Modulation of Antioxidant Enzymes and Programmed Cell Death by n-3 Fatty Acids

Gabriel Fernandes[a,*], Bysani Chandrasekar[a], Xianghong Luan[a], and Dean A. Troyer[b]

Departments of [a]Medicine and [b]Pathology, The University of Texas Health Science Center, San Antonio, Texas 78284-7874

ABSTRACT: Studies from our laboratory indicate that n-3 (fish oil, FO) lipids at 10% (w/w) in a nutritionally adequate, semipurified diet, and supplemented with equal levels of antioxidants, extended the life span of lupus-prone (NZB/NZW)F_1 (B/W) female mice as compared to n-6 (corn oil, CO) lipids. The early rise of autoimmune disease in CO-fed mice was closely linked to the loss of T-cell function. Both IL-2 production and IL-2 receptor expression were reduced due to the loss of naive T-cells and a rise in memory T-cells. Proliferative response to both mitogens and superantigens (staphylococcal enterotoxins A and B) was higher in FO-fed 6.5-mon-old mice. These changes paralleled decreased PGE_2 production by splenic cells from FO-fed mice.

Analysis of mRNA expression in different organs revealed differential effects of dietary lipids. In FO-fed mice, transforming growth factor β1 (TGF β1) expression was decreased in kidneys, but splenic tissues had higher expression of TGF β mRNA. As TGF β promotes programmed cell death (PCD), we studied the effects of CO and FO on PCD rates in lymphocytes. Both propidium iodide staining and DNA fragmentation were elevated in lymphocytes of FO-fed mice when compared to CO-fed mice of similar age. Also, increased PCD correlated closely with increased Fas gene expression. Thus, in addition to various other antiinflammatory effects, dietary FO appears to increase PCD and prevent accumulation of self-reactive immune cells in lymphoid organs. Further studies are required to dissect the pro- and antiinflammatory mechanisms associated with dietary n-3 and n-6 lipids in modulating autoimmune disorders or malignancy during aging.

Lipids 31, S-91–S-96 (1996).

Fish oil (FO) supplementation has antiinflammatory effects in both humans and experimental animals and delays onset of autoimmunity, cardiovascular disease, and cancer, particularly when compared to diets relatively enriched in saturated fats of animal origin (1,2). Because of adverse effects of animal fats, consumption of vegetable oils containing both monounsaturated n-9 and polyunsaturated fatty acids (rich in 18:2n-6) is rising significantly in the United States (1–3). Though vegetable oils rich in n-6 fatty acids reduce the incidence and the severity of cardiovascular disease, these lipids (particularly hydrogenated fats) have been linked to proinflammatory effects and the rise of certain cancers, rheumatoid arthritis, and other autoimmune disorders during aging (4). Though n-6 lipids reduce serum cholesterol levels, they usually tend to elevate linoleic acid (18:2n-6) levels and arachidonic acid (20:4n-6) levels in the tissue phosphoglycerides. Corn oil (CO) is rich in linoleic acid, and its consumption increases production of free radicals and cyclooxygenase metabolites such as thromboxane A_2 and leukotriene B_4 (LTB_4). In contrast, FO contains highly polyunsaturated fatty acids, eicosapentaenoic acid (20:5n-3) and docosahexaenoic acid (22:6n-3), which act synergistically to inhibit prostaglandin E_2 (PGE_2) production and are thus antiinflammatory (5). Because of these beneficial effects, marine oils, especially menhaden oil, are receiving increasing attention as a source of C_{20} and C_{22} carbon n-3 fatty acids.

Animals and diet. We and others have reported that reduced calories and/or low-fat diets preserve T-cell functions, decrease autoantibody production, and reduce immune complex-mediated glomerulonephritis in (NZB/NZW)F_1 (B/W) mice (6–8). B/W mice spontaneously develop a disease similar to human systemic lupus erythematosus (SLE), which has been used as an animal model to elucidate the pathogenesis of the disease (9,10). The majority of the immunological abnormalities associated with human SLE are also seen in B/W mice, including hypergammaglobulinemia (a result of generalized B-cell hyperactivity), production of autoantibodies against a wide variety of endogenous antigens, formation of immune complexes, and a variety of associated histopathological manifestations (e.g., glomerulonephritis) (9,10). Female B/W mice under normal feeding conditions die between 6–10 mon of age, while males die between 10–22 mon of age. Death usually occurs as a result of renal failure. During the past 25 years, we have utilized female B/W mice for dietary

*To whom correspondence should be addressed at University of Texas Health Science Center, 7703 Floyd Curl Dr., San Antonio, TX 782847874.

