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Only minor differences in renal osteodystrophy features between wild-type and sclerostin knockout mice with chronic kidney disease

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Abstract

Renal osteodystrophy affects the majority of patients with advanced chronic kidney disease (CKD) and is characterized by progressive bone loss. This study evaluated the effects of sclerostin knockout on bone in a murine model of severe, surgically induced CKD in both sclerostin knockout and wild-type mice. Mice of both genotypes with normal kidney function served as controls. Tibiae were analyzed using micro-computed tomography, and lumbar vertebrae were analyzed by histomorphometry. Results were tested for statistical significance by 2-way ANOVA to investigate whether bone of the knockout mice reacted differently to CKD compared with bone of wild-type mice. In the tibiae, there was no difference after creation of CKD between wild-type and knockout animals for cortical thickness or cross-sectional moment of inertia. Increases in cortical porosity induced by CKD differed significantly between genotypes in the tibial metaphysis but not in the diaphysis. In the trabecular compartment, no difference in reaction to CKD between genotypes was found for bone volume, trabecular number, trabecular thickness, and trabecular separation. In the lumbar vertebrae, significant differences in response to CKD between wild-type and knockout mice were seen for both bone volume and trabecular thickness. Osteoblast parameters did not differ significantly, whereas osteoclast numbers significantly increased in the wild-type but significantly decreased in knockout mice with CKD. No differences in response to CKD between genotypes were found for bone formation rate or mineral apposition rate. Thus, complete absence of sclerostin has only minor effects on CKD-induced bone loss in mice.

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DISCLOSURE

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Keywords

bone; kidney disease; mouse; renal osteodystrophy; sclerostin

Renal osteodystrophy develops in the majority of patients with advanced chronic kidney disease (CKD).[1] Bone loss is an integral part of renal osteodystrophy.[2] Bone loss occurring mainly at the cortical regions of the appendicular skeleton[3] was found by peripheral quantitative computed tomography (CT) in patients with renal osteodystrophy. Increased cortical porosity was found by bone histology in high turnover renal osteodystrophy, whereas thin cortices and trabeculae were described in patients with the low-turnover form.[4] Patients with CKD have an elevated risk of bone fracture including hip fractures.[5] Compared with the general population, the morbidity and mortality of CKD patients are significantly elevated and further aggravated by fractures to an unacceptably high level.[6] For example, median survival after hip fracture has been reported to be shorter than 1 year in dialysis patients.[7] There is no established therapy for bone loss occurring with declining kidney function. Although *post hoc* analyses of clinical trials studying the bisphosphonates, denosumab and teriparatide have been reported for patients with severe CKD, there is a lack of clinical trials specifically designed to assess changes in fracture risk in this population.[8–10] Thus, novel approaches for the treatment of bone loss associated with CKD are highly desirable.

Sclerostin is a soluble inhibitor of wnt-signaling and a key player in bone physiology. Sclerostin also appears to play a role in renal osteodystrophy. Sclerostin blood levels increase with declining renal function[11] and correlate inversely with bone turnover[12] but positively with bone mineral density[13] in dialysis patients. Loss-of-function mutations of sclerostin lead to a rare heritable disease named sclerosteosis, which is characterized by overgrowth of mechanically competent bone.[14,15] Inhibition of sclerostin by gene knockout (KO) or monoclonal antibodies also results in this high bone mass phenotype.[16,17] Furthermore, treatment with an anti-sclerostin antibody led to increases in bone mineral density in a phase II trial in women with postmenopausal osteoporosis.[18] The current study evaluated whether genetic KO of sclerostin protects against bone loss in a murine model of severe chronic renal failure.

RESULTS

Induction of CKD

Wild-type (WT) and sclerostin KO (SOST-KO) mice underwent subtotal nephrectomy to induce chronic renal insufficiency (CKD). Sham-operated animals served as controls (CTRL). Table 1 shows mouse weight and laboratory parameters of WT-CTRL ($n = 20$), SOST-KO CTRL ($n = 13$), WT-CKD ($n = 23$), and SOST-KO-CKD ($n = 17$) groups. Body weight was significantly lower, whereas creatinine, BUN (blood urea nitrogen) and calcium were significantly higher in CKD mice compared with WT controls (Student's t test). Using 2-way analysis of variance (ANOVA), statistically significant interactions in response to nephrectomy (CTRL and CKD) between genotypes (WT and SOST-KO) were found for serum creatinine ($P = 0.004$) and serum calcium ($P = 0.02$) and by a nonsignificant trend for

BUN ($P = 0.08$). Animal weight, albumin, phosphorus, and parathyroid hormone (PTH) responded similarly to nephrectomy in WT and SOST-KO mice (P = not significant [NS], 2-way ANOVA).

