Calorie Restriction Delays the Crescentic Glomerulonephritis of SCG/Kj Mice (44289)

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Abstract. **Reduced dietary calories can delay the onset and diminish the severity of murine autoimmunities of numerous inbred and hybrid mutant strains. We sought to determine whether the precipitous, autoimmune, crescentic glomerulonephritis of recombinant inbred SCG/Kj mice could be abrogated similarly by calorie restriction. Weanling SCG/Kj mice develop hematuria and proteinuria, and 50% die as 16-week-old young adults. In this study, 113 4-week-old SCG/Kj mice were fed either *ad libitum* a milled chow (Group A, $n = 50$), or a semipurified diet (Group B, $n = 29$), or were fed a calorie-restricted semipurified diet (Group C, $n = 34$), so that mice of Group C consumed approximately 32% fewer calories, but similar amounts of essential dietary constituents as those of Group B. Calorie restriction of Group C provided modest ($P = 0.05$) or substantial survival advantage ($P = 0.001$) compared to the *ad libitum* feeding of Groups B or A, respectively. Progression to severe glomerular pathology was delayed among Group C mice, with more than a 5-week delay to heavy proteinuria (>100 mg/dl), a >4-week delay to hematuria, and a >5-week delay to median mortality, representing a 20% or 25% extension of median life span, compared to *ad libitum*–fed Group B and A mice, respectively. Mean glomerular histopathology scores were also lower in calorie-restricted mice compared to the *ad libitum*–fed cohorts ($P = 0.001$). Titers of anti-ss-DNA, ds-DNA, and ANCA autoantibodies developed in weanlings prior to the full imposition of calorie restriction and were not reduced significantly by calorie restriction.** [P.S.E.B.M. 1998, Vol 218]

Calorie restriction extends the latency to onset and reduces the incidence of strain-specific pathologies in inbred, hybrid, and mutant mice genetically prone at a young age to develop a progressive disease syndrome that ordinarily would not affect the mice until advanced age (1). Limiting dietary energy to levels 30%–35% less than those consumed when diet is freely accessible delays or prevents the immune thrombocytopenic purpura of (NZW X BXSB)F_1 mice (2), the systemic lupus erythematosus of (NZB X NZW)F_1 mice (3), the nephrophthisis of *kd/kd* mice (4), the lymphoproliferative syndrome of MRL/Mp-*lpr/lpr* mice (5), the breast cancer of C3H/HeOu mice (6), the thymic lymphosarcoma of AKR mice (7), and the autoimmunities of both the BXSB mice (8) and NZB mice (9). This extension of healthful longevity depends quite specifically on calorie restriction and is not the consequence of altered dietary composition (10).

Mice of the inbred SCG/Kj strain were derived by selectively inbreeding those progeny of (BXSB × MRL/Mp *lpr/lpr*)F_1 mice that exhibited clinical and histological evidence of acute crescentic glomerulonephritis, including proteinuria and hematuria, beginning as early as 3–4 weeks of age and that were found to have severe glomerular lesions, postmortem, after the weaning of their litter (11). In addition to advanced renal disease, mice of this strain also develop systemic necrotizing vasculitis involving small arteries and arterioles, and lymphoproliferation resulting in splenomegaly and lymph node enlargement. Median mortality occurs when mice are approximately 16 weeks of age.

Renal lesions include fine granular immune deposits along the glomerular basement membrane, hypertrophy of the glomerular epithelium, fibrin depositions, and extraglomerular cell proliferations and hemorrhage within Bowman's space (11). Marked extraglomerular cellular hyper-

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plasia can lead to the accumulation of two or more distinct layers of cells within Bowman's space, forming a characteristic crescent of cells.

Although the pathogenesis of autoimmunity in SCG/Kj mice is not fully understood, a positive association exists between the severity of glomerular lesions, the degree of systemic vasculitis, and the circulating autoantibody titer of antineutrophilic cytoplasmic antibody (ANCA) (11). Titers of anti-ss-DNA and anti-ds-DNA autoantibodies and a polyclonal hypergammaglobulinemia also develop. Lymphoproliferation in SCG/Kj mice is attributable to the accumulation of Thy-1^+, Ly-1^+, CD4–, CD8–, and $B220^+$ T cells (11).

