Lipoic acid dose-dependently stimulates bone formation in ovariectomized rats

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Lipoic acid dose-dependently stimulates bone formation in ovariectomized rats

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Abstract

The study was undertaken to determine the osteotropic effect of different doses of lipoic acid (LA) on the mineralization of bone tissue in female Wistar rats with experimental osteopenia induced by bilateral ovariectomy. Fifty-six rats were randomly selected and submitted to either a sham-operation \((n=8)\) or an ovariectomy \((n=48)\). The ovariectomized rats were randomly placed into two control groups treated subcutaneously either with physiological saline or \(17\beta\)-estradiol in the dose of 4 µg/kg b.w./day, respectively, and into four experimental groups which received LA subcutaneously in the doses of 12.5, 25, 50 and 100 mg/kg b.w/day \((n=8 \text{ in each group})\). After 28 days of experimental treatment, the rats were sacrificed. Thereafter, body weight, total skeletal density and body composition were recorded. Blood serum and isolated femora were stored for further analysis. Our results revealed that osteoprotective effect of LA is dose-dependent and was observed in females treated with 50 and 100 mg/kg of LA. Moreover, the LA applied to the ovariectomized rats in the dose of 50 mg/kg, not only stopped the bone resorption, but stimulated its formation.

Keywords: rats, bone tissue, osteopenia, ovariectomy, densitometry, pQCT, lipoic acid, estradiol
Introduction

Bone loss changes, characterizing osteoporosis, occur during aging or after gonadectomy. Hypo/afunction of gonads results in an imbalance between resorption and formation of bone tissue, but also an imbalance between production and neutralization of reactive oxygen species (Muthusami et al. 2005; Radzki et al. 2012a). A comparative study conducted on gonadectomized rats documented the relationship between hormonal functions of gonads and activity of oxidative stress markers (Gomez-Zubeldia et al. 2000; Kume-Kick et al. 1996). There is substantial evidence that estradiol has an antioxidative effect (Gomez-Zubeldia et al. 2000). Moreover, this effect has been regarded as the main mechanism by which this hormone protects skeletal and cardiac muscle (Persky et al. 2000), the uterus (Diaz-Flores et al. 1999) and the liver (Huh et al. 1994) from oxidative damage. In addition, estradiol deficiency is reported to lower thiol antioxidant defenses in bone cells, thereby increasing reactive oxygen species levels (ROS), which increase the expression of TNF-α, IL-1 and IL-6, and, in consequence, accelerate loss of bone tissue (Jagger et al. 2005; Lean et al. 2003).

Lipoic acid (LA) is present in almost all foods, but slightly more in kidney, heart, liver as well as in spinach, broccoli and in yeast extract. Furthermore, LA is widely available as a nutritional supplement. Additionally, it can be used as a drug in the therapy of diabetes comorbidities (Ziegler 2004). LA, also known as thiocetic acid, is a molecule that assists in acyl-group transfer and acts as a coenzyme in the Krebs cycle. LA has also been described as being a universal antioxidant (Packer et al. 1995). In cells, LA is converted to dihydrolipoic acid (DHLA), and then DHLA can be converted back to LA (Cakatay et al. 2000; Packer et al. 2001). Furthermore, DHLA is capable of regenerating ascorbic acid from dehydroascorbic acid, directly regenerating vitamin C and indirectly regenerating vitamin E (Scholich et al. 1989). Moreover, Busse et al. (Busse et al. 1992) have found that LA increases intracellular
glutathione concentration, while Kagan et al. (Kagan et al. 1990) have demonstrated the elevation of the level of co-enzyme Q10. A few studies have also documented the positive effect of LA on the metabolism of bone tissue (Akman et al. 2013; Polat et al. 2013). Ha et al. have revealed that LA restricts IL-1-dependent osteoclast formation by inhibiting of PGE$_2$ synthesis (Ha et al. 2006), whereas Zhang et al. observed a decrease in the level of TNF-$\alpha$ and in the expression of RANKL (Zhang and Frei 2001).

The present study was aimed toward establishing the effect of treatment with different doses of LA on the mineralization of bone tissue in female rats during the development of experimental osteopenia induced by bilateral ovariectomy. In addition, our study was intended to verify the influence of different doses of LA on particular parameters of body composition in rats after ovariectomy.

