

EXERCISE PREVENTED THE LANSOPRAZOLE-INDUCED REDUCTION OF ANTI-OSTEOPOROTIC EFFICACY OF ALENDRONATE IN ANDROGEN DEFICIENCY RATS

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Abstract: Clinical studies indicate that proton pump inhibitors (PPIs), used long-term in elderly patients, increase the risk of osteoporotic fractures, and decrease the anti-fracture efficacy of alendronate. The aim of the present study was to examine the effect of physical exercise on the anti-osteoporotic efficacy of alendronate administered concurrently with lansoprazole, a PPI, in male rats with androgen deficiency induced by orchidectomy. Male Wistar rats at 3 months of age were divided into: sham-operated control rats, orchidectomized (ORX) control rats, ORX rats receiving alendronate, ORX rats receiving alendronate and lansoprazole, ORX rats receiving alendronate and subjected to exercise, and ORX rats receiving alendronate and lansoprazole and subjected to exercise. The orchidectomy or sham-operation was performed 7–8 days before the start of drug administration. The rats were subjected to the exercise on the treadmill 1 hour/day for 7 weeks (6 days a week). Alendronate sodium (3 mg/kg *p.o.*) and lansoprazole (4 mg/kg *p.o.*) were administered once daily for 7 weeks (6 days a week). Mechanical properties of the tibial metaphysis and femoral neck were assessed. Bone turnover markers, histomorphometric parameters, bone mass and mass of bone mineral were also studied. Lansoprazole weakened the anti-osteoporotic efficacy of alendronate. The exercise increased the alendronate effect. Similar changes were observed in the rats treated with lansoprazole and alendronate, subjected to exercise; no deleterious effects of lansoprazole were observed.

In conclusion, the exercise prevented the lansoprazole-induced reduction the anti-osteoporotic efficacy of alendronate in orchidectomized rats.

Keywords: bone mechanical properties, exercise, alendronate, lansoprazole, orchidectomized rats

Osteoporosis in elderly men is an important although neglected health problem (1–3). It occurs less frequently than in women, and some 5–10 years later, too (4). However, at present, it is considered one of the main causes of morbidity and mortality in elderly men (2, 5). It is assumed that among the total incidence of fractures of the spine, hip, and forearm, some 42, 30, and 20%, respectively, occur in men, while the mortality connected with such fractures is definitely higher than in women (3, 6–8). Moreover, the number of osteoporotic fractures in men increases quickly, which is connected with increasing life expectancy (1, 3). Despite that, presently, less information is available about the efficacy of anti-fracture therapy for men, in comparison with women (1, 5), and all the data hitherto provided are based upon the results of bone mineral density (BMD) examinations (9).

In the anti-fracture therapy for men, the first-line drugs are bisphosphonates. Standard therapy is based upon oral administration of alendronate or risedronate (9–13). Bisphosphonates are strong anti-resorptive drugs. They prevent the loss of BMD and reduce the risk of fracture, by direct inhibition of osteoclast activity (11, 14). The optimum treatment time with bisphosphonates in men has not been studied, though. However, from the studies performed on women it can be gathered that in most cases bisphosphonates should be administered to men for a minimum of 5 years, and – in case no substantial improvement of BMD occurs – for 2 more years (10). Long term application of bisphosphonates increases the risk of undesired effects, including osteonecrosis of the jaw, hypocalcemia, atrial fibrillation, musculoskeletal pain, as well as atypical fractures of the femur. Long term therapy with the use

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of bisphosphonates also aggravates alimentary tract disorders, including gastroesophageal reflux and esophagitis, which may lead to the development of esophageal squamous cell carcinoma (10, 14).

Alimentary tract disorders occurring during therapy with bisphosphonates require simultaneous application of drugs that reduce the hydrochloric acid production, e.g., proton pump inhibitors (PPIs). PPIs are commonly used in case of elderly patients, in the treatment of esophagus reflux. There are data indicating that PPIs applied for a long time in postmenopausal women increase the risk of osteoporotic fractures (15, 16), and increased risk of fracture during PPI therapy may be greater in women than in men (17). Also, results of studies have been published, which indicate that PPIs administered to patients with osteoporosis reduce the anti-resorptive efficacy of alendronate and increase the risk of hip fractures (18, 19). Moreover, our earlier studies demonstrated that PPIs reduced the anti-resorptive activity and anti-osteoporotic efficacy of alendronate also in experimental studies conducted on rats with estrogen deficiency (model of postmenopausal osteoporosis) (20, 21).

On the basis of data provided above we assumed that lansoprazole, being a strong inhibitor of PPI (22), may reduce the anti-osteoporotic efficacy of alendronate also in male rats with experimental osteoporosis induced by orchidectomy. On the basis of reports indicating that exercise reduced the risk of fractures in women after menopause (23–25) as well as in elderly men [26], we examined whether physical exercise is capable of counteracting possible reduction of anti-osteoporotic efficacy of alendronate, induced by lansoprazole, in orchidectomized rats.

