Gerontology *28:* 233–241 (1982)

Effects of Intermittent Feeding Upon Growth and Life Span in Rats

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Key Words. Rat · Growth · Life span · Body weight · Nutrition · Fasting

Abstract. From weaning to death, 28 male Wistar rats were maintained on an ad libitum (AL) diet, and 24 counterparts were provided the diet every-other-day (EOD). The mean life span of the EOD group represented an 83% increase over that of the AL group. Furthermore, a Gompertzian analysis of mortality rates suggested that the rate of aging was retarded in the EOD group. While body weight and growth rate were reduced in the EOD group, their growth duration was 75% longer compared to the AL group. Significant positive relationships emerged between life span and growth rate parameters in the AL group; however, no significant relationships were found between life span and body weight parameters in the EOD group. Therefore, in support of the hypothesis that dietary restriction effects prolongevity through retarded development, evidence was produced only in the between-group comparisons of AL- and EOD-fed animals.

The life span of laboratory rodents has been increased through a variety of dietary manipulations begun early in life. The three basic regimens applied in past studies can be categorized as follows: (a) altering the protein/calorie content of the food [*Fernandes* et al., 1976; *Goodrick,* 1977, 1978; *Leto* et al., 1976; *Miller and Payne,* 1968; *Nakagawa and Masana,* 1971; *Ross,* 1959, 1961; *Ross and Bras,* 1973; *Stoltzner,* 1977]; (b) reducing protein/calorie intake by restricting the amount of food provided [*Berg and Simms,* 1960; *McCay,* 1935; *McCay* et al., 1939, 1943; *Nolen,* 1972; *Riesen* et al., 1947; *Ross,* 1961; *Saxton* et al., 1944; *Stuchlíková* et al., 1975]; and (c) reducing the protein/calorie intake by restricting feeding periods [*Beauchene* et al., 1979; *Carlson and Hoelzel,* 1946; *Cheney* et al., 1980; *Gerbase-DeLima* et al., 1975; *Leveille,* 1972].

Further study has suggested that these manipulations retard many age-related biological changes, including disease and tumor incidence [*Berg and Simms,* 1960; *Bras and Ross,* 1964; *Cheney* et al., 1980; *McCay* et al., 1943; *Ross and Bras,* 1965, 1971, 1973; *Saxton* et al., 1944; *Visscher* et al., 1942], biochemical parameters [*Barrows and Kokkonen,* 1978;

Leto et al., 1976; *Ross,* 1959], skeletal and organ morphology [*Berg,* 1960; *Johnson and Barrows,* 1980; *McCay* et al., 1939], immunological response [*Fernandes* et al., 1976; *Gerbase-DeLima* et al., 1975; *Mann,* 1978] and even fertility [*Berg,* 1960]. Although several hypotheses have been offered and tested, the mechanism for prolongevity induced through dietary means remains unknown [*Barrows and Kokkonen,* 1977].

One classic hypothesis links increased life span to retarded development that results from restricted dietary intake [*McCay,* 1935, 1952; *McCay* et al., 1939]. Studies of laboratory rodents indicate that increases in life span parallel increases in the duration of growth, or body weight gain, and that growth rate is negatively correlated with longevity [*Goodrick,* 1977, 1978, 1980; *Ross* et al., 1976].

In the present study we examined the effects on longevity produced by restricting feeding to an every-other-day schedule during the entire post-weaning life span of male rats. In contrast to an early report of minor effects [*Robertson* et al., 1934], significant life span extension has been demonstrated with this periodic fasting regimen in studies of mice [*Gerbase-DeLima* et al., 1975; *Cheney* et al., 1980] and rats [*Beauchene* et al., 1979; *Carlson and Hoelzel,* 1946]. By recording weekly body weights in the sample, the present study allowed a closer examination of the relationship between growth parameters and life span as a further test of the hypothesis linking prolongevity to retarded development.

