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DIFFERENTIAL EFFECTS OF DIETARY CALORIC AND PROTEIN RESTRICTION IN THE AGING RAT[1]

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Abstract—Numerous studies have shown caloric restriction retards the physiological decline and increases the life span of animals. However, in these studies protein consumption was also reduced; thus, whether the beneficial effects were due to caloric or to protein restriction is unclear. To examine independently the effects of caloric and protein restriction on growth, renal function, and survival, caloric restricted male rats were fed 18, 30 or 42 percent casein diets that provided two-thirds of the quantity of diet consumed by groups fed 12, 20, or 28 percent casein diets *ad libitum*, respectively. Hence, caloric restricted groups consumed the same amount of protein as their paired *ad libitum* fed groups but one-third fewer calories. The results showed that caloric restriction decreased mature body weight, increased the rate of attaining mature body weight, retarded the age-associated decline in renal function, and increased survival. Protein restriction had no effect on mature body weight, decreased maturation rate, improved renal function, and decreased survival. Thus, protein restriction did not contribute to the survival-promoting effects of caloric restriction in rats.

INTRODUCTION

THE LIFE span of experimental animals has been increased by nutritional modification, particularly by caloric restriction (Weindruch *et al.*, 1982; Beauchene *et al.*, 1979; Berg and Simms, 1960; McCay *et al.*, 1939). This increased longevity may have been due, in part, to a restriction in dietary protein since in almost all studies, calorie restriction was accompanied by a parallel reduction in protein intake (Beauchene *et al.*, 1979; Berg and Simms, 1960; McCay *et al.*, 1939). However, data available on the effect of dietary protein on life span are conflicting. Divergent results have been reported (Leto *et al.*, 1976; Miller and Payne, 1968; Nakagawa *et al.*, 1974; Ross and Bras, 1973; Ross and Bras,

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1975) and may be due to the large variation in the levels of dietary protein utilized and the differences in caloric intake.

Restriction of caloric and/or protein intake of experimental animals decreased their body weight (Goodrick, 1978; Berg and Simms, 1960), reduced the activities of certain enzymes (Leto *et al.*, 1976), improved immune responses (Weindruch *et al.*, 1979), and delayed the development of diabetes (Gerritsen, 1976). In addition, dietary restriction delayed the age-associated decline in renal function (Adams and Barrows, 1973; Beauchene *et al.*, 1965; Saxton and Kimball, 1941), decreased the incidence of renal lesions (Bras and Ross, 1966), and reduced age-associated changes in renal morphology (Johnson and Barrows, 1980). In our laboratory, changes in renal function during hypocaloric feeding were quantified as reductions in urinary protein excretion and improved renal transport of para-aminohippuric acid (Tucker *et al.*, 1976).

The present study was designed to examine the independent effects of caloric and protein restriction on aging. Thus, the effects of caloric restriction with or without protein restriction, and of protein restriction with or without caloric restriction were determined on survival, renal function, and growth of rats. The results indicated that survival and renal function were more sensitive to restriction of caloric intake than to that of dietary protein.

METHODS

Animals and diet

Male Wistar rats were obtained as weanlings (National Research Laboratories) and individually housed in hanging wire cages. Animals were provided water *ad libitum* and fed a nutritionally complete 20 percent casein diet (Table 1) *ad libitum* until 32 days of age when they were placed on experimental diets. At the beginning, 36 animals (old) were assigned to each of six dietary treatments and maintained for two years (Table 2). Twelve months later, 12 additional 32-day old rats (young) were assigned and maintained for one year on each of the six dietary treatments. The restricted groups (R) were fed 18, 30, and 42 percent casein diets that provided two-thirds of the mean quantity of diet consumed by groups fed 12, 20, and 28 percent casein diets *ad libitum* (A), respectively. Thus, R groups consumed the same quantity of protein as their paired A groups but one-third less calories. Diets of different protein levels were prepared by substituting amounts of 1:1 mixture of cornstarch and sugar for casein.

Daily feed intakes of restricted groups were based on average intakes of ad libitum-fed animals established over seven-day periods; all intakes were corrected for spillage. Daily feed intakes were measured throughout the first year of life and every other week thereafter. Rats were weighed weekly until six months of age, biweekly until 12 months and monthly thereafter.