EXPERIMENTAL

Male Wistar rats at 3 months age (Center of Experimental Medicine, Medical University of Silesia, Katowice) were used in these studies. The initial rat body mass was 260–280 g. The rats were fed a standard laboratory diet (Labofeed B) *ad libitum* and were allowed free access to water. All procedures of the experiments on animals were approved by the Ethical Commission, Katowice, Poland.

Orchidectomy and sham-operation were performed in general anesthesia induced by intraperitoneal injections of ketamine – Bioketan (Vetoquinol Biowet) and xylazine – Xylapan (Vetoquinol Biowet). After 7–8 days, the rats were divided into six groups ($n = 8$): sham-operated

(Sham) control rats, orchidectomized (ORX) control rats, ORX rats receiving alendronate, ORX rats receiving alendronate and lansoprazole, ORX rats receiving alendronate and subjected to exercise, ORX rats receiving alendronate and lansoprazole and subjected to exercise. The animals were weighed every second day. Lansoprazole (Lanzul, Inter Pharma) and alendronate sodium, substance (Polpharma S.A.) were used in the study. Alendronate (3 mg/kg) and lansoprazole (4 mg/kg) were administered by a gastric tube (*p.o.*) once daily, for 7 weeks (6 days a week), at a volume of 2 mL/kg *p.o.* Alendronate was administered in the morning hours, lansoprazole 2 h after the administration of alendronate. The control rats were administered the vehicle (distilled water) in the same volume of 2 mL/kg *p.o.* daily. Moreover, all rats were given intraperitoneal injection of 20 mg/kg of tetracycline hydrochloride (Sigma-Aldrich), to mark the calcification front (27), one day before the start of drug or exercise or vehicle administration and one day before sacrifice, in order to determine the periosteal and endosteal transverse growth.

On the day following the last administration of drugs, after 24-h fasting, the animals were killed by cardiac exsanguination, under full ketamine and xylazine anesthesia. The adrenal gland and bones: the left and right tibia and right femur were isolated from the sacrificed animals. Immediately after isolation, the left tibia and adrenal gland were weighed (with the accuracy of 0.1 mg). The left tibia and right femur were wrapped in gauze soaked in 0.9% NaCl solution and kept in the temperature of -20°C until the mechanical tests were performed on thawed bones.

Exercise training

The exercise training was performed on a tape treadmill for rats (model BTP-10, Porfex, Białyłstok, Poland). The apparatus consisted of a 10-lane animal exerciser. The dimensions of each exercise lane are $37 \times 13 \times 8$ cm. The animals were placed on a belt facing away from the electrified grid (2 mA intensity). Exercise sessions were always performed between 9 and 11 a.m., started in 7–8 days after the orchidectomy or sham-operation performed. The exercise consisted of a 7-week running, 6 days per week for 60 min. The time was gradually increased in the four first days (by 15 min daily) until 60 min a day was reached, and kept until the end of the training. The velocity was 25 m/min. The treadmill inclination was kept at 7°C uphill during the entire training.

Studies of bone mechanical properties

Mechanical properties of the left tibial metaphysis and the neck of the right femur were assessed using the Instron 3342 500N apparatus with Bluehill 2 software, version 2.14. Mechanical properties of the left tibial metaphysis were studied using bending tests with three-point loading, as previously described (28–30). The load was applied perpendicularly to the proximal tibial metaphysis. The displacement rate was 0.01 mm/s. The load displacement curves, representing the relationships between load applied to the bone and displacement in response to the load, were analyzed. Maximum load and displacement, energy, and stress for the maximum load, as well as fracture load and displacement, energy, and stress for the fracture load were all assessed. Young's modulus was also determined. The moment of inertia in the cross-section, necessary for the calculations of the intrinsic bone mechanical parameters, was also determined, as previously described (31). Mechanical properties of the femoral neck were studied using a compression test. The maximum load (load causing the fracture of the femoral neck) was determined, as previously described (30, 31).

Bone histomorphometric studies

Bone histomorphometric parameters were assessed on histological specimens, prepared as previously described (32, 33). Histomorphometric measurements were made using an Optiphot-2 microscope (Nikon), connected through an RGB camera (Cohu) to a computer, using Lucia G 4.51 software (Laboratory Imaging), with final magnifications of 200 and 500 times, or using Osteomeasure software (magnification 70 times). The width of trabeculae in the distal epiphysis and metaphysis was measured in the longitudinal preparation from the femur. The area of the transverse cross-section of the cortical bone and the area of the transverse cross-section of the marrow cavity were determined in transverse cross-sections made from the tibial diaphysis. The periosteal and endosteal transverse growth of the tibia was also measured.

