Life Span Is Prolonged in Food-Restricted Autoimmune-Prone (NZB × NZW)F(1) Mice Fed a Diet Enriched with (n-3) Fatty Acids[1]

Christopher A. Jolly, Alagarraju Muthukumar, C. P. Reddy Avula, Dean Troyer* and Gabriel Fernandes[2]

*Division of Clinical Immunology, Department of Medicine and *Department of Pathology, The University of Texas Health Science Center at San Antonio, San Antonio, TX 78229-3900*

ABSTRACT Moderate food and/or energy (calorie) restriction delays age-related immune dysfunction and prolongs life span in multiple animal models. The amount and type of dietary fatty acids can also profoundly affect life span. Marine-derived fish oils contain (n-3) fatty acids, which have potent anti-inflammatory properties. We therefore examined the influence of food restriction (40% overall reduction in intake of all dietary components) combined with substitution of fish oil for corn oil in a factorial design. Autoimmune-prone (NZB × NZW)F(1) (B/W) mice, which develop fatal autoimmune renal disease, were used. The food-restricted/fish oil diet maximally extended median life span to 645 d (vs. 494 d for the food-restricted corn oil diet). Similarly, fish oil prolonged life span in the ad libitum–fed mice to 345 d (vs. 242 for the ad libitum/corn oil diet). Increased life span was partially associated with decreased body weight, blunting renal proinflammatory cytokine (interferon-γ, interleukins-10 and -12 and tumor necrosis factor-α) levels and lower nuclear factor-κB (NF-κB). Reductions in NF-κB were preceded by enhanced superoxide dismutase, catalase and glutathione peroxidase activities. These findings demonstrate the profound additive effects of food restriction and (n-3) fatty acids in prolonging life span in B/W mice. These observations may have additional implications in the management of obesity, diabetes, cancer and/or the aging process. J. Nutr. 131: 2753–2760, 2001.

KEY WORDS: • *food restriction* • *(n-3) fatty acids* • *renal disease* • *autoimmunity* • *mice*

Over 65 y ago, food restriction was shown to extend the life span of rodents (1), and subsequent studies have confirmed this in a variety of models (2–4). Our previous studies showed that a 40% food-restricted, corn oil–based diet (CO/FR)[3] delays the onset of autoimmune kidney disease (5), decreases renal platelet-derived growth factor A (PDGF-A) and thrombin receptor expression (6), reduces the expression of serum gp70 and immune complex deposition (7,8), and decreases glomerular expression of plasminogen activator inhibitor type 1 (9).

The autoimmune diseases, rheumatoid arthritis (RA), Sjogren's Syndrome (SS) and systemic lupus erythematosis (SLE) are major causes of disability in the United States. Treatment of these diseases requires high doses of immunosuppressive agents given over prolonged time periods. The side effects of these drugs pose a heavy burden to the patient as well as the economy as a whole, and alternative strategies are required to reduce the dosage and/or diminish the toxicity of these potent drugs. Rodent and human studies have demonstrated that dietary fish oil ameliorates the autoimmune diseases (10–12) RA (13), SS (14) and SLE (15,16) as well as malignancy (17,18) and, most notably, cardiovascular disease (19–21). Nutritional modulation, particularly energy (calorie) and/or food restriction, offers the prospect of delaying or suppressing autoimmune disease and malignancy and has been found to reduce drug toxicity (3,4,22).

An excellent model with which to study SLE is the (NZB × NZW)F(1) (B/W) female mouse (23), which is frequently used to study the influence of diet and immunosuppressive drugs on autoimmunity (24). The B/W female, slightly obese mouse develops nephritis at ~5 mo of age and succumbs to glomerular disease and renal failure at 6–12 mo of age. In B/W mice, T-lymphocyte activation and induction of polyclonal B-cell proliferation result in the production of autoantibodies to histones and double-stranded DNA. The immune complexes are deposited in the kidney, leading to mononuclear immune cell recruitment (25) and release of the proinflammatory cytokines interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α) and PDGF as well as transforming growth factor-β (TGF-β) (26). These cytokines can modulate the

[1] Supported in part by National Institutes of Health grants F32 AG05826 (to C.A.J.) and RO1 AG13693 and RO1 AG14541 (to G.F.).

[2] To whom correspondence should be addressed.
E-mail: fernandes@uthscsa.edu.

[3] Abbreviations used: AA, arachidonic acid; AP-1, alkaline phophatase 1; B/W, (NZB x NZW)F(1); CAT, catalase; CO, corn oil; CO/AL, corn oil–based diet, ad libitum intake; CO/FR, corn oil–based diet, restricted intake; DHA, docosahexaenoic acid ; EMSA, electrophoretic mobility shift assay; EPA, eicosapentaenoic acid; FO, fish oil; FO/AL, fish oil–based diet, ad libitum intake; FO/FR, fish oil–based diet, restricted intake; GAPDH, D-glyceraldehyde-3-phosphate dehydrogenase; GSH-Px, glutathione peroxidase; GST, glutathione-*S*-transferase; IFN-γ, interferon-γ; IL, interleukin; NF-κB, nuclear factor κB; PCR, polymerase chain reaction: PDGF-A, platelet-derived growth factor A; PGE_2, prostaglandin E_2; PUFA, polyunsaturated fatty acids; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; SOD, superoxide dismutase; SS, Sjogren's syndrome; TGF-β, transforming growth factor-β; TNF-α, tumor necrosis factor-α.

0022-3166/01 $3.00
Manuscript received 12 January 2001. Initial review completed 26 February 2001. Revision accepted 2 July 2001.

function of immune cells (macrophages, B lymphocytes and T lymphocytes) and resident glomerular cells (26).

Substitution of fish oil (FO), enriched in the (n-3) polyunsaturated fatty acids (PUFA) for corn oil (CO), which is rich in (n-6) PUFA, delays autoantibody production, resulting in increased life span of B/W mice (27–29). The increased life span of CO/FR mice is accompanied by decreased renal expression of the proinflammatory growth factor TGF-β and cytokines [interleukin (IL)-1β, IL-6 and TNF-α], increased expression of antioxidant enzymes (30) and decreased expression of c-Myc and c-Ha-Ras oncogenes (31).

Although both FR and FO supplementation profoundly suppress autoimmune kidney disease in B/W mice (32), it is not known whether the combination of food restriction (FR) with fish oil (FO/FR) is advantageous over either alone. Therefore, we combined dietary FO with FR in B/W mice and determined the long-term effects on life span and body weight in a large number of mice. In addition, renal pathology, cytokine expression at both the protein and mRNA levels, and antioxidant enzyme activity were examined at 4 (young) and 8 mo (old) of age.

MATERIALS AND METHODS

Materials. All antibodies were obtained from Pharmingen (San Diego, CA). Dietary ingredients were from ICN (Costa Mesa, CA). All other chemicals were reagent grade or better. Fish oil was obtained from the U.S. Department of Commerce, National Marine Fisheries Service, Charleston, NC and contained 8.6 g docosahexaenoic acid (DHA) and 13.3 g eicosapentaenoic acid (EPA)/100 g by gas chromatographic analysis (33).