Methods

Animal procedures

The experimental design was approved by the II Local Animal Welfare Committee in Lublin, Poland. The study was carried out on 56 female Wistar rats at the age of 2.5 months. The rats were housed individually with ad libitum access to food (Agropol-Motycz, Poland) and water. On the day of surgery, the rats were randomized, anaesthetized with an intramuscular injection of Ketaminum (Biowet-Pulawy, Poland), Atropinum sulphuricum (Polfa-Warszawa, Poland) and Rometar (Leciva, Czech Republic) at the doses of 10, 2 and 0.05 mg/kg b.w. respectively and submitted to sham-operation (SHO) ($n$=8) and ovariectomy (OVX) ($n$=48).

Experimental procedures

Seven days after surgery, the OVX rats were randomly placed into control groups treated subcutaneously with physiological saline (OVX-PhS) ($n$=8) and 17$\beta$-estradiol (OVX-E2) (4 $\mu$g/kg b.w./day) ($n$=8), respectively, as well as into four experimental groups of OVX
females. These received subcutaneously LA in the doses of 12.5 (OVX-LA12.5), 25 (OVX-LA25), 50 (OVX-LA50) and 100 mg/kg b.w/day (OVX-LA100) (Thiogamma 600; Wörwag Pharma, Germany) \( (n=8 \text{ in each group}) \). Herein, the usage of estradiol in OVX-E2 rats mimics the changes in bone tissue observed in postmenopausal women during hormonal replacement therapy (HRT). In contrast, the SHO females control were treated subcutaneously with physiological saline. After 28 days of experimental treatment, the rats were killed by cervical dislocation. Blood samples, obtained at the time of their killing, were centrifuged within 30 min, and serum samples were stored at -30°C for further analysis. After exsanguination, their femora were dissected, the soft tissue cleaned off and the femora were stored at -30°C until use.

Markers of bone tissue metabolism

Serum concentration of osteocalcin (OC) and C-terminal telopeptides of type I collagen (CTX-I) were determined by an enzyme-linked immunosorbent assay (ELISA), with the use of Rat-MID Osteocalcin and RatLaps kits (Immunodiagnostic Systems, Denmark), respectively.

Densitometric analysis (DXA) of whole femur.

Bone mineral density (BMD) and bone mineral content (BMC) of total skeleton (Ts.BMD; Ts.BMC) and isolated right femora (f.BMD; f.BMC) were established with the use of a Norland Excell Plus Densitometer (Fort Atkinson WI, USA) equipped with Illuminatus Small Subject Scan software. This enabled an experimental analysis of isolated bones. The DXA method also allowed for the establishment of body composition parameters eg. percentage of total fat content (Total Fat %), percentage ratio of fat tissue content in relation to soft tissue (Soft Tissue Fat %) and percentage ratio between total skeleton BMC and fat free mass of body (%TBMC/FFM).
Peripheral quantitative computed tomography (pQCT).

The right femora were scanned by way of peripheral quantitative computed tomography (pQCT), using the XCT Research SA Plus system with software version 6.2 C (Stratec Medizintechnik GmbH, Pforzheim, Germany). The scans were performed in the distal femur metaphysis (DFM) (5 mm from distal end) for analysis of trabecular bone tissue, and in the mid-shaft femur of diaphysis (MFD) (50% of bone length) for the analysis of cortical bone tissue (Fig. 1). The scan line was adjusted using scout view after the initial scan of the pQCT system. Upon completion of scanning, the following parameters were determined in DFM: trabecular bone area (Tb.Ar), trabecular bone mineral content (Tb.BMC) and trabecular volumetric bone mineral density (Tb.vBMD). Moreover, cortical bone area (Ct.Ar), cortical bone mineral content (Ct.BMC), cortical volumetric bone mineral density (Ct.vBMD), as well as cortical thickness (Ct.Th), periosteal (Peri.C) and endocortical (Endo.C) circumferences in MFD were established.

