05$) (Figure 3(e)), Ct.Ar/Tt.Ar ($p < 0.05$) (Figure 3(f)), and Ct.Th ($p < 0.01$)

(**Figure 3(g)**), and it significantly decreased Ct.Po ($p < 0.01$) (**Figure 3(b)**) compared with those in the CKD group.

3.4. Effects of EC on Micro-CT Trabecular Bone Parameters

Axial micro-CT images of the distal femoral metaphysis showed that the trabecular bone microstructure was degraded in the CKD group compared with the Cont group (**Figure 4(a)**).

Micro-CT analysis of the trabecular bone of the distal femur showed significantly reduced BV/TV ($p < 0.05$) (**Figure 4(b)**) and Tb.Th ($p < 0.05$) (**Figure 4(c)**) and significantly increased Tb.Sp ($p < 0.05$) (**Figure 4(e)**) in the CKD group compared with the Cont group. Although EC treatment for 4 weeks did not show a significant change in these parameters compared to the CKD group, there was no significant difference between the EC group and the Cont group. SMI and Conn D, which are parameters related to bone strength of trabecular bone, did not differ significantly among the three groups (**Figure 4(f)**, **Figure 4(g)**).

3.5. Effects of EC on Bone Mineral Density

The results for femur and lumbar BMDs are shown in **Table 1**. Compared with normal rats (Cont group), the BMDs of the femur at the total, proximal, middle, and distal and lumbar vertebrae were significantly lower in the CKD group and the EC group ($p < 0.05$). Compared with the CKD group, the EC group showed no significant changes in these BMDs.

3.6. Effects of EC on Bone Strength

Representative stress-strain curves from the three-point bending test are shown in **Figure 5(a)**. In the three-point bending test, the CKD group had significantly lower maximum load (**Figure 5(b)**), stiffness (**Figure 5(c)**), and breaking energy (**Figure 5(d)**) compared to the Cont group ($p < 0.05$). Although EC treatment for 4 weeks did not show significant changes in these parameters compared to the CKD group, there was no significant difference between the EC group and the Cont group (**Figures 5(b)-(d)**).

In the compression test at the distal metaphysis of the femur, the parameters related to trabecular bone strength, which are maximum load (**Figure 5(e)**), stiffness (**Figure 5(f)**), and breaking energy (**Figure 5(g)**), were not significantly different among the three groups.

4. Discussion

4.1. Summary of the Present Study

Few reports have investigated the effects of EC on bone, including bone microstructure and bone strength in trabecular and cortical bone. To the best of our knowledge, the present study is the first to report them in a rat model of adenine-induced CKD. In the adenine-induced CKD rat model in the present study, bone microstructures were not altered in cortical bone at the relatively early stage of 12 weeks after CKD induction, but BMD and cortical bone strength were reduced by increased cortical porosity. On the other hand, in trabecular

