

LOW-CALORIE DIET SELECTIVELY REDUCES EXPRESSION
OF RETROVIRAL ENVELOPE GLYCOPROTEIN gp70 IN
SERA OF NZB × NZW F₁ HYBRID MICE*

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NZB × NZW F₁ hybrid (NZB × W)¹ mice are highly susceptible to an autoimmune disease that resembles human systemic lupus erythematosus (SLE), and they die at an early age of the consequent immune complex (IC) glomerulonephritis (1). However, restricting their intake of calories delays the onset of disease and markedly prolongs their life spans (2–4). This beneficial effect is associated with alteration of host immunologic function and decreased deposition of IC in their kidneys (4, 5).

Because SLE-prone mice have high concentrations of the retroviral envelope glycoprotein, gp70, in their sera and deposited in their diseased glomeruli (6–9), endogenous retroviruses and/or their gene products have been implicated in the pathogenesis of this autoimmune disease (6, 10, 11). Apparently, only SLE-prone strains of mice spontaneously develop immune responses to their own serum retroviral gp70 and form antibodies, which subsequently become part of the IC found in their sera and tissues (12, 13). The fact that the appearance of gp70-anti-gp70 IC (gp70 IC) in serum closely parallels the onset of disease in each SLE-prone mouse (12, 14–16) underscores the pathogenic significance of gp70 IC. Therefore, in the present study, we have investigated whether the prophylactic effect of a low-calorie diet in NZB × W mice is attributable to decreased expression of endogenous retroviruses or their gene product, gp70, or to reduction in the immune response to them. Our results indicate that NZB × W mice given low-calorie diets have far lower levels of retroviral gp70 antigen in their sera than similar mice consuming standard diets, but maintain usual levels of the retroviral major structural protein, p30. Additionally, the inhibition of antibody production markedly minimizes the formation of gp70 IC in such mice.

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¹ Abbreviations used in this paper: dsDNA, double-stranded DNA; gp70 IC, gp70-anti-gp70 immune complex; LPS, lipopolysaccharide; NZB × W, NZB × NZW F₁ hybrid; SLE, systemic lupus erythematosus; ssDNA, single-stranded DNA; Staph A, *Staphylococcus aureus* protein A.

Materials and Methods

Mice. NZB × W mice of both sexes were obtained by mating NZB females with NZW males in the animal colony at Sloan-Kettering Institute. Their blood samples were collected by orbital sinus puncture, and the sera were stored at -20°C until use.

Diets. After weaning (1 mo of age), NZB × W mice of both sexes were maintained on one of three different diets. In group I, the animals were fed Purina laboratory chow (Ralston Purina Co., St. Louis, Mo.) *ad libitum* (≈ 20 calories per day); group II was limited to a diet of ~ 20 calories per day; and group III was limited to 10 calories per day, but was provided with supplementary vitamins and minerals to equal those given mice of group II. Composition of diet, source of ingredients, method of preparation, and feeding procedures, as well as housing of animals were identical to those described previously (3). Briefly, the diet was 22% casein, 33% dextran, 33% starch, 5% corn oil, 4% mineral mixture, 2% vitamin mixture, and 1% agar. Exact amounts of food, either 5 g (~ 20 calories) for group II or 2.5 g (~ 10 calories) for group III, were provided at the same time each day between 8 and 10 a.m.

Morphologic Studies. Kidney tissues from NZB × W mice were assessed for morphologic and immunopathologic changes by light and fluorescence microscopy as previously described (14). Renal histopathologic alterations were graded on a semiquantitative scale by criteria adopted from Pirani and Salinas-Madrigal (17). The scale ranged from 0 to 4: 0, normal; 0.5, minimal or questionable; 1, mild; 2, moderate; 3, moderately severe; 4, severe alterations.

Radioimmunoassay for gp70 and gp70 IC. The concentrations of gp70 in serum samples of test mice or gradient fractions from these sera were determined by a serum's ability to inhibit the binding of goat anti-feline leukemia virus antibody to ^{125}I -labeled gp70 from Rauscher murine leukemia virus, as described previously (12).

To determine the amounts of gp70 bound to IgG anti-gp70 antibodies, the sera were first analyzed for gp70 content, then IgG was removed by adsorption with protein A-containing *Staphylococcus aureus* (Staph A, Calbiochem-Behring Corp., La Jolla, Calif.), and finally concentrations of gp70 were compared before and after removal of IgG from sera. The details of this assay were described previously (14). The sedimentation characteristics of serum gp70 were analyzed by sucrose density gradient ultracentrifugation (12).

