Effect of Strontium Ranelate on Femur Densitometry and Antioxidative/Oxidative Status in Castrated Male Rats

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Summary

The studies were aimed at determinatning of the effect of strontium ranelate (SR) on the mineralization processes and selected parameters of oxidative stress in orchidectomized rats during the development of osteopenia. Male Wistar rats were sham-operated (SHO) and orchidectomized (ORX). ORX animals were divided into control (ORX-C) and gavaged with SR (ORX-SR), at a dose of 900mg/kg/b.w. After 60 days the animals were scanned for determination of bone mineral density (BMD) of the whole skeleton. Isolated femora were examined by DEXA and pQCT. Tomographic measurements were performed for a total slice and separately for the cortical and trabecular parts of the distal end of the femora. The intensity of lipid peroxidation (ILP) and total antioxidant capacity (TAC) in blood serum were measured. SR treatment increased vBMD and BMC of total, trabecular and cortical bone in ORX rats compared to ORX-C and SHO rats. ORX significantly increased TAC in control animals, and SR limited this increase. ILP in SHO and ORX-C rats which on a similar level. SR increased ILP by 21.3%, as compared to SHO. SR improved densitometric and geometric parameters of femora by orchidectomized rats what prevented degradation of bone tissue. Beneficial effects of SR were also demonstrated in stabilization of TAC in ORX rats at the level noted in SHO rats.

Introduction

Osteoporosis has been defined as "a progressive systemic disease characterized by low bone density and microarchitectural deterioration of bone tissue, with a consequent increase in bone fragility and susceptibility to fracture" (*Genant et al., 1999*). Bone loss, which characterizes osteoporosis, occurs during aging or after gonadectomy dictated by medical (humans and animals) or breeding (animals) indications (*Cavani et al., 2003; Stubbs et al., 1996; Bonnick, 2006*). Hypo/afunction of gonads results in an imbalance between resorption and formation of bone tissue, but also an imbalance between production and neutralization of reactive oxygen species (*Isomura et al., 2004; Basu et al., 2001*). In females, hormonal hypo/afunction of gonads generates oxidative stress (*Gomez-Zubeldia et al., 2000*). A comparative study conducted on gonadectomized female and male rats documented the relationship between hormonal functions of gonads and activity of markers of oxidative stress (*Kume-Kick et al., 1996*). There is substantial evidence that estrogens have an antioxidative effect (*Gomez-Zubeldia et al., 2000; Lacava & Luna, 1994*). The antioxidant effect of estrogens has been regarded as the main mechanism by which this hormone protects skeletal and cardiac muscle (*Persky et al., 2000*), uterus

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(*Az-Flores et al., 1999*) and liver (*Huh et al., 1994*) from oxidative damage. Chainy et al. (*Chainy et al., 1997*) observed that contrary to the effect of female steroids, testosterone decreased the activities of superoxide dismutse (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px).

Recently, great interest has been focused on strontium (Sr) as a potential antiosteoporotic factor (*Marie, 2005; Grynpas et al., 1996*). Contrary to radioactive strontium, which causes toxicity to bone, stable strontium has a beneficial effect on bone cells (*Pors, 2004; Shorr & Carter, 1952*). Shorr and Carter for the first time, documented that after the administration of a moderate dose of strontium lactate the deposition of Ca in bone was greater than the total Ca storage when Ca was given without Sr. Moreover, they demonstrated that strontium lactate reduced bone pain in patients with osteoporosis with concomitant radiological signs of amelioration (*Shorr & Carter, 1952*).

Our hypothesis is that SR can effectively protect against osteoporosis development in male, orchidectomized rats and that SR may participate in the control of ROS imbalance during hormonal insufficiency.

Our study was aimed at the determining of the effect of SR on densitometric and structural properties of femora in orchidectomized rats during the development of osteopenia.

