

BEATA NOWAK<sup>1, A-D, F</sup>, AGNIESZKA MATUSZEWSKA<sup>1, A, B</sup>, JAROSŁAW FILIPIAK<sup>2, B, C</sup>,  
ANNA NIKODEM<sup>2, B, C</sup>, ANNA MERWID-LĄD<sup>1, B, E</sup>, MAŁGORZATA PIEŚNIEWSKA<sup>1, B</sup>,  
JOANNA KWIATKOWSKA<sup>1, B</sup>, BARTOSZ GROTTTHUS<sup>1, E</sup>, ADAM SZELAĞ<sup>1, E, F</sup>

## The Negative Impact of Selective Activation of Retinoic Acid Receptors on Bone Metabolism and Bone Mechanical Properties in Rats\*

<sup>1</sup> Department of Pharmacology, Wrocław Medical University, Poland

<sup>2</sup> Division of Biomedical Engineering and Experimental Mechanics, Wrocław University of Technology, Poland

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of article

### Abstract

**Background.** Drug-induced osteoporosis is a significant health problem, as many drugs have deleterious effects on bone metabolism. Data from several studies concerning the influence of retinol on bone homeostasis are inconsistent.

**Objectives.** The purpose of this study was to investigate the influence of tazarotene, a selective agonist of the retinoic acid receptor (RAR), on bone metabolism and bone mechanical properties in rats.

**Material and Methods.** Sixteen male Wistar rats were assigned either to the group receiving tazarotene or to the control group. Serum biochemical markers of bone turnover (osteocalcin: OC, tartrate resistant acid phosphatase 5: TRACP5b, and osteoprotegerin: OPG) and the mechanical properties of bones were analyzed.

**Results.** The mean Young's modulus was 24% higher ( $p < 0.05$ ) in the control group than in the group receiving tazarotene. The stiffness of femur bones was 25% lower ( $p < 0.05$ ) in rats receiving tazarotene. Flexural yield stress was slightly (2%) decreased in the tazarotene group, but the difference was not statistically significant. In the tazarotene group significantly lower serum concentration of bone turnover markers were observed (TRACP5b:  $0.86 \pm 0.30$  ng/mL vs.  $2.17 \pm 0.67$  ng/mL, OC:  $7.77 \pm 2.28$  ng/mL vs.  $13.04 \pm 3.54$  ng/mL and OPG:  $0.09 \pm 0.04$  ng/mL vs.  $0.27 \pm 0.10$ ) than in the control group.

**Conclusions.** Tazarotene worsened bone mechanical properties and inhibited bone turnover in rats. These results suggest that tazarotene has a negative impact on bone metabolism and that it exerts osteoporotic activity (*Adv Clin Exp Med* 2016, 25, 2, 213–218).

**Key words:** retinoic acid receptors, osteoporosis, bone turnover, bone mechanical properties, tazarotene.

Osteoporosis is defined as pathological bone microarchitecture making bones prone to low-energy fractures. In recent decades it has become one of the major health and socio-economic problems in many countries. Drug-induced osteoporosis is a significant health problem, as many drugs have deleterious effects on bone metabolism. Glucocorticoids, proton pump inhibitors, selective serotonin receptor inhibitors, thiazolidinediones, anticonvulsants, medroxyprogesterone acetate, aromatase in-

hibitors, androgen deprivation therapy, heparin, calcineurin inhibitors and some chemotherapies are among the drugs increasing the risk of osteoporotic fractures [1].

The mechanisms of the regulation of bone turnover involved in the etiology and pathology of osteoporosis seem to be very complex. Recent papers have revealed that various nuclear receptors are involved in the regulation of bone metabolism, its physiology and pathology. Many studies suggest

\* The study was financed from the grant for Young Researchers (9 Pbm).

that retinoids may also be involved in the regulatory process controlling bone remodeling. However, the role of retinoic acid receptors (RARs) in bone turnover has not yet been fully explained.

