

# *Olea europaea* and its Constituents Promote Bone Health by Enhancing Osteoblast Differentiation and Proliferation: A Review

Ruszymah Bt Hj Idrus<sup>1,\*</sup>, Aminuddin Bin Saim<sup>2</sup>

## ABSTRACT

**Background:** The human bone is in a constant state of balance between bone resorption and bone formation, maintained by the osteoclasts and osteoblasts respectively. Association of Mediterranean diet and bone health has been prevalently studied in recent years. Olive and its constituents have been suggested to be the major contributor to the benefits of the Mediterranean diet in bone health. **Method:** In this review, cellular and molecular mechanism of bone homeostasis and the influence of olive and its constituents were discussed. **Result:** Many studies ranging from clinical, animal and *in vitro* cell culture reported benefits of olive and its constituents in bone health. Olive and its constituent exerted its beneficial effect in bone health through stimulation of bone formation as opposed to inhibition of bone resorption. **Conclusion:** Olive and its constituents promotes osteoblast differentiation and proliferation. **Key words:** Bone, Olive, Oleuropein, Hydroxytyrosol, Osteoblast.

Ruszymah Bt Hj Idrus<sup>1,\*</sup>,  
Aminuddin Bin Saim<sup>2</sup>

<sup>1</sup>Department of Physiology, Universiti Kebangsaan Malaysia Medical Centre, Cheras, 56000, MALAYSIA.

<sup>2</sup>Ear, Nose and Throat Consultant Clinic, Ampang Puteri Specialist Hospital, Ampang, 68000, MALAYSIA.

## Correspondence

Prof. Ruszymah Bt Hj Idrus

Department of Physiology, Universiti Kebangsaan Malaysia Medical Centre, Cheras, 56000, MALAYSIA.

Phone no : +60391458617

E-mail: ruszyidrus@gmail.com

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## INTRODUCTION

The bone structure and the cellular processes that occur within the bone, allows it to simultaneously serve as a calcium reservoir while providing structure support and locomotion. As a result, the human bone is in a constant state of balance, between deposition and resorption of bone minerals to maintain the calcium homeostasis and bone integrity.<sup>1</sup> Bone minerals is deposited by osteoblasts in a process called ossification and is resorbed by osteoclasts to meet physiological calcium demand in the body.<sup>2</sup>

Condition that result in a decrease in bone mass, such as osteoporosis, can either be caused by a decrease in ossification or an increase in resorption.<sup>3</sup> The ossification of bone involves a complex series of events that encompasses osteoblasts proliferation and differentiation.<sup>4</sup> Alternatively, the bone reabsorption process involves the formation and activation of osteoclasts, which is derived from monocytes or macrophages.<sup>5</sup>

Physiologically, bone modelling and remodeling processes are governed not only by heritable traits but also by nutritional, mechanical and hormonal factors. Modification towards dietary patterns has been associated with reduced risk in osteoporosis incidence.<sup>6</sup> A group that studied the osteoporosis incidence among Greece women noted that the osteoporosis incidence in their population is substantially lower than in the US and other European countries. They hypothesized that Mediterranean diet to play a major role in this difference.<sup>7</sup>

The Mediterranean diet is defined by high intake of olive oil, plant products, fish and seafood; a low intake of dairies, meat and meat products; and a moderate

ethanol intake.<sup>8</sup> These food items possessed a complex array of naturally occurring bioactive molecules with antioxidant, anti-inflammatory and alkalinizing properties that may contribute to the bone-sparing effect of the Mediterranean diet.<sup>6</sup> Adherence to the Mediterranean diet has been linked to the prevention of bone diseases via a number of observational studies done across different populations.<sup>7,9,10</sup>

Upon inspection of the studies that associated Mediterranean diet with bone health, olive oil has been considered to be the major contributor to the health benefits associated with this diet.<sup>6</sup> Olive oil is the major source of fats in the Mediterranean diet. Olive oil has been associated with reduction in the incidence of some diseases such as cardiovascular, diabetes and several cancers, among other degenerative pathologies.<sup>11</sup>