μ-CT

Tibia-cortical regions—The diaphysis of the tibia (tibial midshaft) representing the distal appendicular skeleton consisted almost entirely of cortical bone, as expected. The tibial metaphysis represents more proximal appendicular skeleton consisting of both cortical and trabecular regions. The trabecular compartment of the tibial metaphysis was analyzed separately.

In the cortical regions of the tibial diaphyses and metaphyses, SOST-KO and WT mice reacted similarly to CKD. Results are shown in Table 2. Cortical thickness, periosteal perimeter, marrow area, and cross-sectional moment of inertia did not differ significantly between SOST-KO and WT mice (P for interaction = NS). For cortical porosity and mineral density, no significant difference was seen in the diaphysis (P for interaction = NS). In the metaphysis, increases in cortical porosity and decreases in mineral density in CKD mice differed significantly between SOST-KO and WT mice, with SOST-KO mice showing a significant attenuation of increases in cortical porosity and loss of mineral density in CKD (P for interaction < 0.005 and < 0.03, respectively).

Tibia-trabecular region—Results of the trabecular region of the tibial metaphyses are shown in Table 3. There was no significant difference in response to CKD between SOST-KO and WT mice for trabecular bone volume/tissue trabecular number, trabecular thickness, trabecular separation, intra-individual distribution of trabecular separation, and mineral density (P for interaction = NS). For the structure model index (SMI), where higher numbers indicate more plate-shaped trabeculae, WT and SOST-KO mice reacted differently to CKD (P for interaction < 0.001).

Histomorphometry

For histomorphometric examination, lumbar spines were used, which represent the axial skeleton and consist predominantly of trabecular bone. Results of histomorphometric analyses are shown in Table 4.

Significant differences in response to CKD between WT and SOST-KO mice were seen for bone volume/tissue volume (BV/TV), trabecular thickness, and trabecular separation (P for interaction was <0.008, <0.001, and <0.02, respectively). Compared with WT-CTRL, WT-CKD mice had increased BV/TV and decreased trabecular separation, suggesting increases in vertebral trabecular bone mass as a consequence of uremic conditions. Conversely, BV/TV and trabecular separation were similar in SOST-KO-CKD mice compared with SOST-KO-CTRL mice. Osteoid thickness increased in both SOST-KO-CKD and WT-CKD groups, but this increase was blunted in SOST-KO-CKD mice (P for interaction < 0.02). Osteoclast numbers reacted differently to CKD, depending on genotype. Whereas osteoclast numbers increased in WT-CKD mice, they decreased in SOST-KO-CKD mice compared with the genotype healthy controls (P for interaction < 0.01). Double-labeled surfaces

decreased in WT-CKD mice but increased in SOST-KO-CKD mice (*P* for interaction < 0.02).

For all other static or dynamic histomorphometric parameters, SOST-KO and WT mice showed comparable responses to CKD conditions (*P* for interaction = NS).

DISCUSSION

In this study, we investigated whether bone of SOST-KO mice reacted differently to uremic conditions (CKD) compared with bone of WT mice. Induction of chronic renal insufficiency was robust with little variance as demonstrated by significantly higher creatinine and BUN values in CKD mice despite lower body weight. Our results for WT-CKD mice correspond well to the findings reported by Nikolov *et al.*[19] and Gonzalez *et al.*,[20] who also used electrocauterization and nephrectomy in C57/Bl6 mice to induce chronic renal insufficiency. Abnormalities in bone volume in combination with delayed mineralization found in this model are consistent with the formerly used clinical term *mixed uremic dystrophy* found in CKD patients.

