ERYTHROCYTE ANTIOXIDATIVE ENZYMES IN EXPERIMENTALLY INDUCED OSTEOPENIA IN RATS

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Abstract

The study aimed at the determination of dynamic relationship between mineralisation processes and antioxidative/oxidative status during the development of osteopenia. One hundred and two healthy female Wistar rats at the age of 2 months and initial body weight of 200 g were used in the experiment. The rats were divided into control (CON, n=6), sham operated (SHO, n=48), and ovariectomised (OVX, n=48) groups. Animals from SHO (n=6) and OVX (n=6) groups were sacrificed every week during 8 weeks of the experiment in order to detect dynamic changes in examined parameters. The samples were collected weekly from day 7 to day 56. The femora were examined with the use of DXA (bone mineral density) and pQCT (area, mineral content, volumetric density of trabecular and cortical part of distal femora). The pQCT scans were performed 5 mm from distal end of the tibia. The determination of activity of glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) in haemolysates of erythrocytes were performed spectrophotometrically. Obtained data showed wave like changes in both enzyme activities and bone parameters and indicated the importance of the 2nd-3rd and 5th-6th week after surgery as a key moment for bone metabolism and activity of enzymatic antioxidative defence during the development of osteopenia induced by bilateral ovariectomy. The obtained results proved that alterations in activity of GSH-Px and SOD, and pQCT ahead the changes registered by DXA by 7 d.

Key words: rats, oxidative stress, ovariectomy, osteopenia, pQCT, bone.

Osteoclasts and osteoblasts are responsible for metabolic activity of bones, their resorption or synthesis (14). The balance between osteoblastic and osteoclastic activity depends on age as well as physiological conditions and maintains the proper bone density. Remodelling of the skeleton is under the control of numerous cytokines, growth factors, hormones and others, which directly or indirectly regulate the functional activities of osteoblasts and osteoclasts, leading to either sustaining healthy bones or pathological conditions such as an increased bone resorption and, in consequence, osteoporosis and bone fractures. Reactive oxygen species (ROS) and oestrogens are other regulating factors (3, 22).

Reactive oxygen species are produced by osteoclasts during the process of bone resorption (24). There is the evidence that they are involved in bone resorptive processes by facilitating the degradation of bone collagen, and are also engaged in the control of osteoclasts/osteoblasts balance (6, 8). Under physiological conditions, ROS produced by osteoclasts exert positive effect by accelerating the destruction of calcified tissue and participating in bone remodelling. Large amounts of ROS are generated in case of bone fractures (21).

Oestrogens play a crucial role in physiology of bone tissue either during maturation and in adults (4). Post-menopausal deficiency of ovarian hormones alters the acceleration of progressive increase in bone resorption (17). Such an increase is the first step for osteoporosis. The evidence for oxidative stress conditions during post-menopausal osteoporosis was reported previously (1, 12, 18). The authors proved the alterations of antioxidative enzyme activities and lipid peroxidation products in osteoporotic women.

The study was focused on establishing the relationship between metabolism of bone tissue and activity of the enzymatic antioxidative defence in erythrocytes during 8 weeks of post-surgery osteopenia development. The study was also undertaken to determine an early stage indicator (useful in diagnostics) of possible pathological changes in bone metabolism as a consequence of gonadal afunction.

Material and Methods

Animal procedures. The experimental design was accepted by the local Ethic Committee of Animal Welfare. Female Wistar rats with initial body weight of 200 g were used in experiment (n=102). The rats were fed the LSM Standard Rats and Mouse Diet (Agropol-Motycz, Poland) and water was available with no limits. After 14 d of adaptation period (temp. 22 °C, day/night
cycle – 12 h/12 h, humidity 55%), six rats were decapitated and served as control group (CON), 48 rats were sham-operated (SHO) and 48 were ovarioctomised (OVX). Before surgery, the rats were anaesthetised with an intraperitoneal injection of ketaminum (Biotest-Pulawy, Poland), atropinum sulphuricum (Polfa-Warszawa, Poland), and Rometar (Leciva, Czech Republic) at doses of 10, 2, and 0.05 mg/kg b.w., respectively. During ovarioctomy, the ovaries were removed bilaterally from approach in medial line. Special care was taken to prevent accidental reimplantation of ovarian tissue during surgery. Increased body weight and uterine atrophy (observed during dissection) indicated properly made ovarioctomy. During sham-operation, the ovaries were bilaterally isolated from approach in medial line and replaced intact. Because the purpose of the study was to determine the dynamic changes in bone metabolism and oxidative stress, six rats from SHO and six rats from OVX groups were anaesthetised in CO2 and then killed by decapitation every 7 d from the 7th to 56th d of the experiment. Before decapitation, body weight was registered and blood was collected for biochemical analysis. After decapitation, right femora were removed cleaned of soft tissues, and analysed densitometrically by Dual X-Ray Absorptiometry (DXA) as well as tomographically using peripheral quantitative computed tomography (pQCT).

