

## Progress in nutritional immunology

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**Abstract** Optimum but balanced food intake maintains healthy growth and disease-free lifespan. However, imbalanced and over-nutrition promotes obesity, diabetes, malignancy, osteoporosis, infectious diseases, etc. In 1936, McCay reported that calorie restriction prevents weight gain and extend lifespan in rodents. In early 1970, Dr. Good at University of Minnesota and Dr. Walford at UCLA began studies in mice by reducing protein and calorie intake and studying their impact on immune function. Dr. Good's group (Jose, Fernandes, Kramer, Cooper, Day, etc.) reported changes in humoral and cellular immunity at present known as innate and adaptive immune function. Later, much interest was devoted by late Dr. Good on studying the role of calorie restriction (CR) and the role of zinc on immunity, particularly their role on aging, autoimmunity, and malignancy. Both functional role of T-cells, NK-cells and B-cells and their interaction during CR was studied extensively. We recently decided to pursue the beneficial effects of n-3 fatty acids (fish oil) with and without CR on controlling autoimmune-disease in NZB × NZW F1 mice. Our results indicated that n-3 FA when fed ad-libitum prolongs lifespan higher than commonly consumed n-6 FA (corn oil) in these mice. Moreover, n-3 FA + CR is found to be more effective than n-6 FA + CR. Some of the beneficial changes by n-3 FA include enhancing antioxidant enzymes and lowering Th-1/Th-2 cytokines, adhesion molecules, COX-2/PGE<sub>2</sub> levels, pro-inflammatory cytokines (IL-1 $\beta$ , IL-6 and TNF- $\alpha$  etc. The decreased pro-inflammatory cytokines were also found to protect against bone loss in OVX mice. Further, Fat-1 transgenic mice (which make n-3 FA endogenously in vivo from n-6 FA) when fed CR revealed decreased NF- $\kappa$ B and AP-1 activity and increased expression of life-prolonging gene SIRT1. Also CR and n-3 FA decreases body weight and increases insulin sensitivity, as well. Thus, to prevent obesity decreased calorie intake with n-3 FA supplement is far more effective and may have protection against CVD, malignancy, autoimmunity, and osteoporosis. The CR studies undertaken in primates and recently in humans are showing very encouraging results. In order to understand more precisely the role of diet and nutrition, new approaches exploring the link through nutrigenomics,

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proteomics and metabolomics may soon provide insight into controlling age-related diseases by following a balanced food intake.

**Keywords** n-3 fatty acids · Calorie restriction · Pro-inflammatory cytokines · Aging · Bone

## Introduction

The field of nutrition and immunology was originally developed by Dr. Robert A. Good in early 1970 at the University of Minnesota along with Drs. Yunis, Fernandes, Jose, Cooper, Kramer etc [1]. Interestingly, I also studied with Ranadive in early 1960, in India, the role of nutrition, particularly the use of pharmaceutical waste products as a dietary supplements, to increase the yield of eggs and meat in chickens and to improve the breeding performance in rats and mice, primarily because at that time, commercially pelleted diets for feeding laboratory animals were not available [2–5]. Due to this prior experience in nutrition and animal studies, I was fortunate to join Yunis's lab in 1968 and able to continue my nutrition studies with Yunis and particularly with Good and published a series of papers, studying the role of nutrition on breeding of mice and its influence on autoimmunity and renal disease in NZB and NZB × NZWF1 mice [6, 7]. However, most active work on nutrition and cellular immunity was, however, carried out by Jose and Good in early 1970, which received much attention to appreciate the protein deficiency and its relationship to cellular immunity [8–12]. Since Dr. Good is a noted pediatrician, he too immediately recognized and fostered nutrition research to study the impact of either nutrition deficiency during early growth and development or the adverse effects of excess nutrition on obesity, cancer, and particularly on aging. Soon with Yunis and Good, I also undertook a series of studies in the area of calorie restriction to prevent the development of renal disease and to reduce breast cancer in mice [13–17]. Later when Dr. Good moved to New York to head the Sloan Kettering Institute, he persuaded me to join him to continue our close and active collaborative work in the field of calorie restriction and aging. The brief summary of the impact of ad-libitum food intake on diseases of aging (Tables 1, 2) was very much in mind of Dr. Good and he was keen on studying various cellular and molecular mechanisms involved in reducing the development of autoimmune disease, cancer, and delaying the aging process by calorie restriction which he actively pursued with many of his young investigators in Oklahoma and at St. Petersburg, Tampa, Florida as well with me in San Antonio [18–34]. Dr. Good and we also studied in early years using short lived NZB × NZW F1 autoimmune prone mice fed ad-libitum (AL) were found to respond to AL feeding and found to die much earlier with kidney disease by becoming

