Effect of 1-year dietary supplementation with vitaminized olive oil on markers of bone turnover and oxidative stress in healthy post-menopausal women

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Abstract Osteoporosis represents a serious health problem worldwide associated with an increased risk of fractures and mortality. Nutrition should form part of bone disease prevention strategies, especially in the light of the population ageing and the diet effect on bone health. Thus the study aimed at verifying whether 1 year of oral supplementation with either extra virgin olive oil (VOO) enriched with vitamins D$_3$, K$_1$ and B$_6$ (VitVOO) or VOO used as placebo (PlaVOO) is able to modify some bone turnover and oxidative stress markers. Bone mineral density (BMD) was assessed in 60 healthy post-menopausal women together with the bone vitamin K status by measuring undercarboxylated osteocalcine (ucOC) plasma levels, the ratio between ucOC and carboxylated osteocalcine (UCR) and the relations with oxidative stress markers. After 1 year ($T_1$), subjects taking VitVOO showed lower ucOC levels than those taking PlaVOO; the same trend was found for UCR. As far as BMD is concerned, a significant increase in T-score at $T_1$ 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 was significantly increased in VitVOO group compared to PlaVOO group at $T_1$. It might be suggested that the use of VitVOO in the diet of post-menopausal women could represent a proper tool for bone protection and a useful strategy against oxidative stress and related diseases, thus confirming the antioxidant role played by the added vitamins.

Keywords Vitaminized oil · Osteoporosis · Oxidative stress · Osteocalcine · Bone mineral density

Introduction

The financial exposure of national healthcare systems is expected to increase dramatically with the ageing of the population (i.e. the financial impact of the management of the elderly diseases) [1]. Bone diseases will represent a sizable proportion of these costs; in fact, the worldwide financial impact of osteoporotic fractures is estimated to double by 2050 to reach 76.7 billion euros [2–4]. In Europe, it is estimated that 179,000 men and 611,000 women suffer a hip fracture every year [5], and these numbers are expected to double in the next 40 years [6].

Nutrition should form part of bone disease prevention strategies [7], especially in the light of the population ageing and the diet effect on bone health. The amount of...
bone tissue in the skeleton, known as bone mass, can keep growing until around age 30 and, at skeletal sites such as the proximal femur, it declines thereafter with an acceleration after 50 years [1].

Although the value of nutrients such as calcium and vitamin D for bone health is well established, an European consensus on maximum levels to allow in food supplementation is still lacking.

The fat-soluble vitamin D pro-hormones, ergocalciferol (vitamin D₂) and cholecalciferol (vitamin D₃), are essential for the efficient intestinal absorption of calcium and phosphate and the subsequent mineralization of bone. Inadequate actual vitamin D active tissue level leads to chronic secondary hyperparathyroidism and osteoporosis [8]. Furthermore, both dietary calcium intake and intestinal calcium absorption decrease with age [9], whilst vitamin D deficiency is associated with accelerated bone loss and increased fracture risk [10]. Moreover, in a recent study, whilst exploring the antioxidative effects of vitamin D in prostate cells, Bao et al. found that the active form of vitamin D, 1α,25-dihydroxyvitamin D₃, could protect non-malignant human prostate epithelial cell lines from oxidative stress-induced cell death [11].

Another nutrient that has being increasingly linked to bone health and osteoporosis is vitamin K [12]. The justification for a role of vitamin K in bone health spread up from the isolation of a vitamin K-dependent protein from bone, called osteocalcin (OC). OC is produced in osteoblasts, and fully carboxylated OC (cOC) binds the calcium ions of hydroxyapatite [13].

A clear rationale for ensuring optimal dietary intakes of vitamin K is the evidence that a high circulating level of undercarboxylated OC (ucOC) is an independent risk predictor of bone fractures [14] and of low bone mineral density (BMD) [15]. Also, vitamin K has been found an antioxidiant role in lipid [16].

Finally, vitamin B₆ which is well known in its biochemically active form as pyridoxal 5’-phosphate is an essential cofactor of numerous metabolic enzymes. This vitamin is also implicated in numerous human body functions (for example, it modulates hormone function or it can also act as a strong antioxidiant) [17].