Materials and Methods
Experimental procedures
The experimental design was approved by the Local Animal Welfare Committee in Lublin, Poland. The study was carried out on 30 male Wistar rats aged 80 days with initial body weight of 340g±5g. After transportation to the animal house at the Department of Biochemistry and Animal Physiology, Faculty of Veterinary Medicine of Agriculture University in Lublin, rats were adapted to the experimental conditions for 7 days (12h/12h light/dark ratio at 22 °C ± 2 and at 55 % ± 2 humidity). The animals were housed in plastic cages (Tecniplast, Italy) with free access to food (Agropol-Motycz,

Poland) and tap water. On the day of surgery, the rats were randomized, anaesthetized with an intramuscular injection of Ketaminum (Biowet-Puławy, Poland), Atropinum sulphuricum (Polfa-Warszawa, Poland) and Rometar (Leciva, Czech Republic) at doses of 10, 2 and 0.05 mg/kg b.w. respectively. The rats were divided into sham–operated (SHO) (n=10) and orchidectomized (ORX) (n=20) groups. The animals were pair fed throughout the study. Seven days after surgery the orchidectomized animals were randomly divided into a control group (ORX-C) and a group treated with Strontium Ranelate (Protelos® 2g – Servier, France) (ORX-SR) at a dose of 900 mg of active substance per kilogram of b.w. by daily gavage. The dose was chosen based on the results of Ammann (*Ammann et al., 2004*) . SHO and ORX-C animals received physiological saline at a dose of 0.5ml/100g b.w. daily by gavages. The body weight of animals was determined weekly. After 60 days, the rats were anaesthetized with CO_2, weighed and euthanized by cervical dislocation. Blood collected from a cardiac puncture served for the analysis of markers of oxidative stress. Immediately after euthanasia, the animals were scanned to determine the bone mineral density (BMD) of total skeleton using DEXA and then femora were isolated, cleaned of soft tissues and frozen for further analysis.

Densitometric analysis (DEXA) of bone mineral density (BMD).
Bone mineral density (BMD) of total skeleton and isolated right femora were determined using a Norland Excell Plus Densitometer (Fort Atkinson WI, USA) equipped with Small Subject Scan software, which allowed isolated bones to be analysed. The machine was calibrated daily with the use of the quality assurance phantom (QA-Phantom) provided by the manufacturer.

Peripheral quantitative computed tomography (pQCT).
Right femora were scanned with peripheral quantitative computed tomography (pQCT) XCT Re-

search SA Plus system with software version 5.5 D (Stratec Medizintechnik GmbH, Pforzheim, Germany). The femoral epiphysis was scanned 5mm from distal end. The scan line was adjusted using scout view of the pQCT system. During measurement the bones were fixed in a test tube filled with 70% ethanol. Upon completion of scanning, the following parameters were determined: total bone area (Tot.Ar), total bone mineral content (Tot.BMC), total volumetric bone mineral density (Tot.vBMD), trabecular bone area (Tb.Ar), trabecular bone mineral content (Tb.BMC), trabecular volumetric bone mineral density (Tb.vBMD), cortical bone area (Ct. Ar), cortical bone mineral content (Ct.BMC), cortical volumetric bone mineral density (Ct.vBMD) as well as cortical thickness (Ct.Th), periosteal (PERI.C) and endocortical (ENDO.C) circumferences. Analyses of trabecular bone were performed with the threshold $0.450cm^{-1}$, with a contour mode of 2 and a peel mode of 20, whereas the cortical part was with a threshold of $0.900cm^{-1}$ and cortical mode 2. The initial scan was performed with the speed 10mm/s, and CT-scan 4mm/s. Daily calibration of the system with the use of using hydroxyapatite-containing quality assurance phantom ($_p$QCT-phantom) precedes the measurements.

Intensity of lipid peroxidation (ILP) determined by the method described by Alberti et al., 2000
The method was based on the estimation of the radical cation from the reaction of alkoxy and peroxy radicals, derived from the hydroperoxides, with N,N-diethyl-para-phenylene diamine (DEPPD, Sigma, Poznań, Poland). The incubation mixture contained 1 ml acetate buffer (pH 4.8), 10 μl aqueous solution of DEPPD ($0.37 \ mol/dm^3$) and 20 μl blood serum. After 1.5 h incubation at 37° C, the absorbance was read at 505 nm against distilled water. The control sample contained distilled water instead of sample. Calculations were based on a standard curve prepared with different dilutions of H_2O_2. The results were expressed as μmol/g protein (mean±S.E.M.).

