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Phytonutrients for bone health during ageing

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Osteoporosis is a skeletal disease characterized by a decrease in bone mass and bone quality that predispose an individual to an increased risk of fragility fractures. Evidence demonstrating a positive link between certain dietary patterns (e.g. Mediterranean diet or high consumption of fruits and vegetables) and bone health highlights an opportunity to investigate their potential to protect against the deterioration of bone tissue during ageing. While the list of these phytonutrients is extensive, this review summarizes evidence on some which are commonly consumed and have gained increasing attention over recent years, including lycopene and various polyphenols (e.g. polyphenols from tea, grape seed, citrus fruit, olive and dried plum). Evidence to define a clear link between these phytonutrients and bone health is currently insufficient to generate precise dietary recommendations, owing to mixed findings or a scarcity in clinical data. Moreover, their consumption typically occurs within the context of a diet consisting of a mix of phytonutrients and other nutrients rather than in isolation. Future clinical trials that can apply a robust set of outcome measurements, including the determinants of bone strength, such as bone quantity (i.e. bone mineral density) and bone quality (i.e. bone turnover and bone microarchitecture), will help to provide a more comprehensive outlook on how bone responds to these various phytonutrients. Moreover, future trials that combine these phytonutrients with established bone nutrients (i.e. calcium and vitamin D) are needed to determine whether combined strategies can produce more robust effects on skeletal health.

Introduction

Osteoporosis is a skeletal disease characterized by compromised bone strength, predisposing an individual to an increased risk of fractures [1]. A diagnosis of osteoporosis is reached when the bone mineral density (BMD) of an individual, as measured by dual energy X-ray absorptiometry, is 2.5 standard deviations below the mean value for young sex-matched adults [2]. Fractures associated with this disease affect one in three women and one in five men over the age of 50 years. Indeed, osteoporosis is responsible for consuming more hospital days than many other diseases, including diabetes, heart attack and breast cancer [3]. The direct annual costs of osteoporotic fractures are over €31 billion per year in Europe and $20 billion per year in the USA, and these costs are expected to rise substantially by the year 2050 [4, 5]. Thus, it is imperative to promote effective prevention and treatment strategies to counterbalance the significant morbidity, mortality and economic burden associated with this disease.

Several nutritional factors play a role in skeletal health during ageing. Macro- and micronutrients contribute to skeletal health by supporting bone matrix production and mineralization. Of these, calcium, vitamin D and proteins are the most important nutrients for supporting the skeleton and are reviewed extensively elsewhere [6–9]. However, in many developed countries, where the dietary intake of calcium is adequate for most individuals compared with recommended daily allowances, very high rates of osteoporosis are nevertheless observed. These observations suggest that dietary factors independent of calcium and/or vitamin D may influence bone and mineral homeostasis and may be important for long-term bone health. Indeed, dietary patterns consisting of a high consumption of fruits and vegetables, legumes, seafood, nuts, seeds, rice and/or rice dishes have been shown to be directly associated with BMD, independent of dietary calcium intake [10–13]. Albeit not causal, these data have led support to the hypothesis that there may be dietary factors (e.g. phytonutrients) independent from calcium and vitamin D that may be linked to skeletal health.

The primary focus of this review is to discuss the clinical and preclinical efficacy on bone health of novel nonvitamin phytonutrients (e.g. lycopene, polyphenols from tea, grape seed, citrus fruit, olive and dried plum) that are commonly consumed in the diet and that have gained increasing attention for skeletal health during ageing. Phytoestrogens, found in plants such as soy, are excluded from our current discussion because they have been extensively reviewed elsewhere [14–16]. Using PubMed/Medline databases, the present review focuses primarily on the clinical efficacy of phytonutrients commonly found in the daily diet; however, we also include some preclinical data because they provide valuable information on the efficacy of these ingredients when human data are sparse. Given that a significant number of the fractures that occur during ageing occur in individuals with BMD scores that do not meet the diagnostic criteria of osteoporosis [17–19], a secondary focus of this review is to identify other outcome measures of bone health (e.g. bone turnover and bone microarchitecture) that may complement standard BMD testing in future trials related to the skeletal efficacy of phytonutrients.