**Table 1** Nutrition and immune function described originally in 1970s by late Dr. Good

- 
- Protein deficiency, calorie deficiency
  - Impairs macrophage function
  - Increases cytotoxic T-cell function
  - Decreases antibody production
  - Decreases serum inhibitor factors
-

**Table 2** Calorie Restriction (25–40%)

- 
- Prolongs life span
  - Inhibits diseases of aging
  - Prevents obesity and hyperglycemia
  - Increases antioxidant enzymes and DNA repair processes
  - Modulates immune function and alters gene expression
  - Decreases insulin resistance
  - CR studies in primates are encouraging and in humans CR trials are underway
- 

obese, whereas, CR fed mice maintain much less body weight and found to live significantly longer than AL fed high-fat or low-fat fed mice (Fig. 1).

### Calorie restriction

The earliest evidence that CR retards aging and extends median and maximum lifespan in rodents was first presented in the 1930s by McCay et al. [35]. Since then similar observations have been made in several species including mice, rats, fish, etc. [36, 37]. Our own CR studies which had began over 30 years ago with Dr. Good, have investigated the role of CR on mammary cancer [13], autoimmune disease [16], and aging mice and rats [38–40]. Earlier studies undertaken in CR fed mice for changes in immune functions were also carried out at the same period by Walford and Weindruch et al. [41–43]. We also reported marked changes in IL-2 production [20, 44], Th1/Th2 subsets, changes in insulin receptors and an increased long-chain FA [45, 46]. In addition, we reported increased levels of free-radical scavenging antioxidant enzymes in CR fed mice [46]. Recently, caloric or energy restriction was shown to down regulate expression of several genes linked to inflammation by using oligonucleotide microarrays [47]. Masoro et al. has made very important observations in rats fed CR lifelong and has reviewed this subject very often [48–50].

**Fig. 1** NZB/W female mice as SLE disease model

Weindruch et al. have also made several key observations including major changes by CR in gene regulation. Due to earlier studies by both Dr. Good and Walford and their coinvestigators, and based on encouraging results seen in rodents, the CR studies were then undertaken later in primates and appear to show several benefits [51–53]. Longevity remains to be still established in these models but delayed death with CR is anticipated. CR fed monkeys have lower-body temperature and insulin concentration than AL fed monkey [54]. It is quite encouraging to note that moderate CR studies in humans are revealing quite encouraging results even with a 6 month dietary duration [55]. The precise mechanism of CR-induced lifespan extension is not fully known, yet several theories have been proposed which were recently discussed briefly by Masoro [48, 49]. Although restricting individual nutrients can extend lifespan, based on extensive studies, cutting down 20–40% macronutrients or calories but keeping same level of vitamins and micronutrients can extend maximal lifespan [48]. Several theories have been put forth for the primary mechanism involved in the extension of lifespan such as 1. The growth retardation hypothesis; 2. Reduction of body fat hypothesis; 3. Reduction of metabolic rate hypothesis; 4. Oxidative damage attenuation hypothesis; 5. Altered glucose-insulin system hypothesis; 6. Alteration of the growth hormone IGF-I Axis hypothesis; and 7. Hormesis hypothesis. However, at present based on recent supportive data the following two hypotheses 1. Oxidation damage attenuation hypothesis and; 2. Attenuation of insulin-like signaling hypotheses are both gaining much more attention for elucidating the mechanism involved in prolonging the lifespan [48, 56].