Overall, the absence of sclerostin by genetic KO had only a minor influence on uremia-induced bone changes in the murine model of CKD studied. In the cortical regions of the tibia, which represent distal appendicular weight-bearing bone, cortical thickness was not preserved by sclerostin KO. WT-CKD and SOST-KO-CKD mice lost cortical thickness to a similar extent. Increases in cortical porosity found in CKD animals appeared to be blunted in the tibial metaphysis in SOST-KO-CKD mice. Nevertheless, cortical porosity of the tibial diaphysis and metaphysis increased in the CKD groups in both genotypes. Interestingly, no significant differences between CTRL and CKD groups were found for the cross-sectional moment of inertia, which is an estimate of overall mechanical stability, despite lower cortical thickness and higher cortical porosity. This finding can be explained by slight increases in periosteal perimeter in the CKD groups. As cross-sectional moment of inertia increases with the fourth potency of outer bone diameter, even minor increases in periosteal perimeter in the CKD groups can compensate for or even outweigh decreases in cortical thickness. We conclude that sclerostin KO does not have a substantial influence on uremic bone loss in the cortical regions of the distal and proximal tibia.

As far as the trabecular regions are concerned, WT-CKD mice showed increases in BV (BV/TV) in the lumbar vertebrae and the trabecular compartment of the proximal tibia compared with WT-CTRL mice. This increase in trabecular mass in proximal bones, but loss of bone mass in the distal cortical skeleton in WT-CKD mice, is similar to the situation of patients with CKD. Loss of cortical bone in the distal radius and distal tibia with relatively preserved lumbar spine bone mineral density was reported in CKD patients and was related to elevated PTH levels.[3] Additionally, in patients with primary hyperparathyroidism, lumbar vertebral bone mineral content was reported to be increased, whereas bone mineral density of the radius was found to be decreased.[21] Furthermore, increases in tibial and femoral trabecular BV/TV have been reported in mouse[19,20] and rat[22,23] models of kidney disease with secondary hyperparathyroidism as well as in vertebrae of rats treated with continuous PTH infusions.[24] These reports support the impression that in the model reported here,

hyperparathyroidism led to the observed bone phenotype. This increase in BV/TV under uremic conditions seemed to be attenuated in sclerostin KO mice, but statistical significance was only reached in the vertebrae.

The findings presented here suggest that sclerostin KO does not protect bone from uremia-induced changes under conditions that do not aim to decrease PTH levels. In this respect, our findings are in line with the results reported by Moe *et al.*,[25] who studied the effect of anti-sclerostin antibody treatment on bone in a polycystic kidney disease model in rats under low and high PTH conditions. Moe *et al.* reported that anti-sclerostin antibody treatment increased BV/TV of the tibia in uremic rats with low PTH levels but not in the high PTH group. However, the subtotal nephrectomy mouse model used in the current study differs substantially from the rat model used by Moe *et al.* In addition to studying different rodent species, the polycystic kidney disease rat model shows progressively deteriorating kidney function due to actual kidney disease. With the subtotal nephrectomy model used here, the nephron mass of otherwise healthy kidneys is severely reduced by nephrectomy and electrocauterization. Upon recovery from perioperative stress, renal function of subtotally nephrectomized mice remains stable over months.[26] Furthermore, the rat model used by Moe *et al.* does not show bone loss under uremic conditions,[25] whereas cortical bone mass in the CKD mice studied here was lower compared with kidney-healthy control animals.

The major limitation of the current study is the lack of statistically significant increases in PTH levels in the CKD groups compared with CTRL mice. PTH levels showed a pronounced intragroup variability. Although maximum PTH readings were more than 2 times higher in the CKD compared with the CTRL groups (PTH maximum 1818 pg/ml for WT-CKD mice versus PTH maximum 717 pg/ml for WT-CTRL mice), no statistically significant difference in PTH levels between the CTRL and CKD groups was detected. The reason for this is unclear. The particular physiology of C57/Bl6 mice is unlikely to be the explanation as others have reported secondary hyperparathyroidism after subtotal nephrectomy by electrocauterization in mice with a C57/Bl6 genetic background.[27,28] Preanalytic problems interfering with measurements, especially hemolysis that occurred frequently or issues with the PTH enzyme-linked immunosorbent assay, could explain why elevations in PTH levels in CKD mice did not reach statistical significance. This explanation appears most likely to us because PTH values showed a very high variability (as high as 10-fold) when measured with the enzyme-linked immunosorbent assay used in this study in kidney-healthy control animals not included in the current project (data not shown). Although some increase in PTH in the CKD groups might be deduced from typical bone changes, which are in line with previous studies reporting secondary hyperparathyroidism in this model,[19,20] this remains speculative. Furthermore, CKD mice showed increases in serum calcium (which has been previously described by others using this model[19,28]) in conjunction with normophosphatemia. This is in agreement with results of bone parameters, which were indicative of PTH overactivity and might be a characteristic of the mouse model studied. Another limitation is the cross-sectional design of the study. It might be possible that the higher bone mass found in SOST-KO-CKD mice compared with WT-CKD mice is only due to a preexisting high bone mass in SOST-KO mice before induction of CKD rather than resistance to uremia. However, 2-way ANOVA, which was used in the current analysis, tests whether groups react differently to a stimulus. Here, no difference in response to CKD