Mechanical properties

The mechanical parameters of isolated femora were examined through a in 3-point bending test with the use of a ZwickRoell Z010 (ZwickRoell GmbH and Co. KG, Ulm, Germany) universal testing machine equipped with a 1 kN measuring head Xforce HP series. The analyzed bone were investigated as a tube model, and external as well as internal diameters were measured by pQCT. The bones were placed on two holders, and the force was applied downward, perpendicularly to the horizontal axis and at the midpoint of the bone. The load was increased until the bone breaks. The received data were analyzed with testXpert II 3.1 software assistance. The ultimate strength ($F_{\text{max}}$), work to ultimate strength ($W/F_{\text{max}}$), and the Young modulus ($E_{\text{mod}}$) were subsequently determined.

Statistical analysis
The results were presented as mean values ±S.E.M. One-way analysis of variance (ANOVA) was used to test for significant differences among the experimental groups. To detect significant differences between individual experimental groups, significant ANOVA’s were followed by *post hoc* Tukey test for multiple comparisons. Differences were considered significant at $P < 0.05$. Statistical analyses were performed with the use of STATISTICA software v. 8.0 (StatSoft, Inc. Tulsa, USA).

**Results**

Bone mass, body weight and parameters of body composition.

The results of body weight, bones mass and parameters of body composition are shown in Table 1. As evidenced in the table, ovariectomy significantly decreased the mass of the femur in OVX-PhS rats ($P = 0.004$) (vs. SHO). In addition, significantly lower mass of the femur was observed in the OVX-LA12.5 ($P = 0.027$) and OVX-LA25 ($P = 0.005$) groups. What is more, ovariectomy significantly increased the body weight of OVX-PhS rats ($P = 0.040$) vs. SHO. Significantly elevated body weight was also observed in rats from groups OVX-LA12.5 ($P = 0.029$) and OVX-LA25 ($P = 0.014$). The usage of LA in the doses of 50 and 100 mg/kg limited the increase of body weight, and the obtained values were on the level as those in SHO. Lean mass significantly increased in OVX-LA12.5, OVX-LA25 and OVX-LA50, ($P < 0.05$ vs. SHO). Significantly, the lowest fat mass was observed in animals from the OVX-LA100 group ($P = 0.034$ vs. SHO). Moreover, fat mass decreased in rats treated with 17β-estradiol and in the remaining groups which received LA. Interestingly, the fat mass from OVX-PhS increased only by 3.4%, in relation to the SHO. Furthermore, the Total Fat % measured in the OVX-PhS was similar to that from SHO control. In addition, the treatment with 17β-estradiol (OVX-E2) and with the lower doses of LA (OVX-LA12.5 and OVX-LA25) tended to lower but not statistically significant values of the Total Fat %. However, the usage of LA in the higher doses significantly decreased the Total Fat % in rats from group
Densitometric measurements (DXA)

The values of planar BMD and BMC of total skeleton and isolated femur are shown in Fig. 2. Ovariectomy significantly decreased total skeleton BMD (Ts.BMD) in OVX-PhS ($P = 0.031$), OVX-LA12.5 ($P = 0.006$) and OVX-LA25 ($P = 0.019$), as compared to SHO rats. Interestingly, LA applied in the doses of 50 and 100 mg/kg not only significantly increased Ts.BMD ($P = 0.001$ and $P = 0.002$ vs. OVX-PhS, respectively), but, additionally, increased Ts.BMD in relation to the SHO, and, as compared to OVX-E2. Moreover, the Ts.BMD measured in rat treated with LA in the doses of 50 and 100 mg/kg was significantly higher as compared with the data from the rats which received lower doses of LA. What is more, ovariectomy significantly decreased the BMD of femur (f.BMD) from the OVX-PhS, and OVX-LA12.5, OVX-LA25 ($P < 0.0001$ for all), as well as OVX-LA100 ($P = 0.002$), whereas, f.BMD from OVX-LA50, was similar to those from SHO and OVX-E2. Furthermore, f.BMD from OVX-LA50 was significantly higher in relation to OVX-LA12.5, OVX-LA25 and OVX-LA100 rats ($P < 0.0005$ for all). Finally, the BMC of femur isolated from the OVX-LA50 group was significantly higher in relation to OVX-LA12.5 ($P = 0.01$) and OVX-LA25 ($P = 0.009$).(Fig. 2).