Method

Subjects. The subjects were 52 male Wistar rats obtained at weaning from the colony of the Gerontology Research Center (GRC). The animals were doubly housed in suspended metal cages (Wahmann), equipped with an automated watering system. The cage racks were also equipped with excrement pans filled with wood shavings, and these were changed three times weekly. All racks and cages were changed and cleaned once a month or more frequently, if conditions warranted. The cages were located in one room regulated at 22 ± 2 °C, with a 12-hour light/12-hour dark photocycle.

Procedure. One week after weaning, the rats were randomly assigned to one of two groups defined on the basis of feeding schedule. For 28 of the animals, NIH-07 laboratory chow (24% protein) was provided ad libitum (AL) from weaning. For 24 of the animals, this diet was provided on an intermittent basis, every-other-day (EOD). For the EOD group, food was provided in the morning hours and removed from the food hoppers on the following morning. The rats were weighed weekly and were permitted to survive in the cages until death. The EOD group was weighed only on mornings when food was removed. Cages were checked daily and dead animals were removed when discovered.

Results

Intermittent feeding markedly increased life span in these rats. The mean ± standard error life span of the EOD group was 138.2 ± 4.2 weeks compared to 75.5 ± 2.1 weeks for the AL group, a difference that was statistically significant according to Student's t test, $t(50) = 13.9$, $p < 0.0001$. The estimates of mean life span matched estimates of median life span, 75 weeks for the AL group vs. 139 for the EOD group. The survival curves for the two groups are depicted in figure 1. The dramatic difference in survival was also analyzed with the Lee-Desu statistic [*Lee and Desu,* 1972], which confirmed that the groups were probably drawn from different survival distributions, $D(1) = 3.37$, $p < 0.0001$.

To determine if the rate of aging was affected by the dietary manipulation, a Gom-

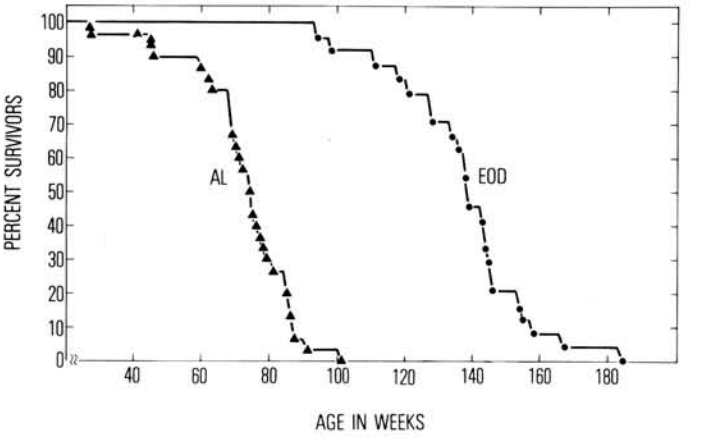

Fig. 1. Percent survivors as a function of age for male Wistar rats fed ad libitum (AL) or every-other-day (EOD).

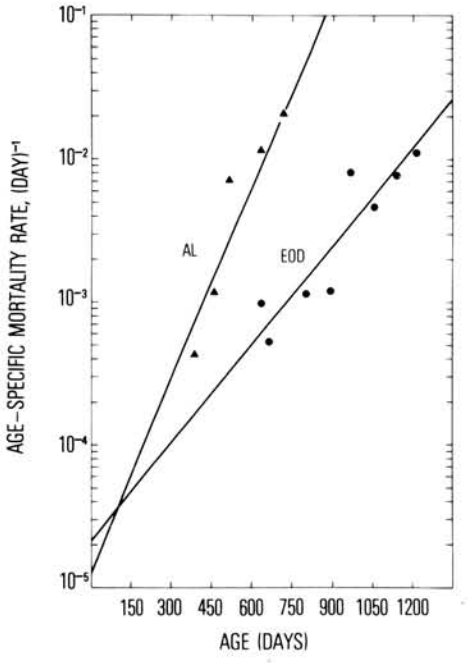

Fig. 2. Gompertzian analysis of mortality rates for male Wistar rats fed ad libitum (AL) or every-other-day (EOD).