Mice and diets. Weanling female B/W mice (4 wk old) were obtained from the Jackson Laboratory (Bar Harbor, ME) and housed and fed as previously described (6). The AIN76A diet was used with the substitution of fish oil for corn oil in two of the dietary groups. The four dietary groups were as follows: 5 g/100 g corn oil, ad libitum consumption (CO/AL); 5 g/100 g corn oil, 40% food restricted (CO/FR); 5 g/100 g fish oil + 0.5 g/100 g corn oil, ad libitum consumption (FO/AL); and 5 g/100 g fish oil + 0.5 g/100 g corn oil, 40% food restricted (FO/FR). Corn oil (0.5 g/100 g) was added to the fish oil diets as a source of linoleic acid to prevent essential fatty acid deficiency (9). The diet consisted of the following (g/kg): casein, 200; dextrose, 300; cornstarch, 315; oil, 50; cellulose, 35; AIN76A mineral mixture, 35; AIN-76 vitamin mixture, 15; DL-methionine, 3; and choline chloride, 2 (34). The oils used in the diets were supplemented to contain 1300 IU α-tocopherol and 1 g *t*-butylhydroquinone/kg oil to prevent oxidation as recommended by the NIH. Diets were prepared weekly and stored at 4°C. Fresh diet was provided daily and any diet remaining from the previous day was discarded. Food intake per gram of body weight in the CO/FR and FO/FR groups was similar to that of AL mice (9). Mice were weighed weekly and, beginning at 5 mo of age, proteinuria was determined weekly using chemstrips (Boehringer Mannheim, Indianapolis, IN) until mice were killed at 8 mo of age. All procedures performed were approved by the institutional animal use and care committee. At 4 (young) or 8 mo (old) of age, mice were killed and whole kidneys removed. One half kidney was placed in OCT 4583 embedding medium for histology studies (Sakura Finetek, Torrance, CA) and the other placed in a 1.5-mL cryovial. Both kidneys were snap frozen in liquid nitrogen and stored at −80°C until further analysis. In each dietary group, ~30 mice were followed until their urinary protein reached 3+ and they had lost 25% of their body weight; at that time, they were killed and the life span recorded. Additional similar groups of mice were utilized for cross-sectional studies at 4 and 8 mo of age.

Histology. Tissue for light microscopic examination was fixed in 1.0 g/L buffered formalin, embedded in paraffin, sectioned at 5 μm and stained with hematoxylin and eosin (H & E) using standard techniques. Histological severity of glomerular disease was graded on a semiquantitative scale from 0 to 4 as described (9,31).

Cytokine Western blotting. Protein isolation was carried out essentially as previously described with a few modifications (35). Briefly, whole-kidney tissue (50 mg) was homogenized in lysis buffer containing 50 mmol/L Tris base, 10 mmol/L EDTA, 150 mmol/L NaCl, 0.01 g/L Tween-20, pH 7.4, with 1 mg/L leupeptin and pepstatin and 100 μmol/L phenylmethylsulfonyl fluoride. An equal volume of lysis buffer was added and the samples incubated for 15 min on ice. The samples were then centrifuged at 13,000 × *g* for 3 min and the supernatant divided into aliquots and stored at −80°C. An aliquot was retained for protein determination using the BioRad Assay (BioRad, Hercules, CA). Western blotting was carried out as previously described (36) except that 50 μg protein was used at a 1:2000 dilution. Visualization of the bands was carried out as described by the manufacturer using SigmaFast diamino benzidine with metal enhancer tablets (Sigma, St. Louis, MO). Band intensity was determined using an AlphaImager 2000 (AlphaInotech, San Leandro, CA).

Cytokine mRNA analysis. Total RNA was isolated from 50 mg of kidney tissue, cDNA synthesized by reverse transcriptase-polymerase chain reaction (PCR) then amplified by PCR as previously described (37). Briefly, the PCR reaction was carried out with 27–30 amplification cycles for IFN-γ, IL-10 and IL-12 or 17 amplification cycles for D-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) of 94°C for 30 s, 59°C for 30 s and 72°C for 1 min.

Electrophoretic mobility shift assay (EMSA) for nuclear factor (NF)-κB. Nuclear extraction and the EMSA were carried out as previously described (38). Briefly, 50 mg of whole kidney tissue was homogenized, incubated on ice for 15 min and 0.625% nonidet P-40 was added; the tubes were vortexed for 10 s and centrifuged at 12,000 × *g* for 30 s. The nuclear pellet was resuspended in a buffer containing 0.4 mol/L NaCl and incubated for 15 min on ice with vigorous shaking. The nuclear extract was centrifuged for 5 min at 12,000 × *g* and protein concentrations determined using the BioRad Assay. Aliquots were stored at −80°C for later use.

The double stranded DNA probes for NF-κB and alkaline phosphatase (AP)-1 were from Promega (Madison, WI). The NF-κB was labeled with [^{32}P-γ]ATP (Amersham, Arlington Heights, IL) using T4 polynucleotide kinase (Promega). Approximately 50,000 cpm of the labeled NF-κB was incubated with 10 μg of kidney protein for 1 h on ice. The mixture was then loaded onto a 5% polyacrylamide gel and subjected to electrophoresis at 20 mA. The dried gel was autoradiographed on Kodak XAR-5 film. The gels were quantitated by densitometry using NIH Image.

Assay for antioxidant enzyme activity. Superoxide dismutase (SOD) activity was measured by the method of Flohe and Otting (39) Catalase (CAT) activity was assayed according to the method of Aebi (40). Glutathione peroxidase (GSH-Px) activity was determined by the method of Tappel (41). Glutathione-*S*-transferase (GST) activity was measured by the method of Habig et al. (42). Protein was measured using the bicinchoninic acid protein assay reagent (Pierce, Rockford, IL); bovine serum albumin was the reference standard.

Statistics. All data were analyzed by factorial ANOVA using NCSS software v. 5.01 (NCSS, Kaysville, UT) or one-way ANOVA with Bonferroni's or Newman-Kuels post-hoc test using GraphPad Prism version 3.00 for Windows (GraphPad Software, San Diego, CA). $P < 0.05$ was considered significant. Survival analysis was conducted using SPSS routines (SPSS, Chicago, IL).

RESULTS

Life span. Similar to our previously published observations, both FO/AL (29) and CO/FR (5) increased the median and maximum life spans of B/W mice compared with CO/AL (242 and 332 d, respectively, $P < 0.0001$) (**Fig. 1**). When FO and FR were combined (FO/FR), median and maximum life spans were extended to 645 and 862 d (2.7- and 2.6-fold), much more than either FO/AL (345 and 466 d, 1.4-fold) or CO/FR (491 and 755 d, 2.0- and 2.3-fold). CO/FR mice had lower body weights relative to the CO/AL and FO/AL groups (**Fig. 2**). Body weights of the AL groups peaked at 8 mo with FO mice reaching the highest weight. The weight gain of FR mice was less pronounced, but FO/FR mice again gained

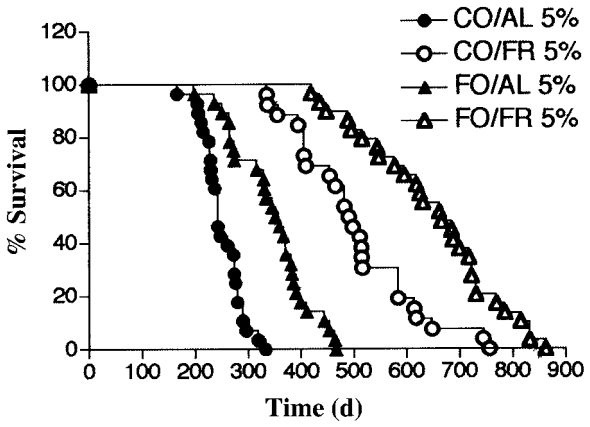

FIGURE 1 Food restriction with dietary (n-3) fatty acids increases life span in female (NZB x NZW)F(1) mice. Female (NZB x NZW)F(1) mice, 6 wk old, consumed a semipurified diet containing 5 g/100 g corn oil, free access (CO/AL); corn oil, 40% food-restricted (CO/FR); fish oil, free access (FO/AL); or fish oil, 40% food restricted (FO/FR). All groups differed from one another, $P < 0.0001$. Values are expressed as the percentage of mice in each dietary group alive ($n = 30$/group initially).

somewhat more weight than CO/FR-fed mice. In both AL and FR mice, body weights declined sharply in the weeks before death due to the severity of the renal disease.

