Prof. dr hab. n. med. Andrzej BOHATYREWICZ[a], dr n. med. Małgorzata WIECHOWSKA[e], Prof. dr hab. n. med. Anna MACHOY-MOKRZYŃSKA[c], dr n. zdr. Alina JUREWICZ[a], dr hab. Katarzyna LEŹNICKA[d], Prof. dr hab. n. med. Katarzyna GROCHOLEWICZ[b], dr n. med. Maciej KARACZUN[a], Prof. dr hab. n. med. Elżbieta WEYNA[b]

Pomeranian Medical University in Szczecin / Pomorski Uniwersytet Medyczny w Szczecinie:
[a] Department of Orthopaedics and Traumatology / Klinika Ortopedii i Traumatologii
[b] Department of Integrated Dentistry / Zakład Stomatologii Zintegrowanej
[c] Department of Experimental and Clinical Pharmacology / Zakład Farmakologii Eksperymentalnej i Klinicznej
[d] Gdansk University of Physical Education and Sport, Faculty of Physical Education / Akademia Wychowania Fizycznego i Sportu w Gdańsku, Wydział Wychowania Fizycznego
[e] Individual Dental Practice, SONOMED, Dentistry, Szczecin / Indywidualna Praktyka Stomatologiczna, SONOMED Stomatologia, Szczecin

EFFECTS OF SODIUM FLUORIDE ON BONE METABOLISM AND STRUCTURAL CHANGES OF THE MANDIBLE IN OVARIECTOMIZED RATS

Abstract

Introduction and aim: The role of fluorides in caries prevention is widely known, however, due to a large number of factors affecting the metabolism of bone tissue (e.g. estrogens), the evaluation of the effect of these compounds on the masticatory system requires comprehensive studies. The aim of the study was to examine the effect of fluorides on the chosen biochemical markers of bone remodelling, on the bone mass and structural changes in the lower jaw (mandible) of castrated rats, after six-week exposure to sodium fluoride in different doses.

Material and methods: The experiment was conducted on an animal model using female Wistar rats divided into four groups: a control group and three research groups (ovariectomized and exposed to fluoride compounds in different doses in the drinking water). In order to evaluate the effect of estrogens shortage induced by double-sided ovariectomy and to analyze the fluorides influence on bone metabolism and structural changes of the mandible, tests of bone tissue metabolism markers, bone mineral content in the mandible, height of a molar tooth, height of alveolar appendix and index of rats' alveolar appendices heights were performed.

Results: Ovariectomy procedure led to the appearance of higher concentrations of bone tissue resorption markers and lower mineral weight of mandible, but did not exert a considerable impact on mineral weight index in animals receiving fluorides. There was no statistical difference in the heights of molar teeth between all experimental groups.

Conclusion: Eestrogens shortage, due to ovariectomy, reduces mineral weight of lower jaw bones and mineral weight index, and the fluoride supply prevents the loss of minerals from an osseous tissue.

Keywords: Rats, ovariectomy, mandible, fluoride, bone remodelling markers, bone mineral content.

(Otrzymano: 15.06.2018; Zrecenzowano: 25.06.2018; Zaakceptowano: 30.06.2018)

WPŁYW FLUORKU SODU NA METABOLIZM TKANKI KOSTNEJ I ZMIANY STRUKTURALNE W ŻUCHWACH SZCZURÓW PODDANYCH OWARIEKTORMII

Streszczenie

Wstęp i cel pracy: *Rola fluorków w profilaktyce próchnicy jest powszechnie znana, jednakże ze względu na dużą liczbę czynników wpływających na metabolizm tkanki kostnej (np. estrogeny), ocena wpływu tych związków na układ żucia wymaga kompleksowych badań.*
Celem tego badania było zbadanie wpływu fluorków na wybrane markery przebudowy kości, na masę kości i strukturalne zmiany w dolnej szczęce (żuchwie) kastrowanych szczurów po sześciotygodniowej ekspozycji na różne dawki fluorku sodu.

Materiał i metody: *Doświadczenie przeprowadzono na modelu zwierzęcym, w którym samice szczurów Wistar podzielono na cztery grupy: grupę kontrolną i trzy grupy badawcze (poddane owariektomii i eksponowane różne dawki związków fluoru podawanych w wodzie pitnej). Aby ocenić wpływ niedoboru estrogenów wywołanego obustronną owariektomią i aby przeanalizować wpływ fluorków na metabolizm kostny i zmiany strukturalne żuchwy, konieczne było przeprowadzenie badań markerów metabolizmu kostnego oraz zawartości związków mineralnych w kości żuchwy, a także dokonanie pomiarów wysokości zęba trzonowego, wysokości wyrostka zębodołowego i wskaźnika wysokości wyrostków zębodołowych u badanych szczurów.*

