Modulation of Gene Expression in Autoimmune Disease and Aging by Food Restriction and Dietary Lipids (42983)

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Abstract. **Several recent observations carried out by many investigators have offered some clues in understanding the mechanism of how food restriction (FR) acts in the prolongation of life-span, but the precise mechanisms involved in modulating the immune system have not been clearly understood. Our own ongoing studies indicate that FR may act at the molecular level and may extend the life-span by modulating functional activities of several genes in various target tissues.**

For instance, while cytochrome P-450 IIB1 and IIB2 expression is known to decline with age in *ad libitum*-fed rats, FR prevented the loss of (drug-inducible) P-450 enzymes in liver tissues. In addition, both α_{2u}-globulin and senescence marker protein 2 expressions, which are regulated by hormones, were also modulated during aging by FR in Fischer 344 male rats. In short-lived autoimmune-prone mice, both FR and omega-3 (*n*-3) fatty acids diet lowered the severity of autoimmune disease both in lupus-prone (NZB × NZW)F_1 mice and in mice prone to develop lymphoproliferative and renal diseases, whereas saturated (*n*-9) and polyunsaturated (*n*-6) dietary lipids not only exacerbated autoimmune disease, but also significantly enhanced expression of several oncogenes in lymphoid tissues. FR and omega-3 fatty acids decreased the expression of certain oncogenes.

Both FR and omega-3 fatty acids may modulate the aging and autoimmune disease processes by not only altering the fatty acid composition, membrane fluidity, and signal transduction, but also by modulating the lymphokine hormone receptors and their functions and thereby modulating expression of several genes in various tissues during the aging process. [P.S.E.B.M. 1990, Vol 193]

In the past two decades there has been considerable new interest in the role of calorie intake on the progression of chronic diseases, aging, and immunologic functions. Advances in immunology have made it possible to dissect changes in lymphoid cell subsets and their receptor functions, changes in antibody subclasses, autoantibodies, and cytokine production.

Although food restriction (FR) has been accepted by several investigators as an effective tool in delaying the aging process, the mechanism involved in the modulation of life-span by FR both in short-lived and/or long-lived strains of mice and rats is not yet clearly understood (1–3). We and others have proposed that FR acts effectively on the immune system, particularly on T cell functions. A progressive decline in T cell immune function, particularly cell-mediated immunity as a function of age, is well established (4, 5). Furthermore, loss of the T cell surface antigen recognition system occurs much earlier than loss of B cell function. We reported that FR prevents loss of interleukin 2 (IL-2) production and maintains vigorous T cell-mediated function in autoimmune-prone mice (6). Other investigators have also reported the action of FR in other T cell and natural killer cell function and life-span extension in long-lived strains of mice (7, 8). FR is also known to inhibit the appearance of age-related diseases such as renal disease, autoimmune disease, and malignancy (9, 10). These changes indicate that the beneficial effects exerted by dietary manipulation may be due to a direct effect at the molecular level. At present, advances in recombinant DNA technology have led to the cloning and sequencing of various genes and to the development of probes needed to study them. This review examines some of the diet-modulated changes occurring at the molecular level.

Dietary alterations are known to have a marked influence on the expression of several genes. Diet, hormones, or their metabolites may cause coordinated changes in the rate of enzyme synthesis and abundance of enzyme mRNA, indicating control at a pretransla-

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tional step (11). Starvation, feeding a carbohydrate-rich diet, insulin, and cAMP all influence the concentration of L-pyruvate kinase, and phosphoenol pyruvate carboxykinase which plays a key role in the regulation of glucose synthesis, by regulating gene transcription (12). Malic enzyme mRNA levels may increase 20-fold when a carbohydrate-rich diet is fed to ducks (13). Dietary and hormonal regulation of fatty acid synthetase is via change in enzyme concentration (14) similar to that observed for other lipogenic enzymes.

