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Nutritional Influences on Aging of Fischer 344 Rats: I. Physical, Metabolic, and Longevity Characteristics[1]

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The aims of this research were (a) to compare food restriction initiated in adult life of male Fischer 344 rats with that limited to early life or involving most of the life span on physical, metabolic, and longevity characteristics and (b) to study a similar level of protein restriction without caloric restriction on these characteristics. Food restriction (60% of the ad libitum intake) initiated at 6 months of age markedly increased life span as did a similar restriction started at 6 weeks of age, but food restriction limited to early life (6 weeks to 6 months of age) and protein restriction caused only a small increase in longevity. Food restriction does not act by reducing the intake of calories or other nutrient per gram of body mass, a finding not in accord with classic views. A progressive decrease in spontaneous locomotive activity with age occurred in ad libitum fed but not restricted rats.

Key Words: Food restriction; Protein restriction; Life span; Systolic blood pressure; Lifetime caloric intake; Metabolic rate; Spontaneous locomotive activity; Median survival time; Tenth percentile survival time

McCAY et al. (1935) reported that restricting the food intake of rats starting at or soon after weaning increases longevity. This study focused attention on the rate of attaining maturity and the rate of growth and its duration as important factors influencing length of life. This focus was supported by the findings of Barrows and Roeder (1965) that food restriction started when rats were 12 months old did not increase longevity but rather resulted in a small but significant decrease in length of life. Since 1965, however, several studies have shown that significant increases in length of life can occur as a result of food restriction started in adult life (Goodrick et al., 1983b; Ross, 1972; Weindruch & Walford, 1982). In none of these studies were the results of the food restriction initiated in adult life quantitatively evaluated in comparison to food restriction started soon after weaning. In this regard, Stuchlikova et al. (1975) reported that food restriction in mice, hamsters, and rats from weaning until 12 months of age was more effective than food restriction started at 12 months of age or lifelong restriction in extending mean length of life. In contrast, Cheney et al. (1983) reported that the greatest mean and maximum survival times were found with mice that were food restricted throughout life. Thus, although it seems established that food restriction started in adult life can extend the length of life, further study is needed to define the relative effectiveness of adult-initiated food restriction compared with that initiated early in life.

The studies (Goodrick, 1978; Leto et al., 1976) showing that decreased intake of dietary protein increases longevity suggest that the extension in length of life by food restriction may, in part, be due to restriction of protein. Other studies, however, do not support this view. For example, Ross and Bras (1973) and Nakagawa and Masana (1971) reported that when fixed levels of protein were fed, the higher the level of dietary protein the greater the longevity. This discrepancy may relate to food intake that was either not carefully measured or the measurements not fully reported. Recently, Davis et al. (1983) addressed this issue in a study that used diets varying in protein content coupled with food restriction and concluded that protein restriction decreases longevity. To resolve this issue fully, however, a study is needed in which caloric intake of ad libitum fed rats remains constant but protein intake is varied.

The aims of our research were (a) to compare food restriction (60% of the ad libitum intake) initiated in adult rats with that limited to early life

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and with food restriction involving most of the life span in regard to a broad spectrum of age-related characteristics and (b) to study the effects of a similar level of protein restriction without caloric restriction in ad libitum fed rats on these same characteristics. In this paper, the effects of these dietary manipulations on physical, metabolic, and longevity characteristics are reported. The accompanying paper (Maeda et al., 1985) contains information on disease characteristics. A number of physiological characteristics also have been explored and are or will be the subject of other papers; see Masoro (1984) for a review of these findings.

METHODS

Rat maintenance and dietary procedures. — A total of 568 weanling (26 to 30 days of age) male Fischer 344 rats were received in a single shipment from the Portage, MI plant of Charles River Laboratories. To maintain the specific pathogen-free condition, the rats, on arrival, were transferred immediately into a barrier facility where they were housed singly in plastic cages with wire mesh floors suspended on the Hazleton-Enviro Rack System (Hazleton Systems, Inc., Aberdeen, MD). Eight rats were killed during the first day for monitoring for the possible presence of infectious disease. The procedures for the operation of the barrier facility including acidification of the drinking water were the same as reported previously (Yu et al., 1982).

The monitoring of the barrier facility involved the following procedures: Twelve random swab samples were taken twice weekly from the floors, doors, and fixtures of the barrier facility to serve as an index of the quality of the operating procedures. They were streaked on brain-heart-infusion agar plates. The outside hallway was used as the control. The plates were incubated for 48 hours at 38 °C. The control swab always gave visible evidence of microbial growth, whereas the swabs from barrier sources routinely did not. When visible evidence of microbial growth was encountered that area of the barrier facility was thoroughly decontaminated. During the course of this study, visible evidence of microbial growth was never observed in the rooms where the rats were housed. Sera from sentinel rats were monitored for murine virus antibodies (Sendai, Reo-3, GD-VII, PVM, KRV, H-1, SDA, LCM, Adeno) and for Mycoplasma antibodies at 4 weeks (8 randomly selected from the Charles River shipment), 11 months (4 Group 5 rats), and 30 months (7 Group 2 rats) of age by Microbiological Associates, Bethesda, MD. All findings were negative except for the 30-month-old rats when 1 out of the 7 rats showed a weak positive response for SDA antibodies. The 30-month-old rats served to monitor the facility for the first 29 months of its operation. No monitoring of rats was carried out after 29 months of operation of the facility except for the histological examinations. All rats that were sacrificed or died spontaneously were necropsied and examined histologically for evidence of infectious disease. Because no evidence of infectious disease was observed, bacteriologic culturing was not done.

The composition of the three diets used is presented in Table 1. All rats were fed Diet A ad libitum until 6 weeks of age at which time the rats were distributed into five dietary groups. Group 1, composed of 130 rats (40 for the longevity study and 90 for various cross-sectional and longitudinal studies), were fed Diet A ad libitum until they died or were sacrificed. Group 2, composed of 110 rats (40 for the longevity study and 70 for the various cross-sectional and longitudinal studies) were fed Diet B at about 60% the mean caloric intake of Group 1 from 6 weeks until 18 months of age with caloric intake thereafter being fixed at the level of intake at 18 months of age. Group 3, composed of 100 rats (40 for the longevity study and 60 for the various cross-sectional studies), were fed Diet B at about 60% of the mean caloric intake of Group 1 until 6 months of age and thereafter were fed Diet A ad libitum. Group 4, composed of 100 rats (40 for the longevity study and 60 for various cross-

Table 1. Composition of Diets

Dietary components	Diet A	Diet B	Diet C
casein (vitamin-free)	21	21	12.6
DL-methionine	0.15	0.15	0.09
sucrose	15	15	15
dextrin	43.65	39.55	52.11
corn oil	10	10	10
Ralston-Purina Mineral Mix[a]	5	7.64	5
Ralston-Purina Vitamin Mix[a]	2	3.33	2
choline chloride	0.2	0.33	0.2
Solka-Floc	3	3	3

Note. Entries are percentages.

[a]The details of the composition of the mineral mix and vitamin mix were reported previously (Bertrand et al., 1980); 96.3% of the mass of the vitamin mix is sucrose. In the case of Diet B the mineral mix was modified to provide the food-restricted rats the same daily intake of calcium, phosphorus, and sodium as the mean intake of the rats eating Diet A ad libitum with the intake of all other minerals being 60% of the mean intake of the rats eating Diet A ad libitum. The concentration of the vitamin mix and choline in Diet B provides a daily intake of these substances in the food restricted rats that is the same as the mean intake of the rats fed Diet A ad libitum throughout life.

sectional studies), were fed Diet A ad libitum until 6 months of age and then Diet B at about 60% the mean caloric intake of Group 1 until 18 months of age with caloric intake thereafter being fixed at the level of intake at 18 months of age. Group 5, composed of 120 rats (40 for the longevity study and 80 for various cross-sectional studies) were fed Diet C ad libitum from 6 weeks of age until they died or were sacrificed. This dietary protocol is summarized in Table 2.

The amount of food ingested by each ad libitum fed rat was measured twice a week for a 3-day and 4-day period, respectively. The amount ingested per day was calculated. For rats fed a restricted amount of food, each was provided the daily food allotment approximately 1 hour before the start of the dark phase of a 12 hour light–12 hour dark cycle. In order to detect spillage of food readily, antibiotic-treated cage boards (Shepherd Specialty Papers Company, Kalamazoo, MI) were placed under the wire mesh cage floor in place of usual bedding materials. The body weight of each rat was measured at 2-week intervals.