Abbreviations: B/W mice, (NZB/NZW)F_1 mice; CAT, catalase; CO, corn oil; CTLL-2, cytotoxic T-lymphocyte line 2; FACS, fluorescence activated cell sorting; FO, fish oil; GSH-Px, glutathione peroxidase; ICAM-1, intercellular adhesion molecule-1; IL-2, interleukin-2; LTB_4, luekotriene B_4; MDA, malondialdehyde; MRL/*lpr* mice, MRL mouse with a lymphoroliferative (*lpr*) gene; PCD, programmed cell death; PGE_2, prostaglandin E_2; PI, propidium iodide; SLE, systemic lupus erythematosus; SOD, superoxide dismutase; TBARS, thiobarbituric acid–reactive substances; TBHQ, *tert*-butylhydroquinone; TGF β, transforming growth factor β; TNF-α, tumor necrosis factor-α.

studies because of early onset of disease and short life span, which we found responsive to dietary manipulations (11–15).

Though dietary supplementation with FO significantly extends life span and alleviates autoimmune symptoms in B/W and MRL mice with lymphoroliferative (*lpr*) gene (MRL/*lpr* mice) (11–18), molecular mechanisms associated with these beneficial effects have not been fully explored. Therefore we sought to determine effects of isocaloric CO- or FO-enriched diets fed *ad libitum* on immunological and molecular parameters associated with onset and progression of disease in B/W mice. We fed weanling B/W mice a nutritionally adequate, semipurified diet supplemented with either CO or FO (10% wt/wt) and equal levels of antioxidants (11). Both oils were supplemented with 2.5 g tocopherol (α and γ) and *tert*-butylhydroquinone (TBHQ)/kg of oil to prevent peroxidation, as recommended by the Fish Oil Test Material Program (National Institute of Alcohol Abuse and Alcoholism). Each diet therefore contained ~215 mg vitamin E/kg (vitamin E from oil and from vitamin mixture) (11).

Immunological studies. We observed that B/W mice become more susceptible to autoimmune disease when fed a CO-supplemented diet (vs. FO) and expressed significantly higher levels of serum anti-dsDNA antibody levels, a parameter known to serve as a highly prognostic marker for onset and severity of renal disease (17). When splenocytes were stimulated with optimal concentrations of mitogens and superantigens, a significantly higher proliferative response was noted in FO-fed mice (vs. CO). When spleen cells were cultured in the presence of Con A for 48 h, and interleukin-2 (IL-2) levels were determined by a bioassay utilizing cytotoxic T-lymphocyte line 2 (CTLL-2) cell line, IL-2 levels (μ/mL) were significantly lower ($P < 0.05$) in FO-fed mice (vs. CO) (17). Though the percentage of $CD4^+$ and $CD8^+$ cells in splenocytes from both CO- and FO-fed mice (FACS analysis) did not differ, a significant decrease in Ig^+ cells ($P < 0.05$) was noted in FO fed group (vs. CO) (17). Also, IL-2 receptor expression fluorescense activated cell sorting (FACS) was significantly increased in FO-fed mice (data not shown), indicating a delay in shift from naive to memory cells in a T-cell subpopulation. In fact, spleens from FO-fed mice had higher naive and lower memory T-cells (17).

We next sought to determine whether FO supplementation alters gene expression in various organs associated with onset and progression of autoimmune disease in B/W mice. We studied expression of both mRNA and protein levels for the oncogenes-c-*myc* and c-*ras*- in spleens, transforming growth factor β (TGF β) in both spleen and kidneys and antioxidant enzymes (catalase, CAT; glutathione peroxidase, GSH-Px; and superoxide dismutase, SOD) in livers (enzyme activity and mRNA) and kidneys (mRNA expression).

Densitometric analysis of total RNA from spleens by Northern analysis (to compensate for any loading differences, values are expressed in ratios ± SEM of c-*myc*, c-*ras* or TGF β to GAPDH) revealed lower expression of c-*myc* and c-*ras* mRNA in FO-fed (vs. CO) mice, while TGF β mRNA was significantly higher (Fig. 1). Furthermore, in FO-fed mice, protein products (Western blotting) of both oncogenes was significantly lower, and for TGF β it was significantly higher (17).