Cellular differentiation, development, growth, and metabolic adaptation are critical biologic processes which are regulated by gene expression. Not much is known about the effect of the nutritional state on the chromatin structure and gene expression. Recent studies (15) indicate that imbalances in dietary carbohydrate, protein, and fat components may alter the higher-order configuration of interphase chromatin. Nutrient deficiencies associated with magnesium and zinc lead to aberrations in the organization of metaphase chromosomes (16). Vitamins like folate, B_{12}, and ascorbic acid are known to play a role in maintaining chromosomal integrity (17). A deficiency of folate or B_{12} may activate malignancy by hypomethylating oncogenes (18). The influence of a protein restriction or a controlled feeding regimen on RNA synthesis and chromatin structure has been examined by several investigators (19, 20). Starvation of rats has been reported to decrease the number of transcribing polymerase II (21). Nutrition-mediated changes in the structure of chromatin may alter in accessibility to several effector molecules like mutagens, xenobiotics, and so forth, and thereby alter the balance of immune surveillance mechanism and could facilitate the onset of several chronic diseases.

Food Restriction and Aging

Cytochrome P-450 Expression. So far, among the several approaches undertaken for comparison of prolongation of life-span, FR and/or calorie restriction is found to be most effective in delaying the aging process. Since Fischer 344 male rats on FR demonstrated significantly less tumors between 18 and 26 months of age (22), we attempted to measure cytochrome P-450 in the livers of 18-month-old *ad libitum* (AL) and FR rats. Cytochrome P-450 is an enzyme system that has been recently thought to play a crucial role in the metabolism of steroids and drugs and the clearance rate of both drugs and carcinogenic compounds (23, 24). It is not clear how a decrease in metabolizing cytochrome P-450 enzymes can facilitate a rise in malignancy. Since inducible cytochrome P-450 mRNA, P-450b (IIB1-gene), and P-450e (IIB2-gene) levels significantly decreased with age (25, 26), we decided to study the influence of FR on the drug-inducible capacity of liver microsomal cytochrome P-450s, IA1, IA2, and IIB1 and IIB2 in 20-month-old AL and FR Fischer 344 rats. Our preliminary data indicate that FR increases the induction rate of mRNA for P-450 IIB1 and IIB2 as well as the level of cytochrome P-450b, c, and e enzymes (Fig. 1). FR is known also to prevent the rise of free radical formation (27) and maintain a prolonged immune system. Since we noted a higher level of inducible mRNA cytochrome P-450 as well as higher IL-2 production (Fig. 2) and natural killer function (Fig. 3) in FR rats (9), we hypothesize that elevation of cytochrome P-450 and higher T cell and natural killer cell function may play a crucial role in delaying the appearance of tumors. However, studies are required to verify whether a close functional relationship exists between spleen and liver tissues for cytochrome P-450 enzymes.

α_{2u}-Globulin Expression. α_{2u}-globulin is a major low molecular weight urinary protein of male rats. The synthesis of α_{2u}-globulin is under complex multihormonal control, e.g., androgens which are stimulatory

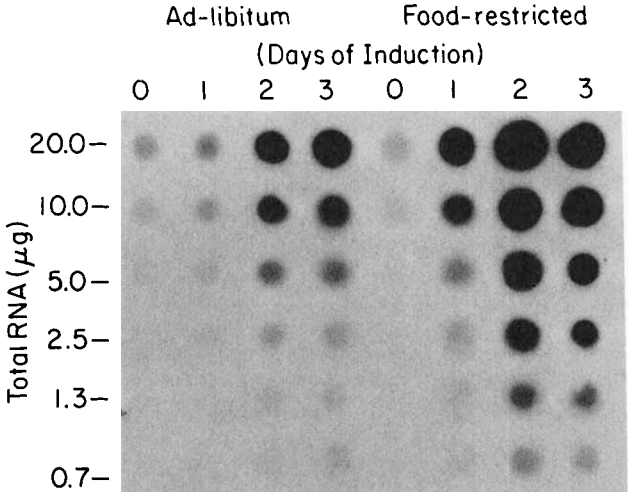

Figure 1. Dot blot showing induction of cytochrome P-450b mRNA by isosafrole in the livers of Fischer 344 rats.