Growth curves

The mature body weight in grams and the rate of attaining mature body weight were calculated from 16 body weights obtained at intervals for each animal during its first year of life. The equation used (Brody, 1964) was as follows: $\mathbf{W = A - Be^{-kt}}$ where **W** = animal weight in grams at a given age, **A** = mature body weight in grams, **B** = integration constant, **e** = base of natural logarithms, **k** = rate of growth with respect to that yet to be made, and **t** = age in weeks. Rats with larger **k** values reach mature body weights more rapidly. Values of **A, B,** and **k** were computed for each rat by the Gauss-Newton method (Bard, 1974), and a non-linear least square procedure available in SAS (Barr and Goodnight, 1979). From these individual data, mean values of **A, B,** and **k** for each experimental group were calculated and used to construct growth curves.

Renal determinations

Within one month prior to sacrifice, each animal was placed in a metabolism cage for 72 hours. Urine was collected daily and centrifuged (2,000 rpm × 15 mintues). Protein was measured in the supernatant using the biuret reagent (Saifer and Gerstenfeld, 1964). Rats were stunned by a blow to the head and decapitated. Kidneys were removed, chilled on ice, decapsulated, and weighed. Right kidneys were sliced with a Stadie-Riggs microtome

fitted with a 0.5 mm head; four slices were used to determine para-aminohippuric acid (PAH) transport (Adams and Barrows, 1963).

Survival

Cages were checked daily for dead animals. Percent survival was calculated at regular intervals until the rats were two years of age at which time all survivors were killed.

Statistical analyses

Data were analyzed using analysis of variance (Snedecor and Cochran, 1967) to test for differences among dietary treatments and ages. Specific effects of dietary protein and caloric levels on each parameter were tested using orthogonal comparisons. If there were no significant interactions between dietary protein and caloric levels, the effect of protein was tested by pooling the variances of the *ad libitum* and restricted groups with the same protein intake. A Chi-square test was used for differences in survival rates among groups. Probability levels of less than 0.05 were considered statistically significant.

RESULTS

Growth and feed intake

The growth curves of all groups were similar in shape, but the A groups were significantly higher than those of the R groups (Figure 1). Also, as shown in Table 3, the mean predicted mature body weights of A were 47% greater than those of R groups ($p < 0.0001$). However, rates of attaining mature body weights (k values) were 7% greater in R than in A groups ($p < 0.005$). The dietary protein levels of either the A or the

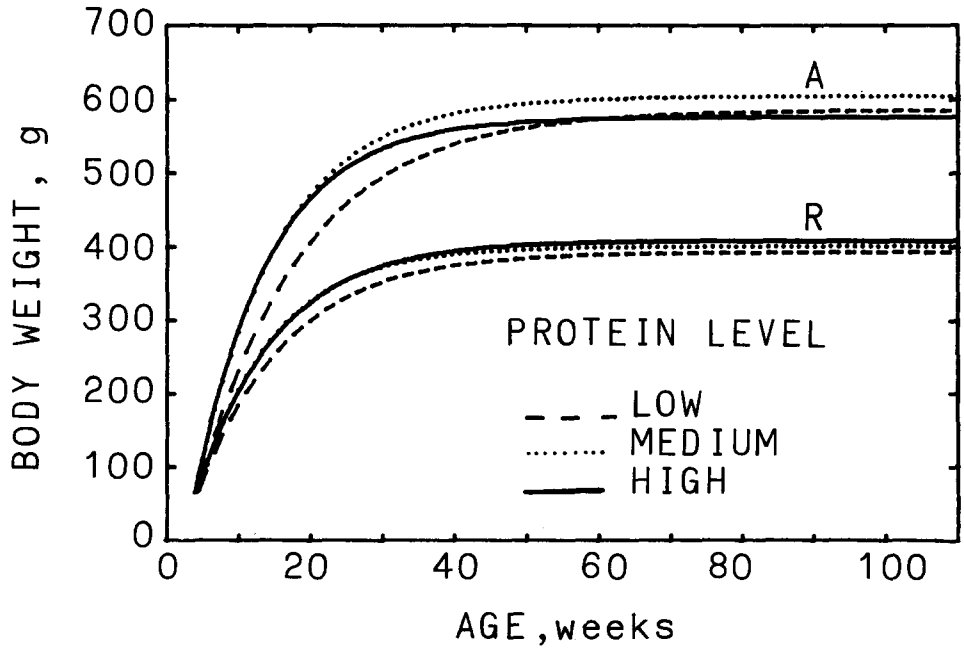

FIG. 1. Influence of level of dietary protein on calculated growth curves (Brody, 1964) of rats fed *ad libitum* (A) and restricted (R) diets.

