Influence of Extremes of Protein and Energy Intake on Survival of B/W Mice[1]

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ABSTRACT Energy restriction increases longevity and life span of B/W mice as it does in mice of other long-lived or short-lived strains. Mice of autoimmune-prone strains that develop certain diseases of aging experience an increase in median longevity when energy intake is restricted early in life. The present experiments analyze the influence of restricting energy intake while feeding constant, adequate and greatly excessive amounts of protein, and constant amounts of minerals and vitamins. The experiments assess the influence of excess protein intake in mice fed ad libitum versus those restricted in energy intake. Ad libitum feeding of diets with protein composition ranging from 15 to 50% did not alter longevity or onset and manifestations of renal disease in B/W mice. In mice consuming a restricted energy intake of a diet providing identical amounts of protein to those consumed by ad libitum–fed mice, whether the protein intake was very high or normal, longevity was equally greatly prolonged. Ad libitum feeding of diets of greatly differing protein content is well tolerated by B/W mice. Both the 15 and 50% protein diets, when ad libitum fed, permitted expression of autoimmune disease and glomerulonephritis in B/W mice but did not adversely influence development or progression of disease. Restriction of energy intake of either the normal protein diet or the high protein diet greatly prolonged the life of mice of the autoimmune-prone, glomerulonephritis-prone B/W strain. *J. Nutr.* 117: 1136–1140, 1987.

INDEXING KEY WORDS:

• *high protein* • *longevity* • *glomerulonephritis* • *energy intake*

In our recent work with the (NZB × NZW) F_1 (BW) female mice (1) we have shown that by restricting the energy intake of mice of this autoimmune-prone strain longevity can be greatly prolonged. The diets used varied widely in concentration of the nonprotein energy component, carbohydrate or fat. In animals maintained on restricted energy intake, reduction in energy intake was achieved by reducing the total food intake while increasing the vitamin and mineral content. Thus, the animals consumed equal amounts of vitamins and minerals when fed ad libitum or when restricted in energy intake. Because mice of this strain usually die between ages of 8 and 10 mo of an immune complex–based glomerulonephritis when fed ad libitum (2) it seemed important to determine whether the food intake restriction, which not only restricted energy intake but also reduced protein intake in each animal, had been protective because of the decreased protein intake or because of the energy restriction (1, 3). The answer to this question seemed especially important in mice that are susceptible to development of nephritis. To compare wider differences in protein intake than had been previously used, levels of protein were chosen for the ad libitum–fed animals that were based on Hamilton's experiments concerning the relationship between dietary protein intake levels and energy utilization (4, 5). Hamilton reported optimal dietary energy utilization of the whole diet at intakes of protein between 12 and 30% using a protein with 100% biological value (whole egg protein). Our previous experiments with ad libitum–fed versus restricted-feeding diets (1, 6) had used protein (casein plus methionine) levels of approximately 30% for the ad libitum groups. As a result, the group restricted to 60% of the energy intake of controls consumed a diet containing approximately 20% protein. In the present experiment protein intake was kept constant (in g/animal·d) between ad libitum–fed mice

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and energy-restricted mice. For the low protein intake group we used animals fed 15% protein, which fell within the lower range of optimal energy utilization of the diet (4, 5). To keep protein intake constant when the energy intake was restricted to 60% of the energy provided to the ad libitum–fed mice required that the energy-restricted group be given a diet of 24.9% protein. For the high protein group it seemed desirable to increase protein intake of the animals as much as possible within the limits of our ability to feed the same amount of protein when the energy intake was restricted to 60% of that of the ad libitum–fed group. For the ad libitum–fed high protein groups use of a diet with 50% protein proved feasible and well above the 30% level of optimal dietary energy utilization and into the level of food energy wastage, according to Hamilton (4, 5). At 60% of the energy intake and under conditions of constant protein intake, a diet containing 83% protein was required. By use of such a diet it was possible to separate clearly the influences of protein and energy intake on the survival and nephritic disease in the B/W mouse.

MATERIALS AND METHODS

Fifty inbred 6-wk-old female B/W mice were obtained from Jackson Laboratories, Bar Harbor, ME, and maintained in the animal facilities at the Oklahoma Medical Research Foundation and later in animal quarters of the University of South Florida, St. Petersburg. The mice were housed and fed individually, as previously specified (1). Animal rooms were operated on a 12-h light and 12-h dark cycle and constant temperature and humidity were maintained. Each group comprised 10 mice except group G_1 which comprised 20 mice.