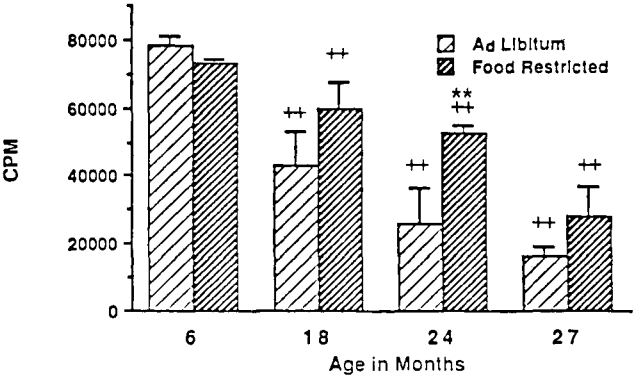

Figure 2. Spleen cells were cultured for 48 hr in the presence of concanavalin A and the supernatants were used to measure IL-2 activity against CTLL-1 cells. ++, Significantly different from 6-month *ad libitum*-fed control rats at <0.05 level. ** Significantly different from age-matched *ad libitum*-fed animals at <0.05 level.

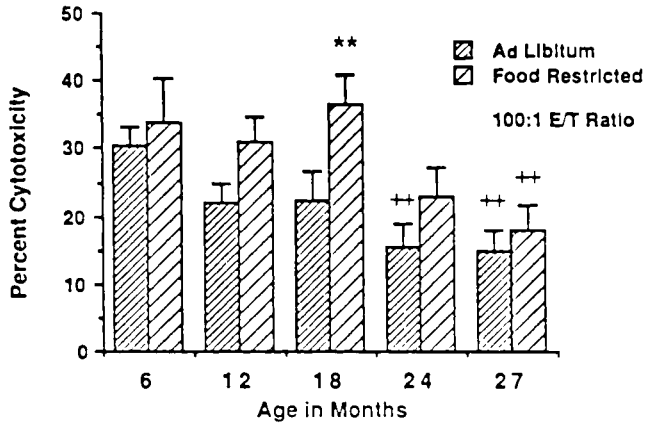

Figure 3. Spleen cells were incubated with ^{51}Cr-labeled YAC-1 tumor cells as targets (1:100 ratio) for 4 hr and the supernatants were measured for ^{51}Cr release in a gamma counter. ++, Significantly different from 6-month *ad libitum*-fed control rats at <0.05 level. ** Significantly different from same age *ad libitum*-fed animals at <0.05 level.

whereas estrogens are inhibitory (28). Our collaborative study with Richardson *et al.* (29) has revealed a significantly decreased α_{2u}-globulin expression in 18-month-old AL-fed rats, whereas FR prevented loss of this protein in the urine. Furthermore, in isolated hepatocytes synthesis of α_{2u}-globulin was markedly greater and the expression of α_{2u}-globulin mRNA was found to be higher in FR rats. However, FR had no effect on the size of the α_{2u}-globulin mRNA, indicating that the regulation of α_{2u}-globulin expression during aging occurs predominantly at the level of transcription. The RNA transcripts that hybridized to the α_{2u}-globulin cDNA probe were revealed to be 2-fold higher for liver nuclei of FR rats and an overall correlation was found between the age-related decline in the synthesis in mRNA in AL-fed rats.

