[Frontiers in Bioscience 13, 4015-4020, May 1, 2008]

Effects of n-3 fatty acids on autoimmunity and osteoporosis

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1. ABSTRACT

Decreased consumption of n-3 fatty acids (FA) and diets rich in animal proteins, saturated fats and n-6 vegetable oils are associated with a higher incidence of cardiovascular disease (CVD), certain malignancies and autoimmune disorders such as rheumatoid arthritis and Systemic Lupus Erythromatous (SLE), and renal disease. Recent studies show that reduced calorie intake and supplementation of diet with n-3 FA delays the onset of autoimmune renal disease, primarily, due to increased antioxidant enzyme activities, decreased NF-kappaB activation and decreased IL-1, IL-6 and TNF-alpha mRNA expression in the kidney tissue. Studies in rodents show that addition of n-3 FA and soy protein to diet affords protection against bone loss induced by ovariectomy in mice due to NF-kappaB expression and decreased activation of osteoclasts. Together, the availale evidence show that increased daily intake of dietary n-3 FA decreases the severity of autoimmune disorders, lessens the chance of developing CVD, and protects against bone loss during post-menopause.

2. REVIEW

2.1. Role of n-3 fatty acids in health and disease

Since the original report of Bang *et al.* (1) on the diet consumed by Greenland Eskimos and the decreased incidence of cardiovascular disease (CVD), there has been considerable interest in the use of n-3 fatty acids (FA) as dietary supplements, and in the clinical setting (2,3). Several studies have shown promising results against inflammatory disorders including cardiovascular and autoimmune disorders (4,5). It is now accepted that the use of n-3 FA may decrease the risk of CVD in the US population (6). n-3 FA also decreased the incidence of several chronic diseases that are closely linked to increased consumption of saturated fat and n-6 vegetable oil in the USA (7). A recent health study in nurses revealed that higher consumption of fish and n-3 fatty acids as well as fruits and vegetables was associated with a lower risk of heart disease (HD), particularly HD related deaths (8). Furthermore, the blood level of n-3 FA is a predictive biomarker for sudden death in men (4,9). Although the mechanism of n-3 FA mediated inhibition of inflammation

and CVD is not fully understood, a great deal of supportive evidence indicates that alterations in arachidonic acid-eicosanoid pathway intermediates, changes in lipid metabolism and/or decreases in pro-inflammatory cytokine production, along with changes in expression of numerous genes and transcription factors are clearly involved (10-16).

The majority of studies on the effects of n-3 FA have been carried out using fish oil (FO) or FO concentrates containing low levels of EPA and DHA. Since there is significant interconversion of EPA and DHA *in vivo*, it is difficult to infer the effects of either of them with absolute certainty even if purified forms EPA or DHA are used. However, effects on brain growth and development and visual acuity in infants are ascribed to DHA, since DHA is preferentially taken up in the brain and found in high concentrations in brain tissue. DHA is reported to promote neurite growth and strengthen periventricular vascular system against hemorrhage (17).

It is widely accepted that FO, rich in n-3 polyunsaturated FA, protect against several types of cardiovascular diseases such as myocardial infarction, arrhythmia, atherosclerosis, and hypertension (18,19). Although, the precise cellular and molecular mechanisms for these beneficial effects are still unknown, one of the mechanisms may be their direct effects on vascular smooth muscle cell functions. These n-3 PUFAs activate K^+ ATP channels and inhibit certain types of Ca^{2+} channels (20). There are at least two mechanisms for these actions: 1) n-3 PUFAs can alter the eicosanoid profile and cytokine-induced expression of inducible nitric oxide synthase and COX-2 through modulation of signaling transduction pathways, and 2) n-3 PUFAs can modulate vascular smooth muscle cell proliferation, migration, and apoptosis. Recent studies strongly suggest that DHA has more potent and beneficial effects than EPA in these systems (21). DHA has been reported to be more potent at decreasing plasma triglyceride levels, as well as DHA supplementation alone significantly increases serum HDL cholesterol levels (22,23), which is associated with reduced risk of coronary heart disease (24) by being a more efficient reverse cholesterol transport pathway. At least a portion of the hypotriglyceridemic effect of n-3 FA has been attributed to increasing circulating apoE levels (25).