Due to our published experience with nutrition, immunity and aging we later initiated studies using n-3 FA alone AL or along with CR, we decided to pursue our recent hypotheses i.e., prevention of oxidative damage, inhibition of inflammation, and inhibition of insulin-like signaling pathway by adopting n-3 FA + CR studies. There is evidence that n-3 FA alone increases insulin sensitivity thus combining n-3 FA + CR should have a synergistic effect during aging. Most importantly, we also have pilot data to support these two hypotheses: that n-3 FA + CR decrease inflammatory mediators and that it decreases insulin and glucose and increases adiponectin in CR fed aging mice. Oxidative damage and ROS production by various immune cells follows during aging in many target tissues and are now linked to the functional loss in metabolic energetics [57]. Recent studies have shown that age-dependent increase in mitochondrial oxidative damage induces rise in ROS production [57], and their attenuation by CR [58].

Indeed, inhibition of ROS generation maybe a major cause for the reduction in the mitochondrial DNA (mtDNA) damage in CR. We strongly feel that because of upregulation of antioxidant enzymes by n-3 FA [59] and also by CR [60], it is now strongly linked to the upregulation of anti-inflammatory genes particularly those encoding ROS scavenging proteins [61]. The capability of long-term CR to induce decrease in ROS production has been shown in various tissues by various investigations such as rat mitochondrial gastrocnemius muscle [58] rat heart mitochondria [62] and rat liver mitochondria [63]. It is well-established that mitochondrial DNA plays a major role during the aging process [64]. A series of studies carried out in AL and CR fed rats have shown that long-term CR decreases the rate of mitochondrial H<sub>2</sub>O<sub>2</sub> production and mtDNA oxidative damage in various tissues [58]. It was also shown that the quantitative reduction of mtDNA oxidative damage was closer to that found for mitochondrial free-radical generation in various tissues. We too have noticed increased antioxidant enzymes and decreased free radicals (MDA production) in CR fed animals.

## Calorie restriction and SIRT1

In the field of CR and aging research many investigators were searching for life prolonging genes and were fortunate to first identify in yeast a silencing information regulator 2 (Sir2) gene [65]. It was induced by CR in yeast and *Drosophila* and shown to have an effect in life prolonging effect of CR [66]. Deletion of this gene led to the elimination of life prolonging effect of CR. It was simultaneously shown to be a histone deacetylase. The deacetylation of histones lead to the closure of the chromatin in the nucleosome with a suppression of gene transcription. Furthermore, acetylation of histones leads to an opening-up of the chromatin in the nucleosome and an increase in gene transcription. The activity of histone acetylation is dependent upon NAD, which accumulates intracellularly in the absence of glycolysis [67, 68]. These life-prolonging genes have been further identified in mammals and have been given the name sirtuins (SIRT1-7 in humans). Among these SIRT1 is the first to have a role linking it to aging and in metabolic regulation. SIRT1 in mammals has now been shown to deacetylate proteins other than histones. They induce p65, a component of NF- $\kappa$ B signaling pathway and is a major mediator of the transcription of NF- $\kappa$ B dependent pro-inflammatory genes [69]. In addition, SIRT1 also deacetylates FOXO-1 [70] and p53 [71] and thus SIRT1 may have a role in the regulation of metabolism and cell proliferation. SIRT1 has recently been shown to have an important role in metabolic regulation [72]. In fasting or CR fed mice SIRT1 has been shown to be activated by increasing concentrations of pyruvate. SIRT1 in turn stimulates genes which regulate gluconeogenesis like PEPCK and SIRT1 suppresses glycolysis simultaneously [73]. These actions would stimulate hepatic glucose production and reduce glucose utilization. In this role it acts through a physical and functional association with PGC-1 $\alpha$  and HNF 4 $\alpha$  [73]. It also deacetylates PGC-1 $\alpha$  to induce gluconeogenesis. Thus, it may have a hyperglycemia inducing effect and may play a role in the regulation of fasting state during CR. However, it appears that the role of SIRT1 is much more complex and needs more research to elucidate its role in regulation and interaction with various factors like oxidative stress and protection induced by CR during aging. We, however, would like to establish whether the expression of SIRT1 varies between n-6 FA and n-3 FA, fed AL and CR mice, and particularly in transgenic Fat-1<sup>+</sup> mice fed CR diet. Elevated SIRT1 is quite apparent in Fat-1<sup>+</sup> + CR mice in our pilot studies (unpublished data), and therefore we plan to measure PGC-1 $\alpha$  and PPAR $\gamma$  expression along with SIRT1 to see its close interaction during aging, particularly, in mice fed n-3 FA + AL and n-3 FA + CR. We anticipate mice fed n-3 fatty acids and CR may live much longer than mice on corn oil and CR diets.

