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Effects of curcumin on the skeletal system in rats

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Abstract:
There is increasing interest in the discovery of natural compounds that could favorably affect the skeletal system. Curcumin is a constituent of turmeric, a plant which has been used for centuries as a dietary spice and a traditional Indian medicine. Curcumin has been reported to affect differentiation, activity and the lifespan of osteoblasts and osteoclasts *in vitro*. The aim of the present study was to investigate the effects of curcumin on the skeletal system of rats *in vivo*.
Curcumin (10 mg/kg, *po* daily) was administered for four weeks to normal (non-ovariectomized) and bilaterally ovariectomized (estrogen-deficient) three-month-old female Wistar Cmd:(WI)WU rats. Ovariectomy was performed seven days before the start of curcumin administration. Bone mass, mineral and calcium content, macrometric and histomorphometric parameters, as well as the mechanical properties of the bone, were examined. Serum total cholesterol and estradiol levels were also determined. In rats with normal estrogen levels, curcumin decreased serum estradiol level and slightly increased cancellous bone formation, along with decreased mineralization. Estrogen deficiency induced osteoporotic changes in the skeletal system of the ovariectomized control rats. In ovariectomized rats, curcumin decreased body mass gain and serum total cholesterol level, slightly improved some bone histomorphometric parameters impaired by estrogen deficiency, but did not improve bone mineralization or mechanical properties.
In conclusion, the results of the present *in vivo* study in rats did not support the hypothesis that curcumin, at doses that are readily achievable through dietary intake, could be useful for the prevention or treatment of osteoporosis.

Key words:
curcumin, ovariectomy, osteoporosis, rats, skeletal system

Introduction

Curcumin is found in the rhizomes of the turmeric plant (*Curcuma longa* L.), a perennial herb belonging to the ginger family that is cultivated extensively in south and southeast tropical Asia. Chemically, curcumin (diferuloylmethane) is a plant polyphenol [30]. Its structure was first described by Miłobędzka et al. in 1910 [24].

Turmeric has been used for centuries as a dietary spice and as a traditional Indian medicine [1, 30]. Most of the activities associated with curcumin seem to be based on its ability to suppress inflammation [30]. Curcumin has been shown to exhibit anti-inflammatory, antioxidant, antiviral, antibacterial, antifungal, and anticancer activities in experimental conditions and in clinical settings [1, 4, 12, 20].

At the cellular level, curcumin modulates important molecular targets: transcription factors (such as nuclear factor-κB, activating protein-1, β-catenin, and peroxisome proliferator-activated receptor-γ), enzymes (cyclooxygenase-2, 5-lipoxygenase, inducible nitric oxide synthase), cell cycle proteins (cyclin D1 and p21), cytokines (tumor necrosis factor-α, interleukin-1,

interleukin-6, and chemokines), receptors (epidermal growth factor receptor, low density lipoprotein receptor, estrogen receptor-α), and cell surface adhesion molecules [1, 4, 12, 20, 30]. Due to these effects, curcumin is now considered to be a possible treatment for cancer, arthritis, diabetes, Crohn's disease, cardiovascular diseases, Alzheimer's disease, psoriasis, and other pathologies [1, 12, 30] such as osteoporosis [1, 3, 30]. However, the potential of curcumin has not been systematically examined through multi-center, randomized, double-blind, placebo-controlled clinical trials yet [1].

Because many of the curcumin targets mentioned above take part in the regulation of bone remodeling, curcumin may affect the skeletal system. In fact, the effects of curcumin on bone cells: osteoclasts (cells which resorb bone) and osteoblasts (cells responsible for bone formation), have previously been investigated *in vitro* [3, 5, 27, 28, 32, 36]. Therefore, curcumin effects on the skeletal system *in vivo* warranted further evaluation. Thus far, only two reports have been published. French et al. [11] examined the long-term effects of curcumin administration in aging ovariectomized rats and found no significant differences in the markers of bone turnover, mineral density or mechanical properties between the rats receiving curcumin and the ovariectomized controls [11]. Very recently, Hie et al. [13] reported on the bone resorption decreasing effect of curcumin in rats with streptozotocin-induced diabetes.

