Crucial Dietary Factors in Maximizing Life Span and Longevity in Autoimmune-Prone Mice[1]

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ABSTRACT When the energy intake of (NZB × NZW)F_1 female mice was reduced to 60% of the intake of simultaneously ad libitum–fed mice, the early death associated with autoimmune-based renal disease in this strain was greatly delayed. The length of prolongation of disease-free life depended not only on the decreased energy intake but also on the energy source. In the group of mice with 60% intake of a carbohydrate-free (i.e., high fat) diet, mean longevity was doubled as compared to that of ad libitum–fed mice. However, when the nonprotein energy was supplied by carbohydrate (sucrose and glycerol) the mean longevity was three times that of the ad libitum–fed groups, although survival times varied widely. With ad libitum feeding the nonprotein energy source did not significantly affect longevity. Clearly, although energy intake restriction provides significant influence on longevity, very high fat diets do not give the same protection as do high carbohydrate diets. The basis for this difference is not entirely clear and several explanations are possible. *J. Nutr.* 117: 1129–1135, 1987.

INDEXING KEY WORDS:

• *high fat* • *high carbohydrate* • *longevity* • *renal disease* • *autoimmunities*

In prior studies, total energy restriction in (NZB × NZW)F_1 (B/W) mice has been shown to increase longevity (1), prevent development of immunologic malfunctions (2) and inhibit the glomerulonephritis that is associated with anti-DNA and anti-GP 70 antibody production (3, 4) and formation of circulating immune complexes (CIC) with these and other molecules (5). It has also been claimed that high fat diets are harmful and foster development of disease (6–8). Therefore, the present experiments were designed to determine whether it is total energy intake or dietary energy composition that represents the crucial variable in prolonging life span, increasing longevity and delaying development of autoimmune disease in autoimmune-prone mice. High fat diets (more than 69% of energy as fat) were compared with high carbohydrate diets (62–65% of energy as carbohydrate) with respect to their influence on longevity and disease development when the diets were fed at two different metabolizable energy levels (**Table 1**). B/W female mice ad libitum–fed diets that differed greatly in energy source began to die before 7 mo of age, and all mice had died before they reached 12 mo of age. By contrast, animals restricted in total energy intake fed either a high carbohydrate or a high fat (lard) diet showed 100% survival beyond 12 mo of age (see Fig. 3).

The experiments reported here show that while the major influence on survival is attributable to total energy intake, dietary composition also exerted a significant influence when the mice were allowed a restricted intake of energy. No significant influence of dietary composition on survival was demonstrated in ad libitum–fed mice on diets of greatly different composition.

MATERIALS AND METHODS

Inbred 6-wk-old female B/W mice were obtained from Jackson Laboratories (Bar Harbor, ME) and maintained in the animal facilities of the Oklahoma Medical Research Foundation and later in the animal quarters of

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TABLE 1

Composition of diets

Component	A_1	A_2	B_1	B_2	C
			g		
Casein	29.4	28.0	17.64	16.24	19.6
Methionine	0.6	0.6	0.36	0.36	0.4
Sucrose or dextrin[1]	47.25	—	26.49	—	57.25
Glycerol	16	—	9	—	16
AIN-76 vitamin premix[2]	1	1	1	1	1
AIN-76 mineral premix[2]	3.5	3.5	3.5	3.5	3.5
Inositol	0.05	0.05	0.05	0.05	0.05
Choline bitartrate	0.2	0.2	0.2	0.2	0.2
Lard	—	30.733	—	18.4	—
Safflower oil	2	—	2	—	2
Total g	100	64.08	60.24	39.75	100
Total kcal[3]	397.6	397.6	238.56	238.56	397.6
kcal/g	3.98	6.20	3.96	6.00	3.98
Ratio of kcal[4]	100	100	60	60	100

[1]Sucrose in all diets except "control diet" C in which sucrose is replaced by dextrin.

[2]The vitamin and mineral premixes for diets A_2 and B_2 were made up in casein (to provide these as carbohydrate-free diets except for glycerol of fat), whereas for diets A_1, B_1 and C these premixes were made up in sucrose (hence, the apparent lowering in the casein column for diets A_2 and B_2) (9).