Radioimmunoassay for p30. Concentrations of the major structural protein of retroviruses, p30, in serum samples were determined in a blocking assay using ^{125}I -p30 from Rauscher murine leukemia virus and guinea pig anti-p30 antibodies (provided by Dr. R. V. Gilden of Flow Laboratories, Inc., Rockville, Md.), as described by Parks and Scolnick (18).

Detection of Anti-DNA Antibodies. Serum levels of antibodies to double-stranded DNA (dsDNA) and single-stranded DNA (ssDNA) were determined by using a modification of the Farr DNA-binding radioimmunoassay (19). The results are expressed as a percentage of 20 ng of ^{125}I -DNA precipitated specifically after correction for nonspecific precipitation in pooled sera from immunologically normal mice.

Quantitation of Haptoglobin and Albumin. Serum concentrations of haptoglobin or albumin were measured by radial immunodiffusion in agar using goat anti-human haptoglobin or anti-murine serum albumin antisera (N. L. Cappel Laboratories, Cochranville, Pa.) according to the method of Mancini et al. (20). Results are expressed as a percentage of values from the pooled sera of C57BL/6 male mice.

Results

Effect of Dietary Restriction on Survival Rate and Renal Disease. Groups of newly weaned NZB × W female and male mice were fed three different diets and the results were charted when survivors were 400 d old. All female mice on an unlimited amount of standard laboratory chow or confined to 20 calories per day had died, whereas $>75\%$ of females fed only 10 calories per day were alive. Of male mice 400 d old, none in the 10 calorie per day group and 50% of two other groups had died.

When evaluated for renal histopathology at 8 mo of age, female mice fed the low-calorie diet had less evidence of IC glomerulonephritis than those fed calorically normal diets. The retardation of renal disease was reflected in histologic mean grades

of disease: renal lesions of mice on standard laboratory chow or on 20 calories were at grade 3.0 or higher, whereas lesions of calorie-restricted mice reached a mean of only 0.9. By immunofluorescence, larger amounts of IgG, IgM, C3, and retroviral gp70 were visible in glomeruli of mice with normal caloric intake compared with mice fed the low-calorie diet.

Effect of Dietary Restriction on Anti-DNA and Retroviral gp70 IC Formation. The percentages of serum dsDNA- and ssDNA-binding activities in 8–10-mo-old NZB × W female mice fed three different kinds of diets are summarized in Fig. 1. A modest but significant decrease in levels of both anti-dsDNA and anti-ssDNA antibodies was observed in the sera of calorie-restricted animals compared with those fed richer diets.

The levels of retroviral gp70 IC in three groups of 8–10-mo-old NZB × W females were compared by measuring amounts of gp70 present before and after IgG was removed from their sera by adsorption to Staph A. The quantities of immunoglobulin (Ig)-bound gp70 were far lower in the group of mice on 10 calories a day (mean ± 1 SD; $2.7 \pm 3.7 \mu\text{g/ml}$) than in those fed laboratory chow ($19.5 \pm 14.1 \mu\text{g/ml}$) or a 20 calorie diet ($16.9 \pm 10.4 \mu\text{g/ml}$) (Fig. 2). As a result of absorption, 13 of 17 mice given their fill of standard laboratory chow and 19 of 25 mice limited to 20 calories per day had $>10 \mu\text{g/ml}$ of gp70 complexed with antibodies in their sera. In contrast, only 1 of 28 mice fed 10 calories per day had $>10 \mu\text{g/ml}$ of Ig-bound gp70.

Reduced amounts of circulating gp70 IC in mice on the low-calorie diet were also documented by sucrose density gradient analysis. Sera from 7 of 9 mice freely fed laboratory chow for 8–10 mo or 9 of 12 mice on 20-calorie diets for the same period had substantial amounts of the rapidly sedimenting, heavy form of gp70, in addition to free 5S gp70. However, sera from mice of the same age and sex restricted to 10 calories had little or no detectable amounts of heavy Ig-bound gp70.

