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The Skeletal Response to Estrogen is Impaired in Female but not in Male Steroid Receptor Coactivator (SRC)-1 Knock Out Mice

U. I. Mödder¹, A. Sanyal¹, J. Xu³, B. W. O'Malley³, T. C. Spelsberg², and S. Khosla¹

¹ Endocrine Research Unit, Mayo Clinic College of Medicine, Rochester, MN

² Department of Biochemistry and Molecular Biology, Mayo Clinic College of Medicine, Rochester, MN

³ Department of Molecular and Cellular Biology, Baylor College of Medicine, Houston, TX, USA

Abstract

Estrogen (E) is critical for the maintenance of bone mass in both female and male mice and steroid receptor coactivator (SRC)-1 has been shown to be important for mediating E effects on bone, at least in female mice. In the present study, we defined the skeletal phenotype of male SRC-1 knock out (KO) mice and compared it with their female littermates. Further, to determine the role of SRC-1 in mediating effects of E on bone in male mice, we examined the skeletal effects of gonadectomy (gnx) with or without E replacement in male mice and placed these findings in the context of our previous studies in female SRC-1 KO mice.

Analysis of a large group of male (WT, n=67; SRC-1 KO, n=56) and female (WT, n=66; SRC-1 KO, n=70) mice showed a significant decrease in trabecular volumetric bone mineral density (vBMD) in SRC-1 KO mice compared to their WT littermates in both genders (male SRC-1 KO, 275 ± 3 vs WT, 295 ± 3 mg/cm³, $P < 0.001$; female SRC-1 KO, 210 ± 2 vs WT, 221 ± 2 mg/cm³, $P < 0.001$). Following gnx and E replacement (10 µg/kg/d), we previously demonstrated that SRC-1 KO female mice have a defect in E action in trabecular, but not in cortical bone. In contrast, we now demonstrate that the same dose of E administered to gnx'd male SRC-1 KO mice was sufficient to prevent trabecular bone loss in these mice. For example, in WT female mice, gnx followed by E replacement maintained spine BMD ($1.2 \pm 3.4\%$ vs baseline) as compared to gnx without E replacement ($-12.7 \pm 2.6\%$, $P < 0.001$ vs sham); this effect of E was absent in SRC-1 KO female mice. By contrast, the identical dose of E was equally effective in maintaining spine BMD in E-treated gnx'd male WT ($-5.2 \pm 5.1\%$ vs baseline) and male SRC-1 KO ($-5.4 \pm 5.3\%$) mice, respectively, as compared to gnx'd mice without E treatment (WT, $-17.6 \pm 2.5\%$, $P = 0.02$; SRC-1 KO, $-28.6 \pm 2.6\%$, $P < 0.001$ vs sham). E treatment was effective in suppressing cancellous bone turnover in both gnx'd WT and SRC-1 KO male mice as determined by significant reductions in osteoblast and osteoclast numbers; however, in female mice, E treatment only suppressed bone turnover in WT but not in SRC-1 KO mice.

Collectively, these findings demonstrate that loss of SRC-1 results in trabecular osteopenia in male and female mice, but in contrast to female mice, this is not due to any detectable resistance to E action in trabecular bone in male SRC-1 KO mice.

Address all correspondence to Sundeep Khosla, M.D., Mayo Clinic, 200 First Street SW, 5-194 Joseph, Rochester, MN 55905. Phone: (507) 255-6788, Fax: (507) 255-4828, E-mail: khosla.sundeep@mayo.edu.

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Keywords

Steroid receptor coactivator; SRC; gender difference; E action; site-specific regulation; gonadectomy

Introduction

Estrogen (E) is known to be the major sex hormone involved in the maintenance of bone mass in women [1–3]. However, there is now accumulating evidence for an essential role for E in regulating bone metabolism in males. The importance of E in the development and maintenance of the male skeleton was clearly demonstrated in the case reports describing a man with homozygous mutations involving the estrogen receptor (ER) gene [4] and two reports of males with aromatase deficiency [5–7]. These patients all had osteopenia, unfused epiphyses, increased markers for bone remodeling and continuing linear growth in adulthood. Treatment of the aromatase deficient patients with E led to closure of the epiphyses and a dramatic increase in bone density.