Renal histology. As described previously (31), substitution of FO for CO modulated the histological lesions in older AL mice. In CO/AL mice, both tubular dilation and tubular atrophy, as well as glomerulosclerosis, were more pronounced than in FO/AL mice (**Table 1**, **Fig. 3***A* and *C*). Both CO/FR and FO/FR mice had normal tubules and glomeruli in most of the kidneys (Table 1, Fig. 3*B* and *D*). When FO supplementation was combined with FR, there was a trend ($P = 0.21$) for a lower score in both young and old mice compared with FR alone (Table 1).

Antioxidant enzyme activity. SOD and CAT activity declined by 28% with age in kidney homogenates of CO/AL mice (**Table 2**) ($P < 0.02$). Both FO/AL and FO/FR, and to a lesser extent CO/FR, normalized the age-dependent reduction. CO/FR, FO/AL and FO/FR maintained SOD activity in young mice at levels 18, 22 and 48%, respectively, greater than those of CO/AL mice. There was a 25% decline in CAT activity with age in the CO/AL group (Table 2). CO/FR marginally blunted the age effect, whereas both FO/AL and FO/FR increased CAT activity to levels 25 and 51% higher than that of the young CO/AL control group. FO/AL and FO/FR increased CAT activity by 45 and 50%, respectively, in the young mice, whereas CO/FR was only marginally effective. GSH-Px activity was not affected by age but was increased 30% by FR or FO feeding in both young and old mice. GST was not affected by age, FR or FO.

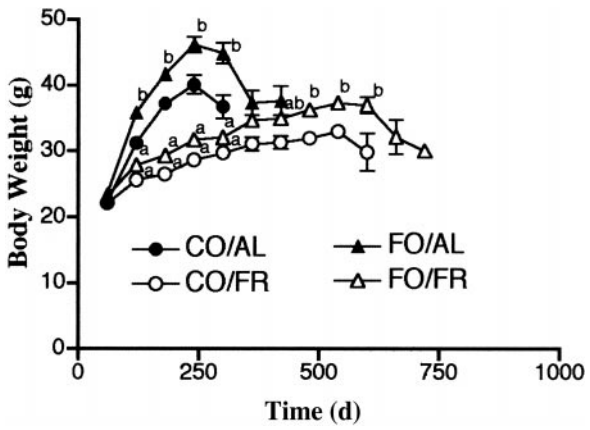

FIGURE 2 Effect of food restriction and dietary (n-3) fatty acids on total body weight in female (NZB x NZW)F(1) mice. Female (NZB x NZW)F(1) mice, 6 wk old, consumed a semipurified diet containing 5 g/100 g corn oil, free access (CO/AL); corn oil, 40% food-restricted (CO/FR); fish oil, free access (FO/AL); or fish oil, 40% food restricted (FO/FR). Mice were weighed every 2 wk throughout their life Values are means ± SEM, $n = 30$, initially. [a]Significantly different from corresponding freely fed mice, $P < 0.05$ (Newman Kuels test). [b]Significantly different from corresponding CO-fed mice, $P < 0.05$ (Newman Kuels test to 420 d and *t* test thereafter).

TABLE 1

Effect of age, food restriction and (n-3) fatty acids on renal histology scores in female (NZB × NZW)F(1) mice[1,2,3]

	Young	Old
CO/AL[4]	1.7 ± 0.3[b]	3.2 ± 0.3[a]
CO/FR	1.7 ± 0.3[b]	1.7 ± 0.3[b]
FO/AL	1.3 ± 0.3[b]	2.2 ± 0.3[b]
FO/FR	1.3 ± 0.3[b]	1.3 ± 0.6[b]

[1] Each value represents the mean ± SD, $n = 3$. A score of 1 indicates a disease-free mouse and 4 indicates severe glomerular nephritis.

[2] Means followed by different superscript letters differ, $P < 0.05$ (Newman Kuels test).

[3] ANOVA for 2 × 2 × 2 factorial design, main effect means: age-young 1.5, old 2.1, $P < 0.001$; oil-corn 2.0, fish 1.5, $P < 0.01$; food intake AL 2.1, CR 1.5, $P < 0.001$.

[4] Diet abbreviations: CO/AL, corn oil–based diet, ad libitum intake; CO/FR, corn oil–based diet, restricted intake; FO/AL; fish oil–based diet, ad libitum intake; FO/FR, fish oil–based diet, restricted intake.

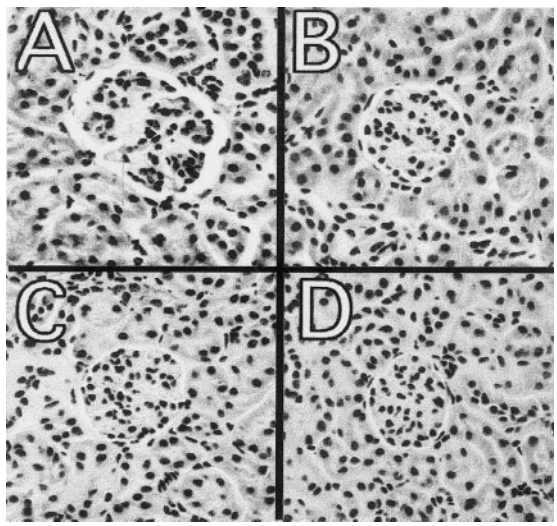

FIGURE 3 Food restriction and dietary (n-3) fatty acids reduced renal interstitial disease and glomerulosclerosis in female (NZB x NZW)F(1) mice. Female (NZB x NZW)F(1) mice, 6 wk old, consumed a semipurified diet containing (*A*) 5 g/100 g corn oil, free access (CO/AL); (*B*) corn oil, 40% food-restricted (CO/FR); (*C*) fish oil, free access (FO/AL); or (*D*) fish oil, 40% food restricted (FO/FR). At 8 mo of age, mice were killed and one kidney was fixed in 1 g/L buffered formalin, sectioned, and stained with hematoxylin and eosin for light microscopic examination as described in Materials and Methods.

