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ORIGINAL ARTICLE

Lycopene influences osteoblast functional activity and prevents femur bone loss in female rats submitted to an experimental model of osteoporosis

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Abstract

Antioxidant properties of several nutrients may influence bone metabolism, affording protection against damaging effects caused by oxidative stress. Thus, we hypothesized that lycopene may benefit bone tissue metabolism and functional activity of osteoblastic cells from bone marrow of osteoporotic female rats. Wistar rats were ovariectomized and paired with sham animals. In vitro evaluations were performed after 60 days of surgery, when cells were cultured in osteogenic medium and divided in control (C), ovariectomized (OVX) and ovariectomized + 1 µmol/L lycopene (OVXL) groups. Besides, in vivo studies were carried out to evaluate femur bone remodeling by histological and histomorphometric analyses after daily intake of 10 mg/kg of lycopene for 30 and 60 days after ovariectomy. Cell proliferation was significantly higher in OVX and OVXL groups after 10 days of culture. Alkaline phosphatase activity (ALP) was higher in OVXL group in later periods of cell culture, whereas its in situ detection was higher for this group in all experimental periods; nevertheless, mineralization did not show significant differences among the groups. There was a significant upregulation of genes *Sp7*, *Runx2* and *Bsp* after 3 days and genes *Runx2* and *Bglap* after 10 days from OVXL when compared to OVX. In vivo results demonstrated that daily intake of 10 mg/kg of lycopene for 60 days decreased bone loss in femur epiphysis in ovariectomized rats by maintaining trabecular bone similar to controls. Data obtained suggest that lycopene might benefit the functional activity of osteoblastic cells from ovariectomized rats, as well as avoid further bone resorption.

Keywords Osteoporosis · Lycopene · Osteoblasts · Genes · Histomorphometry

Introduction

Osteoporosis is a multifactorial disease which has a complex pathophysiology potentially caused by genetic, endocrine disorders, and nutritional factors [1], with no noticeable symptoms until the bone fractures [2]. Despite the availability of many drugs that reduce fracture risk, osteoporosis remains underdiagnosed and undertreated [3]. This metabolic condition induces to a higher bone matrix resorption and oxidative stress caused by estrogen deficiency, which worsens its prognosis [4–6]. Oxidative stress products such as reactive oxygen species (ROS) decrease osteoblastogenesis and inhibit bone formation [7–9]. Nutrition is an important component for treating those with osteoporosis and dietary supplements may be the most important complements to drugs for combination therapy [10]. Considering osteoporosis high prevalence, antioxidant products like lycopene have been studied for its beneficial effects [11, 12]. This carotenoid is notable for its antioxidant activity [12] and it is found in foods with yellow, orange or red pigmentation such as tomatoes, watermelon, peppers, and many others [13, 14]. Its beneficial effect under chronic diseases is noticed because of its ability to eliminate more oxygen molecules than other carotenoids [15, 16], demonstrating its potential as an antioxidant substance. Only few studies about the effects of lycopene on osteoblasts have been reported and most of them show discrepancies in the influence of lycopene on cell

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proliferation or alkaline phosphatase activity when cultured with human (SaOS-2) and mice (MC3T3-E1) cell lines [17, 18]. In the present investigation, we utilized a rat osteoporosis model to evaluate the effects of lycopene on in vitro cell metabolism and on bone formation in vivo. Therefore, two hypotheses for this investigation were addressed: (1) that in vitro administration of lycopene may distinctively influence cell proliferation, alkaline phosphatase activity and potential of mineralization as well as expression of genes associated with bone metabolism in osteoblastic cells from ovariectomized rats; (2) that in vivo administration of lycopene may impair femur bone loss in ovariectomized rats.

Materials and methods

The experimental protocol was approved by Ethics Committee for Animal Experimentation of the University of São Paulo, Ribeirão Preto, São Paulo, Brazil (permit number 2014.1.921.58.6). All applicable institutional and/or national guidelines for the care and use of animals were followed. Thirty-six Wistar female rats weighting approximately 300 g were submitted to bilateral ovariectomy to induce osteoporosis ($n = 18$) or sham operated to serve as control ($n = 18$). From the total of these animals, 18 (9 sham and 9 ovariectomized) were used for cell isolation from femur bone marrow 8 weeks after sham/ovariectomy surgery to perform cell culture assays in contact with lycopene. The other 18 animals received, immediately after sham/ovariectomy surgery, daily administration of lycopene by gavage for 8 weeks until killing, to perform histomorphometric analysis. The animals were selected from the Central Vivarium of the University (USP-Ribeirão Preto) and were kept in a polyethylene box with three animals in each box. The temperature was maintained between 23 and 24 °C with a 12-h cycle of light per day. Throughout the experiment, the animals received solid diet and water ad libitum.