Vitamin A plays an important role in many physiological processes. It is essential for growth, reproduction, ocular health and immune defense. Lohnes et al. described skeletal abnormalities in RAR $\alpha$  and RAR $\gamma$  knockout mice [2]. Several studies have revealed the important impact of retinoids on skeletal development [3, 4]. Vitamin A deficiency may cause different types of bone abnormalities; however, most bone changes related to vitamin A deficiency seem to be reversible [5]. Abnormalities of ossification and calcification have also been described in cases of excessive intake of vitamin A [5]. Some small studies have documented bone resorption, periosteal calcification and hypercalcemia resulting from chronic excessive vitamin A intake [6]. The results of studies evaluating the influence of retinoids on the development of osteoporosis are inconsistent. Some authors suggest that serum retinol concentration may be a good predictor of osteoporotic fractures [7, 8]. However, others did not confirm any negative impact of retinol on bone fragility [9, 10].

It might be anticipated that retinoids used in the treatment of skin diseases and malignancies would cause skeletal toxicity similar to that of vitamin A. However, the results of clinical studies are inconsistent. Whereas some authors report no skeletal effect of synthetic retinoid therapy, others document various toxic effects, including periosteal thickening, ligamentous calcification and decreased mineral bone density [11–13]. Most existing studies involving synthetic retinoids have been small, and definite conclusions about the effect of retinoids on bone health cannot be drawn.

In animal studies, retinol, a non-selective agonist of RARs and retinol receptors X (RXRs), when administered with alendronate, lessened the preventive action of bisphosphonate on the development of osteoporosis in ovariectomized rats [14]. Houhg et al. reported that high doses of retinol led to spontaneous bone fractures in rats [15]. Hotchkiss et al. reported that not only high doses (70 to 120 mg/kg daily) but also lower doses (10 and 15 mg/kg daily) of all-trans retinoic acid (ATRA) induced spontaneous fractures and decreased mineral content in rat bones [16]. However, the mechanism of the metabolic changes in bone tissue caused by retinoids has not been fully explained. Some authors suggest that the osteopenic action of retinol may be the consequence of its interaction with vitamin D, as retinoic acid influence the expression of 25-OH-vitamin-D3-24 hydroxylase [17], and RXRs and vitamin D receptors (VDRs) share

a similar location and structure [18]. Rohde et al. reported that vitamin A antagonized the action of vitamin D in rats [19]. However, other studies suggest that ATRA promotes periosteal bone resorption in rats independently of vitamin D [20].

The results of studies on the effects of retinoids on osteoclast and osteoblasts cultures are inconsistent. Hisada et al. report that retinoic acid regulates the development of mesenchymal cells into osteoblasts and adipocytes [21]. Some authors suggest that retinoic acid stimulates osteoclastic bone resorption [22, 23], while others report that retinoic acid has an inhibitory effect on osteoblastic cell proliferation [24].

Although the effects of retinoids have been examined in rodents in the past, little information about the role of RAR and RXR agonists in the development of bone turnover disorders is available. The aim of the present study is to evaluate the effects of selective RAR (tazarotene) agonists on bone metabolism and on the mechanical properties of bones in rats. Eight weeks of tazarotene administration was planned as it has been demonstrated that an eight-week follow-up allows osteoporotic changes in ovariectomized rats to be detected.

## Material and Methods

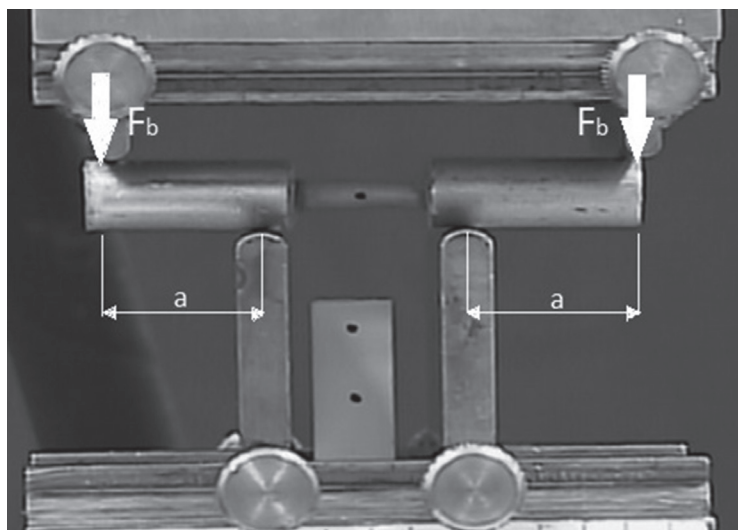
### The Animals and the Experiment

The experiment, which was performed with the approval of the First Local Ethics Committee for Experiments on Animals in Wrocław, Poland, was carried out on 16 male Wistar rats, each weighing about 200 g. The animals were housed individually at a room temperature of 25°C with a 12 : 12 h daily lighting cycle. They were fed a standard diet. Food and water were provided *ad libitum*.

