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REVIEW

Skeletal effects of nutrients and nutraceuticals, beyond calcium and vitamin D

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Abstract There is a need to understand the role of nutrition, beyond calcium and vitamin D, in the treatment and prevention of osteoporosis in adults. Results regarding soy compounds on bone density and bone turnover are inconclusive perhaps due to differences in dose and composition or in study population characteristics. The skeletal benefit of black cohosh and red clover are unknown. Dehydroepiandrosterone (DHEA) use may benefit elderly individuals with low serum dehydroepiandrosterone-sulfate levels, but even in this group, there are inconsistent benefits to bone density (BMD). Higher fruit and vegetable intakes may relate to higher BMD. The skeletal benefit of flavonoids, carotenoids, omega-3-fatty acids, and vitamins A, C, E and K are limited to observational data or a few clinical trials, in some cases investigating pharmacologic doses. Given limited data, it would be better to get these nutrients from fruits and vegetables. Potassium bicarbonate may improve calcium homeostasis but with little impact on bone loss. High homocysteine may relate to fracture risk, but the skeletal benefit of each B vitamin is unclear. Magnesium supplementation is likely only required in persons with low magnesium levels. Data are very limited for the role of nutritional levels of boron, strontium, silicon and phosphorus in bone health. A nutrient rich diet with adequate fruits and vegetables will generally meet skeletal needs in healthy individuals. For most healthy adults, supplementation with nutrients other than calcium and vitamin D may not be required, except in those with chronic disease and the frail elderly.

Keywords Bone density · Fractures · Minerals · Nutraceuticals · Nutrition · Vitamins

Introduction

The general nutritional advice for skeletal health is that adults should follow a varied nutrient-rich diet with adequate protein and fruits and vegetables, and they should also ensure an adequate intake of calcium and vitamin D. Importantly in adults, calcium intake should be 1,000 to 1,200 mg daily (based on gender and age), preferably consumed from diet, with supplements used only if dietary calcium is inadequate [1]. Adults also need at least 800 to 1,000 IU vitamin D per day, that is more difficult to obtain from diet and will in most cases require supplementation in individuals without regular sun exposure [2]. Other minerals such as potassium, magnesium, trace minerals, and vitamins B, K, and C may also be important to bone health. Fruits and vegetables are excellent sources of many of these nutrients and may also create an alkali environment and provide other potentially beneficial antioxidants and compounds. For most healthy adults, supplementation with nutrients other than calcium and vitamin D may not be required, except in those with chronic disease and in the frail elderly where nutrition is often insufficient.

In National Health and Nutrition Examination Survey (NHANES) 1999–2000, 52 % of adults reported taking a dietary supplement in the past month, and rates were higher in individuals with a diagnosis of a chronic disease [3]. In this paper, nutraceuticals are defined as any substance that is a part of a food that may provide medical or health benefits,

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including the prevention and treatment of disease. Nutraceuticals may include isolated nutrients, dietary supplements, herbal products, and medical foods (available only by prescription). The Dietary Supplement Health and Education Act of 1994 in the United States notes that the dietary supplement manufacturer is responsible for ensuring that a dietary supplement is safe and that any representations or claims made about them are substantiated by adequate evidence to show that they are not false or misleading. In this act, a supplement was defined as a product that contains a vitamin, mineral, amino acid, herb, or other botanical or dietary substance that will increase total dietary intake of that ingredient. These products should not make a claim to prevent or cure disease. Unlike drug products that must be proven safe and effective for their intended use before marketing, there are no provisions in the law for the Food and Drug Administration to "approve" dietary supplements for safety or effectiveness before they reach the consumer [4]. In this review, we will provide data regarding the role of phytoestrogens, dehydroepiandrosterone (DHEA), antioxidants such as omega 3 fatty acids, flavonoids, carotene, and several vitamins and minerals in relation to bone health.

Phytoestrogens

Isoflavones

Phytoestrogens are naturally occurring plant compounds that function like estrogen agonist–antagonists. There are three major forms of phytoestrogens: isoflavones (genistein, daidzein, glycitein) that are primarily found in soybeans and soy products, chickpeas, and other legumes; lignans (enterolactone and enterodiol) that are found in oilseeds such as flaxseed, cereal bran, and legumes; and coumestans (coumestrol) that are found in alfalfa and clover. Another compound, black cohosh, may also have estrogenic activity [5]. Many of the phytoestrogens are not only found in foods but are also purified and concentrated into high-dose dietary supplements.

The typical American diet contains only about 1–3 mg/d of isoflavones [6], while typical Asian diets generally have 30 to 60 mg/d of isoflavones [7]. The Dietary Guidelines for Americans (2010) has added fortified soy products as a replacement option for dairy or for protein which may lead to an increased intake in the US [8]. Food sources that are not always fortified include edamame (whole soybeans), tofu (soybean curd), soy milk, soy flour, tempeh (cooked and fermented soy), and miso (fermented soybean paste). Nutritional supplements include isolated soy protein and the soy-derived isoflavones genistein, daidzein, and glycitein.

Phytoestrogens differ in their affinity for estrogen receptor alpha versus estrogen receptor-beta, which alters their effect on the skeleton and other tissues. Phytoestrogens are hypothesized to act on the skeleton by several mechanisms including: their ability to alter the receptor activator for nuclear factor κ B ligand/osteoprotegerin (RANKL/OPG) pathway, inhibit inflammation, enhance antioxidant enzymes, increase calcium absorption, induce osteoclast apoptosis, and modulate insulin-like growth factor levels [9]. Dietary supplements containing genistein-like isoflavones (from soy cotyledon and germ) demonstrated a significant but modest ability to suppress net bone resorption in postmenopausal women, whereas kudzu and red clover did not [10]. These data help to further elucidate a mechanism by which genistein-like isoflavones may improve bone health.

Data on the effect of dietary intakes of phytoestrogens on fracture risk are limited to a longitudinal, observational study of 24,403 postmenopausal Chinese women who were followed for 4.5 years. Women with high dietary soy intake (>13.26 g/day) had a 36 % lower fracture risk as compared with women with low dietary soy intake (<4.98 g/day). A dose response was evident across quintiles of daily soy-rich food intake [11].