Lycopene

Lycopene is a major carotenoid synthesized by many plants and micro-organisms, but not synthesized by animals or humans [20]. This lipid-soluble carotenoid is a highly stable molecule and is responsible for the red colour in many fruits (e.g. tomatoes) and vegetables (e.g. carrots). Lycopene exists in an all-*trans* configuration, which is the most thermodynamically stable form; however, in plasma and tissues, lycopene is present in large amounts as *cis* isomers [21]. The absorption of lycopene is greatest when it is processed into juice, tomato sauce or even ketchup. Unlike other carotenoids, such as α- and β-carotene and β-cryptoxanthin, lycopene has no vitamin A activity. Lycopene has gained attention for its strong antioxidative capabilities and for its potential to play a protective role against a number of chronic diseases, including osteoporosis [22].

Clinical evidence of lycopene for skeletal health

Epidemiological data using various adult populations have demonstrated a positive relationship between the intake levels or serum levels of lycopene and bone mass, bone turnover and/or fracture risk [23–27] (Table 1). In the Framingham Osteoporosis Study, a higher intake of lycopene in elderly men and women was positively associated with a 4 year change in BMD at the lumbar vertebrae and a lower risk for hip and nonvertebral fractures [23, 24]. Other work [27] demonstrated that serum concentrations of lycopene were lower in postmenopausal osteoporotic women compared with their non-osteoporotic counterparts.

Clinical data have supported the epidemiological findings described above. For example, in postmenopausal women supplemented for 4 months with lycopene (as a juice or in a capsule), decreases in N-terminal telopeptide crosslinks of type I collagen and in oxidative parameters (i.e. lipid peroxidation and protein oxidation) were observed [28] (Table 1). In another study, the same research group demonstrated in postmenopausal women that a 1 month restriction in their dietary intake of lycopene-rich foods increased N-terminal telopeptide crosslinks of type I collagen and decreased the antioxidant enzymes catalase and superoxide dismutase [29]. These data, along with *in vitro* work showing that lycopene attenuates the production of osteoclast cells [30], suggest that lycopene protects bone mass during ageing by attenuating bone resorption.

Preclinical evidence of lycopene for skeletal health

Our knowledge concerning the effects of lycopene on bone health stems mostly from epidemiological and clinical trials. Two rodent studies [31, 32] demonstrated that daily administration of lycopene protects against the loss of bone mass and bone strength induced by ovariectomy (Table 1). Given that preclinical research permits us directly to examine biomechanical strength indices of bone and mechanisms of action, as well as toxicological properties of novel ingredients, future studies using preclinical models are needed to characterize the physiological and toxicological effects of lycopene fully.

Polyphenols and polyphenol-rich foods

Polyphenols are plant-based compounds that are present in our daily diet through fruit and vegetables, beans, grains and beverages, such as fruit juices, coffee and green or black tea. To date, around 5000 polyphenols have been identified in the food we consume. Polyphenols are classified according to the number of phenol rings they contain and on the structural elements bound to these rings. Thus, polyphenols have been classified as phenolic acids, flavonoids, stilbenes, tannins, coumarins and lignans [33].

Even though it is hard to quantify the dietary intake of polyphenols, it has been estimated that the average daily diet provides around 1.5 g [33]. Polyphenols, after consumption, are absorbed into the bloodstream as aglycone forms and are further metabolized by the organism and/or microflora enzymes into conjugates of glucuronate or sulfate and/or eventually eliminated [34]. Thus, circulating forms may possess different biological properties within cells and target tissues compared with polyphenol aglycones.

Despite the large number of molecules identified, most research to date on the potential benefits of polyphenols

Table 1

Selected human and animal studies on the efficacy of selected phytonutrients on bone indices during ageing