Some health properties of olive-tree-derived products have been attributed to its phenolic compound, oleuropein. Oleuropein is a key olive-tree phenolic compound with high antioxidant capacity.<sup>12</sup> Therefore, it has interesting health effects, including anti-inflammatory, anti-atherogenic, anti-tumoral, anti-microbial and neuroprotective. They can be found in various parts of the olive tree plant, like fruits and leaves, as well as mill by-products of olive-oil production, including waste water.<sup>13</sup>

Olive, olive oil and its bioactive component has been nominated as a potential candidate for dietary options against osteoporosis. This is based on the growing body of literature focusing on the bone protective effects of olive and its constituents.<sup>14</sup>

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Parallel to the growing body of literature on bone protective effects of olive and its constituents, understanding of its mechanism of action at the molecular level should follow. Hence, this review seeks to elucidate the molecular mechanism utilized by olive and its constituent in promoting bone health.

### Cellular Physiology of Bone Homeostasis

At the hormonal level, calcium homeostasis is regulated by the parathyroid hormone. When the blood calcium level is low, production of parathyroid hormone triggers the physiologic response that leads to the release of bone calcium, increase of intestinal calcium absorption and increase of renal calcium reabsorption. All in the effort of increasing the calcium level back to the normal physiologic level.<sup>15</sup>

Within the bone, bone homeostasis is a dynamic process that involves the interaction between two distinct bone cells, osteoblasts and osteoclasts. It is the balance between the formation of bone and its resorption, executed by the osteoblasts and osteoclasts respectively.<sup>16</sup>

Osteoblast, the cell that is responsible for new bone formation, is derived from osteoprogenitors, the undifferentiated pluripotent mesenchymal cells that are responsible for bone development.<sup>17</sup> Osteoblast differentiation is controlled by the master transcription factor RUNX2 (runt-related transcription factor 2); also known as CBFA1 (core-binding factor A1).<sup>18</sup> Osterix (Osx), is another transcription factor that is also important in osteoblast differentiation.<sup>19</sup> Studies with mice that lack either of these proteins demonstrated the formation of a cartilaginous skeleton at embryonic level but not the bony skeleton, resulting in the morbidity of the mice at birth.<sup>20</sup>

Osteoblasts synthesized collagen and two specialized proteins, osteocalcin and osteopontin.<sup>21</sup> Together, the latter two protein is known as osteoid. Osteoblasts also produce alkaline phosphatase, the enzyme that is required to form the calcium phosphate crystals, hydroxyapatite.<sup>2</sup> This will eventually lead to the formation of mineralized extracellular matrix that made up the structure of the bone.

As mineralization occurs, some osteoblasts will mature into osteocytes and occupy the lacunae, the hollow spaces within the bone, to maintain its structure. Once mineralization is done, active osteoblasts will become flattened and line the surface of the bone. These mature osteoblasts are known as the lining cells. The rest of the osteoblast simply undergo apoptosis.<sup>1</sup>

Osteocytes form a network extending throughout mineralized bone via its long, dendrite-like processes, in order to interact with other osteocytes within the mineralized bone and the osteoblasts on the bone surface.<sup>22</sup> Osteocytes respond to mechanical load and this network is thought to be integral in the detection of mechanical strain and associated bone microdamage that accumulates as a result of normal skeletal loading and fatigue.<sup>22</sup>

On the other hand, the bone resorption involves osteoclasts, which is derived from monocytes or macrophages.<sup>5</sup> Osteoclast is an irregular shaped, giant cell, that break down or reabsorbs the bone. Osteoclasts arise from either macrophages or monocytes from the bone marrow. Monocytes fuse together to form multinucleated osteoclasts cells.<sup>23</sup>