In one review, results from placebo-controlled interventional studies with soy intake through dietary sources (80–90 mg/day) in postmenopausal women (sample sizes 65–203) were inconsistent with no effect in three studies and improved bone mineral density (BMD) in three studies [9]. In a meta-analysis of ten studies (n=608; range, 4.4 to 150 mg/day isolated soy isoflavones), isolated soy isoflavone consumption for 6 months reduced spine bone loss, especially if intake was greater than 90 mg/day [12]. In fact, several reviews have concluded that there is either a lack of effect or only a modest effect of soy protein and isolates on the skeleton [13]. The inconsistent skeletal benefit may be a result of differences in study design and duration, sample size, initial BMD, years since menopause, age, body weight, and the baseline intake of soy, calcium, and other nutrients.

Results subsequent to these reviews continue to be inconsistent, although primarily negative. Information on more recent clinical trials is summarized in Table 1. In general, the trials have studied between 60 and 400 postmenopausal women, with variable compliance, and have shown either no benefit or only a modest benefit on lowering bone turnover or preventing bone loss [13–20]. These studies have used soy protein isolates and/or isoflavones of varying doses and compositions for 9 months to 3 years, and in most cases, calcium and vitamin D were given to all study participants. Isoflavones, whether evaluated as soy proteins or soy isoflavones, that contain varying amounts of genistein, daidzein, and glycitein do not provide a consistent skeletal benefit to postmenopausal women [14–18].

Isoflavones have been marketed as a medical food, which requires data to support an indication for treatment (low

Table 1 Randomized controlled trials of soy isoflavones versus placebo, published 2007–2012

Author	Population	Intervention	Results of soy versus placebo
Alekel 2010 (15)	*n*=255 (224 analyzed), age 45–65 years, 1–8 years postmenopausal, BMD T-score >−1.5 to <+1.0	Placebo versus soy isoflavone extract (80 or 120 mg/day) with genistein/diadzein/glycitein (aglycone) in a 1.3:1.0:0.3 ratio for 3 years 500 mg calcium and 600 IU vitamin D	No impact on change in bone mineral density except femoral neck: less bone loss in 120 mg versus placebo
Brink 2008 (14)	*n*=300 (237 completed), mean age 53 years, 1–5 years postmenopausal, Z-score >−2	Placebo versus biscuits and cereal bars enriched with soy isoflavone aglycone (110 mg) for 1 year 60–70 % daidzein, 1–5 % glycitein	No impact on change in bone mineral density or bone turnover markers
Evans 2007 (19)	*n*=61 (43 completed), age 62±5 years, >2 years postmenopause	25 g protein ±91 mg aglycone units of isoflavones or control and ± exercise for 9 months 900 mg calcium 125 IU vitamin D for 9 months	No impact on change in bone mineral density
Kenny 2009 (17)	*n*=131 (97 completed), age 60–93 years, BMD T-score >−3.0	Soy protein (18 g)+105 mg isoflavone aglycone equivalent tablet versus soy protein + placebo versus control protein + isoflavone tablets versus control protein + placebo tablets for 1 year Counseled for 1,200–1,500 mg calcium a day; gave 315 mg Ca and 200 IU vitamin D when needed	Soy protein and/or soy isoflavones had no impact on bone mineral density or bone turnover markers
Levis 2011 (16)	*n*=248 (182 completed), age 45–60 years, T-score >−2	Placebo versus novasoy 200 mg of isoflavone extracted from soy protein for 2 years Calcium 500–1,000 mg to total 1,500 mg, vitamin D 200–400 IU	No impact on change in bone mineral density at spine or hip
Marini 2007 (22)	*n*=389 (304 completed 2 years), age 49–67 years, T-score <−1.0	Placebo versus 54 mg genistein aglycone tablets for 2 years Calcium 500 mg and vitamin D 400 IU	Increases in bone formation decreases in bone resorption bone mineral density increased at spine and hip (~5 %) versus loss in placebo
Vupadhyayula 2009 (20)	*n*=203 (157 completed), age >55 years (mean 63), T-score >−2.5	25 g soy protein isolate versus 25 g soy protein isolate with 90 mg isoflavones (genistein 1.87 mg/g, daidzein 1.5 mg/g, and glycitein 0.22 mg/g aglycone components) versus 25 g milk protein 500 mg calcium and 125 IU vitamin D for 2 years	No impact on change in bone mineral density in spine or hip
Wong 2009 (18)	*n*=406 (362 analyzed), age 40–60 years, BMD spine T-score>−1.5	Placebo versus 80 mg soy hypocotyl aglycone isoflavones (daidzein 22 mg; glycitein 13.5 mg; genistein 5 mg as glycosides per tablet/3 tablets per day) 400 mg calcium and 400 IU vitamin D for 2 years	No bone mineral density effect except diminished total body bone loss

bone mass) and also show that all ingredients of the product are generally regarded as safe. One medical food is Fosteum, containing 27 mg genistein aglycone, 200 IU vitamin D, and 20 mg citrated zinc bisglycinate (4 mg elemental zinc). In one study [21], 54 mg a day of genistein aglycone (the major component of Fosteum) was compared with hormone therapy (HT) and placebo in 90 Italian postmenopausal women. Women taking genistein aglycone had gains in BMD at the spine and the hip that were equivalent to the gains in BMD seen in the women taking HT. A second trial [22] was a multi-center trial that enrolled 389 Italian postmenopausal women (Table 1). There were modest increases in markers of bone formation and decreases in bone resorption, with concomitant increases in BMD in the treatment group given 54 mg a day of genistein aglycone. Whether the reported benefits of genistein aglycone, in contrast to the negative outcomes with other isoflavone compounds (Table 1), are related to the specificity of this isoflavone compound, its bioavailability and bone affinity, or the dose used needs to be explored. The results showing increased bone mass in women taking genistein aglycone needs to be confirmed in a large head-to-head clinical trial.

Some calcium supplements have now added genistein to create a "product to promote bone health" in the words of one manufacturer. The product includes 27 mg genistein, along with other nutrients, and the reference in support of this product is based on the data mentioned above [21].

