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Original article

Extra virgin olive oil consumption reduces the risk of osteoporotic fractures in the PREDIMED trial

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SUMMARY

Background & aims: The incidence of osteoporotic fractures is lower in countries in the Mediterranean basin. Virgin olive oil, a key component of the Mediterranean Diet (MDiet), with recognised beneficial effects on metabolism and cardiovascular health, may decrease the risk of osteoporotic fractures. The aim to this study was to explore the effect of chronic consumption of total olive oil and its varieties on the risk of osteoporosis-related fractures in a middle-aged and elderly Mediterranean population.
Methods: We included all participants (n = 870) recruited in the Reus (Spain) centre of the PREvención con DIeta MEDiterránea (PREDIMED) trial. Individuals, aged 55–80 years at high cardiovascular risk, were randomized to a MedDiet supplemented with extra-virgin olive oil, a MedDiet supplemented with nuts, or a low-fat diet. The present analysis was an observational cohort study nested in the trial. A validated food frequency questionnaire was used to assess dietary habits and olive oil consumption. Information on total osteoporotic fractures was obtained from a systematic review of medical records. The association between yearly repeated measurements of olive oil consumption and fracture risk was assessed by multivariate Cox proportional hazards.
Results: We documented 114 incident cases of osteoporosis-related fractures during a median follow-up of 8.9 years. Treatment allocation had no effect on fracture risk. Participants in the highest tertile of extra-virgin olive oil consumption had a 51% lower risk of fractures (HR:0.49; 95% CI:0.29–0.81. *P* for trend = 0.004) compared to those in the lowest tertile after adjusting for potential confounders. Total and common olive oil consumption was not associated with fracture risk.
Conclusions: Higher consumption of extra-virgin olive oil is associated with a lower risk of osteoporosis-related fractures in middle-aged and elderly Mediterranean population at high cardiovascular risk.

1. Introduction

Osteoporosis is an age-related progressive bone condition characterised by bone mass loss and microarchitecture degradation that increase the risk of potentially serious fractures. It is a major burden for health care systems as osteoporotic fractures and falls by osteoporotic fractures are associated with a high dependence, morbidity and mortality [1–3]. Osteoporosis is estimated to affect 27.5 million people (22 million women and 5.5 million men) aged

Abbreviations: MedDiet, Mediterranean diet; BMD, bone mineral density; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; EVOO, extra virgin olive oil; FFQ, food frequency questionnaire; BMI, body mass index.

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between 50 and 84 years worldwide and its prevalence is expected to rise to 33.9 million by 2025 [4].

Bone remodeling balance is affected by several factors, such as age, heredity or endocrine diseases [3]. Lifestyle factors, such as smoking, physical activity and diet also affect bone health [5]. Low calcium intake and low exposure to sunlight leading to reduced synthesis of vitamin D have also been identified as common risk factors because of their role in bone mass health [1,6]. In addition, other specific nutrients, foods, or dietary patterns can influence bone health [6–9]. Adhering to a traditional Mediterranean diet (MedDiet), characterized by high intake of fruits, vegetables, nuts and olive oil, has been linked to a lower risk of hip fractures [10–12], which might partly explain the epidemiological evidence of a geographical variation in the incidence of hip fractures across Europe, the highest rates being in North Europe and the lowest in the Mediterranean basin countries or in United States' population where it was associated a lower risk of hip fracture with MedDiet pattern [11,13]. These observations might be attributed to the high content of monounsaturated fats (MUFA) and polyphenols in olive oil, the main fat consumed in the Mediterranean diet. The intake of MUFA has been positively correlated with bone mineral density (BMD) in the Greek and Spanish populations [14–16] and higher circulating levels of bone remodelling osteocalcin have been reported after following a MedDiet enriched with extra-virgin olive oil (EVOO) [17]. Similarly, a high intake of olive extract has also been linked to higher levels of osteocalcin and stabilization of bone mass loss in osteopenic postmenopausal women [18].