Ovariectomy and sham surgery

The rats were weighted and anesthetized by an intramuscular injection of xylazine (10 mg/kg) and ketamine (75 mg/kg) (Agibrands, SP, Brazil). After trichotomy and antisepsis, the ovaries were excised. The tissue suture was performed with silk thread 4.0 (Ethicon, Johnson & Johnson, São José dos Campos, SP, Brazil). Each animal received an intramuscular injection of 0.1 mL/100 g weight of small-size veterinary pentabiotic (Pentabiotic Veterinário Pequeno Porte—Fort Dodge®, Campinas, SP, Brazil) followed by 0.2 mL/100 g of Banamine® injectable (Injetável Pet—Schering-Plough, Cotia, SP, Brazil). The success of ovariectomy was evaluated by analyzing the estrous cycle 2 weeks after the surgical procedure and the atrophy of uterine horns during the euthanasia of the animals. Control animals (sham) were submitted only to ovary exposition to the environment followed by its replacement inside the abdomen. The euthanasia was performed after 60 days and the femurs were collected to isolate osteoblastic cells as described below. The osteoporosis model is in agreement with other reports in the literature [19].

Cell culture

In accordance to previous published protocols [20], osteoblastic cells obtained from femur medullary canals of eighteen female rats divided into sham and ovariectomized groups were cultured in growth medium αMEM (Gibco) supplemented with 10% fetal bovine serum (Gibco), 10-7 M dexamethasone (Sigma), 5 µg/mL ascorbic acid, 0.3 µg/mL fungizone (Gibco), 50 µg/mL gentamycin (Gibco), and 2.16 g/mL β-glycerophosphate. After reaching sub-confluence, the cells were seeded at a concentration of 2×10^4 cells per well in cell culture 24-well plates, and divided into 3 experimental groups: Control (C), ovariectomized (OVX) and ovariectomized with lycopene (OVXL). The lycopene (Sigma-Aldrich®, St. Louis, Missouri, USA) was presented in powder form and mixed in the above-mentioned culture medium to reach a concentration of 1 µmol/L. The dilution was prepared on each culture medium change, which occurred every 2 days.

Cell proliferation

Cell proliferation was assessed by MTT assay (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) at 7, 10, and 14 days. To this end, cells were incubated with 10% MTT (5 mg/mL) in culture medium at 37 °C for 4 h. The medium was then aspirated from the well, and 1 mL of isopropanol (0.04 N HCl in isopropanol) was added to each well. The plates were placed on a shaker for 5 min and 200 µL of this solution was transferred to a 96-well plate. The optical density was read at 570 nm (µQuant, BioTek Instruments, Winooski, VT, USA).

In situ alkaline phosphatase assay

As already formerly described [21], analysis of the enzyme alkaline phosphatase in situ was performed after 7, 10 and 14 days. Cells were washed twice with PBS at 37 °C. Following, 320 mg of Triz (Sigma) was dissolved in 20 mL of deionized water and 7 mg of Fast Red (Sigma). After removal of 2 mL, 8 mg of naphthol was added (Sigma) in 2 mL of dimethyl formamide (Merck) to obtain the work solution. Finally, cells were incubated in 1 mL of this solution in a humidified atmosphere at 37 °C with 5% CO_2 for

30 min and then removed from the wells for subsequent analysis.

Alkaline phosphatase activity

After 10 and 14 days of cell culture, the medium was removed from the plates and the wells were washed three times with PBS (Gibco) heated at 37 °C. The wells were then filled with 1 mL of 0.1% sodium lauryl sulfate solution (Sigma) for 30 min. Activity of ALP was determined by the release of thymolphthalein by the hydrolysis of the substrate thymolphthalein monophosphate, using the commercial kit (Labtest Diagnostica SA, Lagoa Santa, MG, Brazil) and following the manufacturer's instructions. The absorbance was determined in spectrophotometer (Bio-Tek) (590 nm). Total protein content normalized the ALP activity results.

Mineralized matrix formation

Mineralized matrix formation was detected at day 17 by Alizarin Red S (Sigma-Aldrich) staining for areas rich in calcium. Attached cells were fixed in 10% formalin at 4 °C, for 2 h. After fixation, specimens were dehydrated through a graded series of alcohol, which was followed by staining with 2% Alizarin Red S, pH 4.2, for 10 min. The calcium content was evaluated with a colorimetric method formerly described [22].

Quantitative gene expression (real-time PCR)

Quantitative expression of alkaline phosphatase (*Alpl*), osteocalcin (*Bglap*), osteoprotegerin (*Opg*), transcription factor *Sp7/Osterix*, runt-related transcription factor 2 (*Runx2*) and bone sialoprotein (*Bsp*) was performed after 7 and 10 days by quantitative real-time polymerase reaction (rt-PCR) using a TaqMan probe system and StepOne™ System (Thermofisher). Table 1 lists the oligonucleotide primers used in qRT-PCRs primers for mRNAs. To each reaction, 5 µL of TaqMan Universal PCR Master Mix-No AmpErase UNG (2×), 0.5 µL of TaqMan probes for genes of interest (20× TaqMan Gene Expression Assay Mix) and 11.25 ηg of cDNA were added. The reactions were performed with 2 min at 50 °C, 10 min at 95 °C, forty cycles of 15 s at 95 °C and 1 min at 60 °C. Results were analyzed with Ct value (cycle threshold—or threshold cycle) and all samples were subjected to reactions for the mRNA detection of β-actin constitutive gene expression, which was used to normalize the expression levels of the target gene samples. Normalization and relative quantification of gene expression were performed by 2-$\Delta\Delta C_T$ methods.