Osteoclast cells have numerous fingerlike processes known as the ruffled border. The ruffled border is the side where osteoclast attached to the surface of the bone, maximizing the surface area for the resorption of the bone minerals. Osteoclasts bind themselves to the bone via the integrin protein, vitronectin. The site where the osteoclast attached to the bone is known as Howship's lacunae, commemorating the biologist John Howship that first discover it. Osteoclasts secrete tartrate resistant acid phosphates (TRAP) in order to lowers the pH level in the Howship's lacunae to aid the reabsorption of the mineralized bone matrix.<sup>24</sup>

Cathepsin K is a cysteine protease enzyme present in actively resorbing osteoclasts. It is able to cleave the telopeptide region of type 1 collagen allowing for the mineralized bone resorption by the osteoclast. The serum level of cathepsin K reflects the number of osteoclasts and serves as a specific biomarker of osteoclast activity.<sup>24</sup>

The activation of osteoclasts is known to be regulated by two cytokines; receptor activator of nuclear factor- $\kappa$ B ligand (RANKL) and macrophage colony-stimulation factor (M-CSF). The binding of RANKL to its receptor RANK on the surface of osteoclast, leads to the activation of TNF receptor-associated factor 6 (TRAF6), which is linked to nuclear factor  $\kappa$ B (NF- $\kappa$ B) via mitogen-activated protein kinases (MAPKs). RANKL and M-CSF are proteins secreted by osteoblasts and is important for the formation of osteoclast and regulation of its activity.<sup>25</sup>

Osteoblasts also secretes osteoprotegerin (OPG), a soluble decoy receptor for RANKL that blocks its binding to RANK.<sup>25</sup> The OPG secretion by osteoblast prevents osteoclast differentiation, fusion and activation, which causes a decrease in bone reabsorption and destruction.<sup>25</sup> Secretion of transforming growth factor  $\beta$  (TGF- $\beta$ ) by osteocytes, induced the secretion of OPG, resulting in the inhibition of osteoclastogenesis.<sup>26</sup>

### Markers of Bone Health

For bone health assessment and evaluation of therapeutic responses, a number of markers have been used, not just for clinical tests but also applicable to experimental studies using *in vitro* (cell-based) assays or *in vivo* (animal) models. Two categories of bone parameters can be measured to evaluate bone health, which is the physical and biochemical markers.<sup>27</sup>

A major physical parameters of bone health is the bone mineral density (BMD). BMD is the measurement of the amount of calcified tissue in the bone. BMD can be obtained using X-rays. The procedure is painless and non-invasive and involves low radiation exposure. For small animals, mini X-rays equipment is often used. As the prime indicator of bone strength, BMD has become the standard for diagnosis of osteoporosis as recommended by the World Health Organization. However, while BMD is a quantitative assessment of bone health, it does not provide information on bone quality.<sup>28</sup>

BMD results can be supplemented with the measurement of the bone architecture to get an overall picture of bone quality.<sup>29</sup> Bone microarchitecture is accessed via histological observation. Bone microarchitecture is related to the mechanical strength. Deterioration of bone architecture results in bone loss and can be linked to observations such as decreased number of trabeculae, increased inter-trabecular distances, loose connectivity of the trabecular meshwork, reduction of cortical bone thickness and increased porosity. Apart from BMD measurement and bone microstructure assessment, there are plethora of other less common method to measure bone strength and fragility employed in animal models to test for drug effects.<sup>30</sup>

The use of bone biochemical markers to measure and monitor bone turnover and bone loss is considered recent, thanks to the in-depth understanding of the bone physiology from recent years.<sup>31</sup> The markers reflect the metabolic activity of osteoblasts or osteoclasts and are measurable in blood or urine in order to provide a quantitative estimate of the status of bone remodeling.

Information on bone remodeling status is an early indicator of pathological changes or the risk of some bone diseases. These biomarkers are useful not only for clinical assessments as monitors of osteoporosis and predictors of fracture, but also for the evaluation of therapeutic responses in experimental model.

In terms of evaluating therapeutics responses to novel drugs or natural products, bone biomarkers provide immediate information as compare

to measuring the physical bone deposition, which could take some time before significant changes can be detected. Bone biomarkers can be categorized into two, the bone formation markers and the bone resorption markers.