Weight of selected tissues and organs. — Rats were weighed, anesthetized with methoxyflurane and sacrificed by exsanguination. The following tissues and organs were excised from each rat sacrificed in the cross-sectional studies: epididymal fat depots, perirenal fat depots, liver, right lung, left lung, right kidney, spleen, heart, right testis. They were blotted and immediately weighed. The results on liver were presented in a previous report (Yu et al., 1984) as were the results for the fat depots (Bertrand et al., 1984). The data for the other organs will be presented in this report.

Spontaneous locomotive activity. — A longitudinal study was carried out on the spontaneous locomotive activity of rats of Group 1 (n = 8) and Group 2 (n = 8) during the course of their usual daily life. The plastic cage (i.e., the home cage of the rat) containing the rat was placed in a "Digiscan" Optical Animal Activity Monitor (Omnitech Electronic Company of Columbus, OH). The measurements were carried out in the rooms in which the rat colony was maintained. All vertical and horizontal movements of the rats were recorded. Each rat was monitored continuously for 1 week at 4-week intervals. The total number of movements per week was used as the measure of spontaneous locomotive activity.

Table 2. Dietary Protocol

Age	Diet Fed to Group Number				
	1	2	3	4	5
4 to 6 weeks	A	A	A	A	A
6 weeks to 6 months	A	B	B	A	C
6 months to death	A	B	A	B	C

Note. Diets A and C were fed ad libitum and Diet B was fed at about 60% of the mean caloric intake of Diet A by Group 1 rats until 18 months of age and then maintained at the 18 month intake until death. The actual mean food intake of each dietary group is presented in the Results section.

Systolic blood pressure. — Longitudinal measurements of systolic blood pressure were carried out on rats of Group 1 (n = 6) and Group 2 (n = 6). The measurements were made on each rat every other day except for Saturday and Sunday using the photoelectric tail cuff (unheated) and related instrumentation (Innovators In Instrumentation, Inc., Woodland Hills, CA). This provided an indirect measure of systolic blood pressure in the conscious rat. Measurements were initiated at 3 months of age but were initially highly variable requiring about 3 months of time before becoming relatively stable from measurement to measurement. Therefore, recordings are reported from 6 months of age on. On each day, 10 sequential measurements were made in a 10- to 15-min time period. There was no evidence that this testing influenced either the weight or the longevity of the rats involved.

Statistical methods. — The survival curves were estimated using product limit estimates and curves compared using a Wilcoxon test (Gross & Clark, 1975). The median and 10th percentile of survival times of the groups were compared using the quantile test (Conover, 1971).

To analyze the locomotive activity and the blood pressure we fitted linear regression lines for each rat. The fitted parameters were then compared between groups using a weighted analysis of variance (Snedecor & Cochran, 1967) with the weights obtained from the linear regression analysis. Food intake, body weight, organ weights, and lifetime caloric intake were compared for group and age differences using analysis of variance and linear contrasts (Snedecor & Cochran, 1967).

RESULTS

Food intake. — Food intakes by the rats in Groups 1, 2, 3, 4, and 5 are reported in Figure 1. The rats in Groups 1 and 5 had similar food intakes through 19 months of age and thus similar caloric

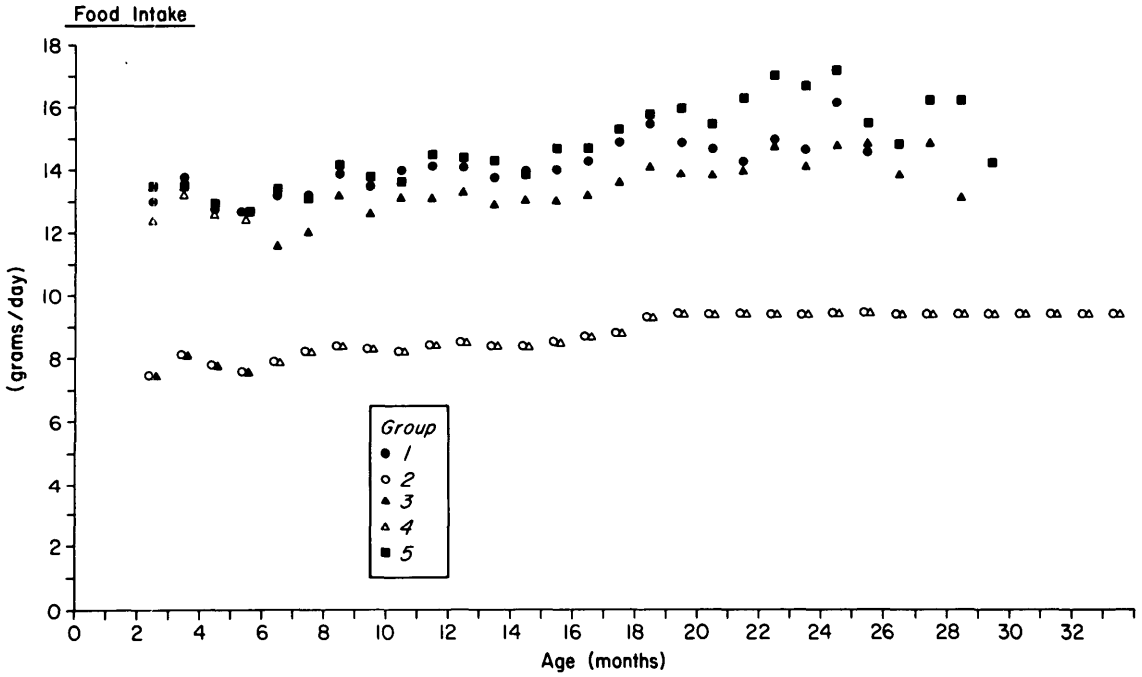

Figure 1. Food intake of the rats in Groups 1, 2, 3, 4, and 5. The presentation of food intake data is discontinued after 26 months of age for Group 1 rats, after 29 months of age for Group 3 rats, and after 30 months of age for Group 5 rats because of the small number of rats living after those ages. The presentation of food intake is discontinued in Group 2 and 4 rats after 34 months of age for convenience since the amount of food provided remained constant after 18 months of age. The values presented for the ad libitum fed groups are the mean intake of the group; in the case of the rats fed the restricted diets the values presented were those consumed by each rat in the group. The number of rats in each group started at 40 and the number of rats represented by each point can be estimated from the survival curves presented in Figures 4 and 6.

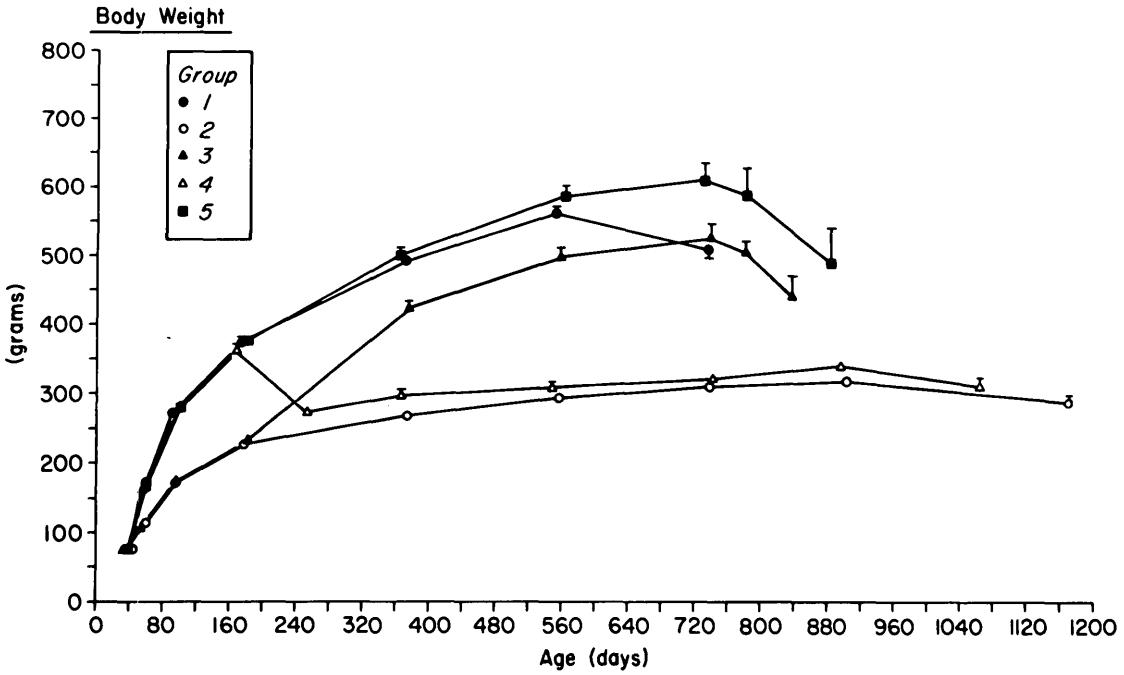

Figure 2. Body weights of the rats in Groups 1, 2, 3, 4, and 5. Values presented are means plus or minus the standard errors of the means. Presentation of the weight data for each group is discontinued when the number of rats became small due to spontaneous deaths. The number of rats represented by each point can be estimated from the survival curves presented in Figures 4 and 6.

