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Olive oil and bone health

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Summary
Objetive: A series of studies in the literature indicate that the incidence of osteoporosis and associated fractures is lower in countries where the Mediterranean diet is predominant. Olive oil is characteristic of the Mediterranean diet, a third of the intake of vegetable fats. We carried out an extensive review of studies showing that the ingestion of olive oil, both in experimental animals, especially ovariectomized rats, and in humans, produces positive actions on the bone. The effects of different components of virgin olive oil such as oleuropein, a phenolic compound, and other phenolic alcohols such as tyrosol and hydrotyrosol have been reviewed. Oleuropein not only exerts actions on the bone of ovariectomized rats, but also enhances the formation of osteoblasts and decreases the formation of "osteoclast-like" cells. The phenolic compounds in olive oil exert anti-oxidant actions *in vitro* and *in vivo*. Tyrosol and hydrotyrosol exert actions on bone loss in ovariectomized rats and inhibit dose-dependent osteoclast formation. Our group's research has shown that virgin olive oil also exerts actions on the biomechanical parameters of the bone such as Young's modulus and fractal dimension in ovariectomized rats. The results of this review indicate that olive oil has a positive action on bone health. Its components have antioxidant and anti-inflammatory properties. Thus they are potential candidates for preventing osteoporosis.

Key words: osteoporosis, virgin olive oil, oleuropein, tyrosol, hydrotyrosol.

Introduction
Osteoporosis is the bone disease that most affects humans and predisposes a person to fractures. It constitutes a serious public health problem due to its impact on patients' quality of life and the economic burden it represents. Osteoporosis reportedly affects more than 200 million people[1]. Therefore, it is extremely important to take all possible measures to mitigate its development.

Along with other factors, bone modeling and remodeling are determined by nutritional status[2]. Nutrition has relevant effects on peak bone mass, bone loss with age, and muscle strength[3]. Of course, the main nutrients for bone are calcium and vitamin D[4], since calcium is the major component of bone and its contribution is regulated by vitamin D, thus optimizing peak bone mass. However, the European Union has indicated the relevance of other nutrients on bone development and the advisability of conducting research into these on bone development[5]. The main advantage of nutrition in assessing its importance for bone health is that it can be modified.

The Mediterranean diet is characterized by a high intake of fruits, vegetables, and olive oil. The incidence of osteoporosis and associated fractures seems to be less in countries where the Mediterranean diet is predominant[6].

In this work we are going to focus on olive oil, which is the main common characteristic of the entire Mediterranean diet, assuming a third of the vegetable fat intake[7].

Olive oil contains oleic acid (C1 8:1) (55 – 83%), palmitic acid (C1 6:0) (7.5 – 20%), linoleic acid (C1 8:2) (3.5 – 21%), and more than 200 additional chemical compounds[8]. Besides triglycerides, we are interested in highlighting phenolic compounds here. Oleuropein is the main phenolic compound in olive leaves, olives and olive oil, with an amount in it between 1 ppb and 11 ppm. A group of very important bioactive compounds in olive oil are phenolic alcohols such as tyrosol and hydrotyrosol[9]. Flavonoids are also abundant, one of which is lutein[10]. In general, in this work we focus on virgin olive oil, because refined olive oil does not contain polyphenols, which, as we will see later, have been shown to exert important positive actions on the bone.

Effect of the Mediterranean diet on bone
Savanelli et al.[11] conducted a study in 418 healthy people (105 men and 313 women) between 50±14 years of age. The results showed a positive correlation between bone health and adherence to the Mediterranean diet (higher consumption of virgin olive oil, vegetables, fruit, legumes, fish), being negatively associated with the consumption of red meat, suggesting that greater adherence to the Mediterranean diet favors bone health.

Silva et al.[12] studied 105 healthy postmenopausal women between 45 and 65 years of age. Those who showed greater adherence to the Mediterranean diet had

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higher lumbar bone mineral density (BMD) values (1.076±0.146 *vs.* 0.997±0.143 g/cm^2, p=0.007). Thus, adherence to the Mediterranean diet is positively associated with higher BMD values in a non-Mediterranean region, since this work was carried out in Brazil. Adherence to the Mediterranean diet has been associated with a decrease in the incidence of fractures in the European Prospective Investigation into Cancer and Nutrition Study, which included 188,795 subjects followed over 9 years[13]. Keiler et al.[6] described that the incidence of osteoporosis and associated fractures is lower in countries where the Mediterranean diet is predominant. Kontogianni et al.[14] showed that adherence to the Mediterranean diet was positively related to bone mass, suggesting its potential bone-preserving properties.