Table 1 TaqMan probe for the evaluated genes

Gene	Gene name	Essay identification
Actb	β-Actin	Rn4352931
Alpl	Alkaline phosphatase	Rn01516028_m1
Bglap	Bone gamma-carboxyglutamate protein (osteocalcina/Oc)	Rn00566386_g1
Sp7	Osterix	Rn01761789_m1
Runx2	Runt-related transcription factor 2	Rn01512298_m1
Bsp	Bone sialoprotein	Rn00561414_m1

In vivo administration of lycopene

The other eighteen rats designed to in vivo study were divided into lycopene-supplemented groups for sham and ovariectomized groups, which were given lycopene (10 mg/kg body weight per day) dissolved in filtrated water by daily intragastric administration for the experimental periods of 30 and 60 days ($n = 3$ for each period). Sham and ovariectomized groups did not receive lycopene, but instead, were given the same volume of filtrated water without lycopene treatment until killing. The group that received 30 days of lycopene had the administration substituted for filtrated water for the other 30 days until killing.

Histological processing

After the rats were euthanized, femurs were excised from each animal and were fixed in buffered (pH 7) 10% formaldehyde solution for 24 h. The specimens were then washed in running water and decalcified by a solution containing 20% sodium citrate and 30% formic acid for 6 days. This solution was replaced every 2 days and neutralized with 5% sodium sulfate. The samples were dehydrated in gradual exposure to ethanol (70% overnight, followed by 80, 85, 90 and 95% for 2 hours each, until 100%). The specimens were then processed with xylol and embedded in paraffin. Longitudinal semiserial sections, 6 µm thick, were stained with hematoxylin and eosin. The analysis was carried out using a Leica DM4000B light microscope (Leica, Bensheim, Germany) outfitted with a Leica DFC310FX digital camera (Leica, Bensheim, Germany).

Histomorphometric analysis

Quantitative analysis was performed by an 80-point grid applied on the images utilizing software ImageJ (version 1.46 plugin/grid). The points localized on trabecular bone and adipocytes were counted. The number of points counted was multiplied by the total points of the test system and is represented in percentage (%).

Statistical analysis

Results were analyzed by analysis of variance (ANOVA) or non-parametric Kruskal–Wallis, with the level of significance set at 5% ($p \leq 0.05$) using the software GraphPad Prism.

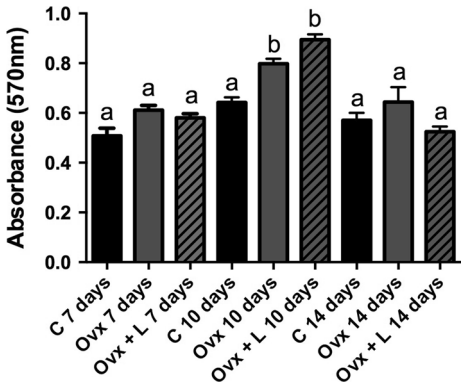

Fig. 1 Cell proliferation of osteoblastic cells from bone marrow of control (C), ovariectomized (OVX) and ovariectomized + in vitro lycopene (OVXL) at 3, 7 and 10 days of cell culture, as assessed by colorimetric assay (MTT). One-way ANOVA statistical test of with significance for $p < 0.05$. Different letters mean statistical difference (sample of 5 wells for each experimental group)

Results

Cell proliferation

Figure 1 shows that cell proliferation had a significant increase after 10 days for OVX and OVX_L experimental groups ($p < 0.0001$), with a subsequent decrease at 14 days of culture for all groups ($p < 0.0001$). At 7 and 14 days, there were no significative differences among experimental groups ($p = 0.1550$).

Alkaline phosphatase in situ assay

In situ detection of alkaline phosphatase shown in Fig. 2 was higher in group OVXL when compared to other groups in all experimental periods.

Detection of ALP

Figure 3 shows that ALP activity at 10 days was very low and similar among the groups ($p = 0.0755$). Nevertheless, at 14 days, it can be seen that group OVXL revealed higher ALP activity when compared to groups C and OVX ($p = 0.0024$).

Mineralized matrix formation

Extracellular calcium deposit at 17 days did not show significative difference among the groups ($p < 0.1330$) (Fig. 4).