intake because Diets A and C are isocaloric; however, at more advanced ages, the food intake of the rats in Group 5 was greater than that of the rats in Group 1 ($p < .005$). As would be expected, the rats in Group 4 had an intake similar to that of the rats in Group 1 until 6 months of age when they were restricted to the food intake of the rats in Group 2. The rats in Group 3, which were restricted to the intake of the rats in Group 2 until 6 months of age, had an ad libitum food intake after 6 months of age that was less than that of the rats in Group 1 ($p < .01$).

Body and organ weights. — The body weights of the rats in Groups 1, 2, 3, 4, and 5 are reported in Figure 2. The weight of the rats in Group 1 increased until 550 days of age with some decline in weight at more advanced ages ($p < .025$). The weight of the rats in Group 5 was similar to that in Group 1 through 550 days of age but in contrast to Group 1 the weight of the rats in Group 5 did not decline until 800 days of age. The weight of the rats in Group 2 was much less than that of the rats in Group 1 at any age greater than 50 days ($p < .01$). There was a rather rapid increase in the weight of the rats in Group 2 until 200 days of age and then a slow increase until 900 days of age with a small drop in weight by 1150 days ($p < .05$). During the first 6 months of life, the rats in Group 4 showed weight gains similar to those of the rats in Group 1. With the initiation of food restriction at 6 months of age, there was a fall in body weight in the rats of Group 4 until 250 days of age when their weight approached that of the rats in Group 2. During the rest of the life span, the weight of the rats in Group 4 paralleled but was somewhat greater than the weight of the rats in Group 2 ($p < .01$).

The changes in weight with age in the heart, spleen, right kidney, right testis, right and left lungs for rats sacrificed for cross-sectional studies are presented in Figure 3. The body weights of these rats have been reported previously (Yu et al., 1984). For each of these organs the weight increased with increasing age ($p < .05$). This is in marked contrast to the liver in which the weight was a constant fraction of body weight throughout life (Yu et al., 1984); that is, liver weight was greatest at the age at which body weight peaked. The weight of the epididymal and perirenal fat depots declined at advanced ages in Groups 1, 3, and 5 (Bertrand et al., 1984). The weight of all organs except testis was greater in the rats in Groups 1, 3, and 5 than in Groups 2 and 4 ($p < .05$). Although at 6 months of age it would be expected that the organ weights of Group 3 rats would be similar to those of the Group 2 rats, the weights of those organs in Group 3 with advancing age tended to approach or reach those of the Group 1 and 5 rats. It is striking that food restriction (even when started at 6 weeks of age) had little effect on the weight of the testis at 6 and 12 months of age. The marked increases in the weight of the testis late in life were as expected because testicular tumors occur in almost all rats at advanced ages (see Maeda et al., 1985). Another striking finding is the marked increase in spleen weight in all groups at advanced ages.

Survival curves. — The survival curves for the rats in Groups 1, 2, 3, and 4 are presented in Figure 4. The survival curves were all significantly different ($p < .01$). The median survival times (Table 3) were different in all the groups ($p < .025$) and, with exception of Groups 2 and 4, the 10th percentile survival times were different ($p < .005$; Group 2 vs. Group 4, $p = .06$).

Because Groups 1 and 2 (each $n = 40$) are a repeat of Groups A and R (each $n = 115$) of a study done some 4 years earlier (Yu et al., 1982), it is of interest to analyze the reproducibility of the survival curves. As is evident from Figure 5, the reproducibility is remarkably good; clearly 40 rats provide reliable information on survival characteristics. The median survival times of 23 to 24 months in our Group A and Group 1 rats compared with the 29 months reported by Coleman et al. (1977) for rats maintained at the Charles River Laboratories probably is the result of a greater caloric intake by the rats in our facility. This is based on the fact that the rats maintained in our facility are heavier at given ages and reach a peak weight 150 g greater than the rats maintained at Charles River Laboratories (Baskin et al., 1979). This proposed difference in caloric intake may be due to the difference in diet (fat content of our diet is 10% by weight compared with 5% for the Charles River diet) or to the fact that our rats are caged singly, whereas those at Charles River Laboratories are caged multiply.

The survival curves for the rats in Groups 1 and 5 are presented in Figure 6. The curves are significantly different ($p < .001$) as are the median and 10th percentile survival times ($p < .025$). The median and 10th percentile survival times as well as the maximum observed lifetimes are reported in Table 3.

Caloric intake and longevity. — The caloric intake per gram body weight per day is summarized

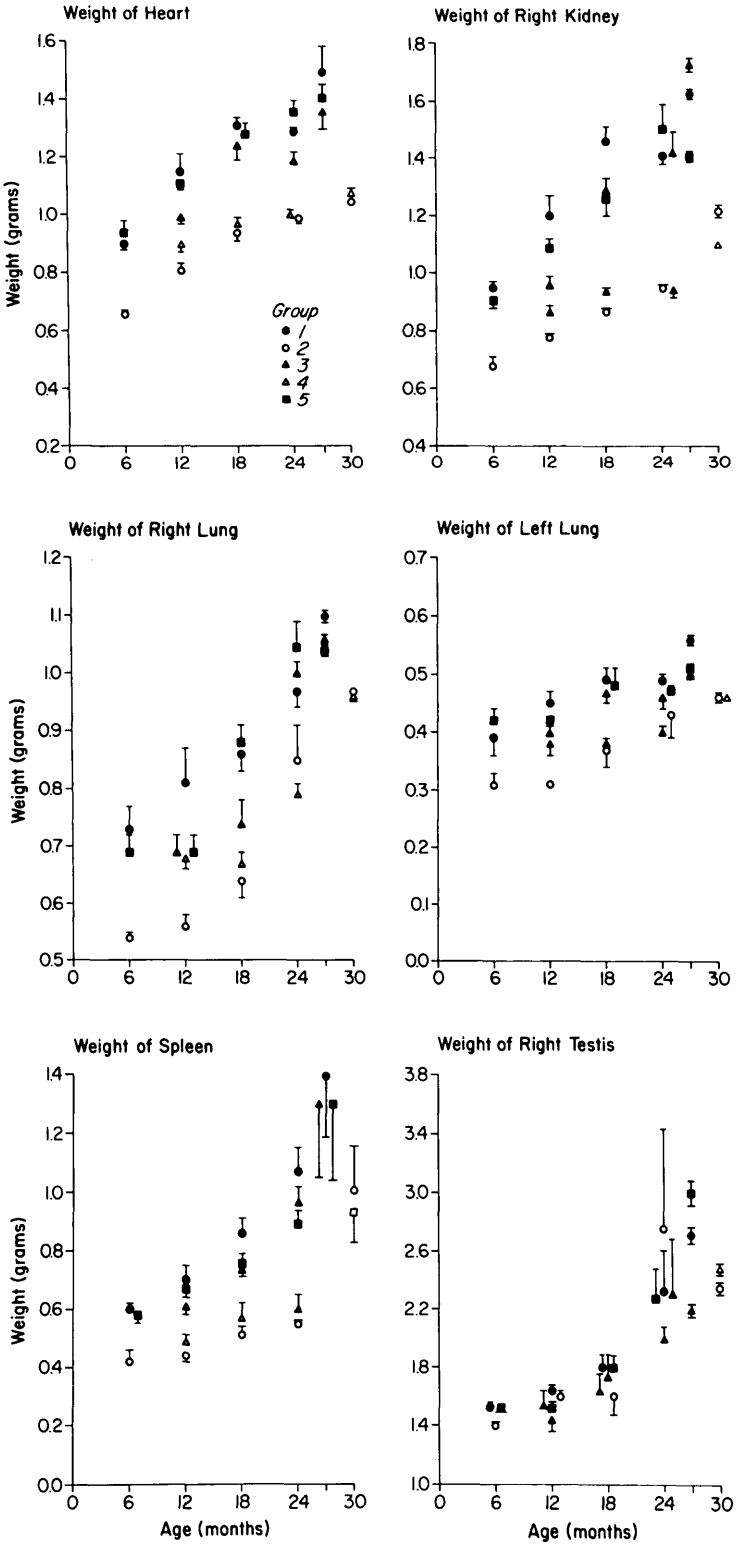

Figure 3. Weights of selected organs: (a) heart, (b) right kidney, (c) right lung, (d) left lung, (e) spleen, and (f) right testis of rats sacrificed in cross-sectional studies. The weights of the rats from which these organs were excised were reported previously (Yu et al., 1984). One of the 27-month-old Group 1 rats had a lymphoma that affected the weight of its spleen.