The biological effects of E are mediated by the nuclear hormone receptors: ER- α and ER- β , which interact with several classes of coactivators/corepressors in a ligand-dependent manner (for reviews, see [8,9]). The members of the steroid receptor coactivator (SRC) family are one class of the key coactivators for estrogen receptors. This SRC family contains three homologous members: SRC-1, SRC-2 (also known as TIF2 and GRIP1), and SRC-3 (also known as p/CIP, AIB1, RAC3, ACTR, and TRAM-1). It is now appreciated that the relative balance of receptors, coactivator, and corepressor proteins is a critical determinant of the ability of the nuclear receptor to regulate gene transcription. Since the relative concentrations of these molecules are cell type specific, sex steroid hormones can have vastly different functions in different tissues. Variations in the recruitment of coregulatory molecules also appears to be a mechanism by which selective ER modulators (SERMs) produce their tissue-specific effects [10].

The physiological importance of SRC-1 for E action has been demonstrated by the generation of SRC-1 KO mice, using conventional gene targeting [11]. The targeting event inserted an in-frame stop codon at the Met³⁸¹ in exon 4, causing a downstream deletion of the genomic sequence and disruption of the SRC-1 functional domains. The homozygous mutants are viable and fertile in both sexes but, at least in females, exhibit significant resistance to E action in a number of tissues, including the uterus and mammary gland. In addition, the androgen-sensitive prostate exhibits slightly decreased growth and development, whereas compensatory increases of SRC-2 were observed in brain and testis [12]. We have previously demonstrated that female SRC-1 KO mice have an impaired response to estradiol (E₂) treatment following ovariectomy (ovx) [13]. However, there was no compensatory up regulation of SRC-2 expression in bone. Interestingly, using independently generated SRC-1 KO mice, Yamada et al. [14] reported osteopenia under basal conditions in the female and male SRC-1 KO mice and resistance to the skeletal effects of E₂ in ovariectomized (ovx'd) female mice and 5 α -dihydrotestosterone effects in orchidectomized (orch'd) male mice. However, there was no information about the effect of E on the skeleton in male SRC-1 KO mice.

In the present study, we characterized the skeletal phenotype of a large number of male SRC-1 KO mice and compared this to findings in the female SRC-1 KO mice. In addition, in order to specifically isolate possible defects in E action, we assessed the effects of gonadectomy (gnx) and E replacement in the male SRC-1 KO mice and placed these findings in the context of our analogous previous study using female SRC-1 KO mice [13]. Our data demonstrate a somewhat surprising gender-specificity regarding the consequences of loss of SRC-1 for E action on trabecular bone.

Materials and Methods

Generation and care of mice

The generation of the SRC-1 KO mice has previously been described [11], and the mice used in the present studies had been extensively back-crossed (for 7 or more generations) into the C57BL/6 background. The animals were housed in a temperature-controlled room ($22 \pm 2^\circ \text{C}$) with a daily light/dark schedule of 12 h. During the experiments the animals had free access to water and were pair fed a standard laboratory chow (Laboratory Rodent Diet 5001, PMI Feeds, Richmond, VA) containing 0.95% calcium. Pups were genotyped at 4 weeks of age by polymerase chain reaction (PCR) as described previously [11]. The Institutional Animal Care and Use Committee approved all animal procedures.

Gonadectomies and E replacement

For the analysis of the skeletal phenotype of male SRC-1 KO mice compared to WT littermates in response to gn_x with and without E₂ treatment, we used an E₂ dose (10 $\mu\text{g/kg/d}$, based on an average body weight of 25g) that was able to restore bone mineral density in female WT mice following gn_x at different skeletal sites [13].

3-month old male SRC-1 KO and WT littermates had baseline bone mineral density scans by DXA and pQCT (see below) and were then divided into three groups ($n = 7\text{--}11$ mice/group). Subsequently, the mice were either sham operated, gn_x'd and implanted with a vehicle pellet or a 10 $\mu\text{g/kg/d}$ E₂ pellet (0.015 mg/60-d pellet (Innovative Research of America, Toledo OH)). For the orchidectomy, mice were anesthetized with 50 mg/kg ketamine and 5 mg/kg xylazine and then placed in a supine position on a sterile working surface. A small incision was made through the skin at the tip of the scrotum. The cremaster muscles were opened with a small incision. The caudal epididymidis was pulled out together with the testis, followed by the caput epididymidis, the vas deferens and the testicular blood vessels. A single ligature was placed around the vas deferens and the testicular blood vessels. The testis was removed and the procedure was repeated for the other testis. The muscle layer was closed by using a resorbable 6-0 suture and the skin with non-resorbable 4-0 suture material. For the sham procedure the testes were exteriorized but not removed. 60 days after the surgical procedure, bone mineral density (BMD) was determined again at the same skeletal sites. The mice were injected with tetracycline (10mg/kg) and calcein (10mg/kg) 11 and 3 days before the mice were sacrificed. The lumbar vertebrae (L1–L4) were excised for bone histomorphometry (see below).