Tomographic measurements (pQCT)

The values of pQCT analysis of cortical and trabecular bone tissue of femur are shown in Fig. 3 and in Table 2. As can be seen, neither ovariectomy, nor treatment with 17β-estradiol or different doses of LA significantly influenced bone mineral content of cortical bone tissue (Ct.BMC) (vs. SHO control) (Fig. 3). What is more, similar effects of LA treatment were seen
after analysis of volumetric bone mineral density (Ct.vBMD) (Fig. 2), cortical bone tissue area (Ct.Ar), pericortical circumferences (Peri.C) and endocortical circumferences (Endo.C) (Table 2). Additionally, Ct.BMC (Fig. 2), Ct.Ar, Ct.Th (cortical bone tissue thickness) and Peri.C (Table 2) of femur isolated from OVX-LA50 rats were significantly higher as compared to rats treated with the LA in the doses of 12.5 and 25 mg/kg. However, ovariectomy significantly decreased the trabecular bone mineral content (Tb.BMC) and trabecular volumetric bone mineral density (Tb.vBMD) ($P = 0.0005$ and $P = 0.0002$, respectively vs. SHO), as measured in the distal femur metaphysis. Furthermore, the values of Tb.BMC and Tb.vBMD of femur from rats treated with the LA in the doses of 12.5 and 25 mg/kg, not only were significantly decreased in relation to the SHO control ($P < 0.0001$), but tended to lower values as compared to those from the OVX-PhS control. The Tb.BMC and Tb.vBMD of femur from OVX-LA50 and OVX-LA100 rats, were, however, not significantly lower as compared to the SHO control (Fig. 3). What is more, the obtained values of tomographic parameters exceeded those from OVX:E2. Moreover, the bone mineral content and volumetric bone mineral density of trabecular compartment of femur from OVX-LA50 and OVX-LA100 rats were significantly higher as compared to the results from the OVX-LA12.5 and OVX-LA25 animals.

Mechanical parameters

The values of the mechanical parameters of femur are shown in Fig. 4. Significantly the lowest Young modulus ($E_{mod}$) was noted in the OVX-PhS rats, ($P = 0.02$ vs. SHO). Ovariectomy also lowered ultimate strength ($F_{max}$) and work to ultimate strength ($W/F_{max}$) in the femur from the OVX-PhS rats. However, these results were not significant, as compared to SHO. The highest Young modulus, ultimate strength and work to ultimate strength were observed in femur from rats treated with LA in the dose of 50 mg/kg. Additionally, the values of mechanical parameters of femur from OVX-LA50 significantly
exceeded the results obtained from the SHO and the OVX-E2 \((P < 0.05)\). It should be noted as well that the values of mechanical parameters of femur isolated from OVX-LA12.5, OVX-LA25 and OVX-LA100 rats were not significantly different from those of SHO control and the OVX-LA50 rats (Fig. 4).

**Biochemical markers of bone metabolism**

The values of the biochemical markers of bone metabolism are shown in Fig. 5. Osteocalcin concentration was significantly elevated in the blood serum drawn from the OVX-PhS control \((P < 0.0001 \text{ vs. SHO})\), while the treatment with 17β-estradiol significantly decreased the serum osteocalcin level in OVX-E2 rats \((P = 0.021)\). Additionally, a significantly elevated osteocalcin concentration was noted in the blood serum of females from OVX-LA12.5 \((P = 0.045)\) and OVX-LA25 \((P = 0.024)\), whereas, in groups treated with higher doses of LA, the concentrations of osteocalcin were similar to those from the SHO and were significantly lower as compared to OVX-LA12.5 and OVX-LA25 rats. Furthermore, ovariectomy significantly increased the serum CTX concentration in OVX-PhS control \((P < 0.0001)\), OVX-LA12.5 \((P = 0.0002)\) and in OVX-LA25 \((P = 0.003)\), as compared to SHO, OVX-E2, OVX-LA50 and OVX-LA100. The lowest CTX concentration was observed in the blood serum from the OVX-LA50 rats, and the obtained results were similar to those from SHO and OVX-E2 (Fig. 5).