The safety of isoflavones has been evaluated in several studies. One potential side effect is that some women develop nausea or gastric irritability, stomach pain, vomiting, and constipation when taking some products [23], but taking isoflavones with food appears to minimize these effects. There has been concern that isoflavones may alter thyroid hormone production, but this is probably limited to those with inadequate iodine intake [24]. Safety data have been reported regarding the effect of genistein and other isoflavones on endometrial thickness or vaginal cytology and changes in breast tissue density or breast cancer, with no negative effects in both epidemiologic studies and clinical trials with up to 3 years in duration in postmenopausal women [25–29].

There are mostly negative reports from clinical trials of the skeletal effects of varying forms of soy isoflavones, but there are also some conflicting reports. This may result, in part, from differences in the composition and dose of the soy product as well as differences in study populations and characteristics. Data are clearly needed to confirm some of the findings.

Red clover (Trifolium pratense)

Red clover is a wild plant belonging to the legume family and is often used to relieve symptoms of menopause, high cholesterol, and osteoporosis. One product containing red clover prevented the loss of lumbar spine, but not hip, bone density in 205 women [30]. However, a review of the potential skeletal benefit of red clover concluded that there was limited evidence of efficacy [31]. For example, in a recent placebo-controlled 3-year trial in 401 women with a family history of breast cancer, 40 mg of red clover produced no effect on BMD [32]. Red clover does seem to be safe for most adults when used for short periods of time. No serious adverse effects have been reported in the literature. However, red clover contains estrogen-like compounds, so there is a possibility that its long-term use would increase the risk of women developing uterine cancer. It is also unknown whether red clover is safe for women who have breast cancer or other hormone-sensitive cancers.

Ipriflavone

Ipriflavone is synthesized from the soy isoflavone, daidzein. In a study of 60 women randomized to 600 mg of ipriflavone or placebo, bone loss was prevented at the spine, and there was a reduction in bone resorption in the ipriflavone group as compared with the placebo group [33]. In a study of 60 women, spine and hip BMD increased with 1 year of ipriflavone treatment (200 mg tablet three times a day) versus placebo [34]. In a 3-year study, ipriflavone (600 and 1,200 mg/day) was no better than placebo in preventing bone loss and fractures in postmenopausal women [35]. Ipriflavone may cause side effects such as stomach pain, diarrhea, or dizziness. There is some concern that ipriflavone may cause a decreased white cell count (lymphocytopenia) in people taking it for greater than 6 months. White cell counts should be monitored, especially in people taking ipriflavone long-term [36].

Black cohosh (Cimicifuga racemosa)

Black cohosh is an herbal member of the buttercup family that is sold as a dietary supplement. It is available as a tablet, capsule, or liquid, at varying doses from120–540 mg and made from the roots and underground stems of the buttercup. It is labeled as black cohosh or *C. racemosa*. Black cohosh may have estrogenic activity [5]. Of recent concern are case reports of hepatitis as well as liver failure in women who were taking black cohosh [37]. Less serious side effects include headaches, gastric complaints, heaviness in the legs, and weight problems. The potential mechanism of action may be related to a report indicating that black cohosh blocked in vitro osteoclastogenesis induced by either RANKL or TNF-alpha [38].

In a small double-blind study of 62 postmenopausal women, with a daily dose of *C. racemosa* corresponding to 40 mg of herbal drug, conjugated estrogen (0.6 mg/day),

or placebo for 12 weeks, bone turnover assessed by serum c-telopeptide (CTX) was stable over 12 weeks while an increase in CTX was noted in the placebo group and a decrease in the estrogen group [39]. A recent study to determine the effect of exercise training with and without *C. racemosa* on BMD in early postmenopausal women found that exercise training limited bone loss. However, no differences were found within the exercise groups with or without *C. racemosa* [40].

Summary

In general, there are more reports stating there is no benefit of isolated soy protein isoflavones with regard to increasing bone density or decreasing bone turnover in postmenopausal women. Phytoestrogen trials in humans have had variability in study design and study sample characteristics such as estrogen status and age, as well as years from menopause. In addition, the metabolism and composition of the phytoestrogen and the impact of baseline soy intake as well as other dietary factors have varied. The variability in skeletal response to isolated soy protein isoflavones that does exist may relate to the specific isoflavone provided. In fact, in the guidance for human research from a National Institutes for Health workshop in July 2009, it was determined that aglycone isoflavone equivalents are the most bioactive form of isoflavones [41]. There are very limited data on either black cohosh and or red clover.

Dehydroepiandrosterone (DHEA)

DHEA is a steroid hormone that serves as a precursor to androgens in men and estrogens in women. Serum levels of dehydroepiandrosterone-sulfate (DHEAS) fall with age in both men and women. The commercial claims for this product include that it is an anti-aging remedy, will increase muscle, decrease fat, and improve energy, strength, and immunity. DHEA can inhibit osteoclastic bone resorption, although the specific mechanism is unknown [42].

Serum levels of (DHEAS) appear to relate to bone loss. In a recent observational cohort study of 1,003 postmenopausal women, high endogenous serum DHEAS at baseline was associated with less bone loss at both the femoral neck and lumbar spine, although the effect diminished over the 15 years of follow-up [36].

There have been several recent clinical trials with conflicting or inconsistent data regarding the skeletal site and gender-specific benefit of DHEA. In one trial, DHEA 50 mg given for 12 months increased BMD at various sites, depending on age, and in another study, 6 months of 100 mg/day produced no change in BMD [43, 44]. In the DHEA and Well-Ness (DAWN) trial, 1 year of 50 mg DHEA led to decreases in CTX and increases in spine BMD in women only, without any benefit to other skeletal sites or to men [45]. Spine BMD improved in women, but not men, who took 50 mg/d DHEA supplements or placebo for 1 year [46]. In a 2-year trial of men with low DHEAS levels [47], men given either DHEA or low-dose testosterone replacement treatment had a BMD increase but only in the femoral neck. In 86 Chinese men given higher-dose DHEA (100 mg per day) or placebo for 6 months, spine and femoral neck BMD increased in the DHEA group as compared with the placebo group [48].