Senescence Marker Protein 2 Expression. To understand the mechanisms of androgen responsiveness to FR, we collaborated with Chatterjee *et al.* (30) to compare the mRNA levels with androgen-inducible α_{2u}-globulin and derepression of the androgen-expressible senescence marker protein 2 (31). Livers obtained from various age groups of AL and FR Fischer 344 male rats were analyzed for mRNAs for α_{2u}-globulin and senescence marker protein 2. The results indicated that in 27-month-old AL-fed rats, α_{2u}-globulin mRNA was only 5% as compared with 6-month-old rats, whereas FR had maintained 45% in 2-month-old rats. In the case of senescence marker protein 2, which is an androgen-repressible protein which rises with age, it was also maintained 45% lower in FR 27-month-old rats than in AL-fed rats. In addition, the effect of FR was also studied for the age-dependent hepatic level of the immunoreactive cytoplasmic androgen-binding protein (32). The results showed that the age-associated loss of cytoplasmic androgen binding and the resultant decreases in hepatic androgen sensitivity were retarded in FR rats. With age, the plasma testosterone level declines both in AL and FR rats, indicating that loss of the plasma testosterone level cannot influence the expression of androgen-responsive genes during aging. Although FR did not alter the plasma testosterone level with age, we, however, did observe the FR prevented a rise in the plasma prolactin level that occurs in AL-fed rats with age (33). Several hormonal and growth factor regulators may play a critical role in the maintenance of membrane stability and function during aging. Age-dependent dysfunction of receptor and postreceptor function along with steroid, androgen, and pituitary hormones have also been studied during aging (34).

***β*-Actin Expression.** Furthermore, expression of β-actin was also measured in the livers of AL and FR rats. Our results show that, although mRNA expression was equal in 6-month-old AL and FR rats (Fig. 4), AL rats showed a rise in β-actin with age while in FR rats the rise was minimal. The implication of higher β-actin mRNA in AL with age is not clear. It is possible that changes in several hormones and growth factors with age may either upregulate or downregulate the β-actin gene expression.

Dietary Lipids and Autoimmune Disease

Variations in the intake of specific dietary factors including vitamins and trace metals are known to influence immune response (35–37), but only relatively recently has much attention been directed to the role of dietary fat. We reported first in 1972 the role of dietary fat on autoimmune disease in NZB mice (38) and later protection from autoimmune renal disease by restricting calorie intake (39). Since then several studies have described adverse effects of dietary lipids in autoimmune-prone animal models. In the past, most studies were directed toward the regulation of immunocompetence by dietary alteration of *n*-6 fatty acids, especially linoleic acid, which is present in high quantity

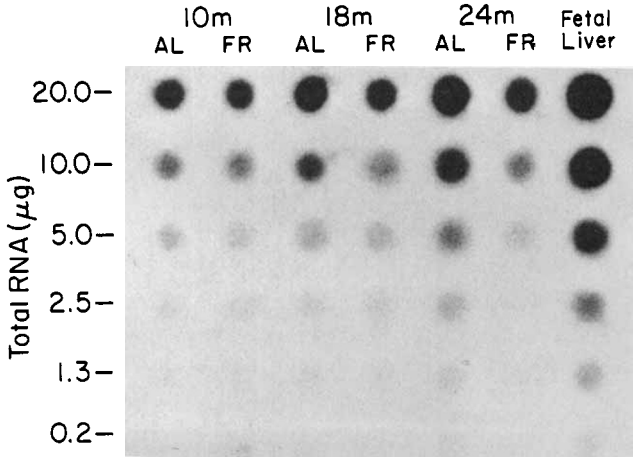

Figure 4. Effect of food restriction on β-actin expression in the livers of aging Fischer 344 rats.

in vegetable oil (safflower oil, corn oil, etc.). Diets enriched with these oils increase the level of linoleic acid in tissue phosphoglycerides and usually tend to elevate arachidonic acid levels (40). Arachidonate is metabolized to other mediators including prostaglandins (PG), thromboxanes, and leukotrienes which may all adversely affect immune function if chronically elevated. Elevated arachidonic acid metabolite levels may in turn alter binding capacity and response of hormones, peptide growth factors, neurotransmitters, and food antigens in specific target tissues. Occupancy of receptors is known to inhibit the production of active messengers including cAMP and cGMP. Current interest is directed at messenger molecules derived from phosphoinositides such as arachidonate, inositol 1,4,5-triphosphate, and 1,2-diacylglycerol.