TABLE 2

Effects of age, food restriction and (n-3) fatty acids in activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px) and glutathione S-transferase (GST) in kidney homogenates from female (NZB × NZW)F(1) mice[1,2]

Age, *mo*	Diet	SOD[3]	CAT[4]	GSH-Px[5]	GST[5]
			EU/mg protein		
4	CO/AL[6]	122 ± 10[c]	106 ± 5[d]	32 ± 2[b]	92 ± 5
	CO/FR	147 ± 7[b]	129 ± 7[c]	41 ± 4[a]	102 ± 3
	FO/AL	153 ± 2[b]	167 ± 8[b]	42 ± 1[a]	99 ± 3
	FO/FR	207 ± 18[a]	180 ± 10[a]	40 ± 3[a]	92 ± 7
8	CO/AL	84 ± 6[d]	74 ± 7[e]	27 ± 1[b]	93 ± 4
	CO/FR	103 ± 12[d]	91 ± 6[de]	36 ± 2[a]	104 ± 3
	FO/AL	121 ± 12[c]	141 ± 4[c]	39 ± 1[a]	93 ± 4
	FO/FR	129 ± 3[c]	189 ± 14[a]	43 ± 2[a]	91 ± 3
			2 × 2 × 2 Factorial ANOVA Main effect means		
Age	Young	157.1	145.5	39.82	96.26
	Old	109.2***	126.4*	35.86	95.82
Oil	Corn	114.1	99.8	34.40	98.36
	Fish	152.1***	172.4***	40.75***	93.73
FR	AL	119.8	124.4	35.31	94.14
	FR	146.4***	147.5**	39.84*	97.95

[1] Values are means ± SEM, $n = 5$.
[2] Means in a column followed by different superscript letters differ at $P < 0.05$ (Newman Kuel's test); significantly different main effects * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.
[3] EU = μmol cytochrome C reduced/min.
[4] EU = μmol H_2O_2 reduced/min.
[5] EU = μmol NADPH oxidized/min.
[6] For diet abbreviations, see Table 1.

Renal cytokine levels. Cytokines exist in hyper- and hypoglycosylated forms, which influence their biological activity (43). **Figure 4** shows a representative Western blot for each cytokine. Individually, IFN-γ, IL-12, IL-10 and TNF-α exhibited a slower migrating hyperglycosylated band in all diet and age groups, whereas the appearance of a faster migrating hypoglycosylated band was present primarily in the CO/AL and FO/AL old groups. In general, FO feeding did not significantly influence cytokine protein expression in young mice. Because the major change due to age and diet existed in the hypoglycosylated band, we have shown data for only the hypoglycosylated forms for each cytokine (**Table 3**). The hypoglycosylated forms of all four cytokines (INF-γ, IL-12, IL-10 and TNF-α) increased with age and with progression of the disease in the AL-fed mice, although the increase in IL-12 was not significant ($P = 0.0613$). This increase was prevented by FR (CO/FR, FO/FR).

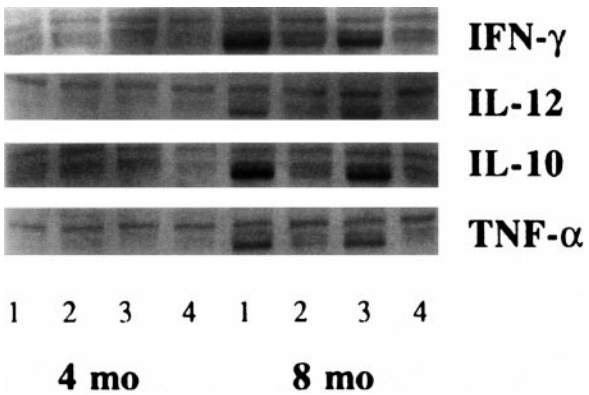

FIGURE 4 Food restriction and to a lesser extent dietary (n-3) fatty acids repressed renal cytokine levels in 8-mo-old B/W mice. Female (NZB x NZW)F(1) mice, 6 wk old, consumed a semipurified diet containing *1*) 5 g/100 g corn oil, free access (CO/AL); *2*) corn oil, 40% food-restricted (CO/FR); *3*) fish oil, free access (FO/AL); or *4*) fish oil, 40% food restricted (FO/FR). The Western blot is representative of 5 independent determinations. Abbreviations: IFN-γ, interferon-γ; IL, interleukin; TNF-α, tumor necrosis factor-α.

Because age and diet affected cytokine protein levels, we next examined the effects on cytokine gene expression. **Figure 5** is a representative ethidium bromide–stained gel for IFN-γ, IL-12, IL-10 and TNF-α; **Table 4** shows the densitometric analysis expressed as the cytokine to GAPDH ratio. Similar to the protein levels, diet did not affect cytokine mRNA expression in young mice. In the CO/AL old mice, however, mRNA expression for all of the cytokines was increased ($P < 0.0001$) from 25% to threefold relative to the CO/AL young group. Both CO/FR and FO/FR diets maintained mRNA expression in the old groups at a level similar to those in the young groups ($P < 0.0001$ vs. AL mice), whereas FO feeding decreased only IL-10 and TNF-α expression significantly ($P < 0.05$) and to a lesser extent than FR.

Nuclear factor-κB. We next examined the influence of diet and age on NF-κB nuclear localization because it plays an important positive role in cytokine gene expression and function during inflammation (44). Diet did not influence NF-κB in the young groups (**Table 5**). A representative gel is shown in **Figure 6***A*. In the old mice, both CO/FR and FO/FR maintained NF-κB at levels comparable to the young groups, whereas FO/AL was only partially effective. Figure 6*B* confirms that the binding was specific because a 50-fold excess of unlabeled NF-κB oligonucleotide competitively eliminated

TABLE 3

Effects of food restriction and (n-3) fatty acids on expression of IFN-γ, IL-12, IL-10, and TNF-α protein, hypoglycosylated form, in kidney from female (NZB × NZW)F(1) Mice[1,2,3]

Age, *mo*	Diet	IFN-γ	IL-12	IL-10	TNF-α
			Relative density		
4	CO/AL	1.18 ± 0.09[b]	1.28 ± 0.11	1.47 ± 0.20[b]	1.37 ± 0.21[b]
	CO/FR	1.33 ± 0.17[b]	1.08 ± 0.08	1.23 ± 0.11[b]	1.22 ± 0.11[b]
	FO/AL	1.17 ± 0.04[b]	1.25 ± 0.07	1.35 ± 0.07[b]	1.60 ± 0.41[b]
	FO/FR	1.18 ± 0.13[b]	1.14 ± 0.07	1.14 ± 0.08[b]	1.24 ± 0.07[b]
8	CO/AL	2.39 ± 0.52[a]	1.44 ± 0.12	3.15 ± 1.01[a]	1.60 ± 0.03[b]
	CO/FR	1.60 ± 0.14[b]	1.26 ± 0.06	1.52 ± 0.19[b]	1.52 ± 0.08[b]
	FO/AL	2.66 ± 0.39[a]	1.61 ± 0.24	3.40 ± 0.27[a]	2.53 ± 0.15[a]
	FO/FR	1.37 ± 0.10[b]	1.17 ± 0.17	1.53 ± 0.18[b]	1.46 ± 0.16[b]
			2 × 2 × 2 Factorial ANOVA main effect means		
Age	Young	1.22	1.19	1.30	1.36
	Old	2.01***	1.37	2.06**	1.78*
Oil	Corn	1.63	1.26	1.67	1.43
	Fish	1.60	1.29	1.69	1.71
FR	AL	1.85	1.39	2.00	1.78
	FR	1.37*	1.16*	1.36*	1.36*

[1] Values are mean ± SEM, $n = 3$.
[2] Means in the same column followed by different superscript letters differ, $P < 0.05$ (Newman Kuel's test); significantly different main effects * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.
[3] See Table 1 for diet abbreviations; IFN-γ, interferon-γ; IL, interleukin; TNF-α, tumor necrosis factor-α.

the NF-κB band and a 50-fold excess of an irrelevant oligonucleotide had no effect.