Bone mineralization studies

The mass of bone mineral (ash) was determined after mineralization. The bones were mineralized at the temperature of 640°C for 48 h in the muffle furnace, and subsequently weighed. The ratio of the mass of bone mineral to the bone mass was also determined as a substitute for bone mineral density measurements.

Biochemical studies

Serum osteocalcin levels were determined using an enzyme immunoassay (Rat-MID Osteocalcin EIA, Immunodiagnostic Systems Ltd.). Serum levels of type I collagen fragments released from bone during bone resorption were determined by an enzyme immunoassay (RatLaps EIA, Immunodiagnostic Systems Ltd.). Moreover, serum total cholesterol was assayed colorimetrically, using a Pointe Scientific reagent set.

Statistical analysis

The results are presented as the arithmetical means \pm SEM. Statistical estimation was carried out on the basis of the analysis of variance. After confirmation of statistically significant differences in one-way ANOVA ($p < 0.05$), further analysis was carried out by means of Duncan's *post hoc* test. In case of a lack of normality (Shapiro-Wilk's test) or of homogeneity of variance (Levene's test), nonparametric tests were used: Kruskal-Wallis ANOVA and Mann-Whitney U test. The results obtained in each experimental group were compared with those of the sham-operated control rats and orchidectomized control rats. The results obtained in rats treated with alendronate and lansoprazole, treated with alendronate and subjected to exercise, as well as treated with alendronate and lansoprazole and subjected to physical exercise were compared with those of the animals treated with alendronate. Moreover, the results obtained in rats treated with alendronate and lansoprazole and subjected to physical exercise were compared with those of the rats treated with alendronate and lansoprazole.

RESULTS

Body mass, adrenal mass and serum total cholesterol level

Androgen deficiency, in the ORX control rats, caused significant increases in the adrenal mass (by 47.4%) and in the adrenal mass expressed as the ratio to the body mass (by 39.5%), and insignificant increases in the serum total cholesterol level (by 30.1%), in comparison with the sham-operated rats. There was no effect of androgen deficiency on the body mass (Table 1). Alendronate did not affect the adrenal mass or serum cholesterol level, in comparison with the ORX control rats. Concurrent treatment with lansoprazole and alendronate led to a significant decrease in the adrenal mass in comparison with the ORX control rats. Exercise significantly increased the ratio of adrenal mass to body

Table 1. Body mass, adrenal mass and serum total cholesterol level in orchidectomized rats.

Parameters	Orchidectomized (ORX) rats					
	Sham-operated rats	Control	Alendronate	Alendronate and lansoprazole	Alendronate and exercise	Alendronate and lansoprazole and exercise
Body mass at the start of exercise and drug treatment [g]	267.3 ± 1.4	266.0 ± 3.2	267.3 ± 3.0	269.6 ± 1.6	265.0 ± 2.4	270.1 ± 3.7
Body mass after 7 weeks [g]	327.9 ± 4.0	343.8 ± 6.1	328.6 ± 7.7	331.1 ± 7.5	318.5 ± 8.2	327.5 ± 5.8
Adrenal mass [mg]	28.17 ± 1.62	41.54 ± 1.93***	36.49 ± 1.93**	34.99 ± 1.88**	46.18 ± 1.45***	47.33 ± 1.78***
Adrenal mass/body mass ratio [mg/100 g of body mass]	8.67 ± 0.54	12.09 ± 0.57***	11.11 ± 0.55**	10.58 ± 0.37*	14.52 ± 0.40***	14.46 ± 0.50***
Serum total cholesterol [mg/dL]	38.65 ± 2.37	50.27 ± 3.08	49.83 ± 3.07	49.03 ± 2.65	45.12 ± 5.03	45.35 ± 2.65

Results are presented as the mean ± SEM (n = 8). One-way ANOVA followed by Duncan's test or, when appropriate, Kruskal-Wallis ANOVA followed by Mann-Whitney U test was used for evaluation of the significance of the results. * – Significantly different from sham-operated rats; ** – p < 0.05, *** – p < 0.001. • – Significantly different from ORX control rats; •• – p < 0.05, ••• – p < 0.01, •••• – p < 0.001. – Significantly different from the rats treated with alendronate; *** – p < 0.001.

mass in rats treated with alendronate or alendronate and lansoprazole in relation to all other groups (Table 1).

Mass and mineral mass of the tibia

Androgen deficiency in the ORX control rats significantly reduced (by 6.7%) the mass of the tibia expressed as the ratio to the body mass, in comparison with the sham-operated rats. There was no significant effect of androgen deficiency on the bone mineral mass (Table 2). Alendronate caused significant increases in the mass of the tibia, mineral mass of the tibia and the ratio of the mineral mass of the tibia to the bone mass, in comparison with the ORX control rats. Those effects of alendronate were weakened by lansoprazole. In rats treated with lansoprazole and alendronate concurrently, the ratio of the mineral mass to the bone mass was increased, in comparison with the ORX control rats. Exercise did not affect the alendronate effect, and counteracted the weakening of the alendronate effect by lansoprazole. Significant increases in the bone mass expressed as the ratio to the body mass and bone mineral mass in the tibia were observed, in comparison with the rats treated with alendronate and lansoprazole (Table 2).

