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REVIEW ARTICLE

Preventive and Curative Effect of Omega-3 Supplementation on Bone Mineral Density in People Aged 60 Years and Older: A Systematic Review

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ABSTRACT

Osteoporosis and osteopenia are common worldwide problems leading to potentially life-threatening consequences. Omega-3 supplementation for treating osteoporosis is less studied and less valued by physicians. We aimed to ascertain the appropriate dosage of omega-3 supplementation to prevent osteoporosis. Google scholar database was searched in May 2017 using the key words: n-3 fatty acids, omega-3 polyunsaturated fatty acids, essential fatty acids, eicosapentaenoic fatty acids, docosahexaenoic acid, docosapentaenoic acid, alpha linolenic acid, linoleic acid, osteopenia, osteoporosis, bone density, and fracture. We reviewed English language reports of randomized controlled trials with intake of omega-3 polyunsaturated fatty acids, in which subjects were over 60 years and supplemented with a quantified dosage of omega-3; and outcome was indicated by bone mineral densitometry medical record of fractures and radiological imaging, and serum biomarker to evaluate bone metabolism. We reviewed 110 papers, which only eight articles met our conclusion criteria and concluded with curative effects. Three articles came up with no prophylactic or curative effect of omega-3 supplementation, three articles suggested a dosage of omega-3 supplement that non-significantly increased bone mineral densitometry or decreased absorption, and thus, had prophylactic effects. One article just concluded the positive effects, not defining the exact results. It is suggested that a dosage of 4.5 to 6 g/d of eicosapentaenoic acid and docosahexaenoic acid can have curative effects, while 900-1000 mg/d can have prophylactic outcomes. N-3 fatty acids have positive effects on bone density, but to confine definitive dosage and formulation of omega-3 supplementation for reducing the risk of osteoporosis, further investigations are required.

INTRODUCTION

Osteoporosis is actually defined as bone strength reduction due to the loss of bone tissue and deterioration in skeletal microarchitecture because of which the bone is at a higher risk of fragility fracture (1). The major complication of osteoporosis is the pathologic fracture of several skeletal sites of the body, such as proximal femur and hip, vertebrae, distal forearm, proximal humerus, and pelvis (2). The susceptibility to low trauma fracture represents a major health burden in rapidly aging global population because it results in significant morbidity and mortality rates (2,3), and high health care costs (2,4). Epidemiologically, in the United States, about 9 million adults have been reported as osteoporotic, and 48 million have low bone mass levels and are at a higher risk of developing osteoporosis (1). As the most expensive fracture, hip fracture is estimated to increase worldwide from 1.26 million in 1990 to 2.6 million by 2025 and to 4.5 million by the year 2050 (2). According to the World Health Organization (WHO), a bone is called "osteoporotic" when its mineral density is 2.5 or more standard deviations (SD) below that of young normal population of the same sex, also referred to as a T-score of –2.5; 1.0 to 2.5 SDs decrease in bone mineral density is defined as "osteopenia", actually a T-score between -1.0 and -2.5; and a bone mineral densitometry (BMD) which is less than one SD below that of mean peak value in young adults of the same gender (T-score between 0.0 and -1.0), is a normal density (1).

There is inverse correlation between BMD and fracture risk; the lower the BMD, the higher the risk of fracture. Although the risk of fracture is lower in the osteopenic group, a great percentage of fractures occur because the number of osteopenic individuals is much greater than that of the osteoporotic individuals (1). There are several risk factors for osteoporosis and osteopenia, such as genetics, low calcium and vitamin D intake, smoking, thinness, menopause

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status, hypercortisolism, hyperthyroidism, hyperparathyroidism, alcohol abuse, immobilization, and the most common factor advanced age (4, 5). Several medications and supplementations are introduced for the prevention or treatment of osteoporosis, including medications like Alendronate and hormone replacement therapy (6) and supplementations like calcium, vitamin D, phosphorus, magnesium, protein, fluorine (7), and omega-3 (8-10).