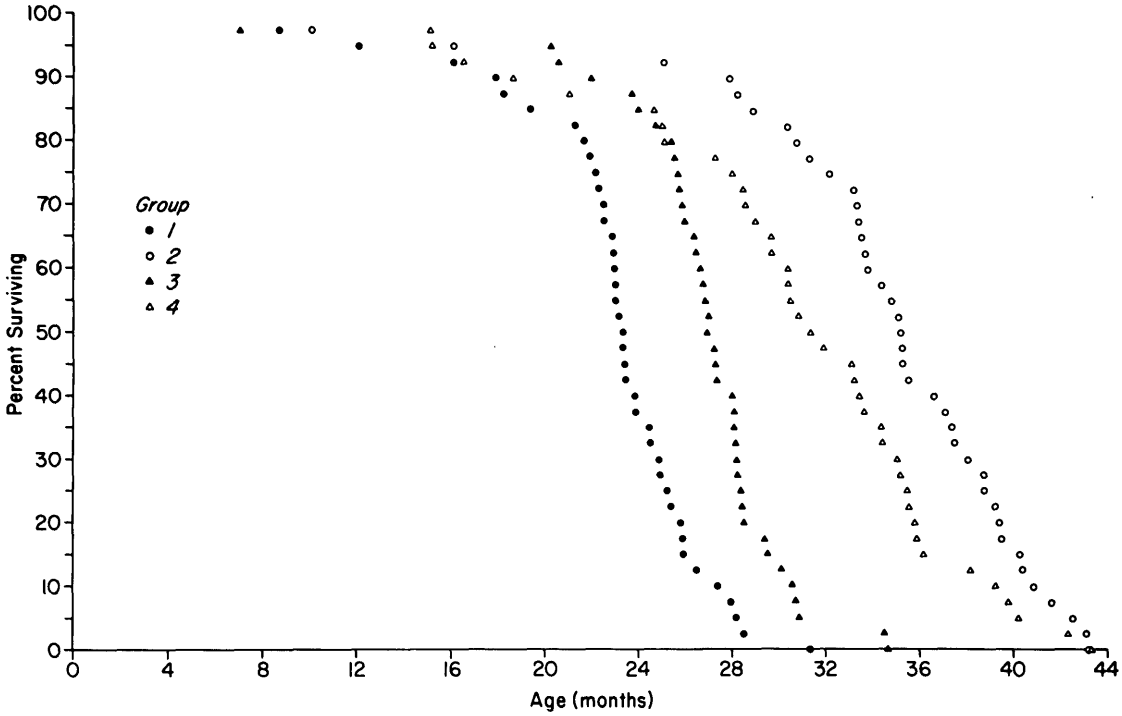

Figure 4. Survival curves for Groups 1, 2, 3, and 4. Each survival curve is generated from 40 rats.

Table 3. Summary of Longevity Findings

Group	*n*	Median	10th Percentile	Maximum
1	40	701 (676–734)	822 (775–941)	941
2	40	1057 (1004–1122)	1226 (1183–1296)	1296
3	40	808 (779–842)	918 (857–1040)	1040
4	40	941 (870–1031)	1177 (1075–1299)	1299
5	40	810 (743–859)	835 (903–969)	969

Note. Entries are days; 95% confidence intervals are in parentheses.

in Figure 7. From 6 through 23 months of life, the caloric intake per gram body weight was greater for the rats in Group 2 than for the rats in Group 1 ($p < .001$). Many rats showed either a large increase or decrease in caloric intake per gram body weight a month or so before death, and this markedly influenced the mean value when the number of animals was small. Until 6 months of age the rats in Group 3 had the same caloric intake per gram body weight as those in Group 2. Upon being fed ad libitum at 6 months of age, predictably the rats in Group 3 had a marked increase in caloric intake per gram body weight which slowly decreased and reached that of the rats in Group 1 by 19 months of age. As expected, the caloric intake per gram body weight of the rats in Group 4 was similar to that of the rats in Group 1 for the first 6 months of life. When these rats were fed the restricted diet at 6 months of age the caloric intake per gram body weight fell markedly. During the rest of the life span the caloric intake per gram body weight of the rats in Group 2 and Group 4 slowly approached each other, but for most of this part of the life span the caloric intake per gram body weight was less in the rats in Group 4 than in those of Group 2 ($p < .05$). The caloric intake per gram body weight in rats of Group 5 was similar to that of the rats in Group 1.

The lifetime kilocalorie intake per gram body mass by the rats in Groups 1, 2, 3, and 4 are reported in Table 4. The value was significantly different for each of these dietary groups ($p < .025$). The value for Group 5 was similar to that of Group 1 but was significantly different from the values for Groups 2, 3, and 4 ($p < .025$).

The lifetime kilocalorie intakes per rat for each of the groups are reported in Table 4. All groups had a similar lifetime caloric consumption except for Group 5 which had a significantly greater intake ($p < .05$).

Spontaneous locomotive activity. — The mean spontaneous locomotive activity of the rats in

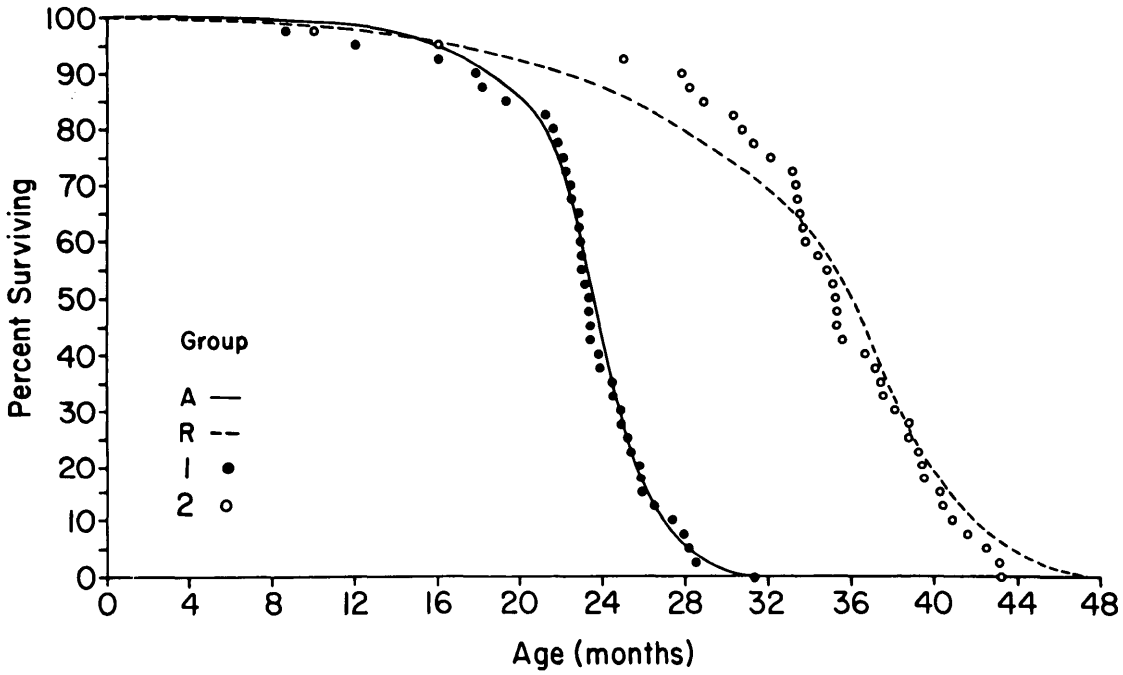

Figure 5. Survival curves for Groups 1, 2, A, and R. The solid curve represents the results from Group A (ad libitum fed) and the broken curve Group R (restricted from 6 weeks of age) from the study of Yu et al. (1982); each curve was generated from 115 rats. The filled circles represent Group 1 and the unfilled circles represent Group 2 of the current study; each survival curve is generated from 40 rats.