One of the most well-known and important characteristic of the Mediterranean diet is the use of virgin olive oil (VOO) as the principal source of dietary fat [18]; it can be used as a food matrix on the basis of its recognized beneficial properties and also because it is a good solvent for lipophilic vitamins. Furthermore, VOO is an useful food carrier on the basis of its recognized beneficial properties ranging from a decreased risk of cardiovascular disease, obesity, metabolic syndrome, type 2 diabetes and hypertension [19].

At moment, the effects of increased intake of micronutrients through fortified foods is poorly known [20]; thus, the aims of the present study were (i) to verify whether 1 year of oral supplementation with either 20 ml/day of VOO enriched with vitamins D₃ (1,25-dihydroxy-cholecalciferol), K₁ (phyloquinone) and B₆ (pyridoxal 5’-phosphate) (VitVOO) or 20 ml/day of VOO used as placebo (PlaVOO) is able to lead to an increase in bone mineral density (BMD) in healthy post-menopausal women; (ii) to compare the bone vitamin K status by measuring ucOC plasma levels, the ratio between ucOC and cOC (UCR) and BMD profiles before and after such intake as previously demonstrated in fertile women [21] where a promptly reduction of both ucOC and UCR in supplemented individuals was found; and (iii) to verify any possible correlations amongst the above-mentioned group of markers also in relation to some oxidative stress markers.

The rationale for the use of VitVOO, in managing of osteoporosis and oxidative stress, is related to its safety, low cost and mainly to the involvement of these vitamins on the above-mentioned metabolisms.

Methods

Design overview

The study was performed according to the guidelines laid down in the Declaration of Helsinki as revised in 2001, and all procedures involving subjects were approved by the Institutional Review Board of Senigallia Hospital. Written informed consent was obtained from all subjects after the procedures had been fully explained.

The study was designed as a 1-year single-centre, randomized, placebo-controlled trial of daily oral supplementation of 20 ml/day of VOO containing vitamin K₁ (0.70 mg/100 ml), vitamin D₃ (50 μg/100 ml) and vitamin B₆ (6.0 mg/100 ml) versus placebo (20 ml/day of PlaVOO) bottled as anonymous containers.

The oil formulation for this trial was prepared by Fattoria Petrini (Monte San Vito, Ancona, Italy). The composition of tested oils is shown in Table 1.

Study participants and intervention protocol

Sixty Caucasian post-menopausal women (aged 50–61 years), enrolled at post-menopausal ambulatory of the Department of Obstetrics, Gynecology and Pediatrics, Senigallia Hospital (Ancona, Italy) for health screening for osteoporosis, were randomly assigned to the two groups. This number of patients achieves 99 % power to test difference in the BMD between the two groups of treatment by a repeated measure of analysis of variance, with a 5 %
significance level and an actual effect standard deviation value of 0.5.

Women were eligible to participate in the study if they were post-menopausal (at least 1 year after last menses). They were excluded if they had (i) any medication affecting bone metabolism in the past 3 months (such as bisphosphonates, selective oestrogen receptor modulators, hormone replacement therapy or calcitonin); (ii) known metabolic bone diseases such as primary hyperparathyroidism or Paget disease; (iii) any corticosteroids use, and disease modifying bone metabolism such as Cushing disease or parathyroid cancer, secreting parathormone; (iv) decompensated diseases of the liver, kidney, pancreas, lung or heart; (v) history of active cancer in the past 5 years; (vi) chronic oral steroid or warfarin use; (vii) chronic disease (e.g. diabetes mellitus, cardiovascular disease, cancer and fat malabsorption syndromes); and (viii) alcohol abuse. They were also excluded if they were on high doses of vitamin A ([10,000 IU/d) or E ([400 IU/d), as these interfere with vitamin K metabolism [22]. Eligible subjects were also required to have normal screening laboratory values. The selected subjects had a body mass index in the range of 22–28 kg/m², consumed a standard Mediterranean Diet (based on a list providing with information on permitted food), that was balanced for each enrolled subject, consisting of 1,500 kcal/day; 15 % proteins, 30 % lipids, 55 % carbohydrates and 34 g of fibre, prescribed 2 weeks before the beginning of the study. They were instructed also to avoid taking dietary supplements other than those allowed throughout the study and food sources of vitamin K such as green vegetables (i.e. spinach, broccoli green beans, asparagus) and fermented foods, like cheese. They were also educated to use the oil only as a dressing, but not for cooking. All dietary recommendations were performed by a physician on individual basis. Dietists took care of giving correct and useful instruction for day-by-day management and food preparation, and meetings were scheduled in a monthly basis to verify that patients were correctly following the diet recommended and that they did not change the dietary supplementation.

