Journal of Trace Elements in Medicine and Biology 62 (2020) 126577

Contents lists available at ScienceDirect

Journal of Trace Elements in Medicine and Biology

journal homepage: www.elsevier.com/locate/jtemb

Review

Pivotal role of boron supplementation on bone health: A narrative review

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ARTICLE INFO

Keywords:
Boron
Bone
Dietary supplementation
Bone mineral density
Nutrients

ABSTRACT

Background: Boron is a trace element that plays an important role in numerous biological functions, including calcium metabolism, growth and maintenance of bone tissue. However, there are still no precise indications regarding a possible role of boron supplementation, and its amount of supplementation, to maintain bone health. So the aim of this narrative review was to consider the state of the art on the effectiveness of boron supplementation (alone or with other micronutrients) on growth and maintenance of bone in humans through control of calcium, vitamin D and sex steroid hormone metabolism in order to suggest a daily dosage of boron supplementation.

Main findings: This review included 11 eligible studies: 7 regarding the supplementation with boron alone and 4 regarding supplementation with boron and other nutrients. Despite the number of studies considered being low, the number of subjects studied is high (594) and the results are interesting.

Conclusions: The studies considered in this narrative review have evaluated the positive effectiveness on bone, in humans, through control of calcium, vitamin D and sex steroid hormone metabolism, considering a dietary supplementation of 3 mg/day of boron (alone or with other nutrients); this supplementation is demonstrably useful to support bone health (in order to prevent and maintain adequate bone mineral density), also considering the daily dose of 3 mg is much lower than the Upper Level indicated by EFSA in the daily dose of 10 mg.

1. Introduction

Boron is a semiconductor element, with metal and non-metal properties, not present on Earth in elementary form, but in the form of borates in sedimentary rocks, clay, oceans, in soil and in coal [1]. Boron is a trace element that plays an important role in numerous biological functions, including calcium metabolism, growth and maintenance of bone tissue. In fact, from what emerged from a review that considered both animal and human studies, boron seems to influence the metabolism of various nutrients and steroid hormones, such as 1.25 (OH) 2 vitamin D, testosterone and 17- β estradiol, according to the hypothesis that this element would be necessary for the hydroxylation reactions of the steroid ring. This role would be particularly evident in case of insufficient dietary intake of vitamin D, magnesium or both [2].

The main sources of boron for humans are food and drinking water [3,4]. The greatest contribution is generally obtained through the intake of coffee (boron content equal to 29 μg / 100 g) and milk (18 μg / 100 g) (for the abundant quantities consumed), peanuts (1700 μg / 100 g) and peanut butter (1450 μg / 100 g), wine (610 μg / 100 g), and sultanas (2200 μg / 100 g) [5]. The most important commercial products containing boron in inorganic form include: borax (or sodium tetraborate), borax pentahydrate, sodium perborate, colemanite (a calcium borate hydrate), boric acid and ulexite (a sodium and calcium borate hydrate) [1]. Among all the numerous active natural organic

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https://doi.org/10.1016/j.jtemb.2020.126577
Received 21 April 2020; Received in revised form 26 May 2020; Accepted 4 June 2020

compounds containing boron present in products of plant origin, sugars and polyalcohol borate esters, pectic polysaccharide borate esters, organic acid-borate esters and aminoacid-borate esters are particularly represented in animal feed and being human [6]. Currently there are also organic boron compounds sold as supplements: calcium fruitate and boron gluconate chelates, boron aspartate, boron citrate, boron ascorbate, boron glycinate [6]. However, only plants are able to metabolize inorganic boron compounds (such as boric acid or borates) and to convert them, for example, to mono- or di-saccharide borate esters (mono- or di-sugar borate esters, SBEs), which are then effectively assimilated by animal and human cells [1]. Calcium fructoborate, in particular, in addition to being produced and marketed as an active component of various dietary supplements, represents the sugar-borate ester complex most commonly found in nature, mainly contained in vegetables, fruit, seeds and honey [1,7].

According to the WHO, the amount of boron absorbed by man through inhaled air is 0.44 μg / day, the quota introduced through the diet is on average 1.2 mg / day while drinking water contributes with an average concentration of 0.1 − 0.3 mg / l of boron [8]. Studies have shown, in both animals and in humans, that boron taken orally is about 90 % absorbed [9]. Absorption through intact skin, on the other hand, is minimal [10].

Boron is then mainly eliminated through urine, to a lesser extent via the stool (2%) and only in small quantities with sweat, breath and bile [11].

Animal studies have shown that boron does not accumulate in significant quantities in the soft tissues, while it tends to reach much higher levels than those found in the blood and soft tissues themselves, in the bone [12]. According to research by Tibbits et al. in dogs, the other tissues at which high levels of boron are reached are mainly the liver, lymph nodes, adrenals and kidneys (minimum concentrations at the level of the tissues of the head region) [13]. In the bone, in particular, this element appears almost completely and exclusively localized at the level of the mineral portion, and the manifestations of its deficiency, completely non-specific, can include arthritis, bone loss and osteoporosis [7].

1.1. Daily intake of boron with the diet

To date, due to insufficient data available, recommended intake levels (RDA) for boron have not yet been established [14,15]; however, the World Health Organization (WHO) has estimated an "boron intake" of 1 − 13 mg / day as an "acceptable safety interval" for adults [16].

Oral exposure to high concentrations of boron in humans was responsible for minimal or little toxicity phenomena, and chronic exposure to ≥ 84 mg B / kg body weight / day could cause neurological, gastrointestinal pathological manifestations, cardiovascular and hepatic, as well as diarrhea, anorexia, kidney damage and testicular atrophy [1].

Based primarily on the negative effects of excess boron on reproductive function and development in animal models, the U.S. Food and Nutrition Board set the tolerable upper limit (UL) for this microelement at 20 mg / day [9]. EFSA (European Food Safety Agency), however, following a critical analysis of boron toxicokinetics and toxicodynamics data, has declared as safe for adults a maximum dose of 10 mg / day (in the form of boric acid and borates) [17].