was found for the majority of parameters using 2-way ANOVA, which strongly suggests that WT and SOST-KO mice react similarly to CKD. This finding is surprising as expression of sclerostin in osteocytes is upregulated in predialysis CKD patients and decreases afterward in patients on hemodialysis.[29] Therefore, one would expect that genetic KO of sclerostin in mice would lead to more pronounced changes in bone under uremic conditions. Whether the lack of sclerostin in SOST-KO-CKD mice is compensated for by alterations in other promotors or inhibitors of canonical wnt signaling such as wnt ligands, sFRP-4, and Dkk-1 needs further investigation.

The findings presented here together with the findings reported by Moe *et al.* may have implications for further research: Currently, anti-sclerostin antibodies are under clinical development for the treatment of postmenopausal osteoporosis. Treatment with either one of the clinically more advanced antibodies (romosozumab and blosozumab) led to impressive increases in bone mineral density in phase II trials in women with normal renal function,[18,30] and phase III trials are ongoing. Given the progressive bone loss encountered in CKD and the osteoanabolic action of anti-sclerostin antibody treatment, CKD patients appear to be good candidates for this potential treatment option. However, inhibition of sclerostin in mice or rats with chronic renal failure without suppression of PTH, as shown here and by Moe *et al.*, does not seem to confer relevant benefits for bone health. Moreover, osteoanabolic efficacy of anti-sclerostin antibody treatment in uremic rats with low PTH was modest compared with kidney-healthy control animals.[25] As sclerostin levels are known to increase with progression of CKD in rodents[25,29] and humans,[11,12] one might argue that the anti-sclerostin antibody was underdosed and therefore efficacy was limited. However, in the current study even genetic KO of sclerostin failed to protect uremic animals from uremic bone disease. These findings raise some concerns as to whether anti-sclerostin antibody treatment will be effective in patients with CKD.

In conclusion, inhibition of sclerostin by genetic KO did not result in the expected improvement of uremia-induced changes in mice with chronic renal insufficiency.

METHODS

Sclerostin KO mice

Female sclerostin KO mice on a C57/Bl6J genetic background as reported previously[31] were provided by Novartis Institutes for Biomedical Research, Basel, Switzerland. Female WT C57/Bl6J mice were used as controls. Mice were fed standard chow containing 19% protein, 0.9% calcium, 0.7% phosphorus, and 1000 IU/kg vitamin D_3 (Altromin 1324 fortified; Altromin, Lage, Germany). The study protocol and all animal interventions were reviewed and approved by the Animal Welfare Committee of the Medical University Vienna, Austria.

Chronic renal failure

Chronic renal insufficiency was induced as described by Gagnon *et al.*[26] with minor modifications. In brief, at 12 weeks of age, the left kidney was mobilized and separated from the adrenal gland. The majority of the renal surface was coagulated by electrocauterization, leaving only the very caudal pole of the kidney intact, which is covered by a fat pad and