Groups 1 and 2 is reported in Figure 8. The measurement of the activity was initiated when the rats were 6 months of age at which time both groups had similar activity. The spontaneous locomotive activity of the rats in Group 1 decreased significantly with increasing age ($p < .01$). In contrast, this activity of the rats in Group 2 did not decline with age.

Systolic blood pressure. — The systolic blood pressure of the rats in Groups 1 and 2 is reported in Figure 9. A gradual increase in systolic blood pressure occurred in both groups from 9 through 18 months of age with little or no change after 18 months of age ($p < .001$). The rats in Groups 1 and 2 had similar systolic blood pressures at all ages.

DISCUSSION

A striking finding of this study is the fact that food restriction initiated in young adult life (6 months of age) was about as effective in extending the life span (based on the maximum length of life of the group and the 10th percentile survival time) as restriction started soon after weaning (6 weeks of age). This result is in line with the findings that food restriction started at 6 months of age is as effective as restriction started at 6 weeks of age in preventing or delaying a spectrum of age changes in the physiological systems (Masoro, 1984) as well as in delaying the appearance of and slowing the progression of age-related disease (see Maeda et al., 1985). There are significant differences, however, between the survival curves of Groups 2 and 4. Through much of the life span the curve of Group 2 is to the right of and nearly parallel to that of Group 4. Apparently it is the food restriction between 6 weeks and 6 months of age that is responsible for this displacement. Does this mean that food restriction influences longevity by two different mechanisms? One, which occurs during the development phase of life, is proposed to cause the displacement of the survival curve to the right. The other, which occurs during adult life, is proposed to cause the slope of the survival curve to decrease (compare the slope of the survival curve of Group 1 with those of Groups 2 and 4).

The findings on the rats of Group 3 (food restriction limited to early life; i.e., 6 weeks to 6 months of age) provide further information in regard to these questions. The displacement of the survival curve of Group 3 to the right of that of Group 1 is similar in magnitude to that observed with Group 2

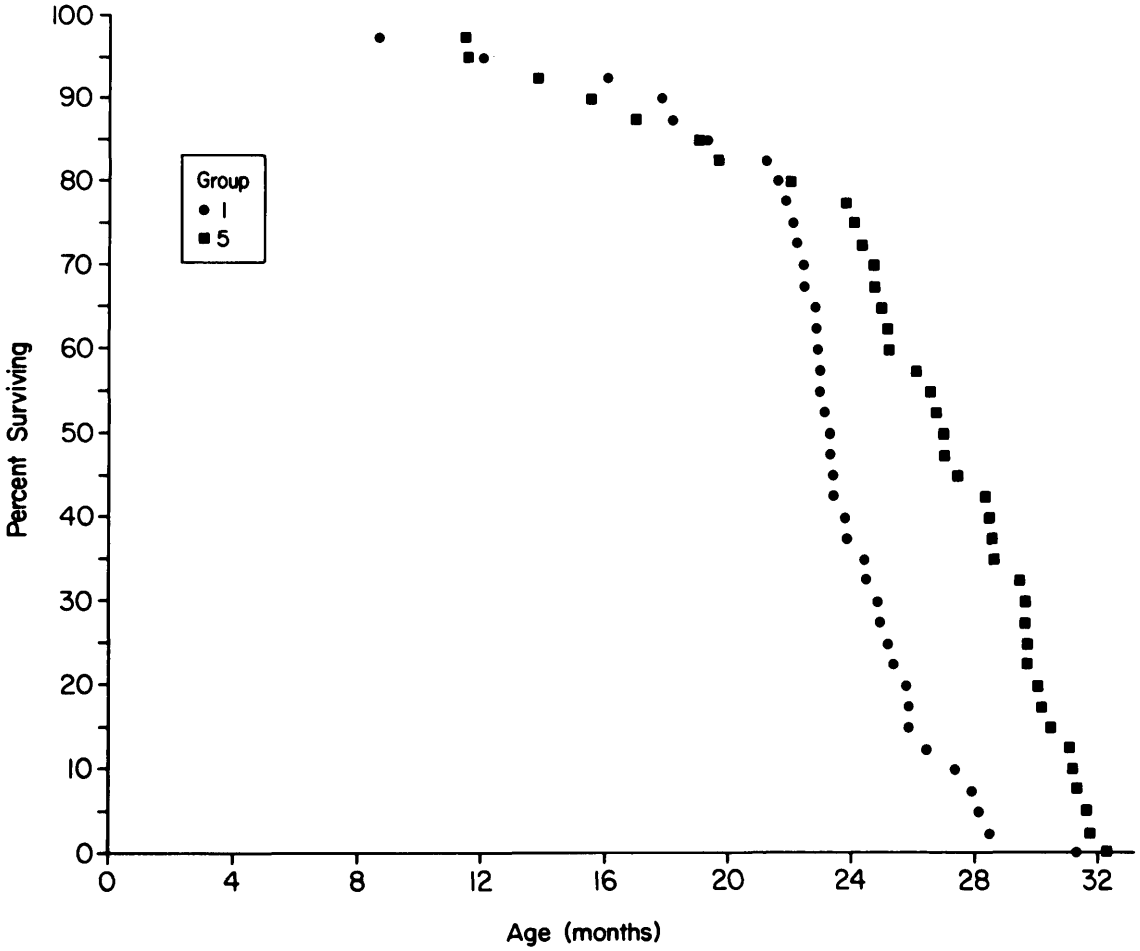

Figure 6. Survival curves for Groups 1 and 5. Each survival curve is generated from 40 rats.

relative to Group 4. This again shows that food restriction during the developmental period of life shifts the survival curve to the right. Unlike food restriction that takes place during adult life, however, the slope of the survival curve of Group 3 is not less than that of Group 1. This observation provides further support for our view that food restriction may act on longevity by different mechanisms during the developmental phase of life compared with adult life. The extent of the increase in longevity in Group 3 was not great in absolute terms, but given the duration of only 4.5 months it appears that food restriction during early life is remarkably effective in extending life. On the other hand, the rats in Group 3 during the long period of ad libitum feeding ate somewhat less than the rats in Group 1 and this may in part contribute to the increased longevity of this group. It should be noted that Stuchlikova et al. (1975) found food restriction until 12 months of age to be the most effective regimen in extending the median length of life of male Wistar rats but life span was not influenced. In that study ad libitum feeding was initiated at 12 months of age and these male Wistar rats became obese. This is in marked contrast to our findings with the male Fischer 344 rats of Group 3 which did not become obese upon being fed ad libitum at 6 months of age (Bertrand et al., 1984).

The findings of the present study clearly show that protein restriction in the absence of caloric restriction results in a significant increase in longevity. The effect was small, however, when compared with the comparable level of protein restriction of the food restriction regimens; that is, the rats in Group 2 and Group 5 had similar daily intakes of protein. It is possible that the somewhat greater food and caloric intake of the rats in Group 5 compared with those in Group 1 after 19 months of