Effects of Dietary Restriction on Expression of Retroviral gp70 and p30. Because, in the foregoing experiments, the effect of calorie restriction on gp70 IC formation was much more striking than on anti-DNA antibody formation, it seemed that a low-calorie

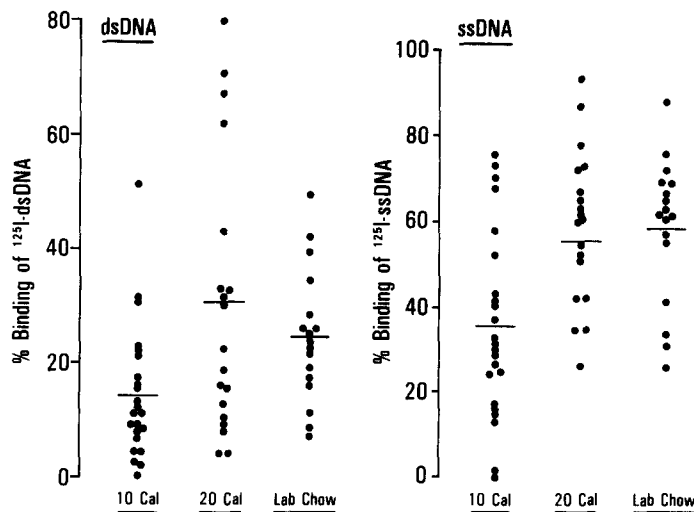


FIG. 1. Serum dsDNA- and ssDNA-binding activities in 8–10-mo-old NZB × W female mice fed three different diets. The mean values are indicated by the horizontal line.

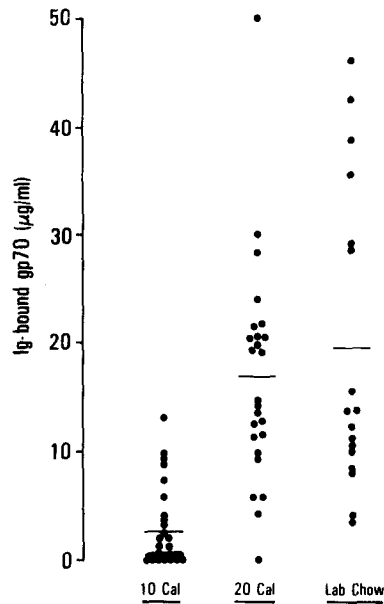


FIG. 2. Concentrations of Ig-bound gp70 in sera of 8-10-mo-old NZB \times W female mice fed three different diets. The mean values are indicated by the horizontal line.

TABLE I
Levels of gp70 in Sera of NZB \times W Female Mice

Age*	10 calories	20 calories	Laboratory chow
<i>mo</i>			
1	43 \pm 9‡ (8)§	44 \pm 12 (8)	46 \pm 10 (8)
1.5	23 \pm 9 (8)	46 \pm 14 (8)	48 \pm 6 (8)
2	21 \pm 4 (16)	53 \pm 16 (23)	47 \pm 16 (17)
6	20 \pm 8 (9)	52 \pm 20 (14)	NT
8-10	21 \pm 10 (47)	44 \pm 15 (58)	43 \pm 15 (17)

* Mice were bled before feedings of 10- or 20-calorie diets.

‡ Serum concentrations of gp70 (μ g/ml). Mean \pm 1 SD.

§ Number of mice tested.

|| Not tested.

intake might affect the expression of retroviral gp70 antigen, thereby causing the more profound reduction in serum levels of gp70 IC. To examine this possibility, the total concentrations of gp70 (free and complexed) detectable in sera were compared before and after mice were fed the three different diets. Concentrations of gp70 were consistently high in sera of mice fed standard laboratory chow at will or 20 calories per day throughout the course of examination (Table I). In contrast, animals fed 10 calories per day had less than half as much gp70 in their sera, although they did have high levels of gp70 equal to other groups of mice before starting the low-calorie diet, i.e., 1 mo of age. These low levels were already evident 2 wk after feeding animals the low-calorie diet and remained virtually unchanged.