The average daily consumption of boron in the US population was assessed by survey, in different age groups, using the "Boron Nutrient Data Base" and a two-day food diary. The average intake of boron was between 0.75 and 0.96 mg / day for school-age children and between 0.87 and 1.35 mg / day for adults [9]. These values are in line with what is highlighted by Biego G.H. et al. for the French population (1.6 mg / day) [18] and slightly lower than those reported by Naghii M.R. et al. for the Australian population (2.23 ± 1.23 mg / day) [19].

By using inductively coupled plasma spectrometry (ICP), Meacham S.L. et al. have identified the 10 richest foods in boron (μg of B / g of dry weight of the food): avocado (14.3 ± 0.4), peanut butter (5.9 ± 0.2), dried peanuts (5.8 ± 0.6), plum juice (5.6 ± 0.0), chocolate powder (4.3 ± 0.4), wine (3.6 ± 0.0), muesli-raisin (3.6 ± 0.3), grape juice (3.4 ± 0.0), pecan nuts (2.6 ± 0.1), and raisin cereals (2.6 ± 0.6) [20]. However, studies conducted on the US population have shown that, in terms of percentage of total daily boron intake, the greatest contribution to the dietary contribution of this element comes from drinks, especially wine (3.52 ± 0.27), coffee (0.24 ± 0.07 μg B / g of food) and beer (0.13 ± 0.06), juices (plum juice 5.19 ± 0.21; apple juice 2.38 ± 0, 10; orange juice 0.17 ± 0.04), milk and dairy products (yogurt 0.46 ± 0.03 whole milk 0.13 ± 0.05), fruit (e.g. cherries 7.0 ± 0, 3; peaches 4.49 ± 0.07; fruit salad with syrup 2.45 ± 0.17) and vegetables and legumes (red beans 3.14 ± 0.04; carrots 2.59 ± 0.09; broccoli 2.47 ± 0.03; spinach 2.40 ± 0.09; and mixed canned vegetables 1.11 ± 0.19) [20,21].

1.2. Boron and bone development: in vitro and in animal model studies

Boron appears to have a positive effect on bone regeneration, as demonstrated by some in vitro and animal research [22,23]. In the study of Hakki et al. an increase in mineralization nodules, an increase in the expression of the gene coding for collagen type I, osteopontin, bone sialoprotein and osteocalcin, and an increase in bone morphogenetic protein levels have been observed on boron-treated pre-osteoblastic cells BMPs-4, -6 and -7, important growth factors that induce bone neoformation [22]. In the study of Uysal et al., conducted on rabbits, daily supplementation of the diet with 3 mg of boron / kg was associated with a significant increase in the mineralization area and in the number of osteoblasts at the level of the median palatal suture [23]. A further 2016 in vitro study showed that boron is able to promote the proliferation and differentiation of mammalian osteoblasts by accelerating the flow of calcium ions [24], and a study conducted on pig bone marrow mesenchymal stromal cells showed how calcium fructoborate can stimulate the differentiation of osteoblasts from the spinal cord by increasing the activity of alkaline phosphatase [25]. Boron deficiency, on the other hand, seems to lead to impaired growth and abnormal bone development [2]. Research conducted on mice has highlighted how reduced dietary boron intake can alter bone remodeling and inhibit bone neoformation: through a histomorphometric analysis on the periodontal alveolar bone, have been observed a reduction in the surface of the osteoblasts and an increase in the quiescent surface in the group of animals subjected to a low boron diet. This suggests that boron introduced with diet may exert a positive action against bone growth and maintenance by affecting the presence and growth of osteoblasts and / or osteoclasts [26].

In a study on ostrich chicks, supplementation of drinking water with boron (0 mg / l, 100 mg / l, 200 mg / l, 400 mg / l) was shown to be useful for bone development, with improvement of several tibial bone parameters, including bone mineral density, length, weight, cortical bone thickness (with the greatest benefit observed for 200 mg / l supplementations) [27]. In the same study, an increase in the serum concentration of leptin, a hormone that affects bone metabolism by reducing its reabsorption and stimulating its turnover, was also observed with high levels of boron introduced in the diet [27].

Furthermore, it would seem that boron supplementation is able to correct the mineral metabolism alterations induced by a deficiency of vitamin D: in fact, the supplementation (3 ppm) of this microelement, in fact, in rats fed a diet low in vitamin D determined an increase in serum calcium levels [28], and the addition of 3 mg of boron / kg of food to the diet stimulated the growth of vitamin D deficient chicks compared to a low boron diet (≤ 0.3 mg / kg of food) [29].

In a study carried out on rabbits, animals fed a high-calorie low-boron diet (3.88 mg B / kg) were compared with animals receiving increasing supplements (10, 30 and 50 mg B / kg body weight). It was thus observed that rabbits with the highest supplementation (50 mg B / kg) had the highest maximum femoral rupture force value and that

supplementations with 30 and 50 mg boron / kg were associated with greater compressive strength of the tibia. The addition of boron to the feeding also resulted in a significant increase in all rabbits' concentrations of calcium and magnesium at the tibial level, with an increase in phosphorus in the groups supplemented with 30 and 50 mg / kg; supplementation with 30 mg B / kg was also associated with an increase in the femoral concentrations of calcium, magnesium and phosphorus [30].

Other animal research has shown that boron deficiency can compromise the bone healing process through a marked reduction in osteogenesis [31,32]. In this study, conducted on male mice after the extraction of the first lower right molar tooth, histological and histomorphometric analyzes were performed on the periodontal alveolar bone after 7 and 14 days of diet containing 3 mg B / kg (+ B, adequate diet) or 0.07 mg B / kg (-B, B-deficient diet). In animals + B, after 7 and 14 days, active osteogenesis was highlighted with the formation of new trabeculae occupying almost entirely the alveolus, unlike the animals -B, in which few trabeculae were present with poor bone formation activity; moreover, the -B mice showed a reduction in the osteoblastic surface and a statistically significant increase in the quiescent surface [31]. Boron deficiency would therefore seem to alter the alveolar bone repair process that begins immediately after a dental extraction, suggesting that this microelement is able to promote normal trabecular formation or bone microarchitecture [32]. In addition, an in vitro study has shown a favorable role of boron towards osteoblastic function, positively regulating proteins such as type I collagen, osteopontin, bone sialoprotein, osteocalcin [22].