In contrast, feeding oils rich in *n*-3 fatty acids, such as fish oil or linseed oil, lowers the level of arachidonic acid in phosphoglycerides of tissue membranes, including those of macrophages (40). This would be expected to reduce PG production, not only by reducing the availability of substrate but also because activation of phospholipase will release *n*-3 polyunsaturated fatty acids, which will compete with the arachidonic acid in the cyclo-oxygenase pathway to reduce levels of the two-series PG or even to lead to the formation of three-series PG with different biologic activities. Nonetheless, due to higher risk of cardiovascular disease caused by saturated dietary fats, there has been a shift toward greater intake of dietary vegetable fats, thus promoting increased production of PGE_2 *in vivo*. This is most likely to decrease IL-2 production as well as to inhibit the response of T cells to IL-2 (41). In addition, PGE_2 may enhance immunoglobulin production and appears to inhibit T cell suppression of B cell functions (42). Such adverse hormonal effects of polyunsaturated fats on normal lymphocytes and on autoimmune-prone mice have also been noted (43, 44).

Years of study have indicated genetic, viral, and various immunologic abnormalities in autoimmune animals (45, 46). In humans, the concordance of lupus disease expression in identical twins is about 67% (47). This implies that external factors may have considerable influence on the expression of this disease. Dietary effects on autoimmunity, therefore, requires close scrutiny. In the case of rheumatoid arthritis and systemic lupus erythematosus in humans, however, the role of diet has not yet received sufficient attention. Nonetheless, in recent years both clinical and experimental studies have focused on the role of food antigens, lipids, and caloric content in modifying immune function (2, 39, 48). Recently, intake of fish and/or fish oil-supplemented diets have been found to protect not only against cardiovascular diseases but also autoimmune disease in humans and animals (49–52).

There is no dispute that calorie restriction ameliorates disease in autoimmune-prone mice (51). However, 40% calorie or food restriction throughout life is not a practical approach, nor is it appealing to those who have active or chronic disease. Thus, alternative approaches are much more attractive. Our recent studies are directed toward identifying dietary fats which are less detrimental to the immune system and ameliorate renal and vascular disease. Several studies by other investigators on autoimmune mice using diets containing either essential fatty acid-deficient, saturated fat (coconut oil), or polyunsaturated fatty acids with an *n*-3 double bond such as fish oil have demonstrated protective effects not seen with either vegetable polyunsaturated fatty acid fats (*n*-6), corn oil or safflower oil, or saturated animal fats (beef or lard) (44, 52). Because polyunsaturated fatty acids lower cholesterol levels and protect against cardiovascular disease, considerably higher levels (8–10 times) of vegetable fat intake have been noted in recent years in the United States (53). As these fats do not protect against experimental autoimmune disease, the mechanism of protection seen with fish oil or coconut oil is considered to be mostly due to decreased production of immunosuppressive PG of the E series (54). Excess PG and decreased IL-2 production may not only alter IL-2 receptor function but may also promote activation of oncogenes.

Diet and Oncogenes. The regulation or structural alteration of cellular oncogenes has been implicated in the development of various malignant tumors in rodents and humans. Oncogene alteration by point mutation can result in a protein product with a strongly enhanced oncogenic potential. Both RNA or DNA of viral origin appear to be involved in tumor formation.

The hallmark of cellular proto-oncogene is its evolutionary conservation and close homology in different species, thereby indicating the most crucial and fundamental roles for many of these genes in normal cellular metabolism (55, 56). At present, proto-oncogenes are thought to encode for proteins that are closely involved with the regulation of cell growth and differentiation from birth to death. Furthermore many proto-oncogenes are found to code for growth factors or growth factor receptors and are expressed during activation in normal cells (Table I) (57). However, since activated lymphoid cells are found in autoimmune disease states, studies undertaken in autoimmune-diseased patients have shown increased expression of several proto-oncogenes, particularly of c-*myc*, c-*myb*, and c-*ras* in peripheral blood lymphocytes.