Bone histomorphometric parameters

In ORX control rats, the transverse cross-section areas of the cortical bone, of the whole diaphysis and of the marrow cavity, were not significantly affected, in comparison with the sham-operated rats, but there was a significant increase in the ratio of the transverse cross-section area of the marrow cavity to the area of the whole diaphysis (by 6.3%), and a significant decrease in the periosteal (by 8.3%) and endosteal (by 12.2%) transverse growth. In the ORX control rats, in comparison with the sham-operated rats, the width of trabeculae in the femoral epiphysis and metaphysis were significantly decreased, by 8.6 and 5.3%, respectively (Table 3). Alendronate counteracted the effect of androgen deficiency, causing significant decreases in the ratio of the transverse cross-section area of the marrow cavity to the area of the whole diaphysis, and increases in the endosteal transverse growth and in the width of trabeculae in the femoral epiphysis and metaphysis, in comparison with the ORX control rats. Lansoprazole weakened the effect of alendronate on all histomorphometric parameters studied. Exercise did not affect the alendronate effect, but counteracted the weakening of the alendronate effect by lansoprazole (Table 3).

Table 2. Mass and mineral mass of the tibia in orchidectomized rats.

Parameters	Orchidectomized (ORX) rats					
	Sham-operated rats	Control	Alendronate	Alendronate and lansoprazole	Alendronate and exercise	Alendronate and lansoprazole and exercise
Bone mass [mg]	627.83 ± 9.13	613.91 ± 10.61	637.23 ± 10.00	610.80 ± 9.18	636.30 ± 8.34	648.86 ± 13.51
Bone mass/body mass ratio [mg/100 g of body mass]	191.54 ± 2.31	178.67 ± 1.81*	193.98 ± 3.08**	184.95 ± 4.56	200.40 ± 3.98***	198.29 ± 3.76***#
Mineral mass [mg]	272.36 ± 4.56	261.02 ± 4.13	279.38 ± 4.83*	269.24 ± 3.58	280.96 ± 4.37**	290.38 ± 5.81***##
Mineral mass/bone mass ratio [mg/100 mg of bone mass]	43.38 ± 0.30	42.53 ± 0.24	43.86 ± 0.50*	44.10 ± 0.37*	44.15 ± 0.26**	44.43 ± 0.87* *

Results are presented as the mean ± SEM (n = 8). One-way ANOVA followed by Duncan's test or, when appropriate, Kruskal-Wallis ANOVA followed by Mann-Whitney U test was used for evaluation of the significance of the results. * - Significantly different from sham-operated rats; ** - p < 0.05. # - Significantly different from ORX control rats; * - p < 0.05, ** - p < 0.01, *** - p < 0.001. # - Significantly different from rats treated with alendronate and lansoprazole; # - p < 0.05, ## - p < 0.01.

Table 3. Bone histomorphometric parameters in orchidectomized rats.

	Orchidectomized (ORX) rats					
	Sham-operated rats	Control	Alendronate	Alendronate and lansoprazole	Alendronate and exercise	Alendronate and lansoprazole and exercise
Transverse cross-section area [mm ²]	Cortical bone	3.63 ± 0.07	3.64 ± 0.03	3.64 ± 0.06	3.73 ± 0.06	3.79 ± 0.07
	Marrow cavity	1.09 ± 0.02	1.13 ± 0.02	1.06 ± 0.02	1.09 ± 0.01	1.08 ± 0.02
	Whole diaphysis	4.88 ± 0.07	4.77 ± 0.08	4.70 ± 0.04	4.73 ± 0.07	4.77 ± 0.09
Transverse cross-section marrow cavity/diaphysis area ratio	0.224 ± 0.003	0.238 ± 0.004*	0.226 ± 0.004*	0.231 ± 0.002	0.219 ± 0.005**	0.223 ± 0.003*
Transverse growth [µm]	Periosteal	94.77 ± 1.61	86.86 ± 2.18*	94.28 ± 5.99	93.14 ± 1.71	106.06 ± 6.14*
	Endosteal	38.62 ± 1.27	33.92 ± 0.88*	39.15 ± 1.26*	38.02 ± 2.57	38.48 ± 0.71**
Width of trabeculae [µm]	Epiphysis	72.13 ± 0.68	66.95 ± 1.22**	72.73 ± 1.26***	70.03 ± 0.73*	71.49 ± 0.93**
	Metaphysis	36.71 ± 0.50	34.79 ± 0.41*	36.64 ± 0.42*	36.09 ± 0.48	37.07 ± 0.51**

Results are presented as the mean ± SEM (n = 8). One-way ANOVA followed by Duncan's test or, when appropriate, Kruskal-Wallis ANOVA followed by Mann-Whitney U test was used for evaluation of the significance of the results. * - Significantly different from sham-operated rats; ** - p < 0.05, *** - p < 0.001. # - Significantly different from ORX control rats; * - p < 0.05, ** - p < 0.01, *** - p < 0.001.