Biomarkers for bone formation are by-products or enzymes used by the active osteoblast during bone formation. Among products of bone formation that can be used as biochemical markers are alkaline phosphatase, osteocalcin and the pro-collagen type 1. Increase in genetic or protein expression of transcription factors that regulate osteogenesis, RUNX2 and Osterix are also useful in measuring bone formation.

In contrast, biomarkers for bone resorption will be the products associated with the activity of osteoclast. Among them are TRAP, RANKL, cathepsin K, osteopontin and hydroxyproline (component of collagen).

### *Olea europaea* and Its Component

Olive or its scientific name, *Olea europaea* is the fruit from the olive tree, which belongs to the family Oleaceae. It is one of the major components in the diet of Mediterranean people. A closer inspection on the health benefits of the olive oil alone suggested that it is the major contributor to most of the health benefits associated with Mediterranean diet. In the review, clinical and epidemiological data that looks into the relevance of olive oil and its components for longevity and against age- and lifestyle-associated pathologies such as cancer, cardiovascular, metabolic and neurodegenerative diseases correlate well with the positive outcomes brought by the Mediterranean diet.<sup>11</sup>

The promising potential of olive health benefits is due to the presence of bioactive phenolics compounds. The major phenolic compounds in olive oil can be divided into three different classes: simple phenols (hydroxytyrosol, tyrosol); secoiridoids (oleuropein, the aglycone of ligstroside and their respective decarboxylated dialdehyde derivatives) and lignans (1-acetoxypinoresinol and pinoresinol).<sup>32</sup> Phenolic compounds of olive contributed to the antioxidative properties of olive.

Traditionally, olive has been used in treating asthma, hemorrhoids, intestinal diseases and use to reduce blood sugar and cholesterol level. Recent human and animal research have shown the importance of olive oil in reducing the incidence of some diseases such as cardiovascular, diabetes and several cancers, among other degenerative pathologies.<sup>11</sup> In terms of bone health, its association with Mediterranean diet has been prevalently studied in recent years. Olive and its constituents have been suggested to be the major contributor to the benefits of the Mediterranean diet in bone health.

### Clinical Studies Reporting Effect of *Olea europaea* and its Constituents in Osteoporosis Prevention

Among the risk factors that can influence osteoporosis, dietary consumption is the factor that can be modified. Hence, researchers are motivated to find the link between dietary consumption and osteoporosis. To date, the most consistently followed approach to examine the potential relation between dietary factors and skeletal health was based on particular micronutrients such as calcium and vitamin D3.<sup>33</sup> However, in reality, people do not eat isolated nutrients but meals consisting of a variety of foods with complex combinations of nutrients and bioactive components.<sup>6</sup>

One of the potential candidates is the Mediterranean diet. The Mediterranean diet is defined by a high intake of olive oil, plant products, fish and seafood; a low intake of dairies, meat and meat products; and a moderate ethanol intake.<sup>8</sup> It has been noted that several studies conducted in the Mediterranean area have reported osteoporosis and osteoporosis-related fractures incidences that is lower among countries across the European Union.<sup>7,10,34</sup> This brings about the interest on linking the Mediterranean diet consumption and osteoporosis prevention.

Adherence to the Mediterranean diet has been shown to reduce rate the hip fracture incidence in Swedish men and women.<sup>34</sup> The study combined two Swedish cohort studies consisting of 37,903 men and 33,403 women (total n=71,333, mean age 60 years) that are free of previous cardiovascular disease and cancer who answered a medical and a food-frequency questionnaire in 1997. A modified Mediterranean diet score (mMED; range 0-8 points) was created based on high consumption of fruits and vegetables, legumes and nuts, whole grains, fermented dairy products, fish and olive/rapeseed oil, moderate intake of alcohol and low intake of red and processed meat. Incident hip fractures between 1 January 1998 and 31 December 2012 were retrieved from the National Patient Register and the result shows an inverse relationship between the higher Mediterranean diet score and low incidence rate in hip fracture.