Oncogenesis is a multistep process that is likely to involve successive activation of several oncogenes. Studies on the involvement of cellular oncogenes in human cancer have contributed to a rapid progress in this field. The role of oncogenes in autoimmune disease states is receiving considerable attention. Several studies have been carried out to measure the activation of various oncogenes in immune cells.

Table I. Oncogene Products and Their Functions (67)

DNA-binding proteins located on nuclei	*-fos, c-myc,* L-*myc, myb, ski,* N-*myc, erb A, ceal*
Proteins in locations other than nuclei	
Protein kinases	
Tyrosine kinases	*-abl, erbB*1, *erbB2, fgr, fms, fps, eph, kit, met, neu, oncD, ros src, syn, lck, lyn*
Serine and threonine kinases	*-mos, raf, pks*
Guanine nucleotide binding protein	-H-*ras,* K-*ras,* N-*ras*
Growth factor-related protein	*-sis*
Integral membrane protein	*-mas*
Unknown	*-dbl, hst, lca, mcf-2, ret, rel*

We have shown that both (NZB × NZW)F_1 and MRL/*lpr* mice, when maintained on a calorie-restricted dietary regimen, have a significantly extended life-span. Disease expression in mice bearing the *lpr* gene is characterized by autoimmunity and massive T cell lymphoproliferation and is thought to be mediated by the effect on B cells caused by T cell factors released from the lymph nodes. The association between abnormal T cell development and increased c-*myb* oncogene expression in *lpr* mice lymphoid tissue was first shown by Mountz *et al.* (59). Their subsequent extensive studies confirmed that selective abnormal proto-oncogene expression was characteristically associated with abnormal cell growth and differentiation of lymphocytes (60).

Recently, much attention has been focused on the role of omega-3 fatty acid supplementation on autoimmune disease, particularly in renal disease and immune dysfunction in autoimmune *lpr* mice. We maintained (NZB × NZW)F_1(B/W) and C3H/*lpr* mice on FR and fish oil-supplemented diets and as Mountz *et al.* (59) described an increase in the expression of several proto-oncogenes in different strains of autoimmune mice, we compared the steady-state levels of oncogene expression in lymph node and spleen cells of *lpr* mice and B/W mice (61–63). The results demonstrated that calorie restriction in general lowered oncogene mRNA expression, but the extent of expression varied in lymph node versus spleen and C57BL/*lpr* versus C3H/*lpr* mice (Fig. 5). The fish oil diet also reduced c-*myb*, c-*ras*, and c-*abl* RNA in C57Bl/*lpr* lymph nodes, but no significant effect was observed in C3H/*lpr* mice. However, using diets supplemented either with 5 or 20% corn oil, and lard, we found that saturated fat had a profound effect on the progression of disease and elevated most of the oncogene expression. The changes in oncogene expression are related to alterations in lymphoid cell subsets. Both FR and fish oil-containing diets prevent a rise in Ia antigen expression, maintain lower Ig^+ cells, and increase T cell functions, including preventing loss of IL-2 production and response (63). In the case of Fischer 344 male rats, FR also modulated V-H-*ras* mRNA expression in 19-month-old rats (Fig. 6).

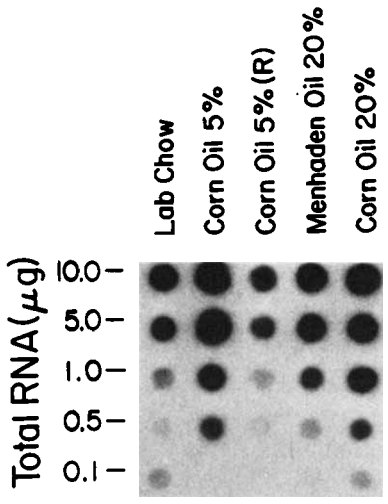

Figure 5. C3H/*lpr* mouse spleen mRNA hybridized with c-*myc* cDNA probe.