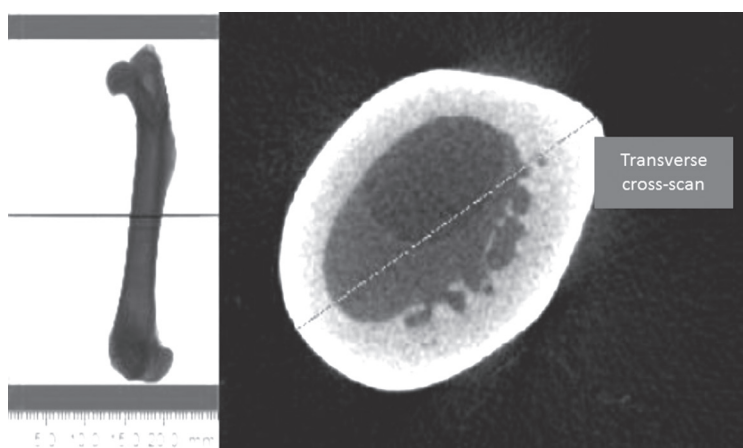
Acclimated rats were randomly assigned to one of two groups (eight animals in each group): Group T, receiving tazarotene (0.075 mg/kg) in sesame oil (4 mL/kg) daily intragastrically (*i.g.*) for 56 days, and Group C, the control group, receiving sesame oil (4 mL/kg daily *i.g.* for 56 days).

Body weights were checked once daily throughout the eight-week experiment period. On day 56 blood samples for serum isolation were collected. Serum was separated by centrifugation (at 1500 × g) and then stored at –70°C until required for bone metabolic marker assays.

After the animals were sacrificed, right femurs were obtained from each rat and the femur index, defined as the ratio of femur weight and body weight ((femur mass [g]/body mass [g]) × 100%) was calculated. Femurs were stored at –70°C until required for mechanical tests.



**Fig. 1.** Set-up for static four-point bending test with the examined femur in aluminum sleeves. Bending moment was calculated as  $M_b = F_b a$



**Fig. 2.** Example of a microtomograph scan used to obtain  $W_b$  transverse cross-sections of femur bones

## Serum Biochemical Markers of Bone Metabolism

Serum osteocalcin (OC) and tartrate resistant acid phosphatase 5 (TRACP5b) levels (both sensitive biochemical markers of bone metabolism) were determined using commercial osteocalcin and tartrate resistant acid phosphatase 5 ELISA kits (Rat Osteocalcin ELISA Kit and Rat Tartrate Resistant Acid Phosphatase 5 ELISA Kit, both from USCN Life Science Inc., Wuhan, China). Serum osteoprotegerin (OPG) levels (a regulatory molecule involved in bone metabolism) were also determined using commercial ELISA kits (Rat Osteoprotegerin ELISA Kit, USCN Life Science Inc.). The ELISA tests were performed according to the manufacturers' instructions.

## Mechanical Tests

In the present study, the mechanical resistance of intact right femurs was studied using a static four-point bending test (Fig. 1). The mechanical properties of the femurs were assessed using an MTS MiniBionix 858 test system (MTS Systems Corp., Eden Prairie, MN, USA). The femurs were

mounted on the station using special-purpose instrumentation appropriate for the specific loading condition executed. The bending moment  $M_b$  was applied perpendicularly to the long axis of the femur on the anatomical frontal plane. The load increased at a rate of 0.5 Nm/min until the breaking load – defined as the load at which the bone actually broke – was reached. The course of each measuring test and measuring data acquisition were controlled using an MTS FlexTest controller.