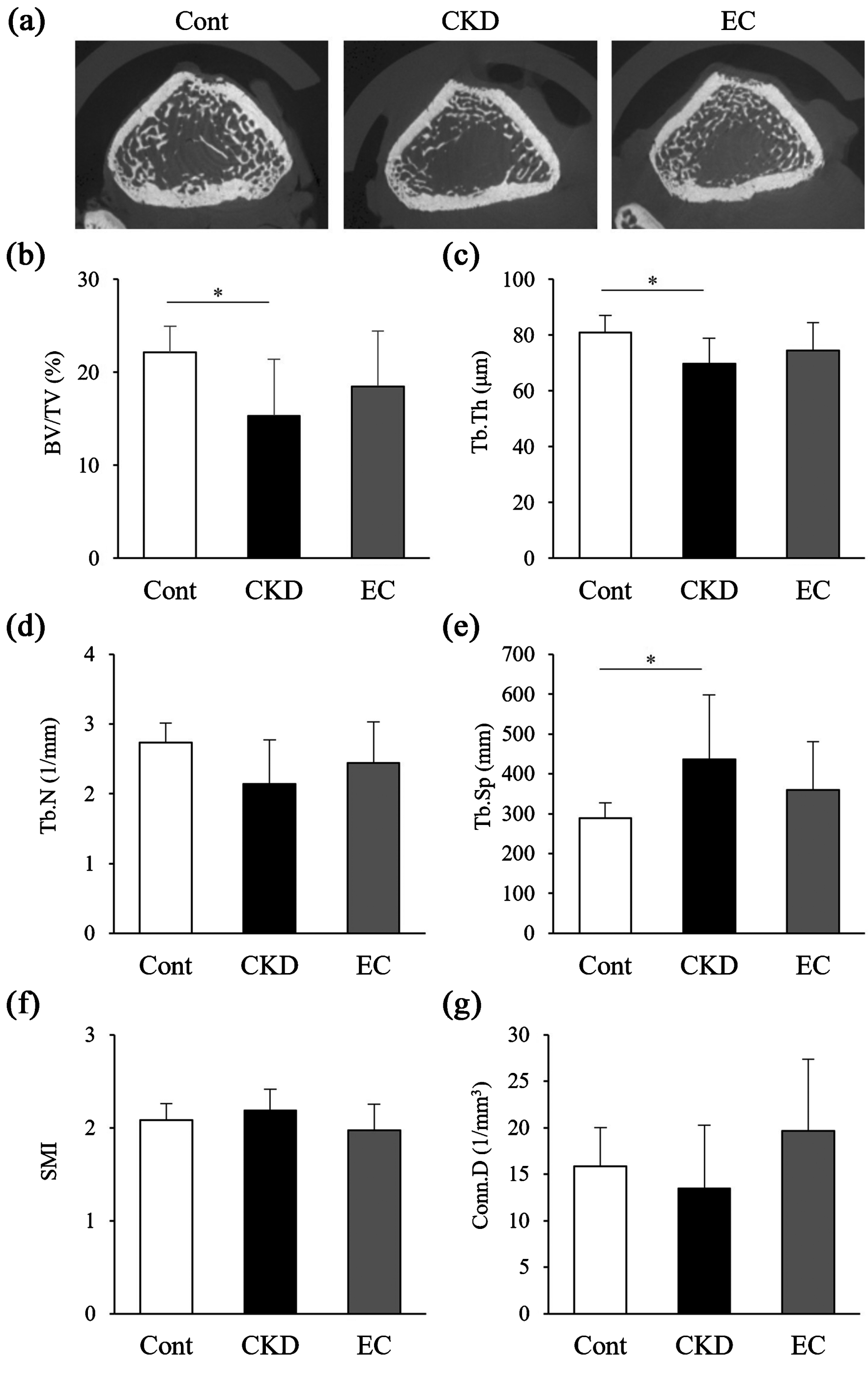

Figure 4. (a) Representative micro-CT images of trabecular bone transverse sections from each group; (b) Bone volume (BV/TV); (c) Trabecular thickness (Tb.Th); (d) Trabecular number (Tb.N); (e) Trabecular separation (Tb.Sp); (f) Structure model index (SMI); (g) Connectivity density (Conn.D). Data are the mean ± SD values (n = 9-11 in each group). $^*p < 0.05$, $^{**}p < 0.01$ (Tukey's multiple comparison test). Cont: non-CKD control rats, CKD: CKD rats administered vehicle, EC: CKD rats administered etelcalcetide.

Table 1. Bone mineral density (mg/cm^2).

	Cont N = 10	CKD N = 11	EC N = 9	ANOVA *p*-value
Femur total	0.266 ± 0.010	0.253 ± 0.011[a]	0.250 ± 0.012[a]	<0.001
Femur proximal	0.269 ± 0.011	0.253 ± 0.012[a]	0.252 ± 0.014[a]	<0.001
Femur middle	0.253 ± 0.008	0.241 ± 0.009[a]	0.240 ± 0.011[a]	<0.001
Femur distal	0.275 ± 0.011	0.256 ± 0.014[a]	0.256 ± 0.011[a]	<0.001
Lumbar	0.272 ± 0.009	0.263 ± 0.005[a]	0.264 ± 0.010[a]	<0.001

Data are mean ± standard deviation values. Cont: non-CKD control rats, CKD: CKD rats administered vehicle, EC: CKD rats administered etelcalcetide. [a]$p < 0.05$ vs Cont group (Tukey's multiple comparison test).

bone, a decrease in BMD and impairment of trabecular bone microstructure were observed, but no significant deterioration in bone strength was observed. In cortical bone, EC improved bone microstructure, reduced porosity, and inhibited the decrease in bone strength after 4 weeks of treatment. In trabecular bone, EC inhibited the progression of degradation of trabecular bone microstructure, but no significant improvement in bone strength was observed.