Table 4. Mechanical properties of the tibial metaphysis (parameters for the fracture point and Young's modulus) in orchidectomized rats.

Parameters	Orchidectomized (ORX) rats					
	Sham-operated rats	Control	Alendronate	Alendronate and lansoprazole	Alendronate and exercise	Alendronate and lansoprazole and exercise
Fracture load [N]	85.19 ± 5.67	68.13 ± 2.25*	93.40 ± 5.04***	74.04 ± 2.50 ^{am}	112.09 ± 5.92*** ^{am}	104.98 ± 4.24*** ^{###}
Displacement for fracture load [mm]	1.22 ± 0.05	1.23 ± 0.06	1.19 ± 0.07	1.12 ± 0.04	1.03 ± 0.05	1.20 ± 0.07
Energy for fracture load [mJ]	73.25 ± 4.39	58.13 ± 3.23*	79.50 ± 6.79**	59.43 ± 4.01 ^{am}	72.00 ± 3.27*	87.00 ± 5.52*** ^{###}
Stress for fracture load [MPa]	75.15 ± 8.19	65.02 ± 3.82	92.14 ± 5.77**	70.36 ± 3.81 ^{am}	108.12 ± 7.64***	97.14 ± 4.89*** ^{##}
Young's modulus [MPa]	3616 ± 293	3077 ± 251	4655 ± 297*	3780 ± 299	4955 ± 562**	5080 ± 652**

Results are presented as the mean ± SEM (n = 8). One-way ANOVA followed by Duncan's test or, when appropriate, Kruskal-Wallis ANOVA followed by Mann-Whitney U test was used for evaluation of the significance of the results. * – Significantly different from sham-operated rats; ** – p < 0.05, *** – p < 0.001. • – Significantly different from ORX control rats; •• – p < 0.05, ••• – p < 0.01, •••• – p < 0.001. ^a – Significantly different from the rats treated with alendronate; ^m – p < 0.01. # – Significantly different from rats treated with alendronate and lansoprazole; ## – p < 0.01, ### – p < 0.001.

Mechanical properties of the tibial metaphysis

Androgen deficiency in the ORX control rats significantly reduced, in comparison with the sham-operated rats, the maximum load (by 22.6%). Energy for the maximum load and the intrinsic mechanical parameters (maximum stress and Young's modulus) were insignificantly reduced (Figure 1, Table 4). The mechanical parameters for the fracture point were also decreased (fracture load and energy for the fracture load – significantly), in comparison with the sham-operated rats (Table 4). Alendronate significantly improved the mechanical parameters for the maximum load and the fracture point, as well Young's modulus. Lansoprazole weakened the alendronate effect on the mechanical properties of tibial metaphysis, significantly decreasing the maximum load, energy accumulated for the maximum load and maximum stress, as well as load, energy and stress for the fracture point, in relation to the rats treated with alendronate alone. Exercise intensified the alendronate effect on the mechanical properties of tibial metaphysis, significantly increasing the maximum load (Figure 1) and fracture load (Table 4) in relation to the rats treated with alendronate alone. Moreover, implementation of exercise significantly counteracted weakening of the alendronate effect by lansoprazole (Figure 1, Table 4).

Mechanical properties of the femoral neck

Androgen deficiency did not significantly affect the strength of the femoral neck in the ORX control rats, in relation to the sham-operated control rats (Figure 2). Alendronate insignificantly increased the maximum load sustained by the femoral neck of the orchidectomized rats. Lansoprazole weakened the alendronate effect on the maximum load sustained by the femoral neck. The exercising rats (both treated with alendronate, and treated with alendronate and lansoprazole) revealed increased strength of the femoral neck, in comparison with the ORX control rats (Figure 2).

Serum biochemical bone turnover markers

Androgen deficiency insignificantly increased the serum level of the biochemical marker of bone resorption (RatLaps, by 44.4%) and significantly increased the marker of bone formation (osteocalcin, by 24.0%), in comparison with the sham-operated controls (Figure 3). Alendronate significantly decreased the biochemical bone turnover markers, in comparison with the ORX control rats. Lansoprazole weakened the alendronate effect on the biochemical bone turnover markers, significant-

ly increasing the osteocalcin level in relation to the rats treated with alendronate alone. The levels of RatLaps and osteocalcin in exercising rats treated with alendronate and lansoprazole were similar to those in rats treated with alendronate alone. The level of osteocalcin in exercising rats treated with alendronate and lansoprazole was significantly increased, in relation to the rats treated with alendronate alone (Figure 3).

