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EFFECTS OF INTERMITTENT FEEDING UPON BODY WEIGHT AND LIFESPAN IN INBRED MICE: INTERACTION OF GENOTYPE AND AGE

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SUMMARY

Beginning at either 1.5, 6 or 10 months of age, male mice from the A/J and C57BL/6J strains and their F_1 hybrid, B6AF_1/J were fed a diet (4.2 kcal/g) either *ad libitum* every day or in a restricted fashion by *ad libitum* feeding every other day. Relative to estimates for *ad libitum* controls, the body weights of the intermittently-fed restricted C57BL/6J and hybrid mice were reduced and mean and maximum life span were incremented when the every-other-day regimen was initiated at 1.5 or 6 months of age. When every-other-day feeding was introduced at 10 months of age, again both these genotypes lost body weight relative to controls; however, mean life span was not significantly affected although maximum life span was increased. Among A/J mice, intermittent feeding did not reduce body weight relative to *ad libitum* controls when introduced at 1.5 or 10 months of age; however, this treatment did increase mean and maximum life span when begun at 1.5 months, while it decreased mean and maximum life span when begun at 10 months. When restricted feeding was introduced to this genotype at 6 months of age, body weight reduction compared to control values was apparent at some ages, but the treatment had no significant effects on mean or maximum life span. These results illustrate that the effects of particular regimens of dietary restriction on body weight and life span are greatly dependent upon the genotype and age of initiation. Moreover, when examining the relationship of body weight to life span both between and within the various groups, it was clear that the complexity of this relationship made it difficult to predict that lower body weight would induce life span increment.

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Marked differences in life span exist among strains of inbred house mouse (*Mus musculus*) as evidence of the genetic determination of aging within a species [10,19,29,33,37]. Goodrick [12] estimated genetic determination of life span to be 48—72% for the A/J and C57BL/6J strains. In spite of this evidence for genetic influence on longevity, it is thus clear that much of the variability in life span within an inbred mouse strain is due to environmental factors.

Nutritional factors appear as a major environmental source of life span determination in laboratory rodents. When compared to the survival of animals on conventional *ad libitum* diets (20—26% protein), weanling counterparts from the same inbred mouse strain fed restricted levels of the diet, either by total caloric reduction or by isocaloric protein reduction, experience increased mean and maximum life spans [4,9,11,13,16,23,36,42].

In addition to the actuarial evidence of the longevity-promoting effects of such nutritional manipulations, other studies have reported that potentially deleterious age-related changes in mice can be altered by dietary restriction (DR). For example, the incidence of tumors is reduced by DR [4,34,39,41,42]. Certain parameters of immune function are improved at older ages [9,11,24,40,42]. Age-related endocrinological and biochemical changes in rodents can also be altered by DR [5,8,23,30,32]. In addition, there is recent evidence that age-associated behavioral impairments, including learning and motor performance, can be reduced by particular regimens of DR in mice [16,18,21].

The apparent effects of DR on the rate of biological aging in laboratory rodents were first considered to result from retarded development [26,27]. Results from several studies refute this hypothesis since improved survival and function and decreased pathology have been demonstrated when DR is initiated in adult animals [2,3,28,31,41,44] even at ages clearly beyond developmental boundaries [15]. However, results of such interventions in juvenile or adult animals have not always been successful [1,16]. Reasons for these discrepant results could include: (a) differences in the DR regimen or the diet composition; (b) age thresholds beyond which DR may be ineffective; (c) differential effects of genotype (including gender) on DR; or (d) interactions among these factors.

We have observed in outbred Wistar rats that an intermittent schedule of feeding, every-other-day, which reduces body weight about 20—30%, produced marked increases in mean and maximum life span when introduced at weaning [14] or at adult ages [15]. In the present study, we examined the effects of every-other-day feeding begun at 1.5, 6 and 10 months of age in two inbred mouse strains, A/J and C57BL/6J, and their F_1 hybrid, B6AF_1/J. Thus, the objective of this parametric approach was to determine the interaction of genotype and age on the survival and growth effects of one regimen of DR in one species. Previous studies have noted that

genotype can differentially influence both mortality and functional measures of aging associated with DR [16,17].

MATERIALS AND METHODS

Subjects

Male mice from the A/J, C57BL/6J (C57) and B6AF$_1$/J (F$_1$) strains had been obtained at 5 weeks of age from the Animal Production Department of the Jackson Laboratory (Bar Harbor, ME). Upon arrival, the animals were doubly housed in standard metal mouse cages (Wahmann) located in a vivarium with ambient temperature controlled at 22 ± 1 °C and with a 12-h light: 12-h dark photocycle. Food (4.2 kcal/g; 24% protein; according to NIH-07 formula) was provided *ad libitum* and water was available *via* an automated and filtered system. Located under the metal grid floors of the cages were stainless steel pans containing wood shavings that were changed three times weekly. All cages and racks were changed and cleaned monthly, or more frequently if conditions warranted.