The effect of consumption of olive oil and its varieties on the risk of osteoporotic fractures has not been studied. Our aim was to examine the association between the amount of total olive oil and its varieties (extra virgin and common olive oil) consumed and the risk of osteoporotic fractures in a sub-sample of middle-aged and elderly Mediterranean participants of the PREDIMED trial. We hypothesized that higher consumption of EVOO containing high amounts of polyphenols would reduce the risk of osteoporosis-related fractures.

2. Materials and methods

2.1. Study design and population

The present study was carried out in the framework of the PREDIMED study, a large, multi-centre, randomized and controlled parallel group trial aimed at assessing the effect of the MedDiet on the primary prevention of cardiovascular diseases in Spain. This trial is registered at http://www.controlled-trials.com as ISRCTN35739639. Osteoporotic fractures were assessed only as part of an ancillary study including all participants (n = 870) recruited in the PREDIMED-Reus centre. Full details of the PREDIMED protocol are published elsewhere [19]. Participants (men aged 55–80 years and women aged 60–80 years) were randomly assigned to 1 of 3 intervention groups: (1) a MedDiet supplemented with EVOO (MedDiet-EVOO group; 50 g or more per day), (2) a MedDiet supplemented with mixed nuts (MedDiet-Nuts; 30 g of nuts daily), or advice on a low-fat diet (Control). Supplemental foods were given for free to participants in the MedDiet groups, while those in the control diet group received non-food gifts. Participants had no history of CVD at baseline but they were at high cardiovascular risk because of the presence of type 2 diabetes or at least three of the following risk factors: current smoker; hypertension; high levels of low-density lipoprotein cholesterol; low levels of high-density lipoprotein cholesterol; overweight or obesity and/or a family history of premature cardiovascular disease. Participants excluded were those with a BMI greater than 40 kg/m^2, severe chronic illness, drug or alcohol addiction, history of allergy or intolerance to olive oil or nuts, and/or a low predicted likelihood of changing dietary habits according to Prochaska and DiClemente's stages-of-change model [20]. The local institutional review board approved the study protocol, and all participants provided written informed consent. Recruitment took place between 1st October, 2003, and 30th June, 2009 and the intervention was terminated in 2010 with an extended follow-up to August 2015. The study was performed according to Declaration of Helsinki about Ethical Principles for Medical Research Involving Human Subjects.

2.2. Measurements

At baseline and at each annual visit until the end of intervention in 2010, data on lifestyle variables, medical conditions and medication use were recorded. Weight and height were measured with light clothing and no shoes, using calibrated scales and a wall-mounted stadiometer, respectively. Waist circumference was measured midway between the lowest rib and the iliac crest using an anthropometric tape. Blood pressure was measured using a validated oscillometer (Omron HEM705CP; Hoofddorp, The Netherlands) in triplicate with a five-minute interval between each measurement, and the mean of these values was recorded. Trained personnel took fasting blood samples for subsequent biochemical analysis. The validated Spanish version of the Minnesota Leisure-Time Physical Activity questionnaire was given at baseline and yearly [21].

2.3. Dietary assessment

A 137-item semi-quantitative validated food frequency questionnaire (FFQ) was given to all participants at baseline and was repeated every year throughout the follow-up period [22]. Energy and nutrient intake were estimated from Spanish food composition tables [23,24]. Data regarding the consumption of different types of olive oil was obtained from the FFQ, which included three different questions on the type of olive oil consumed: (1) EVOO (obtained only by mechanically pressing the olives, acidity <1%), (2) refined oil (refined olive oil, acidity <0.3%) and (3) pomace olive oil (obtained using solvents from the leftovers of pressing the olives and mixed with other refined olive oils, acidity <0.3%). The number of 12 g tablespoons was recorded for each variety in 9 frequency categories as follows: no consumption, one to three times per month, *n* times per week (*n* = one, two to four or five to six) or *n* times per day (*n* = one, two to three, four to six or more than six). The number of tablespoons stated was converted into grams per day. One FFQ item asked about EVOO intake and two other items asked about refined olive oil and pomace olive oil, and these two values were added together for common olive oil intake. Total olive oil intake was then the sum of all three items. Using the Pearson correlation coefficient (r), reproducibility and validity of the FFQ were 0.55 and 0.60, respectively, for total olive oil consumption, and the intraclass correlation coefficients for reproducibility and validity were 0.71 (*P*-value: <0.001) in a population similar to the PREDIMED participants [22].