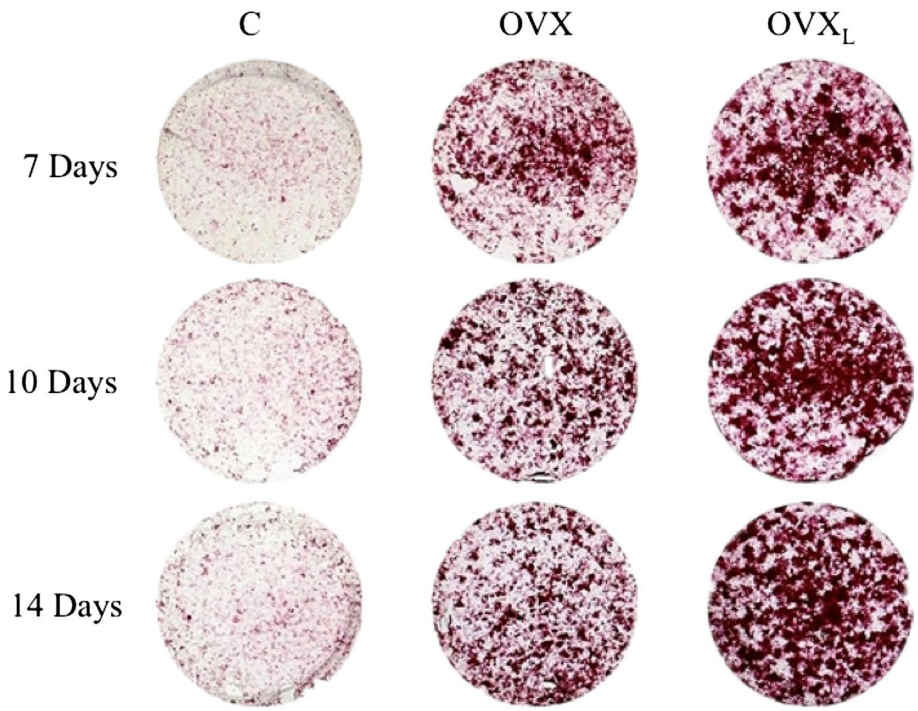

Fig. 2 In situ detection of alkaline phosphatase from bone marrow osteoblastic cells of of control (C), ovariectomized (OVX) and ovariectomized + in vitro lycopene (OVXL) after 7, 10 and 14 days of cell culture (sample of 5 wells for each experimental group)

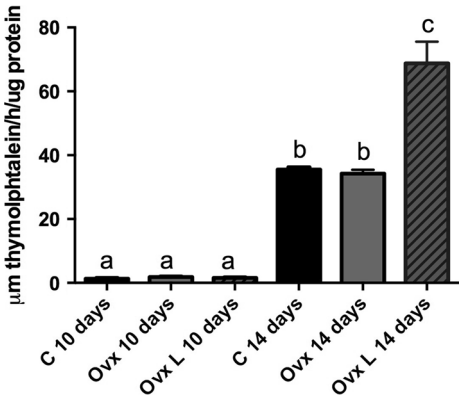

Fig. 3 ALP activity of osteoblastic cells from bone marrow of control (C), ovariectomized (OVX) and ovariectomized + in vitro lycopene (OVXL) at 10 and 14 days of cell culture. Statistical test of Kruskal–Wallis with significance for $p < 0.05$. Different letters mean statistical difference (sample of 5 wells for each experimental group)

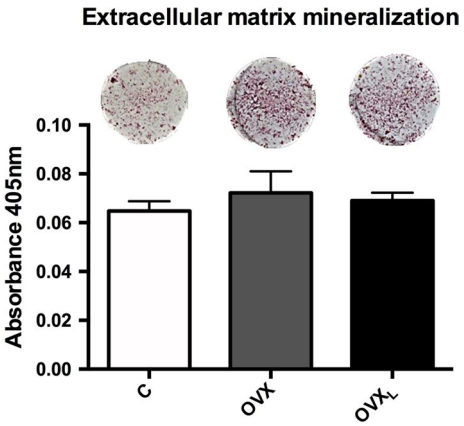

Fig. 4 Detection and quantification of mineralization after 14 days of culture of control (C), ovariectomized (OVX) and ovariectomized + in vitro lycopene (OVXL). No differences were observed among the groups (sample of 5 wells for each experimental group)

Quantitative gene expression (real-time PCR)

After 7 days, expression of genes *sp7*, *Bsp* and *Runx2* was significantly higher in OVX_L groups when compared to OVX group ($p = 0.0271$, 0.0314 and < 0.0001, respectively) and similar to C group for genes *sp7* and *Bsp*. In the same period, *Bglap* and *Alpl* showed a significant increased expression in OVXL group ($p = 0.002$ and $p < 0.0001$) when compared to C group, despite being similar to OVX group. On the other hand, at this period, expression of gene *Opg* was not distinct among the groups ($p = 0.1142$).