Total Antioxidant Capacity (TAC) determined by the method described by Benzie and Strain with modifications (Benzie & Strain, 1996)
TAC was determined by measuring the ferric reducing ability of blood serum. Working reagent, consisting of $300 \ mmol/dm^3$ acetate buffer (pH 3.6), 10 $mmol/dm^3$ 2,4,6-tri-pyridyl-s-triazine (TPTZ, Sigma, Poznań, Poland) in $40 \ mmol/dm^3$ HCl and 20 $mmol/dm^3$ $FeCl_3 \cdot 6H_2O$ mixed in the ratio of 10:1:1, was prepared immediately before use.
The working reagent (2250 μl) was mixed with 25 μl of sample and absorbance was measured at 593 nm against the working reagent alone. After exactly 10 min of incubation at room temperature, the absorbance was read again. The difference in absorbance at time zero and at 10 min was compared with the standard curve prepared with different dilutions of Fe (II) (0 to 1000 μmol/dm³). The results were expressed as μmol/g protein (mean±S.E.M.).

Protein content
Serum protein content was determined by the use of Lowry´s method (*Lowry et al., 1951*)

Statistical analysis
The results were presented as mean values ±S.E.M. One-way analysis of variance (ANOVA) was used to test for significant differences among the 3 experimental groups. To detect significant differences between individual experimental groups, significant ANOVAs were followed by *post hoc* Tukey test for multiple comparisons. Differences were considered significant at *P*<0.05. The relationship between bone tissue parameters and antioxidative/oxidative status, as well as analysis of significant differences, were assessed by the use of STATISTICA software (Stat Soft, Inc. Tulsa, USA).

Results
Body weight, BMD of femora and BMD of total skeleton
Orchidectomy increased the body weight of rats in both groups. Body weight at the end of the study in orchidectomized males increased by 20.7%

Table 1. Body weight, BMD of total skeleton and isolated femora. ą b c - different letters given with results in row indicate significant differences where $P<\cdot0.05$.

Parameter	SHO	ORX-C	ORX-SR
Body weight (g)	368.77 ± 8.66 a	464.80 ± 7.96 b	438.71 ± 9.26 b
BMD of skeleton (g/cm²)	0.141 ± 0.002 a	0.144 ± 0.003 a	0.157 ± 0.003 b
BMD of femora (g/cm²)	0.162 ± 0.001 a	0.152 ± 0.002 b	0.179 ± 0.001 c

compared to sham-operated ($P<0.05$) (Tab. 1). SR treatment limited this increase to 15.9% ($P<0.05$) (Tab. 1). BMD of total skeleton of SHO and ORX-C rats were at a similar level (0.141g/cm² and 0.144 g/cm²). SR increased BMD of total skeleton compared to SHO and ORX-C rats by 10.1% and 8.2%, respectively ($P<0.05$) (Tab. 1). BMD of isolated femora was significantly decreased in ORX-C rats by 6.2% ($P<0.05$) in comparison to SHO rats, whereas in ORX-SR rats BMD of isolated femora increased by 9.5% as compared to SHO and by 15.1% compared to ORX-C reaching the highest value ($P<0.05$) (Tab. 1).

Peripheral quantitative computed tomography (pQCT)
Orchidectomy decreased Tot.vBMD, Tb.vBMD and Ct.vBMD compared to SHO animals. SR treatment significantly increased vBMDs compared to ORX-C by 11. 6% (Tot.vBMD), 17.3% (Tb.vBMD) and 9.2% (Ct.vBMD) and compared to SHO by 7.4%, 13.8% and 4.6% respectively ($P<0.05$) (Tab. 2).
ORX rats of both groups had significantly higher total, cortical and trabecular area at the distal end of femora compared to SHO control ($P<0.05$), but the increase for animals treated with SR was smaller in size (Tab. 2). Total, trabecular as well as cortical mineral content of the distal region of femora in SHO and ORX-C rats were at a similar level. Sixty days of SR treatment significantly increased BMC in the analyzed regions, compared to SHO (16.2%, 20.3% and 14.3%) and ORX-C (12.7%, 19.4% and

Table 2. Total volumetric bone mineral density (Tot. vBMD), cortical volumetric bone mineral density (Ct.vBMD) and trabecular volumetric bone mineral density (Tb.vBMD) measured 5mm form the distal end of the femora. ą b c - different letters given with results in row indicate significant differences where $P<\cdot0.05$.