Study type	Subjects, mean age	Intervention, duration of study	Bone turnover	BMD, BMC	Bone structure	Bone strength	Other findings	Reference
Lycopene								
EPI	Elderly men and women, 75 years old	Food frequency questionnaire completed and records of hip fractures obtained	Not assessed	Not assessed	Not assessed	Not assessed	Higher lycopene intake was associated with lower risk of hip fractures	Sahni *et al.* (2009) [23]
EPI	Postmenopausal women, 50–60 years old	7 day dietary records completed and fasting blood samples taken from participants	Higher serum lycopene was associated with lower NTX levels	Not assessed	Not assessed	Not assessed	Higher serum lycopene was associated with lower protein oxidation	Rao & Rao (2007) [22]
RCT	Postmenopausal women, 50–60 years old	Tomato juice, lycopene-rich tomato juice, tomato Lyc-O-Mato lycopene capsules or placebo for 4 months	↓ NTX in lycopene-supplemented participants (treatment groups pooled)	Not assessed	Not assessed	Not assessed	In pooled lycopene-supplemented groups, was associated with lower protein oxidation and lipid peroxidation	Mackinnon *et al.* (2011) [28]
Animal	OVX rats, 2 months old	Lycopene [0, 20, 30 or 40 mg (kg bodyweight)$^{-1}$ day^{-1}], 8 weeks duration	↓ Alkaline phosphatase *vs.* OVX control with treatments	↑ Femoral BMD and BMC *vs.* OVX control with 30 and 40 mg kg^{-1} treatments	Not assessed	↑ Femoral strength properties *vs.* OVX control with 30 and 40 mg kg^{-1} treatments	↓ Calcium and phosphate *vs.* OVX control with treatments. ↓ Interleukin-6 *vs.* OVX control with treatments	Liang *et al.* (2012) [32]
Green tea and green tea polyphenols								
EPI	Men and women, ≥50 years old who sustained a fracture (cases) or did not (controls)	Questionnaire completed on health and lifestyle, including tea consumption	Not assessed	Not assessed	Not assessed	Not assessed	Tea consumption was inversely associated with hip fracture	Johnell *et al.* (1995) [36] Kanis *et al.* (1999) [73]
RCT	Osteopenic women, mean age between 56.5 and 58.3 years old	Green tea polyphenols (500 mg day^{-1} *vs.* placebo), both with or without tai chi (3 sessions week^{-1}), 6 months duration	↑ BSALP *vs.* placebo in both green tea and tai chi groups. ↔ TRAP	Only assessed for screening purposes	Not assessed	Not assessed	↔ Serum and urinary calcium, inorganic phosphate with any treatments	Shen *et al.* (2012) [39]
Animal	Sham and OVX virgin rats, 14 months old	Green tea polyphenols (0, 0.1 or 0.5% of diet in drinking water), 16 weeks duration	Not assessed	↑ Femoral BMD with treatments compared with respective control groups	Not assessed	Not assessed	↓ Urinary calcium with treatments compared with respective control groups	Shen *et al.* (2008) [41]
Animal	Sham and OVX virgin rats, 14 months old	Green tea polyphenols (0, 0.1 or 0.5% of diet in drinking water), 16 weeks duration	↑ BFR/BS and ↓ ES/BS in tibial shaft with treatments compared with respective control groups	↑ Femoral BMD with treatments compared with respective control groups	Improved bone structure at all skeletal sites with treatments	Not assessed	–	Shen *et al.* (2009) [74]
Hesperidin								
RCT	Healthy postmenopausal women, 50–65 years old	Biscuits containing hesperidin (500 mg day^{-1}) *vs.* placebo biscuits, 2 years duration	↑ PINP/CTX-1 ratio *vs.* placebo	↔ BMD	Not assessed	Not assessed	–	Habauzit *et al.* (2011) [47]
Animal	Sham rats, 3 and 6 months old	0.5% hesperidin diet or control diet, 90 days duration	↓ DPY *vs.* control in younger rats. ↔ Osteocalcin	↑ BMD *vs.* control in younger rats	Not assessed	Not assessed	–	Horcajada *et al.* (2008) [48]
Animal	OVX rats, 3 and 6 months old	0.5% hesperidin diet or control diet, 90 days duration	↓ DPY *vs.* control in both younger and older rats. ↔ Osteocalcin	↑ BMD *vs.* control in younger and older rats	Not assessed	Not assessed	–	Horcajada *et al.* (2008) [48]
Animal	Senescent male rats, 20 months old	0.5% hesperidin, 0.5% naringin, 0.25% hesperidin + 0.25% naringin in diet or control diet, 3 months duration	↓ DPY *vs.* control with 0.5% hesperidin and 0.5% naringin treatments. ↔ Osteocalcin	↑ BMD *vs.* control with 0.5% hesperidin and combination group	↑ BV/TV *vs.* control with 0.5% hesperidin group. ↑ Tb.Th. *vs.* control with combination group	↔ Strength parameters among any groups	↑ BMP-2 and BMP-4 *vs.* control with 0.5% hesperidin	Habauzit *et al.* (2011) [47]