Wyniki: *Na skutek przeprowadzonej owariektomii zaobserwowano wyższe stężenia markerów resorpcji tkanki kostnej oraz niższą masę mineralną kości szczęki dolnej. Zabieg ten jednak nie miał znaczącego wpływu na wskaźnik masy mineralnej u zwierząt otrzymujących fluorki. Nie stwierdzono statystycznej różnicy w wysokości zębów trzonowych pomiędzy grupami eksperymentalnymi.*

Wnioski*: Niedobór estrogenów spowodowany wycięciem jajników prowadzi do obniżenia masy mineralnej kości żuchwy oraz wskaźnika masy mineralnej, a fluorki zapobiegają utracie minerałów z tkanki kostnej.*

Słowa kluczowe: *Szczury, wycięcie jajników (owareiektomia), żuchwa, fluorki, markery przebudowy kości, zawartość składników mineralnych kości.*

(Otrzymano: 20.06.2018; Zrecenzowano: 25.06.2018; Zaakceptowano: 30.06.2018)

1. Introduction

The bone is tissue subjected to the continuous rebuilding process, mainly regulated by hormones and growth factors [1]. Formation, evolution and rebuilding of this tissue are multi-phases processes, including i.e. osteoblasts' maturation, production of extra-cellular matrix elements, activation of osteoclasts, as well as cells' stimulation for emitting of cytokines with paracrinic and autocrinic effect in relation to bones' cells. These processes are coupled together in this way that the activation of one stimulates or breaks other stages of osteogenesis. Metabolism of bone depends on other factors too, one of them are fluorides, used in dentistry since more than half of century. They are strong stimulators of bones formation, what leads to increasing of osseous proteins synthesis on the way of activity stimulation and proliferation of cells of osteoblastic line, and causes an increase of trabecular bone' volume and bone mineral density [2].

Bone metabolism disturbances express in pathological symptoms being clinically determined, of which bones fragility appears the most often. This symptom, together with decrease of bone mass and disturbances of bones micro-architecture, is a definition of generally appearing bones' metabolic disease, as osteoporosis is. This disease can lead to deterioration of life quality, disability, and it requires appreciable financial expenditure concerned with its treatment. The phenomenon of bone mass decrease is the effect of an organism ageing, too, and additional risk factor at women is estrogens' shortage, resulting from from menopause or surgical spays' removal (ovariectomy) [3].

That's why the one of osteoporosis prophylaxis and treatment methods is usage of an estrogenic therapy [4]. Since osteoporosis relates to the mastication organ too, there was noted last years and increase of interest in detection of osteoporosis changes in osseous tissue of jaw bone and lower jaw and their relation with different diseases of mastication organ. Since then the correlation was found between osteoporosis occurrence and paradontium advanced diseases, atrophy of alveolar appendix, post-menopausal teeth's loss and other changes of osseous tissue quality [5]. Clinical researches on remote results of fluorides application in prevention of osteoporosis occurrence within mastication organ are lacking, in spite of common application of fluorides in caries prophylaxis. Tests on animals confirm estrogens shortage impact on acceleration of jaw bones atrophy and osteopenia after teeth removal [6], [7], as well as they describe activity of sodium fluoride used in order to prevention from progress of osteopenia caused by double-sided ovariectomy at rats [8].

2. The aim of the study

The aim of the study was to evaluate an effect of estrogens shortage, induced by double-sided ovariectomy, on osseous metabolism, mineral mass of jaw bones and height of rats' alveolar appendixes, as well as to analyze the impact of fluorides administration on mandibles in ovariectomized rats.

3. Materials and methods

Animals: Analyses were made on 40 six-week old female Wistar rats, coming from licensed animal husbandry of Immunology and Experimental Therapy Institute at Polish Academy of Sciences in Wrocław (Poland). All animals had genetic attestations and bills of health issued by the veterinary. Rats stayed in hutches, 5 specimens in each, in animal quarters, with standard conditions (temperature 22°C, humidity - 60% and 12-hours lighting, alternatively with 12-hours brownout).

Exposure: Animals were randomly divided into 4 groups, each numbering of 10 animals. Group 1 (control one) composed rats getting distilled water to drink, during the period of 6 weeks and then abandoned to the procedure of pseudo-ovariectomy, that is incision of coats without gonads' extraction. In the same time three animals groups received to drink distilled water (group 2), water with concentration of 421 µmol F/L (group 3) and water with concentration of 3158 µmol F/L (group 4). These groups were subjected to the procedure of surgical extraction of ovaries (ovariectomy). This operation was carried out in general anesthetic, using ketamine hydrochloride into the peritoneum, in dose of 50 mg/kg of weight, in the way described by Thorndike et al. [9]. Then, during next 6 weeks, up to the moment of completion of the experiment, all animals received distilled water to drink *ad libitum.*