4.2. Effects of Etelcalcetide on Cortical Bone

Administration of EC to SHPT has an effect on bone by reducing blood PTH levels and improving the overactive bone metabolic turnover. EC has also been reported to inhibit the development and progression of cortical bone porosity in a rat model of CKD [17]. Li *et al.* reported that 6 weeks of EC treatment improved failure energy of cortical bone in a rat model of CKD by subtotal nephrectomy [21]. Furthermore, Damrath *et al.* also demonstrated that calcimimetics may help prevent CKD-induced bone deterioration by improving bone quality in new periosteal bone and in bone tissue near osteocyte lacunae in the Cy/+ rats, which was a model of spontaneous and progressive CKD-mineral and bone disorder [22]. In the present study, EC also significantly reduced cortical bone porosity in the femoral diaphysis and significantly improved cortical bone area and width, but it did not significantly improve BMD in the femoral diaphysis by DXA or bone strength in the femoral diaphysis by the 3-point bending test compared to the CKD group. A slightly longer duration of EC administration may confirm its effect on cortical bone strength.

4.3. Effects of Etelcalcetide on Trabecular Bone

On the other hand, in the trabecular bone area, the 4 weeks of EC treatment had no effect on the parameters of BMD and microstructure in trabecular bone and bone strength. Li *et al.* reported that bone morphometry of trabecular bone in CKD model rats treated with EC for 6 weeks showed a decrease in osteoid volume, but no change in bone volume, trabecular bone microstructure, or osteoclast