Baseline measurements (T₀) were performed in January 2011, and follow-up measurement (T₁) was performed after 1 year. On each occasion, blood samples were drawn between 7:00 and 10:00 a.m. after overnight fast.

Blood sampling

Blood was collected into vacutainers containing heparin, and collection was done as required for each analyte. Samples for routine laboratory measurements were processed immediately.

For the measurement of ucOC and cOC, and for oxidative stress markers, blood was collected on ice and centrifuged at 1,300 × g for 10 min at 4 °C within 2 h from withdrawal, and the middle layer of the plasma was rapidly pipetted off, aliquoted, and frozen at −80 °C until use, as previously described [21].

Assessment of ucOC and cOC

ucOC and cOC, used as indicators of vitamin K status, were determined by separate immunoassays using the respective ELISA kits from Takara Shuzo (Otsu, Shiga, Japan). All measurements were carried out in quadruplicate according to the manufacturers’ instructions with a

| Table 1 Composition of the tested oils: PlaVOO and VitVOO enriched with vitamin K₁ (0.70 mg/100 ml), vitamin D₃ (50 µg/100 ml) and vitamin B₆ (6.0 mg/100 ml) |
|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
|                                | PlaVOO (per 100 ml)             | VitVOO (per 100 ml)             | PlaVOO (% RDA)                  | VitVOO (% RDA)                  |
| Energy value                   | 837 kcal                        | 828 kcal                        | –                               | –                               |
| Proteins                       | 0 g                             | 0 g                             | –                               | –                               |
| Carbohydrates                  | 0 g                             | 0 g                             | –                               | –                               |
| FATS of which:                 | 93.3 g                          | 92.0 g                          | –                               | –                               |
| Saturated                      | 13.3 g                          | 13.8 g                          | –                               | –                               |
| Monounsaturated                | 66.6 g                          | 69.0 g                          | –                               | –                               |
| Polysaturated                  | 13.3 g                          | 9.20 g                          | –                               | –                               |
| Vitamin D₃                     | 0 g                             | 50 µg                           | –                               | 100 %                           |
| Vitamin K₁                     | 0 g                             | 0.70 mg                         | –                               | 100 %                           |
| Vitamin B₆                      | 0 g                             | 6.0 mg                          | –                               | 30 %                            |
| Vitamin E (natural)            | 30 mg                           | 30 mg                           | 30 %                            | 30 %                            |

VitVOO virgin olive oil enriched with vitamins D₃ (1,25-dihydroxy-cholecalciferol), K₁ (phyloquinone) and B₆ (pyridoxal 5′-phosphate), PlaVOO virgin olive oil used as placebo, RDA recommended daily allowance.
Plasma lipid hydroperoxides levels

The extent of lipid peroxidation in plasma was determined using the ferrous oxidation-xylenol orange (FOX) assay [23]. Briefly, aliquots (200 µl) of plasma were mixed with 1,800 µl of FOX-reagent [250 µM ammonium ferrous sulphate, 100 µM xylene orange, 25 mM H2SO4 and 4 mM BHT in 90 % methanol (v/v) in 100 ml]. After incubation at room temperature for 30 min, samples were centrifuged at 2,900×g for 10 min. The supernatant was carefully decanted into a cuvette, and the absorbance was determined at 560 nm. The levels of lipid hydroperoxides were quantified using a stock solution of t-butyl hydroperoxide. The results are shown as µmol/l plasma. Previous studies have reported that the coefficient of variation for individual plasma using the FOX assay is <10 % [23].