At this point, there is suggestive, but not conclusive, evidence that DHEA may have some skeletal benefit, however, the benefit is likely to be limited to elderly individuals with low serum DHEAS. In addition, any potential improvement in BMD does not appear to be consistent across skeletal sites or genders. The safety concerns with the use of DHEA include that it may adversely affect liver function and lead to acne and masculinizing effects.

Antioxidants

Oxidative stress is a potential cause of many diseases. Animal models indicate that oxidative stress is a major mechanism in the loss of bone mass and strength. In fact, aging and the associated increase in reactive oxygen species greatly influence the generation and survival of osteoclasts, osteoblasts, and osteocytes [49]. Therefore, antioxidants have been studied in their role to prevent disease and promote health. Antioxidants that may relate to bone health include vitamin A, vitamin E, vitamin C, carotenoids, and flavonoids such as quercetin. These compounds may also have the potential to create a more alkaline environment, reduce urinary calcium excretion, and provide bioactive components (phenols and flavonoids), or higher intake of these nutrients may simply be a marker for a healthy lifestyle. Any of these factors could result in a skeletal benefit.

Flavonoids

Flavonoids occur in plant-based foods including fruits, vegetables, grains, herbs, tea wine, and juices. Flavonoids are further classified as flavanones, anthocyanins, flavan-3-ols (green tea), polymers, flavonols, flavones, and isoflavones (previously discussed). In a recent cohort study of twins in the UK, higher intakes of total flavonoids was associated with higher BMD at the spine. Anthocyanin and flavone intake was positively associated with spine and hip BMD [50]. These results expand on the findings of a previous study that found that dietary flavonoid intakes were positively associated with BMD at the spine and femoral neck in perimenopausal Scottish women [51].

Quercetin is a member of the flavonoid family and is a strong antioxidant. It can be found in various foods including citrus fruits, apples, onions, parsley, sage, tea, and red wine, and in many food supplements. Quercetin has been shown to decrease the differentiation of osteoclast progenitor cells and inhibit the activity of mature osteoclasts in vitro. In addition, quercetin might act together with the alkaline-forming properties of fruit to inhibit osteoclasts and enhance bone mineral density [52–56]. However, without more data, advice would be to get flavonoid compounds from fruits and vegetables instead of from a supplement.

Omega-3 fatty acids

There are several sources of omega 3 fatty acids including fish, eggs, walnuts, and flax seed. Nutritionally essential omega-3 fatty acids are polyunsaturated fatty acids: α-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA). Omega 3 fatty acids may affect the skeleton, not only by their anti-oxidant function, but they may also upregulate intestinal calcium absorption [57].

The effect of omega-3 fatty acids on BMD has been variable. A positive association was found between dietary intake of omega-3 fatty acids and baseline BMD and BMD accrual (total body, spine) in 78 healthy young men (ages16 and 22 years) [58]. Dietary intake of omega-3 was also positively related to hip BMD in 247 older adults [59, 60] and with lumbar spine and total body BMD in 554 elderly Finnish women [61]. However, intakes with a higher ratio of omega-6 to omega-3 fatty acids has been negatively associated with BMD in 85 healthy young children [62] and with lower hip BMD in older women [63].

In a recent review on the potential benefit of omega-3 fatty acids on skeletal health, it was concluded that any skeletal benefit might be enhanced by concurrent administration of calcium [64]. There are currently insufficient data available to draw conclusions about the beneficial effects of omega-3 on bone health, although there appears to be a clear cardiovascular benefit [65]. It is possible that a lower ratio of omega-6 to omega-3 may be positively associated with bone health [63], so it would be better to obtain omega-3 from dietary sources such as walnuts and fish and flaxseed oils and limit sources of omega-6 by reducing consumption of processed and fast foods and polyunsaturated vegetable oils (corn, sunflower, safflower, soy, and cottonseed).

Carotenoids

Carotenoids are fat-soluble plant pigments that are considered to be antioxidants and are found in vegetables including carrots, sweet potatoes, spinach, kale, collard greens, papaya, bell peppers, and tomatoes. There are four carotenoids (beta-carotene, alpha-carotene, gamma-carotene, and beta-cryptoxanthin) that can be converted to retinol (vitamin A). The other carotenoids lycopene, lutein, and zeaxanthin also act as antioxidants but are not converted to retinol (vitamin A). Data regarding the impact of carotenoids on bone density are inconsistent and are evaluated based on either serum levels or dietary intake of carotenoids. In one study, lower lycopene and cryptoxanthin concentrations were found in the serum of women with osteoporosis compared with women with higher BMD [66]. In the Women's Health Initiative, the only carotenoid that was positively related to higher BMD was beta-carotene [67]. In epidemiologic studies, foods containing higher amounts of carotenoids have been associated with higher BMD [68, 69]. The highest versus lowest tertile of total carotenoids and lycopene intake reduced 15-year fracture incidence by 46 % in women and 34 % in men in the Framingham study [70]. More information is needed regarding the role of carotenoids on bone health. However, with the data that are now available, it would be best to get carotenoids from increasing the intake of the vegetables listed above, since there may be other health benefits to increasing fruit and vegetable consumption, and there are no data on the skeletal benefit of carotenoids as supplements.

Vitamin A

Retinol is the animal form of vitamin A and is found in liver, meats, eggs, milk products, and fatty fish. The daily recommended intake for vitamin A for men is 1,000 mg of retinol equivalents (RE) and for women 800 mg RE. Several studies have shown that excess vitamin A is harmful to bones, based on decreased bone density and increases in hip fracture rates [71–74]. Although retinol has not always been associated with poor skeletal health [75], it is best to avoid cod liver oil and other concentrated fish products as sources of vitamin D due to the higher vitamin A content. In addition, vitamins that contain no more than 2,000 IU to 3,000 IU retinol are preferred. However, for supplements that use the precursor for vitamin A (beta-carotene), there is no concern with the common doses used in supplements or multivitamins.