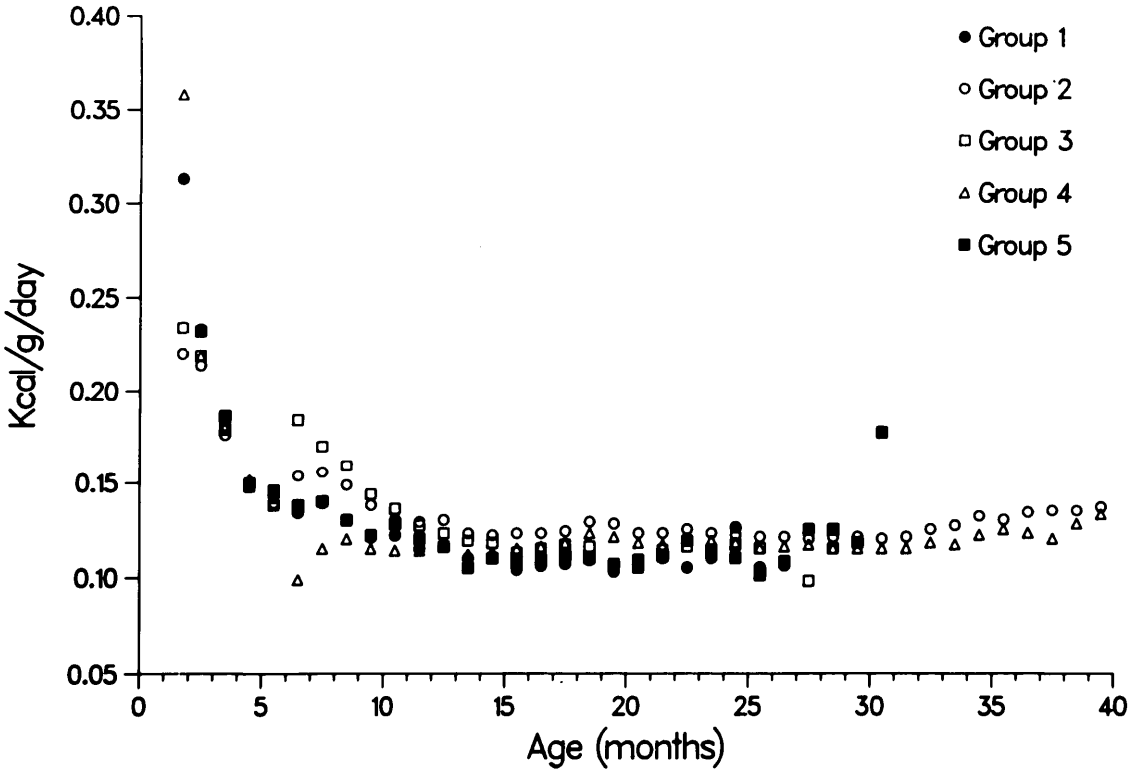

Figure 7. Summary of data on kilocalories of food intake per gram body weight per day. The number of rats in each group started at 40 and the number of rats represented by each point can be estimated from the survival curves presented in Figures 4 and 6. The recording of data was discontinued when the number of rats fell below 5. Values presented are means. In each group the SEM varied with age. For Group 1 it was 0.005 from 1 1/2 to 2 mos, 0.002 from 2 to 3 mos, 0.001 from 3 to 8 mos, 0.002 from 8 to 9 mos, 0.001 from 9 to 17 mos, 0.004 from 17 to 18 mos, 0.002 from 18 to 19 mos, 0.003 from 19 to 20 mos, 0.001 from 20 to 21 mos, 0.005 from 21 to 22 mos, 0.006 from 22 to 23 mos, 0.008 from 23 to 24 mos, 0.007 from 24 to 25 mos, 0.016 from 25 to 26 mos and 0.014 from 26 to 27 mos. For Group 2 it was 0.003 from 1 1/2 to 2 mos, 0.002 from 2 to 5 mos, 0.001 from 5 to 26 mos, 0.002 from 26 to 27 mos, 0.001 from 27 to 32 mos, 0.002 from 32 to 33 mos, 0.003 from 33 to 34 mos, 0.005 from 34 to 35 mos, 0.003 from 35 to 36 mos, 0.004 from 36 to 37 mos, 0.005 from 37 to 39 mos, and 0.006 from 39 to 40 mos. For Group 3 it was 0.005 from 1 1/2 to 2 mos, 0.002 from 2 to 3 mos, 0.001 from 3 to 6 mos, 0.002 from 6 to 7 mos, 0.001 from 7 to 18 mos, 0.002 from 18 to 20 mos, 0.001 from 20 to 21 mos, 0.002 from 21 to 22 mos, 0.003 from 22 to 23 mos, 0.005 from 23 to 24 mos, 0.007 from 24 to 25 mos, 0.005 from 25 to 26 mos, 0.009 from 26 to 27 mos, 0.012 from 27 to 28 mos, 0.021 from 28 to 29 mos, and 0.026 from 29 to 30 mos. For Group 4 it was 0.007 from 1 1/2 to 2 mos, 0.001 from 2 to 24 mos, 0.002 from 24 to 25 mos, 0.001 from 25 to 29 mos, 0.002 from 29 to 31 mos, 0.003 from 31 to 35 mos, 0.005 from 35 to 36 mos, 0.004 from 36 to 37 mos, and 0.008 from 37 to 39 mos. For Group 5 it was 0.001 from 2 to 19 mos, 0.002 from 19 to 21 mos, 0.001 from 21 to 22 mos, 0.004 from 22 to 24 mos, 0.007 from 24 to 25 mos, 0.006 from 25 to 26 mos.; 0.010 from 26 to 27 mos, 0.009 from 27 to 28 mos, 0.011 from 28 to 29 mos, 0.018 from 29 to 30 mos, and 0.040 from 30 to 31 mos.

Table 4. Life Span Caloric Intake

Group	*n*	kcal/g body weight/lifetime	kcal/rat/lifetime
1	40	84.8 ± 2.4	36,285 ± 1189
2	40	133.1 ± 3.9	35,716 ± 1200
3	40	102.5 ± 2.9	37,860 ± 1325
4	40	113.5 ± 3.9	34,516 ± 1269
5	40	91.2 ± 3	42,341 ± 1869

Note. For groups 1, 2, 3 and 4, the intake referred to is from 6 weeks of age until death of the rat; for Group 5 the intake referred to is from 8 weeks of age until death. All rat groups had similar intakes from birth to 6 weeks of age. Entries are mean values plus or minus the standard error of the mean.

age may to some extent have blunted the life-prolonging effect of protein restriction, but this does not seem likely given the extent of the difference in food intake and the advanced age of its occurrence. Unlike the findings with the rats in Groups 2 and 4, there was no modulation in age-related physiologic change in the rats of Group 5 compared with those in Group 1 (Masoro, 1984). Although there was some retardation of age-related disease in the rats of Group 5 compared with those in Group 1, the extent of this effect was much less