A validated 14-item MedDiet screener was also administered to assess the degree of adherence to the MedDiet [25]. Two of the 14 items were related to olive oil intake. To control for the overall dietary pattern, the 2 items related to olive oil were removed from the total score; thus, a 12-point score was used as covariate in the models.

2.4. Outcome

All osteoporotic fractures were adjudicated according to the criteria defined by Warriner and co-workers including fractures scoring over 5, representing those more likely due to osteoporosis

This score consider fracture risk groups according to sex, age and race, and scored from 1 to 9 with higher scores representing those fractures most likely due to osteoporosis [26]. This was also selected in accordance with previous studies regarding new classification of osteoporotic fractures beyond the classical ones (vertebral, hip and wrist-forearm) [27–29]. According to the International Classification of Diseases Clinical Modification (ICD-CM), open clavicle (ICD-CM 810.1–810.3), phalanges (ICD-CM 816.1–816.13 and 826.0–826.1), tarsal/metatarsal (ICD-CM 825.0–825.39), scapula (ICD-CM 811.0–811.19), and skull/facial (ICD-CM 800.00–804.99) fractures were excluded [26]. Incident cases of osteoporotic fractures through 1st December, 2010 were identified initially from a systematic, comprehensive and standardised annual review of all outpatient and inpatient medical records of each participant. Information on osteoporotic fractures was updated yearly using medical records. An independent researcher confirmed all fracture events.

2.5. Statistical analyses

Participants' baseline characteristics were described with means (SD) and percentages (number). To take advantage of the yearly dietary assessments, we averaged the food consumption from the baseline to the end of the follow-up or to the last follow-up FFQ before the occurrence of fractures. Then, participants were categorized into tertiles of total olive oil, EVOO or common olive oil consumption using the mean value of all FFQs from the beginning to the last before the incidence of fracture or the end of follow-up in those not suffering a fracture. Dietary variables were adjusted for total energy intake using the residuals method [30] and they are presented in accordance with energy-adjusted tertiles of EVOO intake. Follow-up time was estimated as the interval from the beginning of the study up to the date of fracture events, death (for any reason) or end of follow-up, whichever came first.

The associations between energy-adjusted tertiles of total olive oil consumption or its different subtypes and the risk of osteoporotic fractures were assessed using time-dependent multivariate Cox proportional hazards models. We tested the proportionality of hazards with the use log-rank test. Results are expressed as hazard ratios (HRs) and 95% confidence intervals (CI). Model 1 was adjusted for age, sex, BMI, education level (primary education, secondary education, academic/graduate), leisure time physical activity (metabolic equivalent of task (MET)-minutes/day), smoking status (never, former, current smoker) and the intervention group. As other covariates can interfere with the risk of fractures, Model 2 was additionally adjusted for prevalence of diabetes (yes/no), prevalence of previous documented osteoporotic fractures (yes/no), use of insulin (yes/no), use of oral antidiabetic medications (yes/no), use of diuretic drugs (yes/no), use of oral glucocorticoids (yes/no), use of anti-osteoporotic drugs (yes/no), use of anticoagulants (yes/no), use of oestrogen (yes/no) and baseline MedDiet adherence (12-point score). Covariates were selected based on their biological plausibility of having an association with the risk of fractures. The same models (excluding the baseline 12-point score) were used to assess the risk of osteoporotic fractures according to the dietary intervention group. The associations between MUFA intake, polyunsaturated fatty acids (PUFA) intake and MUFA:PUFA ratio with the risk of fractures were assessed using the covariates included into the Model 3. Nelson-Alen estimator was used to analyse the increasing failure rates. Sensitivity analysis was conducted excluding early cases observed during the first year of intervention. The level of significance was $P < 0.05$ for all statistical tests for bilateral contrast. Statistical analyses were carried using SPSS 21.0 for windows (IBM, Chicago, IL, USA) and STATA 14 (StataCorp, College Station, TX).