[3]The kcal contents are all calculated on the basis of a ME of 4 kcal/g for casein, sucrose, glycerol, dextrin, methionine, inositol and choline bitartrate and 9 kcal/g for safflower oil and lard.

[4]This is the ratio of kcal actually consumed per day per mouse when the ratio of total grams/diet given (i.e., the Total grams line) was used to calculate the amount to be fed.

the All Children's Hospital at the University of South Florida, St. Petersburg. Except for a control group fed ad libitum (diet C) and housed five or six to a cage, the mice were housed individually and fed as specified. Animal rooms were operated on a 12-h light and 12-h dark cycle. Constant temperature and humidity were maintained. Each group comprised 10 or 11 (group C) mice on each of the different diets. The mice were monitored until death to establish relative survival times.

Diets used in this study were calculated as previously described. Diet A_1 (high carbohydrate) supplied 30% of its energy as protein, 4.5% as fat and 65% as carbohydrate. Diet A_2 (high fat) supplied 30% of its energy as protein and 69.8% of its energy as fat, and was carbohydrate free. Diets A_1 and A_2 were pair fed on an energy intake basis (10). In this pair feeding, diet A_2 at times controlled the energy intake of diet A_1 so that intake was not always strictly ad libitum; however, the energy intake of both groups was equally maintained. As any one of a pair died in either the A_1 or A_2 group the pair mate was fed ad libitum.

Diets B_1 and B_2 were designed to provide similar internal dietary ratios for all nutrients except vitamins, essential fatty acids and minerals; these diets were fed at 60% of the energy intake provided in diets A_1 and A_2. To keep protein intake constant in diets B_1 and B_2, diet B_1 as given in Table 1 provides 62% of its calories as carbohydrate (sucrose and glycerol) instead of the 65% given above for diet A_1, whereas fat supplied 69.4% of the energy in diet B_2. These diets contained increased amounts of vitamins and minerals so that the intake of the latter nutrients per animal was equal for all groups. Protein intake was constant between groups when calculated on the basis of protein per kg body weight to the 0.75 power per day. Animals of group C were fed a synthetic control diet that comprised 73% dextrin plus glycerol, 20% protein and 4.5% fat, somewhat analogous in composition to a commercial mouse diet. These animals were group fed ad libitum as in prior experiments (10).

Blood samples were obtained by bleeding from the retroorbital plexus. Hematocrit and peripheral white blood cells (WBC) were determined by standard methodology. To measure serum levels of CIC the Raji cell radioimmunoassay as adapted for mice was used (5). Serum antibodies specific for double-stranded DNA (ds DNA) were determined by use of a solid-phase, enzyme-linked immunosorbent assay (ELISA) (11). The serum was diluted 1:200 for assay. The concentration of anti–ds DNA was determined by reading optical density (OD) at 410 nm.

Proteinuria was assayed with tetrabromphenol paper (Combistix, Ames Co., Elkhart, IN) on fresh urine samples. The test is graded 1–4+ (neg., <30 mg/100 mL; 2+, >30 < 100 mg/100 mL; 2+, > 100 < 300 mg/100 mL; 3+, > 300 < 2000 gm/100 mL; and 4+, > 2000 mg/100 mL). In this experiment, the animals were considered to have a high grade proteinuria when the urine test was 2% or greater.

Statistical analyses were performed with Student's *t*-test for parametric data and the U-test (Mann-Whitney test) (12) for nonparametric data. The Bonferroni rule

