Omega-3 Fatty Acids and Bone Health

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Dietary fat is an important source of energy for mammals, but it was not until the advent of intravenous feeding that the importance of essential fatty acids was widely accepted. "Essential" fatty acids were identified in research with patients on total parenteral nutrition or with malabsorption syndromes, as they developed dermatitis, changes in visual and neural function, and immunomodulatory disorders. The fatty acids can be divided into two categories: saturated fatty acids (solid at room temperature) and unsaturated fatty acids (liquid at room temperature). The unsaturated fatty acids are of two classes: monounsaturated fatty acids (in olive and canola oil), with only one double bond, and polyunsaturated fatty acids (PUFAs, in corn, sunflower, flax seed, and other oils). Cold-water fish, fish oils, walnuts, wheat germ, and organ meats are good sources of omega-3 PUFAs. The omega-3 family has its first double bond located at the 3rd carbon from the methyl end (designated as omega-3, ω-3, or *n*-3). Chain length is designated by the number of carbons, with long-chain PUFAs having 20 and 22 carbons. Mammalian cells cannot introduce the double bond at the n-3 and n-6 positions; hence, those fatty acids are "essential" and their precursors must be derived from the diet. Omega-3 PUFAs are α–linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA), having 18, 20, and 22 carbons respectively. The omega-6 fatty acids are linoleic acid, gamma-linolenic acid (GLA), and arachidonic acid (AA).

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On the basis of observational and randomized trial data, as well as plausible mechanisms for benefit, the American Heart Association (AHA) [1] and the Institute of Medicine [2] recommend that all adults eat fish, particularly fatty fish, regularly (twice/week) to reduce cardiovascular disease risk. The AHA advises patients with coronary heart disease to consume ~1g/d of EPA and DHA combined. The typical U.S. adult, however, consumes only 100-200 mg/d of EPA+DHA [3]. Many people find it difficult to eat two fish meals per week, and the recommendation of ~1g/d of EPA+DHA translates to more than one fish meal daily. In addition, concerns have been raised about environmental contamination of the fish supply. Thus, fish oil supplements may be a preferable way to achieve compliance.

Calcium, vitamin D, and protein are among the dietary essentials for skeletal health. Concerns about recent changes in the dietary balance of n-6 to n-3 fatty acids in the United States [4] has raised interest in differential cellular effects of dietary fatty acids and in their potential skeletal effects.

ANIMAL INTERVENTION STUDIES

More information is available from animal than clinical intervention studies. Dietary fish oil protected mice from bone loss following ovariectomy, and also prevented the elevation of RANKL that was seen in T-cells from ovariectomized mice supplemented with corn oil [5]. That study supports the view that one of the mechanisms by which dietary n-3 fatty acids reduce bone loss in ovariectormized mice is by inhibition of osteoclast generation and activation. Other research shows direct evidence of beneficial effects on bone formation. Dietary fish oil protected retired breeder rats from bone loss following ovariectomy, with a marked increase in mineral apposition rate [6].

In many animal studies, n-3 fatty acids or a low ratio of n-6 to n-3 fatty acids show a positive influence on bone. In 1994, EPA was shown to inhibit bone loss in ovariectomized rats that were maintained on a low calcium diet [7]. Shortly thereafter, it was reported that both EPA and DHA prevented the increase in bone fragility in diabetic rats, and that EPA prevented osteopenia even in diabetic rats fed a low zinc diet, which was used as a potent accelerator of diabetic osteopenia [8]. Subsequently, it was shown that n-3 PUFA deficiency caused severe osteoporosis in rats. Further, when deficient animals were replenished with n-3 PUFA, the ratio of n-3 to n-6 PUFA in bone compartments was restored and the process of bone degradation was reversed [9]. The beneficial effects of n-3 fatty acids appear to be associated with downregulation of PGE2 formation with a net enhancement of bone formation