Besides, numerous beneficial effects of n-3 FA on several other chronic diseases including osteoporosis are reported. Some clinical FO studies did not show anticipated benefits, maybe because of the variability in the quantity and quality of FO used, the oils used as controls, patient selection, absence of antioxidants to prevent rancidity of oils, and lack of availability of concentrated FO. At present, odor-free oils highly enriched in EPA and DHA are available and only a few capsules per day are needed to produce favorable results (26). A recent study has shown that dietary supplementation with FO may be beneficial in modifying symptomatic disease activity in SLE patients (27).

2.2. n-3 fatty acids on autoimmunity

Since n-3 FA have been found to increase apoptosis (28,29), it is quite possible that combination of various drugs with n-3 FA may increase the death of inflammatory cells, thereby, further increasing the therapeutic benefits of the drugs (30). N-3 FA initially provided beneficial effects in the control of renal disease in animal models and IgA nephropathy in humans (31-34). Our own studies, either with calorie restriction (CR) or FO, or recently with a combination of FO and CR, have shown several beneficial effects on antioxidant enzyme mRNA levels in B/W and MRL/lpr mice (26, 35-42). When B/W mice were fed ad libitum (AL), with either 5% corn oil (CO) or 5% FO, a significant increase in lifespan was noted in FO fed mice. In contrast, when 5% CO diet fed 40% CR mice, they lived much longer (almost double the life-span) whereas, when fed a diet with 5% FO with CR they, lived much longer than CO + CR fed mice . Several recent studies have also shown numerous beneficial effects of n-3 FA on normal strains of mice and rats as well as in healthy humans (43-49). The beneficial effects of n-3 FA have now been linked to changes in various genes and transcription factors including novel bioactive mediators, for example, resolvins, docosatrienes and protectins (11,50-53). Our ongoing studies on T-cell differentiation for Th1 and Th2 cytokine inhibition by n-3 FA and down regulation of NF-kappaB and other transcription factors (T bet) are likely to reveal new mechanistic information.

In the early years of research with n-3 FA, several adverse effects of FO were noted in humans and animals primarily because of oxidation of the oils. We, therefore, undertook detailed studies using vitamin E (vit-E) and we and others also reported much favorable results particularly, elevated antioxidant enzymes in n-3 FA + CR fed mice which may have decreased free radicals and facilitated in increasing the life span (36). Soon afterwards, the value of antioxidants on prevention of oxidation, particularly during storage of the capsules was recognized. Currently, odor-free concentrated FO with added antioxidants is regularly available at health food stores. Very recently, FDA approved OMACOR (FO) capsules are now available as prescription drug to treat hypertriglyceridemia in patients (54). Our recent studies clearly show that concentrated 5/50 DHA enriched FO is far more effective in controlling autoimmune disease and increasing the life-span of B/W mice (submitted for publication). Further, our recent studies using low levels of n-3 FA (5%) and CR not only showed increased longevity but also prevented bone loss with age (ongoing studies).

2.3. n-3 fatty acids on bone

Nutritional or dietary supplements containing anti-inflammatory compounds that are found in plant food or marine source such as fish that is consumed everyday, therefore, have minimal or are free from toxic side effects. One such nutritional supplement that affects pro-inflammatory cytokines and protects bone is n-3 FA. n-3 FA from oily fish decrease cytokines like IL-1beta, IL-6 and TNF-alpha, thereby, reducing bone resorption (28,36,38,55-59) (Table 1). We have shown that n-3 FA when fed to ovariectomized young Balb/C mice,