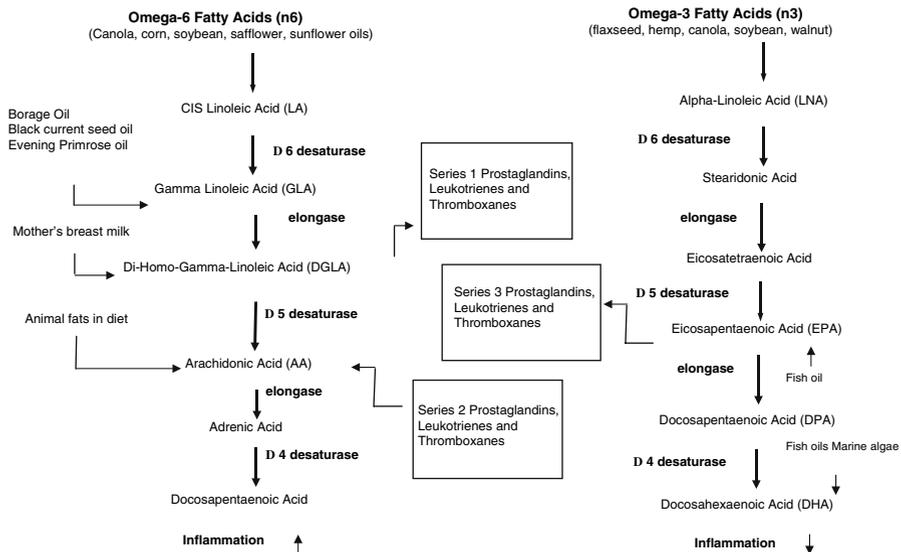
## Role of n-3 fatty acids in health and disease

Since the original report of Bang et al. [74] 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 as dietary supplements, and interest in clinical uses is receiving much attention [75, 76]. Several studies have shown promising results against inflammatory disorders including cardiovascular and autoimmune disorders [77, 78]. It is now accepted that the use of n-3 fatty acids will decrease the risk of CVD in the US population [79]. n-3 fatty acids also decreased the incidence of several chronic diseases that are closely linked particularly to the increase in saturated fat and n-6 vegetable oil consumption in the USA [80]. 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), and particularly HD related deaths [81]. Furthermore, the blood level of n-3 fatty acids is a predictive biomarker for sudden death in men [77, 82]. Although the mechanism of n-3 fatty acid 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 [83–88]. Figure 2 shows the synthesis of higher-chain FA and lists the n-6 and n-3 FA sources [89].

The majority of studies on the effects of n-3 fatty acids have been carried out using FO or FO concentrates containing low levels of EPA and DHA. Even when purified EPA or DHA has been used there is a significant interconversion in the body *in vivo*, therefore, the effects of one without the other cannot be inferred with absolute certainty. 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 is found in high concentrations in brain tissue. DHA promotes neurite growth and strengthens the periventricular vascular system against hemorrhage [90].

It is widely accepted that FO, rich in n-3 polyunsaturated fatty acids, protect against several types of cardiovascular diseases such as myocardial infarction, arrhythmia, atherosclerosis, and hypertension [91, 92]. Although, the precise cellular and molecular mechanisms for these beneficial effects are still unknown, one of the mechanisms may be their direct effect on vascular smooth muscle cell functions. These n-3 PUFAs activate K<sup>+</sup> ATP channels and inhibit certain types of Ca<sup>2+</sup> channels [93]. There are probably 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, 2) n-3 PUFAs also 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 [94]. DHA has also been reported to be the most potent at decreasing plasma triglycerides, and