Bone densitometry

For both the DXA and pQCT measurements, the mice were anesthetized with Avertin (2,2,2 tribromoethanol, 720 mg/kg, ip). For the DXA measurements, they were placed on the animal tray in a prone position on the Lunar PIXImus densitometer (software version 1.44.005, Lunar Corp, Madison, WI). In this position, the head is partially outside the area scanned by the machine. However, in all analyses of the whole body, the bones of the skull were excluded. Calibration of the machine was performed daily using the hydroxyl apatite phantom provided by the manufacturer. After scanning, regions of interest were identified for more specific analyses. Bone density of the lumbar vertebrae was determined in L1 – L3, for the femoral bone density the femur was analyzed in its full length. In repeatedly scanned mice (with repositioning between scans), the coefficients of variation (CVs) for total body, lumbar, and femoral BMD are 4.9%, 2.7%, and 4.3%, respectively. DXA is a two-dimensional method. It determines the areal bone mineral density (aBMD). The results are reported in mg/cm^2 .

For the pQCT measurements the mice were placed in a supine position on the gantry of the Stratec XCT Research SA Plus using software version 5.40 (Nordland Medical Systems, INC., Fort Atkinson, WI). As for the PIXImus, calibration of the machine was performed daily with

the hydroxyl apatite phantom provided by the manufacturer. The mice were positioned so that the total length of the femur and tibia were visible on the scout view. The scout view speed was set at 15.0 mm/sec with a slide distance of 0.5 mm. Once the scout view was completed, the reference line for the CT scans was set at the most proximal point of the tibia. Slice images were set at 1.9 mm (proximal metaphysis of the tibia). The CT speed was set at 3 mm/sec, pixel size was $100\ \mu\text{m} \times 100\ \mu\text{m}$ and slice thickness was 0.5 mm. After scanning, the CT slices were analyzed using peelmode 2, cortmode 1 and contour mode 1 to evaluate trabecular and cortical parameters. To determine the trabecular bone the threshold was set at $214\ \text{mg}/\text{cm}^3$ and for cortical bone at $710\ \text{mg}/\text{cm}^3$. CV is 4.4% for the total tibial volumetric BMD (vBMD). The BMD reported by pQCT are true volume (mg/cm^3) and are less influenced by bone size.

Bone histomorphometry

The lumbar vertebrae (L1 – L4) were fixed in 70% ethanol for at least 72 hours and then dehydrated in 95% ethanol for 1 day and in 100% ethanol for 6 days. The bones were then embedded without demineralization in a mixture of methylmethacrylate-2-hydroxyethyl and methylacrylate 12.5:1 and subsequently sectioned at a thickness of $5\ \mu\text{m}$ on a Reichert-Jung Supercut 2050 microtome using tungsten-carbide tipped steel knives. Sections were taken from the dorsal spine passing through the middle of the lumbar spine. Analysis of bone volume per tissue volume (BV/TV, percentage), osteoblast surface per bone surface (Ob.S/BS, percentage), and number of osteoblast per bone perimeter (N.Ob/BPm, #/mm) was carried out on Goldner Masson Trichrome stained sections in L2 and L3, $350\ \mu\text{m}$ below the cranial growth plate covering an area of $2.2\ \text{mm}^2$ using a light/epifluorescence microscope connected to a digitizing table and the OsteoMeasure histomorphometry system (OsteoMetrics Inc., Atlanta, GA). Osteoclast numbers (N.Oc/BPm, #/mm) and osteoclast surface per bone surface (Oc.S/BS, percentage) were identified by morphology (large multinucleated cells with cytoplasmic vesicles and intimate contact to bone) in the same Goldner Masson Trichrome stained sections.