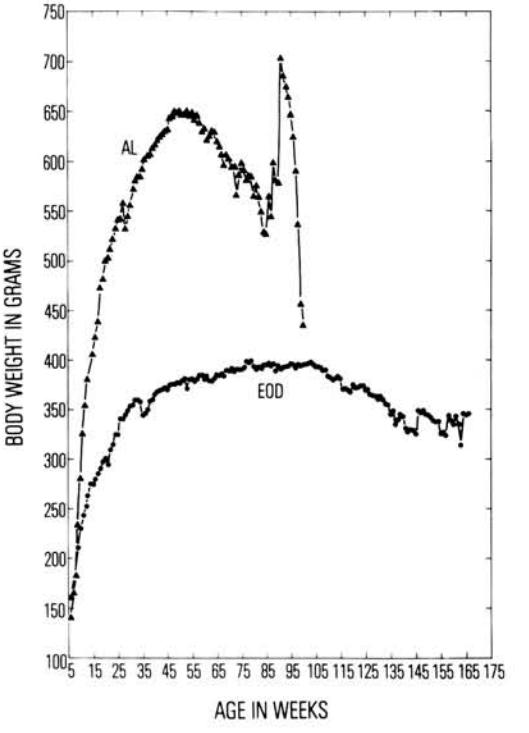

Fig. 3. Mean body weight as a function of age for male Wistar rats fed ad libitum (AL) or every-other-day (EOD).

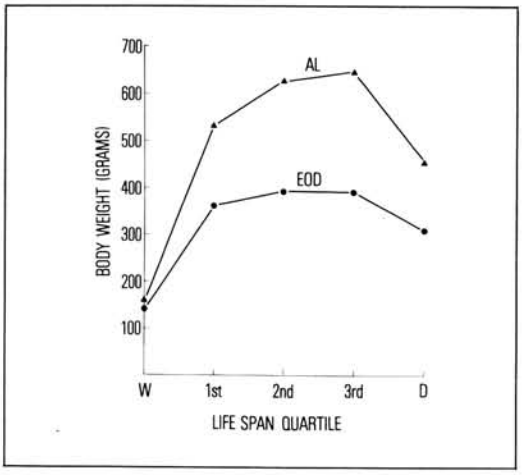

Fig. 4. Mean body weight at weaning (W), death (D), and quartile of individual life span for male Wistar rats fed ad libitum (AL) or every-other-day (EOD).

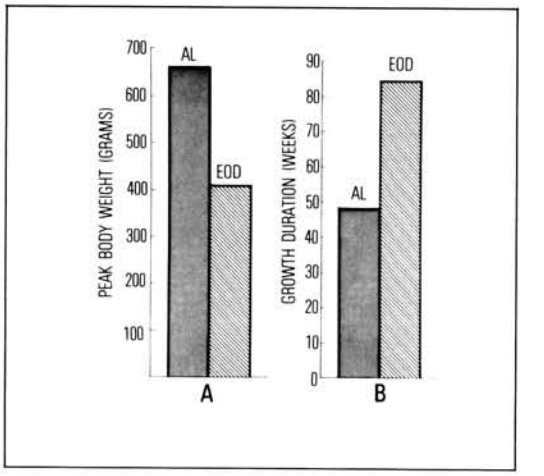

Fig. 5. Mean peak body weight **(A)** and mean growth duration **(B)** of male Wistar rats fed ad libitum (AL) or every-other-day (EOD).

pertzian analysis of mortality rates was also conducted according to the procedures recommended by *Sacher* [1977]. As depicted in figure 2, the computed linear regression lines were nonparallel and thus suggested that the rate of aging had been slowed in the EOD group relative to the AL group. A statistical comparison [*Tallarida and Murray,* 1981] confirmed the significant difference in slopes, $t(11) = 17.08$, $p < 0.0001$. Based on this analysis, the doubling time in mortality, T_d, was also computed [*Sacher,* 1977]. In the AL group, 69.3 days was the estimated doubling time, while in the EOD group, the estimate of 138.5 days was nearly twice as great.