Table 1

Continued

Study type	Subjects, mean age	Intervention, duration of study	Bone turnover	BMD, BMC	Bone structure	Bone strength	Other findings	Reference
Olive oil and olive oil polyphenols								
EPI	Healthy pre-, peri- and postmenopausal women, 48 years old	3 day food records completed and BMD at lumbar spine and total body BMC measured	Not assessed	High consumption of fish and olive oil and low intake of red meat was positively associated with BMC and BMD	Not assessed	Not assessed	–	Kontogianni *et al.* (2009) [57]
Animal	OVX rats, 6 months old	Oleuropein (0.15 g kg^{-1} of diet), olive oil (50 g kg^{-1} of diet), or control diet, 80 days duration, half of the rats induced with inflammation at day 59	↓ DPY *vs.* OVX control with oleuropein treatment in rats with inflammation. ↔ Osteocalcin *vs.* OVX control	↑ Femur BMD *vs.* OVX control with oleuropein and olive oil treatment in rats with inflammation	Not assessed	↑ Peak load at femoral midpoint *vs.* OVX control with oleuropein and olive oil in rats with inflammation. ↔ Peak load at femoral midpoint in rats without inflammation	↓ α-1-Acid glycoprotein *vs.* OVX control with olive oil in rats with inflammation. ↓ Spleen weight *vs.* OVX control with oleuropein and olive oil in rats with inflammation	Puel *et al.* (2004) [59]
Animal	OVX rats, 6 months old	Oleuropein [0, 2.5, 5, 10 or 15 mg (kg bodyweight)$^{-1}$ day^{-1}], or control diet, 100 days duration, half of the rats induced with inflammation at day 79	↔ DPY ↓ Osteocalcin *vs.* OVX controls with 15 mg kg^{-1} dose with inflammation	↑ Femoral BMD *vs.* OVX control with oleuropein in rats with inflammation. ↔ Femoral BMD in rats without inflammation	Not assessed	↔ Peak load at femur midpoint	↓ Spleen weight *vs.* OVX control with 2.5 mg kg^{-1} oleuropein. ↔ Ferric-reducing potential value. ↔ Fibrinongen *vs.* OVX controls	Puel *et al.* (2006) [58]
Dried plums								
RCT	Osteopenic postmenopausal women, 55.6–57.5 years old	Dried plums (100 g day^{-1}) or a dried apple control group (75 g day^{-1}), 12 months duration	↓ BSALP and osteocalcin *vs.* baseline and control group. ↓ TRAP with dried plum *vs.* baseline	↑ Ulnar and spine BMD compared with control group	Not assessed	Not assessed	↓ C-reactive protein levels compared with control group at 3 months	Hooshmand *et al.* (2011) [64]
Animal	OVX mice 3 months old	Dried plums (0, 5, 15 or 25% of diet), 4 weeks duration	↓ PINP *vs.* OVX control group	↑ Spine BMD and BMC compared with OVX control group with 25% dried plums	Improved structural properties at spine and tibia with 15 and 25% dried plums	Improved biomechanical properties at spine with 15 and 25% dried plums	↑ Insulin-like growth factor-1 with 15% dried plums *vs.* OVX control group. ↓ Tumour necrosis factor-α levels with 15 and 25% dried plums	Rendina *et al.* (2012) [65]
Animal	Male intact mice, 6 and 12 months old	Dried plums (0, 15 or 25% of diet), 6 months duration	Diet × age interaction for BFR, trend ($P = 0.08$) for ↑ BFR with treatments in adult mice *vs.* 0%	↔ Femoral BMD with treatments compared with 0% group	Improved structural properties at femur with treatments; effects more pronounced in adult mice	Not assessed	–	Halloran *et al.* (2010) [70]

Abbreviations are as follows: BFR, bone formation rate; BFR/BS, bone formation rate per bone surface; BMC, bone mineral content; BMD, bone mineral density; BMP, bone morphogenic protein; BSALP, bone-specific alkaline phosphatase; BV/TV, bone volume fraction; DPY, deoxypyridinoline; EPI, epidemiology; ES/BS, eroded surface/bone surface; NTX, N-terminal telopeptide crosslinks of type I collagen; OVX, ovariectomized; PINP, procollagen I N-terminal propeptide; RCT, randomized controlled trial; Tb.Th., trabecular thickness; CTX-1, carboxy-terminal collagen crosslinks type 1; and TRAP, tartrate-resistant acid phosphatase. ↑ Significantly higher compared with other groups or a significant increase compared with baseline. ↓ Significantly lower compared with other groups or a significant decrease compared with baseline. ↔ No significant differences among groups or no significant increases/decreases within a group compared with baseline values.

for bone health has focused on the flavonoids subgroup, specifically on isoflavones from soybean, which are extensively reviewed elsewhere [14–16]. The flavonoids subgroup consists of six subclasses, which share a common structure of two aromatic rings. These are flavones, flavonols, flavanones, isoflavones, flavanols (catechins and proanthocyanidins) and anthocyanidins [35]. Summarized below are flavonoids of emerging research interest for their potential roles on protecting skeletal health during ageing. In addition, polyphenol-rich foods (olives and dried plums) that have increasingly gained attention for their implications in supporting bone health are also discussed below.