In the clinical trial PREDIMED, 127 elderly men were divided into three groups, Mediterranean diet with virgin olive oil (at least 50 mL/day), Mediterranean diet with mixed nuts (at least 30 g/day) or a low-fat diet and were followed-up for two years. The virgin olive oil group demonstrated an increased in bone formation marker and decreased in bone resorption markers, suggesting the positive effects that olive have in bone remodeling.<sup>10</sup>

A group of researchers in Italy investigated the effect of 1 year of oral supplementation with either extra virgin olive oil (VOO) enriched with vitamins D3, K1 and B6 (VitVOO) or VOO used as placebo (PlaVOO) on modification of bone turnover and oxidative stress markers.<sup>35</sup> The study revealed that subjects taking VitVOO showed lower plasma osteocalcin levels than those taking the PlaVOO. In terms of bone mineral density, a significant improvement in VitVOO subjects compared to PlaVOO was found. All oxidative stress markers as thiobarbituric acid reactive substances, lipid hydroperoxides and conjugated dienes showed a significant reduction after VitVOO supplementation, whilst plasma total antioxidant capacity values were significantly increased in VitVOO group compared to PlaVOO group. Although all the findings in the study demonstrate the beneficial effect of vitamin on bone health markers compare to the virgin olive oil control, the fact that the placebo resulted in favorable outcome. This is in agreement with the beneficial effects of VOO documented in other studies.<sup>36-38</sup>

### Animal Studies Reporting Effect of *Olea europaea* and its Constituents in Osteoporosis Prevention

In terms of olives fruit, its inclusion in the diet of rat's model of senile osteoporosis, prevented bone loss in the whole femur and at cortical sites of the animals.<sup>39</sup> However, this effect is only seen in the black olives but not green olive, a more common variant of olive. This could be attributed to a higher polyphenol level in the black olives compared to the green olives.

Estrogen, a reproductive hormone has long been associated with osteoporosis. Decreased estrogen predisposed to increased risk of osteoporosis. When discussing the olive oil, it has been associated with prevention of the bone loss caused by the absence of estrogen in ovariectomized rats.<sup>40-42</sup> The effect is associated with olive oil anti-oxidant properties as supported by the decrease in oxidative markers in the serum.<sup>41</sup> The comparison between olive oil supplementation and the synthetic estrogen, suggested the efficacy of olive oil as the dietary alternative to estrogen replacement in preventing post-menopause bone loss.<sup>40</sup>

Positive effect of olive oil in improving bone density can be observed when Liu *et al.* (2014) analyzed the blood samples from patients who regularly consumed olive oil over a year. The bone mineral density of the lumbar spine and left femur of the patients were evaluated by dual energy X-ray absorptiometry and positive correlation between olive oil consumption and bone mineral density was evident.<sup>40</sup>