Adherence to the Mediterranean diet has also been shown to have beneficial effects on BMD in the calcaneus, measured by dual X-ray absorptiometry (DXA) in a sample of healthy women from southern Spain[15]. In the same way, a lower incidence of fractures has been shown in Greece, where a higher proportion of olive oil is consumed than in the USA or in northern European countries[16]. However, in an elderly population in France, a Mediterranean-type diet was not associated with a decrease in the fracture risk[17].

The problem with the interpretation of these data is that we cannot be sure that it is the olive oil that produces the effects of the Mediterranean diet with complete certainty. It contains many fruits, vegetables and fish but there are authors such as Keiler et al.[6] who are almost completely certain that these positive effects are due to the active compounds of virgin olive oil and especially to phenolic compounds. In the works that we present below, we focus on the effects of the oil itself or of its components.

Extensive literature demonstrates the positive effects of olive oil on bone in experimental animals. Ostrowska et al.[18] administered virgin olive oil (19% w/w) to pigs and observed an increase of 6.28 mg/cm^2 of BMD/day in these animals. Bullon et al.[19] demonstrated that a diet based on virgin olive oil prevented alveolar resorption due to age in rats through a mitochondrial mechanism. In an interesting work, Saleh et al.[20] administered 12-14-month-old female Wistar rats with virgin olive oil (1 ml/kg body weight) for 12 weeks, 4 before oophorectomy and 8 weeks after. The ovariectomized rats showed a significant decrease in plasma calcium and an increase in alkaline phosphatase, malondialdehyde, and nitrate levels (the latter two indicating a reduction in oxidative stress). These changes were tempered by olive oil. The tibia of the ovariectomized rats showed a decrease in cortical width and trabecular thickness and a significant increase in the number of osteoclasts. These parameters improved considerably in the group treated with olive oil.

Rezq et al.[21] observed that replacing dietary lipids with olive oil for 6 weeks increased femoral length, volume, and BMD in mice. Liu et al.[22] compared the effectiveness of treatment with oil (1 ml/100 g of diet) and with diethylstilbestrol (25 µg/kg of diet), a synthetic estrogen, to mimic hormone replacement therapy in humans. Both treatments produced an increase in lumbar and femur BMD in ovariectomized rats. This could be attributable to a decrease in oxidative stress in the treated groups, indicated by malondialdehyde and nitrate levels.

In contrast to these results, Tagliaferri et al.[23] found that to alleviate bone loss induced by ovariectomy in rats, virgin olive oil is not enough. Rather, an additional vitamin D supplement is required.

In a study carried out in humans, Roncero Martín et al.[24] administered virgin olive oil to 523 women with a mean age of 50 years (between 23 and 81). The women were divided into two groups: those who ingested more than 18.32 g/day of oil and those who ingested less than that amount. They observed a significant increase in BMD (p<0.001) in the group with the highest olive oil intake.

Liu et al.[22] carried out a study in women between 30 and 50 years old who had undergone a hysterectomy. One group was treated with 50 ml of olive oil daily and another control group received no supplement. After 1 year, the BMD of the L2, L3,L4 and of the left femur decreased significantly in the control group and not in the oil-treated group.

Effects of the different components of olive oil on bone health

Puel et al.[25] evaluated the effects of oleuropein in a model of ovariectomized rats with and without inflammation. This phenolic compound (0.15 g oleuropein/kg/day) was able to exert positive effects on bone loss in rats with inflammation, but not in those without inflammation.

Oleuropein increases the formation of osteoblasts from bone marrow stem cells and decreases the generation of adipocytes and fat cells, suggesting that oleuropein intake could have preventive effects against bone loss associated with osteoporosis and age[26].

In terms of bone resorption, oleuropein at 10 µM decreased the formation of "osteoclast-like" cells (positive tartrate-resistant acid phosphatase) in a spleen cell culture. At a concentration of 50 µM and 100 µM, oleuropein completely suppressed the formation of these cells *in vitro*[27].