Because of the high prevalence of osteoporosis, its noticeable morbidity and mortality rates, and economic burden of its consequences, it is necessary to investigate further medications for preventing and treating the disease. One of the above supplementations that is less studied and less used is omega-3.Animal and human researches indicate that omega-3 in optimal amounts can promote bone formation, inhibit bone resorption, increase BMD (8-11), and modulate markers of bone resorption and formation (12-14). Omega-3 also modulates inflammatory markers, such as tumor necrosis factor alpha (TNF-α), interleukin-1β (IL-1β), (interleukin-6)IL-6, and prostaglandin E2 (PGE2) (15). As the chronic senile disease of osteoporosis is associated with inflammation, inflammatory mediators can modulate the disease (8-10, 12, 15). Omega-3 supplementation is proved to be a treatment for osteoporosis, besides its further beneficial effects on other organs and in preventing other pathological conditions associated with the aging process, like cardiovascular diseases (16), depression (17), impairment of cognition, muscle tonus, and general health status (18).Therefore, the aim of this article is to undertake a review investigating the effect of omega-3 supplementation on modulating osteoporosis in the elderly.

Pathogenesis of Osteoporosis

Bone is a metabolically active organ that changes due to continuous modeling and remodeling process all through the life, modeling in response to biomechanical forces, and remodeling to eliminate old, micro-damaged bone and replace it with a stronger tissue. Decrease in bone density may be due to improper remodeling. Bone tissue include several type of cells, remodeling cells (osteoclasts), support cells (osteoblasts and osteocytes), and matrix proteins (osteoid) in which inorganic minerals are deposited. The precursor of the osteoblasts is mesenchymal stem cells. Osteoblastsare is responsible for matrix formation and its mineralization, and inhibiting osteoclasts' ability in breaking down of the tissue. These cells have receptors for parathyroid hormone, estrogen, thyroid hormone, growth factors, and are also stimulated by physical activity.An organic phosphate-splitting enzyme, which attaches to the external surface of osteoblasts, is alkaline phosphatase. The serum level of alkaline phosphatase reflects bone metabolism.Transforming into osteocytes is seen in some osteoblasts. Among all bone tissue cells, osteocytes function as the mechanoreceptors for bone,signaling osteoclasts fortheirreabsorption activity and osteoblasts for their formation activity. The only cells for bone resorption are osteoclasts, which originated from the hematopoietic stem cells, which also is the precursor of macrophages (19).

Various factors are known to play a role in differentiation of osteoclasts: hormones, growth factors, eicosanoids, immune mediators;the endocrine, paracrine, and autocrine ways; inflammatory cytokines, interleukin-1 (IL-1), IL-6, and TNF-α;receptor activator of nuclear factor-kB ligand (RANKL) and its receptor, reactive oxygen species (ROS), and oxidative stress.IL-1 and TNF-a stimulate IL-6 production, and are potent stimulators of bone resorption and inhibitors of bone formation. These three inflammatory cytokines have been shown to be active in the pathogenesis of osteoporosis. RANKL and RANKL receptor, belonging to the TNF-α family, are present on the osteoblast membrane and their activation leads to osteoclastic differentiation and activation (19-22). Figure1 demonstrates the process of bone remodeling (23).

The Main Mechanism of Action of Dietary Omega-3 Fatty Acids on Bone

Several animal and human studies demonstrate the preventive or curative effects of polyunsaturated fatty acids (PUFAs) on osteoporosis (13, 14, 23-25). Menopause and aging lead to bone loss. Chronic inflammation due to aging can increase bone resorption and inhibit its synthesis (20, 26). The PUFAs are beneficial for bone health because of several mechanisms, such as reducing inflammatory cytokines IL-1, IL-6, and TNF-α, modulation of peroxisome proliferators-activated receptor γ (PPARγ), increasing calcium absorption, increasing skeletal calcium levels, change of membrane function, inhibition of osteoclastogenesis, and promoting osteoblastogenesis (27, 28).