**Discussion**

The development of oxidative stress and impaired antioxidant defense mechanisms are important factors in the pathogenesis of oxidant-related diseases. According to previous work, the levels of biochemical markers of oxidative stress, were found to be negatively associated with both bone mineral density and quantitative ultrasound (Basu et al. 2001). Indeed, numerous evidences have suggest a possible link between oxidative stress and osteoporosis due to, significant decrease of antioxidant defenses in osteoporotic females (Kankofer et al.
Lipoic acid and its reduced form, dihydrolipoic acid, are powerful antioxidants because these have beneficial effects on energy production and because these are also essential cofactor of mitochondrial respiratory enzymes, including the pyruvate dehydrogenase (PDH) complex (Packer et al. 2001). In our work, the influence of LA was assessed so as to verify the hypothesis that LA administration is useful in preventing the development of metabolic bone disorders. In so doing, we set out to establish the most effective doses, base on work with bilaterally ovariectomized rats. It should be noted that ovariectomized rats are a well-known experimental model for the postmenopausal osteoporosis which mimics estrogen deficiency in women (Frost 1992).

The results of our and other studies have demonstrate that gonadectomy significantly affects the mechanical parameters of femur, decreasing of their values (Ferretti et al. 2003). In our study, treatment with the lipoic acid in the doses of 50 and 100 mg/kg increased mechanical resistance of the femur, in relation to that of SHO control. Surprisingly, the usage of the LA in the lower doses prevented the decrease of the mechanical parameters, despite the reduction of the values of the planar BMD. Indeed, numerous studies have shown that planar BMD seems to be a good predictor of bone strength, however, this relationship is not so obvious. For example, the usage of raloxifene reduces the bone fracture without significant affecting of BMD (Ettinger et al. 1999). In contrast, the treatment with fluoride or bisphosphonates elevates BMD, but does not reduce the fragility of bones (Kathleen and Head 1999). Of note, numerous ex vivo studies have documented that only 66-74 % of bone strength variation can be determined by BMD (Ammann and Rizzoli 2003). Therefore, the mechanical competence of cortical bone tissue is dependent not only on BMD, but also on the structural properties of the cortical compartment eg. peripheral circumference (Peri.C), cortical area (Ct.Ar) (Tab. 2) as well as from cross sectional moment of inertia (data not shown) (Augat and Schorlemmer 2006). In our study the structural parameters of the cortical
compartment of femur isolated from the rats treated with the LA, did not reveal statistically significant changes (vs. SHO). Our study, however, has some limitations, and further work is necessary to explain the mechanism by which LA affects the mechanical parameters of the femur. Dual X-ray absorptiometry (DXA) assessment is the criterion standard for the evaluation and diagnosis of osteoporosis. Osteoporosis affects bone tissue quality and brings about a decrease in planar BMD and BMC. In our study, ovariectomy also significantly decreased BMD in OVX-PhS, as measured in total skeleton as well as in isolated femora. This observation is in agreement with other papers (Liu et al. 2009; Radzki et al. 2012b). However, treatment with LA in the dose of 50 mg/kg showed not only a strong osteoprotective effect, but an increased BMD in relation to SHO rats both in total skeleton (3.7 %) and femora (2.9 %). Our observations, in part, also support the earliest results of Polat et al. (Polat et al. 2013). These authors noted, that 50 mg/kg of LA inhibited the resorption of bone tissue. Moreover, Polat and co-authors observed an osteoprotective effect in animals which received LA in the dose of 25 mg/kg. This is in contrast, however, to our study, wherein our data show, that the usage of lower doses (12.5 or 25 mg/kg) is insufficient in exerting any osteotropic effect, and the intensity of resorption and degradation of bone tissue in those groups are similar to that in OVX-PhS.

Measurements made with the use of pQCT are more detailed and allow for separate analysis of cortical and trabecular bone tissue (Fig 1). Ovariectomy affects both the cortical and the trabecular compartments. The trabecular bone tissue is more sensitive to the decreased level of estrogens, and, in consequence, metabolic bone losses are more intensive (Cano et al. 2008). Interestingly, the LA applied in the doses of 12.5 and 25 mg/kg intensified the resorption of bone tissue, and the values of Tb.BMC and Tb.vBMD from OVX-LA12.5 and OVX-LA25 were not significantly lower, as compared to OVX-PhS. However, higher doses of LA curbed the degradation of trabecular bone tissue, and the values of Tb.BMC and
Tb.vBMD were similar to those from SHO. Similarly to the trabecular compartment, the analysis of cortical bone tissue revealed that LA, when used in the dose of 50 mg/kg not only inhibited the resorption of cortical bone tissue, but stimulated its formation, and demonstrated a higher effectiveness than did 17β-estradiol. Our observations are in line with the results reported by Fu et al. (Fu et al. 2015)