Finally, a study conducted on rats by Ghanizadeh et al. Deserves mention. in 2014, where 34 male animals were divided into 5 groups: a control group fed a standard diet containing 650 mg of calcium / 100 g of food and 80 I.U. of vitamin D3 / 100 g of food, and 4 intervention groups supplemented daily with, respectively: fluoride, fluoride + boron, fluoride + calcium + vitamin D and fluoride + boron + calcium + vitamin D. Supplementation provided a total of 0.7 mg of fluorine per animal per day, 1.23 mg of boron, 210 mg of calcium and 55 IU of vitamin D. After 8 weeks of treatment, the measurements carried out on the mechanical properties of the bone showed that the combined intake of fluorine and boron is associated with greater stiffness and breaking force at the femoral level and with a higher maximum load for the lumbar vertebrae compared to the intake of calcium and vitamin D. The blood concentration of calcium was also significantly higher in animals supplemented with fluorine and boron, while higher levels of free testosterone and estradiol were found in all intervention groups at comparison with the control group, indicating the existence of a positive relationship between minerals and steroid hormones. Overall, these results suggest the importance of carrying out further investigations to clarify the role of boron and fluorine, possibly in association with other elements, in influencing bone health [33].

Finally, bioactive glass technology has also shown how boron plays an important role in promoting osteoblast proliferation [34].

1.3. Boron and magnesium

About 60 % of the magnesium present in the human body is found in the bone, where it acts as a cofactor for numerous enzymes that regulate the metabolism of calcium [35]. Boron appears to significantly improve the absorption of magnesium and its deposition at the bone level, as suggested by a study conducted in rats in which a significant increase in the bone content of magnesium (in mg / g) was observed in the animals to which it had been given as part of a diet low in vitamin D, but supplemented in boron compared to those fed a diet low in both vitamin D and boron [36].

Given this background, the aim of this narrative review was to consider the state of the art on effectiveness of boron supplementation (alone or with other micronutrients) on growth and maintenance of bone in humans through control of calcium, vitamin D and sex steroid hormone metabolism, in order to suggest a daily dosage of boron supplementation.

2. Materials and methods

The present narrative review was performed following the steps by Egger et al. [37] as follows:

1 Configuration of a working group: three operators skilled in clinical nutrition (one acting as a methodological operator and two participating as clinical operators).
2 Formulation of the revision question on the basis of considerations made in the abstract: "the state of the art on effectiveness of boron supplementation (alone or with other micronutrients) on growth and maintenance of bone in humans through control of calcium, vitamin D and sex steroid hormones metabolism, in order to suggest a daily dosage of boron supplementation.
3 Identification of relevant studies: a research strategy was planned on PubMed (Public MedIine run by the National Center of Biotechnology Information (NCBI) of the National Library of Medicine of Bethesda (USA)) as follows: (a) Definition of the keywords (boron, bone health, humans, supplementation, bone mineral density), allowing the definition of the interest field of the documents to be searched, grouped in quotation marks ("…") and used separately or in combination; (b) use of: the Boolean (a data type with only two possible values: true or false) AND operator, that allows the establishments of logical relations among concepts; (c) Research modalities: advanced search; (d) Limits: time limits: papers published in the last 20 years; humans; adults; languages: English; (e) Manual search performed by the senior researchers experienced in clinical nutrition through the revision of articles on effectiveness of boron supplementation (alone or with other micronutrients) on growth and maintenance of bone in humans through control of calcium, vitamin D and sex steroid hormones metabolism, in order to suggest a daily dosage of boron supplementation.
4 Published in journals qualified in the Index Medicus.
5 Analysis and presentation of the outcomes: we create paragraphs about effectiveness of boron supplementation alone or in combination with other nutrients, and the data extrapolated from the "revised studies" were collocated in tables; in particular, for each study we specified the author and year of publication and study characteristics.
6 The analysis was carried out in the form of a narrative review of the reports. At the beginning of each section, the keywords considered and the type of studies chosen are reported. We evaluated, as is suitable for the narrative review, studies of any design which considered the effectiveness of boron supplementation (alone or with other micronutrients) on growth and maintenance of bone in humans through control of calcium, vitamin D and sex steroid hormone metabolism.

3. Results

3.1. Effectiveness of boron supplementation on maintenance of bone in humans

This research was conducted based on the keywords: "boron" AND "supplementation" AND "bone" AND "humans".

For the present review we have analyzed a total of seven studies: one observational clinical trial [38], one cross-sectional observational clinical trial [39], two double-blind clinical trials [40,41], one single-blind cross-over clinical trial [42], one experimental controlled study [43] and one follow-up clinical trial [44]. The results of these seven studies have been shown in Table 1.

Table 1
Studies regarding boron supplementation and bone health in humans.