DISCUSSION

We showed previously that FO/AL or CO/FR significantly extends the life span of autoimmune prone B/W mice (5,29). We now extend these observations and show for the first time that the combination of dietary FO and FR (FO/FR) is far more effective than either treatment individually. The effects observed here could be attributed only partially to decreased body weight or obesity. Food-restricted (CO/FR and FO/FR) mice, with 40% lower body weights, did live significantly longer than mice that ate ad libitum (CO/AL and FO/AL). However, in spite of slightly greater body weights, FO/FR mice lived significantly longer than CO/FR mice. Similarly, the FO/AL group lived longer than the CO/AL mice even though their body weights were higher. Thus, the source of dietary fat was a key determinant in suppressing autoimmune renal disease and extending life span significantly. The extension of life span by FR was associated with immune suppression because the disease-dependent increases in IFN-γ, TNF-α, IL-12 and IL-10 were prevented at both the protein and mRNA levels. Furthermore, age-dependent increases in renal NF-κB nuclear localization were equally blunted by (n-3) lipid feeding and reduced food intake. Interestingly, these results were accompanied by increased antioxidant enzyme (SOD, CAT) activity in the kidneys of relatively disease-free young mice.

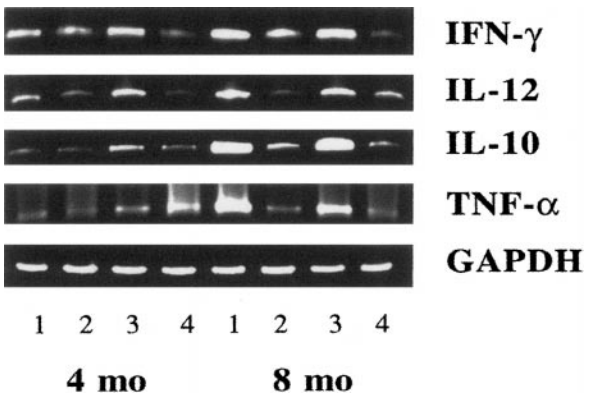

FIGURE 5 Cytokine gene expression is repressed by food restriction and dietary (n-3) fatty acids in 8-mo-old B/W mice. Female (NZB x NZW)F(1) mice, 6 wk old, consumed a semipurified diet containing *1*) 5 g/100 g corn oil, free access (CO/AL); *2*) corn oil, 40% food-restricted (CO/FR); *3*) fish oil, free access (FO/AL); or *4*) fish oil, 40% food restricted (FO/FR). The gel is representative of 5 independent determinations. Abbreviations: IFN-γ, interferon-γ; IL, interleukin; TNF-α, tumor necrosis factor-α.

Several lines of evidence demonstrate the pivotal role of IFN-γ in promoting lupus nephritis. First, IFN-γ receptor knockout B/W mice have reduced renal disease (45). Second, administration of soluble IFN-γ receptor blunts autoimmune nephritis in B/W mice (46). Third, similar results have also been observed in other lupus-prone mice with respect to the proinflammatory actions of IFN-γ (47). Consistent with these observations, we now find that renal IFN-γ expression is also lower in CO/FR and FO/FR mice relative to the age-matched CO/AL and FO/AL groups. Similarly, the reduced levels of IL-10 in CO/FR and FO/FR mice are in agreement with previous findings that administration of anti-IL-10 antibody delays the onset of renal disease in B/W mice (48). It is important to note that both IFN-γ and IL-10 expression are regulated in the same way, i.e., both increase with disease and decrease in response to CO/FR and FO/FR. It is generally thought that these two cytokines are antagonists and if one increases the other decreases. However, in lupus nephritis as

TABLE 4

Effect of food restriction and (n-3) fatty acids on relative expression of IFN-γ, IL-12, IL-10, and TNF-α genes in kidney from female (NZB × NZW)F(1) mice[1,2,3]

Age, *mo*	Diet	IFN-γ	IL-12	IL-10	TNF-α
			Cytokine/GAPDH		
4	CO/AL	1.22 ± 0.05[b]	0.37 ± 0.09[b]	0.48 ± 0.09[b]	1.14 ± 0.07[b]
	CO/FR	0.92 ± 0.28[b]	0.20 ± 0.06[b]	0.43 ± 0.10[b]	0.92 ± 0.09[b]
	FO/AL	1.11 ± 0.14[b]	0.32 ± 0.06[b]	0.55 ± 0.11[b]	1.01 ± 0.11[b]
	FO/FR	0.85 ± 0.28[b]	0.21 ± 0.08[b]	0.39 ± 0.10[b]	1.03 ± 0.19[b]
8	CO/AL	1.69 ± 0.21[a]	0.72 ± 0.11[a]	1.45 ± 0.15[a]	1.91 ± 0.25[a]
	CO/FR	1.10 ± 0.15[b]	0.23 ± 0.14[b]	0.67 ± 0.22[b]	1.06 ± 0.30[b]
	FO/AL	1.82 ± 0.16[a]	0.94 ± 0.05[a]	1.15 ± 0.39[a]	1.57 ± 0.20[a]
	FO/FR	0.82 ± 0.18[b]	0.38 ± 0.16[b]	0.52 ± 0.12[b]	0.89 ± 0.06[b]
			2 × 2 × 2 factorial ANOVA Main effect means		
Age	Young	1.02	0.29	0.46	1.02
	Old	1.42***	0.57***	0.92***	1.36***
Oil	Corn	1.26	0.38	0.74	1.26
	Fish	1.18***	0.48***	0.65*	1.13*
FR	AL	1.49	0.60	0.90	1.41
	FR	0.94***	0.27***	0.48***	0.97***

[1] Values are mean ± SEM in arbitrary units expressed as the ratio of cytokine band intensity to GAPDH band intensity from ethidium bromide–stained agarose gels, $n = 5$.
[2] Means in a column followed by different superscript letters differ, $P < 0.05$ (Bonferroni's test); significantly different main effects * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.
[3] See Table 3 for abbreviations.

well as other autoimmune disorders, it is now becoming evident that both Th-1 and Th-2 cytokines may be involved synergistically (26).

Furthermore, cytokine protein levels were also dramatically increased during the disease process and decreased by FR. Interestingly, the glycosylation state of the cytokines was also significantly reduced in the aging, diseased mice. Cytokines exist physiologically as glycosylated proteins, which present themselves as doublet or multiple bands by Western blot analysis (43). In the diseased kidney, the cytokines we examined existed primarily in a hypoglycosylated form, and both CO/FR and FO/FR significantly blunted this effect. This finding alone is important because, in general, the hypoglycosylated forms of cytokines are more biologically active and typically found at sites or in tissues with severe inflammation (43). The regulation of glycosylation state most likely involves pretranscriptional mechanism(s) because both cytokine mRNA levels and NF-κB nuclear localization were decreased by CO/FR and FO/FR in old mice. Recent findings have shown that NF-κB upregulates IFN-γ (49), IL-10 (49,50) and TNF-α (51) expression and is a pivotal transcription factor in propagating the inflammatory response (44). Furthermore, a recent report showed that mice deficient in NF-κB were resistant to experimental autoimmune encephalomyelitis, establishing a clear link between NF-κB and autoimmunity (52). FR has also been reported to prevent expression of 60–70% of inflammatory and stress response genes typically elevated in the brains of aging mice (53).