The aim of the present study was to investigate the effects of curcumin on the skeletal system of mature rats. We studied normal female rats and rats with estrogen deficiency (bilaterally ovariectomized) as a model of postmenopausal osteoporosis.

Materials and Methods

Experiments were carried out on mature (12- to 14-week-old) female Wistar Cmd:(WI)WU rats, which were fed a standard diet *ad libitum*. The rats were obtained from the Medical Research Center, Polish Academy of Sciences, Warszawa, Poland. The experimental procedures were approved by the Local Ethics Commission, Katowice, Poland. Curcumin (Curcumin from *Curcuma longa* (Turmeric)) was purchased from Sigma-Aldrich.

The animals were divided into four groups (n = 8): I) control rats, II) rats receiving curcumin (10 mg/kg, *po* daily), III) ovariectomized control rats, and IV) ovariectomized rats receiving curcumin (10 mg/kg, *po* daily).

Bilateral ovariectomy was performed seven days before the administration of curcumin started. Curcumin was administered by a stomach tube (*po*) once daily for four weeks, as a suspension, at a volume of 2 ml/kg. The control rats received the vehicle, distilled water, at the same volume. All of the animals were weighed three times a week.

One day before and on the last day that curcumin or the vehicle was administered, the animals were given tetracycline hydrochloride (20 mg/kg, *ip*) in order to mark the calcification front. The next day, blood samples were collected by heart puncture. The rats were fasted overnight before the blood collection. The right and left tibial and femoral bones as well as the L-4 vertebra were excised from sacrificed animals. The mass and macrometric parameters were determined (length, diameter of the diaphysis in the mid-length) for the left bones. The mass of the uterus and the thymus were also determined for each animal.

Serum total cholesterol and estradiol level

Total cholesterol concentrations in the serum were determined colorimetrically using a kit produced by Pointe Scientific Inc. Serum estradiol concentrations were determined by competitive ELISA using a kit produced by DRG Instruments GmbH. The blood samples were processed as previously described [39].

Bone histomorphometry

The right femoral and tibial bones were used to prepare histological specimens as previously described [7, 9, 15]. The histomorphometric measurements were made using an Optiphot-2 microscope (Nikon), connected through an RGB camera (Cohu) to a personal computer (program Lucia G 4.51, Laboratory Imaging), with final magnifications of 200 and 500 times. A lanameter microscope was also used (magnification 50 times).

In transverse cross-sections made from the tibial diaphysis, the periosteal and endosteal transverse growth, the width of endosteal osteoid, the area of the transverse cross-section of the cortical bone and the area of the transverse cross-section of the marrow

cavity were measured. In the longitudinal preparation from the femur, the width of the epiphyseal cartilage as well as the width of trabeculae in the epiphysis and metaphysis were measured.

Bone mechanical properties

Bone mechanical properties were assessed using the apparatus constructed at the Department of Pharmacology, Medical University of Silesia, in cooperation with Hottinger Baldwin Messtechnik GmbH. The mechanical properties of the whole femur and the femoral neck were examined as previously described [8, 15].

The mechanical properties of the whole left femur were studied using a bending test with three-point loading. The load was applied perpendicularly to the long axis of the femur in the mid-length of the bone supported on its epiphyses. The load-deformation curves, obtained for each bone, representing the relationship between the load applied to the bone and deformation in response to the load, were analyzed.

The load-deformation curves can be divided into the elastic deformation region and the plastic deformation region. Within the elastic deformation region, the slope of the load-deformation curve, representing the extrinsic stiffness of bone, was tested. Within the plastic deformation region, the ultimate and breaking load (maximum load sustained by the bone and the load at fracture) as well as the deformation caused by the ultimate and breaking load were determined.