After 10 days, all studied genes showed higher expression in cells from OVXL when compared to the other OVX groups, although only genes *Alpl*, *Runx2*, and *Bglap* showed statistical significance ($p < 0.0001$, 0.0001 and 0.002, respectively). There were no significant differences between OVXL and C groups for all genes but *Bglap*, where the expression was higher in OVXL group. Data can be observed in Fig. 5.

Histological analysis and histomorphometry

Femur epiphysis was submitted to qualitative analysis after histological processing, with ovariectomized rats showing a decrease in trabecular bone and an increase of adipocytes in bone marrow (Fig. 6b), when compared to control group (Fig. 6a). Lycopene daily intake for 30 days resulted in partial maintenance of bone tissue when compared to control (Fig. 6b, e), but still with evidence of bone loss. Figure 6c, f shows a similarity in bone architecture, especially regarding trabecular bone after 60 days of daily intake of lycopene. Histomorphometry confirmed these observations, which can be seen in Figs. 7 and 8, showing that OVX group had a significant decrease of trabecular bone percentage when compared to all other groups ($p < 0.0001$), as well as an increase of adipocytes percentage when compared to controls ($p < 0.0001$). The percentage of trabecular number of OVX60 group was significantly higher than OVX30 ($p < 0.0001$) and similar to all controls. The percentage of adipocytes in all ovariectomized groups was higher than controls ($p < 0.0001$), but lycopene intake suggests a possible decrease in adipocyte percentage, despite the absence of statistical significance.

Discussion

The main finding of the current study is that lycopene may influence bone metabolism in a situation of osteoporosis by in vitro and in vivo evaluations. Chronic diseases such as osteoporosis have been associated with oxidative stress, which disturb bone cell function, inhibiting osteoblastic differentiation and inducing apoptosis [23]. Previous investigations due to lycopene antioxidant properties by in vivo and in vitro cell culture showed that this carotenoid can prevent osteoclast activation, as well as stimulate cell proliferation and differentiation of osteoblasts [12, 17, 24]. Nevertheless, these investigations used bone densitometry measurements and bone turnover biomarkers, as well as cell lines like SaOS-2. Our first hypothesis was confirmed after the biochemical and molecular experiments. The results on cell proliferation emphasize that in vitro administration of lycopene permitted the survival of bone marrow osteoblastic cells without decreasing their multiplication. Kim et al. [17] observed similar results with different concentrations of lycopene, finding a higher proliferative rate statistically significant for SaOS-2 cells cultured with lycopene at 10(− 6) and 10(− 5) M. Costa-Rodrigues et al.