Procedure

From within each genotype, different groups (n = 30—40) were selected randomly to continue on *ad libitum* (AL) feeding or to begin a DR regimen at selected ages. The regimen involved restricting access to food for 24 h every other day (EOD). The food hoppers for EOD groups were removed from the cages in the morning around 0900 h EST and returned the following morning. EOD feeding was initiated at 6—10 weeks (1.5, 1—2 or 2 months), 25 weeks (6 months), or 40 weeks (10 months) of age in each genotype. For comparison to each of these experimental groups, control groups maintained on AL diets were available except for the F$_1$ group begun at 6 and 10 months.

Each animal was weighed weekly throughout life and maintained with the same cage-mate until death. Cages were inspected daily to remove any dead animals.

RESULTS

Life span

Examination of survival data clearly revealed that the effect of EOD feeding was dependent upon genotype and age of initiation. Survival distributions of each group are presented in Fig. 1. For the purposes of statistical analysis of survival in F$_1$ mice, the survival of only one AL-group was compared to that of EOD groups at all three ages. A non-parametric statistical analysis of the survival distributions was applied using the Lee-Desu test [22], and the results are presented in Fig. 1.

In addition, life span data for all mice in each group were submitted to analysis of variance (ANOVA) within each genotype at each age of initiation. The results of this analysis in terms of tests of simple main effects on mean survival distributions of life span [43] are presented in Table I.

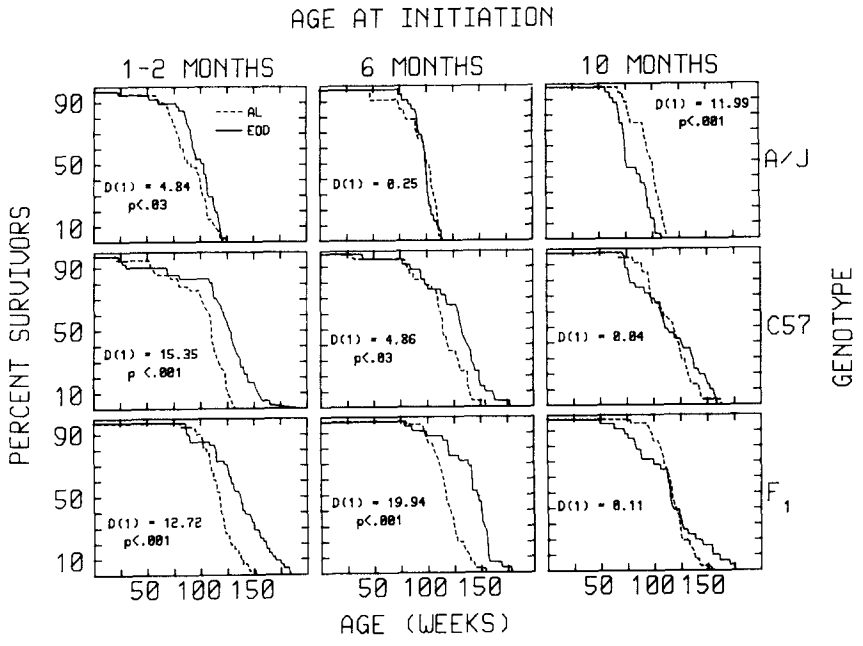

Fig. 1. Survival distributions for male A/J, C57BL/6J (C57) and B6AF$_1$/J (F$_1$) mice fed *ad libitum* (AL) or every other day (EOD) initiated at three ages (ns = 30—40). (*D* statistic represent results of Lee-Desu analysis [22]. Probability values are provided for significant comparisons).

Finally, an analysis of maximum life span was also conducted. Life span data for the longest-lived 20 percent within each group were submitted to ANOVA within each genotype. The results of this analysis in terms of tests of simple main effects on maximum life span are found in Table II.

Comparing the survival of AL to EOD groups within each genotype, the Lee-Desu analysis revealed that intermittent feeding significantly increased survival over

TABLE I

MEAN SURVIVAL (MONTHS) OF MICE FED *AD LIBITUM* (AL) OR EVERY OTHER DAY (EOD)

Starting age (months)	*A/J*				*C57BL/6J*				*B6AF$_1$/J*			
	AL	*n*	*EOD*	*n*	*AL*	*n*	*EOD*	*n*	*AL*	*n*	*EOD*	*n*
1.5	22.5	40	25.3*	40	25.0	40	31.7*	40	27.0	40	32.4*	40
6.0	22.2	30	22.7	30	26.4	30	29.2*	30	27.0	40	32.1*	30
10.0	22.2	30	19.1*	30	26.9	30	27.0	30	27.0	40	26.8	30

n = Sample size.
*Significantly different from AL group, $P < 0.05$.