TABLE 1. COMPOSITION OF 20% CASEIN DIET

Dietary Component	*Percent of Diet*
Casein, High nitrogen[a,b]	20.0
Sucrose	29.0
Cornstarch	29.0
Crisco	6.0
Wesson oil	2.0
Vitamin Mix[a,c]	2.0
Salt Mix[a,d]	3.0
Alphacel[a]	9.0

[a]ICN Nutritional Biochemicals, Cleveland, Ohio 44128.

[b]Determined by the Kjeldahl method to contain 91.5% protein.

[c]Vitamin Diet Fortification Mixture formulated to supply the following amounts of vitamins (g/kg vitamin mix): vitamin A, 4.5; vitamin D, 0.25; thiamin hydrocholoride, 1.0; riboflavin, 1.0; niacin, 4.5; p-aminobenzoic acid, 5.0; calcium pantothenate, 3.0; pyridoxine hydrochloride, 1.0; ascorbic acid, 45.0; inositol, 5.0; choline choride, 75.0; menadione, 2.25; biotin, 0.02; folic acid, 0.09; vitamin B_{12}, 0.00135; alpha-tocopherol, 5.0; and sufficient dextrose to make 1 kg.

[d]Hubbell, R.B., Mendel, L.B. & Wakeman, A.J. (1937). Salt mix formulated to supply the following amounts of minerals (g/kg salt mixture): $CaCO_3$, 543.0; $MgCO_3$, 25.0; $MgSO_4$, 16.0; NaCl, 69.0; KCl, 112.0; KH_2PO_4, 212.0; $FePO_4 \cdot 4H_2O$, 20.5; KI, 0.08; $MnSO_4$, 0.35; NaF, 1.00; $Al_2(SO_4)_2K_2SO_4$, 0.17; and $CuSO_4$, 0.90.

R groups did not affect predicted mature body weights. However, low dietary protein levels at both caloric levels (A and R) resulted in 19% lower k values ($p < 0.0005$) than those obtained for rats fed medium or high levels of protein.

The feed intakes of A groups are shown in Figure 2 (R groups were fed two-thirds of the amount of feed consumed by their paired A groups). The intakes of animals on the low protein diet were 5% less ($p < 0.05$) than those on medium or high levels of protein, which were not significantly different. Feed intakes were age-dependent, peaking at four months of age and decreasing ($p < 0.001$) slightly thereafter.

TABLE 2. DIETARY TREATMENTS[a]

Dietary protein Level	*% Casein in Diet*	
	Ad libitum	*Restricted*[b]
Low	12	18
Medium	20	30
High	28	42

[a]Initially 48 animals per dietary treatment, 12 designated "young," 36 as "old."

[b]Restricted animals were fed the same amount of protein but one-third fewer calories than their ad libitum counterparts.

TABLE 3. BODY GROWTH AS A FUNCTION OF DIETARY PROTEIN LEVEL OF AD LIBITUM-FED AND RESTRICTED RATS

Dietary Group	*Number of Animals*	*Predicted mature Body weight*[a,b] *(Grams)*	*Rate of Attaining mature Body weight*[c,d]
Ad libitum			
Low protein	36	575 ± 13.1	0.0686 ± 0.00191
Medium protein	36	608 ± 13.0	0.0864 ± 0.00351
High protein	36	569 ± 11.6	0.0956 ± 0.00317
Restricted			
Low protein	36	389 ± 4.04	0.0810 ± 0.00215
Medium protein	36	397 ± 4.02	0.0967 ± 0.00176
High protein	36	404 ± 3.74	0.0904 ± 0.00176

[a]A in Brody (1964) equation.
[b]*Ad libitum* greater than restricted ($p < 0.0001$).
[c]k in Brody (1964) equation.
[d]*Ad libitum* less than restricted ($p < 0.005$); directly related to level of dietary protein (orthogonal comparison; $p < 0.0005$).