SCG/Kj mice serve as a model of human, rapidly progressive, crescentic glomerulonephritis, a clear clinical entity that is intractable to therapy (12, 13). Anticoagulants, combined anti-inflammatories, and immunosuppressants may be of some benefit, but most patients with this syndrome exhibit progressive renal dysfunction, and prognosis remains grave.

Herein we describe how, by restricting dietary calories, the progression to glomerular pathology in SCG/Kj mice is delayed by more than a month, the median incidence of mortality occurs more than 5 weeks later, and the severity of glomerular lesions postmortem is significantly modulated. Development of this precipitous autoimmune disease, which expresses itself in nursling mice and can be modified by calorie restriction begun at weaning, even after the appearance of circulating autoantibodies and the development of renal clinical pathology, further supports the suggestion that dietary adjuncts to conventional therapies be considered in the management of autoimmune patients.

Material and Methods

Animals. Mice of the SCG/Kj strain were derived by inbreeding progeny of (BXSB × MRL/Mp *lpr lpr*)F_1 mice, as previously described (11). These studies using animals were conducted using AAALAC-accredited facilities, in compliance with the principles of the *Guide for the Care and Use of Laboratory Animals.*

Diets. In this study, 113 4-week-old female SCG/Kj mice were randomly separated into three dietary groups. Group A mice (n = 50) consumed conventional laboratory chow *ad libitum* (Purina Mills, St. Louis, MO). Group B mice (n = 29) were fed a semipurified diet, the constituents of which were obtained from ICN Biochemicals (Costa Mesa, CA) and the composition of which has been extensively reported (2, 6–9). Group C mice (n = 34) were fed a comparable semipurified diet, but with a 10% reduction in caloric offering per week for 3 weeks, and 40% fewer calories offered beginning at 7 weeks of age and continuing throughout life. The diet consumed by the calorie-restricted mice was enriched so that although less food and energy were consumed, equivalent amounts of vitamins, minerals, essential fatty acids, and 30% of dietary energy as protein were consumed by all mice. To avoid competition for food, calorie-restricted mice were housed individually. Food consumption was determined twice weekly. Calorie-restricted mice consumed approximately 32% fewer calories than those mice offered food *ad libitum.* Body weight and urinalysis were determined weekly.

Clinical Glomerular Pathology. Proteinuria was detected using tetra bromphenol paper, and hematuria was detected by hemoglobin-catalyzing reaction of di-iso-propyl benzene di-hydroperoxide and 3,3′,5,5′-tetra-methyl benzidine (Labstix, Miles, Elkhart, IN). Proteinuria was graded 1+ to 4+. Grades of 2+ indicated 100 mg/dl of urinary protein. Grades 2+ or greater were considered indicative of heavy proteinuria.

Glomerular Histopathology. Histological analysis was performed on all mice. Tissues were fixed in 10% formalin and stained with hematoxylin and eosin (H&E), or Periodic acid Schiff (PAS). Lesions of crescentic glomerulonephritis were graded using the World Health Organization's (WHO) classification of glomerular disease (14). Grade I consisted of mild fibrin deposits and increased cellularity. Grade II consisted of mild hypertrophy of the glomerular lining epithelium, mild to moderate fibrin deposition, and increased glomerular cellularity. Grade III consisted of moderate to severe hypertrophy of glomerular lining epithelium, fibrin deposition, and glomerular hypercellularity. Grade IV consisted of marked glomerular hypercellularity with more than two layers of fusiform cells within Bowman's space forming a distinct crescent of cells, and an associated mononuclear cell infiltration. Twenty glomeruli were graded according to this classification and used to calculate the mean glomerular histopathology score for each mouse, and those scores were used to calculate mean scores for each dietary cohort.

Autoantibody Titers. Autoantibodies to ds-DNA, ss-DNA, and ANCA were quantified using an enzyme-linked immunosorbent assay (ELISA) as described elsewhere (15).