Data Collection: All animals were weighed and subject to the dissection in the general anesthetic in order to collection of biological material for further tests collection, after completion of the experiment. The blood from heart was collected in the quantity of about 5 ml, which was destined for biochemical and enzymatic tests, as well as the bones of lower jaw were dissected free and subjected to radiological tests. ICTP (telopeptide of type I collagen) concentration in blood serum - the marker of osseous tissue disintegration - was tested with the radio-immunological method, with use of set designed for testing of rat serum (Orion Diagnostica - Finland). ALP (alkaline phosphatase) activity – the marker of bones formation, was marked with the kinetic method, with use of Cormay ALP-35 set (Hoffman - La Roche license). Mineral mass of lower jaw bones was densitometrically marked with the technique of absorption of two X-rays energies, with use of DPX-L osteodensitometer (Lunar, Madison, Wisconsin, USA), in the similar way to long bones testing [10], [11]. There was used mineral weight index, to avoid an impact of body weight on mineral weight of lower jaw bones. The indicator was rated at the following formula:

$$\text{Mineral weight index} = \frac{\text{Bone mineral weight (mg)}}{\text{Animal weight (g)}} \qquad (1)$$

Radiological tests of dissected free lower jaw bone at rats were made with use of mammographical plates with X-ray apparatus with punctual source of X-rays (type 401). Measurements of absolute height of alveolar appendix at 1-st molar tooth and of 1-st molar tooth's overall length were made after introduction of photos to the graphic analyzer. The index proposed by Adamowicz-Klepalska [12] was used for the estimation of true height of an alveolar appendix in the lower jaw. The index was a quotient of appendix height measured from its back to root top and total height of 1-st molar tooth.

$$\text{Alveolar appendix index} = \frac{\text{Height of alveolar appendix (mm)}}{\text{Height of molar tooth (mm)}} \qquad (2)$$

Statistical analysis: Dissolutions of tested parameters were analyzed with the help of Shapiro-Wilk's test. Non-parametrical methods were assumed for standardization of calculations, since some of tested parameters had dissolutions different from normal ones. Comparisons of averages for constant variables were made with U - Mann – Whitney's test. The calculations were preceded by Kruskal-Wallis's test, if the comparison of more than two averages was necessary. All average values were calculated for each group, as well as standard deviation (SD). Furthermore minimum (Min) and maximum (Max) values are contained in tables containing descriptive statistics. There was assumed, as boundary one, level of significance $p<0.05$, in all calculations.

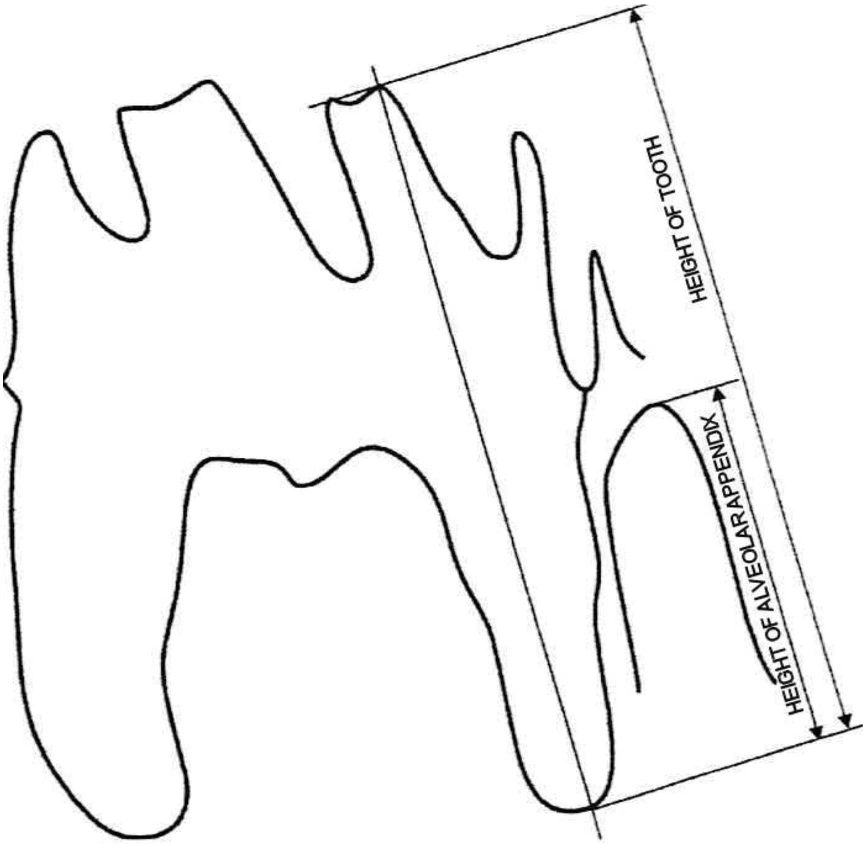

Fig. 1. Diagram of rat's 1st molar tooth fibular section illustrating the principle of measurement of values indispensable for calculation of alveolar appendix height index
Source: Elaboration of the Authors

4. Results

All rats were weighed after completion of the experiment. The lowest body weight was found at animals in the control group - 313 ± 27 g. Higher average body weights were found in all tested groups subjected to ovariectomy procedure, while the difference was statistically important ($p < 0,05$) (Tab. 1) only in the case of group getting fluorides in the concentration of 421 µmol F^{-}/l.