One molecular mechanism involves PGE2 synthesis. Once mechanical load is applied to the bone, cell membranes release arachidonic acid, which then forms PGE2 as the expression of cyclooxygenase 2 (COX-2) increases. The PGE2 stimulates expression of RANK, RANKL and inhibits osteoprotegerin (OPG) expression, leading to osteoclastogenesis. It also activates the Wnt signaling pathway and insulin like growth factor-1 (IGF-1), leading to osteoblastogenesis. So, both formation and resorption processes are stimulated by PGE2. Generally, osteoblastogenesis as well as bone formation is promoted with the low levels of PGE2, while osteoclastogenesis and bone resorption result from high levels of PGE2 [28]. As eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) can modulatethe production of PGE2 and COX-2, osteoclastogenesis is suppressed and osteoblastogenesis is activated, resulting in bone health (16, 29).

PPARγ is a nuclear transcription factor in fat tissue and bone marrow, resulting in suppression of osteoblastogenesis and bone loss. The n−6 polyunsaturated fatty acids can activate PPARγ (30).

There are two categories, structurally, for poly unsaturated fatty acids:n-3 (w-3, in which the third carbon from the methyl terminal is unsaturated) and n-6 (w-6, in which the sixth carbon from the methyl terminal is unsaturated) (30). Linoleic acid (LA) and α-linolenic acid (ALA) are essential fatty acids because humansdo not havethe ability to synthesize them, needing these FAs to be available in daily diet. Linoleic acid, found in high concentrations in various vegetable oils, is an n-6 PUFA that finally forms arachidon-

ic acid (AA). Arachidonic acid is a precursor of prostaglandins, leukotrienes,lipoxines, and P-450 compounds. The ALA, as a forerunner of EPA and DHA, is an n-3PUFA, mainly found in fish and fish oils. Because of low conversion efficiency of ALA to EPA and DHA, supplementation with these FAs is recommended. The conversion of n-6 and n-3 polyunsaturated fatty acids to their longer chain forms is shown in Figure-2 (31).

Omega-6 and omega-3 fatty acids are present in phospholipid membranes of cells and affect gene expression, functioning as precursors of eicosanoids (thromboxanes, prostaglandins, and leukotrienes). The n-6 FAs produce eicosanoids of atherogenic, proinflammatory, and vasoconstrictive activity, more than that made of n-3 PUFAs. EPA and DHA have the most potent anti-inflammatory effects.Presence of EPA and DHA in cell membrane, results in reduced amount of n-6 fatty acids in the membranes; thus, reducing the presence of AA as well. A higher ratio of n-3/n-6 fatty acids in cell membranes can decrease the production of inflammatory cytokines IL-1, IL-6, and TNF-α. So, essential fatty acids have inflammatory modulatory effects, and play a role in the pathogenesis of osteoporosis (3, 28, 30, 32, 33).

MATERIALS AND METHODS

We reviewed randomized controlled trials (RCTs) reported in the literature from 1990 to 2017 in order to ascertain the appropriate dosage of omega-3 supplementation to treat and prevent osteoporosis in the elderly. Google scholar database was searched in May 2017 using the key words: n-3 fatty acids, omega-3 fatty acids, polyunsaturated fatty acids, essential fatty acids, eicosapentaenoic fatty acids, docosahexaenoic acid, docosapentaenoic acid, alpha linolenic acid, linoleic acid, osteopenia, osteoporosis, bone density, and fracture.

We reviewed English language reports of RCTs with intake of omega-3 PUFAs, in which subjects were over 60 years and were supplemented with a quantified dosage of omega-3, not subjective and unmeasurable amounts like questionnaires and intake of seafood, and outcome was indicated by bone mineral densitometry, medical record of fractures and radiological imaging, and serum biomarker to evaluate bone metabolism (bone formation markers like bone specific alkaline phosphatase (BSAP) and osteocalcin (OC), bone resorption markers like N-terminal telopeptide (NTx), C- terminal telopeptide (CTx), urinary pyridinoline (u-pyr) and urinary deoxypyridinoline (u-dpyr), and regulators of bone turnover like osteoprotegerin (OPG), receptor activator of NFkB ligand (RANKL), OPG/RANKL). There were no restrictions based on gender of the participants or race. Articles on cancer, autoimmune and inflammatory diseases, endocrinology diseases like diabetes and thyroid diseases, use of special medications like corticosteroids, hormones, gonadotropin-releasing hormone analogue (GnRH analogs), anticonvulsive drugs, heparin, thyroid hormones, and smoking or alcohol consumption were excluded (Table1).