Flow Cytometry. Suspensions of spleen and lymph node cells from four mice of each dietary cohort were incubated with phycoerythrin-conjugated or fluorescein-conjugated anti-Thy-1, anti-L3T4, or anti-B220 (RA3-6b2) antibodies (Pharmingen, San Diego), and subpopulations of cells were quantified using flow cytometric analysis.

Statistical Analysis. A log-rank Mantel-Haenszel test was used to compare the survival of each dietary cohort and differences in the rate of development of proteinuria and hematuria. Student's t tests were used to compare body weights and glomerular histopathology scores.

Results

Body Weight. All mice grew and gained weight, with mice of Group C (fed restricted dietary calories) gaining weight more gradually than *ad libitum*–fed mice. Mean body weights of *ad libitum*–fed mice of Groups A and B were comparable. Mean body weights of calorie-restricted Group C mice were approximately 30% less those of either Group A or B mice when >14 weeks of age, and signifi-

cantly less than those of mice of either dietary cohort at all intervals of assessment ($P < 0.002$) (Fig. 1).

Survival Analysis. *Ad libitum*–fed mice developed spontaneous and rapidly progressive crescentic glomerulonephritis, vasculitis, and lymphoid hyperplasia and died at an early age. Median mortality occurred among *ad libitum*–fed Group A mice when they were 114 days old, and among Group B mice when they were 121 days old (Fig. 2).

In contrast, an improvement in survival was observed among calorie-restricted Group C mice compared to mice of either Group A or B, with the first mortality occurring among Group C mice 57 days later than the first mortality among mice of either Groups A or B. Median mortality occurred among calorie-restricted Group C mice when they were 152 days of age, a 20% or 25% extension of median life span compared to mice of Groups B or A, respectively. Survival analysis using a log-rank Mantel-Haenszel test found a significant difference among the three dietary cohorts ($P < 0.0001$) and indicated a modest ($P = 0.05$) or substantial survival advantage ($P < 0.0001$) among calorie-restricted mice compared to the *ad libitum* fed Group B or A mice, respectively.

Clinical Glomerular Pathology. Clinical onset of renal pathology as evident by heavy proteinuria (>100 mg/dl) and hematuria occurred earlier in *ad libitum*–fed Group A and B mice compared to calorie-restricted Group C (Fig. 3). By 15 weeks of age, all mice of Group A had developed both hematuria and heavy proteinuria (>100 mg/dl). Similarly, by 15 weeks of age, all mice of Group B developed heavy proteinuria, and 52% had hematuria.

In contrast, when calorie-restricted mice of Group C were 15 weeks of age, only 83% had developed heavy proteinuria, and only 18% had developed hematuria. This delay in the onset of substantive clinical renal pathology among Group C mice was reflected in lower scores of the glomerular lesions among these mice compared to *ad libitum*–fed mice (see below).

Autoantibodies. Serum levels of anti-ss-DNA, anti-ds-DNA, and ANCA autoantibodies were determined for mice from each dietary group when mice were 12, 16, and 20 weeks of age. Serum from 20-week-old C3H/HeJ mice served as control. Mice from each of the dietary cohorts showed an increase in each of the autoantibody titers with age, but a significant difference in titer of any of these three autoantibodies was not noted among each of the dietary cohorts (data not shown).

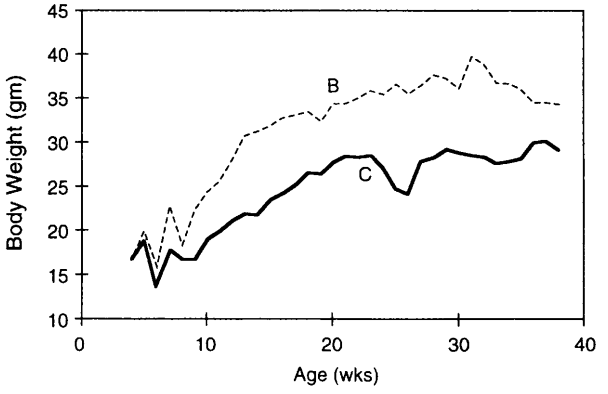

Figure 1. Mean body weights of SCG/Kj mice fed semipurified diets either *ad libitum*, Group B ($n = 29$), or restricted in dietary calories by 32%, Group C ($n = 34$).