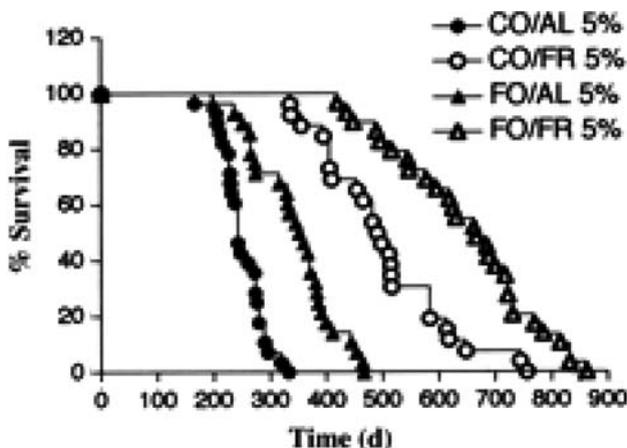


**Fig. 2** Dietary sources and the AA cascade

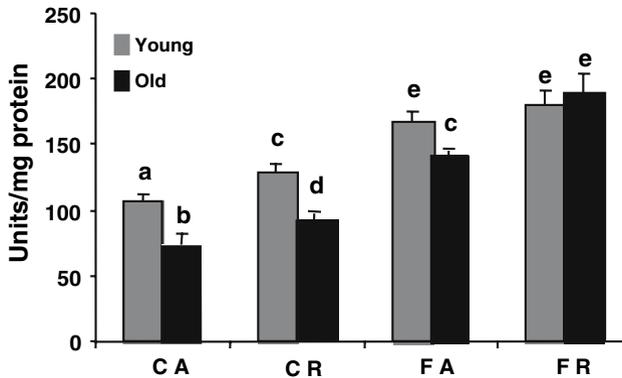
DHA (but not EPA) supplementation significantly increased serum HDL-cholesterol [95, 96] which is associated with more efficient reverse cholesterol transport and reduced risk of coronary heart disease [97]. At least a portion of the hypotriglyceridemic effect of n-3 fatty acids has been attributed to increasing circulating apoE levels [98].

Besides, numerous beneficial effects of n-3 FA on several other chronic diseases including osteoporosis are also encouraging. Even though some earlier clinical fish oil studies did show anticipated benefits, some of the reasons for unfavorable results were the variability in the quantity and quality of FO used, variations in the oils used as controls, variability in patient selection, absence of antioxidants to prevent rancidity of oils, and lack of availability of concentrated FO early on. 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 [99]. A recent study has shown that dietary supplementation with FO may be beneficial in modifying symptomatic disease activity in SLE patients [100].

Since, n-3 fatty acids have been found to increase apoptosis [101, 102], 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 [103]. n-3 FA initially provided beneficial effects in the control of renal disease in animal models and IgA nephropathy in humans [104–107]. Our own studies, either with 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 [59, 99, 108–114]. When B/W mice were fed with either with 5% corn oil or 5% fish oil AL a significantly increased lifespan was noted in FO fed mice. In contrast, when a 5% corn oil diet when fed 40% CR, mice lived much longer almost double the life-span whereas when fed a diet with 5% fish oil with CR they however lived much longer than CO + CR fed mice (Fig. 3). Several recent studies have also shown numerous beneficial effects of n-3 fatty acids on normal strains of mice and rats as well as in healthy humans [115–121]. The beneficial effects of n-3 fatty acids have now been linked to changes in various genes and transcription factors including novel bioactive mediators, for example, resolvins, docosatrienes and protectins [84, 122–125]. Our ongoing studies on T-cell differentiation for Th1 and Th2 cytokine



**Fig. 3** Effect of fat source and calorie restriction on survival in low-fat-fed NZB × NZW F1 mice. Survival curves are significantly different at  $P < 0.0001$  by Logrank test. Median survival CO/AL 242, CO/CR 450, FO/AL 354, FO/CR 665. Source: Jolly, et al., J. Nutr. 131:2753, 2001



**Fig. 4** Catalase activity in kidney homogenates of Young (4 mo) and Old (8 mo) B/W Mice. Source: Jolly, et al., *J. Nutr.* 131:2753, 2001