Tea and grape flavanols

Flavanols exist in both the monomer form (e.g. catechins) and the polymer form (e.g. proanthocyanidins). The main flavanols include catechin, epicatechin, gallocatechin and epigallocatechin. Catechin and epicatechin are found in many types of fruit, but also in red wine, green tea (more than 80% of green tea polyphenols are catechins) and chocolate, whereas gallocatechin, epigallocatechin and epigallocatechin gallate occur in certain seeds of leguminous plants, in grapes and, above all, in tea. Tea, brewed from the dried leaves of the plant *Camellia sinensis*, is the most widely consumed beverage worldwide.

Clinical evidence for tea and grape flavanols

Several epidemiological studies have reported reduced risk of hip fractures or higher bone BMD in habitual tea drinkers [36–38]. Despite these reports on the benefits of tea on human health, the osteoprotective effects of tea polyphenols and flavanols (including grape flavanols) using randomized control trials have been poorly investigated. Indeed, only a recent randomized control trial [39] has been published, in which 171 postmenopausal women with osteopenia received a supplement of green tea polyphenols (500 mg day^{-1}) and/or tai chi exercise for 6 months (Table 1). The findings of this short-term, 6 month clinical trial indicated that the consumption of green tea supplement provided higher values for serum bone-specific alkaline phosphatase (bone formation biomarker) after 4 weeks, while tai chi exercise provided higher values for bone-specific alkaline phosphatase after 12 and 24 weeks [39]. Neither green tea supplementation nor tai chi exercise had any effect on serum levels of tartrate-resistant acid phosphatase (bone resorption biomarker). Although the effects of green tea polyphenols on bone biomarkers are promising, a longer term clinical study assessing BMD is needed to confirm the bone-protective effects of green tea polyphenols in postmenopausal women [39].

Preclinical evidence for tea and grape flavanols

Several lines of evidence concerning the osteoprotective effects of green tea on bone mass and microarchitecture in various induced bone loss models (by ageing, sex hormone deficiency and chronic inflammation) have been reported, as extensively reviewed elsewhere [40]. Green tea polyphenols provided daily in the drinking water of ovariectomized and sham-operated rats for 16 weeks resulted in higher femoral BMD and lower urinary levels of calcium compared with respective ovariectomized and sham control animals [41] (Table 1). These effects were accompanied by significant increases in urinary levels of epigallocatechin and epicatechin. Protective effects of green tea polyphenols on bone have also been observed in a model of bone loss induced by chronic inflammation [42, 43]. Grape seed proanthocyanidins could also have a potential role in skeletal protection. Grape seed proanthocyanidins extract was able to increase bone formation and bone strength at the mandibular bone in developing rats [44, 45]. Furthermore, grape seed proanthocyanidins extract supplementation was more effective in reversing debility of the mandibular condyle bone induced by a low-calcium diet than a standard diet or high-calcium diet alone. It would be of interest in the future to know whether grape seed proanthocyanidins extract supplementation could also protect skeletal mass and strength during ageing.

Citrus flavanones

Hesperidin (hesperetin-7-*O*-rhamnoglucoside) represents one of the most abundant flavanones and is also the most studied flavanone with respect to bone health. The daily intake of hesperidin has not been precisely evaluated in different populations, but arguably it is relatively high for the flavanone class of polyphenols owing to worldwide consumption of citrus products, such as citrus fruits and juices (e.g. in Western countries, intakes of oranges range from 35 to 50 kg per person per year). Indeed, the content of hesperetin in oranges and in orange juice is considerable, ranging from 31 to 43.2 mg $(100\ g)^{-1}$ and from 200 to 700 mg l^{-1}, respectively [34, 46].

Clinical evidence for citrus flavanones

Only one clinical study has been conducted on the ability of hesperidin to protect against postmenopausal bone loss [47] (Table 1). This study was a parallel, double-blind, placebo-controlled, 24 month randomized intervention trial assessing the effect of hesperidin on validated biomarkers of bone turnover and BMD. It was performed in healthy postmenopausal women (50–65 years old) not taking any hormone replacement therapy. Volunteers were assigned to either a hesperidin (500 mg hesperidin day^{-1} in two biscuits) or placebo group (same biscuits without hesperidin). Subjects kept dietary records and minimized their citrus-rich food intake during the study period.