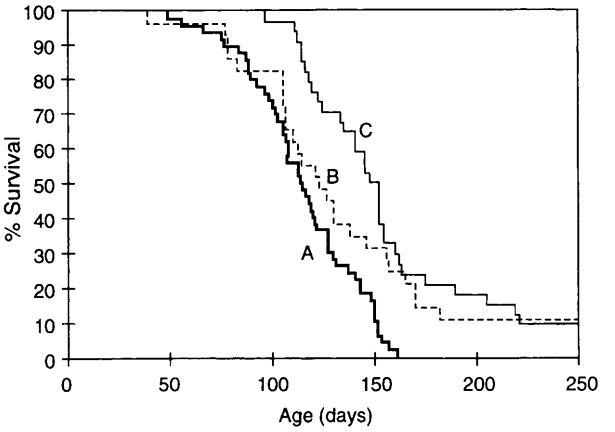

Figure 2. Survival curves of SCG/Kj mice fed either laboratory chow *ad libitum*, Group A, a semipurified diet *ad libitum*, Group B, or a semipurified diet with 32% restriction in calorie intake, Group C.

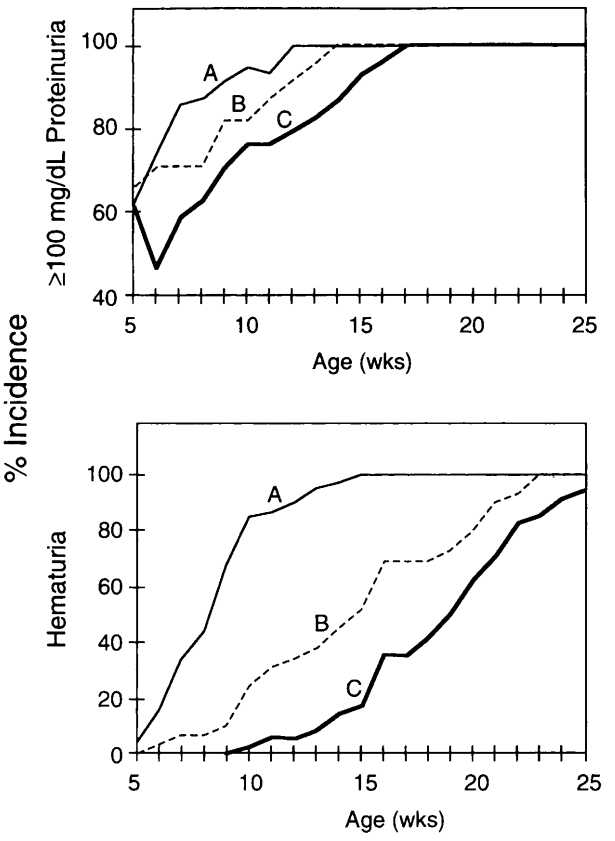

Figure 3. Development of grade 2+ proteinuria (100 mg/dl), and hematuria in SCG/Kj mice fed laboratory chow *ad libitum*, Group A, a semipurified diet *ad libitum*, Group B, or a semipurified diet with 32% restriction in calorie intake, Group C.

Glomerular Histopathology. Twenty glomeruli within each kidney of 20 Group A, 18 Group B, and 20 Group C mice, when mice were 100 days old, were graded according to the WHO classification of glomerular disease. Mean glomerular lesion scores for each mouse were determined, and mean glomerular lesion scores for each dietary cohort were calculated and compared. Glomerular lesions of 100-day-old Group C mice were significantly less severe compared to those of age-matched *ad libitum*–fed mice of either group A ($P < 0.001$) or Group B ($P < 0.001$).