In the undertaken tomographic measurements of the midshaft femur of diaphysis, we also considered an analysis of bone geometry. As a result, we established the existence of an influence due to the ovariectomy as well as due to different doses of LA on the periosteal and endocortical circumferences of the diaphysis. The relationship of changes in circumferences of bone diaphysis was reflected in the thickness of the cortical bone tissue. Our data demonstrated that ovariectomy accelerated the resorption of bone tissue on the periosteal surface, therein, decreasing the periosteal circumference by 3.1 %. In contrast, the endocortical circumferences of femur from the OVX-PhS control decreased by 1.3 %. What is more, while the metabolic bone changes on the peri- and endocortical surfaces were slightly marked, however, the thickness of the cortical bone tissue (Ct.Th) was significantly decreased. Of note our observations are in line with other papers (Saxon and Turner 2005). The decrease of Ct.Th was also observed in female rats treated with the lower doses of LA. The results of our work, therefore, showed that the usage of LA in the dose of 100 mg/kg inhibited the resorption of bone tissue and maintained the Ct.Th at level similar to that of the SHO females. Our work also revealed that the greatest effect was generated by a dose of 50 mg/kg. This not only stopped the metabolic bone disorders induced by bilateral ovariectomy, but increased the thickness of the cortical bone tissue Ct.Th (by almost 3.0 %) as compared to SHO control.

The osteotropic influence of LA treatment supports analysis of the biochemical markers of bone metabolism. Indeed, the concentration of OC, a marker of bone turnover, was
dramatically elevated by ovariectomy. Furthermore, the intensification of bone turnover was observed in rats treated with the lower doses of LA, however, administration of 50 and 100 mg/kg of LA limited the metabolic activity of bone tissue, and the concentration of OC was on a level similar to that of the SHO rats. Similar observations were also reported previously (Aydin et al. 2014; Polat et al. 2013).

In our work, the concentration of CTX in the blood serum drawn from rats treated with LA in the lower doses was significantly elevated. This suggests the intensification of bone resorption. The analysis of the concentration of the CTX, a marker of bone resorption, thus, showed that LA effectively inhibits the resorption of bone tissue in rats treated with LA at doses of 50 and 100 mg/kg.

An important aspect of the present work is the reported effect of different doses of LA treatment on body weight and on parameters of body composition. Our results show that ovariectomy significantly increased the body weight of OVX-PhS. However, the analysis of the parameters of body composition revealed that ovariectomy increased body weight without causing obesity (Clark and Tarttelin 1982), while the significantly higher body weight in the OVX-PhS control came about due to both lean and fat mass that was not insignificantly elevated. In our work, a significant increase of body weight (as the effect of a significant increase of lean mass) was also observed in rats treated with the lower doses of LA, yet, the usage of the higher doses effectively curtailed this increase, in spite of the enhanced lean mass. The inhibition of body weight gain after LA treatment was also described by the other authors (Koh et al. 2011; Prieto-Hontoria et al. 2009). Moreover, the analysis of parameters of body composition demonstrated that all used doses of LA counteracted the accumulation of fat tissue in OVX rats, however the doses of 50 and 100 mg/kg were more effective. Our observations are in line with the results presented by other authors (Prieto-Hontoria et al. 2009, 2013).
For a long time, fat tissue was considered to be merely a passive reservoir of energy, however, it is now known to be an important endocrine organ that plays a key role in bone metabolism. Indeed, in recent literature, the relationship of fat tissue and bone tissue is widely discussed. While fat tissue secretes pro-inflammatory cytokines e.g. pro-inflammatory cytokines such as TNF-α, IL-1 and the receptor activator NF-κB, fat tissue also secretes adipokines beneficial for bone metabolism (leptin, adiponectin). Therefore, the effect of fat tissue on bone tissue is not so clear and is still widely discussed (Cao 2011). However, it can be underlined that after menopause or in a condition of experimental ovariectomy the concentrations of pro-inflammatory cytokines are elevated, the degree of which partially depends on the increased predominately accumulate visceral fat (Yamatani et al. 2014). These pro-inflammatory cytokines stimulate resorption of bone tissue (Le et al. 2012; Shoelson et al. 2007). Hence, we are of the hypothesis that the possible osteoprotective influence of LA comes about from the reduction of the fat tissue. Our supposition seems to be rationale - especially in the light of the results presented by Polat and co-authors (Polat et al. 2013).