served as an anatomic landmark. Two weeks later, mice underwent nephrectomy of the right kidney. Again, the adrenal gland was left intact. Widespread electrocauterization was applied with the aim to induce severe loss of kidney function with its associated renal osteodystrophy. It was estimated that ~30% of animals would die in the CKD groups as a consequence of uremia. However, periprocedural as well as short-term mortality was unexpectedly high, totaling 58% of the animals. Anesthesia had to be quite deep because electrocauterization of the kidney was exquisitely painful. Deep anesthesia led to asphyxia in some animals. After nephrectomy of the contralateral kidney, which led to severe renal insufficiency, some animals were in poor clinical condition and died or had to be killed within days, probably due to overt uremia. Therefore, the experiment had to be repeated to reach sufficient animal numbers in each treatment group. Survival curves of both experiments are shown in Supplementary Figure S1. The data presented here represent the pooled results of both runs. After induction of severe renal insufficiency, mice were kept for another 12 weeks. In total, 73 mice were available for analysis: 20 WT control (sham surgery), 13 sclerostin KO control (sham surgery), 23 WT CKD (subtotal nephrectomy), and 17 sclerostin KO CKD (subtotal nephrectomy). Anesthesia was performed using a combination of local (bupivacaine, 8 mg/kg, s.c.) and systemic (buprenorphine, 0.75 mg/kg, i.p. and 2%– 4% isoflurane insufflation) anesthesia in addition to i.p. administration of 0.5 ml of 5% glucose at 37°C to prevent dehydration and hypothermia during the procedure. Mice were killed 3 months after induction of chronic renal insufficiency.

Laboratory tests

At the end of the study, mice were anesthetized (ketamine 100 mg/kg + xylazine 5 mg/kg, i.p). Blood was drawn by retro-orbital bleeding using heparinized capillaries for routine blood chemistry and cardiac puncture using ethylenediamine tetraacetic acid–primed syringes for PTH testing. Samples were immediately placed on ice, centrifuged at 4°C, and stored at −80°C. Creatinine, BUN, albumin, calcium, and phosphorus were analyzed in heparinized blood using automatized analytic systems (Cobas, Roche Diagnostics, Mannheim, Germany). Mouse intact PTH was analyzed in ethylenediamine tetraacetic acid plasma using an enzyme-linked immunosorbent assay (Cat.# 60-2300, Immutopics, San Clemente, CA, USA).

μCT

For μCT analyses, the right tibiae were used. Soft tissue and fibulae were carefully removed from mouse tibiae, and samples were placed in 70% ethanol at 4 °C on a shaker to optimize fixation of tissue. After removal of air bubbles by vacuum, the right tibiae underwent CT scanning (μCT 40, SCANCO Medical AG, Brüttisellen, Switzerland) using the settings of 70 kVp, 114 mA, 200 ms integration time, and isotropic voxel size of 12 μm.[3] For the tibial diaphyses, 300 slices were recorded starting 3.6 mm distal to the tibia-fibular junction. For the tibial metaphyses, 75 slices were recorded starting 0.9 mm distal to the growth plate. Scan locations are shown schematically in Supplementary Figure S2. Structural parameters were obtained according to current recommendations on μCT scans in rodents[32]: bone volume/tissue volume (analyzed separately for cortical and trabecular regions), cortical thickness, trabecular number, trabecular thickness, trabecular separation, intraindividual distribution of trabecular separation (SD of 1/trabecular number) and mineral density. The

structure model index (SMI) was calculated as a dimensionless parameter where higher numbers indicate a more platelike trabecular structure, whereas low numbers indicate a more rod-shaped structure. Cortical porosity was calculated as the ratio of the intracortical pore volume to the total volume of the cortex.[33] The cortical pore diameter was calculated using a distance transformation approach applied to the pore structures.[34] Polar cross-sectional moment of inertia, which is an estimate for overall mechanical stability of the tibial diaphysis, is given as mean value of all μCT slices taken from the tibial midshaft.

Histomorphometry

Mice were labeled with alizarin (Merck/Millipore, Darmstadt, Germany) 20 mg/kg, s.c. (dissolved in $NaHCO_3$) 10 days and calcein (Fluka/Sigma-Aldrich, Vienna, Austria) 10 mg/kg, s.c. 3 days before killing. Briefly, lumbar spines were fixated in 96% ethanol, dehydrated, and embedded in methyl methacrylate. Sections of 4 μm were obtained and stained with modified Masson-Goldner trichrome. Sequential 7-μm sections were used for fluorescent microscopy.[35] Bone histomorphometry was performed using the Osteoplan II system (Carl Zeiss, Thornwood, NY).[36] Results of histomorphometric analyses are reported as recommended by the American Society for Bone and Mineral Research[37]: BV/TV, cortical thickness, trabecular number, trabecular thickness, trabecular separation, osteoid surface/bone surface, osteoid thickness, osteoid volume/bone volume, number of osteoblast/100-mm bone perimeter, osteoblast surface/bone surface, number of osteoclast/100-mm bone perimeter, osteoclast surface/bone surface, erosion surface/bone surface, mineral apposition rate/day, mineralization lag time, bone formation rate/bone surface, and osteoid maturation time.