3. Results

During a median of 5.2 years of intervention and 8.9 years of follow-up, we documented 114 incident cases of osteoporosis-related fractures (40 in MedDiet-EVOO group, 37 in MedDiet-Nuts group and 37 in control group). Tables 1 and 2 show the baseline anthropometric and dietary characteristics of the study participants according to energy-adjusted tertiles of EVOO consumption. There were not significant differences in age, sex, BMI, previous fractures, prevalence of diabetes, medications, energy intake, protein intake, alcohol intake, vitamin D or fermented dairy products intake between tertiles of EVOO consumption. The mean consumption of total olive oil was 56.5 g/day in participants at the highest tertile and 37.6 g/day in those in the lowest tertile.

Table 1
Baseline characteristics of study participants according to energy-adjusted tertiles of extra virgin olive oil consumption.

Variable	T1 (n = 290)	T2 (n = 290)	T3 (n = 290)
Age (years)[a]	67 ± 6	68 ± 6	67 ± 6
Men, % (n)	46.6 (135)	42.1 (122)	45.9 (133)
BMI (kg/m^2)[a]	29.7 ± 3.2	29.5 ± 3.2	29.6 ± 3.4
Waist circumference (cm)[a]	101.9 ± 9.00	100.6 ± 8.3	101.1 ± 9.1
Leisure-time energy expenditure in physical activity (MET minutes/day)[a]	255.1 ± 265.8	286.3 ± 281.3	244.2 ± 239.8
Smoking status, % (never, current, former)	59.3, 12.8, 27.9	61.7, 14.1, 24.2	64.8, 9.3, 25.9
Educational level, % (n)			
Primary education	5.9 (17)	6.6 (19)	6.6 (19)
Secondary education	14.5 (42)	18.3 (53)	20.0 (58)
Academic/graduate	79.6 (231)	75.2 (218)	73.4 (213)
History of osteoporotic fractures, % (n)	18.3 (53)	14.5 (42)	19.3 (56)
Diabetes, % (n)	51.0 (148)	49.3 (143)	55.5 (161)
Hypertension, % (n)	85.2 (247)	86.2 (250)	85.5 (248)
Medication use, % (n)			
Diuretics	26.9 (78)	23.8 (69)	23.8 (69)
Insulin	5.2 (15)	5.9 (17)	6.6 (19)
Oral glucocorticoids	1.4 (4)	1.0 (3)	1.7 (5)
Osteoporosis drugs	9.7 (28)	11.0 (32)	13.1 (38)
Oral anticoagulants	1.4 (4)	1.4 (4)	0.3 (1)
Oral antidiabetic drugs	36.2 (105)	30.3 (88)	37.2 (108)
Oestrogens	1.7 (5)	2.8 (8)	2.4 (7)

[a] Data are expressed as means ± SD. BMI, body mass index; MET, metabolic equivalent of task.

Table 2
Baseline dietary characteristics of study participants according to energy-adjusted tertiles of extra virgin olive oil consumption.