The mechanical properties of the neck of the right femur were studied using a compression test. The bone was prepared for the measurement by fixing the diaphysis, which was cut in 1.7 cm from the proximal end of the femur, in a methacrylate plate. The load was applied to the head of the femur along the long axis of the femur. The load that caused the femoral neck to fracture was determined.

The L-4 vertebra, left tibia and femur were lyophilized for seven days to determine the dehydrated bone mass. To determine the mineral content of the bone, the L-4 vertebra, left tibia and femur were mineralized at 640°C for 48 h and weighed. Then, the calcium content in mineralized bones, dissolved in 6 M HCl, was determined colorimetrically using a kit produced by Pointe Scientific Inc.

Statistical analysis

The results are presented as the mean ± SEM. One-way ANOVAs followed by Duncan's test were used to evaluate the significance of the results. The results obtained in the non-ovariectomized rats receiving curcumin were compared to the results of the non-ovariectomized control rats. The results obtained in the ovariectomized control rats were compared to the results of the non-ovariectomized control rats and the results of ovariectomized rats treated with curcumin.

Tab. 1. The effects of curcumin (10 mg/kg, *po* daily), administered for four weeks, on the mass of the uterus, thymus and body mass gain in non-ovariectomized and ovariectomized rats

Parameter/Group	Non-ovariectomized control rats	Non-ovariectomized rats treated with curcumin	Ovariectomized control rats	Ovariectomized rats treated with curcumin
Body mass at the start of curcumin administration (g)	205.5 ± 7.2	206.1 ± 3.6	220.6 ± 3.9	219.1 ± 4.3
Body mass gain after four weeks (g)	19.9 ± 1.7	16.5 ± 1.3	49.2 ± 2.3***	40.5 ± 1.3*** ^{00}
Uterus mass (g)	0.488 ± 0.085	0.491 ± 0.051	0.099 ± 0.007***	0.089 ± 0.008***
Thymus mass (g)	0.303 ± 0.018	0.284 ± 0.018	0.746 ± 0.032***	0.681 ± 0.032***
Serum estradiol (pg/ml)	5.35 ± 0.45	1.80 ± 0.86***	3.01 ± 0.51*	1.32 ± 0.47***
Serum total cholesterol (mg/dl)	63.92 ± 5.55	63.35 ± 3.80	94.67 ± 4.15***	80.14 ± 4.00* ^{0}

The results are presented as the means ± SEM (n = 8). One-way ANOVAs followed by Duncan's test were used to evaluate the significance of the results. * $p < 0.05$, *** $p < 0.001$ – significantly different from the non-ovariectomized control rats, ^{0} $p < 0.05$, ^{00} $p < 0.01$ – significantly different from the ovariectomized control rats

Tab. 2. The effects of curcumin (10 mg/kg, *po* daily), administered for four weeks, on bone mass and mineralization in non-ovariectomized and ovariectomized rats

Parameter/Group		Non-ovariectomized control rats	Non-ovariectomized rats treated with curcumin	Ovariectomized control rats	Ovariectomized rats treated with curcumin
Bone mass/body mass (g/100 g of body mass)	Femur	0.280 ± 0.003	0.283 ± 0.005	0.249 ± 0.005***	0.259 ± 0.004**
	L-4 vertebra	0.078 ± 0.002	0.085 ± 0.002*	0.080 ± 0.001	0.078 ± 0.002
Lyophilized bone mass/ body mass (g/100 g of body mass)	Femur	0.191 ± 0.002	0.196 ± 0.003	0.166 ± 0.003***	0.167 ± 0.002***
	L-4 vertebra	0.052 ± 0.001	0.057 ± 0.001**	0.050 ± 0.001	0.048 ± 0.001*
Bone mineral mass/ body mass (g/100 g of body mass)	Femur	0.119 ± 0.002	0.122 ± 0.002	0.102 ± 0.002***	0.102 ± 0.001***
	L-4 vertebra	0.031 ± 0.001	0.032 ± 0.001	0.028 ± 0.001***	0.027 ± 0.001***
Bone mineral mass/ bone mass ratio	Femur	0.425 ± 0.004	0.431 ± 0.004	0.408 ± 0.002**	0.393 ± 0.006*** ^{0}
	L-4 vertebra	0.392 ± 0.007	0.377 ± 0.007	0.344 ± 0.007***	0.351 ± 0.006***
Bone mineral mass/ lyophilized bone mass ratio	Femur	0.622 ± 0.005	0.621 ± 0.003	0.611 ± 0.002	0.607 ± 0.005*
	L-4 vertebra	0.589 ± 0.006	0.560 ± 0.005**	0.551 ± 0.005***	0.566 ± 0.008*
Femur length (mm)		32.0 ± 0.2	32.2 ± 0.3	32.8 ± 0.1**	32.7 ± 0.1*
Femur diameter (mm)		3.19 ± 0.06	3.22 ± 0.05	3.28 ± 0.04	3.28 ± 0.03