Serum measurements of C-terminal telopeptide of collagen type I cross links (CTX)

Serum was collected and analyzed individually in duplicate with the RatLapsTM ELISA assay (Nordic Bioscience Diagnostics, Herlev, Denmark) according to the manufacturer's instructions.

Statistical analyses

All data are presented as mean $\pm$ SEM. The baseline comparison between the WT and SRC-1 KO mice within the gender was determined by Student's t-test. For the E study, the primary, pre-specified comparison in all cases was between the WT, gnx'd plus E₂ versus the SRC-1 KO, gnx'd plus E₂ mice, since the key question was the skeletal response to E₂ in the two groups of mice. Thus, this was analyzed using a t-test. For the remainder of the analyses, which did not test our primary hypothesis, we used an ANOVA followed by the post-hoc Fisher's PLSD test. A P-value of < 0.05 was considered significant.

Results

Basal conditions

The baseline bone phenotype of a large group of 3 month old male SRC-1 KO mice (n=56) was compared with their WT littermates (n=67), and was placed in the context of female SRC-1 KO (n=70) mice and WT mice (n=66). Whole body, femur and vertebral bone mineral density (BMD), as measured by DXA, was significantly decreased in the male SRC-1 KO mice (Figure 1A) compared to the WT male mice. The same significant difference in bone densities at the different skeletal sites were also apparent between female WT and SRC-1 KO mice (Figure 1B).

Table 1 summarizes bone mineral density measurements from the tibial metaphysis as determined by pQCT. This site contains sufficient trabecular bone for analysis, and as is evident, both male and female SRC-1 KO mice had significant deficits in trabecular vBMD at this site. Cortical vBMD and cortical thickness were not significantly different between either male or female SRC-1 KO as compared to their corresponding WT mice. Both genders of the SRC-1 KO had reduced periosteal and endocortical dimensions, suggesting that the loss of SRC-1 leads to smaller bones as compared to the WT littermates.

Effects of Gn α and E replacement on bone in male SRC-1 KO mice

To evaluate if E action was impaired in male mice lacking SRC-1, 3 months old mice were gn α 'd and treated with or without E₂ (0.015 mg/pellet) replacement for 60 days. As shown in Figure 2, gn α resulted in a decrease in vertebral aBMD (lumbar vertebrae L1 –L3) in both the WT and SRC-1 KO male mice; however there was a slightly greater bone loss in the SRC-1 KO male mice compared to the WT littermates. In male WT and SRC-1 KO mice the dose of E₂ administered was sufficient to maintain BMD at levels similar to sham operated mice at the vertebrae (predominantly trabecular bone) (Figure 2). As also shown in Figure 2, this was in marked contrast to our previous findings in female mice [13], where vertebral aBMD was preserved by this dose of E₂ in the WT, but not in female SRC-1 KO mice.

As shown in Figure 3, aBMD of the femur (a site of mixed trabecular and cortical bone) also decreased significantly following gn α in the WT and SRC-1 KO male mice. While femoral aBMD of the WT and SRC-1 KO male mice responded comparably to the treatment with E₂, the same dose of E₂ was previously found to be ineffective in maintaining femur aBMD at sham levels in the female SRC-1 KO mice (Figure 3).

A significant decrease in total and trabecular vBMD (determined by pQCT) at the proximal tibial metaphysis was observed after gn α in the WT and the SRC-1 KO male mice, with a similar trend for cortical vBMD (Table 2). E₂ treatment in both the male and female WT and the SRC-1 KO mice was sufficient to maintain total and cortical tibial vBMD at the level of the sham mice. However, the dose of E₂ used was not sufficient to maintain tibial trabecular vBMD in either the WT or the SRC-1 KO male mice at the tibial metaphysis, even though the identical dose was effective in preventing trabecular bone loss in the female WT mice. These results suggest a differential response to E in trabecular bone at this site between male and female mice (Table 2). As such, the response to E was not different in trabecular bone at the tibial metaphysis in the WT vs. SRC-1 male mice, but was different in the corresponding female mice while E preserved trabecular bone in the tibial metaphysis in WT female mice, since it did not prevent bone loss at this site in the female SRC-1 KO mice (Table 2). Collectively, therefore, the findings at the vertebrae and tibial metaphysis indicate that WT and SRC-1 KO male mice have identical responses to E in trabecular bone, whereas trabecular bone at both sites is resistant to E action in the female SRC-1 KO mice.