TABLE 5

Effect of food restriction and (n-3) fatty acids on electrophoretic mobility shift assay for nuclear factor (NF)-κB activation in female (NZB × NZW)F(1) mouse kidneys[1–4]

	Young	Old
	Arbitrary units	
CO/AL	3.18 ± 0.51[b]	16.69 ± 6.51[a]
CO/FR	3.11 ± 0.55[b]	4.54 ± 0.93[b]
FO/AL	2.80 ± 0.37[b]	10.28 ± 2.86[b]
FO/FR	2.64 ± 0.06[b]	5.68 ± 1.43[b]

[1] Values are mean ± SEM, $n = 3$.
[2] Means followed by different superscript letters differ, $P < 0.05$ (Newman Kuels multiple comparison test).
[3] ANOVA for 2 × 2 × 2 factorial design, main effect means: age-young 2.93, old 9.30, $P < 0.01$; oil-corn 6.88, fish 5.35, $P > 0.05$; food intake-AL 8.23, FR 3.99, $P < 0.05$.
[4] See Table 1 for diet abbreviations.

The mechanism by which NF-κB is suppressed by CO/FR and FO/FR in old B/W mice is unclear, but we speculate that it is due to increased antioxidant enzyme activity. Previously, we found increased antioxidant enzyme activity and expression in mouse liver and kidney of B/W mice fed FO/AL compared with CO/AL mice (30). Free radicals have been shown to activate NF-κB and other cytokines (54). In the kidney, free radicals derived from resident kidney cells as well as from the infiltrating immune cells may play a pivotal role in causing the acute glomerular disease (55). Antioxidant enzymes could reduce free radical levels and thereby block or down-regulate NF-κB activation. This has been observed in response to in vivo antioxidant administration, which suppressed NF-κB activation and subsequent lung inflammation (56). Indeed, we observed a simultaneous increase in NF-κB nuclear levels and decrease in antioxidant enzyme activity in relatively obese CO/AL and FO/AL old mice. In the present studies, SOD, CAT and GSH-Px were increased, but GST was not affected. Interestingly, the CO/FR and FO/FR treatments increased antioxidant enzyme activity before the onset of disease in the young mice as well, which may account for the

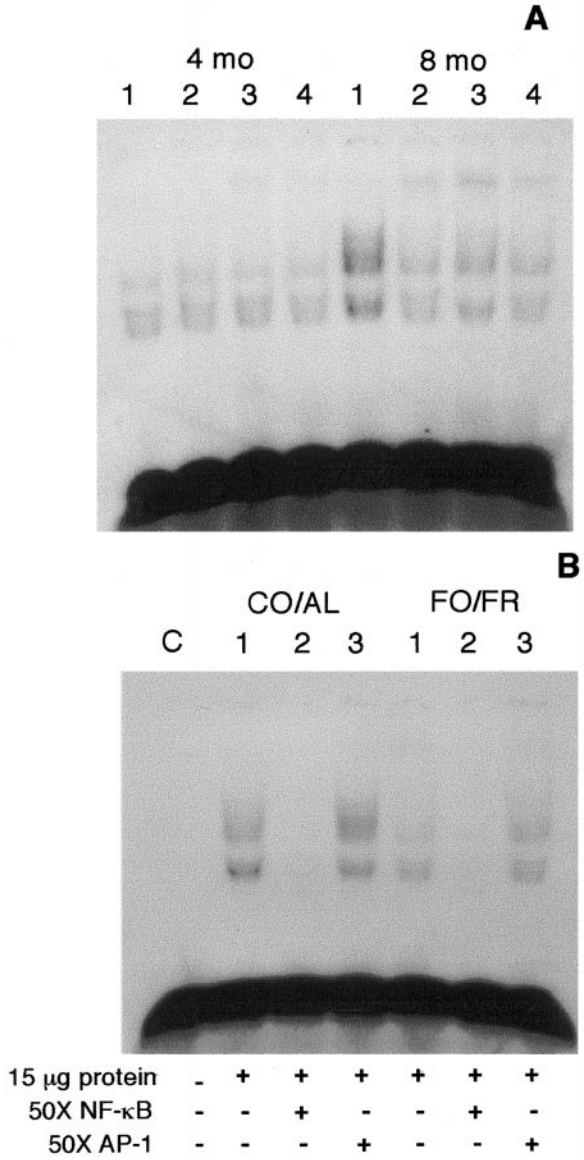

FIGURE 6 Food restriction and dietary (n-3) fatty acids prevented the rise in nuclear factor (NF)-κB activation in female (NZB x NZW)F(1) mice. Female (NZB x NZW)F(1) mice, 6 wk old, consumed a semipurified diet containing *1*) 5 g/100 g corn oil, free access (CO/AL); *2*) corn oil, 40% food-restricted (CO/FR); *3*) fish oil, free access (FO/AL); or *4*) fish oil, 40% food-restricted (FO/FR). (*A*) is representative of 3 independent determinations; (*B*) represents controls; (*C*) indicates no protein: *1*) 15 μg kidney protein, *2*) 50X excess unlabeled probe; *3*) 50X excess unlabeled alkaline phosphatase (AP)-1 probe.

maintenance of low or normal renal cytokine levels in the old lean CO/FR and FO/FR mice. These results are in agreement with others and with our own previous data, which showed that FO/AL and CO/FR increase antioxidant enzyme activity in both healthy (57) and autoimmune-prone mice (58) and rats (59). An alternative mechanism may involve diet-induced changes in fatty acid composition (60). We showed previously that both CO/FR and FO/AL decrease arachidonic acid [AA: (n-6) fatty acid] levels, whereas FO/AL causes a concomitant increase in the (n-3) fatty acids, EPA and DHA (29,61). It was shown recently that AA, through its metabolite prostaglandin E_2 (PGE_2), but not EPA, which inhibits PGE_2 synthesis, could activate NF-κB in vitro, suggesting a mechanism that may be independent of free radical formation (62). The lack of a diet effect on renal cytokine levels in young mice is important because it suggests that FR is not immunosuppressive or detrimental to the point that cytokine steady-state levels are influenced at a young age. It is important to note also that FR and FO had no adverse effects on antioxidant enzyme activity.

Data presented herein show that CO/FR and FO/FR acted similarly with respect to suppressing renal cytokine and NF-κB levels, whereas FO/FR was much more effective at inhibiting renal disease and thereby increasing life span. Indeed, in this study, the analysis of cytokines and NF-κB was conducted simultaneously in both young and old mice as a cross-sectional study, whereas life span was followed longitudinally. The cross-sectional analysis was chosen to allow direct comparison of all diet groups at the same time. The important finding of this study is that the age effect of diet most likely involved increased antioxidant enzyme activity, which was significantly higher even in the young FO/FR mice relative to the CO/FR and FO/AL groups, paralleling the pattern of the survival data. This observation is further supported by a recent report that showed that increasing CAT expression can extend life span (63). Free radical damage is thought to be a major mechanism contributing to aging, and FR may delay this by altering multiple genes that are influenced by age (64). Therefore, early increases in antioxidant enzyme activity in CO/FR and FO/FR mice may represent a key event, one that ameliorates autoimmune kidney disease. In summary, our data indicate that renal disease progression could be a result of stimulation of NF-κB by increased free radical species in AL mice. The increased NF-κB could in turn stimulate production of TNF-α, IFN-γ, IL-10 and IL-12, leading to increased inflammation and failure of kidney function. Both fish oil feeding and food restriction, and especially a combination of the two, increase antioxidant enzyme activity, thereby preventing the rise in NF-κB and consequently the increase in the proinflammatory cytokines, TNF-α, IFN-γ, IL-10, and IL-12, that occurs in these mice.