TGF β was initially characterized on the basis of its ability to induce a transformed phenotype in normal rat kidney fibroblasts in culture (19). TGF β are now recognized as multifunctional, regulatory factors influencing a remarkable array of physiological processes, including embryogenesis, hematopoiesis, angiogenesis, immune function, inflammation, myogenesis, osteogenesis, tissue repair, and remodeling (20). Hence, TGF β impacts virtually every organ system, and it may not be surprising that aberrant expression or regulation of these molecules has been implicated in numerous pathological conditions, including autoimmune disorders, glomerulonephritis, diabetic nephropathy, tumorigenesis, and immunosuppression associated with acquired immunodeficiency syndrome and malignancies (19–21).

TGF β not only affects a plethora of physiological processes, but also elicits diverse cellular responses and may have both inhibitory or stimulatory effects on the same cell, depending on cell type, state of differentiation, and context of other factors or signals in the cellular environment. In FO-fed mice, significantly elevated TGF β expression was observed at both mRNA and protein levels. Also, significantly lower oncogene-c-*myc* and c-*ras*- expression was noted, indicating

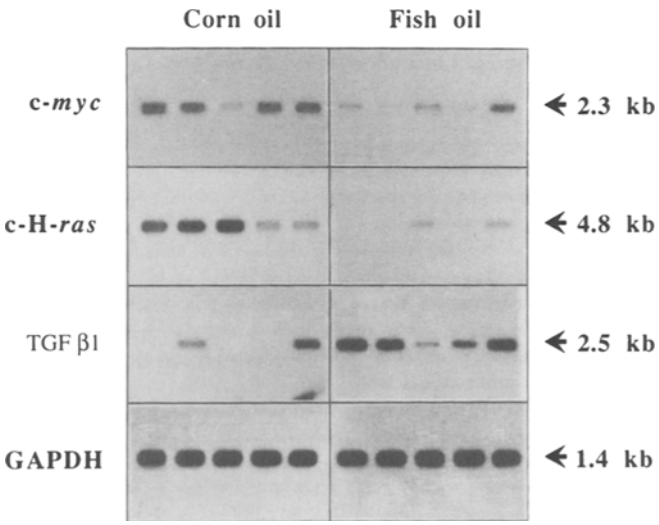

FIG. 1. Inhibition of c-*myc* and c-*ras*, and upregulation of transforming growth factor β (TGF β) mRNA expression by dietary fish oil supplementation (10% w/w) in spleens of 6.5-mon-old B/W mice (18). For Northern blot analysis, 20 μg of total RNA/lane was denatured, electrophoresed through 2.2 M formaldehyde/0.8% agarose gel, and electroblotted onto nitrocellulose. Blots were prehybridized, hybridized, washed, and autoradiographed as described (18). Autoradiographic exposure time was 36 h to 5 d. Blots were reprobed after stripping off previous labeling. GAPDH was used as an internal control and indicates equal levels of RNA loading in all lanes of the gel. Each lane represents an RNA sample from one animal.

that FO supplementation is immunosuppressive by increasing or by maintaining higher levels of the immunoregulatory molecule TGF β in splenic lymphoid cells (17). As Ig^+ cells and the serum anti-dsDNA antibody levels were significantly lower in FO-fed mice, this in turn may be due to higher levels of TGF β. Though it is a significant contribution to the field of nutrition that the source of dietary lipids can modulate TGF β expression, it has been shown previously in various *in vitro* studies that TGF β has immunosuppressive effects on immune cells, particularly on B-cell proliferation and immunoglobulin production (17).

TGF β and lupus nephritis. In contrast to its beneficial immunosuppressive properties, expression of TGF β has been reported to play a pathological role in various models of glomerulonephritis (22). Its role in either the initiation or progression of lupus nephritis is not known, however. Cellular proliferation is an important pathological element in the development of lupus nephritis. In an inflammatory process, local generation of TGF β may promote resolution of inflammation, but persistent production of TGF β, possibly in combination with cytokines, may promote accumulation of extracellular matrix (23). In CO-fed mice, a significantly higher histological score (vs. FO-fed mice, 1-fold lower, $P < 0.05$) and a higher frequency of perivascular infiltration of mononuclear cells was observed (12). Northern and Western blot analyses revealed significantly higher TGF β expression in kidneys from CO-fed mice as compared to FO-fed mice (12). Also, expression of fibronectin, one of the components of ECM, is significantly elevated in kidneys of CO-fed (vs. FO-) mice (12). Intercellular adhesion molecule-1 (ICAM-1), which is normally expressed on endothelial cells of glomeruli in kidneys and whose expression is increased by several fold in inflammatory conditions (24), was also significantly elevated in CO-fed mice (12). IL-1 and TNF α alter endothelial cell morphology and induce expression of adhesion molecules, including ICAM-1 (24). In kidneys from CO-fed mice (vs. FO) significantly lower IL-1, IL-6, and TNF α mRNA and protein levels were observed (14), indicating that higher levels of TGF β in kidneys might have modulated expression of these inflammatory cytokines. Taken together, these results indicate the dual nature of TGF β, behaving as a (beneficial) immunosuppressant in the spleen, but as a pathological agent in kidneys.