DIETS

The composition of the diets fed is given in **Table 1**. As illustrated, G_2 and H_2 were fed at 60.24% of the gram amounts of diets G_1 and H_1 that were actually consumed. These mice, fed a decreased amount of energy as carbohydrates, consumed the same amounts of protein, vitamins, minerals and essential fatty acids as were consumed by the mice fed ad libitum. In other words, for the mice energy-restricted to 60% of this intake, the reduction in energy intake was entirely due to a decreased intake of carbohydrate by the restricted animals. Diet G_1 was prepared so as to derive 50.3% of its energy from protein. Diet G_2 was derived from diet G_1 in the following manner.

The amount of protein was kept constant while the carbohydrate content of the diet was reduced so that the energy content of the G_2 diet intake was 60% of the energy content of the ad libitum–fed G_1 diet intake (Table 1). As a result the percent of energy derived from protein increased from 50.3% in G_1 to 83.8% in G_2. Diet G_1 was fed ad libitum to one group of mice and diet G_2 was fed at 60.24% of the gram intake (60% of the energy intake) of the G_1 group of mice so that protein intake for animals remained constant between G_1 and G_2. Diets H_1 and H_2 were prepared in a similar manner, except that diet H_1 derived 15.1% of its energy from protein and diet H_2 derived 25.15% of its energy from protein because of reduction in carbohydrate energy as in the case of diets G_1 and G_2. H_1 and H_2 were fed in a manner similar to that of diets G_1 and G_2. The survival data were analyzed statistically by the Mann-Whitney test (U-test) (8), and the Bonferroni rule (9) was employed to correct the *P*-values for the multiple comparisons.

TABLE 1

Composition of diets used

Component	G_1	G_2	H_1	H_2
		g		
Casein	49.0	49.0	14.7	14.7
Methionine	1.0	1.0	0.3	0.3
Choline bitartrate	0.2	0.2	0.2	0.2
Sucrose[1]	13.25	0.0	48.25	18.49
Glycerol	30.00	3.49	30.0	20.0
Inositol	0.05	0.05	0.05	0.05
Safflower oil	2.0	2.0	2.0	2.0
AIN mineral mixture[2]	3.5	3.5	3.5	3.5
AIN vitamin mixture[2]	1.0	1.0	1.0	1.0
Total g	100.00	60.24	100.00	60.24
% of protein	50.0	83.0	15.0	24.9
Total kcal	397.6	238.56	397.6	238.56
Ratio of kcal as % of G_1	100	60	100	60
Protein energy as % of total energy	50.3	83.84	15.1	25.15

[1]Both the mineral and the vitamin mixes are made up in sucrose; thus the diets all contain 1.4 g more sucrose than is listed on the sucrose content line.

[2]Reference 7.