**Table 1: Molecular study of olive oil and constituent and bone health.**

No	References	Subject matter	Model	Treatment	Parameters	Outcome	Conclusion
1	Garcia 2014 <sup>37</sup>	Phenolic extracts from Sicilian virgin olive oil of wide variety: Biancolilla Passulunara Tonda iblea Nocellara del belice Organic Nocellara del belice Nocellara etnea 0m Nocellara etnea 100m Nocellara etnea 900m	Human osteosarcoma cell line MG-63	Phenolic extracts at concentrations of 0.001, 0.0001 and 0.00001% were dissolved in fresh culture medium	Cell proliferation assay via MTT	1. a) Phenolic extracts increased the number of cell by 13.77-30.98% compared to controls	Phenolic extracts from different virgin olive oil variety stimulate osteoblast MG-63 proliferation
2	Santiago 2011 <sup>44</sup>	Olive oil phenolic compound: Oleuropein	Human mesenchymal stem cells induced osteoblast and adipocyte differentiation	Oleuropein treatment at concentrations of 10 <sup>-4</sup> , 10 <sup>-5</sup> , 10 <sup>-6</sup> M in induced medium	Gene expression for osteoblastogenesis via RT-PCR RUNXII, osterix, ALP, Collagen type 1, Osteocalcin, OPG/RANKL ALP activity via ELISA and RT-PCR  <i>In vitro</i> mineralisation via Alizarin red S staining Adipogenesis via oil red O staining  Gene expression for adipogenesis PPARγ2, LPL, FABP-4	1) Oleuropein influence on osteoblastogenesis a) Oleuropein increased the expression of RUNXII, osterix and ALP b) Oleuropein increased the expression of collagen type 1 at concentrations of 10 <sup>-6</sup> and 10 <sup>-4</sup> M at day 7, while significantly increased at day 14 at 10 <sup>-5</sup> M. c) Oleuropein increased the expression of osteocalcin at 10 <sup>-6</sup> M. d) Oleuropein showed a higher OPG/RANKL ratio e) ALP activity increased with oleuropein compared to control 2) Oleuropein influence on adipogenesis a) Oleuropein inhibit the expression of PPARγ2, LPL and FAB-4 b) Area occupied by lipid droplets was lower with oleuropein treatment c) Size of the fat particles was smaller with oleuropein treatment	Oleuropein promote MSC induced osteoblast differentiation and inhibit adipogenesis
3	Garcia 2016 <sup>46</sup>	Olive oil phenolic compound: Hydroxytyrosol Tyrosol, Caffeic acid, Vanillic acid, Vanillin, p-coumaric acid, Ferulic acid, Sinapic acid, Oleuropein, Luteolin, (+)-pinoselinol, Apigenin	Human osteosarcoma cell line MG-63	Each phenolic compound was added into medium at 10 <sup>-5</sup> , 10 <sup>-6</sup> , 10 <sup>-7</sup> and 10 <sup>-8</sup> M dose  Phenolic extracts at concentrations of 0.001, 0.0001 and 0.00001% were dissolved in fresh culture medium	Cell proliferation assay via MTT	1. a) Concentration of hydroxytyrosol at 10 <sup>-6</sup> M significantly increase cell proliferation while concentrations lower than 10 <sup>-6</sup> M did not have significant effect on cells. b) Caffeic acid, p-coumaric acid, ferulic acid increased the cell proliferation by 12-16% compared to controls in the range of 10 <sup>-5</sup> to 10 <sup>-9</sup> M c) Luteolin, apigenin increased cell proliferation by 11-15% in all concentrations d) Vanillic acid, Vanillin, Sinapic acid did not affect cell proliferation e) Oleuropein did not induce cell proliferation f) (+)-pinoselinol has no effect on cell proliferation 2) Phenolic extracts in all concentrations stimulate MG-63 cell proliferation	Phenolic compounds and extracts from different extra virgin olive oil stimulate osteoblast MG-63 proliferation

Continued...

Table 1: Cont'd.