**Biochemical analysis.** Blood samples were collected to heparinised tubes from cardiac puncture and centrifuged. The haemolysates were prepared from erythrocytes, after three-folds washing with 0.9% NaCl (in order to remove leukocytes and platelets) by use of cold distilled water. Erythrocyte membranes were removed by centrifugation at 3.000 x g for 10 min. Erythrocytes count and haemoglobin content were determined before haemolysis. These values served for recalculations of enzyme activities. Haemolysates were used for the determination of antioxidative enzyme activities such as glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD).

**GSH-Px determination (16).** Ten microlitre of glutathione reductase (100 U/mg protein, Sigma), 10 µl of 1.125 mol/dm3 sodium azide (Sigma), and 100 µl of 0.02 mol/dm3 glutathione (Sigma), 100 µl of haemolysate and 100 µl of 0.022 mol/dm3 H2O2 were added to incubation mixture consisting of: 2.58 ml of phosphate buffer (0.05 mol/dm3, pH 7.0) and 100 µl of 8.4 mol/dm3 NADPH (Merck). The absorbance was measured between the 2nd and 4th min of reaction at 340 nm (Ultrospec 2000, Pharmacia, Sweden). The calculation was based on standard curve prepared with different concentrations of NADPH. Enzyme activity was expressed in nanokatals (nkat) per mg of haemoglobin (Hgb).

**SOD determination (20).** Quartz cuvette contained 1.8 ml of carbonate buffer (0.05 mol/dm3, pH 10.2), 100 µl of haemolysate and 100 µl of adrenaline (18 mg/10 ml 0.1 mol/dm3 HCl, Sigma). An increase in absorbance at 340 nm during 10 min incubation was compared with control where haemolysate was replaced by 0.9% NaCl. Enzyme activity was expressed in SOD units (U) per mg Hgb.

**Estimation of bone parameters.** Densitometric analysis (DXA) of bone mineral density (BMD) of whole isolated femora, was estimated with the use of Norland Excell Plus (Fort Atkinson, USA) densitometer and Small Subject Scan software.

The right femora were scanned with pQCT. The femoral epiphysis was scanned 5 mm from distal end. The scan line was adjusted using scout view of the pQCT system. During the measurement, the bones were fixed in test tube filled with 70% ethanol. Upon completion of scanning, the following parameters were determined: 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), and cortical volumetric bone mineral density (Ct.vBMD). All trabecular bone analyses were performed with the threshold of 0.450 cm1 with a contour mode 2 and a peel mode 20, whereas cortical part was tested with threshold 0.900cm1 and cortical mode 2. Scout view was conducted with the speed 10 mm/s and CT-scan 4 mm/s. The machines were daily calibrated with phantoms provided by the manufacturers.

**Statistical analysis.** The obtained results are presented as mean values ±S.E.M. Statistical comparison of values between SHO and OVX animals at the same age was performed using U – Mann Whitney test, and significant differences (P<0.05) were marked by asterisks in figures. Analyses of the differences in the values between particular age groups, separately for SHO and OVX rats, were performed using Kruskal-Wallis test. Statistical differences (P<0.05) between SHO and OVX rats at the same age were marked by asterisks. Statistical differences between values from particular age groups, separately for SHO and OVX rats (P<0.05) were indicated by proper letter a, b, c for SHO and A, B, C for OVX animals. Statistical analyses were performed with the use of STATISTICA software v. 8.0 (StatSoft, Inc., USA).