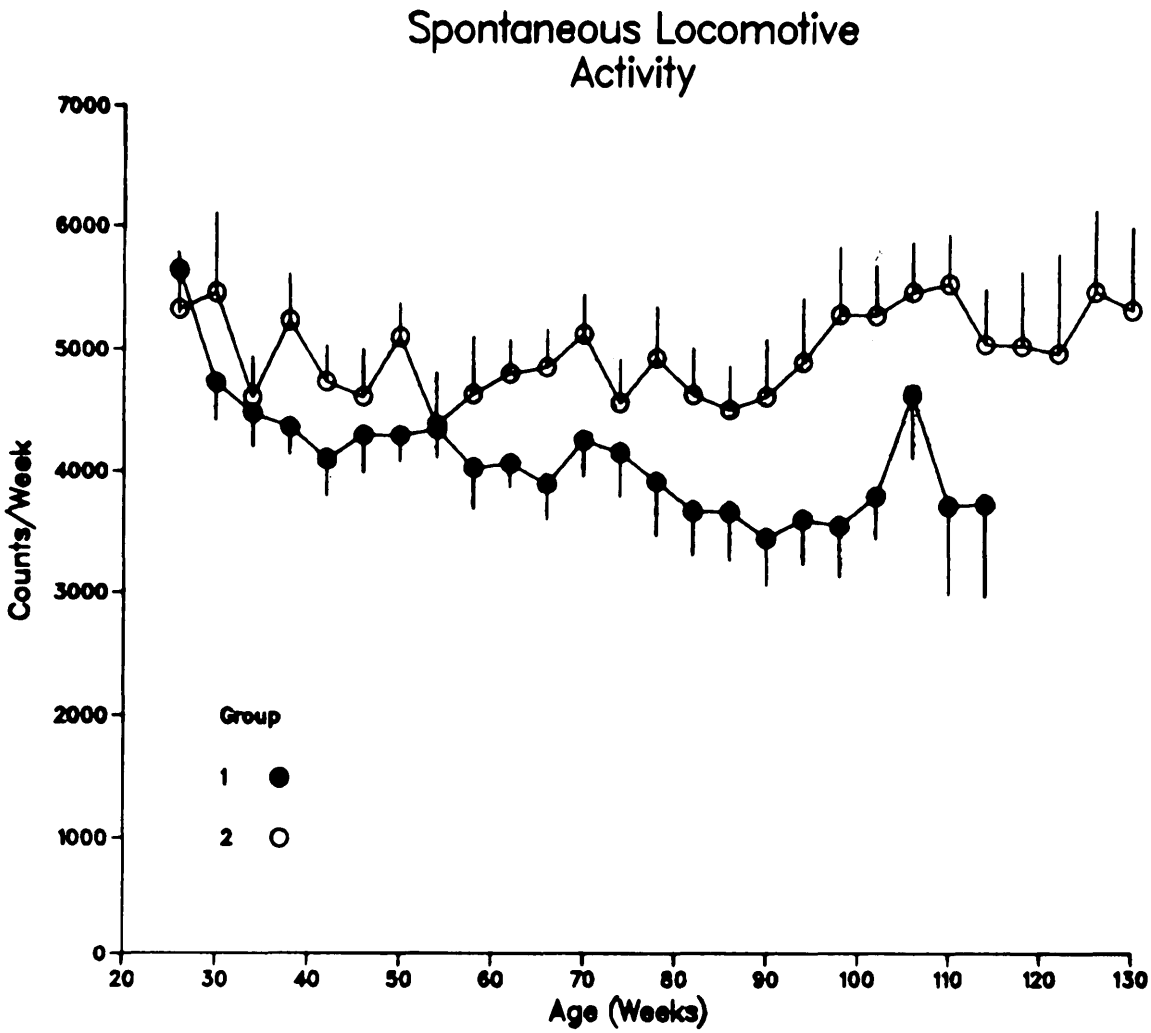

Figure 8. Spontaneous locomotive activity in the rats of Groups 1 and 2. The recording of data was discontinued when the number of rats fell below 4. The values reported are the mean total number of movements per week plus or minus the standard error of the means.

than that observed with the rats in Groups 2 and 4 (see accompanying article, Maeda et al., 1985).

Sacher (1977) proposed that food restriction slows the aging process by reducing the metabolic rate per gram body mass. The basis for this proposal was his analysis of data from a study by Ross (1969) in which five different diets were used to produce a range of longevity characteristics. Sacher calculated that, irrespective of the mean length of life of a dietary group, the mean caloric expenditure per gram body weight per lifetime was about 102 kcal with no group differing from this by more than 4.5%. He concluded from this that food restriction prolongs life by increasing the time required for the rat to reach this 102 kcal per gram body weight per lifetime total. Data from a previous study from our laboratory disagreed with this (Masoro et al., 1982), however. The results from our study showed that rats that were food restricted consumed more kilocalories per gram body weight per day than rats fed ad libitum and that the mean lifetime caloric consumption per gram body mass was much greater for the rats fed the restricted diet than for those fed ad libitum. Because Sacher was not in possession of the original data for his calculations it was necessary for him to make assumptions about the temporal course of food intake and body weight when using the published data of Ross. Indeed, if different assumptions had been made, his results would have been similar to ours.

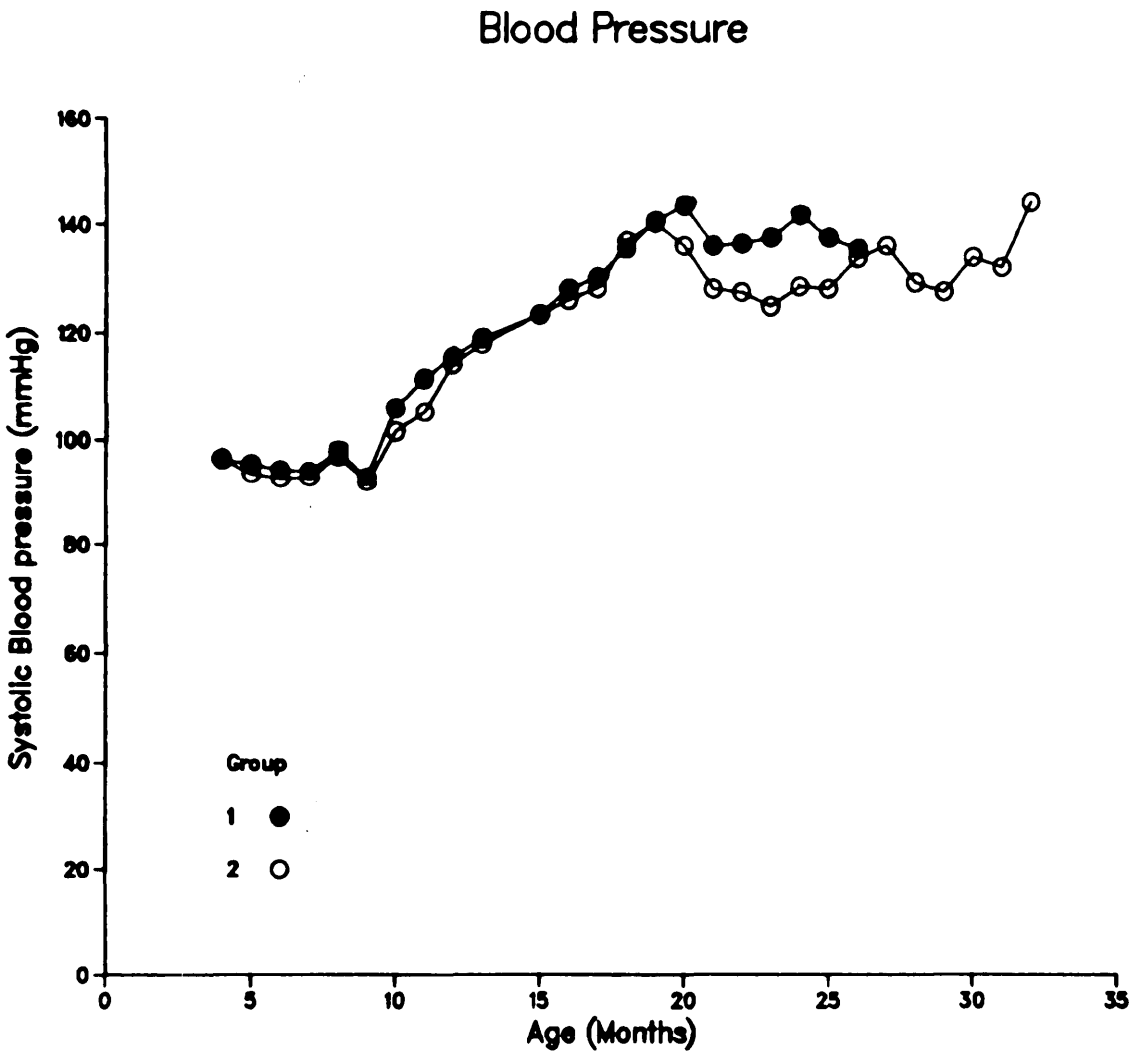

Figure 9. Systolic blood pressures in the rats of Groups 1 and 2. The values presented are averages from 6 rats. The recording of data was discontinued when the number of rats fell below 3.

The relationship between caloric intake and longevity was further explored in the present study. In agreement with our previous findings, the rats in Group 2 were found to have a greater daily caloric intake per gram body mass than those in Group 1. If the data of Bertrand et al. (1980) are used to estimate daily caloric intake per gram lean body mass, no difference is found between the Group 1 and Group 2 rats. Because of the conceptual significance of these findings, it is important to measure energy expenditure in addition to food and energy intake. To this end we have initiated the measurement of caloric expenditure by means of oxygen consumption over a 24-hour period under usual living circumstances. The caloric expenditure for 6-month-old rats of Group 1 and Group 2 was found to be 136 ± 7 and 143 ± 3 kcal per kg lean body mass per day, respectively (McCarter et al., 1985). These data indicate that in rats fed the food-restricted diet there is not a reduction in caloric expenditure or in the input of any nutrient per gram lean body mass; that is, there is a reduction in lean body mass that is proportional to the reduction in food intake. Thus the classic view that food restriction acts by reducing caloric or other nutrient input per unit protoplasmic mass must be reexamined.

In support of our earlier findings, dietary groups 1 through 4 in the present study had considerably different kilocalorie intakes per gram body mass per lifetime. What was unexpected, however, is the

fact that the rats in Groups 1, 2, 3, and 4 had similar mean total caloric intakes per lifetime per rat; that is, the mean of the four groups were within 5% of 36,100 kcal per rat per lifetime. Indeed, only the rats fed the low protein diet (Group 5) had a significantly different mean total caloric intake per lifetime per rat.