Plasma lipid peroxidation measurement

The degree of plasma oxidation was determined by measurement of the hydroperoxide level of TBARS. TBARS were determined using the method of Yagi [24]. Results are expressed as nanomoles malondialdehyde per 100 µg protein.

Determination of plasma conjugated dienes

The extent of lipid peroxidation of plasma was evaluated by measuring the level of conjugated dienes formation monitoring the increase in the optical density (OD) at 232 nm in controls and patients as previously described [25]. Results are expressed as arbitrary absorbance units (AAU).

Determination of total antioxidant capacity (TAC)

Total antioxidant capacity was evaluated in plasma with Total Antioxidant capacity assay kit (Biovision, USA). The assay relies on the ability of antioxidants in the sample to reduce the Cu2+ ion, which is then chelated by a colorimetric probe giving a broad absorbance peak at around 570 nm, proportional to the total antioxidant capacity. The capacity of the antioxidants in the sample to prevent Cu2+ ion reduction is compared with that of Trolox, a water-soluble tocopherol analogue. The results are expressed as nmol/µl or mM Trolox equivalents. Inter-assay and intra-assay values are 3 and 3.4 %, respectively.

Bone mineral density (BMD) assessment

BMD at the proximal phalanx of the second, third, fourth and fifth finger of right hand was measured using a DBM Sonic Bone Profiler (IGEA S.p.A., Carpi, Modena, Italy). Bone ultrasonometry was always performed in the morning by the same operator, and the mean values of BMD collected at the four fingers were calculated. Results were expressed as an absolute value (g/cm², bone mineral content relative to projected area) and transformed to a T-score (number of standard deviations above or below the mean). The coefficient of variation (CV) was 1.78 % determined by repeated measurements in a subgroup of 15 subjects (three measurements per person on two different days).

The range indicative of high risk of osteoporosis was based on the results of a large epidemiologic study of European women indicating as high risk of osteoporosis a T-score value < −3.2 SD [26].

Statistical analysis

Statistical analysis was performed using the SAS statistical package (Statistical Analysis System Institute, Cary, NC). The results are expressed as means ± standard deviations.

The Kolmogorov–Smirnov test was used to determine normality of distribution of the examined variables. Because all variables were found to be normally distributed, parametric tests were applied in all further analyses.

Differences between baseline (T0) and end-point (T1) values within the subjects were tested by analysis of variance (ANOVA) followed by the Bonferroni t multiple comparisons test to reduce the probability of significant differences arising by chance. Differences were considered significant at P < 0.05.

Results

All 60 participants completed the study, and as summarized in Table 2, their baseline (T0) characteristics were similar in the two groups. There was a slightly decrease, but not significant, in BMI, fasting plasma glucose levels, fasting plasma cholesterol levels, LDL cholesterol levels and fasting plasma triglycerides levels in both groups at T1 compared to T0, whilst there was a statistically significant increase in HDL cholesterol levels in VitVOO and PlaVOO groups at T1 compared to T0, 65.7 ± 13.6 mg/dl for VitVOO and 64.4 ± 12.5 mg/dl for PlaVOO vs. 58.6 ± 14.1 mg/dl and 57.6 ± 15.3 mg/dl, respectively; P < 0.05).

Bone metabolism markers

At T0, both groups showed not significant differences in ucOC plasma levels (3.05 ± 0.15 vs. 3.08 ± 0.12 ng/ml; P = NS) (Fig. 1). On the contrary, after 1 year of oral supplementation, subjects taking VitVOO showed lower ucOC plasma levels than those taking PlaVOO.
(2.60 ± 0.14 vs. 3.12 ± 0.19 ng/ml; P < 0.001) and those at T0 (P < 0.001).

The same trend was found for UCR ratio, where no significant differences in both groups at T0 and a significant decrease after 1 year of oral supplementation (T1) were found in subjects taking VitVOO compared to those taking PlaVOO and those at T0 (Fig. 2).