**Results**

**Body weight.** During the 1st week of experiment, no significant changes in body weight of CON and SHO animals were observed. During the next three weeks, the weight of SHO females increased dynamically up to 245.24 g (P<0.05). From the 4th to 8th week of the study, no significant changes in weight within this group, were observed. The weight of OVX rats increased by 16.27 g during the first week of experiment as compared to CON. Seven days later, the weight of OVX rats increased to 252.68 g (P<0.05). On the 3rd and 4th week of the experiment, a decrease in the weight to 222.23 g and 236.87 g, respectively, in comparison to that of females from the 2nd week, was observed. From the 5th to 8th week, the progressive increase in this parameter was noted (284.8 g, 313.33 g, 311.5, 348.5 g) (Fig. 1). This increase was statistically significant (P<0.05).
Bone mineral density of the femora. Bone mineral density (BMD), measured by DXA, of CON was 0.07 g/cm². The similar value was noted in SHO animals one week later, whereas in OVX rats, the BMD was slightly higher (0.08 g/cm²). Higher BMD of the femora in OVX, than in SHO rats in the 2nd week of the experiment was observed as well (P<0.05). From the 3rd to 8th week of the study, BMD of the femora in OVX rats was lower than in SHO group with statistical confirmation of differences at the 4th and 5th week (P<0.05) (Fig. 2).

Trabecular and cortical bone area. Area of trabecular (Tb.Ar) part of the study, BMD of the femora in OVX rats was lower than in SHO group with statistical confirmation of differences at the 4th and 5th week (P<0.05). Two weeks after the surgery, Tb.Ar of distal femora in SHO females was the lowest (5.31 mm²). Two weeks after the surgery, Tb.Ar of distal femora in SHO animals was 6.46 mm² and progressively increased up to the 5th week of the experiment, where the highest area was noted (7.97 mm²) (P<0.05). From the 6th to 8th week, trabecular area decreased from 7.71 mm² to 7.57 mm² (P<0.05). In OVX rats, Tb.Ar increased from 6.5 mm² (1st week) to 9.05 mm² (6th week) of the experiment (P<0.05). On the 7th and 8th week, the Tb.Ar decreased and was 8.91 mm² and 8.62 mm², respectively (P<0.05 in relation to CON) (Fig. 3).

Fig. 1. Body weight of SHO and OVX rats (g).

Fig. 2. BMD of whole femora of SHO and OVX rats (g/cm²).

Fig. 3. Area of trabecular part of distal femora of SHO and OVX rats (mm²).

Fig. 4. Area of cortical part of distal femora of SHO and OVX rats (mm²).

Fig. 5. Bone mineral content (Tb.BMC) of trabecular part of distal femora of SHO and OVX rats (mg/mm).

Fig. 6. Bone mineral content (Ct.BMC) of cortical part of distal femora of SHO and OVX rats (mg/mm).
A similar tendency in cortical bone area (Ct.Ar) was observed. The Ct.Ar of CON animals was 7.26 mm\(^2\). One week later, the Ct.Ar of distal femora in SHO animals was 6.46 mm\(^2\) and in OVX animals 7.94 mm\(^2\). From this moment, the progressive increase in the Ct.Ar was observed in both SHO animals to the 5\(^{th}\) (9.75 mm\(^2\)) (P<0.05) and in OVX rats to the 6\(^{th}\) week of the study (11.09 mm\(^2\)) (P<0.05). After the 5\(^{th}\) week in SHO and 6\(^{th}\) week in OVX, Ct.Ar slowly decreased and in the 8\(^{th}\) week was 9.26 mm\(^2\) and 10.55 mm\(^2\), respectively (Fig. 4).

Trabecular and cortical bone mineral content. Trabecular bone mineral content (Tb.BMC) in rats of CON group was 2.23 mg/mm. One week after surgery, Tb.BMC was 1.46 mg/mm in SHO and 2.74 mg/mm in OVX rats. Beginning from the 2\(^{nd}\) week of the study, the Tb.BMC of distal femora of OVX rats was lower than in SHO animals. Between the 2\(^{nd}\) and 5\(^{th}\) week, Tb.BMC of distal femora of SHO rats ranged from 3.08 mg/mm to 5.40 mg/mm (P<0.05). At the same time, the values of the analysed parameter in OVX rats remained relatively stable and ranged from 2.74 mg/mm to 3.18 mg/mm. At the 6\(^{th}\) week of the experiment, Tb.BMC of distal femora in SHO females decreased to 4.75 mg/mm (P<0.05), and at the 7\(^{th}\) week to 4.54 mg/mm (P<0.05), whereas in the 8\(^{th}\) week, the value of this parameter increased to 4.88 mg/mm (P<0.05). In opposition to SHO, in OVX rats, Tb.BMC measured in the 6\(^{th}\) week increased to 4.27 mg/mm (P<0.05) and it was the highest value (Fig. 5). Cortical BMC (CT.BMC) of distal metaphysis of CON rats was 5.9 mg/mm. One week later, Ct. BMC was 5.61 mg/mm in SHO and 6.35 mg/mm in OVX animals. Additionally, in the 2\(^{nd}\) week, the value of Ct.BMC in OVX rats was higher than in SHO animals. Beginning with the 3\(^{rd}\) week, mineral content of distal metaphysis was higher in SHO than in OVX rats. The highest Ct.BMC of distal metaphysis was observed in 8\(^{th}\) week in SHO and OVX animals and was 9.55 mg/mm (P<0.05) and 9.21 mg/mm, respectively (P<0.05) (Fig. 6).