At 6 months of age, Group 1 and Group 2 rats were found to have similar spontaneous locomotive activity. Although in our study activity measurements were not made at ages younger than 6 months, Goodrick et al. (1983a) measured wheel activity from 2 through 6 months of age and found that, except for 2 months of age, ad libitum fed rats had a greater activity than food restricted rats. After 6 months of age, spontaneous motor activity declined in ad libitum fed rats and food restriction was found to modulate this loss of spontaneous locomotive activity with increasing age. As a consequence, the rats in Group 2 had more spontaneous locomotive activity during most of their life than the rats in Group 1. This raises the question of the extent to which the increased longevity in food restricted rats is secondary to this greater motor activity. From the data available in the literature it appears that this difference in spontaneous locomotive activity plays little or no role. Several studies, reviewed by Goodrick (1980), have shown that exercise can increase longevity of rodents, but the effect is small compared with that of food restriction. Also, Goodrick et al. (1983b) found that exercise initiated at either 10.5 months of age or 18 months of age in ad libitum-fed or food restricted rats did not influence the length of life. Probably the work of Holloszy et al. (in press) most directly applies to our studies. Holloszy found that rats would not exercise throughout their life span unless their food was restricted, the average restriction necessary being a 10% reduction in food intake. He found that the maximum life span of the exercised, slightly food restricted rats to be the same as the pair-fed sedentary control rats.

The finding that food restriction did not retard the age-related increase in systolic blood pressure in male Fischer 344 rats was surprising. Most age-associated physiologic changes studied by us and others were found to be retarded or prevented by food restriction (Masoro, 1984). Systolic blood pressure is clearly an exception. Moreover, it should be noted that Baskin et al. (1979) did not observe an age-related increase in systolic blood pressure in a cross-sectional study of adult male Fischer 344 rats.

The following conclusions emerge from the findings of this study and from the relationship of these findings to other recent work. Food restriction initiated in young adult rats is as effective in extending life span as food restriction initiated soon after weaning. Food restriction limited to early life (6 weeks to 6 months of age) causes a small but significant increase in life span. The mechanism of action of this dietary manipulation during the developmental period of life may be different from that occurring during adult life. Protein restriction in the absence of caloric restriction increases longevity but is much less effective than a similar level of protein restriction in the food restriction regimens involving caloric restriction as well. Reduced caloric intake or nutrient intake per gram body weight, reduced metabolic rate per unit metabolic mass, and increased physical activity do not appear to play a role in life extension by food restriction. The age-related increase in arterial blood pressure is not influenced by food restriction, thus showing that not all age-related physiological change is modulated by this dietary manipulation.

References

Barrows, C. H., Jr., & Roeder, L. M. (1965). The effect of reduced dietary intake on enzymatic activities and life span of rats. *Journal of Gerontology, 20,* 69–71.

Baskin, S. I., Roberts, J., & Kendrick, Z. V. (1979). Effect of age on body weight, heart rate and blood pressure in pair-caged, male, Fischer 344 rats. *Age, 2,* 47–50.

Bertrand, H. A., Lynd, F. T., Masoro, E. J., & Yu, B. P. (1980). Changes in adipose mass and cellularity through the adult life of rats fed ad libitum or a life-prolonging restricted diet. *Journal of Gerontology, 35,* 827–835.

Bertrand, H. A., Stacy, C., Masoro, E. J., Yu, B. P., Murata, I., & Maeda, H. (1984). Plasticity of fat cell number. *Journal of Nutrition, 114,* 129–133.

Cheney, K. E., Liu, R. K., Smith, G. S., Meredith, P. J., Mickey, M. R., & Walford, R. L. (1983). The effect of dietary restriction of varying duration on survival, tumor patterns, immune function, and body temperature in B10C3F$_1$ female mice. *Journal of Gerontology, 38,* 420–430.

Conover, W. J. (1971). *Practical nonparametric statistics.* Wiley, New York.

Coleman, G. L., Barthold, S. W., Osbaldiston, G. W., Foster, S. J., & Jonas, A. M. (1977). Pathological changes during aging in barrier-reared Fischer 344 male rats. *Journal of Gerontology, 32,* 258–278.

Davis, T. A., Bales, C. W., & Beauchene, R. E. (1983). Differential effects of dietary caloric and protein restriction in the aging rat. *Experimental Gerontology, 18,* 427–435.

Goodrick, C. L. (1978). Body weight-increment and length of life: The effect of genetic constitution and dietary proteins. *Journal of Gerontology, 33,* 184–190.

Goodrick, C. L. (1980). Effects of long-term voluntary wheel exercise on male and female Wistar rats. I. Longevity, body weight and metabolic rate. *Gerontology, 26,* 22–33.

Goodrick, C. L., Ingram, D. K., Reynolds, M. A., Freeman, J. R., & Cider, H. L. (1983a). Effects of intermittent feeding

upon growth, activity and lifespan of rats allowed voluntary exercise. *Experimental Aging Research, 9,* 203–209.

Goodrick, C. L., Ingram, D. K., Reynolds, M. A., Freeman, J. R., & Cider, N. L. (1983b). Differential effects of intermittent feeding and voluntary exercise on body weight and life span in adult rats. *Journal of Gerontology, 38,* 36–45.

Gross, A. J., & Clark, V. A. (1975). *Survival distributions: Reliability applications in the biomedical sciences.* Wiley, New York.

Holloszy, J. O., Smith, E. K., Vining, M., & Adams, S. (in press). Effect of voluntary exercise on longevity of rats. *Journal of Applied Physiology.*

Leto, S., Kokkonen, G., & Barrows, C. (1976). Dietary proteins, life spans, biochemical variables in female mice. *Journal of Gerontology, 31,* 144–148.

Maeda, H., Gleiser, C. A., Masoro, E. J., Murata, I., McMahan, C. A., & Yu, B. P. (1985). Nutritional influences on aging of Fischer 344 rats: II. Pathology. *Journal of Gerontology, 40,*

Masoro, E. (1984). Nutrition as a modulator of the aging process. *The Physiologist, 27,* 98–101.

Masoro, E., Yu, B. P., & Bertrand, H. A. (1982). Action of food restriction in delaying the aging process. *Proceedings National Academy of Sciences USA, 79,* 4239–4241.

McCarter, R., Masoro, E. J., & Yu, B. P. (1985). Does food restriction retard aging by reducing the metabolic rate? *American Journal of Physiology, 248,* E488–E490.

McCay, C. M., Crowell, M. F., & Maynard, L. A. (1935). The effect of retarded growth upon the length of life span and upon the ultimate body size. *Journal of Nutrition, 10,* 63–79.

Nakagawa, I., & Masana, Y. (1971). Effect of protein nutrition on growth and life span in the rat. *Journal of Nutrition, 101,* 613–620.

Ross, M. H. (1969). Aging, nutrition and hepatic enzyme activity patterns in the rat. *Journal of Nutrition, 97,* Supplement, Part II, 563–602.

Ross, M. H. (1972). Length of life and caloric intake. *American Journal of Clinical Nutrition, 25,* 834–838.

Ross, M., & Bras, G. (1973). Influence of protein under- and over-nutrition on spontaneous tumor prevalence in the rat. *Journal of Nutrition, 103,* 944–963.

Sacher, G. A. (1977). Life table modification and life prolongation. In C. Finch & L. Hayflick (Eds.), *Handbook of biology of aging.* Van Nostrand Reinhold, New York.

Snedecor, G. W., & Cochran, W. G. (1967). *Statistical methods.* Iowa State University Press, Ames, IA.

Stuchlikova, E., Juricova-Horakova, J., & Deyl, Z. (1975). New aspects of the dietary effects of life prolongation in rodents. What is the role of obesity in aging? *Experimental Gerontology, 10,* 141–144.

Weindruch, R., & Walford, K. L. (1982). Dietary restriction in mice beginning at 1 year of age: Effect on life-span and spontaneous cancer incidence. *Science, 215,* 1415–1418.

Yu, B. P., Masoro, E. J., Murata, I., Bertrand, H. A., & Lynd, F. T. (1982). Life span study of SPF Fischer 344 male rats fed ad libitum or restricted diets: longevity, growth, lean body mass and disease. *Journal of Gerontology, 37,* 130–141.

Yu, B. P., Wong, G., Lee, H., Bertrand, H., & Masoro, E. J. (1984). Age changes in hepatic metabolic characteristics and their modulation by dietary manipulation. *Mechanisms of Ageing and Development, 24,* 67–81.