As far as BMD is concerned, at T0, both VitVOO and PlaVOO groups showed no statistically significant differences in T-score (−2.41 ± 0.30 vs. −2.38 ± 0.35, respectively; P = NS) (Fig. 3), whilst a significant increase in T-score at T1 in subjects taking VitVOO compared to those taking PlaVOO (−1.28 ± 0.18 vs. −2.43 ± 0.32; P < 0.05) and those at T0 was found (Fig. 3).

Oxidative stress markers

All oxidative stress markers as TBARs, lipid hydroperoxides and conjugated dienes assessed in plasma showed a significant reduction after VitVOO supplementation, whilst there were no significant differences between the two groups at T0. Specifically, TBARs plasma levels decreased significantly, after 1 year of oral supplementation, in subjects taking VitVOO with respect to those taking PlaVOO (12.10 ± 1.70 vs. 41.68 ± 3.68 nmol/ml; P < 0.001) and those at T0 (P < 0.05) (Table 3).

Lipid hypoperoxide plasma levels showed no statistically significant differences in both groups at T0 (9.30 ± 0.70 vs.

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Table 2 Characteristics of the study subjects at the time of recruitment (T0) and after supplementation with either VitVOO or PlaVOO (T1)

<table>
<thead>
<tr>
<th></th>
<th>T0</th>
<th>T1</th>
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<tbody>
<tr>
<td></td>
<td>VitVOO (n = 30)</td>
<td>PlaVOO (n = 30)</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Age (years)</td>
<td>55.6</td>
<td>2.6</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>25.9</td>
<td>3.1</td>
</tr>
<tr>
<td>Fasting plasma glucose (mg/dl)</td>
<td>82.5</td>
<td>9.0</td>
</tr>
<tr>
<td>Fasting plasma cholesterol (mg/dl)</td>
<td>219.6</td>
<td>34.3</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>58.6</td>
<td>14.1</td>
</tr>
<tr>
<td>Fasting plasma triglycerides (mg/dl)</td>
<td>125.2</td>
<td>46.9</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dl)</td>
<td>133.1</td>
<td>33.7</td>
</tr>
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VitVOO virgin olive oil enriched with vitamins D3 (1,25-dihydroxy-cholecalciferol), K1 (phylloquinone) and B6 (pyridoxal 5′-phosphate), PlaVOO virgin olive oil used as placebo, SD standard deviations, HDL high-density lipoproteins, LDL low-density lipoproteins

* P < 0.05 (T1 vs. T0)
9.38 ± 0.80 nmol/mg prot \( P = \text{NS} \), whereas a significant decrease, after 1 year of oral supplementation, in subjects taking VitVOO compared to those taking PlaVOO (3.10 ± 0.30 vs. 8.8 ± 0.6 nmol/mg prot; \( P < 0.01 \)) was found (Table 3).

Conjugates dienes plasma levels showed the same trend: there were no significant differences in both groups at \( T_0 \) (4.70 ± 0.40 vs. 4.30 ± 0.35 arbitrary absorbance numbers; \( P = \text{NS} \)) and a significant decrease, after 1 year of oral supplementation, in subjects taking VitVOO compared to those taking PlaVOO (2.10 ± 0.30 vs. 4.5 ± 0.41 arbitrary absorbance numbers; \( P < 0.01 \)) (Table 3).

In accordance with the previously mentioned results, plasma TAC values showed no differences at \( T_0 \) (4.070 ± 0.130 vs. 3.997 ± 0.110 nmol/\( \mu \)l Trolox equivalents; \( P = \text{NS} \)) and a significant increase in VitVOO group compared to PlaVOO group at \( T_1 \) (4.631 ± 0.110 vs. 4.067 ± 0.14 nmol/\( \mu \)l Trolox equivalents; \( P < 0.01 \)) (Table 3).

**Discussion**

Osteoporosis has a huge burden on public health through the increased morbidity, mortality and economic costs associated with resultant fractures of vertebrae, hip, spine, femur and wrist, and with the pharmacological treatments [27]. Moreover, during menopause, oxidative stress occurs due to loss of estrogens [28].