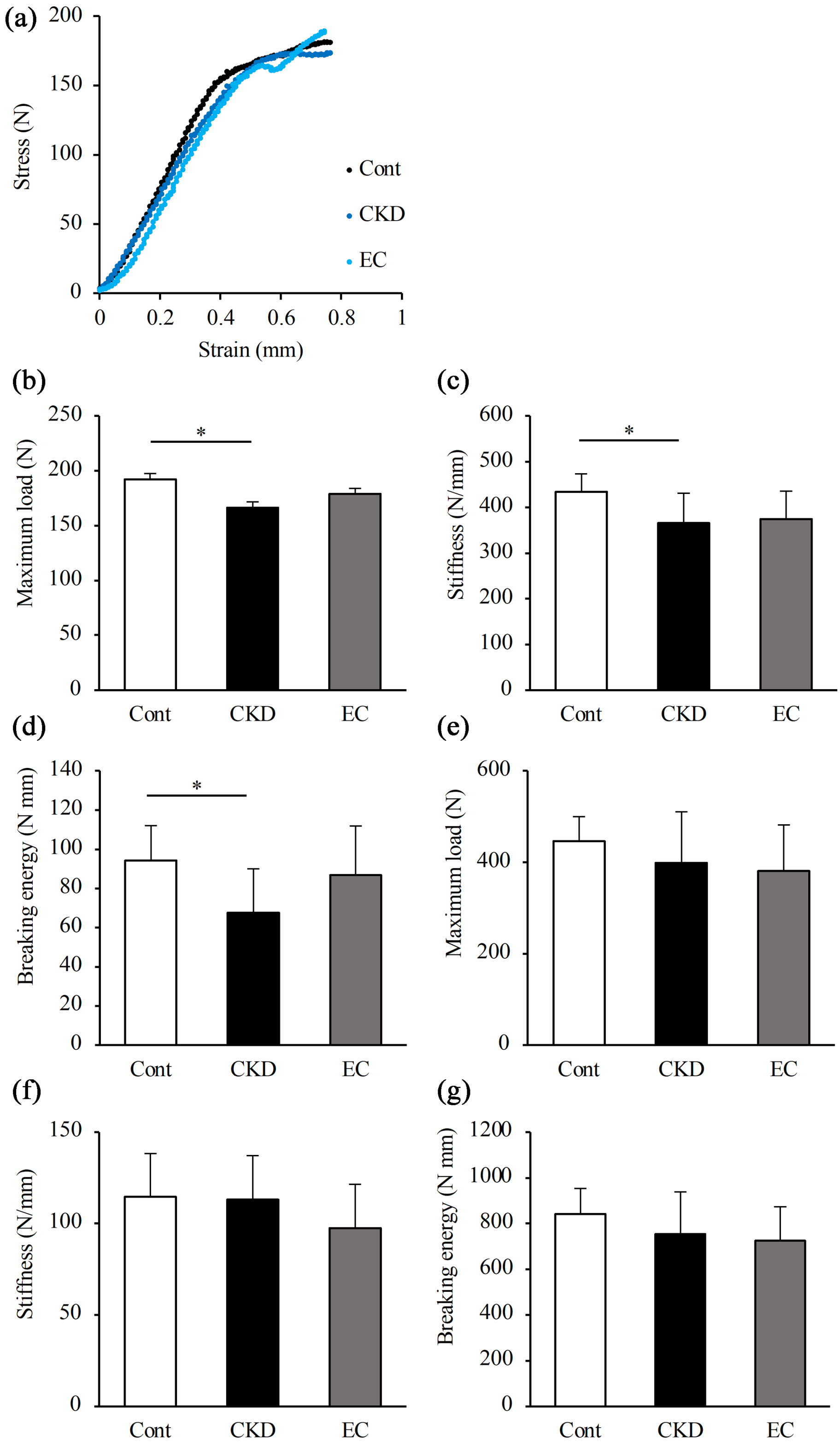

Figure 5. (a) Representative stress-strain curves of the three-point bending test at the mid-shaft of the femur. (b) Maximum load, (c) Stiffness, and (d) Breaking energy of the three-point bending test at the mid-shaft of the femur. (e) Maximum load, (f) Stiffness, and (g) Breaking energy of the compression test at the distal metaphysis of the femur. Data are the mean ± SD values (n = 9 - 11 in each group). $^*p < 0.05$, $^{**}p < 0.01$ (Tukey's multiple comparison test). Cont: non-CKD control rats, CKD: CKD rats administered vehicle, EC: CKD rats administered etelcalcetide.

surface [21]. A recent study showed that EC treatment improved BMD and trabecular bone quality and reduced bone metabolism without altering bone material properties [23]. Although a simple comparison between humans on hemodialysis and CKD model rats may not be possible, a clearer effect on trabecular bone may be confirmed in rats by extending the duration of etelcalcetide treatment.

4.4. Possibility of Combined Treatment with Etelcalcetide and Osteoporosis Medicine

In the present study, EC alone did not significantly improve BMD or bone strength in CKD model rats, and it was found that EC inhibited CKD-induced bone strength loss. Swallow *et al.* pointed out that EC mitigates, but does not reverse the progression of cortical bone deterioration in CKD, so combination therapy with osteoporosis drugs should be considered to more effectively improve bone loss in CKD [17]. However, there have been no randomized, controlled trials investigating the effects of osteoporosis medications in hemodialysis patients. In addition, there are no reports detailing the effects of the combination of calcimimetics and osteoporosis medications in CKD with SHPT. Osteoporosis medications include anti-resorptive agents and osteoanabolic agents, the most common of which are bisphosphonates and denosumab, and teriparatide [24]. However, in CKD or hemodialysis patients, bisphosphonates may cause over-suppression of bone turnover and development of adynamic bone disease, denosumab may cause severe hypocalcemia, and teriparatide may affect the pathophysiology of hyperparathyroidism [24] [25]. Furthermore, hypocalcemia is one of the side effects of etelcalcetide, so measures against hypocalcemia are necessary when etelcalcetide is used in combination with those bone resorption inhibitors. Iwasaki *et al.* reported that, in rats with SHPT induced by 5/6 nephrectomy, the elastic mechanical properties of cortical bone deteriorated regardless of bone metabolism or bone mass, and the factors determining bone elasticity were associated with the severity of uremia [26]. Therefore, normalization of PTH secretion alone is insufficient to improve bone fragility in CKD, and a more beneficial effect may be confirmed by concurrent treatment of uremic toxins. However, there is a report that the combination with the nicotinamide adenine dinucleotide phosphate oxidase (NOX1/4) inhibitor GKT-137831, which has a suppressive effect on oxidative stress, had no effect in combination with EC [27]. In the future, the combined effect of EC with a treatment that improves bone metabolism should be studied, rather than the effect of EC alone on bone.