Flexural yield stress ( $\sigma_b$ ) was calculated using the formula:  $\sigma_b = M_b/W_b$  [MPa] ( $M_b$ : bending moment [Nm],  $W_b$ : index of cross-section deformity in response to bending [ $m^3$ ]).  $W_b$  was determined for the transverse cross-section of the medial part of femur. It was assumed that the analyzed cross-sections were elliptic. To obtain  $W_b$ , transverse cross-sections of the femurs were examined with a SkyScan 1172 100kV microtomograph (Bruker Corp., Billerica, MA, USA) (Fig. 2).

Young's modulus ( $E$ ) was calculated using the formula  $E = ((F \times a^2)/(6 \times f_1 \times I) \times (3a + 2a))$  [MPa] ( $F$ : maximal force [N],  $I$ : moment of inertia [ $m^4$ ],  $f_1$ : bending arrow [m],  $a$ ,  $b$ : distance between holders [m], Fig. 1).

Bone stiffness ( $k$ ) was calculated using the formula  $k = E \times I$  ( $E$ : Young's modulus [MPa],  $I$ : moment of inertia [ $m^4$ ]).

## Statistical Analysis

The significance of differences between values was calculated using Student's  $t$ -test. A  $p$ -value of less than 0.05 was considered statistically significant. The results are presented as the mean  $\pm$  standard deviation (SD) unless otherwise noted.

All statistical analysis were performed using STATISTICA software v. 10 (StatSoft, Inc., Tulsa, OK, USA).

## Results

### Body Weight and Bone Parameters

Body weights were checked once daily throughout the eight-week experimental period. At the start of experiment, body weights were similar in both the groups. On day 56 there was also no significant difference in body weights in the two groups ( $303.3 \pm 15.5$  g in the control group vs.  $298.6 \pm 21.2$  g in the tazarotene group,  $p > 0.05$ ).

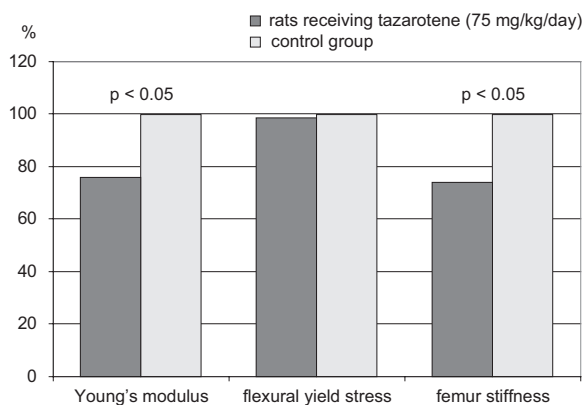


Fig. 3. Relative bone mechanical properties

The femur index was significantly lower in rats receiving tazarotene than in the controls ( $0.37\% \pm 0.02\%$  vs.  $0.50\% \pm 0.02\%$ ,  $p < 0.05$ ).

The mean Young's modulus was 24% higher in the control group than in the group receiving tazarotene ( $17.49$  GPa vs.  $13.27$ ,  $p < 0.05$ ).

Flexural yield stress was slightly (2%) decreased in the tazarotene group, but the difference was not statistically significant.

The stiffness of femur bones was 25% lower in the rats receiving tazarotene than in the control group ( $0.1145$  N/m<sup>2</sup> vs.  $0.1525$  N/m<sup>2</sup>,  $p < 0.05$ ).

### Serum Biochemical Markers of Bone Turnover

In the rats receiving tazarotene, there were lower serum concentrations of bone turnover markers (TRACP5b, OC and OPG) than in the control group (Table 1).

## Discussion

Increased risk of skeletal fractures has been described by some authors as a possible side effect of the use of retinoids in the treatment of dermatological conditions and in cancer treatment or prophylaxis. Both retinoic acid receptors (RARs) and retinoid X receptors (RXRs) have been detected in the cells of bone tissue [25, 26]. As RARs have been detected in osteoblasts and osteoclasts, they have to be taken into account when bone pathology is considered. The aim of the present study was to establish the effects of tazarotene, a selective RAR agonist, on bone metabolism and bone mechanical properties.