Autoimmunity and apoptosis. Within the immune network, TGF β mediates a negative feedback circuit inhibiting activation, proliferation, and effector functions. TGF ß decreases c-*myc* oncogene expression in lymphokine-stimulated T-cells and $CD4^+$ cells are affected more than are $CD8^+$ T-cells. Since TGF β modulates proliferation or function of every immune cell type, it is not surprising that its absence has devastating consequences as seen in TGF β knock-out mice (25,26). Accumulation of autoreactive B- and T-cells and severity of inflammation observed in some tissues of these mice raises the question of whether this pathology is somehow associated with aberrant cell death as a result of lower apoptosis or programmed cell death (PCD) in the immune system. Excess accumulation of cells can be due to either increased proliferation or decreased apoptotic cell death. In fact, TGF β expression has been associated with negative regulation of growth. It inhibits DNA synthesis in liver, mammary gland, and uterine endometrium (27). Enhanced expression of TGF β was found in castration-induced regression of prostate and in regressing tumors, suggesting its involvement in PCD (27). In primary cultures of uterine endometrial cells and of hepatocytes, TGF β induced cell death (28). In our dietary studies in B/W mice, spleens from FO-fed mice (vs. CO) expressed significantly higher levels of TGF β and lower levels of c-*myc*. Hence we studied the degree of apoptosis in the splenocytes from both CO- and FO-fed mice.

Reliable methods to assess apoptosis include endonuclease-mediated DNA fragmentation (ladder formation; extensive degradation of chromosomal DNA into oligomers of about 180 b.p.) and propidium iodide (PI) uptake and flow cytometric analysis of PI-stained apoptotic cells. Splenocytes from young FO-fed mice (vs. CO) did not show differences in PI staining, but old mice at 8–10 mon of age showed higher PI-positive cells (Fig. 2). Also in older mice, splenocytes exhibited higher DNA fragmentation (cultured for 18 h in the presence or absence of dexamethasone at 10^{-7} M; data not shown). Although differences in PCD appear to be small, *in vivo* physiological PCD may play a role in modulating autoimmune disease. Encouraged by these results, we have studied the effects of dietary CO and FO on expression of Fas, one of the positive modulators of apoptosis, in splenocytes.

The Fas antigen belongs to a receptor supergene family that includes TNFR, B-cell antigen CD40, NGFR, and T-cell Ag OX40 (29). Fas is broadly expressed on myeloid and lymphoid cells, on fibroblasts, liver cells, heart muscle, and in the ovarian follicle (29). The cytoplasmic domain of Fas probably interacts with signal transduction molecules such as G-proteins, kinases, and/or phospholipases since it has no apparent intrinsic enzymatic activity. Its importance is further emphasized by the fact that the Fas gene was found to be defective in mice carrying the lymphoproliferation (lpr) mutation, closely resembling human SLE (29). This defect in Fas expression leads to excessive numbers of self-reactive T-cells escaping from negative selection in the thymus, resulting in autoimmune disease. FO supplementation significantly increased or maintained higher levels of Fas gene expression in spleens from B/W mice (30). Also, Western blot analysis complemented gene expression, and significantly higher Fas protein levels were observed in the spleens of FO-fed (vs. CO-) mice (data not shown; 30). Thus, increased survival of B/W mice on FO may be due to higher apoptosis of autoreactive T-cells and elimination of self-reactive cells in the periphery. Further, higher PCD was also noted in lymphoid cells from both spleen and thymic tissues from MRL/*lpr* mice on calorie restriction (31).

Autoimmunity and antioxidant enzyme activity and gene expression. Since autoimmune diseases have multifactorial etiologies including genetic, hormonal, viral, and immunological factors, inability of the antioxidant defense system to

cope with oxidative stress has also been reported to contribute to disease progression (32,33). Unsaturated fatty acids can undergo free radical-initiated chain reactions. GSH-Px acts to protect cellular components such as unsaturated fatty acids from free radical oxidation by converting hydrogen peroxide, a potent endogenous free radical generator, to water. Though CAT plays a similar role as that of GSH-Px, GSH-Px is reported to be more important than CAT in hydroperoxide catabolism. Conversely, SOD detoxifies superoxide radicals, and all three enzymes play an important role in the host's antioxidant defense mechanism. Vitamin E, a lipophilic free radical scavenger, protects unsaturated fatty acids by terminating chain reactions involving fatty acid peroxyradicals. In-