Tab. 1. Rat's body weight after experiment

Animal Group	Control - (n = 10) Average ± SD (Min - Max)	Ovariectomy 0 µmol F^{-}/l (n = 10) Average ± SD (Min - Max)	Ovariectomy 421 µmol F^{-}/l (n = 10) Average ± SD (Min - Max)	Ovariectomy 3158 µmol F^{-}/l (n = 10) Average ± SD (Min - Max)
Body weight (g)	313 ± 27 (282 – 352)	333 ± 16 (305 – 365)	342 ± 30 (300 – 395)*	332 ± 32 (280 – 370)
* p < 0.05 in comparison with the control group				

Source: Elaboration of the Authors

The results of tests of bone tissue metabolism markers: ICTP concentrations and ALP activity are all presented in table 2.

Tab. 2. ICTP concentration and ALP activity in rat's serum

Animal Group	Control - (n = 10) Average ± SD (Min - Max)	Ovariectomy 0 µmol F¯/l (n = 10) Average ± SD (Min - Max)	Ovariectomy 421 µmol F¯/l (n =10) Average ± SD (Min - Max)	Ovariectomy 3158 µmol F¯/l (n = 10) Average ± SD (Min - Max)
Concentration of ICTP (µg/l)	12.48 ± 1.15 (11.4 – 13.6)	18.20 ± 1.47 (16.8 – 20.4) *	16.05 ± 1,49 (13.2 – 18.1) *, **	16.53 ± 1.55 (13.9 – 19.4) *, **
Activity of ALP (j./l)	59.24 ± 11.36 (49.5 – 84.5)	71.69 ±12.35 (58.3 – 94.8) #	90.23 ± 10,80 (74.5 –110.1) ##, ###	87,46 ± 17.32 (64.5 – 114.8) ####, ##

* p < 0.05 in comparison with the control group
** p < 0.01 in comparison with group receiving 0 µmol F¯/l
# p < 0.05 in comparison with the control group
p < 0.0001 in comparison with the control group
p < 0.01 in comparison with group receiving 0 µmol F¯/l
p < 0.05 in comparison with group receiving 0 µmol F¯/l

Source: Elaboration of the Authors

Ovariectomy procedure led to appearance of really higher concentrations of bone tissue resorption marker - ICTP in all groups in comparison with the control group. The highest concentrations of ICTP were found in the group of animals receiving distilled water. Serving of fluorides in concentrations of 421 and 3158 µmol F¯/l in earlier period, led in groups of ovariectomized rats to appearance of lower ICTP concentrations in comparison with animals receiving distilled water to drink. While analyzing activity of bone tissue formation marker -ALP, its increase was noted in all groups in comparison with the control group. ALP highest activity was found in the group of animals getting distilled water containing 421 µmol F¯/l, before ovariectomy procedure. ALP values in groups of rats subjected to ovariectomy and getting before the procedure 421 and 3158 µmol F¯/l, were significantly higher from ALP activities stated both in the control group, and at animals after ovariectomy, receiving exclusively distilled water to drink. The highest activity of osseous tissue formation index (ALP) and the lowest concentration of osseous tissue dissolution index (ICTP) appeared at animals receiving fluorides in small concentrations - i.e. 421 µmol F¯/l before the operation, among rats subjected to ovariectomy, as it results from table 2. The results of the analysis of lower jaw mineral weight, made in the end of experiment, are shown in table 3.

Executed measurements showed significantly lower mineral weight of lower jaw bones at rats subjected to ovariectomy and receiving distilled water to drink, in comparison with the control group. Ovariectomy at animals earlier receiving fluorides in a drinking water in concentration of 421 and 3158 µmol F¯/l caused gaining oflaw jaw mineral weight not different from bone mineral content of animals in the control group. Mineral weight index, determining mineral weight of lower jaw, falling on animal body mass unit, was the lowest in the group of rats subjected to ovariectomy and receiving distilled water to drink, and it differed statistically from the index calculated for remaining experimental groups.