It was evident that intermittent feeding had a marked effect upon body weight parameters as well as life span. Figure 3 presents the mean body weight of the EOD and AL groups as a function of age. Body weight gain in the EOD group was reduced in comparison to the AL group. The difference in body weight was evident 5 weeks after the dietary treatment began. By 1 year, the mean body weight of AL-fed rats was nearly twice that of the EOD-fed rats. Because attrition due to death increases as a function of time, however, this presentation of body weight data is necessarily biased. This accounts for the erratic appearance of mean body weight at older ages in figure 3.

For more accurate and relative comparisons of body weight gain, figure 4 provides estimates of mean body weight at weaning, at death, and at quartiles of individual life span. These comparisons could be subjected to statistical analysis. An analysis of variance with repeated measures across life span intervals confirmed the main effect of diet on body weight, $F(1,50) = 223.4$, $p < 0.0001$, the main effect of life span interval, $F(4,200) = 890.4$, $p < 0.0001$, and a significant interaction between diet and life span interval, $F(4,200) = 116.4$, $p < 0.0001$.

Further analysis of the simple main effects [*Winer,* 1971] verified that the mean body

weight of the AL group was significantly higher ($p < 0.0001$) than that of the EOD group at all points except weaning ($p > 0.05$). It is also evident in figure 4 that both groups tended to lose body weight over the last quartile of life span. In the AL group there was a 30% decrease in mean body weight between the third quartile and death, and in the EOD group the decrease was about 21%.

Presented in figure 5A is the comparison for peak body weight (PKBW), or the highest body weight that each rat obtained; and the comparison of growth duration (GD), or the age at which PKBW was obtained, is presented in figure 5B. According to the results of a t test, the mean PKBW of the AL group was significantly higher, about 62%, compared to the EOD group, $t(50) = 15.98$, $p < 0.0001$. Conversely, mean GD in the EOD group was about 75% longer compared to the AL group, $t(50) = 12.32$, $p < 0.0001$.

Table I provides data to permit examination of the relationships between life span and various growth parameters within each diet group. Among AL animals many parameters of body weight growth were correlated with longevity; whereas, in the EOD group there were no significant relationships. Among AL animals there was a significant negative correlation between body weight at weaning and life span; however, body weight at the first and second quartile of life was a significant positive correlate of life span in this group. Similarly, the rate of body weight gain in the AL group was a significant positive correlate of life span during the first 3 months after weaning and after 10.5 months of age. In general, then, longevity in AL-fed rats was associated with a lower body weight at weaning, a relatively higher rate of body weight gain shortly after weaning, and greater body weight gain after 10.5 months of age. The resulting pattern of growth also produced a very high correlation between GD and life span. PKBW was also positively correlated with life span, but failed to reach statistical significance ($p < 0.07$). Among EOD animals the correlation between GD and life span was also positive but did not exceed statistical significance ($p < 0.14$).