From the clinical point of view, the most dangerous consequence of osteoporosis and other bone metabolism disorders is the increase in bone fractures [27]. For this reason, the assessment of tazarotene's influence on bones was based, among others things, on an investigation of mechanical properties. Decreased mechanical properties (mean Young's modulus, flexural yield stress and stiffness)

Table 1. Serum biochemical markers of bone turnover

	Rats receiving tazarotene (0.75 mg/kg/day) (n = 8)	Control group (n = 8)	p-value
TRACP5b [ng/mL]	$0.86 \pm 0.30$	$2.17 \pm 0.67$	$< 0.001$
OC [ng/mL]	$7.77 \pm 2.28$	$13.04 \pm 3.54$	0.010
OPG [ng/mL]	$0.09 \pm 0.04$	$0.27 \pm 0.10$	0.002

n – number of animals; TRACP5b – tartrate resistant acid phosphatase 5b; OC – osteocalcin; OPG – osteoprotegerin; results presented as mean  $\pm$  SD.

were detected in the bones obtained from the rats receiving tazarotene, as compared to the control group. Decreases in flexural yield stress, stiffness and the mean Young's modulus indicate changes of an osteoporotic nature similar to those observed in ovariectomy-induced osteoporosis [28].

Markers of bone turnover are widely used to assess the influence of different substances on bone metabolism. Their contribution to the diagnosis of osteoporosis is low. However, some studies suggest that they may be useful in predicting the risk of fracture [29]. In the present study, the administration of tazarotene decreased the serum levels of TRACP5, OC and OPG, suggesting that tazarotene has an inhibitory influence on bone turnover.

OPG is an osteoblast-derived paracrine cytokine that is essential for osteoclast functions. It acts as a decoy receptor for the receptor activator of nuclear factor kappa B ligand (RANKL). By binding RANKL, OPG prevents RANK-mediated nuclear factor kappa B activation, which is a key regulator for many physiological processes, among others cell differentiation. OPG binds to RANKL on osteoblasts/stromal cells, blocking interactions between osteoblasts/stromal cells and osteoclast precursors. It inhibits the differentiation of osteoclast precursors into mature osteoclasts. An increased RANKL-to-OPG ratio favors osteoclast differentiation and bone resorption [30]. The decreased serum OPG

level found in the present study may at least partially explain the detected changes in bone mechanical properties. A decrease in OPG synthesis is also observed in glucocorticoid-induced osteoporosis [31]. Cai et al. reported that all-trans retinoic acid induced hyperactivity of the hypothalamus-pituitary-adrenal (HPA) axis [32], so it cannot be excluded that the observed changes in bone turnover and bone mechanical properties are at least partially caused by increased secretion of endogenous corticoids.

TRACP5 is highly expressed by activated osteoclasts [33]. However, its exact physiological role is unknown. OC, on the other hand, is a bone matrix protein produced by osteoblasts that is widely used as a marker of bone formation, as its level increases during the events characterized by rapid bone turnover [34]. High OC serum levels correlate with increases in bone mineral density (BMD) during the treatment of osteoporosis with drugs stimulating bone formation [35]. The decreased serum OC and TRACP5 levels observed in the present study suggest that tazarotene has an inhibitory effect on bone turnover. It seems to inhibit both bone resorption and bone formation. The effect of tazarotene observed in this study might be a consequence of the inhibitory influence of retinoids on osteoclast and osteoblast progenitors [36, 37].

In conclusion, tazarotene decreases femoral mechanical properties and inhibits bone turnover.