Cellular bone histomorphometric parameters in E₂-treated SRC-1 KO mice

Histological sections showed that gn α 'd SRC-1 KO male mice with or without E₂ treatment exhibited overall comparable bone tissue organization to the corresponding WT littermates. As shown in Figure 4 different bone histomorphometry parameter were determined at the lumbar vertebrae (L2 and L3). These data clearly show that E₂ treatment resulted in significant higher BV/TV in both the male WT and SRC-1 KO mice as compared to the vehicle-treated, gn α 'd mice (Figure 4A). Osteoblast parameters (Ob.S/BS, N.Ob/BPm) were significantly decreased in both the E₂-treated SRC-1 KO and WT mice compared to the respective gn α + V group (Figure 4B, and C). Similarly, there was a significant decrease in osteoclast parameters (Oc.S/BS, N.Oc/BPm) in these mice receiving E₂ (Figure 4D, and E). However, there was no difference in BV/TV, osteoblast and osteoclast parameter comparing the E₂-treated SRC-1 KO

mice with the E₂-treated WT mice. Interestingly, there was a significant increase in osteoclast number and surface in gn_x'd SRC-1 KO mice compared to the gn_x'd WT mice, which might explain the greater loss of lumbar vertebrae trabecular bone in the SRC-1 KO mice (Figure 4D, and E). Finally, serum CTx values did not differ between these groups, and were not different in the gn_x vs. E₂-treated mice, perhaps because it was obtained 60 days following gn_x, when the rapid turnover of bone had abated (Figure 4F).

A clearly different response to E treatment was observed in the female SRC-1 KO mice in terms of the bone histomorphometric findings. As shown in Figure 5A, treatment with E following gn_x resulted in a significant increase in BV/TV in the female WT, but not the SRC-1 KO mice. Osteoblast surface (Ob.S/BS, Figure 5B) and osteoblast numbers (N.Ob/BPm, Figure 5C) were reduced in gn_x + E treated WT female mice compared to the vehicle treated female WT mice, although these differences did not achieve statistical significance; these effects of E were less evident in the SRC-1 KO mice. Osteoclast parameters (Oc.S/BS, N.Oc/BPm) were significantly suppressed following E treatment in the WT mice but not in the SRC-1 KO mice (Figures 5D and E). Thus, in contrast to male SRC-1 KO mice, treatment with a physiological dose of E following gn_x was not effective in suppressing cancellous bone turnover in female SRC-1 KO mice.

Effects on reproductive organs

Seminal vesicle weights were not formally documented because it was macroscopically evident that there was no difference between the gn_x'd WT and SRC-1 KO male mice treated with the vehicle pellet and the gn_x'd mice receiving E₂. However, as reported earlier the dose of E₂ was biologically meaningful because it restored uterine weight in the gn_x female mice to near the level of the sham mice in the WT animals. This effect was completely abolished in the female SRC-1 KO mice [13].

Changes in body weight

There was no significant difference in body weights at the beginning of the study between the WT and SRC-1 KO mice in both the male and female mice (Table 3). Interestingly gn_x'd male WT mice lost body weight, whereas there was no significant change in body weight in the gn_x'd male SRC-1 KO mice and in contrast, the gn_x'd female mice gained body weight. However, there was no difference in body weight between the SRC-1 KO mice and the WT littermates when the mice received a physiological dose of E₂.

Discussion

SRC-1 is an important coactivator not only for the ER, but also for a number of other nuclear receptors. Our studies of the basal phenotyping of a large number of male (n=56) and female (n=70) SRC-1 KO mice clearly establish that loss of SRC-1 adversely impacts trabecular bone in both genders. The present findings do differ from our previous report [13] where, using a smaller number of animals, we were unable to detect significant differences in trabecular vBMD in the female SRC-1 KO mice under basal conditions (n = 9–10 mice analyzed in that study). The present studies, however, establish that there are subtle but detectable deficits in trabecular bone in female and in male SRC-1 KO mice. Our findings are now consistent with those of Yamada and colleagues [14] where the SRC-1 KO mice were generated somewhat differently, using a Cre-loxP system, and both female and male SRC-1 KO mice were found to have a significant decrease in trabecular vBMD under basal conditions.