Table I. Relationship of body weight and growth rate parameters to life span (LS) in rats fed ad libitum (AL) or every-other-day (EOD)[1]

Parameter	AL	EOD
Body weight		
Weaning (1.5 months)	–0.43*	–0.03
LS quartile 1	0.49**	0.16
LS quartile 2	0.42*	0.06
LS quartile 3	0.34	–0.17
Peak body weight	0.35	–0.01
Growth rate[2]		
1.5–4.5 months	0.47*	0.02
4.5–7.5 months	0.12	0.21
7.5–10.5 months	0.02	–0.18
10.5–13.5 months	0.72***	0.26
13.5–16.5 months	0.63***	0.15
16.5–19.5 months	0.83***	0.03
Growth duration	0.88***	0.31

[1] Values reported are the Pearson product-moment coefficients for the correlations between life span and respective parameters. For the AL group, d.f. = 26, except for the last three growth rate intervals, where d.f. = 25, 23, and 15, respectively. For the EOD group, d.f. = 22, except for the last two growth rate intervals, where d.f. = 21.

[2] Growth rate = (BWT2 – BWT1)/BWT1, where BWT1 = body weight at younger age, and BWT2 = body weight at older age, for the intervals specified. This growth rate parameter was found to correlate 0.95 with other measures of body weight gain [*Ingram* et al., 1982].

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

Discussion

The present study replicated earlier reports of life span extension in laboratory rodents, produced through a regimen of intermittent feeding begun early in life [*Beauchene* et al., 1979; *Carlson and Hoelzel,* 1946; *Cheney* et al., 1980; *Gerbase-DeLima* et al., 1975]. The 83% increase in mean life span relative to that of AL-fed rats observed in the present study was more dramatic than previously recorded. Several explanations exist for the difference in the magnitude of the dietary effect on life span. First, different strains and species were used in all but one of the earlier studies. *Beauchene* et al. [1979] represents the one previous experiment that also used male Wistar rats. In their study both the control (AL) and experimental (EOD) groups had mean life spans in excess of those observed in the respective groups in the present investigation. This difference might reflect the use of different diets. Wayne Lab Blox was used in the previous study, whereas NIH-07 Laboratory Chow was used in the present study. When we compared constituents of the two diets, little difference was found in the main ingredients, including protein, fat, fiber, ash; but there were several substantial differences in vitamin and trace mineral content, including niacin, pyridoxine, folic acid, manganese and iron, with differences ranging from 36 to 150% in parts per million.

Another possible explanation for the surprisingly dramatic dietary effect obtained in the present study is one that always exists, namely, the general health of the animal colonies under investigation. The median life span of the AL group was 75 weeks in the present study, which was lower than the median of 95 weeks that has been recorded in the GRC colony of male Wistar rats [unpublished data]. However, even if the AL group in the present study had attained this higher median level, it remains quite evident that the 139-week median life span of the EOD group was substantially longer.

According to *Sacher's* [1977] recommendation, mortality rates in the samples were subjected to a Gompertzian transformation to determine whether the dietary manipulation had affected the rate of aging. The present study confirmed previous observations [*Sacher,* 1977] that dietary restriction retards the rate of aging in addition to increasing the mean life span relative to AL-fed animals. Moreover, our estimates of doubling time in mortality rates, 69 days for the AL group and 139 days for the food-restricted group, closely corresponded to the respective estimates of 66 and 130 days that *Sacher* [1977] extracted from several previous studies.

Consistent with past findings using the same or other nutritional manipulations [*Barrows and Kokkonen,* 1977, 1978], post-weaning body weight and growth rates were reduced among EOD-fed animals in the present study. Examining body weight at equivalent quartiles of life span, we found that the mean body weights of the EOD group were about 30–40% less than comparable mean body weights of the AL group at the first, second, and third quartiles. The PKBW obtained in the AL group also tended to be higher, about 62%, than observed in the EOD group. Conversely, the period of body weight gain in the EOD group, or GD, tended to be considerably longer, by about 75%. Both groups showed a decline in body weight over the last quartile of the life span.