## References

- [1] Panday K, Gona A, Humphrey MB. Medication-induced osteoporosis: Screening and treatment strategies. *Ther Adv Musculoskelet Dis* 2014, 6, 185–202.
- [2] Lohnes D, Mark M, Mendelsohn C, Dollé P, Dierich A, Gorry P, Gansmuller A, Chambon P: Function of the retinoic acid receptors (RARs) during development (I). Craniofacial and skeletal abnormalities in RAR double mutants. *Development* 1994, 120, 2723–2748.
- [3] Lee K, Skromme I: Retinoic acid regulates size, pattern and alignment of tissues at the head-trunk transition. *Development* 2014, 141, 4375–4384.
- [4] Koyama E, Golden EB, Kirsch T, Adams SL, Chandraratna RA, Michaille JJ, Pacifici M: Retinoid signaling is required for chondrocyte maturation and endochondral bone formation during limb skeletogenesis. *Dev Biol* 1999, 208, 375–391.
- [5] Ahmadiéh H, Arabi A: Vitamins and bone health: Beyond calcium and vitamin D. *Nutr Rev* 2011, 69, 584–598.
- [6] Binkley N, Krueger D: Hypervitaminosis A and bone. *Nutr Rev* 2000, 58, 138–44.
- [7] Michaëlsson K, Lithell H, Vessby B, Melhus H: Serum retinol levels and the risk of fracture. *N Engl J Med* 2003, 348, 287–294.
- [8] Barker ME, McCloskey E, Saha S, Gossiel F, Charlesworth D, Powers HJ, Blumsohn A: Serum retinoids and beta-carotene as predictors of hip and other fractures in elderly women. *J Bone Miner Res* 2005, 20, 913–920.
- [9] Ambrosini GL, Alfonso H, Reid A, Mackerras D, Bremner AP, Beilby J, Olsen NJ, Musk AW, de Klerk NH: Plasma retinol and total carotenes and fracture risk after long-term supplementation with high doses of retinol. *Nutrition* 2014, 30, 551–556.
- [10] Ambrosini GL, Bremner AP, Reid A, Mackerras D, Alfonso H, Olsen NJ, Musk AW, de Klerk NH: No dose-dependent increase in fracture risk after long-term exposure to high doses of retinol or beta-carotene. *Osteoporos Int* 2013, 24, 1285–1293.
- [11] Lawson JP, McGuire J: The spectrum of skeletal changes associated with long-term administration of 13-cis-retinoic acid. *Skeletal Radiol* 1987, 16, 91–97.
- [12] McGuire J, Lawson JP: Skeletal changes associated with chronic isotretinoin and etretinate administration. *Dermatologica* 1987, 175, Suppl 1, 169–181.
- [13] Margolis DJ, Attie M, Leyden JJ: Effects of isotretinoin on bone mineralization during routine therapy with isotretinoin for acne *vulgaris*. *Arch Dermatol* 1996, 132, 769–774.