Vitamin C and vitamin E

Ascorbic acid, a water-soluble vitamin, is also called vitamin C. Dietary sources include citrus fruits and juices, green vegetables (especially peppers, broccoli, cabbage), tomatoes, and potatoes. The dietary reference intakes for vitamin C are 75 mg/day for adult women and 90 mg/day for adult men. Vitamin C is an essential cofactor for collagen formation and synthesis of hydroxyproline and hydroxylysine. Vitamin C may also provide skeletal benefit by its antioxidant properties. In a clinical trial of 34 women, the results

suggested that vitamin E (400 mg) and vitamin C (1,000 mg/day) supplements taken for 6 months might reduce spinal bone loss [76]. In an observational study, those women who reported taking both vitamin C and E supplements had reduced bone resorption as measured by serum CTX, compared with the rest of the cohort [69]. Other epidemiological studies have shown a positive association between vitamin C and bone mass, although the results are not always significant. In these studies, low intakes of vitamin C are associated with a faster rate of bone loss in women of varying ages, and one study found that higher vitamin C was associated with fewer fractures [77–87].

Tucker and colleagues [88] evaluated bone density for 4 years in 213 men and 393 women (average age, 75 years) to determine if any association existed between vitamin C intake and bone health, but smoking and hormone replacement therapy use were accounted for in the analysis. Men with the highest vitamin C intake (>300 mg/day; >3 times the recommended intake in men) had significantly less bone loss than men in the lowest group of vitamin C intake (106 mg). A similar finding in women was not significant, and it was shown that vitamin C may interact with estrogen use, calcium, and vitamin E in women [89].

The associations of vitamin C intake (total, dietary, and supplemental) with incident hip fracture and non-vertebral osteoporotic fracture was evaluated over a 15- to 17-year follow-up period in the Framingham Osteoporosis Study. In this study with 100 incident hip fractures, subjects in the highest tertile of total vitamin C intake had significantly fewer hip fractures (P trend=0.04) and non-vertebral fractures (P trend=0.05) compared with subjects in the lowest tertile of intake; this was found to be a result of supplemental rather than dietary vitamin C [90]. In the Women's Health Initiative Observational Study, increasing intakes of antioxidants (including vitamin C) were not independently associated with BMD, after adjustment for important covariates [67]. Clearly, there is a need for further study that will control for important covariates and allow a more clear evaluation of the effect of vitamin C on bone density.

In many studies, it is not possible to separate the effects of vitamin C supplements from vitamin C in fruits and vegetables [77, 89]. Therefore, the recommendation is to obtain the needed amounts of vitamin C from fruits and vegetables, rather than from supplements. In fact, higher fruit and vegetable intakes may have positive effects on bone mineral status at all ages, so that this recommendation should cover the lifespan [86]. Recommended intakes of five or more servings of fruits and vegetables per day should supply enough vitamin C for bone health. Future studies of vitamin C and bone health need to take into account gender, estrogen use, and intake of other nutrients to assess any potential interactions.

Vitamin K

The major forms of vitamin k are phylloquinone (vitamin K1) found in green leafy vegetables such as spinach, kale, broccoli, and menaquinone (vitamin K2) found in meat, eggs, dairy, cheese, and natto. Dietary K2 accounts for about 10 % of total vitamin K intake. The Dietary Reference Intake (DRI) for vitamin K is 90 μg. Osteocalcin is a vitamin K-dependent protein that is unique to bone. Vitamin K deficiency leads to a decrease in the carboxylation of osteocalcin, and vitamin K treatment will reduce undercarboxylated osteocalcin [91]. It is unclear whether K1 and K2 have differential effects on bone density.

Serum vitamin K has been found to be lower in persons with osteoporosis or fractures, while higher vitamin K intake is positively related to bone health in epidemiologic studies [92–94]. Randomized controlled trials using vitamin K supplementation have found either no effect on BMD [93, 95] or a positive effect, but only on hip BMD [96]. In 334 Norwegian women, 360 μg MK-7, a form of K-2, was given in the form of natto capsules for 1 year. After 12 months, there were no statistical differences in bone loss rates between the groups at the total hip or any other measurement site, although carboxylated osteocalcin was increased [97].

There have been several recent clinical trials of vitamin K. In a 2-year randomized clinical trial, women given 200 μg vitamin K-1 (phylloquinone), vitamin D, and calcium had an increase in ultradistal BMD that was more than in the group of women given vitamin D and calcium alone [98]. In another 3-year randomized clinical trial, women given 500 μg vitamin K-1 (phylloquinone), vitamin D, and calcium had no difference in BMD change as compared with the group given vitamin D and calcium alone [99]. In the Evaluation of the Clinical Use of Vitamin K Supplementation in Post-menopausal Women with Osteopenia (ECKO) trial, 5 mg of daily vitamin K1 supplementation for 2 to 4 years did not protect against age-related decline in BMD but protected against clinical fractures [95]. Clearly, these fracture results need to be confirmed as there were very few incident fractures in the 4 years (nine in vitamin K1 versus 20 in placebo).

In a recent review, it was noted that most studies where subjects were given 45 mg of K2 found modest increments in BMD at various skeletal sites [100]. In a meta-analysis of seven trials that reported fracture effects, all subjects were Japanese and used high-dose vitamin K-2, typically menaquinone. Pooling the fracture data from the seven trials in a meta-analysis, the odds ratio favoring menaquinone over placebo was 0.40 (95 % confidence interval [CI], 0.25–0.65) for vertebral fractures, 0.23 (95 % CI, 0.12–0.47) for hip fractures, and 0.19 (95 % CI, 0.11–0.35) for all non-vertebral fractures [100]. In another study, 15-mg menatetrenone capsules (at supraphysiologic doses; n=2,185)

versus placebo (n=2,193) was found to prevent vertebral fracture (4.4 versus 3.4 % of participants fractured) and limit height loss in women with a prior fracture [101], similar to findings from other studies in Japanese women [102, 103].

In general, patients should be encouraged to consume a diet rich in vitamin K, which is chiefly obtained from green leafy vegetables and certain vegetable oils. Although vitamin K may be related to fracture prevention, the benefit appears limited to high doses of vitamin K2 as menaquinone and in specific populations (Japanese women) [100–104]. Therefore, supplementation is not recommended until beneficial results are confirmed in larger trials with varying populations.