DISCUSSION

Orchidectomy in rats is a standard model used in examination of the influence of androgen deficiency upon the osseous system, reflecting the changes occurring in men with hypogonadism (34, 35). It causes a reduction of testosterone level in rat blood serum, by 80–95% (34, 36, 37). Reduction of mass and weakening of the bone microarchitecture may be observed as early as four weeks after the procedure, their intensity depends upon rat age and the duration of androgen deficiency (38, 39). Due to

the bone mass dependence on age, orchidectomy is most often performed in fast growing rats, between third and twelfth month of life (34, 40–42). In the model applied in the study, orchidectomy was performed in three-month old rats, and the androgen deficiency lasted for 8 weeks.

Androgen deficiency increases the rate of bone turnover, causing loss of cancellous and cortical bone (34, 43–45). Also in the study reported here, the blood serum of orchidectomized control rats revealed increased levels of biochemical markers of bone turnover. Eight weeks after the procedure of orchidectomy, the mass and mass of bone mineral in the tibia were diminished. Those results indicate that the reduction of trabeculae width induced by androgen deficiency was connected with the increased resorption of cancellous bone. Bone loss was also observed in compact bone, which has been demonstrated through reduced transverse growth of the cortical bone in tibia diaphysis, from the periosteum and marrow cavity side. The loss of compact bone was less profound, and no significant changes of the

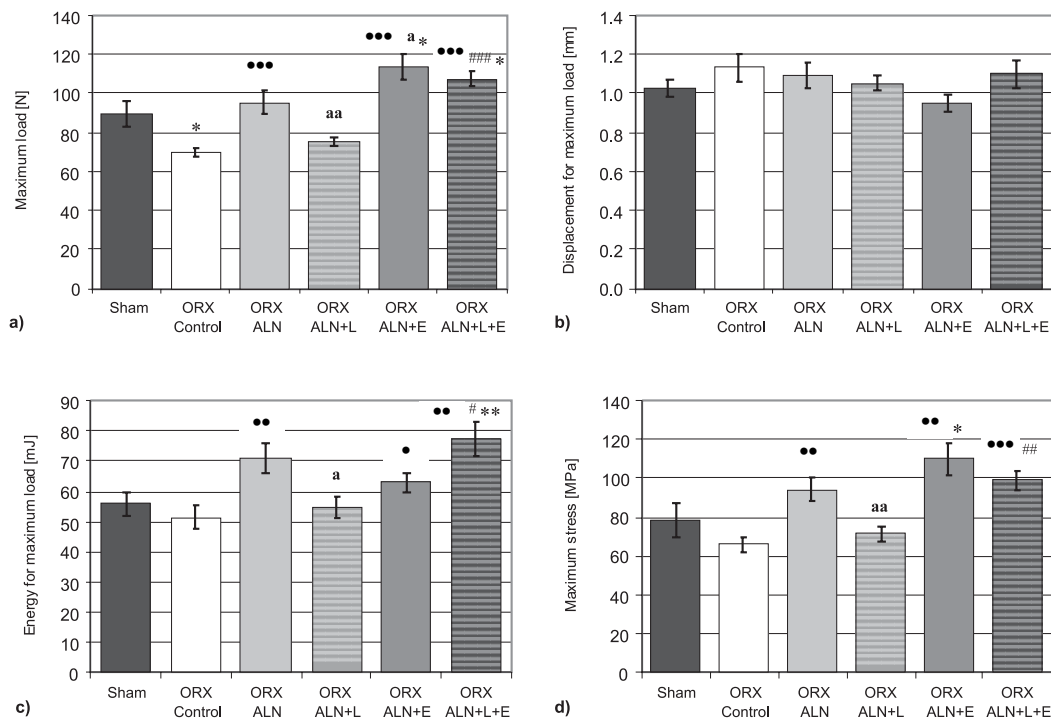


Figure 1. Mechanical properties of the tibial metaphysis (parameters for the maximum load point) in orchidectomized (ORX) rats. Results are presented as the mean \pm SEM ($n = 8$). Kruskal-Wallis ANOVA followed by Mann-Whitney U test was used for evaluation of the significance of the results. * – Significantly different from sham-operated rats; * – $p < 0.05$, ** – $p < 0.01$. • – Significantly different from ORX control rats; • – $p < 0.05$, •• – $p < 0.01$, ••• – $p < 0.001$. ^a – Significantly different from the rats treated with alendronate; ^a – $p < 0.05$, ^{aa} – $p < 0.01$. # – Significantly different from rats treated with alendronate and lansoprazole; # – $p < 0.05$, ## – $p < 0.01$, ### – $p < 0.001$