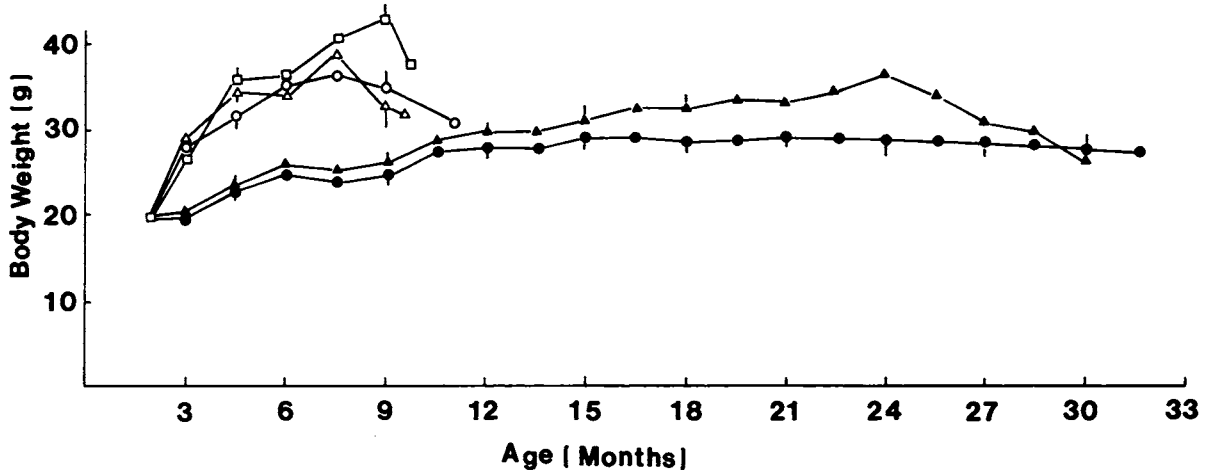

FIGURE 1 Body weights of B/W mice fed A_1 (○), A_2 (△), B_1 (●), B_2 (▲) or C (□) diets. Diet A_1 represents a high carbohydrate, relatively low fat diet. Diet A_2 represents a high energy, high fat, no carbohydrate diet. Diet B_1 represents the high carbohydrate, low fat diet consumed at 60% of energy of A_1. Diet B_2 represents a high fat, no free carbohydrate diet consumed at 60% of energy of A_2. Diet C represents a high dextrin glycerol diet used as control and fed ad libitum in these experiments.

(13) for multiple comparisons was employed and *P*-values <0.05 were considered significant.

RESULTS

Growth curves. The growth of mice fed the various diets is shown in **Fig. 1**. Each pair-fed mouse of groups A_1 and A_2 had a mean calorie intake of approximately 16 kcal/d. With 40 g as their mean body weight, this calculates to 310 kcal per day per $kg^{0.75}$ of body weight. The mice on the high energy intakes (A_1, A_2 and C) gained weight rapidly, reaching approximately 35 g by 5 mo, and thereafter gained weight more slowly up to about 40 g. These mice all lost weight prior to death after the onset of autoimmunities and chronic renal disease. The mice restricted to 60% of the intake of A_1 or A_2 showed a slower weight gain for 15 mo. These mice fed diets B_1 and B_2 consumed an average 9.6 kcal/(d·mouse). On the basis of an average 25-g adult body weight this calculates to 276 kcal per day per $kg^{0.75}$ of body weight. As the mice in groups A_1 and A_2 died and pair feeding was no longer possible, the mice of the B_1 and B_2 diet groups were fed 9.6 kcal/(d·mouse). When calorie intakes were the same, body weights were almost identical during the first year (e.g., A_1 vs. A_2 vs. C and B_1 vs. B_2). However, the restricted mice fed the high fat diet (B_2) gained slightly more weight than the restricted mice fed the high carbohydrate diet (B_1) after 1 yr of age even though their energy intake on the basis of metabolic body mass ($kg^{0.75}$) was similar.

Proteinuria. The mice on the high energy intakes began to develop severe proteinuria at 5 mo of age and all mice developed proteinuria by 9 mo (**Fig. 2**). All mice with restricted energy intake, regardless of dietary composition, were all negative for proteinuria over more than 12 mo. However, the energy-restricted mice fed a