Renal responses

Kidney weights were smaller in caloric restricted young (18%, $p < 0.05$) and old (41%, $p < 0.0001$) rats as compared to their *ad libitum* fed controls as shown in Table 4. When expressed as a percent of body weight, kidney weights were 18% greater in young R than in young A ($p < 0.05$). Kidney weights in grams or as percent of body weight were unaffected by the dietary protein level. Old rats had higher ($p < 0.001$) kidney weights than young animals when expressed in grams (26%) or as a ratio to total body weight (28%).

PAH transport, a measure of the kidney's ability to perform osmotic work (Adams and Barrows, 1963), 25% less ($p < 0.07$) in old A than in old R (Table 5). Urinary protein excretion was 250% greater in old A than in old R rats ($p < 0.0001$). PAH transport was unaffected by dietary protein level, but urinary protein excretion increased as dietary protein increased in old rats ($p < 0.05$). As compared to young rats, old animals decreased PAH transport 39% ($p < 0.001$) and increased urinary protein excretion 118% ($p < 0.001$). PAH transport and urinary protein excretion were inversely correlated in old A rats fed medium ($r = -0.92$, $p < 0.01$) or high ($r = -0.90$, $p < 0.01$) protein diets.

Survival

Survival data for all groups are presented in Figure 3 and Table 6. At two years of age (104 weeks) restricted rats fed high, medium and low levels of dietary protein had survival values of 53%, 61% and 31%, respectively; corresponding values for *ad libitum* fed rats were 33%, 31% and 19%. Survival values for restricted rats were significantly higher than those of *ad libitum* fed rats ($p < 0.0005$). Low levels of dietary protein resulted in a decrease in survival in both *ad libitum* fed and restricted rats ($p < 0.05$). The highest survival rate was observed in R animals fed the medium level of dietary protein.

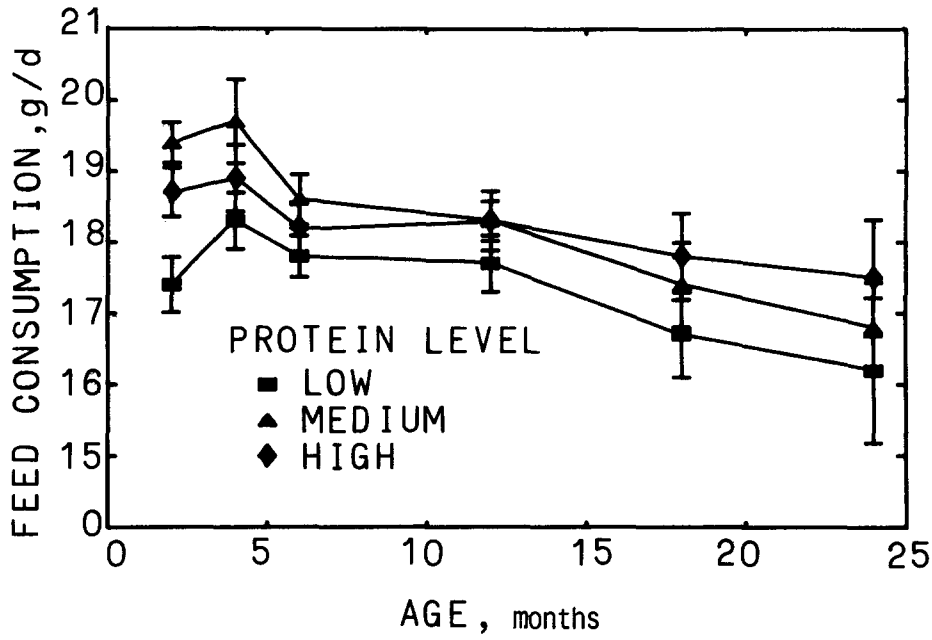

FIG. 2. Feed intake of *ad libitum* fed rats as a function of dietary protein level and age. Values plotted are mean intakes per day per rat ± SEM.