Tab. 3. Bone mineral content of mandible and mineral weight index in rats

Animal Group	Control - (n = 10) Average ± SD (Min - Max)	Ovariectomy O µmol F^-/l (n = 10) Average ± SD (Min - Max)	Ovariectomy 421 µmol F^-/l (n = 10) Average ± SD (Min - Max)	Ovariectomy 3158 µmol F^-/l (n = 10) Average ± SD (Min - Max)
Bone mineral content of mandible (mg)	266 ± 24 (221 – 300) *	186 ± 57 (105 – 273)	282 ± 44 (233 – 358) **	299 ± 40 (256 – 377) ***
Mineral weight index	0.850 ± 0.081 (0.764 – 1.007) **	0.548 ± 0.175 (0.326 – 0.787)	0.824 ± 0.126 (0.668 – 1.102) **	0.945 ± 0.169 (0.721 –1.239) **

* $p < 0.01$ in comparison with the control group 0 µmol F^-/l
** $p < 0.001$ in comparison with group receiving 0 µmol F^-/l
*** $p < 0.0001$ in comparison with group receiving 0 µmol F^-/l

Source: Elaboration of the Authors

Mineral weight index in the group of animals subjected to ovariectomy, increased alongside with the concentration of fluorides being received in a drinking water and it was the highest in the group receiving 3158 µmol F^-/l. Ovariectomy execution at animals receiving fluorides didn't considerably impact on mineral weight index, which didn't differ from the value of index in the control group. The results of radiological tests being executed after completion of an experiment are showed in table 4.

Tab. 4. Measurements of molar tooth heights, of alveolar appendix heights and index of rats' alveolar appendices heights

Animal Group	Control - (n = 10) Average ± SD (Min - Max)	Ovariectomy 0 µmol F^-/l (n = 10) Average ± SD (Min - Max)	Ovariectomy 421 µmol F^-/l (n = 10) Average ± SD (Min - Max)	Ovariectomy 3158 µmol F^-/l (n = 10) Average ± SD (Min - Max)
Height of molar tooth (mm)	43.90 ± 1.97 (41.45 – 46.63)	43.78 ± 2.12 (40.80 – 47.28)	42.88 ± 1.42 (41.45 – 45.34)	42.29 + 1.93 (38.86 – 44.04)
Height of alveolar appendix (mm)	24.95 ± 1.00 (23.96 – 27.20) **	24.94 ± 1.65 (23.32 – 28.50) *	22.22 ± 2.41 (18.13 – 24.61)	24.61 ± 2.38 (20.73 – 27.20) ***
Alveolar appendix index	0.57 ± 0.03 (0.53 – 0.61) ***	0.57 ± 0.04 (0.53 – 0.63) ***	0.52 ± 0.05 (0.44 – 0.59)	0.58 ± 0.05 (0.49 – 0.64) **

* $p < 0.01$ in comparison with group receiving 421 µmol F^-/l
** $p < 0.02$ in comparison with group receiving 421 µmol F^-/l
*** $p < 0.05$ in comparison with group receiving 421 µmol F^-/l

Source: Elaboration of the Authors

Heights of molar teeth didn't statistically differ in all experimental groups. Ovariectomy execution caused considerably decrease of alveolar appendix height, as well as alveolar appendix index in the group receiving 421 µmol F^-/l, in comparison with the rest of experimental groups, while it didn't considerably impact on the same parameters at ovariectomized animals.

5. Discusion

The fundamental function of bone tissue is a support function, because this tissue has to be made of complete osseous matrix, which would be able to non faulted mineralization. The structure of an osseous tissue plays an important part in correct running of mastication organ, the exponent of what can be alveolar appendicitis' structure and size. Bone atrophy and destruction in the course of peridontopathy can lead to rapid exposure of teeth' roots and their progressive and irreversible hypermobility. The effect is quicker teeth' loss and reduction of occlusion stability. The course and intensity of bones recession processes within lower jaw depend on many factors, both local and extracorporeal [13]. For example, the reduction of estrogens number causes intensified loss of osseous mass and can be a reason of typical osteoporosis changes. One of the most important parameters of osseous tissue estimation in dentistry is, from the clinical point of view, a rate of alveolar appendicitis atrophy, while the atrophy of lower jaw's alveolar part occurs four times faster than in the jaw bone [14].

An animal model was used in presented thesis, in order to estimate of changes occurring in the osseous tissue of lower jaw induced by estrogens' shortage caused by ovariectomy. Routine biochemical analyses are used for the estimation of the dynamics of bones rebuilding process, using measurements of enzymatic activity of cells creating and resorbing an osseous tissue and analyzing components of osseous matrix getting in blood and excreted with urine during resorption and bones formation processes [15]. The estimation of osseous markers is commonly used non-invasive method, possible to use both in experimental test, and in mesh tests. They provide information concerning an intensification of osseous rebuilding process and describe the balance of bones' formation/resorption [16], which is an exponent of systemic changes [17]. The marker of bone tissue resorption was used in the thesis being presented (ICTP), composing the product of bones' degradation [18]. Thanks to elaboration of determination methods of aminoacids sequences', being specific for collagen I released during bones resorption [19], the increase of ICTP concentration at rats subjected ovariectomy shows intensification of resorption processes (Tab. 2). Second marker, being used in related own tests, is alkaline phosphatase. It's not an enzyme specific for an osseous tissue, because it appears in the form of numerous isoenzymes, coded by different genes. However the measurement of total activity in serum is readily available and from that reason it is still used in diagnostics and treatment [20]. ALP total activity in the serum of animals after ovariectomy, as it results from table 2, was much higher in comparison to values being obtained in the control group. This is an evidence of increased rate of an osseous tissue rebuilding at rats with estrogens shortage being caused experimentally [14].