Lymphoid Hyperplasia. Lymphoid hyperplasia as evidenced by a peripheral leukocytosis and an increase in splenic and lymph node weights was present in mice of all groups. Although lymphoid and other organ weights of group C mice were less than those of *ad libitum*–fed Group A or B mice, organ to body weight ratios were comparable. Splenic and lymph node leukocytes were evaluated using flow cytometric analysis. No differences in the proportion of leukocyte phenotypic subpopulations was noted among dietary groups (data not shown).

Discussion

Investigations of whether the pathogenesis of human autoimmune disease can be influenced by nutritional interventions have been limited to evaluations of the dietary modulation of systemic lupus erythematosus or rheumatoid arthritis (16, 17). The therapeutic potential of calorie restriction in arresting acute crescentic glomerulonephritis has not been investigated.

In the experimental setting, limiting dietary calories to levels 30%–35% less than those consumed *ad libitum,* while ensuring adequate consumption of all dietary essentials, delays or abrogates the development of systemic autoimmunity in autoimmune-prone strains of mice (18), including the (NZB X NZW)F_1 (3), NZB (9), *kd/kd* (4), MRL/MP *lpr/lpr* (5), BXSB (8), and (NZW X BXSB)F_1 (2) strains. Whether calorie restriction can abrogate the progression of the acute crescentic glomerulonephritis of SCG/Kj mice has not previously been addressed. In each of these different autoimmune-prone strains of mice, calorie restriction has been shown to prevent the strain-specific pathologies, including glomerulonephritis and vasculitis (3), suppress the production of platelet-associated autoantibodies (2) and anti-DNA autoantibodies and the formation of circulating immune complexes (8), and delay the appearance of CD-5/Lyt-1 + B-lymphocytes linked to autoantibody formation (9). Calorie restriction also lowers the rates of vegetative cellular proliferation within the intestine, skin, thymus, spleen, and lymph nodes (19, 20), while preserving or even enhancing adaptive cellular proliferation such as in the regenerative hyperplasia of hepatocytes following partial hepatectomy (21) or in the responses of lymphoid cells to antigens or phytomitogens (18). Limiting dietary protein without calorie restriction does not extend life span or limit disease incidence in autoimmune-prone mice to the degree that calorie restriction does (10).

In the present study, we determined that calorie restriction could delay the development of the acute crescentic glomerulonephritis of SCG/Kj mice. The modest survival advantage shown in calorie restricted SCG/Kj mice compared to the dramatic doubling or tripling of life span shown with other autoimmune strains of mice (1, 8) may be attributable in part to the shorter latency to onset and more aggressive nature of this strain-specific pathology. Even nursling and weanling SCG/Kj mice have detectable proteinuria and circulating autoantibodies (11; Kinjoh K, personal communication). That the pathologies of other autoimmune-prone strains of mice are more amenable to prevention by calorie restriction may be partially attributable to the longer latency to onset of pathology in those strains.

It is logistically difficult to limit the dietary calories of nursling mice while ensuring an adequate intake of all essential nutrients, and it is doubtful whether such a restriction imposed by limiting the calories offered the dam contributes significantly to the abrogation of the development of strain-specific pathologies or extends the healthful longevity of the progeny (22). Restricted calorie offerings to weanling mice are best imposed gradually over several weeks, so that a 30%–35% restriction in calorie consumption occurs when mice are approximately 6–7 weeks of age. Hence, the beneficial consequences of reducing dietary calories may not be present until mice are at 7–8 weeks of age, an age in SCG/Kj mice when significant morbidity, and even mortality, related to glomerular pathology have already developed.

A positive association between the severity of glomerular lesion and titer of ANCA has been described (11), but was not noted in the present study. Additional analyses are required to elucidate the pathogenesis of crescentic glomerulonephritis and means by which it can be modulated by dietary calories.

That calorie restriction delayed the development of clinical signs relating to renal disease, diminished the severity of terminal glomerular lesion scores, and extended the life span of SCG/Kj mice 20%–25% is an additional testament to the therapeutic potential of calorie restriction, and provides further evidence for considering the potential benefits of nutritional adjuncts to conventional therapies available to autoimmune patients.

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