Statistics

Data are expressed as mean ±SD for normally distributed data and as median with the 25th to 75th percentile for nonnormally distributed data. Nonnormally distributed data were natural log-transformed (Supplementary Table S1 for list of ln-transformed parameters). To examine whether induction of CKD was successful and to characterize this CKD model in more detail, differences between WT and SOST-KO mice and between CTRL and CKD mice were analyzed using Student's *t* test. *P* values < 0.05 were considered statistically significant. To study the influence of CKD compared with CTRL in WT and SOST-KO mice, 2-way ANOVA (2 levels ×2 factors) was used for weight, laboratory, and bone parameters. Results from the 2-way ANOVA show whether SOST-KO mice react differently to CKD compared with WT mice. *P* values for interaction are reported. A *P* value for interaction < 0.05 indicates that there is a statistically significant difference in response to CKD between SOST-KO and WT mice. *P* values for interaction > 0.05 suggest that SOST-KO and WT mice show similar responses to CKD. Statistical analysis was performed using SPSS 20 (IBM, Armonk, NY, USA). *P* values < 0.05 were considered statistically significant.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Mouse weight and laboratory parameters

	WT-CTRL	WT-CKD	SOST-KO-CTRL	SOST-KO-CKD	*P* WT-CTRL versus SOST-KO-CTRL	*P* WT-CTRL versus WT-CKD	*P* SOST-KO-CTRL versus SOST-KO-CKD	*P* for interaction (2-way ANOVA)
Weight (g)	24.8 ± 1.2	20.8 ± 3.4	28.3 ± 1.6	24.1 ± 1.9	<0.001	<0.001	<0.001	0.8
Creatinine (mg/dl)	0.25 (0.21 – 0.27)	0.45 (0.42–0.54)	0.27 (0.21–0.29)	0.35 (0.29–0.43)	0.4	<0.001	<0.001	0.004
BUN (mg/dl)	25 ± 4	89 ± 21	24 ± 3	75 ± 26	0.7	<0.001	<0.001	0.08
Calcium (mmol/l)	2.56 ± 0.18	3.02 ± 0.07	2.65 ± 0.10	2.88 ± 0.12	0.3	<0.001	0.002	0.02
Albumin (g/l)	38 ± 5	38 ± 2	37 ± 1	38 ± 2	0.7	0.8	0.3	0.5
Phosphorus (mmol/l)	1.77 ± 0.46	1.92 ± 0.37	1.92 ± 0.33	1.99 ± 0.35	0.3	0.2	0.6	0.6
PTH (pg/ml)	264 (119–314)	274 (70–833)	150 (105–245)	151 (101–271)	0.2	0.5	0.4	0.8

ANOVA, analysis of variance; BUN, blood urea nitrogen; CKD, chronic kidney disease; CTRL, control; PTH, parathyroid hormone; SOST, sclerostin.

Data are shown as mean ± SD or median (25th to 75th percentiles).