García Martínez et al.[28] investigated the effects of the phenolic extracts of Sicilian virgin olive oil on the growth of osteoblasts, using the MG-63 osteosarcoma line. Treatment of osteoblast cells with phenolic extracts increased the number of cells between 13.77 and 30.98%, compared to controls.

Cells of the same MG-63 line were cultured for 24 h with 10^{-6} M of the phenolic compound phenyl acid, caffeic acid, coumaric acid, apigenin or luteolin. The expression by MG-63 cells of growth markers and differentiation/maturation was modified after treatment with 10^{-6} M of the aforementioned phenolic compounds, increasing the gene expression of transforming growth factor β1 (TGF-β1), the TGF1, 2 and 3 receptor, the bone morphogenetic protein 2 and 7, the transcription factor-run 2, the alkaline phosphatase, osteocalcin, type I collagen and osteoprotegerin. The phenolic components of virgin olive oil reportedly have a beneficial effect on the bone by modulating the osteoblast's physiology, which supports its protective effect against bone diseases[29].

The phenolic compounds in olive oil have been shown to possess antioxidant properties *in vivo* and *in vitro*[30,31]. Taking phenols can influence BMD by acting as free radicals, preventing oxidation-induced damage to bone cells.

An extensive review carried out from 2001 to 2014 in the databases of MEDLINE L´EMBASE and the Cochrane Library, using as entries "Mediterranean diet", "virgin olive oil", "phenols", "bone", "osteoblasts" and "osteoporosis," suggest that phenols in olive oil may be beneficial

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in preventing bone loss. They are reported to induce the proliferative capacity and cell maturation of osteoblasts by increasing alkaline phosphatase activity and depositing calcium ions in the extracellular matrix[28].

We previously mentioned phenolic alcohols, tyrosol and hydroxytyrosol, as components of olive oil. Hydroxytyrosol has been shown to eliminate trabecular bone loss in femurs of ovariectomized rats[27]. On the other hand, hydroxytyrosol at concentrations between 50 µM and 100 µM inhibits the formation of multinucleated osteoclasts in a dose-dependent manner. In a culture of spleen cells, hydroxytyrosol (50 and 100 µM) and tyrosol (100 µM) reduced the formation of acid phosphatase-tartrate-cell resistant[27].

Although, as we have mentioned, there are various studies in the literature that show that treatment with olive oil increases BMD, it has not been possible to demonstrate that there has been an increase in biomechanical parameters[32,33]. However, in a recent work carried out by our group[34] we have treated a group of ovariectomized 6-month-old Wistar rats with olive oil by oral gavage for 3 months (100 µl/day or 200 µl/day). Our results show that the treatment with 100 µl of olive oil recovered the value of Young's modulus in the x-axis, which had decreased with oophorectomy, and the treatment with 200 µl of oil produced an improvement in the z-axis of Young's modulus. with respect to the ovariectomized rats, that is to say, that the olive oil influenced the biomechanical parameters. In this same work, we found that the groups treated with 200 µl of olive oil presented a value of the fractal dimension D2D and D3D greater than that of the ovariectomized rats. The fractal dimension expresses the degree of complexity of the outline of a structure in filling a surface or volume. These results indicate that the bone composition of rats treated with 200 µl of virgin olive oil is more complex and more irregular and, thus, more similar to normal bone. Despite these improvements in bone health, in our work we did not find differences in the BMD of the treated rats or in the micro-morphometric parameters, but the results obtained in Young's modulus and in the fractal dimension that indicate an improvement cannot be disregarded in the bone quality of the treated ovariectomized rats. It is important to highlight that in our work we give the rats 100 µl or 200 µl of virgin olive oil/day. Taking into account that the rats weighed 320 g at the beginning of the study, this would be equivalent to giving 18.7 or 37 ml of olive oil/day to a 60 kg person, a dose that could be consumed normally. Many of the published experimental works supply rats with a very high quantity of oil relative to what a normal human diet might be.

The results of this review show, without a doubt, that virgin olive oil exerts a positive action on bone health. This is possibly due to the action of its phenolic components, which include oleuropein, tyrosol and hydroxytyrosol. These agents have been shown to have anti-oxidant and anti-inflammatory properties, and therefore may be potential candidates for the prevention of osteoporosis.

Conflict of interests: The authors declare no conflict of interest.

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