and diminished bone resorption [10]. Not all studies, however, show beneficial skeletal effects of n-3 in ovariectomized rats [11], but it has been suggested that study design and the type of fatty acid in the diet may be more important than the overall ratio of n-3 to n-6 PUFA [12]. A very recent study with intact female mice indicates that long-term intake of n-3 fatty acids, especially EPA, improved structural and mechanical properties of cortical bone in the femur, without detectable effects on age-related loss of trabecular bone or bone mineral density [13]. Those effects of EPA were accompanied by increased leptin and osteocalcin levels, and no effect on a marker of bone resorption. A recent review concluded that most animal studies confirm the beneficial effects of n-3 fatty acids on bone health, with many differences in study design, type and dose of feeding, and measured bone markers [14]. Underlying differences in lipoprotein metabolism between animals and humans may limit the utility of certain animal models to reveal the biochemical basis of some effects of n-3 fatty acids and observed differences between marine and plant n-3 fatty acids [15, 16].

MECHANISM STUDIES

Studies directed at revealing mechanism of actions of n-3 fatty acids on inflammation and cardiovascular disease indicate their effects on arachidonic acid-eicosanoid pathway intermediates, changes in lipid metabolism, and decreases in pro-inflammatory cytokine production [17]. Cytokines, such as Interleukin(IL)-1, IL-6, and TNF, are well known pro-inflammatory mediators and also play key intermediate and interacting local roles in bone remodeling. Several studies with humans showed dramatic decreases in cytokine production following n-3 fatty acid supplementation. A six-week trial of fish oil with EPA and DHA significantly reduced inducible production of IL-1β, IL-1α, and TNFα in peripheral blood mononuclear cells [18]. A three-month trial of daily n-3 supplementation significantly suppressed IL-1, IL-6, TNFα, and IL-2 production in peripheral mononuclear cells from groups of pre-menopausal and postmenopausal women; although baseline levels of all cytokines were similar in both groups, the magnitude of suppression by n-3 was greater in the postmenopausal women [19]. The n-3 fatty acids also promote intestinal calcium absorption, but this may occur only when dietary calcium is low [20]. The mechanism by which n-3 fatty acid affects bone metabolism is complicated and may depend upon the interactions of immune and bone cells. *In vitro* studies showed that some n-3 fatty acids decrease NFκB expression, RANKL signaling, and osteoclastogenesis [21]. Experiments with MC3T3-E1 osteoblast-like cells showed that n-3 PUFA modulated COX-2 protein expression, reduced prostaglandin E_2 production, and increased alkaline phosphatase activity [22]. The PUFAs regulate transcription of a number of genes via the action of peroxisome proliferator activator receptors (PPARs); in other words, they are natural PPAR ligands [23]. New evidence from studies with related strains of mice indicates that the beneficial effects of n-3/n-6 on bone microarchitecture in aging mice depends upon the level of PPARγ expression [24].

EPIDEMIOLOGICAL STUDIES.

Some clinical information has become available that suggests that certain dietary patterns have bone-preserving properties. For example, data from the Framingham Study indicated significantly higher bone mass in elderly men with high consumption of fruit, vegetables, and breakfast cereal, compared with a diet rich in meat, bread, and potatoes [25]. Results from the National Health and Nutrition Examination Survey III revealed that saturated fat (the main fat of animal foods) intake was inversely associated with bone density, with the strongest effects shown for men <50 years old [26]. In a study of pre-menopausal Japanese women, those with the highest BMD scores consumed more fish and shellfish, fruit, mushrooms, and dark-yellow vegetables than those women with greater intake of meat, fats, and oils [27]. A cross-sectional analysis of dietary patterns in healthy Mediterranean pre- and post-menopausal women showed a positive association between bone mineral density and a modified Mediterranean diet (characterized as high consumption of fish and olive oil and low intake of red meat) [28].