In conclusion, for a long time LA was regarded as an anti-resorptive factor which prevented bone loss exclusively via its anti-oxidative capacity. However, the results of our study provide documentation that LA treatment effectively stimulates the formation of bone tissue in OVX rats in a dose-dependent manner. Nevertheless, the mechanisms by which LA affects bone tissues are probably multidirectional and further examination is necessary. Still, the present findings may have important clinical implications, and, hence, motivate the further testing of LA for the purposes of prevention and therapy of osteopenia and osteoporosis.
Acknowledgements

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References


Table 1. Final body and femur weight as well as parameters of body composition

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<th>SHO</th>
<th>OVX-PhS</th>
<th>OVX-E2</th>
<th>OVX-LA12.5</th>
<th>OVX-LA25</th>
<th>OVX-LA50</th>
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<tr>
<td>Body weight (g)</td>
<td>298.00±9.75</td>
<td>325.00±9.87#</td>
<td>289.38±7.28*</td>
<td>325.09±1.97#</td>
<td>328.71±10.82#‡</td>
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<td>309.50±7.81</td>
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<td>Femur mass (g)</td>
<td>0.82±0.024</td>
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<td>0.80±0.024*</td>
<td>0.74±0.016#</td>
<td>0.72±0.03#‡</td>
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<td>Lean mass (g)</td>
<td>187.96±8.28</td>
<td>207.87±4.26</td>
<td>189.45±5.22</td>
<td>209.50±8.71#</td>
<td>211.16±10.04#‡</td>
<td>208.66±7.35#</td>
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<td>Fat mass (g)</td>
<td>37.81±6.31</td>
<td>39.09±9.13</td>
<td>25.95±2.46</td>
<td>26.50±4.12</td>
<td>35.28±6.82</td>
<td>24.61±3.57</td>
<td>21.80±4.47**</td>
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<td>Total fat % (%)</td>
<td>0.1668±0.025</td>
<td>0.1538±0.032</td>
<td>0.1205±0.012</td>
<td>0.1142±0.020</td>
<td>0.1430±0.028</td>
<td>0.0883±0.020#</td>
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<td>Soft tissue fat %</td>
<td>0.1671±0.025</td>
<td>0.1540±0.032</td>
<td>0.1207±0.012</td>
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<td>0.1432±0.029</td>
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<td>%Tot.BMC/FFM (%)</td>
<td>0.0433±0.002</td>
<td>0.0370±0.001#‡</td>
<td>0.0417±0.001*</td>
<td>0.0356±0.002#‡</td>
<td>0.0365±0.002#‡</td>
<td>0.0394±0.001#</td>
<td>0.0403±0.001a</td>
</tr>
</tbody>
</table>

Results are the means ± S.E.M. (n=8); # P ≤ 0.05 vs. SHO; * P ≤ 0.05 vs. OVX-PhS; ‡ P ≤ 0.05 vs. OVX-E2; a P ≤ 0.05 vs. OVX-LA12.5; b P ≤ 0.05 vs. OVX-LA25; c P ≤ 0.05 vs. OVX-LA50. Abbreviations: Total Fat % – percentage of total fat content; Soft Tissue Fat % – percentage ratio of fat tissue content in relation to soft tissue; %Tot.BMC/FFM – percentage ratio between total skeleton BMC and fat free mass of body,
### Table 2. Tomographic (pQCT) measurements of architectonical parameters of femur