TABLE 1
Effect of 10% (w/w) Corn Oil–Krill Oil–or Fish Oil–Based Diets on Activities of Hepatic Cytosolic Antioxidant Enzymes in B/W Mice (11)

Enzyme	Corn oil	Krill oil	Fish oil
Catalase[c]	59.1 ± 4.4[a]	529.9 ± 27.5[b]	472.0 ± 55.8[b]
GSH-Px[d]	25.5 ± 2.0[a]	98.7 ± 5.9[b]	106.0 ± 3.4[b]
SOD[e]	7.2 ± 0.3[a]	.9 ± 0.4[b]	10.2 ± 0.2[b]

[a,b]Values are mean ± SEM of 5 mice/group. Values with different superscript letters (a,b) in the same row are significantly different at $P < 0.05$ level as analyzed by Student's *t*-test with Bonferroni adjustment.
[c]Catalase activity is expressed as μmoles H_2O_2 reduced/mg protein/min.
[d]Glutathione peroxidase (GSH-Px) activity expressed as μmoles NADPH oxidized/g protein/min.
[e]Superoxide dismutase (SOD) activity is expressed in units/mg protein.

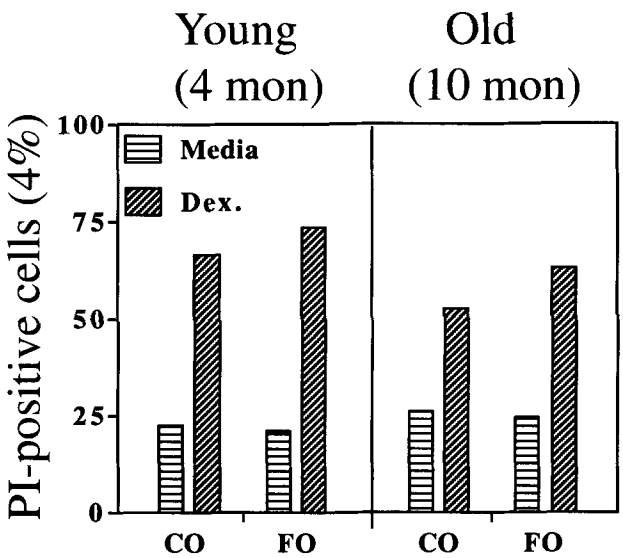

FIG. 2. Effects of dietary corn oil (CO) and fish oil (FO) (10% w/w) on programmed cell death (PCD) in splenocytes from young and old B/W mice as assessed by propidium iodide staining. PCD was lower in old mice on both diets as compared to that in young. Dexamethasone-induced PCD was higher in FO-fed mice (vs. CO) in both young and old mice. PI, propidium iodide.

adequate levels of vitamin E in the presence of low selenium uptake may also result in higher frequency of tumor development. We have demonstrated the importance of supplementing FO-containing diets with vitamin E. Both increased membrane vitamin E and decreased thiobarbituric acid-reactive substances (TBARS) generation was found by supplementing 500 IU of vitamin E with FO-containing diets (34). We have also studied the effects of dietary supplementation of CO and FO on free radical generation and the status of antioxidant defenses in livers and kidneys of B/W mice. TBARS in liver cytosolic fractions were significantly higher in CO-fed mice, indicating higher free radical generation (11). Hence we became interested in testing whether or not the degree of peroxidation in subcellular membrane lipids in autoimmune mice was due to defects in the antioxidant defense system. For this purpose, we analyzed enzyme activity for CAT, SOD, and GSH-Px in the liver cytosolic fraction, and mRNA expression for these enzymes in livers from CO- and FO-fed mice. Both enzyme activity (Table 1) and mRNA expression of these key antioxidant enzymes were significantly lower in the livers (Fig. 3) and kidneys (Fig. 4) of CO-fed mice (vs. FO) (11,12), indicating weaker antioxidant defense mechanisms in various organs in B/W mice fed a CO diet. As described earlier (11), the diets as well as the oils (CO and FO) were supplemented with equal levels of antioxidants, so that our results could not have been compromised because of differences in dietary antioxidant levels.

In summary, the use of highly refined FO with adequate antioxidant supplementation appears to delay onset of autoimmune disease when compared to n-6 (CO)-containing diets. FO also modulates PCD and enhances antioxidant enzyme mRNA levels and decreases free radical-induced tissue damage *in vivo*. Further studies at the molecular level are needed to dissect the protective mechanisms of FO.