TABLE 4. INFLUENCE OF LEVEL OF DIETARY PROTEIN AND AGE ON KIDNEY WEIGHTS[a] OF *AD LIBITUM* AND RESTRICTED RATS

	Kidney wt[b]		$\frac{\textit{Kidney wt}}{\textit{Body wt}} \times 100$[b]	
Dietary group	*Young*[c]	*Old*[d]	*Young*[e]	*Old*
	g		%	
Ad libitum				
Low protein	2.51 ± 0.118 (8)[f]	3.54 ± 0.388 (5)	0.498 ± 0.0142 (8)	0.648 ± 0.0821 (5)
Medium protein	2.59 ± 0.098 (9)	3.67 ± 0.802 (5)	0.504 ± 0.0268 (9)	0.742 ± 0.212 (5)
High protein	2.89 ± 0.148 (8)	4.33 ± 0.621 (7)	0.553 ± 0.0282 (8)	0.785 ± 0.114 (7)
Restricted				
Low protein	2.23 ± 0.051 (9)	2.17 ± 0.211 (5)	0.623 ± 0.0141 (9)	0.708 ± 0.0523 (5)
Medium protein	1.89 ± 0.043 (10)	2.22 ± 0.069 (8)	0.574 ± 0.0239 (10)	0.771 ± 0.0377 (8)
High protein	2.46 ± 0.079 (11)	2.43 ± 0.047 (7)	0.639 ± 0.0201 (11)	0.696 ± 0.0254 (7)

[a]Weights of right plus left kidney.
[b]Old greater than young ($p < 0.001$).
[c]*Ad libitum* greater than restricted ($p < 0.05$).
[d]*Ad libitum* greater than restricted ($p < 0.0001$).
[e]*Ad libitum* less than restricted ($p < 0.05$).
[f]Number of rats per group in parentheses.

TABLE 5. URINARY PROTEIN EXCRETION IN MALE RATS AS A FUNCTION OF DIET AND AGE[a]

Dietary group	PAH[b] (μg/g)		Urinary protein[b] (mg/day)	
	Young	Old[c]	Young	Old[d,e]
Ad libitum				
Low protein	211 ± 9.86	95 ± 32.1	10.8 ± 1.03	31.3 ± 18.1
Medium protein	218 ± 19.9	106 ± 45.1	16.9 ± 3.82	81.2 ± 31.1
High protein	184 ± 25.0	98 ± 22.7	23.5 ± 2.19	46.2 ± 14.8
Restricted				
Low protein	186 ± 13.7	139 ± 27.0	10.3 ± 1.76	14.4 ± 4.13
Medium protein	185 ± 20.3	125 ± 22.9	9.5 ± 0.94	13.5 ± 1.49
High protein	171 ± 17.3	135 ± 22.3	22.7 ± 3.33	17.3 ± 3.79

[a]Number of rats per group ranged from 5 to 11.
[b]Old vs. young ($p < 0.001$).
[c]Ad libitum vs. restricted ($p < 0.07$).
[d]Ad libitum vs restricted ($p < 0.0001$).
[e]Directly associated with dietary protein level (orthogonal comparison; $p < 0.05$).

DISCUSSION

The results of this study demonstrate that rats fed calorically restricted diets (67% of *ad libitum* fed controls) survived longer than those fed *ad libitum* even when both caloric groups consumed the same amount of protein. In previous studies (Beauchene *et al.*, 1979; McCay *et al.*, 1939; Miller and Payne, 1968; Ross and Bras, 1973; Ross and Bras, 1975), reduction in caloric intake was accompanied by a proportional decrease in protein intake, thus making it difficult to determine whether the increase in survival was related primarily to caloric or to protein restriction. Although caloric intake had a greater effect