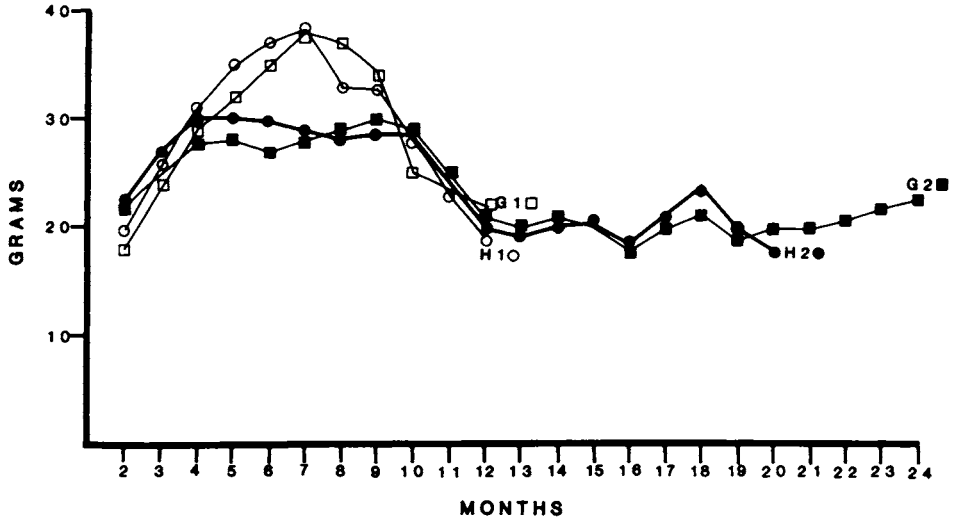

FIGURE 1 Growth curves on female B/W mice on the four different diets obtained by weighing the mice weekly. One set of curves compares mice fed ad libitum on a very high (50%) protein intake (G_1) or fed ad libitum on the diet lower in protein (15%) (H_1). Growth curves were not significantly different from each other. These curves, which terminate at 12 mo with the death of the last mouse in each of these groups, are compared with another set of growth curves for the mice that consumed restricted amounts of energy of diets relatively normal in protein content (25%) (H_2) versus diets very high in protein (83%) (G_2); this provides approximately the same total protein intake per mouse whether calorie consumption was restricted or ad libitum. Weights of the mice fed the very high protein content diet with reduced energy intake and those of the mice fed the more than adequate but much lower protein content diet with low energy intake were approximately the same at the end of the experiment as when the diets were imposed at age 2 mo. Diet H_1, ad libitum–fed high protein; diet G_1, ad libitum–fed lower but adequate protein; diet H_2, energy restricted lower but adequate protein; diet G_2, energy restricted high protein.

RESULTS

Figure 1 shows the growth curves of the mice fed the different diets. The mice maintained on restricted energy intake reached weights of about 60% of those maximally reached by the ad libitum–fed mice. As the individual ad libitum–fed mice showed evidence of proteinuria they rapidly and drastically lost weight as is reflected in the weight curves.

Figure 2 depicts survival data of the groups of mice. The graphs show that survival of the group fed the high protein diet ad libitum averaged 9.0 mo. The mice fed the same high protein intakes but restricted in energy intakes had a marked prolongation in longevity approximately twice (mean 18 mo) that of the ad libitum–fed mice. The comparative data for the groups fed a lower but adequate protein intake are 9.8 mo for mice fed ad libitum and 16.73 mo for mice fed the same protein intake but restricted in energy.