Individuals on oral anticoagulants such as warfarin should avoid high intakes of vitamin K through diet or supplements. Any differences between vitamin K1 and K2 need to be further explored.

Summary

In several studies, higher fruit and vegetable intakes have been found to reduce bone turnover and increase bone mineral density [77, 86, 105]. There are several potential mechanisms whereby fruit and vegetables may improve bone health. Fruits and vegetables may reduce urinary calcium excretion, create an alkaline environment, provide specific nutrients, provide an antioxidant effect, and provide bioactive components (phenols and flavonoids), or they may simply be a marker for a healthy lifestyle. Therefore, foods, rather than supplements, may be the best way to get the benefit of antioxidant compounds and vitamins, because they may also provide skeletal benefit through other mechanisms.

Bicarbonates

An acidic environment may have a negative impact on the skeleton [106, 107]. Therefore, potassium and other bicarbonates have been studied for their skeletal effect. Bicarbonate (HCO_3) may improve bone health by decreasing 24-h urinary calcium and perhaps by lowering plasma C-terminal cross-linking telopeptide of type 1 collagen [108]. In a 41-day randomized, placebo-controlled, double-blind study of potassium bicarbonate ($KHCO_3$; up to 90 mmol/day) or placebo, treatment with KHCO3 appeared to promote calcium absorption [109]. In a double-blind, controlled trial, 171 men and women age 50 years and older were randomized to receive placebo or 67.5 mmol/day of potassium bicarbonate, sodium bicarbonate, or potassium chloride for 3 months. Subjects taking either bicarbonate had significant reductions in urinary *N*-telopeptide and calcium excretion, but potassium alone had no effect on these markers [110]. Potassium citrate supplementation for 2 years did not reduce bone turnover or increase BMD in healthy postmenopausal women as compared with placebo [111]. Therefore, it is unclear whether the alkali content of the diet can benefit the skeleton and whether this may depend on the baseline acidogenicity of the diet [111]. Further study may clarify these differences and further elucidate the effect of the bicarbonate as compared with the effect of potassium.

B-vitamins and homocysteine

Vitamins B_6 (pyridoxine), B_9 (folic acid), and B_{12} (cyanocobalamine) are water-soluble vitamins that have been reported to have a skeletal benefit. These B vitamins are available as supplements either in combination or individually. Food sources of pyridoxine (B6) are whole grains, fortified cereals, liver, soybeans, and beans. The DRI for adults for pyridoxine (B6) is 1.5 mg for females and 1.7 mg for males. Folate (vitamin B9) occurs naturally in food, and dietary sources include dark green leafy vegetables, wholegrain breads, nuts, and fortified cereals. Folic acid is the synthetic form of folate which is used in supplements and added to fortified foods. The DRI for vitamin B_9 (folate, folic acid) is 400 μg. Vitamin B12 is found in liver, shellfish, fish, beef, lamb, cheese, eggs, and some fortified foods. The recommended dietary allowance (RDA) is 2.4 μg for adults.

Homocysteine has recently been linked to fragility fractures, including hip fractures in older men and women [112–115], and elevated serum homocysteine levels may be caused by deficiencies of folate (folic acid), vitamin B_{12}, or vitamin B_6. The role of B vitamins in skeletal health has been the subject of many recent research studies.

Incident osteoporosis-related fractures were assessed in a study with 702 Italian participants aged 65–94 years with a mean follow-up of 4 years. In this study, low serum folate was shown to be responsible for the association between higher homocysteine levels and greater risk of osteoporosis-related fractures in elderly persons [116].

In other studies, poor vitamin B_{12} status was associated with low bone mass or osteoporosis in both women and men [117–120]. This association might reflect vitamin B_{12} deficiency or may be an indicator of overall poor nutrition and increased frailty both of which are negatively related to BMD and positively related to fracture risk.

In 1,002 men and women (mean age, 75 years) in the Framingham Osteoporosis study, low serum B vitamin concentrations, particularly folate, were noted to be a risk factor for decreased bone health, although these low concentrations did not fully explain the relationship between elevated homocysteine and hip fracture [121]. The Hordaland Homocysteine Study is a population-based study of more than

18,000 men and women in Western Norway. Among women in this study, raised homocysteine levels were associated with decreased BMD and an increased risk of osteoporosis [122].

In the Rotterdam Study, a reduction in the risk of fracture was related to dietary pyridoxine (vitamin B6) intake in the cohort of over 5,000 people who were followed over 7 years. A positive and independent relation between dietary intakes of pyridoxine (B6) with BMD was also found [123].

In some studies, folate is more strongly related to BMD than any other B vitamin [123–126]. A 2-month study of 2.5 mg folic acid supplementation in 61 healthy individuals did not affect biochemical bone markers in subjects without osteoporosis but who had a low folate status [127]. In a Japanese study, 5 mg folic acid and 1,500 μg mecabolamin (a form of vitamin B 12) daily for 2 years reduced hip fractures as compared with placebo; of note, the population consisted of stroke patients, and the doses were pharmacologic [128].

Controlled trials to determine whether treatment with any of the B vitamins would reduce fracture rates among community-dwelling cohorts would be ideal [129]. One trial has been initiated in The Netherlands to assess the efficacy of oral supplementation with vitamin B12 and folic acid to prevent fractures in elderly people with elevated homocysteine concentrations (B_PROOF study) [130]. Clearly, a need exists for further research, including long-term clinical trial data, to determine the role of each B vitamin in skeletal health. Without further evidence of a benefit for B vitamins, it would be best to promote a healthy varied diet to assure adequate intake of B vitamins.

Minerals

Magnesium

Magnesium is the fourth most abundant mineral in the human body, and food sources include green vegetables such as spinach, legumes (beans and peas), nuts and seeds, and whole, unrefined grains. The RDA for magnesium is 420 mg/day in adult males and 320 mg/day in adult females. According to recent data from NHANES, magnesium is a shortfall nutrient, and over half of adults do not meet this RDA [131].