First author, year	Study design	Setting	Inclusion criteria	Intervention	Parallel treatments/ placebo	Number of subjects (M-F)	Duration of the intervention	Primary outcomes	Secondary outcomes	Results
Beattie J.H., 1993	Observational clinical trial	Human Nutrition Unit of the Rowett Research Institute, Buckburn, Aberden	Helthy; no history of alcohol or drug abuse; no more than 20 years since the menopause; BMI 25 kg/m^2); postmenopausal	Low boron diet (LBD, 0.33 mg/d) for the first 3 weeks; then daily supplement of 3 mg B/ 8 MJ energy intake for other 3 weeks	Self-selected diet (acclimation-period diet, APD)	6 healthy postmenopausal volunteers	3 weeks + 3 weeks	Bone mineral absorption and excretion; plasma sex steroid levels; urinary excretion of pyridinium crosslink markers of bone turnover	Urinary calcium, magnesium and phosphorous	During LBD: increased urinary Ca and Mg excretion; hyperabsorption of Ca (positive Ca balances in combination with elevated urinary Ca Excretion). Changing boron intake: no effect on minerals, steroids or crosslinks.
Boyacioglu O., 2018	Cross-sectional observational study	Two different regions of Turkey, with natural exposure to high (≥1 mg/L) or low (< 1 mg/L) boron concentration in drinking water	All su bjects free from spontaneous menses for at least a year	25 subjects consuming drinking water with a boron concentration of 1.59 ± 0.04 mg/L	28 subjects consuming drinking water with a boron concentration of 0.012 ± 0.05 mg/L	53 postmenopausal women (50 − 60 years old)	All subjects in the boron exposed group living in the boron-rich area for an average of 57.5 ± 12.0 years	Serum osteocalcin levels; expression of osteocalcin gene rs1800247 polymorphism	//	Serum osteocalcin levels in the boron exposed regions significantly higher (27.55 ± 3.20 ng/mL) compared to that of control group (23.47 ± 12.55 ng/mL).
Hunt C.D., 1997	Double-blind (except for boron) clinical trial	Metabolic unit under close supervision for 167 days	Healthy postmenopausal women; normal bone, kidney and liver function; normal blood pressure; negative lung scan; normal plasma Ca and Mg levels	Basal diet supplemented with 3 mg B/d for 48 days: 6 women with a boron diet low in Mg, 5 women with a boron diet supplemented with 200 mg Mg/d	Conventional basal diet (daily average intake of 0.36 mg of B) for the first 119 days	11 women (48 − 82 years)	48 days	Fecal and urinary B and Ca loss	Serum levels of B, Ca, Mg and K	During B supplementation: increased faecal B loss, regardless of Mg intake; decreased urinary Ca loss without supplemental Mg; increased urinary Ca loss with supplemental Mg; increased plasma B concentrations (1.5-folds). Supplemental B without supplemental Mg: decreased serum Mg concentrations. Supplemental B with supplemental Mg: increased serum Mg concentrations.
Naghii M.R., 1997	Single blind cross-over trial	Free-living subjects	Healthy subjects	5 mg of B (as sodium tetrabaorate) twice a day	Placebo tablets (containing lactose only)	8 healthy male subjects	4 weeks	Boron and calcium excretion in 24-h urine sample	Plasma lipids and susceptibility to oxidation; plasma steroid hormones (estradiol and testosterone)	In the treatment group: increased mean concentration of urinary B; reduced calcium excretion. No difference in plasma lipids or the oxidizability of LDL. Significant increase in plasma estradiol concentration; trend for plasma testosterone levels to be increased.
Naghii M.R., 2011	Experimental controlled study	Free-living subjects	apparently healthy non-smoker subjects	10 mg of boron (as sodium tetraborate)/d	Habitual diet and, for only one day, a capsule containing lactose powder as placebo	8 male volunteers (mean age 41.3 ± 7.5 years)	7 days	Plasma boron concentrations	Plasma levels of total and free testosterone, DHT, estradiol, SHBG, cortisol, LH, vitamin D; hsCRP, IL-6, TNF-α	Following hours and weekly B consumption: significant increase in plasma B. After six hours B supplementation: decrease of SHBG, hsCRP and TNF-α; after one week: increase of mean plasma free testosterone and decrease of mean plasma estradiol. After supplementation: increase of

(*continued on next page*)

Table 1 (*continued*)

First author, year	Study design	Setting	Inclusion criteria	Intervention	Parallel treatments/ placebo	Number of subjects (M-F)	Duration of the intervention	Primary outcomes	Secondary outcomes	Results
										all inflammatory biomarkers concentrations; non significantly higher levels of DHT, cortisol and vit. D.
Nielsen F.H., 1987	Observational clinical trial	Metabolic unit for 167 days	Postmenopausal Caucasian women in good health and emotionally suited for the study; informed consent	Basal diet supplemented with 3 mg of B (as sodium borate)/day: 7 women fed with a B diet low in Mg, 5 women fed with a B diet supplemented with 200 mg of Mg/day	Diet low in boron (0.25 mg/2000 kcal/ d) for an initial period of 119 days	12 women (48 – 82 years old)	48 days	Urinary excretion of Ca, Mg and P; serum concentrations of 17β-estradiol and testosterone	//	After B supplementation: decreased Ca and Mg urinary excretion (more marked depression when dietary Mg was low); decreased P urinary excretion only by the low-Mg women; marked elevation of 17β-estradiol and testosterone serum concentrations (more marked when dietary Mg was low).

3.2. Effectiveness of boron in association with other nutrients, supplementation on maintenance of bone in humans

This research was conducted based on the keywords: “boron” AND “supplementation” AND “nutrients” AND “bone” AND “humans”.

Four studies have included about nutritional supplements containing boron in combination with other nutrients: two comparative effectiveness research (CER) studies [45,46], one single-blind, uncontrolled, prospective clinical trial [47] and one longitudinal trial [48]. The results of these four studies have been shown in Table 2.

Fig. 1 shows the flow chart of literature research.

4. Discussion

4.1. Effectiveness of boron supplementation on calcium metabolism, growth and maintenance of bone in humans

A study conducted on 12 postmenopausal women, aged between 48 and 82 years, showed that a 3 mg / day boron supplementation (as sodium borate) for 48 days, after 119 days of low boron diet (0.25 mg / die), is able to reduce the urinary excretion of calcium and magnesium and to increase the serum levels of 17-β estradiol and testosterone, to a greater extent in case of low dietary intake of magnesium [40]. On the other hand, a diet low in boron would induce an increase in urinary calcium excretion, as emerged from a study conducted on 6 post-menopausal women (53–65 years), after administration of a diet containing 0.33 mg / day of boron for 3 weeks: urinary calcium excretion increased rapidly, in some cases, to double, following the transition from a free diet to a low boron diet [38].

In order to further investigate the effects of a boron supplement on serum levels and on the excretion of boron, calcium and magnesium, Hunt C.D. and colleagues enrolled 11 postmenopausal women, aged 48–82, who were given a low boron diet (on average 0.36 mg / day) for 119 days, followed by an integration period with 3 mg B (as disodium tetraborate decahydrate) / day for 48 days. During this second period of the study, 5 of the 11 volunteers also received supplementation of 200 mg / day of magnesium. The data collected showed that a 9-fold increase in boron introduced with the diet was associated with a 1.5-fold increase in its plasma concentration; the urinary and faecal losses of boron also more than 100 % compensated the doses taken with the diet, thus indicating that boron was affected, in the human body, by homeostatic regulation phenomena. In women not supplemented with magnesium, boron supplementation decreased serum Magnesium concentrations, while in women who received 200 mg / day of Mg, supplementation with boron tended to increase circulating levels. Furthermore, boron supplementation decreased urinary calcium losses only in women in whom Mg was also not integrated, while it was associated with an increase in urinary calcium excretion in women taking magnesium supplementation, thus suggesting that the effects of boron on calcium excretion depended on the simultaneous intake or not of magnesium [41].