<table>
<thead>
<tr>
<th></th>
<th>SHO</th>
<th>OVX-PhS</th>
<th>OVX-E2</th>
<th>OVX-LA12.5</th>
<th>OVX-LA25</th>
<th>OVX-LA50</th>
<th>OVX-LA100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ct.Ar (mm²)</td>
<td>6.09±0.15</td>
<td>5.63±0.09</td>
<td>6.33±0.19*</td>
<td>5.7±0.23‡</td>
<td>5.71±0.23‡</td>
<td>6.51±0.23ab</td>
<td>5.95±0.11‡</td>
</tr>
<tr>
<td>Ct.Th (mm)</td>
<td>0.71±0.011</td>
<td>0.66±0.001#</td>
<td>0.73±0.011*</td>
<td>0.67±0.014#‡</td>
<td>0.67±0.015#‡</td>
<td>0.73±0.023*ab</td>
<td>0.71±0.014*ab</td>
</tr>
<tr>
<td>Endo.C (mm)</td>
<td>6.37±0.20</td>
<td>6.29±0.17</td>
<td>6.33±0.19</td>
<td>6.36±0.10</td>
<td>6.26±0.24</td>
<td>6.70±0.22</td>
<td>6.13±0.15</td>
</tr>
<tr>
<td>Peri.C (mm)</td>
<td>10.83±0.19</td>
<td>10.50±0.13</td>
<td>10.94±0.21</td>
<td>10.62±0.14</td>
<td>10.53±0.25</td>
<td>11.26±0.18*ab</td>
<td>10.60±0.11c</td>
</tr>
<tr>
<td>Tb.Ar (mm²)</td>
<td>8.81±0.25</td>
<td>9.06±0.24</td>
<td>8.38±0.17</td>
<td>8.69±0.19</td>
<td>8.89±0.36</td>
<td>9.03±0.30</td>
<td>9.07±0.27</td>
</tr>
</tbody>
</table>

Results are the means ± S.E.M. (n=8); # P ≤ 0.05 vs. SHO; * P ≤ 0.05 vs. OVX-PhS; ‡ P ≤ 0.05 vs. OVX-E2; a P ≤ 0.05 vs. OVX-LA12.5; b P ≤ 0.05 vs. OVX-LA25; c P ≤ 0.05 vs. OVX-LA50. Abbreviations: Ct.Ar – Cortical bone tissue area; Ct.Th – Thickness of cortical bone tissue; Endo.C – endocortical bone circumference; Peri.C – pericortical bone circumference; Tb.Ar – Trabecular bone tissue area.
Figure captions:

**Figure 1.** Representative peripheral quantitative computed tomography (pQCT) scan images of femur performed in distal metaphysis and in midshaft of diaphysis.

**Figure 2.** Densitometry (DXA) of total skeleton and isolated femur. Results are the means ± S.E.M. (n=8); # P ≤ 0.05 vs. SHO; * P ≤ 0.05 vs. OVX-PhS; ‡ P ≤ 0.05 vs. OVX-E2; a P ≤ 0.05 vs. OVX-LA12.5; b P ≤ 0.05 vs. OVX-LA25; c P ≤ 0.05 vs. OVX-LA50

**Figure 3.** Tomographic (pQCT) analysis of cortical and trabecular bone tissue of femur. Results are the means ± S.E.M. (n=8); # P ≤ 0.05 vs. SHO; * P ≤ 0.05 vs. OVX-PhS; ‡ P ≤ 0.05 vs. OVX-E2; a P ≤ 0.05 vs. OVX-LA12.5; b P ≤ 0.05 vs. OVX-LA25; c P ≤ 0.05 vs. OVX-LA50

**Figure 4.** Mechanical parameters (3-point bending test) of femur. Results are the means ± S.E.M. (n=8); # P ≤ 0.05 vs. SHO; * P ≤ 0.05 vs. OVX-PhS; ‡ P ≤ 0.05 vs. OVX-E2; a P ≤ 0.05 vs. OVX-LA12.5; b P ≤ 0.05 vs. OVX-LA25; c P ≤ 0.05 vs. OVX-LA50

**Figure 5.** Biochemical markers of bone tissue metabolism. Results are the means ± S.E.M. (n=8); # P ≤ 0.05 vs. SHO; * P ≤ 0.05 vs. OVX-PhS; ‡ P ≤ 0.05 vs. OVX-E2; a P ≤ 0.05 vs. OVX-LA12.5; b P ≤ 0.05 vs. OVX-LA25; c P ≤ 0.05 vs. OVX-LA50
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182x72mm (300 x 300 DPI)