RESULTS

We reviewed 110 papers, of which onlyeight articles met our conclusion criteria. Only one concluded the curative effects (25). Three articles (34-36) came up with no prophylactic or curative effects of omega-3 supplementation; three articles (9, 10, 37) suggested a dosage of omega-3 supplement non-significantly, increasing BMD or decreasing absorption, and thus, having prophylactic effects. One article just concluded the positive effects, not defining the exact results (38). Studies are summarized in table 1, with conclusion of studies and final results, which are further discussed in next part.

Several factors play a role in the results of the trials: the duration of taking supplementation, the exact components of supplementation and dosage, sufficient levels of vitamin D and daily calcium intake, sample size, heterogeneity of subjects including age, gender, race, and their pre-intervention nutritional status.

DISCUSSION

According to our search and results, the only study with conclusion of the curative effect, conducted by Kruger et al. has several points to consider regarding its conclusion of having positive effects. Duration of intervention and follow-up of the subjects is an important factor, as bone turnover is a gradual process and the resorption and formation activity in adult human bone takes about 3 to 4 months (39). In the first 18 months, only prophylactic effect of supplementation was observed, and in the next 18 months, the curative effect was achieved. This shows that supplementation should continue for several months to observe results in bone density. Another point is participants' osteoporotic or osteopenic status and their mean age of 79.5, taking a background diet low in calcium. The fact that these old participants were somewhat malnourished, can explain the positive response to calcium and omega-3 supplementation in this study. Regarding ethnicity, they were Caucasian South African women. So, the data and exact changes might not be applicable to other races, but it is not in conflict with the positive effects of omega-3 on skeletal health, because both treatment and placebo groups were from the same ethnicity, as its effect is proved in other animal and human researches.

According to results, lumbar spine density was the first to show increase in BMD in the first 18 months in 65 individuals, and in the next 18 months, femoral bone density increased in treatment group of 21 participants. Maybe the combination of fatty acid supplementation and 600 mg of calcium carbonate preferentially impact hip BMD. But it is not completely reliable because of the small number of samples (65 and 21 subjects). On the other hand, the placebo was coconut oil containing 97% saturated fat which might increase inflammatory factors in the placebo group, overestimating the effect of omega-3 supplementation (8).

The study by Chen et al. (37) comes up with the prophylactic effects. Over the two-year period, subjects received high dose (4.5g/d) and low dose (0.45 g/d) of omega-3 fish oil. This study can be compared with Kruger's (25). In Kruger's study, significant prophylactic effect was ob-

served in lumbar spine and curative effects in femoral neck by 6 mg/d of supplementation for 18 months, while in this study, the non-significant increase in lumbar spine density and constant density of femoral neck was the outcome of 4.5 mg/d supplementation for 24 months. The fact that BMD did not decrease in 2 years and remained constant revealed the prophylactic effect of this dosage. It seems that Kruger's dosage and formulation of supplement was more effective, but the smaller sample size of Kruger's and the differences in gender, nationality, and the underlying disease of participants (osteoporosis and osteoarthritis) in both the studies should be taken into consideration.

The research conducted by Tartibian et al. (10) represents non-significant increase in BMD at lumbar site (L2-L4) and femoral neck by 24 weeks of 1000mg daily supplementation of omega-3. Levels of TNF-α, IL-6, PGE2, PTH, and C-telopepide decreased non-significantly, and calcitonin levels demonstrated a significant increase from the baseline. Although these changes were almost non-significant, the trend increased the bone formation, decreased resorption, and attenuatedthe inflammatory factors, indicating the prophylactic, but not curative effects of 1000mg/day consumption of omega-3 for 24 weeks for post-menopausal women. Maybe longer supplementation shows curative effects, similar to Kruger's.