The yearly rates of bone loss (1–2%) were equivalent in the two groups. Evolution in BMD during the 2 years was not statistically different between the two groups.

However, the subjects consuming hesperidin presented a better balance in bone metabolism, as reflected by the bone turnover index (procollagen I N-terminal propeptide: carboxy-terminal collagen crosslinks type I ratio), during the second year of follow-up at the 18 and/or 21 month time points [47].

Preclinical evidence for citrus flavanones

Dietary hesperidin at a level of 0.5% can improve bone mass in intact 3-month-old rats and protect against ovariectomy-induced bone loss in 6-month-old rats [48] (Table 1). These findings are in accordance with data obtained in ovariectomized mice fed with the same dose of hesperidin [49]. A further study examining hesperetin-7-glucoside, an intestinal metabolite of hesperidin which is more bioavailable than hesperidin itself, also demonstrated a greater efficiency than hesperidin in inhibiting bone loss resulting from ovariectomy in 6-month-old rats [50]. Positive effects of oranges as well as hesperidin on skeletal health have also been observed in growing [51] and older male rats [52]. These findings are in accordance with male orchidectomized rats consuming hesperidin through citrus juice [53]. Thus, hesperidin has the potential to play a protective role against the development of osteoporosis in both women and men.

The beneficial effects of hesperidin on bone mass have been mainly related to a slowing down in bone resorption (urinary free deoxypyridinoline). However, as first suggested by Chiba *et al*., hesperidin could not only modulate bone resorption but could also affect bone formation [49, 54]. Hesperidin may also exert protective effects on bone by modulating the production of inflammatory products [52].

Olive polyphenols

Olives contain over 30 phenolic compounds, such as oleuropein, oleocanthin, tyrosol and hydroxytyrosol. The main phenolic compound of olive is oleuropein, and it is estimated that Mediterranean populations consume approximately 1.16 mg of oleuropein per day [55].

Clinical evidence for olive polyphenols

Mediterranean populations are reported to have lower incidences of bone fractures compared with other European populations [56]. Epidemiological evidence suggests that adherence to certain dietary patterns of the Mediterranean diet (i.e. a high consumption of olive oil and fish and low consumption of red meat) and not to the Mediterranean diet *per se* (i.e. high consumption of plant foods and olive oil, low consumption of meat and dairy products, and moderate intake of alcohol) is associated with greater bone mass [57] (Table 1). Human data on the efficacy of olive polyphenols on bone health are still lacking; however, a number of preclinical data (described in the next subsection) demonstrate that polyphenolic compounds derived from olive may protect bone mass, especially in the presence of inflammation.

Preclinical evidence for olive polyphenols

The effect of oleuropein was investigated by Puel *et al*. using a rat model of bone loss, associating ovariectomy and acute inflammation [58] (Table 1). All doses of oleuropein used [2.5, 5, 10 and 15 mg (kg bodyweight)$^{-1}$ day^{-1}] elicited protective effects on bone mass. It is interesting to note that no dose–response pattern was seen in this study, and the maximal bone effect was achieved at the lowest dose. Observations of a lower spleen weight *vs*. the respective control let to the hypothesis that oleuropein may exert its bone-sparing effect by modulating inflammation rather than by acting directly on bone metabolism. Neither oleuropein nor whole olive oil was able to affect BMD in ovariectomized rats when inflammation was not induced [59] (Table 1). Furthermore, a study to determine whether olive fruits might improve bone loss in ovariectomized rats and in ovariectomized rats with granulomatous inflammation was performed [60]. It was shown that black olive but not green olive was able to prevent bone loss in an experimental model of senile osteoporosis. Indeed, no protective effect was reported when rats were ovariectomized without induction of inflammation, as previously shown with pure oleuropein [59].

Dried plum polyphenols

Dried plums, also known as prunes, the dried fruits of *Prunus domestica* L., are known to be rich in several polyphenols, including phenolic acid derivatives, flavonoids and coumarins. The total polyphenol content in dried plums has been reported to be 184 mg (100 g)$^{-1}$ [61]. The major components in these fruits are chlorogenic acid isomers (i.e. neochlorogenic acid, cryptochlorogenic acid and chlorogenic acid, which are esters of caffeic acid with quinic acid). The mean concentration of these chlorogenic acid isomers is as high as 174.2 mg (100 g)$^{-1}$, which represents more than 94% of total phenolics [61].