Parameter	SHO	ORX-C	ORX-SR
Tot.vBMD (mg/cm³)	679.91 ± 18.88 ab	648.85 ± 15.35 a	734.16 ± 19.87 b
Tot.BMC (mg/cm)	15.13 ± 0.85 a	15.75 ± 0.45 a	18.05 ± 0.41 b
Tot.Ar (mm²)	21.3 ± 0.20 a	25.01 ± 0.32 b	23.63 ± 0.21 b
Tb.vBMD (mg/cm³)	423.66 ± 43.00 a	406.28 ± 17.95 a	491.23 ± 17.24 b
Tb.BMC (mg/cm)	4.32 ± 0.55 a	4.37 ± 0.23 a	5.42 ± 0.18 b
Tb.Ar (mm²)	9.99 ± 0.22 a	11.23 ± 0.17 b	11.06 ± 0.10 b
Ct.vBMD (mg/cm³)	889.44 ± 18.89 ab	846.32 ± 16.23 a	932.10 ± 23.53 b
Ct.BMC (mg/cm)	10.82 ± 0.32 a	11.7 ± 0.25 a	12.63 ± 0.25 b
Ct.Ar (mm²)	12.23 ± 0.19 a	13.78 ± 0.19 b	13.57 ± 0.12 b
External diameter (mm)	16.08 ± 0.16 a	17.71 ± 0.11 b	17.59 ± 0.09b
Internal diameter (mm)	9.48 ± 0.13 a	11.19 ± 0.18 b	9.22 ± 0.08 a
Ct.Th (mm)	1.15 ± 0.02 a	1.04 ± 0.01 b	1.33 ± 0.02 c

7.4%) ($P<0.05$) (Tab. 2).
Orchidectomy significantly ($P<0.05$) increased PERI.C compared to SHO animals by 9.2% (ORX-C) and by 9.4 % (ORX-SR) (Tab. 2), while ENDO.C increased only in ORX-C animals (15.3%) ($P<0.05$) (Tab.2). Orchidectomy significantly ($P<0.05$) decreased Ct.Th compared to SHO animals. The highest Ct.Th was noted in SR treated castrated rats and was statistically significant ($P<0.05$) compared to SHO and ORX-C rats (Tab. 2).

Parameters of oxidative stress
In our study, orchidectomy resulted in a significant increase ($P<0.05$) of TAC in ORX-C animals. This increase, compared to SHO animals, corresponded to 17.2%. An increase of TAC in of ORX-SR animals was also noted, but in contrast to ORX-C animals, 60 days of SR treatment recovered this increase to 9.0% (Tab. 3).
Analysis of ILP in blood serum of SHO and ORX-C animals showed practically similar values. The

Table 3. Total protein (Tot Prot), Total Antioxidative Capacity (TAC), Intensity of Lipid Peroxidation (ILP). ạ b c - different letters given with results in row indicate significant differences where $P<\cdot0.05$.

Parameter	SHO	ORX-C	ORX-SR
Total Protein	72.49 ± 3.37 a	54.88 ± 3.48 b	55.15 ± 2.73 b
TAC	3.35 ± 0.19 a	4.77 ± 0.52 b	3.68 ± 0.16 b
ILP	1.40 ± 0.06 a	1.44 ± 0.16 a	1.69 ± 0.08 a

usage of SR increased the value of this parameter by 21.3% (in relation to SHO rats) (Tab 3). Total protein was significantly lower in orchidectomized rats, both in control (24.3%) and those treated with strontium ranelate (23.9%) ($P<0.05$) (Tab. 3).

The increase of TAC in ORX-C rats was negatively correlated (r= -0.8904; P=0.0301) with BMD of isolated femora. Treatment with RS reversed this relationship, and a slightly positive correlation between BMD of femora and TAC was observed (r= 0.1588; P=0.0799).

Discussion

Over the last few years, numerous *in vitro* and *in vivo* studies have investigated the anti-osteoporotic effect of SR. SR is composed of an organic moiety (ranelic acid) and of two atoms of stable non-radioactive Sr and is regarded as a bone-forming agent due to its stimulation of the replication of osteoprogenitor cells and collagen synthesis (*Marie et al*., 2001; *Fernandez-Garcia et al.*, 2005). Moreover Sr can stimulate non-collagenic protein synthesis in osteoblasts as well as inhibit the ^{45}Ca release from pre-labeled bone *in vitro* (*Su et al.,* 1992).