A study by Boyacioglu O. and colleagues on 53 postmenopausal women aged between 50 and 60 also investigated the relationship between daily intake of boron, blood levels of osteocalcin and polymorphisms of the gene coding for it. Osteocalcin is a sensitive and specific indicator of osteoblastic function; its levels, in fact, appear high in all those clinical conditions characterized by an accelerated bone turnover, such as hyperparathyroidism, hyperthyroidism, acromegaly and Paget's disease [39]. In the study in question 25 women, from regions of Turkey considered "rich in boron", took drinking water with concentrations of B (types of boron compounds not specified) equal to 1.59 ± 0.04 mg / L (average daily intake of boron 6.99 ± 2, 90 mg), while the remaining 28 women, coming from "poor boron" regions, took drinking water with concentrations of this microelement equal to 0.012 ± 0.05 mg / L (average daily intake of boron 1.20 ± 0.12 mg). Analysis of the data showed significantly higher serum levels of

Table 2
Studies regarding boron, in association with other nutrients, supplementation and bone health in humans.

First author, year	Study design	Setting	Inclusion criteria	Intervention	Parallel treatments	Number of subjects (M-F)	Duration of the intervention	Primary outcomes	Secondary outcomes	Results
Cook A., 2002	Single-blind, uncontrolled, prospective trial	Free-living subjects	Women within 1–10 years after menopause, no congenital or other disease known to affect bone density, no medication that depletes or enhances bone density, T score < -1.0 SD at lumbar spine, hip, or forearm (measured by DXA)	Product 1, a vitamin/mineral blend containing Ca, Mg, Mn, Cu, Cr, Se, Mo, B (2 mg), Si, Sr, Zn, vitamin B1, B2, B3, B6, B12, D, K, pantothenic acid, folic acid, ascorbic acid, betaine HCl; and Product 2, an herbal blend containing dong quai extract, licorice root extract, chaste tree berry (Vitex agnus-castus), black cohosh (Cimicifuga racemosa), false unicorn extract (Helonias opulus), fennel seed extract (Foeniculum vulgare), hesperidin complex.	//	12 women (mean age 53.4 years, SD 4.96)	2 years	BMD of the lumbar spine (L1-L4), total hip, and distal one third of the forearm (nondominant, unfractured) (by DEXA); annualized percent BMD loss	Relief of menopausal symptoms	No effects on BMD over a 2-year period of treatment. Mean annualized bone loss: 1.42 % in the spine; 1.42 % in the forearm; no significant losses at the hip (0.42 %) (no protective effect of the treatment against the predictable acceleration of bone mineral losses associated with early post-menopause). Relief of menopausal symptoms: reported by 4 of 4 women experiencing symptoms at the start of the trial.
Kaats G.R., 2011	Comparative effectiveness research study	Free-living subjects	no medical conditions precluding participation	Plan 1: plant-sourced calcium (AlgaeCal, AC), 750 mg; Mg, 65 mg; vit D-3, 1000 IU; no activity program; no health literacy information	Plan 2: AlgaeCal, 720 mg; Mg, 72 mg; vit D-3, 800 IU plus vit K-2 and Sr citrate; activity program; Plan 3: AlgaeCal, 756 mg; Mg, 350 mg; vit D-3, 1600 IU plus vit K-7, Sr citrate, B (3 mg), vit C; activity program;	172 healthy women (> 40 years)	1 year	Mean annualized percent change (MAPC) of BMD among highly compliant subjects; safety in all three plans (assessed by a Quality of Life inventory, a blood chemistry panel and daily tracking self-reports)	MAPC of BMD in the entire cohort for each plan	In the compliant sub-groups, increase in MAPC of BMD: plan 1 = 1.30 %; plan 2 = 2.00 %; plan 3 = 4.1 %. In all subjects, increase in MAPC of BMD: plan 1 = 1.20 %; plan 2 = 0.33 %; plan 3 = 2.5 %. In all three groups, no statistically significant differences between baseline and ending blood chemistry tests or the QoL self-reports.
Kaats G.R., 2016	Longitudinal trial	Free-living subjects	Healthy adult women; reported use of AlgaeCal for one to 7 years; at least one total body DEXA during the past 7 years; no bisphosphonates or other bone building medications.	AlgaeCal (AC) supplement containing: elemental Sr (from citrate), Ca, Mg, vitamin D3, vitamin K-7, vitamin C, B (3 mg).	//	172 females (mean age 65.1 y, SD 7.8) to assess efficacy; 125 females to assess safety	From 1–7 years	Baseline-ending BMD annualized changes. Safety assessed by 45-measurement blood chemistry panel.	//	AC supplement associated with a statistically significant annualized and linear increase in BMD (1.04 % per year, 7.3 % over the 7-year study period). No adverse effects or safety concerns.
Michalek J.E., 2011	Comparative effectiveness research study	Free-living subjects	no medical conditions precluding participation	Bone-health plan 1 (AlgaeCal 1): pedometer-based activity program; health	Bone-health plan 2 (AlgaeCal 2): pedometer program; Sr citrate, AlgaeCal,	AlgaeCal 1: 125 healthy adults (86.4% F; mean age 55.2 ± 11.2 years).	6 months	Changes in BMD (MAPC, mean annualized percent changes, measured by DXA total body)	Measures of safety, volunteer biases, dropout effects, and	Positive MAPC in BMD compared to baseline in both groups, but significant change only

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Table 2 (*continued*)

First author, year	Study design	Setting	Inclusion criteria	Intervention	Parallel treatments	Number of subjects (M-F)	Duration of the intervention	Primary outcomes	Secondary outcomes	Results
				literacy information; Sr citrate, AlgaeCal, trace minerals in AlgaeCal, Ca, Mg, vitamin D-3, vitamin K-2.	trace minerals in AlgaeCal, Ca, Mg, Mg from Mg carbonate, vit D-3, vit K-7, vit C, B (3 mg).	AlgaeCal 2: 51 healthy adults (78.4% F; mean age 56.7 ± 13.2 years).			effects of compliance	in AlgaeCal 2 group. Significantly greater MAPC in AlgaeCal 2 than in AlgaeCal 1. Significant MAPC contrast between compliant and partially compliant subjects for both plans. No significant changes in blood chemistry panel and in self-reported QoL in either group.

osteocalcin in postmenopausal subjects naturally exposed to higher concentrations of food boron than those found in subjects from "poor boron" regions, while no correlation was found statistically significant between the levels of exposure to boron and the presence of particular polymorphisms of the gene coding for osteocalcin [39].