4.5. Limitations

This study has several limitations. First, the duration of EC treatment was only four weeks. The effect of EC on cortical and trabecular bone over a longer period of time needs to be investigated. Second, the effect of EC treatment on bone metabolism has not been investigated. We expect to elucidate these limitations in future studies. Finally, we did not evaluate the effect sizes of intergroup differences of all parameters. We should consider the effect sizes in the small number of each group in this study.

5. Conclusion

In adenine-induced CKD model rats, EC improved cortical bone microstructure and prevented bone strength deterioration. In trabecular bone, although EC did not improve the bone histomorphometric parameters and BMD, EC inhibited the progression of bone structure deterioration in the adenine-induced CKD model rats. Future studies are needed to investigate combination therapy with medicines that can more effectively restore bone loss and deterioration in CKD.

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Authors' Contributions

Shun Igarashi: Investigation, Validation, Visualization, Writing—Original Draft. Yuji Kasukawa: Conceptualization, Methodology, Project administration, Writing—Review & Editing. Koji Nozaka: Conceptualization, Methodology, Writing—Review & Editing. Hiroyuki Tsuchie: Investigation, Formal analysis. Kazunobu Abe: Investigation. Hikaru Saito: Investigation. Ryo Shoji: Investigation. Fumihito Kasama: Investigation. Shuntaro Harata: Investigation. Kento Okamoto: Investigation. Keita Oya: Investigation. Naohisa Miyakoshi: Conceptualization, Funding acquisition, Supervision, Writing—Review & Editing.

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Ethics Approval

The protocols for all animal experiments were approved in advance by the Animal Research Committee of our institute, and all subsequent animal experiments adhered to the "Guidelines for Animal Experimentation" of our university (approval number: a-1-0271).

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Levey, A.S., Atkins, R., Coresh, J., Cohen, E.P., Collins, A.J., Eckardt, K.U., *et al.* (2007) Chronic Kidney Disease as a Global Public Health Problem: Approaches and

Initiatives—A Position Statement from Kidney Disease Improving Global Outcomes. *Kidney International*, **72**, 247-249. https://doi.org/10.1038/sj.ki.5002343

[2] Klawansky, S., Komaroff, E., Cavanaugh, P.F., Mitchell, D.Y., Gordon, M.J., Connelly, J.E., *et al.* (2013) Relationship between Age, Renal Function and Bone Mineral Density in the US Population. *Osteoporosis International*, **14**, 570-576. https://doi.org/10.1007/s00198-003-1435-y

[3] Alem, A.M., Sherrard, D.J., Gillen, D.L., Weiss, N.S., Beresford, S.A., Heckbert, S.R., *et al.* (2000) Increased Risk of Hip Fracture among Patients with End-Stage Renal Disease. *Kidney International*, **58**, 396-399. https://doi.org/10.1046/j.1523-1755.2000.00178.x

[4] Tentori, F., McCullough, K., Kilpatrick, R.D., Bradbury, B.D., Robinson, B.M., Kerr, P.G., *et al.* (2014) High Rates of Death and Hospitalization Follow Bone Fracture among Hemodialysis Patients. *Kidney International*, **85**, 166-173. https://doi.org/10.1038/ki.2013.279

[5] Moe, S., Drüeke, T., Cunningham, J., Goodman, W., Martin, K., Olgaard, K., *et al.* (2006) Definition, Evaluation, and Classification of Renal Osteodystrophy: A Position Statement from Kidney Disease: Improving Global Outcomes (KDIGO). *Kidney International*, **69**, 1945-1953. https://doi.org/10.1038/sj.ki.5000414