In vitro beneficial effects of SR have been supported by *in vivo* observations. Studies in mice indicated that SR increased the formation and decreased the resorption of bone tissue in vertebra, increasing bone mass as a result (*Delannoy et al*., 2002). The potential usefulness of SR was investigated using oophorectomized and immobilized rats. The obtained results proved that treatment with SR decreased bone loss, improved mechanical strength of the vertebrae and peripheral bones, with no alteration in elasticity (*Naveau, 2004*). An improvement in mechanical properties is associated with an, shaft diameter and volume of bone (*Ammann et al., 2004*). These results indicate the usefulness of SR in the prophylaxis and therapy of metabolic bone diseases, but the majority of experiments were conducted in females (*Dimai, 2005; Reginster et al., 2003; Boivin et al., 1996*). There is a lack of information about effect of SR on bone tissue in males with osteopenia.

The most important feature of a therapeutic agent in osteoporosis treatment is its ability to improve the quality of bone tissue. Studies conducted on ovariectomized rat proved that 8 weeks of preventive treatment with different doses of SR inhibited bone loss caused by lack of estrogens, as demonstrated by DEXA, bone ash, bone mineral content and histomorphometry (*Marie et al., 1993*). Moreover, the same team of scientists tested SR and documented a positive effect in rats with osteopathy developed by immobilization (*Hott et al., 2003*). An increase of BMD in vertebrae and femur after treatment with SR in intact mice, rats and monkeys was also observed (*Ammann et al*., 2004; *Delannoy et al., 2002*). The clinical studies with postmenopausal women receiving SR showed a 6.8% increase in bone density, while placebo group had 1.3% decrease. The authors concluded that half the increase in bone density (measured by x-ray densitometry) was attributed to the high atomic weight of Sr compared with Ca, whereas the other half was assumed to be a true increase in BMC (*Meunier et al., 2004*). In mouse calvaria and osteoclast culture, SR inhibited bone resorption by approximately 30%, as assessed by calcium release (*Baron & Tsouderos, 2002*) and pit assay, respectively. Other observation documented that SR decreased by 30% the activity of osteoclasts, as measured by the pit assay in isolated rat cells (*Takahashi et al., 2003*). Using DEXA, we analyzed the BMD of whole skeleton as well as isolated femora. Sixty days of lack of gonadal steroids is too short period to induce significant changes of BMD in skeleton analyzed totally;

therefore, the mineral density of whole skeleton in ORX-C animals was at a similar level to that in SHO rats. Interestingly, our prophylactic treatment of orchidectomized rats with SR at a dose of 900mg/kg b.w./day for 60 days improved the quality of bone tissue, which was expressed by an increase of the BMD value of whole skeleton in compared to SHO and ORX controls.

Peripheral quantitative computed tomography (pQCT) allows for non-invasive measurements of bone tissue and its characteristic by numerous parameters in freely selected region of bones. Additionally, all measurements can be performed not only for a complete slice, but also on indicidual cortical and trabecular areas. Our analyses were focused on the determination of the effect of SR on periosteal and endocortical circumferences as well as vBMD, BMC and Ar of total slice of bone, but also of trabecular and cortical bone. Distal femur methaphysis (DFM) was chosen for analysis because it has much trabecular bone, and is the bone region where metabolic changes are especially evident after gonadectomy.

It is well known that the lack of gonadal hormones accelerates bone metabolism. Increased endoosteal bone resorption and periosteal bone formation are observed in both sexes. This leads to decreased bone-wall thickness poorer mechanical properties. Also in the presented studies orchidectomy accelerated periosteal formation. Simultaneously, an increase of endoosteal bone resorption was observed, but exclusively in ORX-C rats. The usage of SR during 60 days of osteopenia development inhibited endoosteal bone resoption maintaining the ENDO.C at a similar level to SHO-C rats.

The relationship between of periosteal/endocortical circumferences was reflected in the observed cortical thickness (Ct.Th.). Strontium ranelate not only stopped the thinning of cortical bone, but additionally increased the Ct.Th, surpassing the values noted in SHO-C rats. Area of total, cortical and trabecular bone increased in ORX-C group by 11.2%, 11.2% and 11.0% respectively.