Trabecular and cortical bone volumetric mineral density. Volumetric mineral density of trabecular (Tb.vBMD) part of distal femora in CON animals was 357.18 mg/mm\(^3\). During one week, the density decreased to 255.25 mg/mm\(^3\) in SHO and increased to 419.2 mg/mm\(^3\) in OVX animals, and the difference between SHO and OVX rats was statistically significant (P<0.05). Beginning with the 2\(^{nd}\) week of the study, the Tb.vBMD of distal femora was lower in OVX in relation to SHO animals, and from the 3\(^{rd}\) week, the significance of differences was statistically significant (P<0.05). The progressive increase in the Tb.vBMD of distal femora of SHO rats was observed up to the 4\(^{th}\) week of experiment when the highest density in SHO rats was noted (716.66 mg/mm\(^3\)) (P<0.05). The Tb.vBMD of distal femora decreased from the 5\(^{th}\) to 7\(^{th}\) week, when 603.04 mg/mm\(^3\) (P<0.05) was noted. During the next week, the Tb.vBMD of distal metaphysis increased up to 637.94 mg/mm\(^3\) (P<0.05). Tb.vBMD of distal femora in OVX females, between the 2\(^{nd}\) and 5\(^{th}\) week of the experiment, ranged from 368.92 mg/mm\(^3\) (2\(^{nd}\) week) to 379.97 mg/mm\(^3\) (5\(^{th}\) week). On the 6\(^{th}\) week, Tb.vBMD of distal end of the femora reached 471.88 mg/mm\(^3\) (the highest value of Tb.vBMD in OVX rats) and then slowly decreased to 411.0 mg/mm\(^3\) in the 8\(^{th}\) week (Fig. 7). Volumetric density of cortical (Ct.vBMD) part of distal femora of SHO animals was higher than OVX rats during the whole study period. Ct.vBMD of the femora in CON animals was 818.32 mg/mm\(^3\). Beginning with the 4\(^{th}\) week, the differences between SHO and OVX rats were significant (P<0.05). The density in SHO animals in the 1\(^{st}\) week was 870.87 mg/mm\(^3\), and values of this parameter progressively increased up to the 8\(^{th}\) week when 1038.2 mg/mm\(^3\) (P<0.05) was noted. In OVX animals, Ct.vBMD of distal femora remained relatively stable and ranged from 782.8 mg/mm\(^3\) in the 4\(^{th}\) week (the lowest value) to 879.58 mg/mm\(^3\) in the 8\(^{th}\) week (the highest value) (Fig. 8).

Markers of antioxidative defence mechanisms. The results of enzyme activities are presented graphically in Figs 9 and 10. Control group showed the highest activity of SOD — 0.389 U/mg Hgb. The results of SOD activity after the 1\(^{st}\) week were similar in SHO and OVX groups and ranged between 0.293 U/mg Hgb (P<0.05) and 0.302 U/mg Hgb, respectively. After the 2\(^{nd}\) week, the activity raised to 0.556 U/mg Hgb (P<0.05) in SHO group and to 0.367 U/mg Hgb in OVX group. After the 3\(^{rd}\) week, enzyme activity decreased to 0.198 U/mg Hgb (P<0.05) in SHO group and remained stable in OVX group. After the 4\(^{th}\) and 5\(^{th}\) week, SOD activity remained stable in SHO group — 0.204 U/mg Hgb and 0.23 U/mg Hgb, respectively. At the same time, in OVX group, the activities significantly decreased (P<0.05) and were — 0.138 U/mg Hgb and 0.164 U/mg Hgb, respectively. In the 6\(^{th}\) week, the activity of SOD in SHO group decreased to 0.15 U/mg Hgb (P<0.05) and then increased to 0.246 U/mg Hgb (P<0.05) in the 7\(^{th}\) and 0.329 U/mg Hgb (P<0.05) in the 8\(^{th}\) week. In OVX group, the activity increased to 0.324 U/mg Hgb (P<0.05) in comparison to data noted in the 4\(^{th}\) and 5\(^{th}\) week. In the 7\(^{th}\) and 8\(^{th}\) week, it significantly decreased to 0.101 U/mg and 0.148 U/mg, respectively.