In previous work we [13] and Yamada et al. [14] defined the effects of ovx and replacement with a physiological E₂ concentration on bone parameters in female SRC-1 KO mice. Some of the data from our previous study in female SRC-1 KO mice [13] were presented again and

additional bone histomorphometry data for a side-by-side comparison with the data from the male SRC-1 KO study were included in the present study. The findings in the SRC-1 female study clearly established a defect in E action in trabecular bone in these mice. Since E is important for the maintenance of bone mass in females as well as males [15–17], we now report the results of treating the male SRC-1 KO mice and their WT littermates with E₂. The key finding of the present work is that, in contrast to findings in the female SRC-1 KO mice, gnx of the male SRC-1 KO mice and treatment with E₂ delivered using slow release pellets (the same pellets which were used in the female experiment; 10 µg/kg/day), resulted in identical responses to E in trabecular bone in male SRC-1 KO and WT mice. The possible reasons for this gender-specificity in the consequences of loss of SRC-1 for E action in trabecular bone are unclear but may have to do with the possible preferred interactions of SRC-1 with ERβ vs. ERα in bone cells [18]. Thus, work from our group has previously demonstrated that at least *in vitro*, SRC-1 enhances mainly the activity of ERβ or coexpressed ERα/ERβ in osteoblastic cells, with little or no enhancement of ERα activity in these cells [18]. Moreover, we [13] and others [19] have shown that while cortical bone contains predominantly (or exclusively) ERα, trabecular bone contains mostly ERβ, with lesser amounts of ERα. In addition, there is now increasing evidence that, at least in mice, while E utilizes both ERα and ERβ signaling in trabecular bone in females, ERβ appears to play virtually no role in mediating E effects on bone in males [20]. Thus, loss of SRC-1 (which may interact predominantly with ERβ or ERα/β in bone cells) [18] would be expected to have a much greater impact on E action in female as compared to male mice, as demonstrated by the present studies. While a plausible hypothesis, we recognize that additional work needs to be done to further test this and to also define why ERβ signaling seems to be active in female but not in male mice.

Another possible explanation for the relative resistance to E action in trabecular bone in female but not male SRC-1 KO mice may be that there is a gender-related difference in SRC-1 steady state mRNA levels in bone [21] or even in SRC-1 protein activity. Significant gender- and site-specific differences in the expression of SRC-1 in the brain and pituitary gland under basal conditions and following acute stress have previously been noted in rats by Northern blot analysis [22]. Finally, an additional possibility for the differential effects of loss of SRC-1 on trabecular bone in female vs. male mice could be related to compensation for loss of SRC-1 by SRC-2 in male, but not in female mice. While SRC-2 was found to be overexpressed in the brain and testis of SRC-1 KO mice [12], testing this is considerably more difficult for trabecular bone, given the inability to isolate RNA from purely this bone compartment and this will require further studies using more complicated techniques such as *in situ* hybridization.

In summary, our studies clearly establish basal skeletal deficits in trabecular bone in both male and female SRC-1 KO mice, consistent with previous work by Yamada and colleagues [14]. The novel finding of the present work is the demonstration of preserved responses to E in trabecular bone in male SRC-1 KO mice, which contrasts with the deficits in E action in trabecular bone we previously observed in the female SRC-1 KO mice [13]. Since SRC-1 interacts both with E and androgen receptors [23,24], the basal deficits in trabecular bone in male SRC-1 KO mice, despite preservation of the response to E in this bone compartment, is consistent with impaired androgen action in trabecular bone in SRC-1 KO mice, as previously demonstrated by Yamada et al. [14]. Further defining the mechanism(s) underlying the gender specificity of the defect in E action on bone in SRC-1 KO mice, may provide insights into differential ERα vs. ERβ expression, utilization, or interactions with SRC-1 in male vs. female bones.

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Figure 1.

Osteopenia in SRC-1 KO mice. BMD measurements by DXA revealed a significant decrease of the whole body (WB), femur, and vertebral bone density of A) male WT (n= 67, open bars) and male SRC-1 KO (n= 56, solid bars) and B) female WT (n= 66, open bars) and female SRC-1 KO (n=70, solid bars) mice. Bars represent means $\pm$ SEM. The P values for the comparison between the WT and SRC-1 KO mice are noted, **, P<0.01 and ***, P<0.001.

Figure 2.