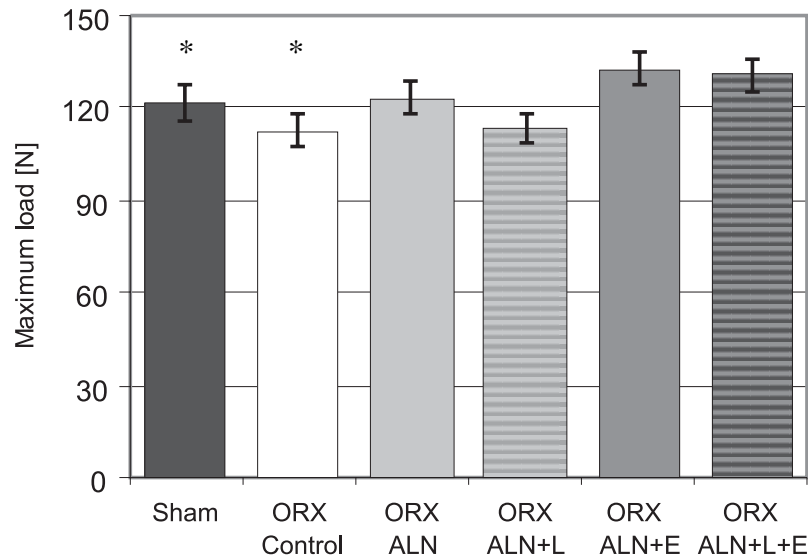


Figure 2. Mechanical properties of the femoral neck in orchidectomized (ORX) rats. Results are presented as the mean \pm SEM (n = 8). Kruskal-Wallis ANOVA followed by Mann-Whitney U test was used for evaluation of the significance of the results. * – Significantly different from sham-operated rats; * – $p < 0.05$

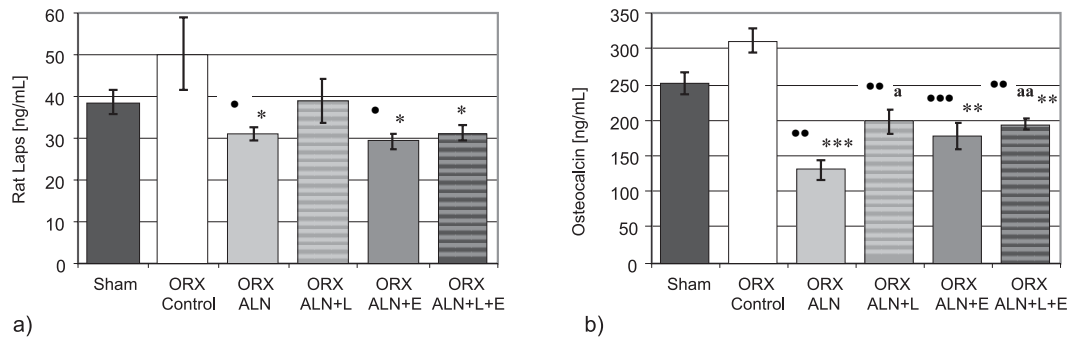


Figure 3. Serum bone turnover markers in orchidectomized (ORX) rats. Results are presented as the mean \pm SEM (n = 8). Kruskal-Wallis ANOVA followed by Mann-Whitney U test was used for evaluation of the significance of the results. * – Significantly different from sham-operated rats; * – $p < 0.05$, ** – $p < 0.01$, *** – $p < 0.001$. • – Significantly different from ORX control rats; •• – $p < 0.01$, ••• – $p < 0.001$. ^a – Significantly different from the rats treated with alendronate; ^a – $p < 0.05$, ^{aa} – $p < 0.01$

area of cortical bone or the marrow cavity were observed. Nevertheless, the ratio of the marrow cavity the whole diaphysis area increased significantly, indicating a greater increase of resorption in the cortical bone. Those changes led to significant deterioration of mechanical properties in the spongy bone of tibia metaphysis, but not that of the femoral neck. Susceptibility to fracture of femoral neck is related mainly with the reduction of thickness of the cortical layer (46, 47). Thus, the absence of significant influence of androgen deficiency upon mechanical

properties of femoral neck may be due to the lower reduction of compact bone layer. The latter has been confirmed by the study performed by Shuid et al. (34), who also failed to find a significant influence of androgen deficiency upon the mechanical properties of compact bone in femoral shaft in rats.

Preventive activity of alendronate has been examined at the dose efficient in preventing the consequences of estrogen deficiency upon the osseous system in ovariectomized rats (20, 21, 48). Alendronate is a potent antiresorptive nitrogen-containing bisphos-

phonate. It inhibits the mevalonate pathway in osteoclasts, by inhibiting farnesyl pyrophosphate synthase. This leads to a decrease of the formation of isoprenoid lipids, such as farnesyl pyrophosphate and geranylgeranyl pyrophosphate, required for the post-translational prenylation of proteins. The lack of geranylgeranyl pyrophosphate in osteoclasts is responsible for inhibiting activity and induction of osteoclast death by apoptosis (49). In the study reported here, the anti-resorptive activity of alendronate was reflected in the reduced level of a biochemical marker of bone resorption. Alendronate occurred to prevent the orchidectomy-induced, reduction of trabeculae width in cancellous bone, what is more, it also inhibited the influence of androgen deficiency upon the growth of compact bone, counteracting bone mass loss and reduction of mechanical strength of the tibia metaphysis and femoral neck in orchidectomized rats.