Thus, in mice fed both very high and relatively low

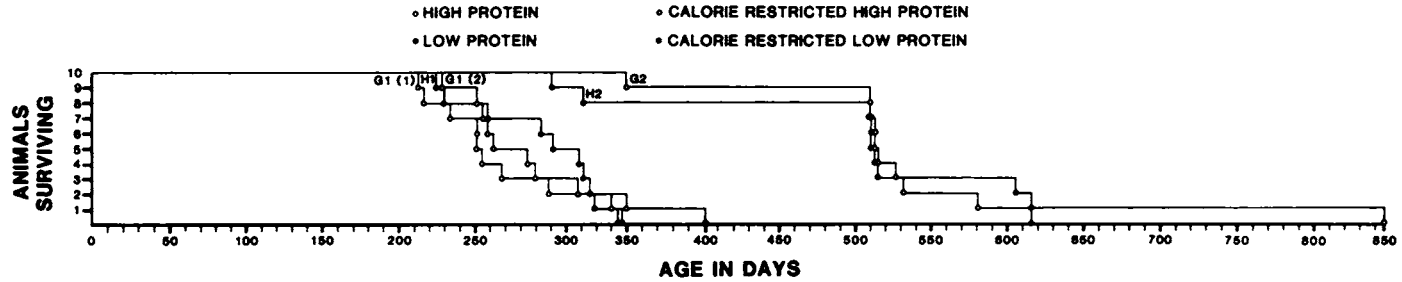

FIGURE 2 Survival curves of mice either fed ad libitum or fed a restricted amount of energy of diets of very different protein composition. One group of 20 mice (G_1 plotted as two groups of 10 mice each) was fed ad libitum the diet of higher protein composition (50% protein) while 10 mice were fed ad libitum a diet (H_1) of lower protein composition (15%). Survival curves for mice of each of these groups were equally short, regardless of the proportion of protein in the diet and the total protein intake. The mice of each group developed autoimmune disease, fulminant renal disease and rapid progression of renal disease. Their disease progressed to death at essentially the same times. Survival curves are shown for the mice fed diets G_2 or H_2, which were calculated to provide protein intakes equal per day to those of mice fed either G_1 or H_1 diets, respectively, but in which the total energy consumption per day was restricted to 60% of that provided on the G_1 or H_1 regimens. Mice fed the H_2 diet received over 83% of their energy as protein; those on the H_1 diet received approximately 25% of their energy as protein. Energy restriction of either of these two diets of very different protein contents when fed in energy-only–restricted amounts showed greatly greatly prolonged longevity, even though protein intake per day was equal to that of the ad libitum–fed mice. The mean survival (in d) for mice fed diets G_1, G_2, H_1 and H_2 was 279 ± 48 (n = 20), 540 ± 116 (n = 10), 295 ± 41 (n = 10) and 502 ± 105 (n = 10). P-value of differences in survival time of mice consuming G_1 vs. G_2: <0.01. P-value of differences in survival time of mice consuming H_1 vs. H_2: <0.01. P-values of mice consuming G_1 vs. H_1 and G_2 vs. H_2: not significant.

but adequate protein diets, the survival time was increased approximately twofold by energy (carbohydrate) intake restriction alone with no harmful nephritic effect of very high protein intake.

DISCUSSION

The results of these experiments show that a diet that derives as much as 83.8% of its energy from protein when consumed in restricted energy amounts is well tolerated by B/W mice. Providing even an extremely high proportion of the diet as protein did not lead to earlier renal disease onset, whereas a restricted energy intake greatly increased the length of time before disease onset and greatly increased the longevity of B/W mice. This energy intake restriction delayed strikingly the onset of autoimmune disease, especially inhibiting development of the lupus nephritis that characterizes mice of this strain (10).

Further, it is shown in these experiments that ad libitum-fed mice consuming diets representing extremes of total protein intake had almost identical survival curves. Similarly, B/W mice with a restricted energy intake of diets differing greatly in protein composition (adequate or excess protein) exhibited prolonged but similar survival times.

These observations are consonant with earlier studies (11, 12) from our laboratories that showed that even severe restriction in protein intake did not delay disease onset in B/W mice, and more recent studies by Yu, Masoro and McMahon (13) confirm that with rats, diets as low in protein as 12.6% are not protective. Thus, great variations of protein intake do not significantly influence the life span of this autoimmune-prone mouse strain. On the other hand, the intake of protein does influence significantly survival of mice with other models of renal disease (14).

Of particular interest are comparisons of these results with those from other experiments with B/W mice carried out at the same time in our laboratories. Mice fed in restricted amounts a diet of high carbohydrate and very low fat content that contained optimal amounts of vitamins, minerals and essential fatty acids (2) lived the longest lives. Mice fed ad libitum high fat, high carbohydrate, high protein or lower protein diets all had essentially identical short lives. Mice fed ad libitum–intake restricted amounts of very high protein diets or lower protein diets or high fat diets in restricted amounts all showed impressively increased life span and the life span with each of these diets was prolonged almost equally (1, 3, 4, 11, 12, 15).

From the previous work (1, 3, 4, 11, 12, 15) (and unpublished data) and also from the present experiments, one can conclude that restriction of energy intake from diets differing greatly in major macronutrient composition regularly prolongs life of B/W autoimmune-prone mice (15). One also sees these same influences with each of the other major autoimmune-prone strains of mice, e.g., MRL/lpr/lpr (3), BXSB, NZB and *kd/kd*. These findings make pressing the question of how energy restriction so greatly influences survival time, longevity and maximum life span and increases the disease-free interval in these short-lived, autoimmune-prone mice. Energy restriction is the variable crucial to the influences observed. Could energy restriction operate in vivo to influence cell proliferation as has been shown by Prescott from studies with mammalian cells in vitro (16), or is the effect related to the way nutrition influences gene expression (e.g., refs. 17 to 21)?

Gabrielsen's earlier findings showed that interference with proliferation of cells by biochemical suppression of proliferation or by biological feedback inhibition of proliferation of certain cell populations produced increases in life span in this same strain of autoimmune-prone mice. Gabrielsen's findings may be relevant to the present observations, and studies to evaluate this issue seem in order (22–24).

Whatever mechanism is ultimately found to explain the tremendous increase on longevity brought about by energy intake restriction in this experimental system, high protein intake clearly does not exert a significant effect on longevity of (NZB × NZW)F_1 mice or on the associated development of destructive renal disease in these animals. The result is totally due to restricted energy intake.

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