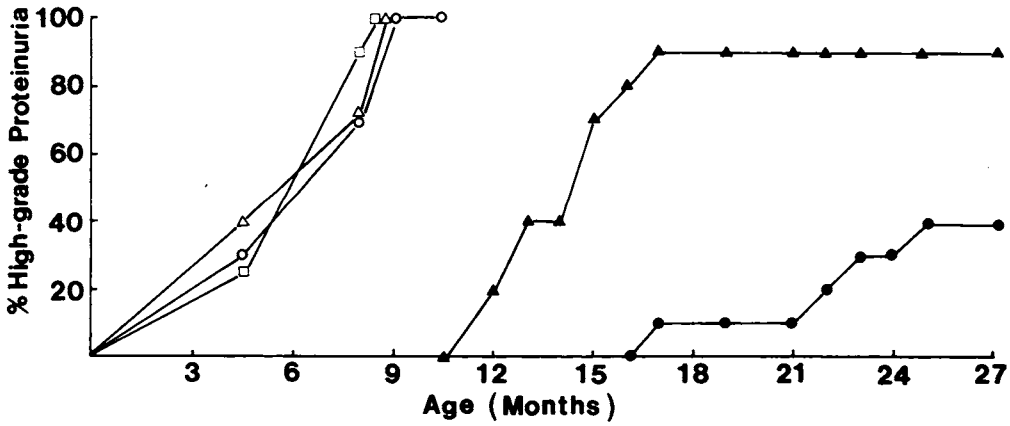

FIGURE 2 Effect of various diets on the cumulative progression to high grade proteinuria (>100 mg/100 mL) up to age 21 mo. A_1 (○), A_2 (△), B_1 (●), B_2 (▲) and C (□) diets. The U-test (12) was used to compare the first date of appearance of a proteinuria of 2+. The Bonferroni rule (13) was employed to calculate the following *P*-values: A_1 vs. C, not significant; A_2 vs. C, not significant; B_1 vs. C, $P < 0.001$; B_2 vs. C, $P < 0.001$; B_1 vs. B_2, $P < 0.01$.

high fat diet (B_2) gradually developed proteinuria after 12 mo, and 90% showed proteinuria by 17 mo of age. On the other hand, the mice with the restricted energy intake of the high carbohydrate diet (B_1) had a very low incidence of proteinuria even at 21 mo of age (1/10) but did develop proteinuria progressively after 21 mo of age. Some long-lived mice from the B_2 and B_1 groups died without developing proteinuria. Among the mice that consumed the very high fat diet (B_2), but at the level of 60% of the calories consumed by the ad libitum–fed mice, proteinuria was exhibited in much larger amounts and at an earlier time than in mice that were equally restricted in energy intake but fed the high carbohydrate, low fat diet (B_1).

Although dramatically protected in comparison to ad libitum–fed mice, on average the energy-restricted mice fed a diet high in fat died at an earlier age than the energy-restricted mice fed the restricted intake of the high carbohydrate, low fat diet.

Hematologic parameters. Serum levels of CIC and anti-DNA antibody were also found to be dramatically different in mice consuming the different energy levels. The mice with a low energy intake regardless of dietary composition had markedly lower levels of both CIC and anti-DNA antibody than mice with a higher energy intake of either dietary source at 4.5 and 6.5 mo of age (10). These parameters were also assayed at 14 mo of age for survivors of both restricted dietary groups (B_1 and B_2) (**Table 2**).

These data show that the mice with a low energy intake of the high carbohydrate diet (B_1) had significantly lower levels of CIC and anti–ds DNA antibody than the mice consuming the high fat diet at a restricted level of energy intake (B_2) ($P < 0.01$). B/W mice of the B_2 group also showed significantly lower hematocrit values than mice fed the B_1 diet ($P < 0.01$). Both groups with restricted energy intakes had lower leukocyte counts as compared to mice of a control CBA/H autoimmune-resistant strain ($P < 0.05$). No significant differences in leukoctye counts between groups B_1 and B_2 were observed at 14 mo ($P > 0.2$ nonsignificant).

Longevity. Although no differences in proteinuria or hematologic parameters were observed within the first year among the ad libitum–fed groups, or between energy intake–restricted groups B_1 and B_2, differences between energy intake–restricted mice fed the B_1 (high carbohydrate) and the B_2 (high fat) diets became apparent during the second year. Differences in proteinuria were directly related to longevity (**Fig. 3**). The mice that had a low energy intake of the high fat diet (B_2) began to die shortly after reaching 1 yr of age, and 90% of these mice had died by 18 mo. By contrast, 100% of the mice consuming the same energy restriction but fed a diet high in carbohydrate survived to 23 mo of age. Mean survival times (in days) of the mice fed the different diets were A_1, 283 ± 52; A_2, 261 ± 35; C, 250 ± 40; B_1, 860 ± 195; B_2, 524 ± 153. With use of the U-test (12) the significance of the difference in longevity between B_1 and B_2 is $P < 0.001$.