Clinical evidence for dried plum polyphenols

Most evidence on the consumption of dried plums and skeletal health status stems from preclinical data [62]; however, two clinical trials conducted in postmenopausal women have been carried out. A short-term clinical study (3 months) with postmenopausal women demonstrated that dietary supplementation with 100 g of dried plums per day positively influenced the bone formation markers, bone-specific alkaline phosphatase and insulin-like growth factor-1 [63] (Table 1). A more recent clinical trial, 1 year in duration, using postmenopausal women fed 100 g of dried plums per day observed significantly increased BMD at the spine and ulna compared with baseline and

the dried apple control group [64] (Table 1), which also supports a beneficial effect linked to consumption of dried plums. A discrepancy was observed between these two trials, however, in relationship to the bone-specific alkaline phosphatase data. While the 3 month study observed an increase in bone-specific alkaline phosphatase, the 1 year study observed a decrease [64]. It is unknown why this discrepancy was found, but it may be due in part to differences in study designs, because the women in the short-term trial were advised to adjust their diets to account for the additional energy, protein and fat provided from the dried plums, while the long-term trial did not include this advice.

Preclinical evidence for dried plum polyphenols

Most of the effects of dried plums on bone metabolism have been demonstrated using preclinical models of bone loss. In mice, consumption for 4 weeks of 25% dietary dried plums protected against ovariectomy-induced loss of BMD at the spine, while both 15 and 25% dietary dried plums protected against the deterioration of bone structure at both the spine and proximal tibial metaphysis [65] (Table 1). This study also demonstrated positive effects on bone strength at the spine using these doses. In addition, dried plums as both 15 and 25% of the diet restored some bone marrow myeloid and lymphoid populations and suppressed splenocyte activation, which occurs following ovarian hormone deficiency [65]. Thus, dried plums may protect ovariectomy-induced bone loss and deterioration of bone tissue and strength, in part by suppressing immune cell activation. In ovariectomized rats fed a standard diet for 40 days prior to treatments to establish bone loss, subsequent consumption of dried plums restored femoral and tibial bone density at doses as low as 5%. In addition, 5% dried plums resulted in higher trabecular microarchitecture in comparison with ovariectomized control animals at the end of the 60 day treatment [66]. Moreover, it was shown that the combination of 5% fructo-oligosacharride with 7.5% dried plums is capable of reversing ovariectomy-induced bone loss in 3-month-old female Sprague–Dawley rats, and this effect was enhanced when both compounds were added to soy-based diet [67]. Likewise, dried plums exert positive effects on bone mass, bone microarchitecture and bone strength in preclinical models of male osteoporosis [68–70], suggesting that this food may be an attractive strategy to explore further in both female and male clinical trials assessing skeletal health.

Perspectives for future trials

Table 2 summarizes outcome measures that are commonly used in clinical and preclinical bone studies. The information obtained by each outcome measure offers insight into how the determinants of bone strength (i.e. bone quantity and bone quality) respond to various agents. Indeed, many preclinical studies, such as those described in the present review, are able to measure the mineral, material, structural and strength properties of bone directly, because *ex vivo* samples are easily obtained. In the clinical setting, however, the assessment of a comprehensive set of bone outcome measures, even in the most ideal conditions, may be limited by various factors, including the degree of invasiveness and costs associated with the outcome measure. Thus, BMD, the gold standard to determine skeletal responsiveness to various agents in clinical trials, and/or biochemical markers of bone turnover are commonly included as primary outcome measures to assess treatment response in clinical trials. Bone mineral density, however, is not always a reliable marker in predicting fracture risk because approximately half of all fractures that occur, at least in postmenopausal women, occur in women with BMD scores that do not meet the diagnostic criteria of osteoporosis [17–19]. It is, however, impossible to measure bone strength directly in humans because strength tests are invasive and destructive. In addition, measurement of bone turnover markers can be limited by biological and laboratory variations, as well as multiple methodologies used for the same analyte. Technological advances in quantitative computed tomography, which examines bone microarchitecture to predict the deformation of bone, can also predict fracture risk (Table 2) [71, 72]; however, it cannot be used to diagnose osteoporosis, and it is more expensive than measuring BMD by dual energy X-ray absorptiometry. Thus, BMD remains the gold standard in clinical bone studies and should be used when possible, along with biochemical markers of bone turnover, in future clinical trials that investigate the skeletal effects of phytonutrients or phytonutrient-rich foods. When feasible, other determinants of bone strength (e.g. bone structure) may provide valuable insight into the skeletal response of nutritional agents when BMD remains unchanged.