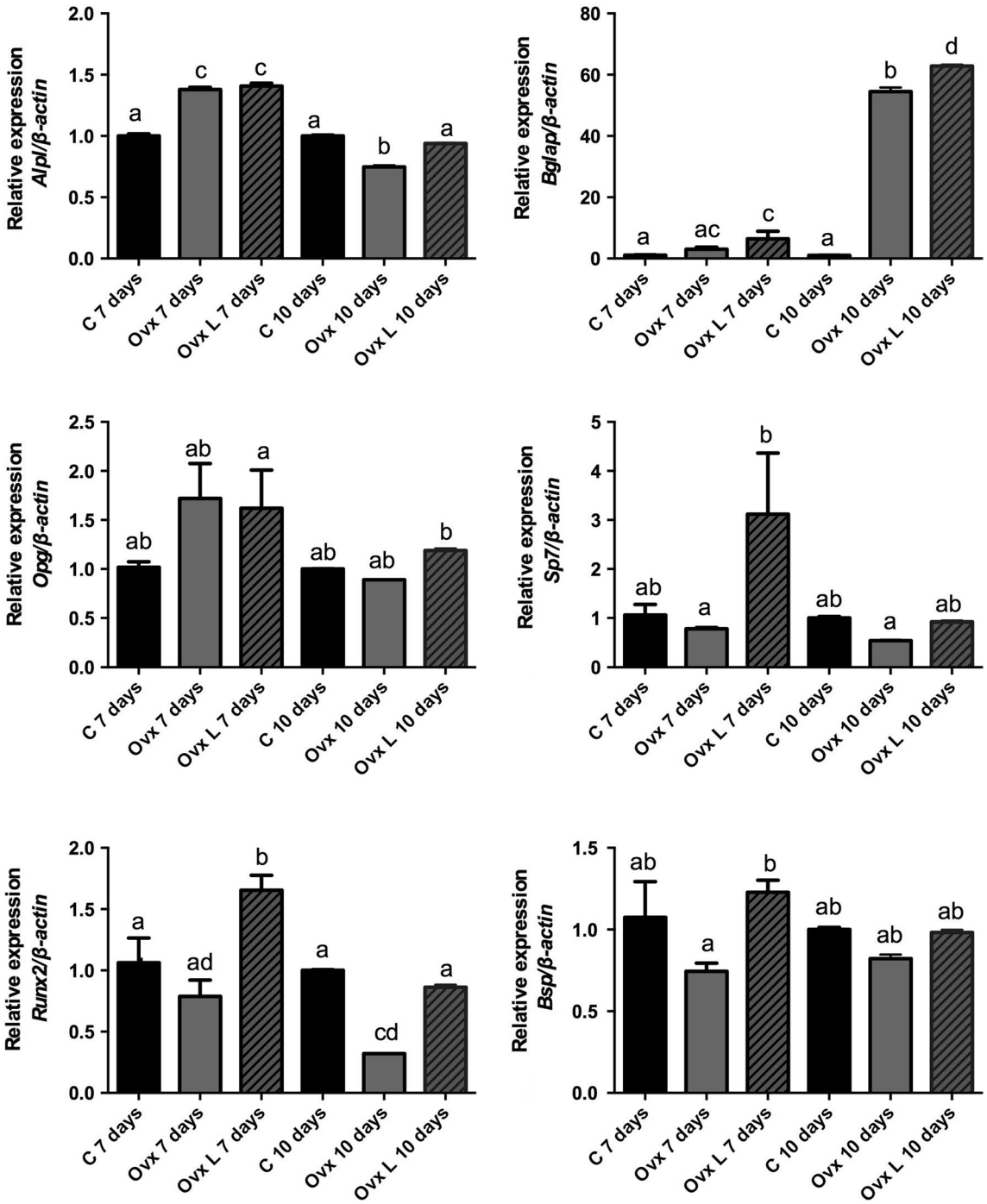

Fig. 5 Quantitative expression through PCR real time of mRNAs of osteoblastic cells from bone marrow of control (C), ovariectomized (OVX) and ovariectomized + in vitro lycopene (OVXL) at 10 and 14 days of cell culture. The expression levels were normalized using the β-actin gene. The difference between groups was assessed using one-way ANOVA for $p < 0.05$. Different letters mean statistical difference (sample of 5 wells for each experimental group)

[25] observed a significant activation of osteoblast cell proliferation and differentiation at levels ≥ 500 nM of lycopene in the presence of dexamethasone, suggesting that a potential anabolic effect of lycopene on bone cellular metabolism.

Alkaline phosphatase (ALP) activity is an active protein in the process of mineral deposition like phosphate and calcium for the formation of crystals of hydroxyapatite, also acting as an osteoblastic marker. Similar to Kim et al. [17], we observed an increase in ALP activity after