The results are presented as the means ± SEM (n = 8). One-way ANOVAs followed by Duncan's test were used to evaluate the significance of the results. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ – significantly different from the non-ovariectomized control rats, ^{0} $p < 0.05$ – significantly different from the ovariectomized control rats

Results

Effects of curcumin on the skeletal system of non-ovariectomized rats

Administration of curcumin at a dose of 10 mg/kg, *po* daily for four weeks to normal female rats did not affect the body mass gain, total cholesterol level, the uterus mass or the thymic mass (Tab. 1). However, curcumin treatment statistically significantly decreased the serum estradiol level. Curcumin did not affect the mass, bone mineral mass, calcium content or macrometric parameters of the long bones that were investigated (the tibia and femur; data for the tibia not shown). The mass of the L-4 vertebra immediately after isolation and after lyophilization tended to increase after administration of curcumin in comparison with the control rats (data not shown). The ratio of the vertebral mass to the body mass also significantly increased (Tab. 2). However, curcumin did not affect the bone mineral mass in the vertebra. Consequently, the ratio of bone mineral mass to lyophilized bone mass significantly decreased after administration of curcumin. Calcium content in the bone mineral of the vertebra was not significantly affected (data not shown).

Curcumin did not affect the investigated histomorphometric parameters of the cortical bone in the tibial diaphysis (Tab. 3). In the cancellous bone of the femur, curcumin administration caused statistically significant increases in the width of epiphyseal and metaphyseal trabeculae compared to control rats.

Curcumin treatment did not affect the mechanical properties of the femur in normal female rats (Tab. 4).

Effects of estrogen deficiency on the skeletal system of female rats

The bilaterally ovariectomized rats were estrogen-deficient, as demonstrated by statistically significant decreases in serum estradiol level and the uterus mass as well as increased thymic mass compared to the non-ovariectomized controls. The body mass gain and total cholesterol level in ovariectomized control rats

Tab. 3. The effects of curcumin (10 mg/kg, *po* daily), administered for four weeks, on bone histomorphometric parameters in non-ovariectomized and ovariectomized rats

Parameter/Group	Non-ovariectomized control rats	Non-ovariectomized rats treated with curcumin	Ovariectomized control rats	Ovariectomized rats treated with curcumin
Width of endosteal osteoid in the tibia (μm)	9.46 ± 0.33	9.67 ± 0.13	12.84 ± 0.33***	10.89 ± 0.20*** ^{000}
Periosteal transverse growth of the tibia (μm)	31.25 ± 3.34	31.40 ± 1.75	46.45 ± 3.95**	37.93 ± 1.71^{0}
Endosteal transverse growth of the tibia (μm)	21.49 ± 2.12	20.26 ± 0.54	30.70 ± 2.50**	25.83 ± 2.23
Transverse cross-sectional area of the cortical bone in the tibial diaphysis (mm^2)	3.333 ± 0.104	3.474 ± 0.076	3.791 ± 0.139**	3.561 ± 0.096
Transverse cross-sectional area of the tibial marrow cavity (mm^2)	0.897 ± 0.059	1.002 ± 0.046	1.072 ± 0.052	0.986 ± 0.044
Width of epiphyseal trabeculae in the femur (μm)	65.48 ± 0.56	71.02 ± 0.55***	61.83 ± 0.63***	63.28 ± 0.59*
Width of metaphyseal trabeculae in the femur (μm)	48.58 ± 0.25	52.30 ± 0.43***	43.34 ± 0.32***	46.08 ± 0.44*** ^{000}
Width of epiphyseal cartilage in the femur (μm)	42.26 ± 3.40	40.41 ± 1.12	53.07 ± 2.48*	57.63 ± 3.47**