Because of the suggestive evidence from animal studies, two types of dietary polyunsaturated fatty acids (PUFAs), n-6 and n-3, have received increasing attention with regard to human skeletal health. In the Rancho Bernardo cohort of elderly, community dwelling men and women, self-reported food-frequency dietary information was converted into nutrient intake with use of the food-composition database of the US Department of Agriculture [29]. It was reported that an increasing ratio of dietary n-6 to n-3 fatty acids was significantly associated with lower BMD. Another cohort study with healthy young men evaluated serum fatty acids and change in BMD between baseline age of 16 years and second measurements at age 22 and third measurements at age 24 [30]. The key findings were positive associations between serum n-3 fatty acids and BMD of the total body and spine and the accumulation of BMD at the spine between 16 and 22 years of age. In addition, BMD of the spine measured at 22 y of age showed an inverse association with the ratio of serum n–6 to n–3 fatty acids.

CLINICAL INTERVENTION TRIALS.

Intervention trials in humans are limited. Results from a small number of experimental studies of the effects of PUFA supplementation on bone in humans are inconclusive and vary in type, concentration, and dosing of n-3. In a randomized trial in 40 patients with osteoporosis, subjects taking a supplement rich in n-3 PUFA showed better calcium absorption and increased markers of bone formation, while those taking a placebo showed no improvement [31]. In a clinical trial of 65 elderly women whose diet was low in calcium, supplementation with a properly balanced ratio of n-6 and n-3 PUFA plus calcium resulted in decreased bone degradation and increased bone mineral density, with a significant fall in osteocalcin and Dpyd, indicative of a decrease in bone turnover. The subjects receiving GLA+EPA treatment for 18-months showed an increase of 3.1% in lumbar spine density and 4.7% in femoral-bone mineral density [32]. In contrast, 12-month trials featuring a

combination supplement of n-6 PUFA, n-3 PUFA, and calcium in pre- and post-menopausal women failed to show an effect on bone mineral density [33]. The lack of effect in that study may be attributable to it being shorter, 12-months in contrast to 18-months, and in using only total body bone mineral density measurements. The results of one investigation suggested that PUFAs are harmful to bone in perimenopausal women [34]. To control for confounders due to background diets, a three-period cross-over feeding trial was designed with 3 test diets, an average American diet and two high-PUFA diets with different ratios of n-6/n-3 fatty acids [35]. The measure of bone turnover, serum NTx, was lowest for all subjects following the period when they were taking the diet with the lowest n-6/n-3 composition. The authors conclude that short-term consumption of the highest PUFA diet induced a reduction in bone turnover and maintenance of bone formation. In that study, most subjects were middle-aged men and plant sources of PUFAs were used.

In summary, based upon mechanisms of action and data from animal and small clinical studies, the consumption of n-3 fatty acids may be beneficial to the skeleton, but compelling data are not available for long-term effects in large numbers of subjects. Further, it is not known what amount or type of PUFA may be required to enhance bone health at different lifestages and, at this time, evidence is lacking to support a recommendation of n-3 PUFAs as a component in the primary prevention of osteoporosis. The national VITAL trial (*VIT*amin D and Omeg*A*-3 Tria*L*) may provide answers to these questions. This national trial will enroll 20,000 men aged ≥60 and women aged ≥65 for randomization to 4 groups: vitamin D_3 (1600 IU/d) and fish oil (EPA+DHA, 1 g/d); vitamin D_3 and fish oil placebo; placebo vitamin D_3 and fish oil; and placebo vitamin D_3 and placebo fish oil. The parent grant will test effects of the supplements on risk of cancer and cardiovascular events, with future ancillary studies planned for musculoskeletal health.

Foods higher in omega-3 fatty acids	**Foods lower in omega-3 fatty acids**
Fish (cold-water, oily):	Meat
salmon, mackerel, herring, anchovies, sardines	Farm-raised tipalia
Walnuts	Dairy foods
Flaxseed (linseed)	Cereals
Omega-3 fortified foods:	Processed and fast food
bread, mayonnaise, pizza, yogurt, orange juice,	Olive, peanut, and canola oils (monounsaturated, with neither n-3 or n-6)
children's pasta, salad dressings, milk, eggs	Sunflower, grapeseed, corn oils
Tofu, soybeans	Evening primrose oil, borage oil
Grass-fed rather than grain-fed beef, chicken, lamb	

Table. Relative Contents of Omega-3 Fatty Acids in Foods

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