The animals treated with strontium ranelate also had a higher area of total, cortical and trabecular part of distal methaphysis in analyzed slice, but the treatment with SR limited these increases to 9.9%, 9.9% and 9.7% respectively. Orchidectomy decreased vBMD of distal femora of ORX-C rats, compared to the SHO control group, by 4.10% in trabecular bone, whereas in cortical bone this decrease was 4.9%. Sixty days of SR treatment not only made equal the volumetric density in relation to SHO rats, but also increased vBMD both in trabecular and cortical regions of DFM. Because of the higher metabolic activity of trabecular bone, this increase corresponds to 13.8%, whereas in cortical bone only to 4.6%. Interestingly, SR treatment, restored vBMD of cortical bone in gonadectomized rats to the level noted in SHO rats, whereas trabecular bone was not only fully restored, but markedly improved. Sixty days of osteopenia development did not affect bone mineral content (BMC) of total DFM or in trabecular and cortical bone in ORX-C rats compared to SHO. Gonadectomized animals treated with SR demonstrated significant increases of BMC as compared to SHO and ORX controls, of total, trabecular and cortical BMC. Similarly to Tb.vBMD, BMC of trabecular part showed the highest percentage increase in relation to SHO; this clearly indicates higher sensitivity of the trabecular part to the SR effect. Higher reactivity of trabecular compared to cortical bone after influence of SR was previously documented by Dakl (*Dahl et al., 2001*).

Due to the position of Sr in the Mendeleev table, close to Ca, it is obvious that these two elements are likely to be incorporated into bone with similar speed. Sr content and distribution is, however, dose and sex dependent. There are a few possible hypotheses concerning beneficial effects of Sr on bone. Sr may activate signals via extracellular cation-sensing receptor (CaSR) (*Quarles, 1997; Brown & MacLeod, 2001; Yamaguchi et al., 2000*) or may regulate cell differentiation as was observed in murine keratinocytes. It is also supposed to participate in apoptosis processes of bone cells (*Li et al., 1993*).

Reactive oxygen species (ROS) are produced by osteoclasts during of bone resorption (*Ries et al., 1992*). Those processes have been widely documented by observations on laboratory animals and postmenopausal women (*Muthusami et al., 2005; Maggio et al., 2003*). There is limited information about the effect of gonadal steroids on oxidative stress in castrated males (*Yalin et al., 2005; Basu et al., 2001*). Depending on the aim, oxidative stress can be determined either directly by measurement of the concentration of free radicals, indirectly by measurement of enzymatic and non-enzymatic antioxidants, as well as by TAC or the intensity of peroxidative processes (ILP) (*Sies, 1993*). TAC determination, together with ILP, may describe not only antioxidative power but also oxidative intensity (*Janaszewska & Bartosz, 2002*).

There is evidence that castration results in a decrease of antioxidative enzyme activities in rat macrophages, and is not changed by testosterone replacement (*Azevedo et al., 2001*). Although direct comparison with our results is difficult, due to different lengths of experiments, these data might suggest not only the influence of gonadal insufficiency on antioxidative defences, but also the result of osteopenia development.

The activities of antioxidative enzymes (GSH-Px, SOD, GSH-Tr), determined in bone homogenates, decreased at 60 days after bilateral ovariectomy in rats confirming the imbalance between production and neutralization of ROS during hormonal insufficiency. Moreover lipid peroxidation intensity, measured by thiobarbituric acid reactive substances, as well as H_2O_2 concentrations, increased when compared to control animals (*Muthusami et al., 2005*)

In our study, however, TAC in ORX-C group significantly increased with no ILP increase, compared to SHO animals but SR treatment limited this increase. Observations of Yalin and co-workers documented a negative correlation between SOD activity and BMD, and concluded that "oxidative stress plays an important role in primary male osteoporosis" (*Yalin et al., 2005*). Also in the presented study, negative correlation between TAC in ORX-C and BMD of

isolated femora was reversed by SR treatment. SR treatment, apart from the beneficial effects on bone properties, resulted in maintaining antioxidative/ oxidative status comparable to SHO animals. This may have clinical significance, but further experiments -including a longer study period - are necessary.

In conclusion, male osteoporosis is an increasing medical problem. The obtained results of this study shows the protective influence of strontium ranelate on densitometric and geometric parameters of femur, measured in distal methaphysis, for osteopenia induced by bilateral orchidectomy. SR not only preserved the bones against accelerated resorption, caused by the lack of gonadal steroids, but markedly improved the quality of both cortical and trabecular tissue. Another beneficial effect of SR in castrated rats was the stabilization of TAC at the level noted in SHO animals, which suggests antioxidative properties of SR.

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