The results are presented as the means ± SEM (n = 8). One-way ANOVAs followed by Duncan's test were used to evaluate the significance of the results. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ – significantly different from the non-ovariectomized control rats, ^{0} $p < 0.05$, ^{000} $p < 0.001$ – significantly different from the ovariectomized control rats

were significantly higher than the values in the non-ovariectomized controls (Tab. 1).

In the ovariectomized control rats, estrogen deficiency caused statistically significant decreases in the ratio of bone mass to body mass in the long bones, the ratio of bone mineral mass to body mass and the ratio of bone mineral mass to bone mass in the long bones as well as the L-4 vertebra compared to non-ovariectomized controls (Tab. 2). Calcium content in the bone mineral was not significantly affected (data not shown).

Estrogen deficiency in the ovariectomized control rats caused significant increases in the width of the endosteal osteoid, the periosteal and endosteal transverse growths as well as the transverse cross-sectional area of the cortical bone in the tibia and an insignificant increase in the transverse cross-sectional area of the tibial marrow cavity compared to non-ovariectomized controls (Tab. 3). In the cancellous bone of the femur, the width of the epiphyseal and metaphyseal trabeculae significantly decreased, and the width of the epiphyseal cartilage was increased.

Mechanical properties of the whole femur in the ovariectomized control rats in the present study did not statistically significantly differ from those of the non-ovariectomized control rats. Only the load causing the fracture of the femoral neck tended to decrease (Tab. 4).

Effects of curcumin on the skeletal system of ovariectomized rats

Administration of curcumin at a dose of 10 mg/kg, *po* daily for four weeks to the ovariectomized rats did not affect the mass of the uterus and the thymus, but the body mass gain was significantly inhibited and total cholesterol level decreased compared to the ovariectomized control rats (Tab. 1). Curcumin did not significantly affect the serum estradiol level in ovariectomized rats.

Curcumin did not affect the investigated parameters of bone mass and mineralization in ovariectomized rats (with the exception of decreasing the ratio of bone mineral mass to bone mass in the femur, Tab. 2).

Tab. 4. The effects of curcumin (10 mg/kg, *po* daily), administered for four weeks, on the mechanical properties of the femur in non-ovariectomized and ovariectomized rats

Parameter/Group		Non-ovariectomized control rats	Non-ovariectomized rats treated with curcumin	Ovariectomized control rats	Ovariectomized rats treated with curcumin
Extrinsic stiffness (N/mm)		234.7 ± 12.6	229.6 ± 6.5	222.1 ± 11.7	220.7 ± 8.9
Load (N)	Ultimate	101.3 ± 3.5	101.1 ± 2.0	104.6 ± 2.5	98.0 ± 3.8
	Breaking	91.0 ± 4.0	90.0 ± 4.5	91.5 ± 4.4	82.0 ± 5.8
Deformation (mm)	At ultimate load	0.512 ± 0.017	0.537 ± 0.022	0.569 ± 0.025	0.542 ± 0.028
	At breaking load	0.577 ± 0.041	0.610 ± 0.044	0.686 ± 0.038	0.683 ± 0.061
Load at fracture of the femoral neck (N)		90.9 ± 4.9	94.6 ± 4.9	78.6 ± 5.4	74.3 ± 4.4*

The results are presented as the means ± SEM (n = 8). One-way ANOVAs followed by Duncan's test were used to evaluate the significance of the results, * $p < 0.05$ – significantly different from the non-ovariectomized control rats

Curcumin slightly improved some bone histomorphometric parameters that were impaired by estrogen deficiency: curcumin counteracted the increase in the width of the endosteal osteoid and the periosteal transverse growth in the tibia and the decrease in the width of metaphyseal trabeculae in the femur (Tab. 3).