Sharif et al. (9) and Dong et al. (34) performed two studies of the same duration, but with a different formulation of supplement and individuals' bone density status, placebo, sample size, and supplementation with calcium and vitamin D. Dong et al. (34) supplemented both groups with calcium and vitamin D, while Sharif did not. Maybe these two supplements' effect on bone health resulted in not having between-group difference in Dong's trial. And maybe the effect of calcium and vitamin D is equal to omega-3 in 6 months, as it can also be concluded from Vanlint's study (35). Sharif did not supplement the individuals with calcium and vitamin D, and the change in bone resorption markers was significantly different from control group, concluding that omega-3 has prophylactic effects.

LeBoff, et al. (38) conducted a VITAL-Bone Health study. The conclusion was that there is a relationship between supplemental vitamin D and / or omega-3 FAs on bone health outcomes. But no data was published in the article and no information on its effect on bone is available.

The studies by Vanlint and Appleton (35, 36) show no effect of supplementation and dosage on bone turnover. Another study by Appleton showed that the short duration of the study and individuals' young ages may play a role in the result. In the study by Vanlint's (35), the results showed that optimal supplementation of calcium and vitamin D can prevent osteoporosis, and addition of omega-3 did not demonstrate further effect which may be due to the small sample size.

CONCLUSION

Considering previous animal and human studies, there is great evidence that n-3 fatty acids have positive effects on bone density, but the form of FAs, dosage, and other factors seem to alter the effect.

Because of the small number of RCTs, short duration of supplementation, and the heterogeneity of studies, we are unable to make strong conclusions regarding the effect of n-3 FAs supplementation.

Notably, sufficient vitamin D and calcium intake is necessary to achieve the greatest effect of omega-3 supplementation or even intake of calcium and vitamin D can have the same effect as omega-3, according to some articles previously mentioned (35,36,38).

It is important that subjects be supplemented and followed for several months, preferentially for several years and evaluated by BMD as well as serum biomarkers. Since exercise has synergic effect with omega-3, active or sedentary individuals should be evaluated in different groups. It is suggested that BMD be evaluated before starting supplementation, because normal, osteopenic, and osteoporotic persons may respond differently to omega-3 intake, and the same DXA (dual X-ray absorptiometry). machine be used for all subjects throughout the study. Usage of medications like bisphosphonates should be considered.

Considering that supplementation needs to be continued for several months, the adverse effects of omega-3, even mild disturbances, should be noticed in order to prevent discontinuation of the treatment. Gastrointestinal upsets due to high doses of supplements was reported in some articles (25, 3). It is suggested that taking supplements every other day, or weekly intake of high dose of omega-3 be evaluated, which seems to be more applicable for osteopenic or normal densityindividuals who want to prevent osteoporosis.

Recent studies have compared the efficacy of DHA and EPA. Some have concluded that DHA is more effective than EPA in modulating inflammatory markers (40, 41), while some concluded similar effects (42). Optimization of the ratio of EPA/DHA of supplements may provide enhanced protective potential.

We need more researches to see which formulation and dosage of omega-3 supplementation can have prophylactic and curative effects. It is suggested that the dosage of 4.5 to 6 g/d of EPA and DHA can have curative effects, while 900-1000 mg/d can have prophylactic outcomes. As the aim of omega-3 supplementation is to prevent or cure osteoporosis, it is recommended that the effects of higher dosage of supplementation (4.5-6g/d) for osteoporotic individuals as a curative drug, and lower dosage (900-1000mg/d) for osteopenic individuals and normal ones as a prophylactic supplement be evaluated in future studies.