The activity of GSH-Px in CON animals was 0.0515 nkat/mg Hgb. One week after surgery, the activity of GSH-Px increased in SHO animals to 0.0882 nkat/mg Hgb (P<0.05) and was significantly higher than that noted in OVX rats (0.0702) (P<0.05). In the 2\(^{nd}\) week of the study, the inverted relationship was observed. The activity of GSH-Px was significantly higher in OVX than in SHO rats and was 0.0874 nkat/mg Hgb and 0.0680 nkat/mg Hgb (P<0.05), respectively. From the 3\(^{rd}\) to 7\(^{th}\) week, the activity of GSH-Px in SHO was relatively stable and ranged from 0.0614 nkat/mg Hgb to 0.0644 nkat/mg Hgb, with exception of the 5\(^{th}\) week when the value increased to 0.0864 nkat/mg Hgb (P<0.05). The stabilisation of GSH-Px activity was observed also in OVX rats. In the 8\(^{th}\) week of the study, the activity of GSH-Px increased significantly in SHO and OVX rats reaching 0.156 nkat/mg Hgb (P<0.05) and 0.1325 nkat/mg Hgb (P<0.05).
Discussion

Osteoporosis may develop as a consequence of gonadal hypofunction leading to a rapid decline in circulating oestrogens. With advancing age of both humans and animals, the processes causing the degradation of bone tissue reach higher or unaltered metabolic activity, whereas formation of new bone is gradually diminished, what results in net bone loss. The metabolism of bone tissue remains under control of numerous hormones, cytokines, growth factors, and ROS. It has been demonstrated that ROS are involved in resorption of bone, with a direct contribution of osteoclast-generated superoxide to bone degradation (18, 26). It is widely known, that osteoblasts produce antioxidants such as GSH-Px to protect against ROS (5). Additionally, osteoblastic cells produce TGF-β, occurring during bone resorption (7).

The mechanism of antioxidant effect of oestrogens is still not fully understood and may vary in different tissues. It is supposed that oestrogens protect the level of endogenous antioxidants (2). On the other hand, oestrogens might play an inhibitory role either in production and/or responsible for scavenging of ROS.

Ovariectomised rats are suitable and commonly used as an animal model for the evaluation of experimental osteoporosis (23). Widely observed consequence of hormonal hypo- or afunction of gonads is a progressive increase in body weight in humans and animals (11). In our study, body weight was also higher in ovariectomised animals when compared to those provided with properly functioning ovaries. It is worth mentioning that the weight of SHO rats increased progressively from day 0 to the 4th week of the study and then the mean weight stabilised on relatively similar level, whereas in OVX animals, the body weight increased up to the 8th week. The highest percentage difference between SHO and OVX rats on the last day of the study was observed and reached 38.7%.

In present experiment, the relationship between dynamic changes in the activity of antioxidative enzymes in erythrocytes as well as BMD and volumetric mineral density, mineral content, and area of trabecular and cortical part of distal femora in rats with developing osteopenia induced by bilateral ovariectomy, has been evaluated for the first time. Wave-like alterations in the activity of GSH-Px and SOD, used as markers of antioxidative defence mechanisms, as well as, in the analysed bone parameters, were found not only in OVX but also in SHO groups.