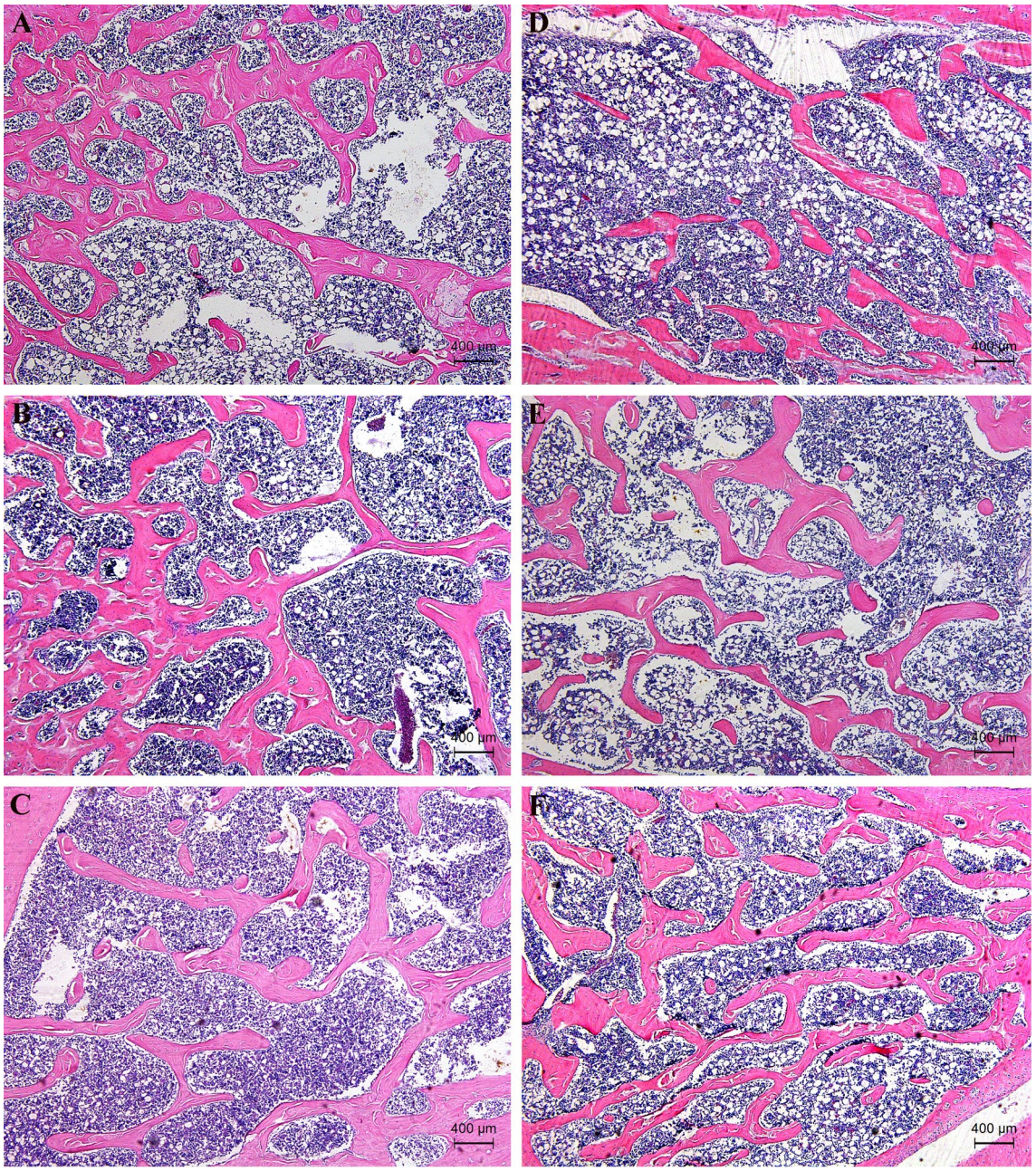

Fig. 6 Histological sections of femur epiphysis. **a** Sham group, **b** sham + daily intake of 10 mg/kg of lycopene for 30 days, **c** sham + daily intake of 10 mg/kg of lycopene for 60 days, **d** OVX group, **e** OVX + daily intake of 10 mg/kg of lycopene for 30 days, **f** OVX + daily intake of 10 mg/kg of lycopene for 60 days (sample of 3 femurs for each experimental group). Hematoxylin and eosin staining, bar = 400 µm

14 days of culture in the presence of lycopene, suggesting its effect in osteoblast differentiation. Iimura et al. [9] also demonstrated that serum bone-type alkaline phosphatase activity was significantly higher in female rats that received 100 ppm of lycopene content in their diet, suggesting that lycopene intake improves bone formation and inhibits bone resorption. ALP could also be detected by in situ staining, revealing a higher detection of this protein in the group OVXL when compared to the other groups. It is interesting to observe that OVX group showed an intense staining, suggesting an effort to produce mineralized nodules despite its similar activity to control group. Yu et al. [26] suggested that a higher energy metabolism caused by a higher generation of ATP that occurs in the presence of osteoporosis might explain the abnormal performance of osteoblasts from osteoporotic animals in these experiments. These authors suggest

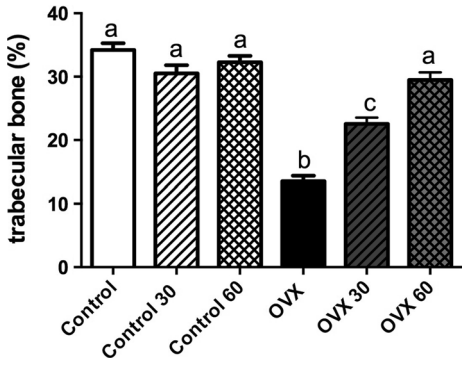

Fig. 7 Trabecular bone percentage evaluated in sham group (Control); sham + daily intake of 10 mg/kg of lycopene for 30 days (Control 30); sham + daily intake of 10 mg/kg of lycopene for 60 days (Control 60); OVX group (OVX); OVX + daily intake of 10 mg/kg of lycopene for 30 days (OVX 30); OVX + daily intake of 10 mg/kg of lycopene for 60 days (OVX 60). The difference between groups was assessed by Kruskal–Wallis test for $p < 0.05$ was considered significant. Different letters mean statistical difference (sample of 3 femurs for each experimental group)

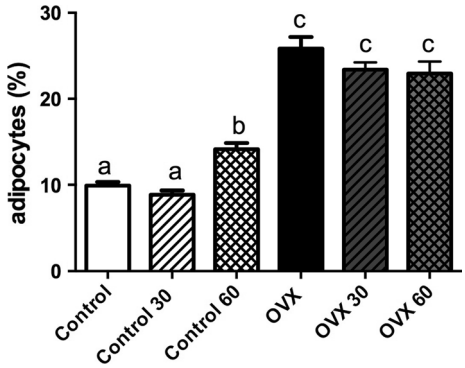

Fig. 8 Adipocytes percentage evaluated in sham group (Control); sham + daily intake of 10 mg/kg of lycopene for 30 days (Control 30); sham + daily intake of 10 mg/kg of lycopene for 60 days (Control 60); OVX group (OVX); OVX + daily intake of 10 mg/kg of lycopene for 30 days (OVX 30); OVX + daily intake of 10 mg/kg of lycopene for 60 days (OVX 60). The difference between groups was assessed by Kruskal–Wallis test for $p < 0.05$ was considered significant. Different letters mean statistical difference (sample of 3 femurs for each experimental group)