4.2. Effectiveness of boron supplementation on vitamin D and sex steroid hormones metabolism in humans

Studies in middle-aged men and women have shown that boron is capable of inducing an increase in serum levels of 25 (OH) vitamin D (similar to estrogen therapy in postmenopausal women) [44,49]. In the study of Nielsen et al., 15 subjects between 44 and 70 years of age (including 5 men, 5 postmenopausal women on estrogen therapy, 4 postmenopausal women not on estrogen therapy and 1 pre-menopausal woman) followed a 2000 kcal / Western diet die containing 0.23 mg of boron for 63 days and, subsequently, a diet supplemented with 3 mg / day of boron (in the form of sodium borate) for 49 days; serum values of ionized calcium and 25-hydroxycholecalciferol were lower during the boron-poor diet than that associated with its supplementation [44]. In a pilot study conducted in Serbia on 13 middle-aged subjects deficient in vitamin D (25 (OH) vitamin D < 12 ng / ml), following boron supplementation of 6 mg / day (in the form of calcium fructoborate) for 60 days, a significant average increase in 25 (OH) vitamin D levels of 20 % was observed [49].

In another double-blind placebo-controlled pilot clinical trial of 20 volunteers with osteoarthritis of the knee, aged 44–65 years, daily administration of 108 mg of calcium fructoborate for 14 days was associated with an increase significant levels of 125 (OH) 2 vitamin D compared to baseline, an effect not observed in the placebo group. However, no significant changes were found in serum 25 (OH) vitamin D levels [50].

Furthermore, several authors have observed that boron introduced in the diet can lead to an increase in the plasma concentrations of 17-β estradiol and / or testosterone [40,42,43]. In a 1997 study by Naghii and colleagues, a supplement of 10 mg of boron (as sodium tetraborate) was administered to 18 healthy male subjects for 4 weeks: at the end of this period, a statistically significant increase was found of plasma estradiol levels and a growth trend (albeit not statistically significant) of free testosterone levels [42]. In a second 2011 study by Naghii et al., the diet of 8 healthy male volunteers, aged between 29 and 50 years, was supplemented for 7 days with 10 mg / day of sodium tetraborate. At the end of the supplementation, a significant increase in plasma levels of boron and free testosterone was observed, while the concentration of estradiol was shown to be reduced; dihydrotestosterone, cortisol and vitamin D were elevated at the end of the study, but not statistically significantly [43]. To explain these effects, it has been hypothesized that boron can act as a powerful inhibitor of some microsomal enzymes that catalyze the insertion of hydroxyl groups in steroids such as, for example, 24-hydroxylase (which catalyzes 25OH vitamin D) and estradiol -hydroxylase (responsible for the catabolism of 17-β estradiol). It may therefore be reasonable in the future to test this hypothesis through in vitro studies on hepatocytes or other cells expressing 24-hydroxylase activity [49].

4.3. Effectiveness of boron supplementation in combination with other nutrients on calcium metabolism, growth and maintenance of bone in humans

Nutraceutical products (represented by individual nutrients, dietary supplements, herbal products, etc.) have recently been strongly promoted and recommended, as they are considered to be able to bring health benefits, including bone health [51]. Research and studies aimed at evaluating the effects of different nutritional supplements on biomarkers associated with chronic diseases and health status are therefore increasing. Among these, bone mineral density (BMD) is often

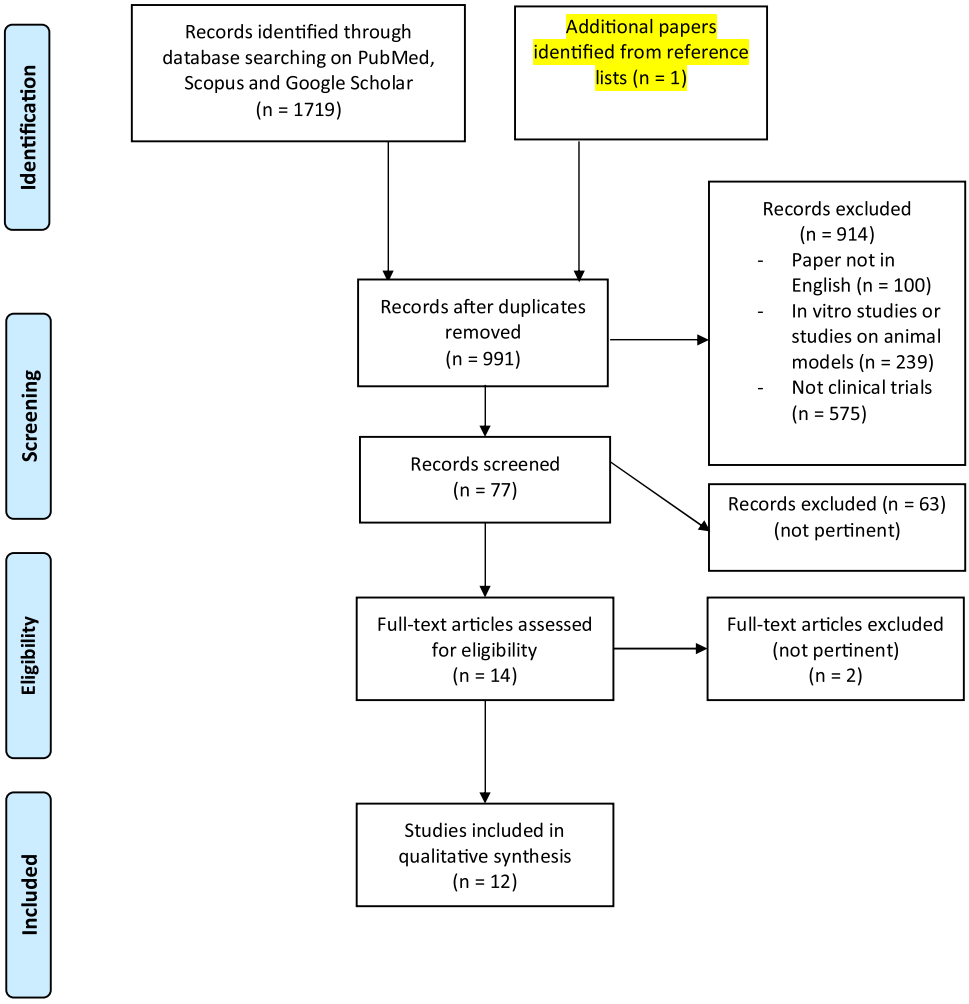

Fig. 1. Flow chart of literature research.