Variable	T1 (n = 290)	T2 (n = 290)	T3 (n = 290)
Nutrients			
Total energy intake (kcal/day)[a]	2314.3 ± 625.2	2327.2 ± 580.7	2291.5 ± 571.6
Proteins (g/day)[a]	95.1 ± 21.8	96.3 ± 22.5	93.2 ± 22.4
Carbohydrates (g/day)[a]	240.9 ± 79.5	234.3 ± 73.4	219.7 ± 67.0
Total fat (g/day)[a]	100.4 ± 30.2	105.0 ± 30.1	108.9 ± 30.5
Saturated fatty acids (g/day)[a]	26.9 ± 9.3	27.8 ± 9.3	27.5 ± 9.4
Monounsaturated fatty acids (g/day)[a]	48.5 ± 15.8	52.4 ± 16.2	56.4 ± 15.9
Polyunsaturated fatty acids (g/day)[a]	16.7 ± 6.8	16.5 ± 5.9	16.4 ± 6.1
Fibre (g/day)[a]	22.6 ± 6.9	24.1 ± 8.5	23.6 ± 7.6
Alcohol (g/day)[a]	9.5 ± 14.9	8.5 ± 13.6	8.4 ± 12.6
Vitamin D (μg/day)[a]	5.8 ± 3.4	6.0 ± 3.6	5.7 ± 3.1
Calcium (mg/day)[a]	1044.6 ± 362.7	1051.3 ± 364.4	992.3 ± 341.7
Food			
Total olive oil (g/day)[a]	34.9 ± 16.9	40.8 ± 17.6	48.0 ± 15.9
Extra virgin olive oil (g/day)[a]	20.0 ± 19.0	35.2 ± 19.2	46.3 ± 17.3
Common olive oil (g/day)[a]	14.8 ± 19.4	5.3 ± 12.7	1.5 ± 6.1
Legumes (g/day)[a]	17.7 ± 8.0	18.1 ± 9.2	17.4 ± 8.5
Vegetables (g/day)[a]	284.7 ± 116.3	313.2 ± 137.0	322.1 ± 134.9
Cereals (g/day)[a]	256.6 ± 101.7	254.0 ± 98.0	238.1 ± 90.4
Fruit (g/day)[a]	299.5 ± 178.0	315.6 ± 177.2	319.3 ± 160.7
No fermented dairy (g/day)[a]	274.83 ± 186.98	258.26 ± 186.93	234.96 ± 173.84
Fermented dairy (g/day)[a]	114.66 ± 96.61	113.54 ± 95.39	105.29 ± 89.80
Meat (g/day)[a]	142.7 ± 54.9	146.6 ± 55.7	146.3 ± 65.5
Fish (g/day)[a]	101.0 ± 42.0	103.3 ± 45.6	102.1 ± 42.8
Nuts (g/day)[a]	10.7 ± 12.6	14.3 ± 14.9	13.6 ± 15.5
Modified MedDiet score (12-point score)	6.4 ± 1.6	6.6 ± 1.8	6.6 ± 1.7

[a] Data are expressed as means ± SD.

According to the intervention group, no significant differences in the risk of osteoporotic fractures were observed (HR (95%CI)) 1.13 (0.71–1.79) and 1.05 (0.66–1.67) in the MedDiet-EVOO and MedDiet-Nuts groups compared to control group (Supplemental file).

Figure 1 shows the survival curve of osteoporotic fractures and the number of participants at risk by energy-adjusted EVOO tertiles at different time points. Table 3 shows the HR and 95% CIs for the association between total olive oil consumption and the specific subtypes and osteoporosis-related fractures. Total olive oil and