LITERATURE CITED

1. McCay, C. M., Crowell, M. F. & Maynard, L. A. (1935) The effect of retarded growth upon the length of the life span and upon the ultimate body size. J. Nutr. 10: 63–79.

2. Masoro, E. J., Yu, B. P. & Bertrand, H. A. (1982) Action of food restriction in delaying the aging process. Proc. Natl. Acad. Sci. U.S.A. 79: 4239–4241.

3. Weindruch, R. & Walford, R. L. (1988) Dietary restriction: effects on biological parameters. In: The Retardation of Aging and Disease by Dietary Restriction (Weindruch, R., ed.), pp. 117–230. Charles C. Thomas, Springfield, IL.

4. Fernandes, G. (1984) Nutritional factors: modulating effects on immune function and aging. Pharmacol. Rev. 36: 123S–129S.

5. Fernandes, G., Yunis, E. J. & Good, R. A. (1976) Influence of diet on survival of mice. Proc. Natl. Acad. Sci. U.S.A. 73: 1279–1283.

6. Troyer, D. A., Chandrasekar, B., Barnes, J. L. & Fernandes, G. (1997) Calorie restriction decreases platelet-derived growth factor (PDGF)-A and thrombin receptor mRNA expression in autoimmune murine lupus nephritis. Clin. Exp. Immunol. 108: 58–62.

7. Fernandes, G., Friend, P., Yunis, E. J. & Good, R. A. (1978) Influence of dietary restriction on immunologic function and renal disease in $NZBxNZWF_1$ mice. Proc. Natl. Acad. Sci. U.S.A. 75: 1500–1504.

8. Izui, S., Fernandes, G., Hara, I., McConahey, P. J., Jensen, F. C., Dixon, F. J. & Good, R. A. (1981) Low-calorie diet selectively reduces expression of retroviral envelope glycoprotein Gp70 in sera of $NZBxNZW_{F1}$ hybrid mice. J. Exp. Med. 154: 1116–1124.

9. Troyer, D. A., Chandrasekar, B., Thinnes, T., Stone, A., Loskutoff, D. A. & Fernandes, G. (1995) Effects of energy intake on type I plasminogen activator inhibitor levels in glomeruli of lupus-prone B/W mice. Am. J. Pathol. 146: 111–120.

10. Simopoulos, A. P. (1991) Omega-3 fatty acids in health and disease and in growth and development. Am. J. Clin. Nutr. 54: 438–463.

11. Fernandes, G. & Venkatraman, J. (1993) Role of ω-3 fatty acids in health and disease. Nutr. Res. 13: S19–S45.

12. Kehn, P. & Fernandes, G. (2001) The importance of omega-3 fatty acids in the attenuation of immune-mediated diseases. J. Clin. Immunol. 21: 99–101.