Generally used, from among currently used diagnostics methods serving for quantitative marking of mineral components contents in an osseous tissue, is densitometry. This method characterizes with good repeatability, short duration of measurement, low radiation dose and high speed. This is additionally one of graphic methods, which can show the earliest changes of mineral components contents in an osseous tissue. The lowest density of lower jaw bone were recorded in group 2, that is at animals subjected to ovariectomy, without administering of fluorides, as it is showed in table 3. This matter is a confirmation of the impact of estrogens shortage on lowering of mineralization rate of low jaw bones at animals in that group. Also the mineral weight index, accepting animals' weights (Tab. 1) was the lowest for animals from group 2. Other authors researches executed on animals, confirm the impact of estrogens shortage on acceleration of jaw's bones and osteopenia after teeth extraction too [6], [7].

A number of researches, made on ovariectomized rats, were published in the world-wide literature, where the lowering of bones mineral density was found [21]. In presented own studies, classical radiological tests were used for the estimation of ovariectomy impact on the possibility of rat lower jaw alveolar part atrophy. However they showed no ovariectomy impact on height of alveolar appendix at rats. Some investigators claim that the ovariectomy itself doesn't lead to change of mineral density of lower jaw bone [22], as well. One didn't show the correlation between measurements of lower jaw's alveolar appendix and the indicator determining its density, as well. Other authors described similar situations [11], [23]. However an essential negative correlation between lower jaw bones' mineral weight and the level of ICTP concentrations for the control group (group 1) and the group with executed ovariectomy (group 2) (Fig. 2), was noted in the thesis being presented. Presented correlation indicates strong intensification of bones' resorption processes.

The second of thesis's realized objectives was the estimation of the impact of two different fluoride's doses on the quality of an osseous tissue at rats subjected to ovariectomy (groups 3 and 4). The results of these tests were presented in tables 2, 3 and 4. The concentration of 421 μmol F^-/l (8 mg F^-/l) in a water administered for rats equals the concentration of fluoride present in fluoridated water designed for food objectives for people (1 mg F^-/l). While the concentration of 3158 μmol F^-/l, that is 60 mg F^-/l in drinking water leads to the achievement of fluorides concentration in serum, being obtained in serum of patient treated for osteoporosis (10 mg F^-/l) [24], [25]. It is worth to notice, that supply of fluorides in the conditions of executed experiment, didn't negatively impact on rats' bodyweights, and there was noted even small body weight increase in the case of 421 μmol F^-/l dose, statistically characteristic ($p<0,05$) in comparison with the control group (table 1). Relevant data from the literature are different depending on fluorides doses and kind and duration of an experiment [26]. Assuming, as other authors do, that administration of fluorides in drinking water reduces rat's body weight [27], [28], [29], and then body weight of ovariectomized rats, being exposed on fluorides, grows up (Tab. 1).

An analysis of osseous markers at animals with executed ovariectomy, which were earlier administered with fluorides with water, showed significant decrease of the concentration of bones resorption marker and increase activity of bones formation marker (Tab. 2). This remarks especially relates to animals from group 3, being exposed to fluorides in concentration of 421 μmol F^-/l. Similar results, showing intensified osseous metabolism at rats exposed to ovariectomy, were obtained by other authors [30], [31]. The results presented in table 3 show, that estrogens shortage, due to ovariectomy, reduces mineral weight of lower jaw bones and mineral weight index, and the fluoride supply prevents from loss of minerals from an osseous tissue. Such an observation was described by Phipps et al. [32], too. However Cheng et al. notice that such dependence doesn't come true at old, 12-months rats, watering with fluoridated water, and then exposed to ovariectomy [33].