To study whether the reduction of serum gp70 by this low-calorie diet was related to the expression of endogenous retroviruses, we measured amounts of the major structural protein of retroviruses, p30, in sera from groups of 6-mo-old NZB \times W

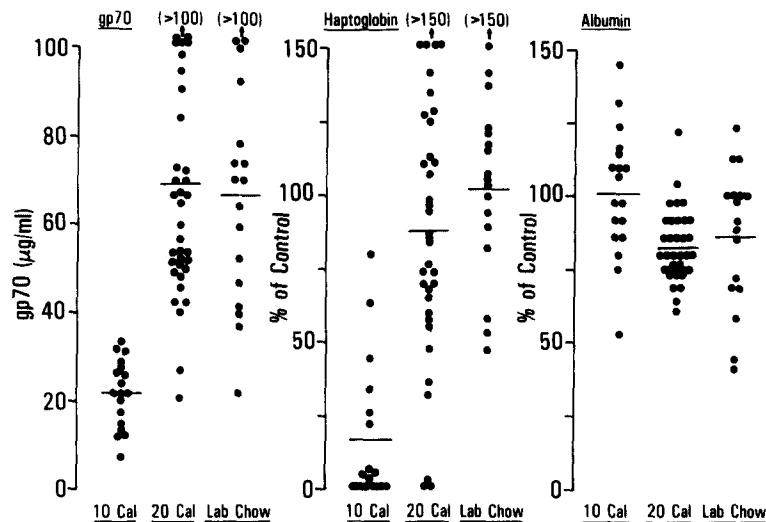


FIG. 3. Serum concentrations of retroviral gp70, haptoglobin, and albumin in 6-mo-old NZB × W male mice fed three different diets. The mean values are indicated by the horizontal line.

female mice fed either 10 calories per day or 20 calories per day. In contrast to their reduced expression of gp70, the calorie-restricted animals had equivalent levels of p30 (119.3 ± 20.8 ng/ml) to those calorically unrestricted animals (120.8 ± 16.4 ng/ml).

Effect of Dietary Restriction on Serum Levels of Haptoglobin and Albumin. Because gp70 found in sera could be synthesized in the liver, as with many other serum proteins as suggested recently (21–23; and I. Hara, S. Izui, and F. J. Dixon, manuscript in preparation), we evaluated the effect of low-calorie intake on the serum levels of another glycoprotein, haptoglobin, and of a nonglycoprotein, albumin, both of which are synthesized by hepatic cells (24–25). In this experiment, we analyzed sera from 1.5-, 6-, and 8-mo-old NZB × W male mice, because sera from male mice generally have much higher levels of haptoglobin and do not lose significant amounts of serum albumin until the mice are 12 mo old and dying of renal failure. Low-calorie intake greatly reduced serum concentrations of haptoglobin compared with those in mice on the other two dietary protocols. Representative results in 6-mo-old mice of three groups are shown in Fig. 3. In mice on 20-calorie diets, mean values were 102.0% of normal pooled values, which were comparable to those of mice fed standard laboratory chow (100.3%). By contrast, the mean values of calorie-restricted mice were only 16.7%. Clearly, this decrease in serum levels of haptoglobin correlated well with a parallel reduction in serum concentrations of retroviral gp70 in mice fed the low-calorie diet. In spite of dramatic decreases in the concentrations of the glycoproteins, gp70 and haptoglobin, levels of nonglycoprotein, albumin, were not lower, but rather higher in mice restricted to 10 calories per day.

Discussion

We have examined the effect of calorie restriction on the expression of serum retroviral gp70 antigen and on the formation of circulating gp70 IC in SLE-prone NZB × W mice. Calorie intake restricted to half the normal amount from the time of weaning lowered the serum concentrations of gp70 to less than half of those in control

mice fed diets established as average. Subsequent formation of circulating gp70 IC was greatly suppressed. Consequently, the present studies confirm and extend previous observations (4) that restricting calorie intake from infancy onward significantly inhibits the spontaneous production of autoantibodies such as antibody to DNA in NZB \times W mice. This alteration of host immunologic function seems to suppress the production of anti-gp70 antibodies and thereby substantially lessen levels of gp70 in sera, although reduced expression of retroviral gp70 antigen might partially cause the reduced formation of antibody against gp70 in calorie-restricted mice. Clearly, generalized suppression of autoantibody synthesis decreased the formation of pathogenic IC containing such anti-gp70 or anti-DNA antibodies and prevented the development of glomerulonephritis in these mice. In fact, deposition of Ig and gp70 antigen in renal glomeruli was minimal in mice on the low-calorie diet. However, one should note that the decrease in serum gp70 IC concentrations was much more drastic than that in anti-DNA antibodies. This notable correlation between serum levels of gp70 IC and the development of glomerulonephritis in the calorie-restricted mice further confirms the pathogenic significance of gp70 IC in murine SLE (12, 14–16).