Conclusion

The role of nutrition is of increasing interest for the support of skeletal health and for the prevention of osteoporosis, a disease which imposes significant health and financial burdens worldwide. While evidence to define a clear link between intakes of phytonutrients, in particular flavonoids, and bone health is currently insufficient to generate precise dietary recommendations, accumulating data suggest that the current public health guidance of 'five servings of fruit and vegetables each day' may also apply as a preventive strategy to slow down the development of osteoporosis. Indeed, the current guidance of five servings of fruit and vegetables each day highlights the possibility and probability that nutrition supports bone metabolism as a consortium of phytonutrients and other nutrients

Table 2

Bone outcome measures used in clinical and preclinical trials

Outcome measure	Technology	Species	Invasiveness	Description
BMD BMC	Dual energy X-ray absorptiometry (DEXA)	Humans and animals	Not invasive; however, subjects are exposed to low doses of X-rays	Measures the BMC of a region of interest, after which the BMD can be calculated as follows: BMD = BMC (in grams)/area (in square centimetres). A T-score is obtained and is compared with the BMD values of young healthy adults. Most widely used technology for measuring BMD. Up to 50% of fractures occur in postmenopausal women with normal BMD values, highlighting that BMD is not always a reliable marker for predicting fracture risk
BMD Microarchitecture of cortical and trabecular bone Prediction of bone strength using finite element analysis	Computed tomography (quantitative computed tomography for humans, mico- or nano-computed tomography for animals)	Humans and animals	Not invasive; however, subjects are exposed to low doses of X-rays	Measures the BMC of a region of interest, after which the volumetric BMD can be calculated as follows: BMD = BMC (in grams)/volume (in cubic centimetres). Produces a three-dimensional image of bone, from which microarchitectural properties can be evaluated (e.g. trabecular number, trabecular separation, trabecular thickness, cortical surface area and cortical thickness). Predictions of skeletal strength can be made using complex geometrical algorithms
Speed of sound (SOS) Broadband ultrasound attenuation (BUA) Stiffness Index (SI)	Quantitative ultrasound	Humans	Not invasive; no exposure to radiation	Provides an estimation of bone mass and skeletal quality. Predictive ability of quantitative ultrasound is similar to that of DEXA . -Low cost and portability of the instrument make quantitative ultrasound an attractive measure in various trials (e.g. children, remote locations where DEXA is not accssible or too costly to use)
Biochemical markers of bone turnover	Analytical instruments or kits (e.g. enzyme-linked immunosorbent assays and radioimmunoassays)	Humans and animals	Blood or urine collection required	Measures markers of bone formation (e.g. osteocalcin, alkaline phosphatase and type I collagen) and bone resorption (e.g. deoxypyridinoline, C-telopeptide of type I collagen, N-telopeptide of type I collagen and pyridinoline). Some biochemical markers have a predictive value for fracture risk (e.g. C-telopeptide of type I collagen and procollagen I N-terminal propeptide)
Osteoblast number, osteoclast number, etc. Bone formation rate, mineral apposition rate, etc.	Static and dynamic histomorphometry	Humans and animals	Invasive, because a bone biopsy is required	Static histomorphometry measures structural parameters of bone. Dynamic histomorphometry measures rates of bone formation and bone resorption
Fracture	DEXA or radiography for confirmation. Report of fracture to study investigators	Humans	Exposure to radiation if DEXA or radiography is used to confirm fracture	Used in long (≥2years) trials to determine whether interventions are effective in reducing the number of fractures at various skeletal sites (e.g. hip, spine and radius)
Bone strength parameters	Biomechanical strength-testing machine	Human cadavars and animals	None, because this destructive test is performed on bones excised from animals or human cadavers	Measures the amount of force a bone can withstand before it fractures. Measures the elastic and plastic properties of bone

Abbreviations are as follows: BMC, bone mineral content; and BMD, bone mineral density.

rather than in isolation. Thus, trials which examine the combined effects of various nutritional approaches, like those mentioned above, may provide more robust results regarding their effects on bone quantity and bone quality. In addition, human trials that include outcome measures related to bone quantity (BMD), bone quality (e.g. bone microarchitecture and bone turnover) and bone strength (using finite element analysis) should be implemented when possible to gain a more comprehensive outlook on how bone responds to these various nutritional factors.

Competing Interests

All authors have completed the Unified Competing Interest form at www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare; no support from any organisation for the submitted work, AS and CS have received funding from Soho Flordis International (SFI) in the previous 3 years; there are no other relationships or activities that could appear to have influenced the submitted work.

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