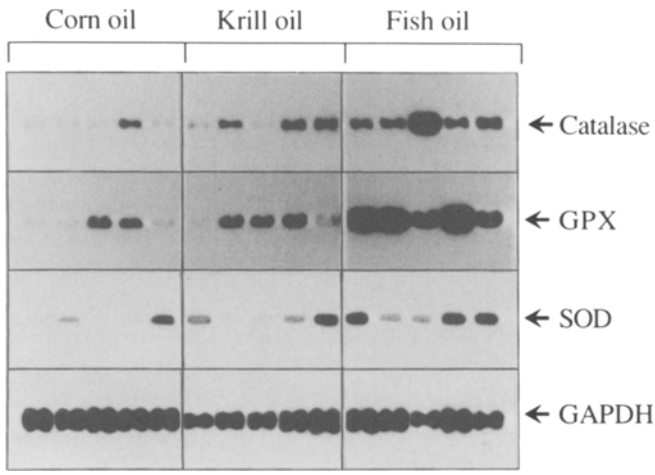

FIG. 3. Higher hepatic antioxidant enzyme gene expression (Northern blot) in 6.5-mon-old B/W mice fed a diet supplemented with CO or FO (10% w/w) (12). Autoradiographic exposure time was 8 d for the enzymes and 36 h for GAPDH. Similar to fish oil, krill oil is a source of n-3 lipids, and krill oil also increased (vs. CO) enzyme gene expression significantly. Each lane represents an RNA sample from one animal. Abbreviations as in Figure 2.

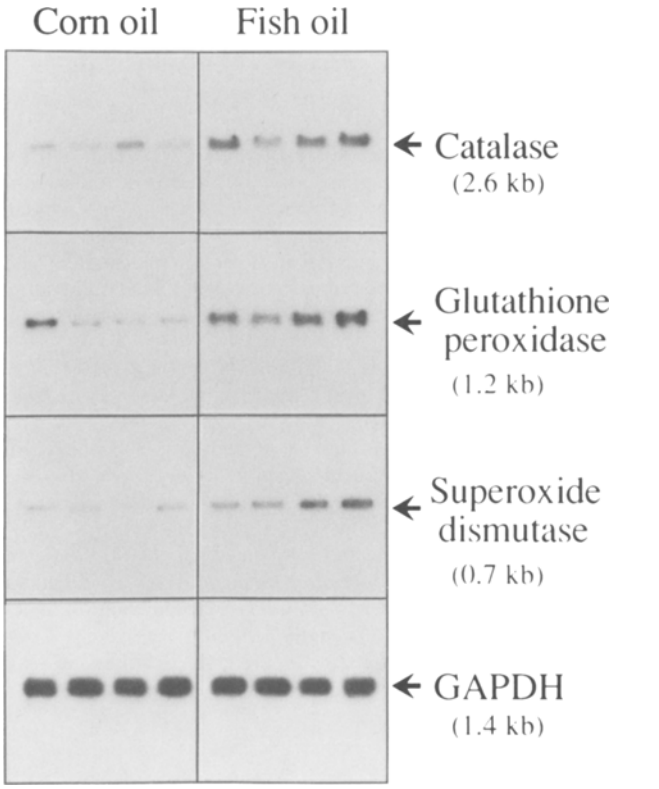

FIG. 4. Northern blot analysis of total RNA isolated from kidneys of 6.5-mon-old B/W mice fed a diet supplemented with CO or FO (10% w/w) (15). Autoradiographic exposure time was 4 d for the enzymes and 36 h for GAPDH. Each lane represents an RNA sample from one animal. Abbreviations as in Figure 2.

ACKNOWLEDGMENTS

This research was supported by National Institutes of Health grants AG 03417, AG 10531, and DE 10863 to Gabriel Fernandes.