As we assumed, the activity of alendronate was significantly reduced by the lansoprazole, which was applied in a dose lower than the effective dose enabling protection against gastric ulcers induced by acidified ethanol and indomethacin in rats (22). Lansoprazole administered in orchidectomized rats together with alendronate weakened the preventive activity of alendronate upon the examined parameters in compact and cancellous bone, reducing its anti-osteoporotic efficacy. Lansoprazole abolished the beneficial influence of alendronate upon all the examined mechanical parameters of tibia metaphysis, and weakened its influence upon the strength of femoral neck. A similar effect has been observed after the administration of omeprazole or pantoprazole to ovariectomized rats (20, 21). The mechanism mediating the attenuation of anti-osteoporotic efficacy of alendronate by PPIs has not been recognized. Earlier reports suggested that increased risk of fracture after the application of PPIs may be connected with inhibition of calcium absorption from intestines (50), later reports – however – did not confirm the influence of PPIs upon calcium absorption (51, 52). It seems that also interaction between PPIs and alendronate at the absorption stage may be excluded, since ranitidine, another drug that reduces the secretion of hydrochloric acid, enhanced the bioavailability of alendronate (53). The latest *in vitro* studies demonstrated that omeprazole decreases the activation of osteoclasts and increases the activation of osteoblasts, which may induce a state resembling osteopetrorickets [54]. Also the studies of the skeletal phenotype in H^+/K^+ -ATPase β -subunit knockout female mice revealed increased OPG/RANKL ratio and PTH, as well as reduced BMD, and inferior mechanical bone strength (55).

The exercise completely prevented the loss of anti-osteoporotic efficacy of alendronate, caused by lansoprazole. What is more, we demonstrated a positive interaction that occurred between the exercise and alendronate. The application of the exercise together with alendronate significantly increased the maximum and fracture load of tibia metaphysis, in comparison with rats treated with alendronate only. It also caused a significant increase of the force causing femoral neck fracture, in comparison with control orchidectomized rats. However, the most significant observation of the present study is that application of exercise to rats treated with alendronate and lansoprazole restored the lansoprazole-reduced anti-osteoporotic efficacy of alendronate. The exercise normalized the rate of bone turnover, promoting bone formation in compact and cancellous bone. The exercise is known to increase mechanical loading to bones, preventing apoptosis of osteocytes, that are main regulators of bone remodeling (56, 57). In response to mechanical load caused by the exercise, activation of the Wnt/ β -catenine pathway also occurs, which is of key importance for differentiation and bone formation activity of osteoblasts (57, 58). Mechanical loading is a potent anabolic stimulus that strengthens bones and a major regulator of bone mass, geometry and microarchitecture (56, 57). In the study reported here, the exercise increased the width of trabeculae in cancellous bone and growth of compact bone from periosteum and marrow cavity, as well as bone mass, counteracting the lansoprazole-induced reduction of anti-osteoporotic efficacy of alendronate. Physical effort that rats treated with alendronate and lansoprazole have been exposed to significantly increased the load, energy, and stress in the points of the maximum and fracture loads in the tibia metaphysis, in reference to rats treated with alendronate and lansoprazole concurrently. It also significantly increased the load causing femoral neck fracture in comparison with orchidectomized control rats.

The data obtained so far indicate, however, that exercise is known to intensity- and duration-dependently induce the activation of the hypothalamus-pituitary-adrenocortical axis, as well as the sympatho-adrenomedullary system (59–61). Indicators that enable verification of exercise intensity in the study reported here may be the mass of adrenals and rat body mass (60, 61). Exercise causes intensity-dependent increase of the adrenal mass in mice (60) and in rats (61). We also noted an increase of adrenal mass, yet which was not statistically significant, at a level observed by Bartalucci et al. (60) in mice subjected to low-

intensity physical exercise. On the other hand, the adrenal mass to body mass ratio in our study was significantly greater than that in orchidectomized control rats, yet lesser than that observed by da Costa Lana et al. (61) in rats subjected to low-intensity exercise. Moreover, we failed to notice significant changes in rat body mass. Those results may indicate that exercise applied in the study reported here did not induce significant adaptive changes caused by the activation of the hypothalamus-pituitary-adrenals axis and the sympatho-adrenomedullary system.

In conclusion, the exercise prevented lansoprazole-induced reduction of anti-osteoporotic efficacy of alendronate in rats with androgen deficiency. Those results indicate that exercise may reduce the disadvantageous influence of PPIs upon alendronate activity and increase its anti-fracture efficacy in the treatment of osteoporosis in men.

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