that this could be a consequence of the reduction of estrogen concentrations due to removal of ovaries, creating a need for the enhancement of osteoblast function to compensate for the effects of estrogen decrease. In spite of that, quality and density of this bone might not be equal when compared to that of a healthy bone. Our results show that even with higher values of ALP detection, there was no statistical difference among the groups when detecting and quantifying mineralized nodules, in agreement with other investigations [27]. To further elucidate the underlying effects of lycopene on osteoblast metabolism, we evaluated the expression of genes associated with osteogenesis. Genes such as *Sp7* and *Runx2* are very important to osteoblast differentiation. Osteoblast progenitors first differentiate through one or several steps into preosteoblasts with *Runx2* playing an essential role in this process. These *Runx2* expressing preosteoblasts then differentiate in one or more steps into mature osteoblasts and express characteristic osteoblast marker genes, a process that requires *Sp7* [28]. Both genes were upregulated in OVXL when compared to OVX, suggesting that lycopene enhanced cell differentiation in later periods, as it could be seen in ALP and MTT assays. A correlation between *Sp7* and *Bsp* genes has been demonstrated by Yang et al. [29], supporting the evidence that *Sp7* targets Bsp directly in osteoblasts. Our data are in agreement with this report, suggesting a positive regulation of *Sp7* on *Bsp* expression after 3 days, facilitating its important role in the initial mineralization of bone. *Bglap* gene encodes a highly abundant bone protein, i.e., osteocalcin, which is an important factor in the regulation of bone metabolism and in the implication of bone mineralization and calcium ion homeostasis [30]. Our data have demonstrated that the presence of lycopene in cell culture upregulated this gene and our results are in agreement with Park et al. [31], that observed a significantly increased expression of *Alpl*, *Runx2*, and *Bglap* after treatment with 10 µg/mL of *Lycii Radicis Cortex* (LRC) herbal extract for 3 days in C3H10T1/2 and MC3T3-E1 cell lines. The significantly downregulation of *Alpl* gene in OVX group after 10 days may reflect in features which where observed in our in vivo experiments, such as reduced osteoblast activity, decreased bone formation, and lower extracellular matrix (ECM) mineralization as suggested by Balla et al. [32]. The presence of lycopene in cell culture upregulated *Alpl* expression to similar values of control, suggesting its effect on osteogenesis.

Besides the role of some genes on osteoblast differentiation, others like *Opg* can regulate osteoclast differentiation, activation, and survival through the *Opg–Rankl–Rank* axis, thereby affecting skeletal metabolism in the body [33]. The anti-osteoclastic activity of *Opg* might enhance bone neoformation in a situation of osteoporosis, yet we have not seen significant differences in its modulation after lycopene treatment. Lycopene is an important intermediate in the biosynthesis of many carotenoids, including β-carotene, which is known to bind to retinoic acid receptors (RARs) and induce the differentiation of a variety of cells, including osteoblasts [34, 35]. These authors showed a significant main effect of β-carotene on enhanced *Runx2*, osteopontin, and *Alpl* mRNA expression of MC3T3-E1 preosteoblastic cells. Other carotenoids like β-cryptoxanthin have been shown to induce the expression of caspase-3 mRNA or *Apaf-2*, which involves apoptosis in osteoclastic cells, in the presence or absence of M-CSF and *Rankl* [36]. Recent investigations observed that osteoblast proliferation via a decrease in apoptosis and

differentiation were increased in the presence of lycopene and suggest that this effect may be related to significant changes in *Mek* signaling pathway [25].

The second hypothesis was also confirmed, demonstrating by in vivo experiments that 10 mg/kg of daily lycopene may prevent bone loss in ovariectomized rats. There are few articles in the literature showing the effect of daily lycopene intake on bone metabolism after ovariectomy, and most of them studied parameters other than histological analysis, as well as utilized different lycopene concentrations [9, 12]. The histomorphometry performed in the present investigation showed that lycopene significantly impaired bone loss in ovariectomized rats after its long-term administration for 60 days. Nevertheless, the increase in adipocyte percentage seen in ovariectomized rats did not return to control values as seen in the qualitative analysis. The bone-protective effects of lycopene were demonstrated by Ardawi et al. [12] by microtomography parameters, including osteogenesis promotion, trabecular bone thickening, and trabecular connectivity strengthening, after 12-week lycopene supplementation ranging from 15 to 45 mg/kg body weight. Our results show positive effects of this carotenoid with a lower concentration but still effective to exert its antioxidant capacity.

In conclusion, data obtained suggest that in vitro administration of lycopene has a positive effect on the functional activity and gene modulation of osteoblastic cells derived from bone marrow of ovariectomized rats. Besides, in vivo results demonstrated that daily intake of lycopene for 30 or 60 days decreased bone loss in femur epiphysis. Thus, lycopene might be a potential adjuvant to drug therapy used in prevention and treatment of osteoporosis.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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