REFERENCES

1. Fernandes, G. (1989) Effect of Dietary Fish Oil Supplement on Autoimmune Disease: Changes in Lymphoid Cell Subsets, Oncogen mRNA Expression and Neuroendocrine Hormones, in *Health Effects of Fish and Fish Oils* (Chandra, R.K., ed.), ARTS Biomedical Publications, St. John's, Newfoundland, Canada, pp. 409–433.
2. Fernandes, G. and Venkatraman, J.T. (1993) Role of Omega-3 Fatty Acids in Health and Disease, *Nutr. Res. 13*, S19–S45.
3. Fernandes, G., and Venkatraman, J.T. (1991) Modulation of Breast Cancer Growth in Nude Mice by Omega-3 Lipids, in *World Review of Nutrition and Dietetics*, Vol. 66, *Health Effects of ω-3 Polyunsaturated Fatty Acids in Seafoods* (Simopoulos, A.P., Kifer, R.R., Martin, R.E., and Barlow, S.M., eds.), Karger, New York, pp. 488–503.
4. Mensink, R.P., and Kafan, M.B. (1990) Effect of Dietary Trans Fatty Acids on High-Density and Low-Density Lipoprotein Cholesterol Levels in Healthy Subjects [see comments], *N. Engl. J. Med. 223*, 439–445.
5. Robinson, D.R., Xu, L.L., Tateno, S., Guo, M., and Colvin, R.B. (1993) Suppression of Autoimmune Disease by Dietary n-3 Fatty Acids, *J. Lipid Res. 34*, 1435–1444.
6. Kubo, C., Johnson, B.C., Day, N.K., and Good, R.A. (1984) Calorie Source, Calorie Restriction, Immunity and Aging of (NZB/NZW)F_1 Mice, *J. Nutr. 114*, 1884–1889.
7. Jung, L.K.L., Palladino, M.A., Calvano, S., Mark, D.A., Good, R.A., and Fernandes, G. (1982) Effect of Calorie Restriction on the Production and Responsiveness to Interleukin-2 in (NZB/NZW)F_1 Mice, *Clin. Immunol. Immunopathol. 25*, 295–301.
8. Morrow, W.J.W., Ohashi, Y., Hall, J., Pribnow, J., Hiroshe, S., Shirai, T., and Levy, J.A. (1985) Dietary Fat and Immune Function. I. Antibody Responses, Lymphocyte and Accessory Cell Function in (NZB/NZW)F_1 Mice, *J. Immunol. 135*, 3857–3863.
9. Talal, N., and Steinberg, A.D. (1974) The Pathogenesis of Autoimmunity in New Zealand Black Mice, *Curr. Topics Microbiol. Immunol. 64*, 79–103.
10. Theofilopoulos, A.N., and Dixon, F.J. (1985) Murine Models of Systemic Lupus Erythematosus, *Adv. Immunol. 37*, 269–390.
11. Venkatraman, J.T., Chandrasekar, B., Kim, J.D., and Fernandes, G., Effects of n-3 and n-6 Fatty Acids on the Activities and Expression of Hepatic Antioxidant Enzymes in Autoimmune-Prone NZB/NZW F_1 Mice, *Lipids 29*, 561–568.
12. Chandrasekar, B., Troyer, D.A., Venkatraman, J.T., and Fernandes, G. (1995) Dietary Omega-3 Lipids Delay the Onset and Progression of Autoimmune Lupus Nephritis by Inhibiting Transforming Growth Factor β mRNA and Protein Expression, *J. Autoimmun. 8*, 381–393.
13. Troyer, D.A., Chandrasekar, B., Thinnes, T., Stone, A., Loskutoff, D.J., and Fernandes, G. (1995) Effects of Energy Intake on Type 1 Plasminogen Activator Inhibitor Levels in Glomeruli of Lupus-Prone B/W Mice, *Am. J. Pathol. 146*, 111–120.
14. Chandrasekar, B., and Fernandes, G. (1994) Decreased Proinflammatory Cytokines and Increased Antioxidant Enzyme Gene Expression by Omega-3 Lipids in Murine Lupus Nephritis, *Biochem. Biophys. Res. Commun. 200*, 893–898.
15. Fernandes, G., Friend, P., Yunis, E.J., and Good, R.A. (1978) Influence of Dietary Restriction on Immunologic Function and Renal Disease in NZB/NZW F_1 Mice, *Proc. Natl. Acad. Sci. USA 75*, 1500–1504.
16. Venkatraman, J.T., Chandrasekar, B., Kim, J.-D., and Fernandes, G. (1994) Genotype Effects on the Antioxidant Enzymes Activity and mRNA Expression in Liver and Kidney Tissues of Autoimmune-Prone MRL/MpJ-lpr/lpr Mice, *Biochim. Biophys. Acta 1213*, 167–175.
17. Fernandes, G., Chandrasekar, B., Venkatraman, J.T., Tomar, V., and 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.
18. Fernandes, G. (1994) Dietary Lipids and Risk of Autoimmune Disease, *Clin. Immunol. Immunopathol. 72*, 193–197.
19. Sporn, M.B., and Roberts, A.B. (1990) The Transforming Growth Factor-Betas: Past, Present, and Future, *Ann. NY. Acad. Sci. 593*, 1–6.
20. Roberts, A.B., and Sporn, M.B. (1990) The Transforming Growth Factor-ßs, in *Peptide Growth Factors and Their Receptors, Handbook of Experimental Pharmacology*, Vol. 95, (Sporn, M.B., and Roberts, A.B., eds.), Springer-Verlag, Berlin, pp. 419–472.
21. Del Giudice, G.D., and Crow, M.K. (1994) Role of Transforming Growth Factor Beta (TGF ß) in Systemic Autoimmunity, *Lupus 2*, 213–220.
22. Sharma, K., and Ziyadeh, F.N. (1993) The Transforming