DISCUSSION

In many experiments we have now shown that dietary energy restriction greatly prolongs maximum life span and longevity of mice of each of the autoimmune-prone strains studied (10, 11, 14, 15) (and unpublished observations from our laboratories). In the present report the source of energy made no significant difference in survival time of mice of the B/W hybrids studied when the animals were fed ad libitum diets that were very high in fat or very high in carbohydrate as their nonprotein energy source. We found, however, that with energy intake restricted and hence with disease onset greatly delayed this delay is much greater when sucrose plus glycerol serves as primary energy source than when fat (fatty acids combined with glycerol) is the primary energy source (diet B_1 vs. diet B_2). Although restriction of energy intake prolonged life twofold in mice fed the high fat diet, mean prolongation of life of intake-restricted mice fed the high carbohydrate diet was threefold. Only when the animals have a restricted energy

TABLE 2

***Dietary influence on hematologic parameters in female B/W mice at 14 mo of age*[1]**

Group	CIC[2]	Anti–ds DNA[3]	Hematocrit	Peripheral white blood cells
	μg/ml	*O.D.*	%	*per mm³*
B_1	608 ± 170[4]	0.05 ± 0.01[4]	49.5 ± 0.5[4]	3367 ± 583[5]
B_2	4290 ± 1174	0.42 ± 0.15	43.0 ± 1.0	3849 ± 398[5]
CBA/H[6]	390 ± 161	0.04 ± 0.02	50.2 ± 0.8	8878 ± 2124

[1]Each group consisted of nine mice. Values are means ± SEM.
[2]Results are expressed as microgram equivalents of aggregated murine IgG/ml of murine serum.
[3]Determined by an enzyme-linked immunosorbant assay (ELISA).
[4]$P < 0.01$ compared with B_2 group by Student's *t*-test.
[5]$P < 0.05$ compared with CBA/H group by Student's *t*-test.
[6]CBA/H mice were fed commercial nonpurified mouse diets ad libitum for 14 mo.