Table 2

µCT of the tibia-cortical regions

	WT-CTRL	WT-CKD	SOST-KO-CTRL	SOST-KO-CKD	*P* WT-CTRL versus SOST-KO-CTRL	*P* WT-CTRL versus WT-CKD	*P* SOST-KO-CTRL versus SOST-KO-CKD	*P* for interaction (2-way ANOVA)
Diaphysis								
Ct.Th (µm)	247 ± 160	205 ± 23	484 ± 54	430 ± 57	<0.001	<0.001	0.01	0.5
Ct.Po (%)	3.3 (2.9–3.5)	3.8 (3.4–4.9)	1.7 (1.6–2.1)	2.2 (1.8–2.3)	<0.001	0.003	0.009	0.7
Mineral density (mg HA/cm^3)	1390 ± 82	1384 ± 96	1434 ± 64	1410 ± 57	0.1	0.8	0.3	0.6
Periosteal perimeter (mm)	4.29 (4.21 –4.73)	4.45 (4.15– 4.74)	5.86 (5.58 –5.96)	6.01 (5.85–6.15)	<0.001	0.9	0.5	0.5
Marrow area (mm^2)	0.26 (0.24–0.32)	0.38 (0.31–0.41)	0.09 (0.07–0.26)	0.26 (0.22–0.32)	<0.001	0.01	0.002	0.025
CSMI (mm^4)	0.14 (0.13–0.19)	0.14 (0.11–0.17)	0.41 (0.39–0.44)	0.44 (0.41–0.51)	<0.001	0.2	0.9	0.4
Metaphysis								
Ct.Th (µm)	182 ± 15.4	163 ± 27	318 ± 23.9	307 ± 33.4	<0.001	0.01	0.3	0.5
Ct.Po (%)	9.1 ± 1.2	12.3 ± 3.5	2.8 ± 0.7	3.0 ± 1.0	<0.001	<0.001	0.5	0.005
Mineral density (mg HA/cm^3)	1243 ± 46	1098 ± 94	1342 ± 106	1279 ± 38	<0.001	<0.001	0.03	0.025

ANOVA, analysis of variance; CKD, chronic kidney disease; CSMI, cross-sectional moment of inertia, polar; Ct.Po, cortical porosity; CTRL, control; Ct.Th, cortical thickness; KO, knockout; mg HA, mg hydroxyapatite; SOST, sclerostin; WT, wild-type.

Data are shown as mean ±SD or median (25th to 75th percentiles).

Table 3

µCT of the tibia-trabecular region (metaphysis)

	WT-CTRL	WT-CKD	SOST-KO-CTRL	SOST-KO-CKD	*P* WT-CTRL versus SOST-KO-CTRL	*P* WT-CTRL versus WT-CKD	*P* SOST-KOCTRL versus SOST-KO-CKD	*P* for interaction (2-way ANOVA)
BV/TV (%)	3.7 ± 1.3	5.4 ± 1.9	40.1 ±11.5	39.9 ± 13.6	<0.001	0.003	0.9	0.6
Tb.N (1/mm)	2.07 (1.89–2.45)	2.23 (1.91–2.42)	3.43 (3.17–4.58)	3.42 (2.63–3.97)	<0.001	0.6	0.3	0.2
Tb.Th (µm)	0.048 (0.031–0.052)	0.047 (0.019–0.051) 0.107	(0.054–0.116)	0.053 (0.047–0.068)	<0.001	0.2	0.009	0.3
Tb.Sp (µm)	484 ± 72	487 ± 80	285 ± 85	323 ± 84	<0.001	0.9	0.2	0.3
Tb1/NSD	0.18 ± 0.03	0.18 ± 0.04	0.15 ± 0.05	0.16 ± 0.05	0.04	0.9	0.5	0.5
Mineral density (mg HA/cm^3)	1131 ± 41	1026 ± 58	1170 ± 77	1101 ± 38	0.1	<0.001	0.017	0.053
SMI	2.9 ± 0.4	2.5 ± 0.3	0.4 ± 0.8	0.9 ± 0.7	<0.001	<0.001	0.08	0.001

ANOVA, analysis of variance; BV/TV, bone volume/total volume; CKD, chronic kidney disease; CTRL, control; mg HA, mg hydroxyapatite; NSD, standard deviation of trabecular number; SMI, structure model index; µCT, micro computed tomography; SOST, sclerostin; Tb.N, trabecular number; Tb.Th, trabecular thickness; Tb.Sp, trabecular separation; Tb1/NSD, SD of 1/trabecular number; WT, wild-type.

Data are shown as mean ±SD or median (25th to 75th percentiles).