Growth Factor-Beta System and the Kidney, *Semin. Nephrol. 13*, 116–128.
23. Bruijn, J.A., Roos, A., DeGeus, B., and deHeer, E. (1994) Transforming Growth Factor-Beta and the Glomerular Extracellular Matrix in Renal Pathology, *J. Lab. Clin. Med. 123*, 34–37.
24. Kawasaki, K., Yaoita, E., Yamamoto, T., Tamatani, T., Miyadaka, M., and Kihara, I. (1993) Antibodies Against Intercellular Adhesion Molecule-1 and Lymphocyte Function-Associated Antigen-1 Prevent Glomerular Injury in Rat Experimental Crescentic Glomerulonephritis, *J. Immunol. 150*, 1074–1083.
25. Shull, M.M., Ormsby, I., Kier, A.B., Pawlowski, S., Diebold, R.J., Yin, M., Allen, R., Sidman, C., Proetzel, G., Calvin, D., Annunziata, N., and Doetschman, T. (1992) Targeted Disruption of the Mouse Transforming Growth Factor-Beta 1 Gene Results in Multifocal Inflammatory Disease, *Nature 359*, 693–699.
26. Kulkarni, A.B., Huh, C.-G., Becker, D., Geiser, A., Lyght, M., Flanders, K.C., Roberts, A.B., Sporn, M.B., Ward, J.M., and Karlsson, S. (1993) Transforming Growth Factor Beta 1 Null Mutation in Mice Causes Excessive Inflammatory Response and Early Death, *Proc. Natl. Acad. Sci. USA 90*, 770–774.
27. Bursch, W., Oberhammer, F., Jirtle, R.L., Askari, M., Sedivy, R., Grasl-Kraupp, B., Purchio, A.F., and Schulte-Hermann, R. (1993) Transforming Growth Factor-Beta 1 as a Signal for Induction of Cell Death by Apoptosis, *Br. J. Cancer* 67, 531–536.
28. Oberhammer, F.A., Pavelka, M., Sushitra, S., Tiefenbacher, R., Purchio, A.F., Bursch, W., and Schulte-Herman, R. (1992) Induction of Apoptosis in Cultured Hepatocytes and in Regressing Liver by Transforming Growth Factor-Beta 1, *Proc. Natl. Acad. Sci. USA 89*, 5408–5412.
29. Ogawa, N., Dang, H., and Talal, N. (1995) Apoptosis and Autoimmunity, *J. Autoimmun. 8*, 1–19.
30. Fernandes, G., Chandrasekar, B., Mountz, J.D., and Zhao, W. (1995) Modulation of Fas Apoptotic Gene Expression in Spleens of B/W Mice by the Source of Dietary Lipids with and Without Calorie Restriction, *FASEB J. 9*, A787 (4559).
31. Luan, X., Zhao, W., Chandrasekar, B., and Fernandes, G. (1995) Calorie Restriction Modulates Lymphocyte Subset Phenotype and Increases Apoptosis in MRL/lpr Mice, *Immunol. Lett. 47*, 181–186.
32. Ames, B.N., Shigenaga, M.K., and Hagen, T.M. (1993) Oxidants, Antioxidants, and the Degenerative Diseases of Aging [Review], *Proc. Natl. Acad. Sci. USA 90*, 7915–7922.
33. Weindruch, R. (1984) Dietary Restriction and the Aging Process, in *Free Radicals in Molecular Biology, Aging, and Disease* (Amstrong, D, Sohal, R., Cutler, R., and Slater, T.F. eds.), Raven Press, New York, pp. 181–202.
34. Laganiere, S., Yu, B.P., and Fernandes, G. (1990) Studies on Membrane Lipid Peroxidation in Omega-3 Fatty Acid Fed Autoimmune Mice: Effect of Vitamin E Supplementation, in *Antioxidant Nutrients and Immune Functions, Advances Exper. Biol. and Med.*, Vol. 262, (Bendich, A., Phillips, M., and Tengerdy, R., eds.), Plenum Press, New York, pp. 95–102.