As from results in the present study, we demonstrated that 1 year of oral supplementation with vitamin-enriched extra olive virgin oil was able to reduce both ucOC and UCR in supplemented subjects compared to the placebo group. The decrease in ucOC and UCR reflects improved vitamin K status.

Such results are in accordance with a previous pilot study by our group: a beneficial effect of VitVOO on OC metabolism in fertile women [21] with a promptly reduction of both ucOC and UCR in supplemented individuals was found.

Population studies in different healthy groups have determined that a low dietary consumption of vitamin K is associated with an increased fracture risk [29, 30] or a lower bone mass [30], and that an impaired vitamin K status is associated with low bone mass [31] or increased bone turnover [32].

It is known that elevated values of ucOC and UCR are associated with low bone mass and increased risk of osteoporotic fractures [33], and that vitamin K insufficiency contributes to the development of osteoporosis [34].

It has also been reported that phyloquinone plasma concentration is inversely associated with ucOC fraction

![Fig. 3 BMD (T-score) in subjects supplemented with VitVOO (black line) versus subjects supplemented with placebo oil (grey line), before \( T_0 \) and after 1-year oral supplementation (\( T_1 \)). Means ± standard deviations (SD) are shown. BMD bone mineral density; VitVOO virgin olive oil enriched with vitamins D\(_3\) (1,25-dihydroxy-choleccalciferol), K\(_1\) (phylloquinone) and B\(_6\) (pyridoxal 5’-phosphate), PlaVOO virgin olive oil used as placebo.](image)

<table>
<thead>
<tr>
<th>( T_0 )</th>
<th>VitVOO (( n = 30 ))</th>
<th>PlaVOO (( n = 30 ))</th>
<th>( T_1 )</th>
<th>VitVOO (( n = 30 ))</th>
<th>PlaVOO (( n = 30 ))</th>
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<tbody>
<tr>
<td>FOX (( \mu )mol/l plasma)</td>
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<td>0.70</td>
<td>9.38</td>
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<tr>
<td>TBARs (nmol MDA/100 ( \mu )g prot)</td>
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<td>2.80</td>
<td>47.39</td>
<td>3.11</td>
<td>12.10</td>
</tr>
<tr>
<td>Conjugated dienes (AAU)</td>
<td>4.70</td>
<td>0.40</td>
<td>4.30</td>
<td>0.35</td>
<td>2.10</td>
</tr>
<tr>
<td>TAC (nmol/( \mu )l trolox equivalent)</td>
<td>4.070</td>
<td>0.130</td>
<td>3.997</td>
<td>0.110</td>
<td>4.631</td>
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</table>

Data are shown as Mean ± SD

VitVOO virgin olive oil enriched with vitamins D\(_3\) (1,25-dihydroxy-choleccalciferol), K\(_1\) (phylloquinone) and B\(_6\) (pyridoxal 5’-phosphate), PlaVOO virgin olive oil used as placebo, FOX ferrous oxidation-xylene orange, TBARs thiobarbituric acid reactive substances, MDA malondialdehyde, AAU arbitrary absorbance units, TAC total antioxidant capacity

* \( P < 0.001 \) (VitVOO at \( T_1 \) vs. PlaVOO at \( T_1 \), VitVOO and PlaVOO at \( T_0 \)
and is positively associated with BMD in both men and post-menopausal women not taking oestrogen replacement therapy [35].

In accordance with our results, a number of studies showed that low intake of vitamin K is associated with a high amount of circulating ucOC and that supplementation with vitamin K is able to decrease the amount of circulating ucOC [36].

In recent years, vitamin B₉ has become a focus of research interests describing the compound’s critical function in cellular metabolism and stress response. However, it became clear that it is also a potent antioxidant that effectively quenches reactive oxygen species and is thus of high importance for cellular well-being [17].

However, there is no evidence of a correlation between vitamin B₉ and OC, whereas animal and cell studies have shown that 1,25-dihydroxy vitamin D induces the synthesis of OC by promoting transcription of its gene, and that vitamin K is responsible for its post-transcriptional activation [37]. Furthermore, vitamin D induces the synthesis of OC, but if vitamin K intake is low, OC remains undercarboxylated, thereby increasing the levels of ucOC in blood and bone [34].