No	References	Subject matter	Model	Treatment	Parameters	Outcome	Conclusion
4	Melguizo 2018 <sup>47</sup>	Olive oil phenolic compound: luteolin, apigenin or p-coumaric, caffeic or ferulic acid	Human osteosarcoma cell line MG-63	Each phenolic compound was added into osteogenic medium at 10 <sup>-6</sup> and 10 <sup>-8</sup> M dose	1. Alkaline phosphatase (ALP) was evaluated by spectrophotometry 2. Antigen expression (CD54, CD80, CD86 and HLA-DR) by flow cytometry	1. ALP activity increases in all treatment of apigenin, ferulic acid, p-coumaric acid, luteolin and caffeic acid except for the lowest doses of luteolin and caffeic acid 2. a) Decrease in CD54 expression versus untreated control cells (p≤0.001) after treatment with each dose of each phenolic compound tested. b) Decrease in CD80 expression was observed after treatment with apigenin or ferulic or p-coumaric acid at doses of 10 <sup>-6</sup> M (p=0.006, p=0.025 and p=0.012, respectively) and 10 <sup>-8</sup> M (p=0.007, p=0.04 and p=0.007 respectively). c) Ferulic acid increased the expression of CD86 or HLA-DR at all doses (p<0.001) and caffeic acid increased CD86 expression at doses of 10 <sup>-6</sup> and 10 <sup>-8</sup> M (p<0.001)	Phenolic compounds studied induce cell maturation <i>in vitro</i> , favoring formation of the extracellular matrix, as indicated by the increase in ALP synthesis and loss of the expression of antigens involved in osteoblastic immune function.
5	Casado 2017 <sup>49</sup>	Olive oil by-products (distillate 6; D6) that is rich in squalene	Human mesenchymal stem cells induced osteoblast and adipocyte differentiation	MSC culture medium was supplemented with 10% of serum obtained from women that consumed olive oil by-products D6	1. Alkaline phosphatase (ALP) was evaluated by spectrophotometry 2. Gene expression of osteogenic markers (RUNX2 and ALPL) or adipogenic markers (PPARG2 and LPL) via RT-PCR 3. Calcium deposit (osteoblast) detection using Alizarin red or fat droplet (adipocyte) via	1) Higher ALP compares to control 2) Upregulation of RUNX2 and ALPL while PPARG2 and LPL were inhibited 3) Calcium deposit was non-significant compare to control but fat droplet was lower compare to control	Serum from postmenopausal women supplemented with olive oil by-product promotes MSC induced osteoblast differentiation and inhibit adipogenesis

The correlation between olive oil antioxidant properties and bone remodeling can be observed when osteoporosis patients, supplemented with extra virgin olive oil diet, presented an increased in bone formation marker and decreased in bone resorption markers, together with the increased in antioxidant capacity and decreased levels of oxidative products.<sup>35</sup>

Oleuropein when given to inflamed ovariectomized rats, prevented the decline of total, diaphyseal and metaphyseal bone mineral density. This is due the attenuation of bone resorption, indicated by the decrease of serum osteocalcin and a marginal decrease of urinary deoxyypyridinoline level.<sup>43</sup>

Taken together, the bone protective effects of olive and its derivative is well established via clinical and pre-clinical model. Hence, elucidating the intracellular molecular signaling involved in the olive effects on both osteoblasts and osteoclasts is of paramount importance.

### *In vitro* Studies Reporting Effect of *Olea europaea* and its Constituents in Osteoporosis Prevention

From the literature review, 5 studies that investigated the effect of olive and its constituent on *in vitro* culture of human cells has been identified and listed in Table 1. Two studies used bone marrow derived mesenchymal cell to investigate the effect that oleuropein<sup>44</sup> and olive oil by-products<sup>45</sup> have on osteoblast differentiation. Another three studies, used human osteosarcoma cell line MG-63 to investigate the effect of olive oil phenolic compounds on the cell proliferation of the osteoblast<sup>37,46</sup> and expression of bone differentiation markers.<sup>47</sup>

Bone formation is dependent on the cell proliferation of the osteoblast. Evaluation of olive oil and its constituent was reported by Garcia *et al.* In the earlier study, they reported significant increase in proliferation with phenolic extract from a number of Sicilian virgin olive oil variants.<sup>37</sup> In a more recent study, they supplemented the osteoblast cell with both extra virgin olive oil extracts and isolated phenolic compounds namely hydroxytyrosol, tyrosol, caffeic acid, vanillic acid, p-coumaric, ferulic acid, sinapic acid, oleuropein, luteolin, pinoresinol and apigenin. They also reported stimulation of the osteoblast proliferation with phenolic compounds and extracts.<sup>46</sup> The authors suggested that the beneficial effect of olive oil and its constituent on the proliferation of osteoblast in culture is mainly due to its anti-oxidant effect.