13. James, M. J. & Cleland, L. G. (1997) Dietary n-3 fatty acids and therapy for rheumatoid arthritis. Semin. Arthritis Rheum. 27: 85–97.
14. Chandrasekar, B., McGuff, H. S., Aufdermorte, T. B., Troyer, D. A., Talal, N. & Fernandes, G. (1995) Effects of calorie restriction on transforming growth factor beta 1 and proinflammatory cytokines in murine Sjogren's syndrome. Clin. Immunol. Immunopathol. 76: 291–296.
15. Donadio, J.V.J., Bergstralh, E. J., Offord, K. P., Spencer, D. C. & Holley, K. E. (1994) A controlled trial of fish oil in IgA nephropathy. Mayo Nephrology Collaboratory Group. N. Engl. J. Med. 331: 1194–1199.
16. Fernandes, G. & Jolly, C. A. (1998) Nutrition and autoimmune disease. Nutr. Rev. 56: S161–S169.
17. Cave, W. T., Jr. (1991) Dietary n-3 (ω-3) PUFA effects on animal tumorigenesis. FASEB J. 5: 2160–2166.
18. Rose, D. P. & Connolly, J. M. (1999) Omega-3 fatty acids as cancer chemopreventive agents. Pharmacol. Ther. 83: 217–244.
19. Guthy, E. (1996) Enteric-coated fish oil in Crohn's disease. N. Engl. J. Med. 335: 1397–1398.
20. Leaf, A. (1995) Fish intake and the risk of coronary disease. N. Engl. J. Med. 333: 937–938.
21. Nestel, P. J. (2000) Fish oil and cardiovascular disease: lipids and arterial function. Am. J. Clin. Nutr. 71: 228S–231S.
22. Hart, R. W., Leakey, J. E., Chou, M., Duffy, P. H., Allaben, W. T. & Feuers, R. J. (1992) Modulation of chemical toxicity by modification of caloric intake. Adv. Exp. Med. Biol. 322: 73–81.
23. Foster, M. H. (1999) Relevance of systemic lupus erythematosus nephritis animal models to human disease. Semin. Nephrol. 19: 12–24.
24. Fernandes, G., Troyer, D. A. & Jolly, C. A. (1998) The effects of dietary lipids on gene expression and apoptosis. Proc. Nutr. Soc. 57: 543–550.
25. Theofilopoulos, A. N. & Dixon, F. J. (1985) Murine models of systemic lupus erythematosus. Adv. Immunol. 37: 269–390.
26. Kelley, V. R. & Wuthrich, R. P. (1999) Cytokines in the pathogenesis of systemic lupus erythematosus. Semin. Nephrol. 19: 57–66.
27. Prickett, J. D., Robinson, D. R. & Steinberg, A. D. (1981) Dietary enrichment with the PUFA eicosapentaenoic prevents proteinuria and prolongs survival in $NZBxNZW_{F1}$ mice. J. Clin. Investig. 658: 556–559.
28. Prickett, J. D., Robinson, D. R. & Steinberg, A. D. (1983) Effects of dietary enrichment with EPA upon autoimmune nephritis in female $NZB/NZWF_1$ mice. Arthritis Rheum. 8: 437–439.
29. Fernandes, G., Bysani, C., Venkatraman, J. T., Tomar, V. & Zhao, W. (1994) Increased TGF-beta and decreased oncogene expression by omega-3 fatty acids in the spleen delays onset of autoimmune disease in B/W mice. J. Immunol. 152: 5979–5987.
30. Chandrasekar, B. & Fernandes, G. (1994) Decreased pro-inflammatory cytokines and increased antioxidant enzyme gene expression by ω-3 lipids in murine lupus nephritis. Biochem. Biophys. Res. Commun. 200: 893–898.
31. Chandrasekar, B., Troyer, D. A., Venkatraman, J. T. & Fernandes, G. (1995) Dietary omega-3 lipids delay the onset and progression of autoimmune lupus nephritis by inhibiting transforming growth factor beta mRNA and protein expression. J. Autoimmun. 8: 381–393.
32. Fernandes, G. (1995) Effect of calorie restriction and omega-3 fatty acids on autoimmunity and aging. Nutr. Rev. 53: S72–S79.
33. Venkatraman, J. T., Angkeow, P., Satsangi, N. & Fernandes, G. (1998) Effects of dietary n-6 and n-3 lipids on antioxidant defense system in livers of exercised rats. J. Am. Coll. Nutr. 17: 586–594.
34. Lim, B. O., Jolly, C. A., Zaman, K. & Fernandes, G. (2000) Dietary (n-6) and (n-3) fatty acids and energy restriction modulate mesenteric lymph node lymphocyte function in autoimmune-prone (NZB × NZW)F1 mice. J. Nutr. 130: 1657–1664.
35. Shaw, C. E. & Zheng, J. (1998) Western Immunoblot Analysis. In: Bird I. M., ed. Phospholipid Signaling Protocols. Totowa, NJ: Humana Press.
36. Jolly, C. A., Hubbell, T., Behnke, W. D. & Schroeder, F. (1997) Fatty acid binding protein: stimulation of microsomal phosphatidic acid formation. Arch. Biochem. Biophys. 341: 112–121.
37. Muthukumar, A. R., Jolly, C. A., Zaman, K. & Fernandes, G. (2000) Calorie restriction decreases proinflammatory cytokines and polymeric Ig receptor expression in the submandibular glands of autoimmune prone (NZB x NZW)F1 mice. J. Clin. Immunol. 20: 354–361.
38. Flescher, E., Ledbetter, J. A., Schieven, G. L., Vela-Roch, N., Fossum, D., Dang, H., Ogawa, N. & Talal, N. (1994) Longitudinal exposure of human T lymphocytes to weak oxidative stress suppresses transmembrane and nuclear signal transduction. J. Immunol. 153: 4880–4889.
39. Flohe, L. & Otting, F. (1984) Superoxide dismutase assays. Methods Enzymol. 105: 93–104.
40. Aebi, H. (1984) Catalase in vitro. Methods Enzymol. 105: 121–126.
41. Tappel, A. L. (1978) Glutathione peroxidase and hydroperoxides. Methods Enzymol. 52: 506–513.
42. Habig, W. H., Pabst, M. J. & Jakoby, W. B. (1974) Glutathione *S*-transferases. The first enzymatic step in mercapturic acid formation. J. Biol. Chem. 249: 7130–7139.
43. Van den Steen, P., Rudd, P. M., Dwek, R. A., Van Damme, J. & Opdenakker, G. (1998) Cytokine and protease glycosylation as a regulatory mechanism in inflammation and autoimmunity. Adv. Exp. Med. Biol. 435: 133–143.
44. Barnes, P. J. & Karin, M. (1997) Nuclear factor-kappaB: a pivotal transcription factor in chronic inflammatory diseases. N. Engl. J. Med. 336: 1066–1071.
45. Haas, C., Ryffel, B. & Le Hir. M. (1998) IFN-gamma receptor deletion prevents autoantibody production and glomerulonephritis in lupus-prone (NZB × NZW)F1 mice. J. Immunol. 160: 3713–3718.
46. Ozmen, L., Roman, D., Fountoulakis, M., Schmid, G., Ryffel, B. & Garotta, G. (1995) Experimental therapy of systemic lupus erythematosus: the treatment of NZB/W mice with mouse soluble interferon-γ receptor inhibits the onset of glomerulonephritis. Eur. J. Immunol. 25: 6–12.
47. Schwarting, A., Wada, T., Kinoshita, K., Tesch, G. & Kelley, V. R. (1998) IFN-gamma receptor signaling is essential for the initiation, acceleration, and destruction of autoimmune kidney disease in MRL- Fas(lpr) mice. J. Immunol. 161: 494–503.
48. Ishida, H., Muchamuel, T., Sakaguchi, S., Andrade, S., Menon, S. & Howard, M. (1994) Continuous administration of anti-interleukin 10 antibodies delays onset of autoimmunity in NZB/W F1 mice. J. Exp. Med. 179: 305–310.
49. Sica, A., Dorman, L., Viggiano, V., Cippitelli, M., Ghosh, P., Rice, N. & Young, H. A. (1997) Interaction of NF-kappaB and NFAT with the interferon-gamma promoter. J. Biol. Chem. 272: 30412–30420.
50. Mori, N. & Prager, D. (1997) Activation of the interleukin-10 gene in the human T lymphoma line HuT 78: identification and characterization of NF-kappa B binding sites in the regulatory region of the interleukin-10 gene. Eur. J. Haematol. 59: 162–170.
51. Ghosh, S., May, M. J. & Kopp, E. B. (1998) NF-kappa B and Rel proteins: evolutionarily conserved mediators of immune responses. Annu. Rev. Immunol. 16: 225–260.
52. Hilliard, B., Samoilova, E. B., Liu, T. S., Rostami, A. & Chen, Y. (1999) Experimental autoimmune encephalomyelitis in NF-kappa B-deficient mice: roles of NF-kappa B in the activation and differentiation of autoreactive T cells. J. Immunol. 163: 2937–2943.
53. Lee, C. K., Weindruch, R. & Prolla, T. A. (2000) Gene-expression profile of the ageing brain in mice. Nat. Genet. 25: 294–297.
54. Ginn-Pease, M. E. & Whisler, R. L. (1998) Redox signals and NF-kappaB activation in T cells. Free Radic. Biol. Med. 25: 346–361.
55. Shah, S. V. (1989) Role of reactive oxygen metabolites in experimental glomerular disease. Kidney Int. 35: 1093–1106.
56. Blackwell, T. S., Blackwell, T. R., Holden, E. P., Christman, B. W. & Christman, J. W. (1996) In vivo antioxidant treatment suppresses nuclear factor-kappa B activation and neutrophilic lung inflammation. J. Immunol. 157: 1630–1637.
57. Demoz, A., Willumsen, N. & Berge, R. K. (1992) Eicosapentaenoic acid at hypotriglyceridemic dose enhances the hepatic antioxidant defense in mice. Lipids 27: 968–971.
58. Venkatraman, J. T., Chandrasekar, B., Kim, J. D. & Fernandes, G. (1994) Effects of n-3 and n-6 fatty acids on the activities and expression of hepatic antioxidant enzymes in autoimmune-prone NZBxNZW F1 mice. Lipids 29: 561–8.
59. Ruiz-Gutierrez, V., Perez-Espinosa, A., Vazquez, C. M. & Santa-Maria, C. (1999) Effects of dietary fats (fish, olive and high-oleic-acid sunflower oils) on lipid composition and antioxidant enzymes in rat liver. Br. J. Nutr. 82: 233–241.
60. Laganiere, S. & Fernandes, G. (1991) Study on the lipid composition of aging Fischer-344 rat lymphoid cells: effect of long-term calorie restriction. Lipids 26: 472–478.
61. Venkatraman, J. & Fernandes, G. (1992) Modulation of age-related alterations in membrane composition and receptor-associated immune functions by food restriction in Fischer 344 rats. Mech. Ageing Dev. 63: 27–44.
62. Camandola, S., Leonarduzzi, G., Musso, T., Varesio, L., Carini, R., Scavazza, A., Chiarpotto, E., Baeuerle, P. A. & Poli, G. (1996) Nuclear factor κB is activated by arachidonic acid but not by eicosapentaenoic acid. Biochem. Biophys. Res. Commun. 229: 643–647.
63. Taub, J., Lau, J. F., Ma, C., Hahn, J. H., Hoque, R., Rothblatt, J. & Chalfie, M. (1999) A cytosolic catalase is needed to extend adult lifespan in *C. elegans* daf-C and clk-1 mutants. Nature (Lond.) 399: 162–166.
64. Sohal, R. S. & Weindruch, R. (1996) Oxidative stress, calorie restriction and aging. Science (Washington, DC) 273: 59–63.