An important parameter, helpful in the estimation of the quality of mastication organ osseous tissue is, from the clinical point of view, the rate of lower jaw alveolar appendix atrophy. This phenomenon is a problem in dentistry, because it is connected with diminished resistance on injuries and lower stability of prosthetic restorations. The lack of comprehensive studies in this matter is noticed in literature. Individual works concern mainly the relation between fluorides supply and paradontium diseases. Radiological studies, carried out in the present thesis, show that in the group of ovariectomized animals and administered with 421 μmol F^-/l, came about significant height decrease of an alveolar appendix of lower jaw bone and reduction of an alveolar appendix height index – in comparison with other groups. The experiments on animals confirm the impact of estrogens shortage on significant acceleration of jaws' bones atrophy after teeth' extraction [6], [7], [34]. However Louridis et al. [35] excludes the impact of different doses of fluorides' concentrations in a drinking water on an alveolar appendix height. Obtained results of own studies, as well as other authors' reports, confirm diverse and multidirectional influence of fluorine compounds on an osseous tissue, including an alveolar appendix height [36], [37]. One should also remember that, despite recognition the fluoride as a strong stimulator of bones formation, they directly increased osteoblasts quantity not in every experimental model. Stimulation of osseous cells' proliferation by fluorides, can depend on the presence of such factors as parathormon, calcitonin, insulin or insulin-alike growth factor. Moreover, fluoroapatites rising under the influence of fluorides are soluble and prone to resorption in a lesser degree than hydroxyapatite is, but bones containing numerous fluoroapatites crystals are more fragile and sensitive to stretching and rotation, at the same time, despite increased osseous weight. Also, taking fluorine compounds' toxicity under consideration and ambiguous impact on an osseous tissue, one should continue to look and answer for the question concerning purposefulness and effectiveness of fluorides application in osteoporosis prophylaxis and treatment. Potential benefits from such a therapy seem currently to by disputable.

6. Conclusions

- Estrogens shortage, due to ovariectomy, reduces mineral weight of lower jaw bones and mineral weight index.
- The fluoride supply prevents loss of minerals from an osseous tissue.

References

[1] Hadjidakis D.J.: *Androulakis II. Bone remodeling.* Ann N Y Acad Sci. 2006 Dec; 1092: 385-96.

[2] Kanis J.A.: *Treatment of symptomatic osteoporosis with fluoride.* Am J Med 1993; 95 (suppl. 5A): 53-61.

[3] Kalu D.N.: *The ovariectomized rat model of postmenopausal bone loss.* Bone Minera1 1991; 15: 175-92.

[4] Duursma S.A.: *Oestrogens and osteoporosis.* Post Nauk Med 1999; 3(4): 179-83 *(in Polish).*

[5] Ozola B., Slaidina A., Laurina L., Soboleva U., Lejnieks A.: *The influence of bone mineral density and body mass index on resorption of edentulous jaws.* Stomatologija. 2011; 13(1): 19-24.

[6] Elovic R.P., Hipp J.A., Hayes W.C.: *Maxi11ary molar extraction decreases stiffness of the mandible in ovariectomized rats.* J Dent Res 1994; 73: 1735-41.

[7] Hsieh Y.D., Devlin H., McCord F.: *The effect of ovariectomy on the healing tooth socked of the rat*. Archs Oral Bio1 1995; 40: 529-31.

[8] Pytlik M., Nowińska B., Janiec W., Czubak E.: *Effects of sodium fluoride on development of osteopenia induced by ovariectomy in rats).* Post Osteoartrol 1998; 10: 99-108 *(in Polish).*

[9] Thorndike E.A., Turner A.S.: *In search of an animal model for postmenopausal diseases.* Front Biosci. 1998 Apr 16; 3: c17-26.

[10] Govindarajan P., Schlewitz G., Schliefke N., Weisweiler D., Alt V., Thormann U., Lips K.S., Wenisch S., Langheinrich A.C., Zahner D., Hemdan N.Y., Böcker W., Schnettler R., Heiss C.: *Implications of combined ovariectomy/multi-deficiency diet on rat bone with age-related variation in bone parameters and bone loss at multiple skeletal sites by DEXA.* Med Sci Monit Basic Res. 2013 Feb 28; 19: 76-86.

[11] Gasser J.A., Ingold P., Venturiere A., Shen V., Green J.R.: *Long-term protective effects of zoledronic acid on cancellous and cortical bone in the ovariectomized rat.* J Bone Miner Res. 2008 Apr; 23(4): 544-51.

[12] Adamowicz-Klepalska B.: *Computerized morphometry of periodontium and humerus in albino rats after administration of low and high fIuorine concentrations in drinking water.* Czas Stomat 1994; 47(4): 267-71 *(in Polish).*

[13] Gołębiewska M., Bielaczyc A.: *Scanning electron microscopy of the influence of dietary calcium and vitamin D-deficiency on periodontium in the adult rats.* Ann Acad Med Bio 1997; 42 Suppl 2: 159-65.

[14] Tallgren A.: *The continuing reduction of the residual alveolar ridges in complete denture wearers: a mixed-longitudinal study covering 25 years.* J Prosthet Dent 1972; 27:120-32.

[15] Grabowska S.Z., Rogowski F., Citko A.: *Selected biochemical markers in the process of bone remodeling.* Czas Stomat 1996; 49(6): 436-40 *(in Polish).*

[16] Singer F.R., Eyre D.R.: *Using biochemical markers of bone turnover in clinical practice.* Cleve Clin J Med. 2008 Oct; 75(10): 739-50.