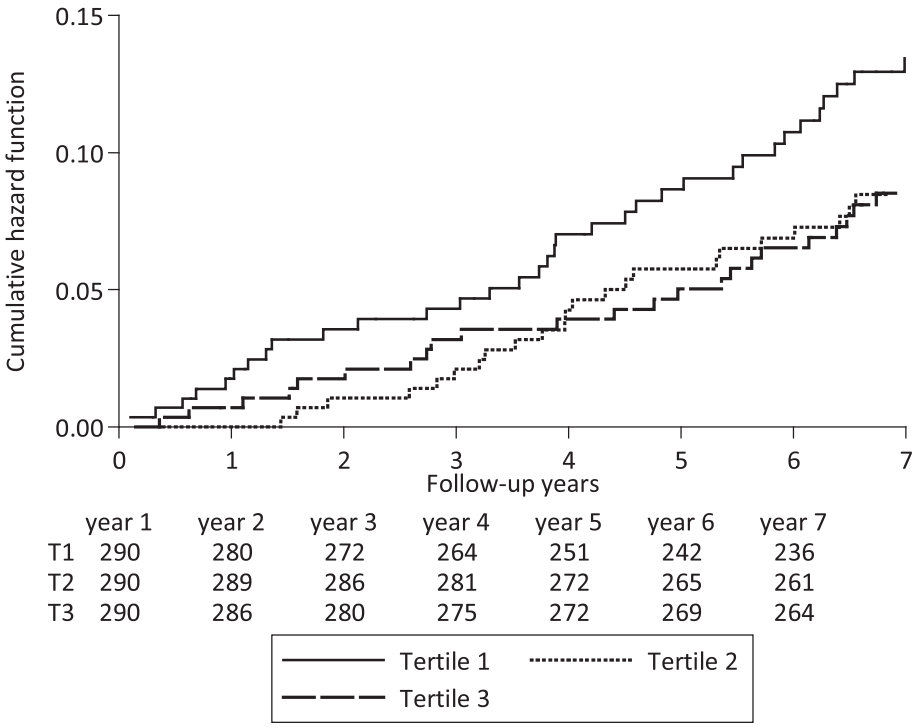

Fig. 1. Nelson-Aalen curves of cumulative hazard for osteoporotic fracture by tertiles of energy adjusted extra-virgin olive oil intake.

Table 3
Risk of osteoporotic fracture according to energy-adjusted tertiles of cumulative olive oil intake.

	T1 (n = 290)	T2 (n = 290)	T3 (n = 290)	P for trend
Mean total olive oil intake (g/day)	37.60 ± 6.76	48.23 ± 1.99	56.52 ± 4.32	
Fracture event, % (n)	13.80 (40)	13.80 (40)	11.70 (34)	
Mean total energy intake (kcal/day)	2240.19 ± 450.80	2254.16 ± 354.91	2236.28 ± 361.21	
Crude model	1 (Ref.)	0.93 (0.60, 1.44)	0.81 (0.51, 1.27)	0.367
Multivariate model 1[a]	1 (Ref.)	0.78 (0.49, 1.23)	0.73 (0.45, 1.19)	0.202
Multivariate model 2[b]	1 (Ref.)	0.74 (0.47, 1.18)	0.69 (0.42, 1.14)	0.141
Mean common olive oil intake (g/day)	−0.13 ± 0.12	0.63 ± 0.85	12.49 ± 8.90	
Mean total energy intake (kcal/day)	2000.56 ± 209.33	2516.34 ± 352.67	2213.73 ± 396.01	
Fracture event, % (n)	15.90 (46)	10.30 (30)	13.10 (38)	
Crude model	1 (Ref.)	0.63 (0.40, 1.00)	0.81 (0.53, 1.25)	0.950
Multivariate model 1[a]	1 (Ref.)	0.88 (0.54, 1.42)	0.94 (0.61, 1.46)	0.955
Multivariate model 2[b]	1 (Ref.)	0.96 (0.59, 1.56)	1.00 (0.64, 1.55)	0.952
Mean extra-virgin olive oil intake (g/day)	28.77 ± 10.27	45.11 ± 2.99	55.35 ± 4.62	
Mean total energy intake (kcal/day)	2229.47 ± 446.80	2254.69 ± 352.28	2246.47 ± 368.47	
Fracture event, % (n)	15.90 (46)	12.80 (37)	10.70 (31)	
Crude model	1 (Ref.)	0.73 (0.48, 1.13)	0.63 (0.40, 0.99)	0.037
Multivariate model 1[a]	1 (Ref.)	0.62 (0.39, 0.97)	0.52 (0.31, 0.85)	0.007
Multivariate model 2[b]	1 (Ref.)	0.59 (0.37, 0.95)	0.49 (0.29, 0.81)	0.004

Cox regression models were used to evaluate the risk of osteoporotic fracture event by energy-adjusted tertiles of total olive oil (g/day), energy-adjusted tertiles of common olive oil (g/day) and energy-adjusted tertiles extra-virgin olive oil (g/day). Results were expressed as Hazard Ratios (95% CI) and means ± SD or percentage (n).

[a] Model: adjusted for age (years), sex, body mass index (BMI) (kg/m^2), educational level (illiterate/primary education, secondary education, academic/graduate), leisure time physical activity (Metabolic Equivalent of Task (MET)-minutes/day), the intervention group and smoking status (never, former, current smoker).