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AUTHOR CONTRIBUTIONS

Niloofar Khodabandehloo: Conceptualization & design,

Table 1. Summary of the finding

Study	Year	Subjects	Diet	Measurment	Study Period	Result/Conclusion
LeBoff, et al. (38)	2015	men aged ≥50 women aged ≥55 25,875 participants in VITAL-Fracture 773 participants in VITAL-Bone structure/Architecture	vitamin D3 (2000 IU/d) and omega-3 FA (1000mg/d)	medical records and radiological images of hip and femur at baseline and 2 years post- randomization	2 years	clarify the relationship between supplemental vitamin D and/or omega-3 FAs on bone health outcomes
Chen et al. (39)	2015	202 Australian participants aged ≥40 with knee osteoarthritis (mean age 61.0 ± 10.0 years, 49% female)	high dose EPA acid and DHA (4.5 g/d) vs low dose (0.45 g/day)	BMD at lumbar spine and femoral neck	2 years	Non-significant changes: femoral neck BMD decreased in low dose group/ constant in high dose group lumbar spine BMD remained constant in low dose group/increased in high dose group)
Dong, Hongli, et al. (40)	2014	126 postmenopausal women (mean age 75 ± 7 years)	EPA + DHA (1.2 g/d) vs. olive oil calcium citrate (315 mg/d) cholecalciferol (1000 IU/d) for all women	BSAP Osteocalcin RBC ratio of DHA+EPA	6 months	BSAP and osteocalcin decreased in the n-3 LCPUFA group ($P < 0.05$) with no between-group difference Bone turnover decreased, but not statistically compared to placebo
Vanlint et al. (41)	2012	40 individuals with osteopenia	algal oil containing docosahexanoic acid (400 mg /d) vs unidentified placebo calcium carbonate (1200 mg/d) for all vitamin D3 (1000 IU/d) for all	BMD serum CTx	12 months	CTx suppressed with no difference in effect size between groups non-significant trend towards rising BMD in both groups no effect demonstrated from the addition of DHA
Appleton, K. M. et al. (42)	2011	113 individuals (26 males and 87 females, aged 18–67 years)	EPA + DHA (1·48 g/d) vs olive oil placebo	*n*-3 PUFA status in serum samples β-CTx	12 weeks	*n*-3 PUFA supplementation may be unlikely to be of benefit in preventing bone loss
Tartibian, et al. (10)	2011	79 healthy sedentary post- menopausal women aged 58- 78 years	omega-3 FA (1000 mg/d) vs exercise, supplement and exercise	TNF-α, IL-6, prostaglandin E2, estrogen, osteocalcin, 1, 25 Vit D, C-telopeptide, PTH, calcitonin, BMD at lumbar spine (L2-L4) and femoral neck.	24 weeks	slight but non-significant increases in BMD attenuation of inflammatory markers (TNF-α, IL-6) exercise training plus N-3 supplementation have a synergistic effect (augmentation of estrogen, osteocalcin and 1, 25 Vit D levels and profound changes in BMD)
Sharif, Pooneh Salari, et al. (9)	2010	25 osteoporotic post-menopausal women	omega-3 FA (900mg/d) vs unidentified placebo	osteocalcin, BSAP, calcium, Vit D, PTH, urine concentration of pyridinoline	6 months	Decrease bone resorption but could not affect bone formation significantly
Kruger, M. C. et al. (25)	1998	65 women with mean age of 79.5 for the first 18 months & 21 continue the next 18 months	6g of a mixture of (8%GLA, 60%LA, 4%EPA,3%DHA) vs coconut oil placebo Calcium carbonate (600 mg/day) for all	BMD osteocalcin dpyr BSAP	18 months and then 36 months	Over the first 18 months, lumbar spine density remained the same (prophylactic for lumbar spine) femoral bone density increased (curative for femoral bone) second period of 18 months: lumbar spine and femoral density increased

Abbreviations: FA: fatty acid, BMD: bone mineral densitometry, EPA: eicosapentaenoic acid, DHA: docosahexaenoic acid, BSAP: bone specific alkaline phosphatase, LCPUFA: long-chain polyunsaturated fatty acids, RBC: red blood cell, CTx: c-terminal telopeptides, PUFA: polyunsaturated fatty acids, TNF-α tumor necrosis factor alpha (TNF-α), IL-6: interleukin-6, PTH: parathyroid Hormone, Vit D: vitamin D, GLA: Gamma-linolenic acid, LA: linoleic acid, αCTx: C-terminal cross-linking telopeptide of type 1 collagen, dpyr: deoxypyridinoline

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CONFLICT OF INTERESTS

The authors declare that they have no conflict of interest to declare.

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