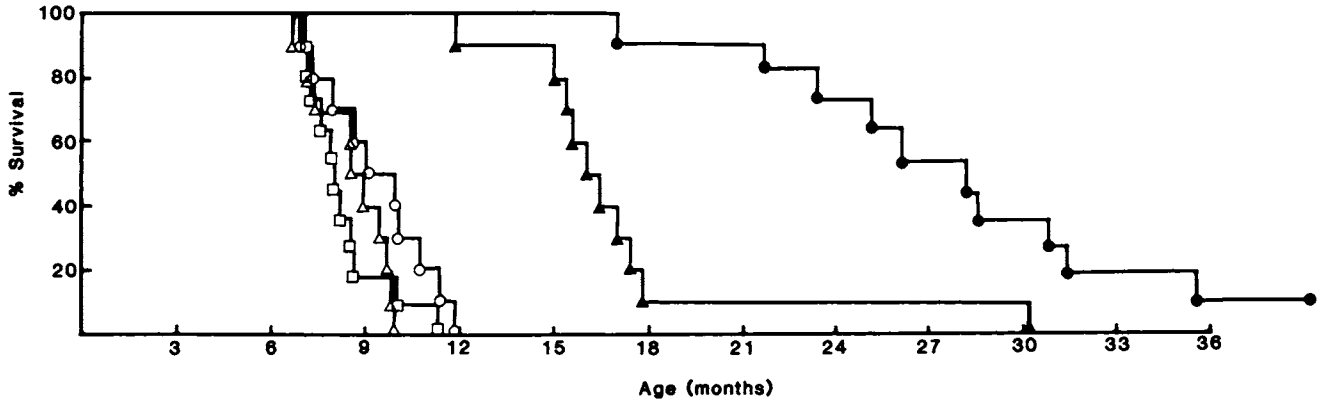

FIGURE 3 Survival curves for B/W mice fed A_1 (○), A_2 (△), B_1 (●), B_2 (▲) and C (□) diets. The survival curves for the mice fed ad libitum the high fat and both high carbohydrate diets (A_1, A_2 and C) are not different from each other. The mice all developed autoimmunity early in life and died of their autoimmune disease and progressive renal destruction before 1 yr of age. By contrast, mice consuming energy-restricted amounts (ratio given in Table 1) of diets B_1 and B_2 in which either the fat or carbohydrate was high survived much longer than mice of any of the other groups. However, mice restricted in energy intake of a diet especially high in fat (B_2) and with no added carbohydrate showed a survival curve significantly shorter than that exhibited by mice with a restricted energy intake of a diet low in fat and high in carbohydrate (B_1). The U-test (12) was used to compare the survival data of the four groups. The Bonferroni rule (13) was employed to calculate *P*-values of difference between groups: A_1 vs. C, not significant; A_2 vs. C, not significant; B_1 vs. C, $P < 0.001$; B_2 vs. C, $P < 0.001$; B_1 vs. B_2, $P < 0.01$.

intake and hence live very much longer is this difference in survival time seen between B/W mice fed diets high in fat and those fed diets high in carbohydrate.

Nevertheless, in comparing longevity of mice fed fat and carbohydrate energy sources in the energy intake–restricted groups, by the time the last animal consuming the high fed diet in restricted amounts had succumbed, 64% of the restricted mice fed the high-carbohydrate diet had also died. However, as is clear from Fig. 3, at the time 90% of the mice equally restricted in energy intake of the diet high in fat content had died, over 91% of the mice being fed the energy-restricted, high carbohydrate diet were still alive. The statistical analysis of these data (see Fig. 3) based on the U-test clearly supports the conclusion that the differences in these survival curves are significant. This is the first time such widely varied energy sources have been used to inhibit the onset of autoimmune disease and immunmologically based glomerulonephritis by energy intake restriction.

The difference may be due to the rapidity of soluble carbohydrate absorption in contrast to slower fat absorption. For example, high fat food may remain in the intestinal tract for much longer periods, thus supplying additional energy to the mice consuming restricted amounts of a high fat diet than the high carbohydrate diet.

These results may support the postulation of investigators (16–20) who have long claimed that lipid peroxides are formed and build up to toxic levels over time. In the present experiment time was made available by restriction of energy intake, and differences in the two diets that had not been evident with a higher energy intake became apparent. These findings suggest the possible use of antioxidants along with the high fat diet and of restriction of energy intake in an effort to eliminate the difference between fat and carbohydrate revealed herein.

Another factor that is demonstrated, especially in the results with the high carbohydrate diet but also apparent with the mice fed the high fat diet, when the animals consumed markedly restricted energy intakes permitting greatly extended longevity, is the probable genetic heterogeneity still present even though the ad libitum–fed mice had uniform mortality times. These findings with ad libitum–fed mice imply genetic homogeneity but are in striking contrast to the great spread of times of death in the energy-restricted mice of both groups and are especially apparent in the group fed a diet high in carbohydrate and low in fat. The findings may contradict the statement of Kay (21) that inbred strains are genetically more homogenous with respect to age than are rats; however, this may only be a matter of how long one is able to keep the animals alive.

As shown in Fig. 1, the mice fed the high fat diet with restricted energy intake were heavier than the mice fed the high carbohydrate diet with restricted energy intake. They apparently received effectively more energy although they had equal energy intakes using the 4,4,9 values for kcal ME/g carbohydrate, protein and fat for calculation of the diets.