Table 1. Bone Loss and the Immune System

• The immune system has recently been linked to bone loss • Pro-inflammatory cytokines such as IL-1, IL-6 TNFα, GM-CSF and prostaglandin E2 increase osteoclast proliferation • Estrogens and TGF-β decrease production of these cytokines and inhibit osteoclast activation and bone resorption

Table 2. n-3 fatty Acids Prevents Bone Loss

• Lack of certain fatty acids in the diet contributes to bone loss • Mammals cannot synthesize fatty acids with a double bond past the Δ9 position • Dietary intake of EFAs determines membrane composition in all cells • Membrane EFAs determine the production of various cytokines by lymphoid cells

downregulated the expression of RANKL and inhibits activation of NF-kappaB suggesting that n-3 FA can inhibit osteoclastogenesis (60). In the case of ovariectomized (OVX) mice fed casein + CO, 20% bone was lost, whereas, casein + FO fed OVX mice had 10% bone loss. Interestingly, mice fed soy protein and CO had 13% bone loss, whereas, soy + FO had only 3% bone loss indicating that soy proteins + FO had far more protection against bone loss (Table 2).

The mechanism by which n-3 FA affects bone metabolism is probably multifaceted. In young male rats, n-3 FA have been reported to increase alkaline phosphatase activity (61) and in growing rats, IGF-1 and IGFBP levels increased (62,63). In another study, long term feeding of n-3 FA to MRL/lpr mice (6 weeks to 12 months of age) also showed that BMD increased at the end of the treatment period (64). n-3 FA are reported to modulate osteoblast functions by influencing the expression of core binding factor alpha 1 (cbfa1). Arachidonic acid (AA) and linoleic acid (LA) also increased the expression of cbfa1 (65). These studies suggest that n-3 FA may play a role in increasing bone formation. Studies on rats (F344 x BNF1) when fed n-3 FA showed higher serum IGF-I, parathyroid hormone, vitamin D, bone specific alkaline phosphatase and decreased urinary calcium suggesting that n-3 FA not only assists in bone formation but also in calcium absorbtion (66). Bone loss can be attributed to increased bone resorption by increased expression of NF-kappaB activity leading to osteoclastogenesis. *In vitro* studies have showed that some n-3 FA are linked to decreased NF-kappaB expression and modulation of RANKL signaling (67), whereas fatty acids from CO (LA+ Arachidonic) showed high NF-kappaB expression. So, n-3 FA may also reduce bone resorption by decreasing osteoclastogenesis.

It is well established that loss of body weight, seen after CR, is associated with lower bone mass. Similarly, male F344 rats, on 40% food restricted (FR) di*et al*so showed lower BMD. This study further reported that with age, rats fed AL lost bone whereas rats fed FR did not loose bone (68). Middle aged female F344 rats on 40% FR diet had lower cancellous bone mineral content in the proximal tibia, distal femur and the fourth lumbar vertebra when compared to that of AL rats (69). In the tibia-fibula junction there was increased bone resorption in the endocortical surface, thereby, increasing bone marrow space (70). In aged female Sprague Dawley rats, fed 40% energy restricted diet for 9 weeks, bones showed reduced BMD (71). In male Wistar rats, fed 80% food for 4 weeks, lower bone mass and strength were reported when compared to their AL fed counterparts (72). In male rhesus monkeys on 30% FR for 6 years, there was significantly reduced bone mineral content (73). The mechanism by which FR reduced bone mass is not yet clear, but bone remodeling in FR animals, especially in the cortical bone, seems to be envelope specific, since endocortical bone formation rates increased significantly but there was no change in the periosteal bone formation rates (70). We strongly feel that n-3 FA when fed either AL or moderate CR will prevent bone loss during aging.

3. ACKNOWLEDGMENTS

This work is supported by NIH grants - AG023648 and AT004259-01.

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Key Words: N-3 Fatty Acids, Autoimmunity, Osteoporosis, Review

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