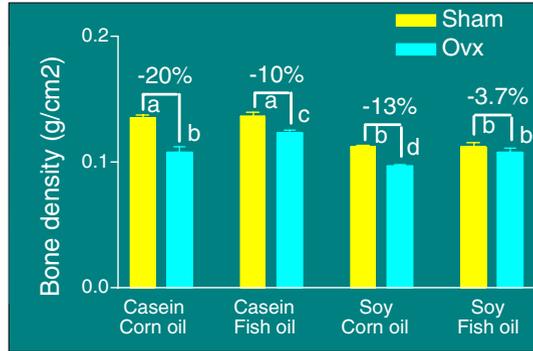
inhibition by n-3 fatty acids and down regulation of NF- $\kappa$ B and other transcription factors (T bet) likely to reveal new mechanistic information.

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, the elevated antioxidant enzymes in n-3 FA + CR fed mice which may have decreased free radicals and facilitated in increasing the life span [59] (Fig. 4). 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 (fish oil) is available to treat hypertriglyceride in patients [126]. Our recent studies clearly showed that concentrated 5/50 DHA enriched fish oil 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 is also not only showing increased longevity but also showed to prevent bone loss with age as well (ongoing studies).

### n-3 fatty acids on bone

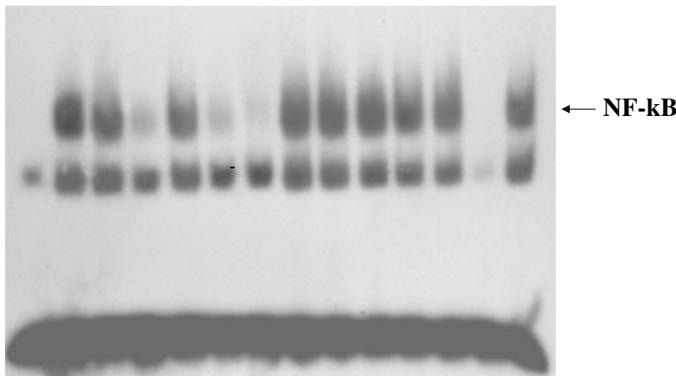
Nutritional supplements are compounds that are found in food material that is consumed everyday, therefore any side effects are minimal or none. One such nutritional supplement that affects the pro-inflammatory cytokines and protects bone is n-3 FA. n-3 FA decrease cytokines like IL-1 $\beta$ , IL-6 and TNF- $\alpha$ ? thereby, reducing bone resorption [39, 59, 60, 101, 110, 127–129]. We have shown that n-3 FA when fed to ovariectomized young Balb/C mice downregulated the expression of RANKL and inhibits activation of NF- $\kappa$ B suggesting that n-3 FA can inhibit osteoclastogenesis [130]. In the case of mice fed casein and corn oil and when ovariectomized 20% bone was lost whereas casein and FO fed mice had 10% bone loss. Interestingly, mice fed soy protein and corn oil had 13% bone loss whereas, soy + FO had only 3% bone loss indicating that soy proteins + FO had far more protection against bone loss (Fig. 5). Further, the mechanism of bone loss was also linked to increased NF- $\kappa$ B expression. In-vitro when EPA+DHA added to bone marrow cells showed

**Fig. 5** Effect of ovariectomy, protein source and fat source on bone density in distal left femur of Balb/C mice. Source: Fernandes et al., Prostaglandins Leukot Essent Fatty Acids 68:361–372, 2003



markedly less NF-κB expression, whereas, fatty acids from corn oil (LA + Arachidonic) showed high NF-κB expression(Fig. 6). 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 [131]. In other studies using young male rats, n-3 FA have been reported to increase alkaline phosphatase activity [132] and in growing rats, IGF-1 and IGFBP levels increased [133, 134] suggesting that n-3 FA may play a role in increasing bone formation as well. In Table 3 and 4 briefly the immune mechanism involved and the role of n-3 FA in preventing bone loss are listed.