considered to be the "gold standard" for assessing bone health [46].

In this context, the study by Michalek et al. is inserted, a comparative effectiveness research (CER) in which a first group (AlgaeCal1) of 125 healthy volunteers (average age 55.2 ± 11.2 years) of both sexes (86.4 % women) a calcium-based supplement of plant origin (AlgaeCal, obtained from a seaweed from the coasts of South America) was daily administered for 6 months, also containing magnesium, strontium citrate, 800 IU of cholecalciferol and vitamin K-2. A second group (AlgaeCal2) of 51 volunteers (average age 56.7 ± 13.2; 78.4 % female) was instead given, again for a period of 6 months, an alternative version of the same supplement based of AlgaeCal, which contained similar amounts of strontium citrate, calcium and magnesium, but 1600 IU of cholecalciferol, vitamin K-7 instead of vitamin K-2 and, in addition, 275 mg of magnesium carbonate, 3 mg of boron (type of boron compound not specified by the authors) and 50 mcg of vitamin C. To evaluate the effectiveness, the BMD value at the baseline and at the end of the 6 months of treatment was obtained by DXA examination (dual-energy x-ray absorptiometry). In both groups there was a positive increase in BMD compared to the baseline (calculated as the "mean annualized percentage change", mean annualized percent change or MAPC), but only in the second group was this change statistically significant. In both groups, moreover, greater compliance with integration has been shown to be associated with higher BMD values [46].

In a second comparative efficacy research (CER) by Kaats G.R. et al., 172 healthy volunteer female subjects over 40 years of age were divided into three groups and assigned to as many plans for promoting bone health: Plan 1 included the only intake of a supplement based of calcium of vegetable origin (AlgaeCal) also containing 65 mg of Mg and 1000 IU cholecalciferol; Plan 2 included integration with AlgaeCal together with 72 mg of Mg, 800 I.U. cholecalciferol, strontium citrate and vitamin K-2; finally, Plan 3 provided for the intake of AlgaeCal together with 350 mg of Mg, 1600 I.U. of cholecalciferol, strontium citrate, vitamin K-7, vitamin C and 3 mg of boron (form of boron compound not specified). In addition, both Plan 2 and Plan 3 included a physical activity program (measured by pedometer) and the delivery of bone health information material. Within each group, the subjects were further divided also according to the degree of compliance with the treatment (two subgroups, above the median: "compliant"; below the median: "partially compliant"). Since the duration of Plan 1 was 1 year, while that of Plans 2 and 3 was 6 months, in this study the changes in the BMD were calculated as "mean annualized percentage change" or MAPC. Lacking a control group, the study sponsor chose to compare the changes in the BMD in the three intervention groups with data deriving from previous studies in the literature, according to which after middle age there would be an annual loss of bone mass linked to the same age, in both sexes, of about 1% and an even more accelerated loss, up to 2% per year, in women during the first 14 years after menopause; also based on additional data from three different databases (DXA machinery manufacturer, researchers themselves and National Osteoporosis Foundation), a change in BMD was also estimated in the absence of treatment of 0.75 % / year. At the end of the study, a significantly greater increase in BMD was observed in all three intervention groups compared to the expected change values (MAPC Plan 1: 1.20 %; Plan 2: 0.33 %; Plan 3: 2, 5%) and the group belonging to Plan

3, the most complete from a nutritional point of view, showed far greater values than the other two, especially considering, for each group, the only voluntary subjects with greater compliance (MAPC Plan 1: 1.30 %; Plan 2: 2.00 %; Plan 3: 4.1 %) [45].

The increase in BMD values that emerged in all intervention groups in both of these research appears, therefore, in contrast to previous studies according to which calcium and vitamin D3 supplementation was associated with a slowdown in age-related BMD loss, but not to an increase [45,46].

In 2016 Kaats G.R. et al. then published a further study on the efficacy and safety of the vitamin-mineral supplement based on AlgaeCal (containing 680 mg of strontium, 720 mg of calcium, 72 mg of magnesium, 1600 IU of vitamin D3, 100 mcg of vitamin K-7, 3 mg of boron (form of boron compound not specified) and 50 mg of vitamin C). A total of 172 healthy adult women (average age 65.1 years) were recruited who had hired the integrator for 1–7 years and who had performed at least one assessment of BMD via DXA in the same time interval. After making a second BMD measurement (not less than one year and no more than 7 years from the first), the annualized average percentage change (MAPC) was calculated, which was then compared with the expected or "normal" values obtained, similar to the previous study, from data from three different databases (Centers for Disease Control (CDC), GE Lunar Norms, Integrative Health Technologies, Inc. (IHTI)). To assess the safety of the same supplement, a total of 125 women from the same intervention group performed a panel of 45 blood chemistry tests on two occasions, 1–4 years apart. The results showed a statistically significant increase in BMD during the entire study period, both in comparison with the baseline data and in comparison with the expected values obtained from the databases (-0.4 % per year): BMD MAPC was in fact 1.04 % per year (7.3 % in the 7 years of study). However, no adverse effects or safety problems related to the intake of this supplement have emerged [48].