Comparable response to E₂ treatment in male WT and SRC-1 KO mice. The percent changes of lumbar vertebral BMD, measured by DXA, were determined following either sham surgery, gonadectomy (gnx) and implantation of a vehicle pellet (V) or gonadectomy (gnx) and implantation of an E₂ pellet (10 µg/kg/d) for 60 days. Males are indicated by squares, and for comparison, data from our previous study in females [13,14] are shown with the female mice represented by circles. The P-values for the main comparison (SRC-1 KO, gnX + E₂ versus WT, gnX + E₂) are as indicated. ANOVA P-values for within group comparisons: male WT, 0.03 and male SRC-1 KO, < 0.001, for both female WT and SRC-1, ≤0.001. *P<0.05 and ***P<0.001 for direct comparison with the respective sham group. ‡P<0.05 for comparison between SRC-1 KO, gnX + V, and WT, gnX + V.

Figure 3.

The percent changes of femoral BMD, measured by DXA, were determined following either sham surgery, gonadectomy (gnx) and implantation of a vehicle pellet (V), or gonadectomy (gnx) and implantation of an E₂ pellet (10 µg/kg/d) for 60 days. Males are indicated by squares, and for comparison, data from our previous study in females [13] are shown with the female mice represented by circles. The P-values for the main comparison (SRC-1 KO, gnX + E₂ versus WT, gnX + E₂) are as indicated. ANOVA-P values for within group comparisons: male WT, 0.01 and male SRC-1 KO, 0.003, for female WT, 0.03 and SRC-1, <0.001. *P<0.05, **P<0.01 and ***P<0.001 for direct comparison with the respective sham group.

Figure 4.

Bone histomorphometry data were determined at the lumbar vertebrae of male WT ($n = 8-10$, open bars) and SRC-1 KO ($n = 7-11$, solid bars) mice either gnax'd and treated with a slow release E₂ pellet or vehicle for 60 days. (A) Trabecular bone volume expressed as percentage bone volume/tissue volume (BV/TV). (B) Percentage of osteoblast surface per bone surface (Ob.S/BS). (C) Number of osteoblast per bone parameter (N.Ob/BPm). (D) Percentage of osteoclast surface per bone surface (Oc.S/BS). (E) Number of osteoclast per bone parameter (N.Oc/BPm). (F) C-terminal telopeptide of collagen type I cross links (CTx) was determined in serum of $n = 7$ male WT and SRC-1 KO mice. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ for direct comparison with the respective gnax + vehicle group. In addition the gnax + V, WT and SRC-1 KO mice were compared with each other. § $P < 0.01$, and £ $P < 0.001$.

Figure 5.

Bone histomorphometry data were determined at the lumbar vertebrae of female WT (n = 5, open bars) and SRC-1 KO (n = 3–6, solid bars) mice either gn x'd and treated with a slow release E₂ pellet or vehicle for 60 days. (A) Trabecular bone volume expressed as percentage bone volume/tissue volume (BV/TV). (B) Percentage of osteoblast surface per bone surface (Ob.S/BS). (C) Number of osteoblast per bone parameter (N.Ob/BPm). (D) Percentage of osteoclast surface per bone surface (Oc.S/BS). (E) Number of osteoclast per bone parameter (N.Oc/BPm). *P < 0.05 for direct comparison with the respective gn x + vehicle group. WT and SRC-1 KO gn x + E groups were compared with each other; the P values are noted in the figure. For the statistical analyses the post-hoc Fisher's PLSD test was used.

BMD parameters from male and female WT and SRC-1 KO mice under basal conditions at the proximal tibial metaphysis.

Table 1

	Male		Female	
	WT (n=67)	SRC-1 KO (n=56)	WT (n=66)	SRC-1 KO (n=70)
Total vBMD (mg/cm ³)	432 ± 5	419 ± 6	414 ± 3	415 ± 3
Trabecular vBMD (mg/cm ³)	295 ± 3	275 ± 3 ^c	221 ± 2	210 ± 2 ^c
Cortical vBMD (mg/cm ³)	815 ± 3	816 ± 3	824 ± 2	829 ± 3
Cortical Thickness (mm)	0.065 ± 0.003	0.062 ± 0.003	0.080 ± 0.003	0.080 ± 0.003
Periosteal Circumference (mm)	7.38 ± 0.03	7.11 ± 0.04 ^c	7.01 ± 0.04	6.62 ± 0.04 ^c
Endocortical Circumference (mm)	7.00 ± 0.04	6.72 ± 0.05 ^c	6.52 ± 0.05	6.13 ± 0.05 ^c

BMD parameters were determined by pQCT at the proximal tibial metaphysis in 3-month old mice. Data are expressed as mean ± SEM. P values between WT and SRC-1 KO mice of the same gender (

^c P< 0.001) were calculated by t-test.