In our study, the BMD of the femora were controlled weekly and it was found that ovariectomy decreased bone mineral density, which has found its justification with the results presented in other reports (10). Peripheral quantitative computed tomography is the most modern and extremely sensitive method allowing the determination of true volumetric BMD. The analysis of the distal metaphysis with the use of pQCT supports the observations obtained from DXA. The fact that trabecular was more affected by OVX than cortical bone has been documented earlier (9, 25). In our study, it has been noted that ovariectomy affects both trabecular and cortical part of distal metaphysis, but
obviously trabecular part was more receptive to the lack of oestrogens. Higher sensitivity of pQCT allowed the documentation, that first symptoms of changes in bone metabolism appear on the 2nd week of the study, that is one week earlier than observed after DXA measurements. These observations relate to volumetric BMD of trabecular and cortical part of distal femora. In opposition to DXA analysis, volumetric BMD demonstrated a progressive decrease in cortical and trabecular bone tissue up to the 4th or 5th week of the study. In the 6th week of the experiment, an increase in the analysed parameter in both cortical and trabecular part of distal femora was observed. Mechanism of this phenomenon is unknown. A possible explanation relates to activation, under condition of developing osteopenia caused by the lack of oestrogens, the processes allowing a momentary and compensatory increase in bone mineral density. This momentary effort aiming at the improvement of bone tissue quality was stopped in the 7th and 8th week, when a slow decrease in total, cortical, and trabecular vBMD of distal femora was observed.

Content of minerals in the femora of OVX rats decreased in the 2nd week of the study, in relation to SHO, but only in trabecular part of distal femora. Interestingly, changes in trabecular bone mineral content were observed prior to changes in cortical BMC. Similarly to the results from DXA, the inversion of the relationship between SHO and OVX animals in the 3rd week was observed. The development of pathological influences on metabolism of cortical bone appeared one week later. Similarly to vBMD, in cortical and trabecular BMC, the highest differences between SHO and OVX rats in the 4th or 5th week were also observed (P<0.05). In the 6th week, the differences were minimised due to a decrease in the values in SHO animals and increase in OVX rats. In the 7th and 8th week, the differences between SHO and OVX animals slowly increased.

Wave-like relationship in the activity of antioxidative enzymes was also observed. The activity was lower in OVX and SHO animals with exception of the 2nd week for GSH-Px, 3rd week for SOD, and 6th week for both parameters. It may suggest that the imbalance between production and neutralisation of ROS appeared during experimental period in OVX group. Biochemical analysis supports the importance of the 2nd-3rd and 6th week as a period having key role in both healthy SHO rats as well as castrated females with developing osteopenia.

Moreover, antioxidative defence systems react in accordance to current challenge in dynamic way confirming the presence of imbalance between production and neutralisation of ROS. In accordance to Strehlow et al. (19) oestrogen deficiency in OVX mice caused the downregulation of ecSOD and MnSOD expression, which could directly support the results observed in OVX group.

Sontakke and Tare (18) determined a significant decrease in GSH-Px and SOD activities in osteoporotic postmenopausal women as well as significant increase in malondialdehyde (MDA) concentration in comparison to controls. Similar results regarding antioxidative enzyme activities were described by Maggio et al. (12). These authors did not confirm an increase in MDA concentration but they observed a significant decrease in plasma content of vitamins A, C, and E in osteoporotic women as compared to healthy ones. The activity of GSH-Px measured by Massafr et al. (13) was higher in premenopausal than in postmenopausal women and was sensitive to oestriol treatment. The results cited here proved that postmenopausal period, related to a decrease in oestrogens, cause a decrease in efficiency of antioxidative system.

Basu et al. (1) reported a negative relationship between lipid peroxidation intensity, measured by the level of 8-iso-PGF2α, and bone mineral density. Narasimha et al. (15) determined antioxidative/oxidative status in femora homogenates in rats with osteopenia induced by ovariectomy. The authors registered only end point values of GSH-Px, SOD, glutathione transferase, as well as the concentration of MDA and hydrogen peroxide. Similarly to our results, a decrease in enzyme activities and increase in lipid peroxidation intensity were observed after 8 weeks of osteopenia development.

To conclude, the study has proven that the analysis of bone tissue performed by peripheral quantitative computed tomography is it possible to get information on disturbances of bone metabolism one week earlier than after measurement by DXA. Similarly to tomographical measurements, the determination of activities of antioxidative defence markers can significantly hasten the establishment of proper diagnosis and withdrawal of metabolic bone diseases therapy in humans and animals. The presented study has also proved wave-like changes of bone metabolism and indicated the importance of the 2nd - 3rd and 5th - 6th week, after gonadectomy, as the significant moment of bone metabolism turnover in healthy and castrated rats.

References

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