In contrast to the results of the studies just described are those that emerged from a prospective, uncontrolled, single-blind, two-year trial conducted in 2002 by Cook A. et al. on a sample of 12 post-menopausal initial women (average age 53.4 years). Two products were administered daily to each subject: the first, a vitamin-mineral mixture containing calcium 600 mg, magnesium 250 mg, manganese 20 mg, copper 2 mg, chromium 200 mcg, selenium 100 mcg, molybdenum 50 mcg, boron (chelated) 2 mg, silicon 1 mg, strontium 500 mcg, zinc 20 mg, vitamins B1 20 mg, B2 20 mg, B3 50 mg, B6 25 mg, B12 20 mcg, D 200 IU, K 300 mcg, pantothenic acid 20 mg, folic acid 800 mcg and betaine hydrochloride 20 mg; the second represented by a mixture of herbs including Angelica sinensis, licorice root extract, Vitex agnus-castus, Cimicifuga racemosa, Helonia opulus, Foeniculum vulgare, hesperidin. The study participants were then subjected to BMD detection of the lumbar spine (L1-L4), hip and distal third of the forearm via DXA at the baseline and then at 6, 12 and 24 months. Lacking a control group, the comparison of the data obtained was made with the placebo groups of other trials performed on women in the initial post-menopause and compared to a "non-response" (modification of the BMD equal to zero). The bone mineral loss detected, on an annual basis during the study, was -1.42 % per year for the lumbar spine, -0.43 % for the hip and −1.42 % for the forearm; these values were not significantly different from those of the controls of the other trials used for the comparison. The authors therefore concluded that a combined treatment based on vitamins, minerals and herbs would seem ineffective in countering the acceleration of bone mineral loss that occurs in the early stages of post-menopause [47].

One of the limitations of the literature available today could be represented by the fact that studies conducted in humans do not consider the intake of boron as the only ingredient of the dietary supplement (the products previously described and compared, in fact, contained, for example, also quotas different cholecalciferol); in this way it is difficult to establish whether the addition of boron to multivitamin and mineral supplements for bone health can be responsible for providing further beneficial effects.

4.4. Boron and bone: possible mechanisms of action

The various boron-containing compounds (BCCs) can each exercise different biological actions, from the modulation of enzymatic activities to the interactions with proteins and molecules containing ribose [6]. Some of them (such as boric acid and borate) have shown affinity towards hydroxyl groups in the cis position, such as those contained in some biologically important sugars (in particular ribose), and this could, at least in part, explain the mechanism behind some biological effects of boron [1,52]. For example, the function of ribose-containing molecules, such as S-adenosylmethionine, diadenosine phosphate, NAD + and its metabolite ADP cyclic ribose (cADPR), could be modified by boron; these biochemical entities are involved, in addition to cardiovascular health and neurological functions, also in the formation and maintenance of bone, to which boron seems to bring beneficial effects [52].

Boron also appears to interfere in the metabolism of human steroid hormones, thus affecting the levels of estrogen and testosterone; in fact, it has been hypothesized that it can facilitate their hydroxylation and protect them from rapid degradation. Vitamin D3, which contributes to joint and bone health, is also affected by boron and its compounds, which tend to increase serum concentrations [53]. As already mentioned, boron can form covalent complexes with compounds containing two hydroxyl groups close together in the cis position; it has therefore been hypothesized that it may inhibit a series of microsomal enzymes that catalyze, in steroid compounds, the insertion of hydroxyl groups close to existing hydroxyl groups (such as 24-hydroxylase and estradiol-hydroxylase, responsible for the catabolism of the vitamin D 25 OH and estradiol), or forming complexes that act as competitive inhibitors or, alternatively, causing a down-regulation of the expression of these enzymes [49].

Considering the evidence currently available, it seems reasonable to assume that boron also plays a favorable role in calcium metabolism, a figure of great importance for the prevention of osteoporosis and bone loss. A connection between boron and the signal pathways mediated by calcium (Ca^{2+}) was also hypothesized, as the latter would counteract the expression of some genes also influenced by a boron deficiency; further investigations are necessary to determine whether boron deficiency can alter the influence and release system of Ca^{2+} ions [53].

Finally, calcium fructoborate can exert a protective effect against inflammatory molecules at cellular and enzymatic level, being able to chemically bind to specific glycoprotein receptors of cytokines present on the surface of cell membranes; administered orally, it appears to be effective in improving the manifestations of the physiological response to stress (including, among other things, discomfort and joint stiffness and bone loss), modulating the serum levels of reactive protein C (CRP) and other cytokines [7]. In fact, there is a strong association between chronic inflammation and bone loss: inflammation can help decouple the formation from bone resorption in favor of excessive resorption, thus leading to decreased BMD. In fact, proinflammatory cytokines such as IL-1, IL-6 and TNF-α seem to stimulate osteoclastogenesis while the levels of reactive protein C (CRP) are positively associated with the total serum concentration of alkaline phosphatase (ALP) and with higher rates of bone turnover [54]. However, further studies are needed to improve understanding, even at the molecular level, of the various possible mechanisms of action with which boron and its compounds affect bone health.

5. Conclusions

To date, unfortunately, due to insufficient data available, recommended intake levels (RDA) for boron have not yet been established [14,15]; however, the World Health Organization (WHO) has estimated an "boron intake" of 1 − 13 mg / day as an "acceptable safety

interval" for adults [16]. EFSA (European Food Safety Agency), however, following a critical analysis of boron toxicokinetics and toxicodynamics data, has declared as safe for adults a maximum dose of 10 mg / day (in the form of boric acid and borates) [17].

The average intake of boron was between 0.87 and 1.35 mg / day for American adults [9]. These values are in line with what is highlighted by Biego G.H. et al. for the French population (1.6 mg / day) [18] and slightly lower than those reported by Naghii M.R. et al. for the Australian population (2.23 ± 1.23 mg / day) [19].

Considering that the studies published to date have evaluated the positive efficacy on bone metabolism considering a supplementation of 3 mg / day, this supplement is considered useful to support bone health (in order to prevent and maintain adequate bone mineral density), also in consideration of a daily dose of 3 mg is much lower than the Upper Level indicated by EFSA in the daily dose of 10 mg. Finally, given the recent discoveries on the various beneficial effects of boron on human health, it may be necessary to evaluate whether to classify it among the essential microelements for humans.

Declaration of Competing Interest

None.

Acknowledgments

None. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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