[6] Komaba, H., Kakuta, T. and Fukagawa, M. (2011) Diseases of the Parathyroid Gland in Chronic Kidney Disease. *Clinical and Experimental Nephrology*, **15**, 797-809. https://doi.org/10.1007/s10157-011-0502-5

[7] Isakova, T., Wahl, P., Vargas, G.S., Gutiérrez, O.M., Scialla, J., Xie, H., *et al.* (2011) Fibroblast Growth Factor 23 Is Elevated before Parathyroid Hormone and Phosphate in Chronic Kidney Disease. *Kidney International*, **79**, 1370-1378. https://doi.org/10.1038/ki.2011.47

[8] Sherrard, D.J., Hercz, G., Pei, Y., Maloney, N.A., Greenwood, C., Manuel, A., *et al.* (1993) The Spectrum of Bone Disease in End-Stage Renal Failure—An Evolving Disorder. *Kidney International*, **43**, 436-442. https://doi.org/10.1038/ki.1993.64

[9] Nickolas, T.L., Stein, E.M., Dworakowski, E., Nishiyama, K.K., Komandah-Kosseh, M., Zhang, C.A., *et al.* (2013) Rapid Cortical Bone Loss in Patients with Chronic Kidney Disease. *Journal of Bone and Mineral Research*, **28**, 1811-1820.

[10] Alexander, S.T., Hunter, T., Walter, S., Dong, J., Maclean, D., Baruch, A., *et al.* (2015) Critical Cysteine Residues in both the Calcium-Sensing Receptor and the Allosteric Activator AMG 416 Underlie the Mechanism of Action. *Molecular Pharmacology*, **88**, 853-865. https://doi.org/10.1002/jbmr.1916

[11] Martin, K.J., Pickthorn, K., Huang, S., Block, G.A., Vick, A., Mount, P.F., *et al.* (2014) AMG 416 (Velcalcetide) Is a Novel Peptide for the Treatment of Secondary Hyperparathyroidism in a Single-Dose Study in Hemodialysis Patients. *Kidney International*, **85**, 191-197. https://doi.org/10.1038/ki.2013.289

[12] Parfrey, P.S., Drüeke, T.B., Block, G.A., Correa-Rotter, R., Floege, J., Herzog, C.A., *et al.* (2015) The Effects of Cinacalcet in Older and Younger Patients on Hemodialysis: The Evaluation of Cinacalcet HCl Therapy to Lower Cardiovascular Events (EVOLVE) Trial. *Clinical Journal of the American Society of Nephrology*, **10**, 791-799. https://doi.org/10.2215/CJN.07730814

[13] Moe, S.M., Chertow, G.M., Parfrey, P.S., Kubo, Y., Block, G.A., Correa-Rotter, R., *et al.* (2015) Cinacalcet, Fibroblast Growth Factor-23, and Cardiovascular Disease in Hemodialysis: The Evaluation of Cinacalcet HCl Therapy to Lower Cardiovascular Events (EVOLVE) Trial. *Circulation*, **32**, 27-39. https://doi.org/10.1161/CIRCULATIONAHA.114.013876

[14] Saito, H., Miyakoshi, N., Kasukawa, Y., Nozaka, K., Tsuchie, H., Sato, C., *et al.* (2021) Analysis of Bone in Adenine-Induced Chronic Kidney Disease Model Rats. *Osteoporos and Sarcopenia*, **7**, 121-126. https://doi.org/10.1016/j.afos.2021.11.001

[15] Yokozawa, T., Zheng, P.D., Oura, H. and Koizumi, F. (1986) Animal Model of Adenine-Induced Chronic Renal Failure in Rats. *Nephron*, **44**, 230-234. https://doi.org/10.1159/000183992

[16] Ferrari, G.O., Ferreira, J.C., Cavallari, R.T., Neves, K.R., dos Reis, L.M., Dominguez, W.V., *et al.* (2014) Mineral Bone Disorder in Chronic Kidney Disease: Head-to-Head Comparison of the 5/6 Nephrectomy and Adenine Models. *BMC Nephrology*, **15**, Article No. 69. https://doi.org/10.1186/1471-2369-15-69