[b] Model: additionally adjusted for prevalence of diabetes (yes/no), prevalence of previous fractures (yes/no), use of insulin (yes/no), use of oral antidiabetic drugs (yes/no), use of diuretic drugs (yes/no), use of glucocorticoids drugs (yes/no), use of osteoporotic drugs (yes/no), use of anticoagulant drugs (yes/no), use of estrogen drugs (yes/no) and baseline Mediterranean diet adherence (12-point score).

common olive oil consumption were not associated with a lower risk of fractures despite a non-significant trend to a lower reduction of bone fracture risk was observed in subjects allocated in the highest tertiles of total olive oil consumption. In contrast, a 51% reduction in the risk of osteoporosis-related fractures was observed in the fully-adjusted model for individuals in the highest tertile of EVOO consumption compared to the reference tertile (HR: 0.49; 95% CI: 0.29 to 0.81). The highest tertile compared to the reference tertile of MUFA intake (HR: 1.04; 95% CI: 0.66 to 1.65), PUFA intake (HR: 1.20; 95% CI: 0.76 to 1.90) or the MUFA:PUFA ratio (HR: 0.87; 95% CI: 0.55 to 1.38) showed no association with fracture risk.

The results of the sensitivity analysis were consistent with the general analysis. When early cases occurred during the first year (7 events were excluded), the risk in the higher tertile of EVOO consumption was relatively 46% lower (HR: 0.54; 95% CI: 0.32 to 0.92, *P* for trend = 0.050) than the reference tertile.

4. Discussion

The novel finding of this longitudinal study in an older Mediterranean population at high risk for cardiovascular disease is that high EVOO consumption is associated with a reduced risk of osteoporotic fractures, whereas a non-significant trend to a lower risk was also observed for total olive oil consumption.

The prevalence of osteoporosis and osteoporosis-related fractures is highly variable within European regions, with the lowest prevalence in the Mediterranean area [31]. These differences might be attributed to environmental factors and dietary regimens [10–12,32]. The MedDiet is based on a combination of foods comprising a complex array of nutrients and bioactive phytochemicals with anti-inflammatory, antioxidant and alkalinising properties that could all contribute to bone health. Olive oil is one of the key foods in the MedDiet and its consumption accounts for one to two thirds of total vegetable fat intake, where MUFA, in the form of oleic acid, is the most abundant fatty acid consumed. In a cross-sectional study conducted in Greece, MUFA intake was associated with a higher BMD [33]. Another study conducted in adult Greek women found higher total and spine BMD in those whose diet contained a combination of olive oil and fish with little meat, but not in association with the full MedDiet pattern [34]. A higher dietary MUFA:PUFA ratio has also been related to a lower risk of osteoporotic-related fractures produced by a same-level fall in elderly subjects [16].

However, in the present study, we found no associations of MUFA intake or the MUFA:PUFA ratio with fracture risk. These differences might be due to our study population displaying narrow ranges of MUFA intake and the MUFA:PUFA ratio compared to previous studies. In fact, results from prior studies showed no significant protection against fractures from MUFA intake or MUFA:PUFA ratios in the ranges of our study population. Moreover, the differences in the risk of osteoporosis-related fractures between different types of olive oil observed in our study cannot be explained by differences in its fatty acid profile, as the fatty acid composition is not affected by the extraction method used, since all olive oils are produced from the same variety of olives [35]. This suggests that other compounds present in olive oil, beyond the fatty acid composition, might play an important role in bone health.

Common olive oil is a mixture of virgin and (usually) more than 80% of refined oil, with fewer antioxidant and anti-inflammatory compounds. In contrast, EVOO is the best quality oil, produced by mechanically pressing ripe olives, and contains the highest amounts of bioactive and antioxidant components, such as polyphenols, that by different mechanisms might exert favourable effects on bone metabolism [35]. Several studies conducted *in vitro* and in animal models have assessed the beneficial role of olive oil phenols on the formation and maintenance of bone through its modulation of both bone cell differentiation and function [36–38]. Oleuropein, tyrosol and hydroxytyrosol, the most abundant polyphenols in olive oil, have been related to several beneficial effects on bone metabolism *in vitro* an in animal models [39]. In humans, osteopenic subjects who consumed 250 mg/day of a polyphenol extract from *Olea europaea* for 12 months significantly increased their osteocalcin levels and stabilized lumbar spine BMD compared to a control group [18]. Similarly, in a prior PREDIMED sub-study, we found higher serum levels of osteocalcin and the bone remodelling marker procollagen amino-terminal pro-peptide after 2 years of intervention