It has been reported that B/W mice fed ad libitum high fat diets have more severe immune complex nephritis and tend to die earlier than mice fed a low fat diet (6–8); however, this is not true in this study using a diet of a much higher fat content. A high intake of fat in ad libitum–fed mice also has been found to enhance the development of elevated serum cholesterol levels and promote development of arteritis and fatty-proliferative lesions of the aorta or its branches, particularly the coronary vessels (22, 23). High fat intake

has also been reported to promote the development of renal inflammatory lesions in ad libitum–fed mice (8). In the present experiments, B/W mice with ad libitum energy intake showed no significant differences in longevity or maximum life span regardless of dietary composition (mean survival days: high fat diet group, 261 ± 35; high sucrose and glycerol diet group, 283 ± 52; high dextrin group, 250 ± 40). Mice consuming 60% of this energy level lived longer than ad libitum–fed mice regardless of dietary composition (10). However, a greatly increased fat content of the diet with a restricted energy intake was associated with significantly (see Figs. 2 and 3) earlier development of renal disease and earlier death than was observed in mice restricted in energy intake of a diet high in carbohydrate energy and low in energy from fat. The mechanisms underlying the rate of development of the glomerulonephritis and the earlier deaths observed with restricted feeding of a diet of very high fat composition are obscure. Several possibilities must be considered:

1) Hyperlipidemia might promote accumulation of lipid in areas that have been damaged by CIC. This deposition might contribute to vascular injury and thus favor the additional localization of CIC within the glomerular capillaries and even in other locations in mice restricted in energy intake (8).

2) Hyperlipidemia might enhance focal sclerosis in the glomeruli and in blood vessels elsewhere leading to chronic renal or other organ failure (24). Minick, Murphy and Campbell (25) have shown that hyperlipidemia enhances the development of atherosclerosis induced by immunologic injury to the arteries.

3) The fat in the diet may actually enhance autoantibody production in some way either indirectly by influencing either T or B cells or macrophage function (6–8).

4) Lipid absorption is much slower than sucrose–glycerol absorption, with the lipids remaining "layered out" in the stomach and being gradually passed on to the intestine where they are hydrolyzed and absorbed. With lowered energy intake, absorption of fat energy in particular seems to be improved as indicated by efficiency of energy utilization per unit of metabolic body mass. This has recently been studied in detail with vitamin A as an indicator and an increased absorption of vitamin A demonstrated in energy-restricted mice (26). This slower absorption might then be important with respect to energy or lipids available to mice restricted in intake of diets high in fat (26). It does appear most probably from the weight data that changes in utilization of dietary energy (27) may occur in mice restricted in diet intake and that this change may be greater in the mice fed the diet high in fat. Thus, such mice might be getting an effective energy intake greater than that provided in the mice consuming a restricted intake of a diet high in carbohydrate. Indeed, the mice restricted in intake of a high fat diet did gain more weight than the mice that consumed an equal number of calories of the diet high in carbohydrates. This observation could be compatible with either an increased absorption of fat as mentioned above or more efficient utilization of energy consumed. We can already infer from prior studies (10) that a higher energy intake accelerates the development of autoimmune disease and that these analyses might become crucial if a difference of utilization of energy exists in mice restricted in intake of different diets. The increased efficiency of utilization of per gram metabolic mass between the ad libitum–fed and the energy intake–restricted groups is shown in the difference between 310 kcal per day per $kg^{0.75}$ of body weight required for ad libitum–fed mice and only 276 kcal per day per $kg^{0.75}$ of body weight required for energy intake–restricted mice (see Materials and Methods) to maintain their respective body weights.

5) Finally, the mortality of B/W mice with advancing age may be influenced not only by the major disease that seems genetically programmed to occur in mice of this autoimmune-prone strain but also by other diseases associated with aging, such as atherosclerosis. Total energy intake by itself may affect primarily the expression of the genetically determined autoimmunities, immune complex formation and renal disease; this influence may occur very early in the lives of these mice. Events determined by reduced energy consumption may play an important role by providing more time for the development of other disease processes in these mice that are independent of or less closely related to the mechanisms responsible for the early deaths. Studies of the influence of fat consumption on metabolism of icosanoids must also be considered, particularly since feeding of fats of different compositions, and most notably those diets high in certain fish oils, has already been shown to influence both disease expression and longevity (28–30). Careful pathologic analysis of the influences of these two different diets in mice consuming diets in restricted amounts now seems very much in order.

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