According to BMD results, the values of T-score are consistent with OC results, being increased between T₀ and T₁ in VitVOO supplemented subjects. Different studies showed that supplementation with vitamin K is not associated with increased BMD at the femoral neck, but is associated with increased BMD at the lumbar spine [38]. Ushiroyama et al. suggested that combined therapy with vitamin K₂ and D₃ is able to increase bone turnover and promote bone construction and calcification, resulting in marked increases in BMD and improvement in bone quality [39].

Indeed there is growing evidence that olive oil consumption has also a beneficial effect on oxidative stress-associated diseases such as coronary heart diseases, cancer and ageing [19, 40, 41].

A recent study highlighted that daily consumption of a high-phenol extra virgin olive oil reduces oxidative DNA damage in post-menopausal women [42]. The biological benefits of olive oil consumption in preventing oxidative damage could be linked both to its antioxidant content, mainly phenolic compounds and vitamin E, and to its high monounsaturated fatty acid content.

In the present work, after 1 year of oral supplementation with 20 ml/day of VitVOO, it has been observed a decrease in oxidative stress markers in healthy post-menopausal women, confirming our previous results on fertile women (2012 submitted).

In particular, using VitVOO, a significant increased plasma HDL cholesterol concentration and plasma TAC was demonstrated, as well as a significant decrease of both lipid peroxidation markers (TBARS and conjugated dienes).

In addition, the results of the present study regarding formation of organoperoxides as determined by FOX and TBARS assays might provide a more consistent index of oxidative stress. In fact, oxidative degradation of lipids proceeds by a variety of pathways in which organoperoxides are early products and malondialdehyde is the later product of lipid oxidation [43]. Organoperoxides measured by the FOX assay have also been shown to provide a temporal view of changes in oxidative stress.

Our results are fully in agreement with those present in the literature where beneficial effects of VOO are documented in the young, middle-aged and old healthy population by means of increased plasma antioxidant capacity, modified lipid profile, prevention of oxidative damage or improved antioxidant enzyme function [19, 44, 45]; indeed, the reduction of oxidative stress markers is more striking in the VitVOO group due to the antioxidant role of the added vitamins.

Finally, although not statistically significant, the VitVOO group shows a slightly decrease, in the BMI. Our results indicate the presence of a reduction of oxidative stress parameters associated with dietary supplementation with VitVOO, which is independent of BMD as documented by the absence of any correlation between the BMI and the oxidative stress markers (data not shown).

Strength of the present study is the nutritional approach given to the work, due to the fact that VOO is the principal source of dietary fat of the Mediterranean Diet; as a matter of fact, no participant withdrew as a consequence of reported side effects. There are, however, also some limitations. Our study population consisted of healthy women without major complaints, all of Caucasian origin, from Senigallia or surrounding areas. Thus, they do not represent the general population of post-menopausal women 55 years of age or older. A second limitation of this study is that BMD was measured at the proximal phalanx of the second, third, fourth and fifth finger of right hand, instead of at the lumbar spine (LS) at L1 to L4 and femoral neck (FN), so this technique does not take into account also the role of gravity on osteoporotic lesions.

Since there are now a variety of approaches available for the management of osteoporosis, in the present study, we have demonstrated that 1-year oral supplementation with VitVOO is able to counteract bone loss by reducing both ucOC concentration and UCR and increasing the T-score values.

Considering all the results taken together, we propose that VitVOO supplementation counteracts estrogens loss menopause-associated, by reducing also oxidative stress markers and increasing total antioxidant capacity. We can then hypothesize that the use of VitVOO in the diet of
menopausal women could represent, on the one hand, a proper tool for bone protection and might signify a useful intervention to slow menopausal bone loss and, on the other hand, a useful strategy against oxidative stress and related diseases, thus confirming also an antioxidant role played by the added vitamins.

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Conflict of interest The authors of this research article do not have any conflict of interest at the time of submission.

References