In preadolescent girls, a positive relationship between ultrasound determination of bone mass of the calcaneus and dietary magnesium intake was found [132], suggesting that this mineral may be important in skeletal growth and development. Another study in young girls found those supplemented with magnesium had greater accumulation of bone mineral at the hip [133]. Magnesium in serum and hair was associated with BMD in premenopausal women, and the ratio of serum calcium to magnesium was a significant indicator of BMD [134]. The intake of fruits and vegetables, containing vitamin C, magnesium, and potassium, may protect against premenopausal bone loss, but magnesium alone may not be protective against declines in bone mineral content and BMD [77]. In other studies of premenopausal women, magnesium intake was related to lumbar spine BMD in a cross-sectional evaluation [135], and a significant relationship was found over a 1-year period between dietary magnesium intake and rate of change of BMD of the lumbar spine and total body calcium [136].

A study of postmenopausal women showed a positive correlation between BMD of the forearm and magnesium intake [137]. Several small epidemiological studies have found that higher magnesium intakes are associated with higher BMD in elderly men and women, although rates of bone loss over 4 years were only related to magnesium intake in men [105]. In 2,038 older black and white men and women (aged 70 to 79 years) enrolled in the Health, Aging and Body Composition Study, a greater magnesium intake was significantly related to higher BMD in white women and men, but not in black women and men, an effect that may have been related to differences in dietary assessment or to baseline intakes of magnesium [138].

Only a few small controlled clinical trials of magnesium supplementation and its effects on bone have been conducted [139, 140]; supplementation was primarily effective in magnesium-depleted subjects based on baseline serum magnesium levels. Overall, observational and clinical trial data concerning magnesium and BMD or fractures are inconclusive, and, in fact, one recent study from the Women's Health Initiative surprisingly reported higher intakes of magnesium were associated with a higher risk of wrist fracture [105, 139–143].

There is little evidence that magnesium is needed to prevent osteoporosis in the general population. Results relating magnesium to BMD are often confounded by coexisting limited intakes of other nutrients that are also important for skeletal health. Clearly, a magnesium supplement may be required in those with low magnesium levels including: frail elderly with poor diets [142], persons with intestinal disease, alcoholics, or persons on treatment with diuretics or chemotherapy that depletes magnesium. In addition, as calcium supplements sometimes result in constipation, a supplement with magnesium might be useful in helping to keep bowel habits regular. More research is needed before magnesium supplements can be routinely advised in other than cases of magnesium deficiency.

Boron

Boron is not an essential nutrient, so therefore recommended intakes have not been determined. Boron is present in

several foods, such as fruits, vegetable (potato and avocado), legumes, nuts, eggs, milk, wine, and dried foods [144]. A significant number of people, however, do not consistently consume more than 1 mg per day of boron [145] and whether a low intake is of clinical concern is unknown. The daily requirement of boron has yet to be defined, and the boron content of most diets is around 1.5–3 mg/day [145]. Many daily multivitamin and mineral supplements contain between 3 and 9 mg.

A small study noted that urinary boron excretion changes rapidly with changes in boron intake, indicating that the kidney is the site of homeostatic regulation [146]. Although a few studies have found that 3 mg daily of boron may have a positive effect on bone [139, 147], controlled trials are lacking. In a study of postmenopausal women, supplementation with boron (3 mg/day) decreased urinary calcium loss [148]. Large intakes may increase riboflavin B2 excretion. Many of the foods that contain boron are likely to have beneficial effects on bone, therefore it is suggested that foods such as fruits, vegetables, and legumes be the source of boron.

Silicon

Silicon is a trace mineral that may be essential for skeletal and connective tissue formation [149]. Cereals provide the greatest amount of silicon in the US diet (about 30 %), followed by fruit, beverages (based on the silicon in water), alcoholic beverages (beer), high fiber grains, bananas, and vegetables. There was no DRI set for silicon, and average intakes are 19 and 40 mg/day in women and men, respectively. [150]. However, some believe silicon intakes may be suboptimal [151], and there is a decrease in serum silicon concentrations that occurs with age, especially in women [152]. The biological role of silicon in bone health remains unclear, although a number of possible mechanisms, including effects on the synthesis of collagen and/or its stabilization and on matrix mineralization, have been suggested [149].

Dietary silicon intake was found to have a positive association with BMD [149]. In a cross-sectional study (n=2,847) of participants in the Framingham Offspring cohort (age, 30–87 years), dietary silicon correlated positively and significantly with hip BMD in men and premenopausal women, but there was no relationship in postmenopausal women [153]. Silicon may be responsible for the positive association between beer and BMD in the Framingham Offspring cohort (age, 29–86 years) [154]. Results that indicate a potential positive association between silicon and skeletal health require further follow-up and confirmation.

Strontium

Strontium is a mineral that is absorbed in the body like calcium. Common salts of strontium include strontium ranelate, strontium citrate, and strontium carbonate. Strontium ranelate is approved in several European countries for the treatment of osteoporosis, but it is not approved in the US. It increases bone mass throughout the skeleton and in clinical trials; the drug reduced the risk of vertebral fractures by 41 % [155] and nonvertebral fractures by 15 % [156]. Strontium appears to be a modest antiresorptive agent. Strontium is incorporated into hydroxyapatite, replacing calcium, a feature that might contribute to dramatic increases in bone mineral density. During clinical trials, the most common side effects were nausea, diarrhea, headache, and skin irritation. Small increased risks of venous thrombosis, seizures, and abnormal cognition have been seen and require further study [155, 156].

There are many strontium salts that are available through the Internet, although their long-term safety and efficacy have not been evaluated in humans in large-scale clinical trials. Websites for many strontium compounds reference data from strontium ranelate clinical trials as proof of efficacy of their particular compound, even though they are often marketing a different strontium salt. The manufacturers of these compounds go on to explain that strontium is the active component and not the carbonate, ranelate, lactate, or citrate cation, although data to support this statement are not presented. Furthermore, various formulations often contain amounts of elemental strontium, which vary from the strontium ranelate used in clinical trials. In addition, the dose, absorption, and bioequivalence of the many forms of strontium available for purchase are not known. There are no clinically relevant data to support that any of these compounds available, without a prescription, will build bone mass or prevent fracture. In addition, the safety of these compounds is unknown.