A particularly striking influence of the calorie restriction in NZB \times W mice was the reduction in serum concentrations of gp70, the major envelope glycoprotein of their endogenous retrovirus. This gp70 circulates in a form that is not associated with viral particles but is closely related structurally to the gp70 of xenotropic virus (13, 26). Apparently, the decreased amounts of retroviral gp70 in the sera of our test mice were unrelated to the production of complete viral particles, because serum levels of the major structural viral protein, p30, were not reduced by low-calorie diet. This is also supported by the previous finding that the expression of endogenous retroviruses in tissues from calorie-restricted mice was equal to that of control mice (27). In fact, certain G_{IX}^+ mice, such as strain 129, expressed substantial amounts of gp70 in their sera but did not produce the related virions according to assays used thus far (8, 26, 28). Furthermore, we recently found (23) that a single injection of bacterial lipopolysaccharide (LPS) greatly increased serum levels of xenotropic viral gp70 without apparent activation of xenotropic virus in several strains of mice, including strain 129.

Because virion-free gp70 is also present on the surfaces of lymphocytes (29–31) and activation of such lymphocytes enhances its expression (32, 33), any reduction in the expression of serum gp70 could relate to generalized suppression of the immune system in calorie-restricted mice. However, several lines of evidence dispute such a possibility. Increased levels of polyclonal antibody production observed in all SLE-prone mice did not correlate with serum levels of gp70 (34), and gp70 concentrations remained unchanged throughout the course of disease despite the progression of autoimmune disease (12). Further, neither thymectomy, splenectomy, nor exchange of hematopoietic tissues between 129- G_{IX}^+ and 129- G_{IX}^- mice changed levels of serum gp70 in either strain (21). Finally, our recent study showed that the enhancement of serum gp70 expression by LPS was independent of the activation of B lymphocytes and of the presence of T lymphocytes (22; and I. Hara, S. Izui, and F. J. Dixon, manuscript in preparation).

Besides the reduction of serum gp70, low-calorie intake markedly reduced concentrations of another serum glycoprotein, haptoglobin, although albumin levels did not decrease in these mice. The decrease in haptoglobin levels was evident 2 wk after the

low-calorie diet began, at the same time that gp70 levels dropped. It should be noted that serum levels of haptoglobin, as well as gp70, vary considerably among murine strains and are always higher in male mice than in females (12; and I. Hara, S. Izui, and F. J. Dixon, manuscript in preparation). The combined data suggest that the expression of serum retroviral gp70 seems to be controlled by a mechanism similar to that for other serum glycoproteins, such as haptoglobin, but different from that for albumin. Additionally, like many other serum glycoproteins (24, 35, 36), gp70 may be synthesized by hepatic cells. In fact, the dependence of serum gp70 concentrations on the male sex hormone, testosterone (21), supports this possibility, because the liver is a known source of several proteins whose serum concentrations depend markedly on the host's sex (37). More directly, our preliminary studies demonstrated that the injection of LPS greatly elevated the amounts of gp70 recovered only from the liver with a parallel increase in serum gp70 (I. Hara, S. Izui, and F. J. Dixon, manuscript in preparation). All these results strongly support the hypothesis that hepatic cells are the major source of serum xenotropic viral gp70. If so, the low-calorie diet given mice predisposed to autoimmune disease in the present study might have selectively suppressed the production of these glycoproteins in hepatic cells.

Summary

The effect of dietary restriction on the expression of retroviral envelope glycoprotein, gp70, and the formation of gp70-anti-gp70 immune complexes was investigated in lupus-prone NZB \times NZW F₁ hybrid mice. Restricting total calorie intake from the usual 20 to only 10 calories per day after weaning markedly reduced serum levels of both free and antibody-complexed gp70, prevented renal disease, and increased the life spans of these mice. The reduction in serum gp70 was evident after only 2 wk of feeding these animals the low-calorie diet, and the concentration remained virtually unchanged throughout the course of 10 mo experimentation. However, serum concentrations of the major structural protein, p30, of endogenous retroviruses were not altered by restricting calories. Amounts of the serum glycoprotein, haptoglobin, decreased parallel to those of gp70 but amounts of albumin did not. These results suggest that the expression of gp70 in serum is controlled independently of the production of complete viral particles, and regulated by a mechanism similar to that for other serum glycoproteins, such as haptoglobin.

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