- [14] Śliwiński L, Janiec W, Pytlik M, Folwarczna J, Kaczmarczyk-Sedlak I, Pytlik W, Cegiela U, Nowińska B: Effect of administration of alendronate sodium and retinol on the mechanical properties of the femur in ovariectomized rats. *Pol J Pharmacol* 2004, 56, 817–824.
- [15] Hough S, Avioli LV, Muir H, Gelderblom D, Jenkins G, Kurasi H, Slatopolsky E, Bergfeld MA, Teitelbaum SL: Effects of hypervitaminosis A on the bone and mineral metabolism of the rat. *Endocrinology* 1988, 122, 2933–2939.
- [16] Hotchkiss CE, Latendresse J, Ferguson SA: Oral treatment with retinoic acid decreases bone mass in rats. *Comp Med* 2006, 56, 502–511.
- [17] Rhinn M, Dollé P: Retinoic acid signalling during development. *Development* 2012, 139, 843–858.
- [18] Long MD, Sucheston-Campbell LE, Campbell MJ: Vitamin D receptor and RXR in the post-genomic era. *J Cell Physiol* 2015, 230, 758–766.
- [19] Rohde CM, Manatt M, Clagett-Dame M, DeLuca HF: Vitamin A antagonizes the action of vitamin D in rats. *J Nutr* 1999, 129, 2246–2250.
- [20] Rohde CM, DeLuca H: Bone resorption activity of all-trans retinoic acid is independent of vitamin D in rats. *J Nutr* 2003, 133, 777–783.
- [21] Hisada K, Hata K, Ichida F, Matsubara T, Orimo H, Nakano T, Yatani H, Nishimura R, Yoneda T: Retinoic acid regulates commitment of undifferentiated mesenchymal stem cells into osteoblasts and adipocytes. *J Bone Miner Metab* 2013, 31, 53–63.
- [22] Hu L, Lind T, Sundqvist A, Jacobson A, Melhus H: Retinoic acid increases proliferation of human osteoclast progenitors and inhibits RANKL-stimulated osteoclast differentiation by suppressing RANK. *PLoS One* 2010, 5, e13305.
- [23] Conaway HH, Pirhayati A, Persson E, Pettersson U, Svensson O, Lindholm C, Henning P, Tuckermann J, Lerner UH: Retinoids stimulate periosteal bone resorption by enhancing the protein RANKL, a response inhibited by monomeric glucocorticoid receptor. *J Biol Chem* 2011, 286, 31425–31436.
- [24] Ohishi K, Nishikawa S, Nagata T, Yamauchi N, Shinohara H, Kido J, Ishida H: Physiological concentrations of retinoic acid suppress the osteoblastic differentiation of fetal rat calvaria cells *in vitro*. *Eur J Endocrinol* 1995, 133, 335–341.
- [25] De Luca F, Uyeda JA, Mericq V, Mancilla EE, Yanovski JA, Barnes KM, Zile MH, Baron J: Retinoic acid is a potent regulator of growth plate chondrogenesis. *Endocrinology* 2000, 141, 346–353.
- [26] Harada H, Miki R, Masushige S, Kato S: Gene expression of retinoic acid receptors, retinoid-X receptors, and cellular retinol-binding protein I in bone and its regulation by vitamin A. *Endocrinology* 1995, 136, 5329–5335.
- [27] Delmas PD: Different effects of antiresorptive therapies on vertebral and nonvertebral fractures in postmenopausal osteoporosis. *Bone* 2002, 30, 14–17.
- [28] Peng ZQ, Väänänen HK, Zhang HX, Tuukkanen J: Long-term effects of ovariectomy on the mechanical properties and chemical composition of rat bone. *Bone* 1997, 20, 207–212.
- [29] Younes M, Hachfi H, Ouertani D, Jguirim M, Hassine Neffati F, Zrouer S, Bejia I, Touzi M, Najjar MF, Bergaoui N: Utility of biochemical markers of bone turnover in diagnosis of osteoporosis and fracture risk prediction. *Tunis Med* 2014, 92, 304–310.
- [30] Hofbauer LC, Kühne CA, Viereck V: The OPG/RANKL/RANK system in metabolic bone diseases. *J Musculoskelet Neuronal Interact* 2004, 4, 268–275.
- [31] Canalis E: Clinical review 83: Mechanisms of glucocorticoid action in bone: implications to glucocorticoid-induced osteoporosis. *J Clin Endocrinol Metab* 1996, 81, 3441–3447.
- [32] Cai L, Yan X, Chen X, Meng Q, Zhou J: Chronic all-trans retinoic acid administration induced hyperactivity of HPA axis and behavioral changes in young rats. *Eur Neuropsychopharmacol* 2010, 20, 839–847.
- [33] Minkin C: Bone acid phosphatase: tartrate-resistant acid phosphatase as a marker of osteoclast function. *Calcif Tissue Int* 1982, 34, 285–290.
- [34] Kanbur NO, Derman O, Sen TA, Kinik E: Osteocalcin. A biochemical marker of bone turnover during puberty. *Int J Adolesc Med Health* 2002, 14, 235–244.
- [35] Mokuda S, Sawada N, Matoba K, Yamada A, Onishi M, Okuda Y, Jouyama K, Murata Y, Takasugi K: Serum undercarboxylated osteocalcin level increases with 48 weeks of teriparatide treatment in pre-treated elderly rheumatoid arthritis patients who use anti-resorptive drugs. *J Endocrinol Invest* 2012, 35, 796–799.
- [36] Wang A, Ding X, Sheng S, Yao Z: Retinoic acid inhibits osteogenic differentiation of rat bone marrow stromal cells. *Biochem Biophys Res Commun* 2008, 375, 435–439.
- [37] Conaway HH, Persson E, Halén M, Granholm S, Svensson O, Pettersson U, Lie A, Lerner UH: Retinoids inhibit differentiation of hematopoietic osteoclast progenitors. *FASEB J* 2009, 23, 3526–3538.

### Address for correspondence:

Beata Nowak  
Department of Pharmacology Department  
Wroclaw Medical University  
Mikulicz-Radeckiego 2  
50-345 Wrocław  
Poland  
E-mail: beata.nowak@umed.wroc.pl

Conflict of interest: None declared

Received: 8.04.2015

Revised: 3.05.2015

Accepted: 25.05.2015