In bone marrow derived mesenchymal cell culture, oleuropein has been reported to enhance osteoblastogenesis but suppress adipogenesis.<sup>44</sup> This suggested that oleuropein play a role in the lineage specific differentiation of bone marrow stem cell. Oleuropein-treated cells demonstrated increased expression of osteoblastogenesis genes such as runt-related transcription factor II, osterix, collagen Type I, alkaline phosphatase and osteocalcin.<sup>44</sup> The role of olive phenolic compound in selective induction of osteoblastogenesis is supported by the experiment using serum from post-menopausal women supplemented with olive oil by-product. The diet of post-menopausal women was supplemented with olive oil distillate that is high in squalene, a component of olive oil, for two months and serum was collected. Treatment of human bone marrow-derived mesenchymal stem cell with the serum demonstrated an induction of osteogenesis and suppression of adipogenesis.<sup>48</sup>

Role of olive oil phenolic compound in osteoblast differentiation was also reported in human osteosarcoma cell line MG-63. Different phenolic compounds of olive oil which are luteolin, apigenin, p-coumaric, caffeic acid and ferulic acid were supplemented to the bone cell culture to investigate the expression of bone formation and immunological markers. All phenolic compounds tested were able to induce cell maturation *in vitro* as indicated by the increase in ALP synthesis and loss of the expression of antigens involved in osteoblastic immune function.<sup>47</sup> This suggested

that olive and its constituent beneficial effect on bone health by inducing bone formation while simultaneously inhibiting bone resorption process.

## CONCLUSION

The literature review reveals numerous evidences supporting the role of olive and its constituents on bone health. The evidence is available in a wide spectrum of experimental studies, including *in vitro* cell culture, animal models and clinical studies. Knowledge on the molecular mechanism of bone homeostasis has been well established, enabling scientist to understand the influence of olive and its constituents on these mechanism of action at the molecular level. Olive and its constituents seem to exert beneficial effect on the bone homeostasis via inducing the bone formation process as opposed to the inhibition of bone resorption process. In particular, olive and its constituent has been shown to promote osteoblast differentiation and proliferation.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## ABBREVIATIONS

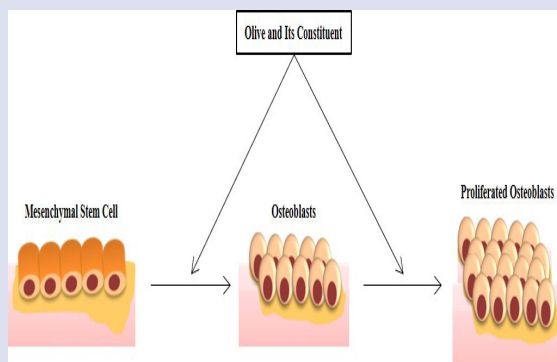
**RUNX2:** Runt-related transcription factor 2; **CBFA1:** Core binding factor A1; **Osx:** Osterix; **TRAP:** Tartrate resistant acid phosphatase; **RANKL:** Receptor activator of nuclear factor- $\kappa$ B ligand; **M-CSF:** Macrophage colony-stimulation factor; **TRAF6:** TNF receptor-associated factor 6; **NF- $\kappa$ B:** Nuclear factor  $\kappa$ B; **MAPK:** Mitogen-activated protein kinase; **OPG:** Osteoprotegerin; **TGF- $\beta$ :** Transforming growth factor  $\beta$ ; **BMD:** Bone mineral density; **mMED:** Modified Mediterranean diet score.

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## GRAPHICAL ABSTRACT



## SUMMARY

- Bone homeostasis is the balance between bone resorption and bone formation that is regulated by the plethora of signaling molecules including transcription factors, cytokines and surface receptor proteins. Association of Mediterranean diet and bone health has been the subject of interest among scientists in recent years. Olive and its constituents have been suggested to be the major contributor to the benefits of the Mediterranean diet in bone health. Many studies ranging from clinical, animal and *in vitro* cell culture has reported benefits of olive and its constituents in bone health. In this review, cellular and molecular mechanism of bone homeostasis and the influence of olive and its constituents were discussed. Olive and its constituent exerted its beneficial effect in bone health through stimulation of bone formation as opposed to inhibition of bone resorption. Specifically, olive and its constituent has been reported to promote osteoblast differentiation and proliferation.

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