Curcumin did not affect the investigated bone mechanical properties of the whole femur in the ovariectomized rats (Tab. 4). The strength of the femoral neck in ovariectomized rats treated with curcumin was significantly lower than the strength of the femoral neck in the non-ovariectomized control rats.

Discussion

The incidence of osteoporosis-related hip fractures is significantly lower in Southern and Eastern Asian women than in Western women [21]. This fact is often attributed to Asian diets, which are typically rich in soya based foods, as it has been hypothesized that an isoflavone-rich diet may have bone protective effects [21, 23]. However, it seems likely that high intake of turmeric, containing curcumin, may also be of value. As mentioned above, curcumin is considered to be a potential antiosteoporotic agent [1, 3, 30].

There are several reports concerning the effects of curcumin on the differentiation, activity and death of osteoclasts and osteoblasts.

Curcumin significantly inhibited the proliferation of murine osteoclast precursor cells, specifically, the granulocyte-macrophage colony-forming unit [14]. Curcumin inhibited receptor activator of nuclear factor-κB ligand (RANKL)-induced osteoclastogenesis in murine monocytic cells RAW 264.7 and in bone marrow-derived macrophages [3]. It also inhibited parathormone-stimulated formation of osteoclast-like cells in mouse bone marrow cultures [36]. A suppressive effect of curcumin on human osteoclast differentiation and function was reported [32]. Curcumin also had a potent stimulatory effect on apoptosis in rabbit osteoclasts. This curcumin stimulation of osteoclast apoptosis was dose- and treatment time-dependent [28]. Although these data indicated decreasing numbers of osteoclasts, which should lead to inhibition of bone resorption, the data on the effects of curcumin on bone resorption are ambiguous. Curcumin was reported to strongly inhibit bone resorption by rabbit osteoclasts [28] and *Porphyromonas gingivalis* fimbria-stimulated bone resorption in the mouse calvarial system [26]; however, curcumin did not inhibit the stimulatory effect of parathormone on bone resorption in rat bone tissue cultures *in vitro* [36]. Curcumin also did not affect the diaphyseal calcium content in tissue cultures *in vitro* [36].

There are few reports concerning the effects of curcumin on osteoblasts and bone formation. Curcumin markedly inhibited the proliferation of rat calvarial osteoblastic cells [27]. In human osteoblast cell line (HFOb 1.19), curcumin treatment induced two distinct types of cell death: apoptosis at concentrations of

12.5–25 μM and necrosis at concentrations greater than 50 μM [5]. However, curcumin can both stimulate and inhibit apoptotic signaling, and the treatment period as well as the dosage may determine the effects of curcumin on various cell types [5].

Taken together, there was a need to verify the effects of curcumin on the skeletal system in an *in vivo* experimental model.

Curcumin has been consumed as a dietary spice at doses of up to 100 mg/day [30], i.e., approximately 1.5 mg/kg/day, assuming that human body mass is 65–70 kg. Although phase I clinical trials indicated that humans can tolerate curcumin even at a dose of 8 g/day [6], and, in experimental conditions, curcumin is often used at high doses [13, for example], in the present study, we used the moderate dose of 10 mg/kg daily. We chose this dose because we wanted to examine the skeletal effects of curcumin administered at reasonable doses that moderately exceed the readily achievable dietary dose. We also thought that a potential antiosteoporotic drug, as a long-term treatment, would only be of value if it was effective at doses that are safe and easily acceptable for patients. Our notion is consistent with that presented by López-Lázaro [20] on curcumin use in cancer prevention. Although the use of curcumin at the maximum tolerated dose is a valid approach in cancer chemotherapy, it may not be appropriate for cancer prevention because it may produce toxicity in the long term. So, doses of curcumin equivalent to those found in diets rich in turmeric should be used in future cancer chemoprevention clinical trials [20]. The duration of curcumin treatment (four weeks) was sufficient to develop significant skeletal changes both due to estrogen deficiency and drug treatment in our previous studies [7–10, 15].