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) diet also showed lower BMD. This study further reported that with age, rats fed AL lost bone whereas rats fed FR did not loose bone [135]. 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 [136]. In the tibia-fibula junction there was increased bone



RANKL	-	+	+	+	+	+	+	+	+	+	+	+	+	+
DHA(μM)	-	-	10	40	-	-	40	-	-	-	-	-	-	-
EPA(μM)	-	-	-	-	10	40	40	-	-	-	-	-	-	-
LA(μM)	-	-	-	-	-	-	-	10	40	-	-	40	-	-
AA(μM)	-	-	-	-	-	-	40	-	-	10	40	40	-	-
50×NF-κB	-	-	-	-	-	-	-	-	-	-	-	-	+	+
50×AP-1	-	-	-	-	-	-	-	-	-	-	-	-	-	+

**Fig. 6** Effect of fatty acids on NF-κB activation in bone marrow derived macrophages. Source: Sun, et al. (2003) J Bone Miner Res 18: 1206, 2003

**Table 3** Bone loss and the immune system

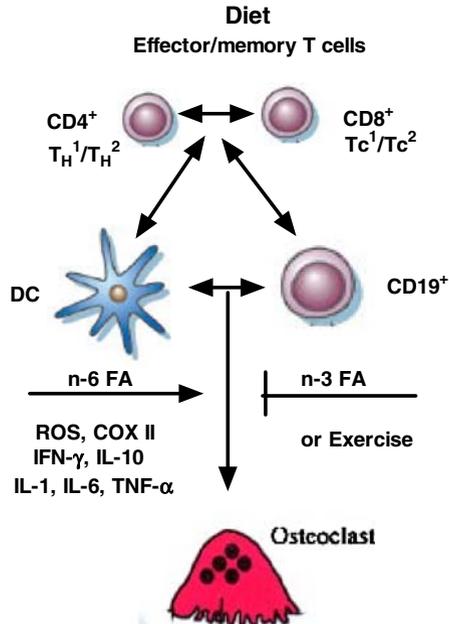
- The immune system has recently been linked to bone loss
- Pro-inflammatory cytokines such as IL-1, IL-6, TNF $\alpha$ , GM-CSF, and prostaglandin E2 increase osteoclast proliferation
- Estrogens and TGF- $\beta$  decrease production of these cytokines and inhibit osteoclast activation and bone resorption

**Table 4** 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  $\Delta 9$  position
- Dietary intake of EFAs determines membrane composition in all cells
- Membrane EFAs determine the production of various cytokines by lymphoid cells

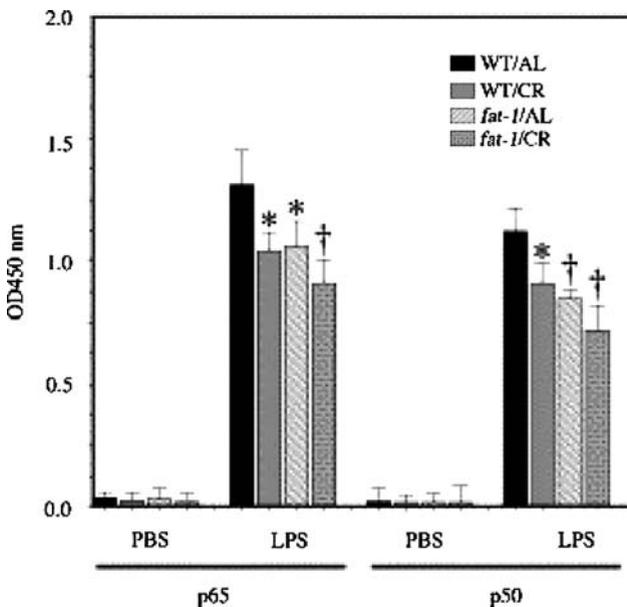
resorption in the endocortical surface thereby increasing bone marrow space [137]. In aged female Sprague Dawley rats, fed 40% energy restricted diet for 9 weeks, the bones showed reduced BMD [138]. In male Wistar rats, fed 80% food for 4 weeks, lower-bone mass and strength were reported when compared to their AL fed counterparts [139]. In male rhesus monkeys on 30% FR for 6 years, there was significantly reduced bone mineral content [140]. The mechanism by which FR reduces bone mass is not yet clear, but bone modeling 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 [137]. We strongly feel that n-3 FA when fed either AL or moderate CR will prevent bone loss during aging. In Fig. 7, we summarize also the immune and molecular mechanism involved in preventing bone loss by n-3 FA.