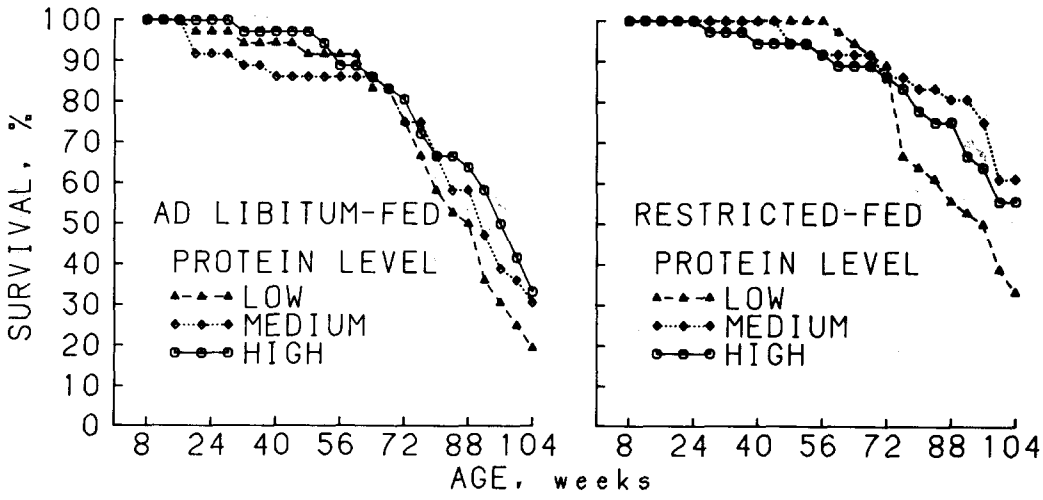

FIG. 3. Influence of protein and caloric restriction on survival of rats (n = 36 per dietary treatment initially).

TABLE 6. SURVIVAL RATES OF RATS FED *AD LIBITUM* AND RESTRICTED DIETS

	Percent Survival After	
Dietary group	*1 year*	*2 years*[a,b]
Ad libitum		
Low Protein	97	19
Medium protein	86	31
High protein	94	33
Restricted		
Low protein	100	31
Medium protein	94	61
High protein	94	53

[a]Ad libitum vs. restricted ($p < 0.0005$).
[b]Directly associated with dietary protein level (orthogonal comparison; $p < 0.05$).

on survival than protein intake in the present study, low levels of dietary protein in both calorie-restricted and *ad libitum* fed groups were associated with decreased survival rates. While caloric restriction (33%) increased survival, the concomitant reduction in feed intake (5%) by both the *ad libitum* fed and restricted rats fed the low protein diets was associated with decreased survival.

Reductions in feed intake in animals fed low protein diets have been reported previously (Leto *et al.*, 1976; Ross *et al.*, 1970). Ross and Bras (1973) reported higher survival rates in rats fed high protein diets. Other studies have reported conflicting results but these utilized levels of protein that resulted in a stunting of growth (Goodrick, 1978; Leto *et al.*, 1976; Miller and Payne, 1968).

Higher rates of attaining mature body weight (k values) may be related to increased survival. Although caloric restriction increased survival and decreased body weight, k values of restricted rats were greater than those *ad libitum* fed. In both A and R groups, however, a low level of dietary protein resulted in decreased survival and less rapid growth rates. In contrast to our findings, Goodrick (1978) reported that a reduction in growth rate and a longer growth duration enhanced survival. Growth rates are not expressed in the same terms in the present study and that of Goodrick, however.

Dietary factors which prolong survival may vary in their effects on renal function. For example, caloric restriction increased percent survival and also improved renal function. On the other hand, protein restriction decreased survival and yet improved renal function. Thus, it appears that while high levels of dietary protein can adversely affect the function of a specific biological system, that is, the kidney, moderately high protein diets are beneficial for other systems in these animals, and therefore, favorably influence their survival.

Renal function and survival appear to be more sensitive to the calories consumed than to the quantity of dietary protein ingested. While caloric restriction improved renal function, low levels of dietary protein decreased urinary protein excretion that becomes elevated with age, but did not significantly affect renal PAH transport. However, these two measures of renal function were inversely correlated in old *ad libitum* fed animals.

Survival was enhanced to a greater extent by caloric restriction than by increasing the level of protein in the diet.

SUMMARY

The independent effects of caloric and protein restriction on survival, growth, and renal function were assessed in aging adult male rats fed for up to two years on various diets. Restricted groups were fed 18, 30, and 42 percent casein that provided two-thirds of the quantity of diet consumed by groups fed 12, 20 and 28 percent casein *ad libitum,* respectively. Thus, the restricted groups consumed the same amount of protein as their paired *ad libitum* fed groups but one-third fewer calories.

Calorie restriction decreased the mature body weight but increased the rate of attaining mature body weight. Also, the age-associated decline in renal function was improved by calorie restriction as indicated by *in vitro* transport of para-aminohippuric acid and excretion of urinary protein. Finally, the percent survival at two years was significantly increased by calorie restriction.

Low levels of dietary protein had no effect on mature body weight but decreased the rate of maturation. Also, dietary protein restriction improved renal function, but unlike caloric restriction, decreased survival.

Caloric restriction was more effective than protein restriction in altering mature body weight, renal function, and survival of rats.

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