Phosphorus

Phosphorus is a mineral with a recommended intake of 700 mg a day for adult men and women. Phosphorus is a component of dairy foods, meat, eggs, cereal, and processed foods. Phosphoric acid is found in cola-type sodas. The diets of many individuals have a disproportionately high intake of phosphorus as compared with calcium [157].

Data from the Framingham study demonstrated that higher consumption of cola beverages was associated with lower BMD in women but not men [158]. In a recent study of Brazilian men and women, an increase in phosphorus intake was related to higher fracture rates. This was quantified as a 9 % increase in fracture per 100 mg intake of phosphorus in the diet [159]. These data are preliminary and require further study to determine the impact of dietary phosphorus, particularly as phosphoric acid in soda, on skeletal health.

Calcium agents

Adequate calcium intakes throughout life are needed to attain maximal peak bone mass and prevent bone loss at older ages. Foods are a preferred source to maintain calcium balance because there are other essential nutrients that are found in high calcium foods. For those individuals where there is inadequate calcium intake from diet, supplemental calcium can be used. Calcium intakes in excess of the required 1,000–1,200 mg per day have limited potential for benefit and may possibly increase the risk of developing kidney stones or cardiovascular disease. Calcium supplements may contain different calcium compounds such as calcium acetate, calcium carbonate, calcium citrate, calcium citrate malate, calcium gluconate, calcium lactate, calcium lactogluconate, tricalcium phosphate, and others. Calcium bioavailability from these various formulations is fairly similar, especially if supplements are taken with food. Calcium carbonate and calcium citrate are the most common. Calcium carbonate has the highest amount of calcium per dose, although calcium citrate may be preferred for patients with constipation or for persons taking acid blocking medication. Supplemental or dietary calcium should be spread out throughout the day with 500 mg or less being consumed at each meal in order to optimize absorption.

There are several studies that indicate a potential risk of excessive calcium supplementation on cardiovascular health, although this risk is not evident when calcium is consumed in a food [160–162]. The benefits of calcium for skeletal health have been recently reviewed, and based on meta-analyses, calcium appears to prevent bone loss [163, 164]. However, the potential for calcium to reduce bone loss is less clear, and often, a benefit is only seen in compliant women or in women with a low calcium diet at baseline [163–168].

There are many forms of calcium available that claim to have benefit beyond standard calcium supplementation, but there are often no data or only limited data presented for these products. Many calcium products also come with vitamin D and other nutrients. Although there is a rationale to consume a product that also contains vitamin D (see below), there is no reason to add other nutrients to the calcium supplement.

There is no benefit for healthy individuals to consume more calcium than what is recommended. More calcium is not better, and chronic high calcium intakes may even be harmful. Most people can easily get at least half of the calcium they need from food. In patients with low dietary intake of calcium, supplementation may be needed, but the first choice is to promote food sources for calcium. It is also important to educate patients that their recommended daily calcium intake, minus the estimated daily calcium in their diet, will determine how much calcium they need to take from a supplement.

Supplemental calcium should be taken with a full glass of water and at a low calcium meal.

Vitamin D

Vitamin D is a fat-soluble vitamin that is needed for calcium metabolism. Food sources of vitamin D are limited and include milk (100 IU per glass), salt-water fish, and liver. Some calcium supplements and most multivitamin tablets also contain vitamin D. Vitamin D from foods, supplements, and/or multivitamins can be used to meet the vitamin D requirement. In fact, a supplement will likely be needed to meet the requirement in individuals without regular sun exposure. The Institute of Medicine recommends 600 IU of vitamin D for people between the ages of 1 and 70 years and 800 IU for people age 71 years and older [8]. Vitamin D intakes of 800 to 1,000 IU each day for people over 50 years are recommended by the National Osteoporosis Foundation [169]. Vitamin D plays a major role in calcium absorption, bone health, muscle performance, balance, and risk of falling [166, 170–173]. There is accumulating evidence that vitamin D insufficiency is more prevalent than previously thought, particularly among the elderly; those living in northern latitudes; and in individuals with poor nutrition, malabsorption, or chronic liver or renal disease. Dark-skinned individuals are also at high risk of vitamin D deficiency. The role of vitamin D in skeletal health is clear and has been recently reviewed [166, 174, 175]. It may in fact be the combination of calcium and vitamin D that leads to the greatest fracture reduction [176]. Vitamin D2 is called ergocalciferol and comes from vegetarian sources. Vitamin D3 is called cholecalciferol and comes from animal sources. Any difference in clinical efficacy between these compounds, if administered similarly and provided at the same dose, is minimal.

Conclusion

Clearly, calcium and vitamin D play an important role in bone health. In cases of low dietary intake of calcium, supplementation may be needed, but the first choice is to consume calcium from foods. In many cases, vitamin D supplementation will be needed to ensure adequate intake. Nutraceutical products are heavily promoted and advertised, leading many individuals to believe they will benefit bone health. However, potential safety issues must be recognized when these products are taken in amounts that greatly exceed dietary recommendations. Higher fruit and vegetable intakes may be associated with higher BMD. However, the evidence for the skeletal benefit of isolated nutrients such as flavonoids, carotenoids, omega-3-fatty acids, and vitamins A, C, E, and K are limited to observational data or a few small clinical trials, sometimes investigating pharmacologic doses. Data are also

limited for the role of nutritional levels of magnesium, boron, strontium, silicon, and phosphorus in bone health. Although high homocysteine levels are related to fracture risk, the skeletal benefit of each individual B vitamin is unclear. The relationship between soy compounds and bone density and bone turnover are inconclusive. Skeletal effects of supplemental dehydroepiandrosterone are inconsistent and may only provide benefit in elderly with low serum levels. The general nutritional advice for skeletal health is that adults should follow a varied nutrient-rich diet with adequate protein and fruits and vegetables, while ensuring an adequate intake of calcium and vitamin D. Clearly, for patients with osteoporosis or with a high risk for fracture, nutraceuticals will not replace a medication proven to prevent fractures.

Conflicts of interest None.

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