**Fig. 7** Prevention of osteoporosis by diet



## Fat-1 mouse

Recently, Kang et al. developed a transgenic (Tg) mouse model that can endogenously synthesize n-3 FA and lowers in vivo n-6 FA. The synthesis of n-3 FA was achieved through the expression of the Fat-1 transgene encoding for an n-3 desaturase from *Caenorhabditis elegans* which utilizes n-6 FA as a substrate [141, 142]. In wild type mice, the polyunsaturated FA (n-6 FA) found in the tissues are mainly (98%) the linoleic (LA, 18:2n-6) and arachidonic acids (AA, 20:4n-6) with trace amounts of n-3 FA. In contrast, in these transgenic mice, a large amount of n-3 FA including  $\alpha$ -linoleic (ALA, 18:3n-3), EPA (20:5n-3) and DHA (22:6n-3) are found in all the tissues [143, 144]. Thus, the levels of n-6 FA LA and AA, in Tg mice tissues are significantly reduced indicating the conversion of n-6 FA to n-3 FA, and hence the ratio of n-6/n-3 is reduced from 20:1 to nearly 1:1. This reduction of n-6/n-3 FA ratio leads to an anti-inflammatory state instead of pro-inflammatory state without feeding any exogenous n-3 FA. We feel that this animal model will be useful to study the role of n-3 FA parallel to exogenously supplied n-3 FA. Last year, we obtained Fat-1 breeding pairs from Dr. Kang, and are bred in-house. We recently crossed Fat-1<sup>+</sup> mice with B6 mice and after genotyping the progeny, mice were separated into Fat-1<sup>+</sup> x B6 and Fat-1<sup>-</sup> X B6 mice. These mice have similar genetic background except that Fat-1 gene was found in ~50% of the mice. This mouse model may be an invaluable tool to establish the role of n-3 FA on longevity both in the absence and in combination with CR. Our pilot data indicate that Fat-1 gene prevents bone loss in OVX mice. Moreover, elevated SIRT1 protein levels and decreased IL-6 and TNF- $\alpha$  cytokines were also observed in Fat-1<sup>+</sup> + CR mice (unpublished observation). Thus, we are extremely excited to undertake our proposed CR studies both in B6 mice fed n-3 FA as well as Fat-1 mice with CR. Our recent published studies [145] indicates significantly decreased NF- $\kappa$ B



**Fig. 8** Effect of fat-1 transgenic mice fed calorie restricted on LPS-induced NF- $\kappa$ B p65/p50 activity in cultured splenocytes. WT/AL = wild type ad libitum, WT/CR = wild type calorie restricted, fat-1/AL = fat-1 mice ad libitum, fat-1/CR = fat-1 mice calorie restricted. Bhattacharya et al., BBRC 349:525–530, 2006

in Fat-1<sup>+</sup> + CR fed mice than in Fat-1<sup>-</sup> + CR fed mice indicating the anti-inflammatory role of n-3 FA particularly along with CR (Fig. 8). Thus, new studies are needed to establish whether moderate CR diet along with consumption of fish or supplementing with fish oil capsule will provide benefits in decreasing age-related on set of diseases particularly CVD and bone loss during aging.

## Summary

In summary, soon after the discovery of the importance of bone marrow and the thymus in the development of B-cells and T-cells, Dr. Good also established the importance of nutritional deficiency and excess in altering the cellular and humoral immunity and later the changes in innate and adaptive immunity to prevent infection and to enhance the cellular immunity during the protein-calorie deprivation using various animal models. Further, the role of moderate caloric or food restriction in increasing life-span of short lived and long lived animals and in the reduction of mammary cancer was also unfolded by Dr. Good and his coinvestigators.

In later years the functional role of n-3 fatty acids and caloric restriction on reducing the autoimmunity and inflammation particularly the role of n-3 fatty acids on osteoclastogenesis was also established by my coinvestigators and myself. It is with great respect, we recognize the foresight of Dr. Good in pursuing the role of nutrition and diet on immunological function and its relationship on the development of diseases of aging.

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