[17] Swallow, E.A., Metzger, C.E., Newman, C.L., Chen, N.X., Moe, S.M., and Allen, M.R. (2022) Cortical Porosity Development and Progression Is Mitigated after Etelcalcetide Treatment in an Animal Model of Chronic Kidney Disease. *Bone*, **157**, Article ID: 116340. https://doi.org/10.1016/j.bone.2022.116340

[18] Miyakoshi, N., Fujii, M., Kasukawa, Y. and Shimada, Y. (2019) Impact of Vitamin C on Teriparatide Treatment in the Improvement of Bone Mineral Density, Strength, and Quality in Vitamin C-Deficient Rats. *Journal of Bone and Mineral Metabolism*, **37**, 411-418. https://doi.org/10.1007/s00774-018-0941-0

[19] Kasukawa, Y., Miyakoshi, N., Itoi, E., Tsuchida, T., Tamura, Y., Kudo, T., *et al.* (2004) Effects of h-PTH on Cancellous Bone Mass, Connectivity, and Bone Strength in Ovariectomized Rats with and without Sciatic Neurectomy. *Journal of Orthopaedic Research*, **22**, 457-464. https://doi.org/10.1016/j.orthres.2003.08.017

[20] Kanda, Y. (2013) Investigation of the Freely Available Easy-to-Use Software "EZR" for Medical Statistics. *Bone Marrow Transplantation*, **48**, 452-458. https://doi.org/10.1038/bmt.2012.244

[21] Li, X., Yu, L., Asuncion, F., Grisanti, M., *et al.* (2017) Etelcalcetide (AMG 416), a Peptide Agonist of the Calcium-Sensing Receptor, Preserved Cortical Bone Structure and Bone Strength in Subtotal Nephrectomized Rats with Established Secondary Hyperparathyroidism. *Bone*, **105**, 163-172. https://doi.org/10.1016/j.bone.2017.08.026

[22] Damrath, J.G., Moe, S.M. and Wallace, J.M. (2022) Calcimimetics Alter Periosteal and Perilacunar Bone Matrix Composition and Material Properties in Early Chronic Kidney Disease. *Journal of Bone and Mineral Research*, **37**, 1297-1306. https://doi.org/10.1002/jbmr.4574

[23] Khairallah, P., Cherasard, J., Sung, J., Agarwal, S., Aponte, M.A., Bucovsky, M., *et al.* (2023) Changes in Bone Quality after Treatment with Etelcalcetide. *Clinical Journal of the American Society of Nephrology*, **18**, 1456-1465. https://doi.org/10.2215/CJN.0000000000000254

[24] Khairallah, P. and Nickolas, T.L. (2018) Updates in CKD-Associated Osteoporosis. *Current Osteoporosis Reports*, **6**, 712-723. https://doi.org/10.1007/s11914-018-0491-3

[25] Hiramatsu, R., Ubara, Y., Sawa, N., Hoshino, J., Hasegawa, E., Kawada, M., *et al.* (2015) Denosumab for Low Bone Mass in Hemodialysis Patients: A Noncontrolled Trial. *American Journal of Kidney Diseases*, **66**, 175-177. https://doi.org/10.1053/j.ajkd.2015.03.012

[26] Iwasaki, Y., Kazama, J.J., Yamato, H., Matsugaki, A., Nakano, T. and Fukagawa, M. (2015) Altered Material Properties Are Responsible for Bone Fragility in Rats with Chronic Kidney Injury. *Bone*, **81**, 247-254. https://doi.org/10.1016/j.bone.2015.07.015

[27] Damrath, J.G., Chen, N.X., Metzger, C.E., Srinivasan, S., O'Neill, K., Biruete, A., *et al*. (2022) Non-Additive Effects of Combined NOX1/4 Inhibition and Calcimimetic Treatment on a Rat Model of Chronic Kidney Disease-Mineral and Bone Disorder (CKD-MBD). *JBMR Plus*, **6**, e10600. https://doi.org/10.1002/jbm4.10600