Table 4

Histomorphometry of lumbar vertebrae

	WT-CTRL	WT-CKD	SOST-KO-CTRL	SOST-KO-CKD	*P* WT-CTRL versus SOST-KO-CTRL	*P* WT-CTRL versus WT-CKD	*P* SOST-K OCTRL versus SOST-KO-CKD	*P* for interaction (2-way ANOVA)
BV/TV (%)	21 ± 5	23 ±5	49 ±8	42 ±5.1	<0.001	0.220	0.02	0.01
Tb.Th (μm)	39 ± 4	34 ± 4	63 ± 11	46 ± 5	<0.001	0.002	<0.001	0.001
Tb.Sp (μm)	158 ± 49	114 ± 27	68 ± 19	64 ± 13	<0.001	0.006	0.5	0.02
OV/BV (%)	0.6 (0.2–1.1)	2.3 (1.6–6.7)	1.1 (0.1–1.4)	1.8 (0.7–2.9)	0.7	<0.001	0.028	0.2
OS/BS (%)	5.7 (1.4–8.1)	9.5 (7.0–19.1)	7.9 9 (1.6–10.9)	9.1 (4.6–14.2)	0.4	0.004	0.2	0.2
O.Th (μm)	2.5 (2.1–3.0)	4.8 (4.1–5.7)	4.0 (2.7–4.6)	4.8 (4.0–5.6)	0.01	<0.001	0.01	0.02
Ob.N (1/100 mm)	142 (47–319)	95 (64–202)	117 (73–202)	74 (40–99)	0.6	0.5	0.2	0.6
Ob.S/BS (%)	2.2 (0.7–3.5)	1.4 (0.8–2.8)	1.6 (0.9–3.2)	1.0 (0.3–1.2)	0.5	0.5	0.1	0.5
ES/BS (%)	2.0 (1.2–2.9)	2.1 (1.3–5.1)	1.6 (1.1–2.1)	1.3 (0.6–1.8)	0.5	0.3	0.2	0.1
Oc.N (1/100 mm)	52 (34–78)	76 (51–153)	53 (40–77)	33 (21–45)	0.9	0.08	0.06	0.01
Oc.S/BS (%)	1.7 (1.1–2.7)	1.6 (1.2–4.4)	1.2 (0.8–2.0)	1.1 (0.7–1.3)	0.6	0.6	0.1	0.1
MAR (μm/d)	1.0 (0.6–1.4)	0.9 (0.7–1.6)	1.4 (0.8–1.8)	0.8 (0.7–1.0)	0.2	0.5	0.1	0.1
DLS (%)	6.7 ±3.0	4.4 ±3.5	8.9 ±4.5	9.1 ± 2.7	0.2	0.08	0.9	0.02
SLS (%)	5.2 ±2.7	4.8 ±2.3	3.7 ±0.8	3.2 ± 1.6	0.08	0.7	0.4	0.6
MS/BS (%)	8.7 (6.4–12.2)	5.4 (4.0–7.8)	10.6 (6.4–15.1)	10.3 (8.1–12.8)	0.6	0.03	0.7	0.07
BFR/BS ($mm^3/cm^2/y$)	2.5 (2.0–6.1)	2.1 (1.8–3.8)	4.1 (3.1–6.2)	3.6 (2.8–4.3)	0.1	0.3	0.3	0.8
Mlt (d)	1.2 (0.7–2.6)	9.2 (2.3–18.1)	1.9 (0.7–3.2)	6.1 (2.7–7.5)	0.4	<0.001	0.04	0.06
Omt (d)	3.2 ±1.3	5.7 ±2.6	3.4 ± 1.4	5.5 ±1.7	0.7	0.004	0.003	0.8

ANOVA, analysis of variance; BFR/BS, bone formation rate/bone surface; BV/TV, bone volume/total volume; CKD, chronic kidney disease; CTRL, control; DLS, double-labeled surface; ES/BS, erosion surface/bone surface; KO, knockout; MAR, mineral apposition rate; Mlt, mineralization lag time; MS/BS, mineralizing surface/bone surface; Ob.N, osteoblast number/bone length; Ob.S/BS, osteoblast surface/bone surface; Oc.N, osteoclast number/bone length; Oc.S/BS, osteoclast surface/bone surface; Omt, osteoid maturation time; OS/BS, osteoid surface/bone surface; O.Th, osteoid thickness; OV/BV, osteoid volume/bone volume; SLS, single-labeled surface; SOST, sclerostin; Tb.Sp, trabecular separation; Tb.Th, trabecular thickness; WT, wild-type.

Data are shown as mean ±SD or median (25th to 75th percentiles).