In mature non-ovariectomized female rats, curcumin affected only the cancellous bone, increasing the width of femoral trabeculae. Consistently, curcumin increased the mass of the vertebra (containing mainly cancellous bone). However, the quality of bone decreased. Bone mineralization in the vertebra of normal rats was impaired because the ratio of bone mineral mass to lyophilized bone mass was significantly lower than the ratio in the control rats. This observation was consistent with the results of Notoya et al. [27], who demonstrated that curcumin reduced the rate of deposition of calcium and the formation of mineralized nodules by rat osteoblastic cells *in vitro*. However, curcumin seemed to increase cancellous bone formation *in vivo*, which is inconsistent with reports on the effect of curcumin on the proliferation and apoptosis of osteoblasts *in vitro* [5, 27]. The increased trabeculae width could result also from inhibition of bone resorption, which agrees with the demonstrated effects of curcumin on osteoclastogenesis *in vitro* [3, 32, 36] as well as the recent report of Hie et al. [13] on the suppressive effect of high-dose curcumin (approximately 120 mg/day for 14 days) on bone resorptive activity in rats with streptozotocin-induced diabetes.

In the present study, we observed a statistically significant decrease in the serum estradiol levels caused by curcumin administration to rats with normal estrogen levels (non-ovariectomized). To our knowledge, estradiol decreasing activity has not been reported for curcumin to date. The decreased estrogen level, which we observed after a four week period of curcumin administration, could have contributed to the impairment of bone mineralization in the vertebra. However, the overall changes caused by curcumin administration differed from the changes induced by estrogen deficiency from bilateral ovariectomy. The model of bilaterally ovariectomized rats mimics the accelerated bone loss observed in postmenopausal women due to estrogen deficiency [16]. Estrogen deficiency leads to increased rates of bone remodeling (both resorption and formation) with an imbalance between bone resorption and formation that favors the former [22].

In the present study, estrogen deficiency induced osteoporotic changes in the skeletal system of the ovariectomized control rats, which manifested as decreased bone mass and bone mineral mass, expressed as their ratios to the body mass. The mineralization of bones was significantly worse in the ovariectomized rats because the ratio of bone mineral mass to bone mass was significantly decreased. The histomorphometric measurements demonstrated that bone resorption was intensified in the cancellous bone (decreased width of trabeculae) and in the cortical bone (increased transverse cross-sectional area of bone marrow cavity). Increases in the width of osteoid, periosteal and endosteal transverse growths as well as the transverse cross-sectional area of the cortical bone in the tibia indicated that there was also intensified bone formation in the cortical bone.

Estrogen deficiency also caused an increase in body mass gain, accompanied by an increased total cholesterol level in ovariectomized rats. Those effects were counteracted by curcumin administration, which

is consistent with previous reports on the hypocholesterolemic properties of curcumin [2, 17]. However, curcumin did not exert any favorable effect on bone mineralization, which was impaired by estrogen-deficiency in the ovariectomized rats. A slight favorable effect was observed in the cancellous bone of the femur (increase in the width of trabeculae). This effect on bone trabeculae was likely an antiresorptive. However, the effect was not confirmed by improvements in the mechanical properties of the femoral neck, which mainly consists of cancellous bone. In fact, the strength of the femoral neck in ovariectomized rats treated with curcumin was significantly lower than the strength of the femoral neck in non-ovariectomized control rats. The decrease in the width of endosteal osteoid in the tibia of the curcumin-treated ovariectomized rats could be the result of inhibition of bone matrix formation that led to the inhibition of transverse growth. These slight effects of curcumin on the bone histomorphometric parameters are consistent with previous *in vitro* studies [3, 5, 26–28]. However, there was lack of favorable effects of curcumin on bone mineralization and mechanical properties in the ovariectomized rats. Taken together, the favorable effects of curcumin (10 mg/kg, *po*) on the bones of estrogen-deficient rats were negligible. This conclusion was consistent with results of a study by French et al. [11], who did not find significant changes in the skeletal system of much older ovariectomized rats, except for an increase in the size of the femur after a six month period of curcumin administration (15 mg/day in the diet). However, they did observe a significant positive correlation between curcumin dose and the energy to fracture of the femur [11].