[17] Lotz J., Streeger D., Hatner G., Erhenthal W., Heine J., Prellwitz W.: *Biochemical bone markers compared with bone density measurements by energy x-ray absorptiometry*. Calcif Tissue Int 1995; 57: 253-7.

[18] Bettica P., Moro L.: *Biochemical markers of bone metabolism in the assessment of osteoporosis.* JFCC 1995; 7(1): 16-22.

[19] Eriksen H.A., Sharp C.A., Robins S.P., Sassi M.L., Risteli L., Risteli J.: *Differently cross-linked and uncross-linked carboxy-terminal telopeptides of type I collagen in human mineralised bone.* Bone. 2004 Apr; 34(4): 720-7.

[20] Romagnoli E., Minisola G., Camevale V., Scillitani A., Frusciante V., Aliberti G., et al.: *Assessment of serum total and bone alkaline phosphatase measurement in clinical practice.* Clin Chem Lab Med 1998; 36(3): 163-8.

[21] Gaumet N., Seibel M.J., Braillon P., Giry J., Lebecque P., Oavicco M.J., et al.: *Influence of ovariectomy on bone metabolism in very old rats.* Calcif Tissue Int 1996; 58: 256-62.

[22] Elovic R.P., Hipp J.A., Hayes W.C.: *Ovariectomy decreases the bone area traction of the rat mandible.* Calcif Tissue Int 1995; 56: 305-10.

[23] Johnston B.D., Ward W.E.: *The ovariectomized rat as a model for studying alveolar bone loss in postmenopausal women.* Biomed Res Int. 2015; 2015: 1-12.

[24] Bohatyrewicz A., Białecki P., Larysz D., Gusta A.: *Compressive strength of cancellous bone in fluoride-treated rats.* Fluoride 2001; 34: 236-41.

[25] Reginster J.Y., Felsenberg D., Pavo I., Stepan J., Payer J., Resch H., Glüer C.C., Mühlenbacher D., Quail D., Schmitt H., Nickelsen T.: *Effect of raloxifene combined with monofluorophosphate as compared with monofluorophosphate alone in postmenopausal women with low bone mass: a randomized, controlled trial*. Osteoporos Int. 2003 Sep; 14(9): 741-9.

[26] Guna Sherlin D.M., Verma R.J.: *Vitamin D ameliorates fluoride-induced embryotoxicity in pregnant rats*. Fluoride 2001; 34(3): 184-5.

[27] Chinoy N.J., Sharma M., Michael M.: *Beneficial effects of ascorbic acid and calcium on reversal of fluoride toxicity in male rats.* Fluoride 1993; 26(1): 45-56.

[28] Banu Priya C.A.Y., Anitha K., Murali Mohan E., Pillai K.S., Murthy P.B.: *Toxicity of fluoride to diabetic rats*. Fluoride 1997; 30(1): 51-8.

[29] Bords J., Keszler P., Csikos G., Kalasz H.: *Fluoride intake, distribution and bone content in diabetic rats consuming fluoridated drinking water*. Fluoride 1998; 31(1): 33-42.

[30] Calero J.A., De la Piedra C., Oiaz Curiel M.: *Usefulness of serum pyridinoline cross- linked carboxy-terminal telepeptides of type I collagen and osteocalcin in the study of the bone resorption rate in oophorectomized rats*. Miner Electrolyte Metab 1998; 24: 303-6.

[31] Srivastava A.K., Bhattacharyya S., Castillo G., Wergedal J., Mohan S., Baylink D.J.: *Development and application of serum C-telepeptide and osteocalcin assay to measure bone turnover in an ovariectomized rat model.* Calcif Tissue Int 2000; 66: 435-42.

[32] Phipps K.R., Orwoll E.S., Mason J.D., Cauley J.A.: *Community water fluoridation, bone mineral density, and fractures: prospective study of effects in older women.* BMJ. 2000 Oct 7; 321(7265): 860-4.

[33] Cheng P.T., Huang L., Low N.: *Sodium fluoride does not build bone in aged ovariectomized rats.* Fluoride 1995; 28(3): 157-8.

[34] Li X., Nishimura J.: *Altered bone remodeling pattern of the residua' ridge in ovariectomized rats.* J Prosthet Dent 1994; 72: 324-30.

[35] Louridis O., Bazopoulou-Kyrkanides, Demetriuo N.: *Histometric studies on the periodontium of albino rats drinking fluoridated water.* J Periodont 1970; 41: 483-6.

[36] Chachra D., Limeback H., Willett T.L., Grynpas M.D.: *The long-term effects of water fluoridation on the human skeleton.* J Dent Res. 2010 Nov; 89(11): 1219-23.

[37] Klemetti E., Kröger H., Lassila L.: *Fluoridated drinking water, oestrogen therapy and residual ridge resorption.* J Oral Rehabil 1997; 24: 47-51.