Table 2

Percentage changes in vBMD at the tibial metaphysis (determined by pQCT) in male and female WT vs. SRC-1 KO mice following sham surgery, gnrx plus vehicle, or gnrx plus E₂ for 60 d.

Male		Total vBMD (%)		gnx + vehicle		gnx + E ₂		ANOVA	
WT	SRC-1 KO Female	Sham		gnx + vehicle		gnx + E ₂		ANOVA	
		2.55 ± 2.24	**	-19.70 ± 1.52	**	3.65 ± 9.94	0.004		
		-0.40 ± 2.17	**	-16.00 ± 1.95	**	-1.95 ± 6.20	0.002		
		Sham		gnx + vehicle		gnx + E ₂		ANOVA	
WT	SRC-1 KO Female	Sham		gnx + vehicle		gnx + E ₂		ANOVA	
		2.02 ± 2.50	*	-12.82 ± 1.38 ^(0.06)	*	18.48 ± 9.66	0.002		
		5.34 ± 1.64	**	-10.68 ± 1.58	**	4.56 ± 4.64	0.003		
		Cortical vBMD(%)		gnx + vehicle		gnx + E ₂		ANOVA	
WT	SRC-1 KO Female	Sham		gnx + vehicle		gnx + E ₂		ANOVA	
		2.20 ± 0.39		-0.63 ± 0.89		6.11 ± 2.41	0.006		
		1.61 ± 0.89		0.58 ± 0.95		7.02 ± 1.66	0.002		
		Sham		gnx + vehicle		gnx + E ₂		ANOVA	
WT	SRC-1 KO Female	Sham		gnx + vehicle		gnx + E ₂		ANOVA	
		3.74 ± 1.18	*	-0.09 ± 0.68	*	7.65 ± 1.95	0.002		
		5.15 ± 0.86	**	-0.46 ± 0.85	**	5.09 ± 1.41	0.002		
		Trabecular vBMD (%)		gnx + vehicle		gnx + E ₂		ANOVA	
WT	SRC-1 KO Female	Sham		gnx + vehicle		gnx + E ₂		ANOVA	
		-14.84 ± 2.01	***	-39.71 ± 2.67	***	-24.20 ± 7.88	0.001		
		-15.96 ± 2.39	***	-34.59 ± 2.34	***	-31.90 ± 3.17	<0.001		
		Sham		gnx + vehicle		gnx + E ₂		ANOVA	
WT	SRC-1 KO Female	Sham		gnx + vehicle		gnx + E ₂		ANOVA	
		-12.78 ± 2.0	^(0.06)	-26.93 ± 1.77	^(0.06)	9.48 ± 9.20	<0.001		
		-15.85 ± 3.13		-20.12 ± 2.35		-13.57 ± 3.28 ^a	0.322		

P values for the main comparison (SRC-1 KO gnrx + E₂ vs WT gnrx + E₂) as follows:

^a, P < 0.05. ANOVA P values for within group comparisons are as indicated.

*, P < 0.05; and

***, P < 0.001 for direct comparison with the respective sham group.

Table 3 Body weights of male and female WT vs. SRC-1 KO mice following sham surgery, gnx plus vehicle, or gnx plus E₂ for 60 d.

Male	Sham	gnx + vehicle	gnx + E ₂	ANOVA
WT SRC-1 KO Female	31.43 ± 0.83	28.45 ± 0.75 [*]	30.2 ± 0.88	0.04
	30.25 ± 0.70 Sham	29.19 ± 0.67 gnx + vehicle	29.07 ± 0.84 gnx + E ₂	0.46 ANOVA
WT SRC-1 KO	22.98 ± 0.59	28.08 ± 0.59 ^{***}	25.91 ± 0.62 ^{**}	<0.001
	23.21 ± 0.90	26.51 ± 0.95 [*]	26.48 ± 0.86 [*]	0.02

ANOVA P values for within group comparisons are as indicated.

^{*}, P < 0.05;

^{**}, P < 0.01, and

^{***}, P < 0.001 for direct comparison with the respective sham group.