with the MedDiet-EVOO compared to theMedDiet-Nuts or the control diet [17]. In contrast, we found no significant protective effect on bone fractures in subjects allocated to the MedDiet-EVOO group compared to the control diet, as would be initially expected. This apparent discrepancy could be explained because the difference in the total consumption of either total olive oil or extra-virgin olive oil between participants in the MedDiet-EVOO group or control group was substantially lower than differences between tertiles of olive oil consumption, as participants had a high MedDiet score at baseline with olive oil as the main culinary fat. It is also plausible that exposure time to the intervention diets was not long enough to improve or delay the age-related changes in bone structure. Thus far, no other studies have been conducted to assess the relationship between olive oil consumption and bone-related markers. Our findings extend the potential beneficial role of EVOO consumption demonstrated on bone biochemical markers to a lower risk of osteoporotic-related fractures as clinical outcome. Moreover, our results also suggest a beneficial role of the phenolic compounds present in EVOO, as no association was found for the common refined olive oil, which is depleted of these bioactive compounds.

The strengths of our study are a well-characterized cohort with long-term follow-up, controlled by several potential confounders, the analysis of different varieties of olive oil and the use of cumulative mean across all the available FFQs to improve the precision of the exposure. For the fracture identification we used an objective score, however, this classification has some potential limitations as was based on fracture categories identified by standard diagnostic codes which identifies accurately a total of 94% of cases compared with the gold standard of medical record review [26]. There are also limitations to our study. First, the generalizability of our results may be limited, as the study population was made of older Mediterranean individuals at high cardiovascular risk which increased their risk for osteoporotic fractures [40]. Second, because of the observational nature of the study, residual confounding remains a possibility even though our analyses were extensively adjusted for a wide range of potential confounders. Third, no bone biochemical markers or data on BMD were available. Fourth, due to the low number of fractures and the relative small study size, we cannot exclude a potential beneficial effect of total olive oil consumption on the risk of bone fractures as the hazard ratio clearly indicates a lower risk, although not strong as for EVOO. Finally, although the FFQ used was validated, measurement errors cannot be discarded, especially regarding the self-reporting of different varieties of olive oil. Still, our findings are consistent with the potential beneficial effects of olive oil on bone health previously described.

In summary, we found that greater consumption of EVOO is associated with a lower risk of osteoporosis-related fractures in an older Mediterranean population at high cardiovascular risk. Our findings highlight the consumption of EVOO, one of the key foods of the MedDiet, in the prevention of osteoporosis-related fractures.

Authors' contributions

The authors' responsibilities were as follows—MB, MAM, RE, MF, DC, ER and JS-S: contributed to the conception, design, and implementation of the project; JGG, SC, SG and MB contributed to data collection and analytical procedures; JGG, SC, SG and MB: conducted the statistical analysis, interpreted data, and wrote the manuscript; and all authors: read and approved the final version of the manuscript.

Conflict of interests

JSS reports grants from RTIC G03/140 ISCIII, Spain, grants from CIBER obn ISCIII, Spain, other from California Walnut Commission, Sacramento CA, USA, other from Patrimonio Comunal Olivarero, Spain, other from La Morella Nuts, Spain, other from Borges S.A., Spain, other from Nut and Dried Fruit Foundation, personal fees from Nuts for Life, other from Nut and Dried Fruit Foundation, other from Nut and Dried Fruit Foundation, during the conduct of the study; personal fees from Danone S.A., personal fees from Font Vella Lanjaron, personal fees from Eroski Distributors and personal fees from Instituto Danone, outside the submitted work.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.clnu.2016.12.030.

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