One possible explanation for the discrepancies between the *in vitro* and *in vivo* effects of low-dose curcumin on the skeletal system is that by targeting numerous molecules in different cells, curcumin leads to specific effects in bone cells *in vitro*, but different effects may occur *in vivo*. For example, in murine monocytic cells RAW 264.7, curcumin (0.5×10^{-5} M and 10^{-5} M) prevented osteoclast formation stimulated by RANKL, inhibiting RANKL-induced IκB kinase activation, which led to the suppression of nuclear factor-κB activation [3]. Similarly, in human preosteoclast cultures, inhibition of osteoclastogenesis was accompanied with the inhibition of IκB phosphorylation and nuclear factor-κB activation [32]. However, nuclear factor-κB is a ubiquitous, inducible, nuclear transcriptional activator that acts in many different cell types [19] and its suppression in cells other than osteoclast precursors may have differential effects.

Another problem may be the concentration of curcumin in the bone environment of the rats; the local concentration of curcumin may have been lower than the amount reported in the *in vitro* studies because the oral bioavailability of curcumin in rats is about 1% and the elimination half-life of curcumin is rather short [38]. In most of the *in vitro* studies on bone cell number and activity, the effects were observed with curcumin concentrations of at least micromolar range [3, 26–28, 32]. In humans, very high curcumin doses, 4–8 g, are necessary to achieve average peak serum concentrations of $0.51–1.77 \times 10^{-6}$ M, respectively [6]. However, curcumin significantly inhibited parathormone-stimulated formation of osteoclast-like cells in mouse bone marrow cultures at 10^{-8} M [36]. In the mouse model of collagen-induced arthritis, curcumin (4 and 20 mg/kg, *po*) exhibited anti-arthritis effect in the joints, indicating that the concentration of curcumin reached there effective levels [25]. Similar low oral doses were reported to be effective in ameliorating diabetic nephropathy [29], delaying diabetic cataract [31], inhibiting chemical-induced liver inflammation and fibrosis [33], exerting antidepressant effects in models of depression [35] and reversing the effects of chronic stress on the behavior and the hypothalamic-pituitary-adrenal axis [34] in rats. It is possible that not only curcumin, but also its metabolites, may exert biological activity. Metabolites of dietary polyphenols, even if less potent than the parent compounds, may contribute to the overall biological activity of the compound *in vivo* [37].

Nevertheless, the aim of the present study was to investigate curcumin treatment with a moderate dose, and the experiments demonstrated that the utilized dose of curcumin affected the rat skeletal system, serum estradiol, total cholesterol and body mass gain. The effects on the skeletal system were significant, but ambiguous.

There is a possibility that turmeric, which is routinely used in diet, may express better effects that curcumin alone. Turmeric contains three analogs of curcumin (curcumin, demethoxycurcumin and bisdemethoxycurcumin). Yet, it is unclear whether the analogs exhibit equal activity [1]. Curcumin, which comprises 0.3–5.4% of raw turmeric, is the best researched constituent of turmeric [18]. The remaining

constituents are volatile oils (turmerone, atlantone, and zingiberone), sugars, proteins, and resins [18] which may affect curcumin absorption and metabolism. Suryanarayana et al. [31] observed that turmeric (0.5% in the diet) was more effective in delaying streptozotocin-induced cataract than a corresponding level of curcumin (0.01% in the diet) [31]. This issue needs to be investigated further.

In conclusion, the results of the present *in vivo* study in rats do not support the hypothesis that curcumin at a dietary achievable dose may be useful for the prevention or treatment of osteoporosis.

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