The above observations, based upon comparisons between AL and EOD groups, sup-

port the hypothesis that the mechanism for life span extension produced by dietary restriction might be linked to the retardation of growth rate [*Goodrick,* 1977, 1978, 1980; *McCay,* 1935; *McCay* et al., 1939]. In past studies, within-group correlations between life span and various growth parameters have also supported the hypothesis [*Goodrick,* 1977, 1978, 1980; *Ross* et al., 1976]. For example, within groups of rats and mice on various dietary regimens, *Goodrick* [1977, 1978, 1980] observed that life span tended to be positively correlated with GD and PKBW but negatively correlated with growth rate, when growth rate was computed as the ratio of PKBW to GD. Further analysis of this measure of growth rate, however, revealed that the parameter lacked construct validity, i.e., it was not related to other measures of growth, and it was also a redundant measurement of GD [*Ingram* et al., 1982]. Using more conventional estimates of growth rate between specified age intervals, *Ingram* et al. [1982] showed that there were no significant negative correlations between life span and growth rate among *Goodrick's* [1980] groups. In fact, among AL-fed rats housed in both standard cages and wheel-activity cages, growth rate after about 9 months of age was significantly correlated with life span, but the direction of the relationship was positive.

The positive correlation of growth rate with life span was also made in the AL group in the present study. After 10.5 months of age, estimates of growth rate correlated highly with life span, and the relationship was consistently positive. In contrast, no significant relationships between life span and growth rate were observed in the EOD group. PKBW was also unrelated to longevity in this group; and, although positive in direction, the correlation between GD and life span failed to meet the accepted level of statistical significance ($p < 0.14$). These findings also conflict with those of *Ross* et al. [1976], who observed that growth rate during several early developmental periods was negatively correlated with life span in rats; however, their animals were maintained on a self-selected diet. These investigators also reported that GD, but not PKBW, was positively related to longevity.

Thus, the only evidence in the present study supporting the hypothesis that prolongevity is associated with retarded development was observed in the between-group comparisons. The rate of body weight gain was reduced in the EOD group, while the duration of body weight gain was prolonged. It might be expected that variability in the developmental mechanism affecting longevity would also be expressed within groups. When examining the intra-group correlations in the present study, however, there was little evidence in support of the hypothesis. Instead of the hypothesized negative relationship between growth and longevity, only positive relationships were observed between growth rate and life span within the AL group, and there were no significant relationships in the EOD group.

The most damaging evidence against the retarded-growth-rate hypothesis of prolongevity has been produced in studies in which the nutritional manipulation was begun in adult laboratory rodents and was successful in extending life span [*Barrows and Kokkonen,* 1975; *Stuchlíková* et al., 1975; *Ross,* 1976]. *Everitt* [1959] provided additional experimental evidence against the hypothesis, when he demonstrated that injections of growth hormone failed to increase life span in rats. Thus, while dietary restriction obviously alters growth patterns, it is likely that the relation-

ship to life span extension is coincidental. A causative link has yet to be confirmed.

A reduction in protein synthesis has been an alternatively proposed mechanism of life span extension in restricted animals [*Barrows and Kokkonen,* 1975, 1978]. The concentrations of proteins and DNA and the activities of several enzymes were found to be reduced in the livers and kidneys of young and adult mice fed a low-protein (4%) diet in comparison to groups fed normal levels (24%). However, there were no differences in these biochemical variables between groups of AL-fed mice and groups fed on intermittent basis (3 out of 5 days). Therefore, it is possible that different mechanisms are involved in the different dietary manipulations producing life span extension. Other mechanisms that are being explored include alterations of immune responsivity and susceptibility to disease [*Berg and Simms,* 1960; *Fernandes* et al., 1976; *Gerbase-DeLima* et al., 1975; *Saxton* et al., 1944; *Ross,* 1976; *Ross and Bras,* 1971; *Weindruch* et al., 1979] and effects of prolongevity-assurance systems, such as natural antioxidants [*Cutler,* 1978]. At present, what remains is the empirically reliable but unexplainable product of dietary restriction in laboratory animals – an increased life span relative to animals fed AL under conventional laboratory conditions.

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Received: December 4, 1981
Accepted: December 11, 1981

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