Interleukin 2 Receptors. FR has facilitated both the induction of higher IL-2 receptor expression and increased IL-2 production as compared with AL-fed rats (64). It is not clear how FR selectively causes an increase in HA IL-2 receptor. However, FR does appear to increase both α chain and β chain levels on cell membranes. It is well known that IL-2 receptors appear in three different forms on the lymphocyte membrane. It has been reported that high affinity IL-2 receptor promotes the rapid receptor-mediated internalization of IL-2 receptor complex by the mediation of β chains and that this internalization causes DNA synthesis and mitosis in cells (65). A similar receptor mechanism may operate in maintaining several other hormone actions on other cells. Loss in various receptor activities during aging such as loss of insulin receptors in diabetes and loss in low-density lipoprotein receptors could lead to accelerated aging phenomena and a rise in the severity of renal and cardiovascular disease states.

The possible changes in various growth factors and cytokine production of IL-1, IL-2, IL-4 and so forth, by diet may have an effect on maintaining a reduced level of various oncogene expressions *in vivo*. Our on-

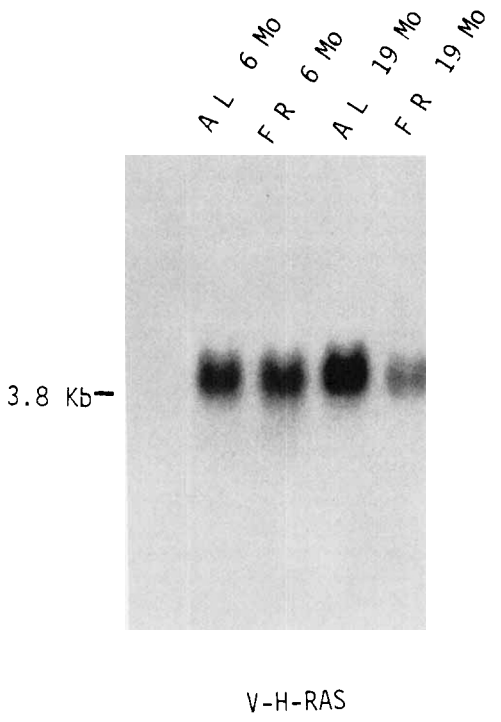

Figure 6. Northern blot of mRNA extracted from spleen tissues of 6- and 18-month-old *ad libitum*-fed and food-restricted rats. mRNA hybridized with V-H-*ras* cDNA probe.

going studies strongly suggest that dietary lipids not only modulate membrane fatty acid composition but also could modify the signal transduction mechanism, including membrane receptors and membrane fluidity. Increases in fluidity, IL-2 receptor expression, IL-2 production, and decrease in cholesterol level were noted on spleen cells in FR and fish oil-fed B/W old mice (9). These changes appear to be due to less incorporation of *n*-6 fatty acids and an increased level of *n*-3 fatty acids into cell membranes. Future nutritional and aging studies using more defined molecular approaches with genes of interest, particularly by using transgenic mice (66), may contribute in understanding the role of individual genes in inducing accelerated aging, malignancy, and/or autoimmune diseases in different dietary regimens. Although we and others have reported changes in the gene expression by diet, still several more crucial experiments have to be undertaken soon to understand the mechanism of altered expression of proteins regulated by hormones and oncogenes with age and thereby reduce renal and autoimmune disease states in these animals.

This research was supported by NIH Grants AG01188 and AG03417 and The Kleberg Foundation.

We would like to thank Drs. Masoro, Yu, Richardson, Chatterjee, Roy, and Mountz for their collaborative help in these studies and Vikram Tomar for his expert technical assistance. We also would like to thank Kay Meerscheidt for her excellent typing assistance.

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