TABLE II

MEAN LIFE SPAN (MONTHS) OF MAXIMUM LIVING 20 PERCENT OF MICE FED *AD LIBITUM* (AL) OR EVERY-OTHER-DAY (EOD)

Starting age (months)	*A/J*		*C57BL/6J*		*B6AF$_1$/J*	
	AL	*EOD*	*AL*	*EOD*	*AL*	*EOD*
1.5	28.1	29.0*	31.1	38.3*	34.3	38.2*
6.0	27.7	26.9	34.6	38.8*	34.3	39.4*
10.0	27.6	25.4*,**	35.2**	37.6*	34.3	39.0*

*Significantly different from AL counterparts, $P < 0.05$.
**Significantly different from 1.5-month-old counterparts, $P < 0.05$.

the AL condition when begun at 1.5 months of age in all three strains of mice. When initiated at 6 months of age, EOD feeding significantly increased survival in C57 and F_1 mice but not in A/J mice. When initiated at 10 months of age, the EOD diet had no significant effect on survival in C57 or F_1 mice; however, the treatment significantly decreased survival in A/J mice.

Analysis of mean life spans within groups confirmed these findings. As observed in Table I, the EOD regimen significantly increased mean life spans over the AL condition in all three mouse strains when introduced at 2 months of age. The increment in mean life span that EOD feeding induced was smallest for the A/J strain (2.8 months) and greatest for the C57 genotype (6.7 months). When initiated at 6 months of age, EOD feeding had no significant effect on mean life span among A/J mice, while mean life span was incremented by 2.8 and 5.1 months in the C57 and F_1 genotypes, respectively. When the EOD diet was begun at 10 months of age, there was no significant effect on mean life span among C57 and F_1 genotypes, while the treatment reduced mean life span among A/J mice by 3.1 months.

Based on inspection of data in Table I, there is clear reliability of the estimates of mean life span within the AL group of A/J mice with a range in estimates of 22.2—22.5 months. These comparisons are spurious for the F_1 group as only one control group was assessed. Nonetheless, these estimates replicate well previous estimates of mean life span of this genotype fed AL in this laboratory [12]. Estimates of mean life span for AL-fed C57 mice appear somewhat less reliable. Apparently, the 25-month estimate of mean life span for the 1.5-month cohort of C57 mice is an underestimate when compared to the other cohorts and to previous estimates of mean life span from this laboratory [12]. Thus, assuming a mean life span of closer to 26.5 months for AL mice of this genotype, the increment in mean life span induced by EOD feeding is probably closer to the 5 months effect observed in the hybrid strain. This increment appears to drop to about 2.8 months for C57 mice begun on the EOD diet at 6 months of age which would be lower than the 5.1 months increment observed for F_1 mice introduced to this treatment at the same age.

Although neither the Lee-Desu analysis of survival curves nor the ANOVA of life spans for all mice within each genotype revealed a significant effect of EOD feeding at 10 months of age for the C57 and F_1 genotypes, examination of the survival distributions (Fig. 1) indicates a possible effect on maximum life span. EOD feeding introduced at this age apparently had an early detrimental effect on survival, which persisted in the A/J strain. For the C57 and F_1 genotypes, however, the survival curves cross around the 70 percent survival point and diverge thereafter to favor survival of EOD mice over the longest-lived AL mice. Results of the analysis of life span of the longest-lived 20 percent from each group confirms this observation. As noted in Table II, maximum life span was affected in all three genotypes when the EOD regimen was initiated at 10 months. EOD feeding significantly reduced maximum life span in the A/J strain, while increasing it in the C57 and F_1 strains with the greatest effect in the latter genotype. These findings contrast with the analysis of mean life span discussed above. Consistent with the results of the analysis of mean life span were the observations of increased maximum life spans for the C57 and F_1 genotypes on EOD feeding since 1.5 or 6 months of age and for the A/J strain on the EOD diet since 1.5 months of age. Thus, according to this analysis, the only group in which maximum life span was not affected by intermittent feeding was for A/J mice begun at 6 months of age.

Mean body weight

The extent of body weight loss associated with EOD feeding was again dependent upon genotype and age of initiation. Mean body weight as a function of chronological age is presented for each genotype-age group in Fig. 2. These data are also presented on a life span relative basis by comparing body weights at individual survival quartiles in Table III. Statistical comparisons at each age were made using a 3 (genotype) by 2 (diet) factorial analysis of variance (ANOVA) of body weight at each survival quartile with $P < 0.01$ as the accepted level of significance because of the use of multiple tests [43]. When significant main effects or interactions occurred, further tests of main effects or simple main effects were conducted [43]. The results of these are indicated in Table III. Again only one control group was compared to the three experimental groups of F_1 mice. Finally, to clarify further the effect of EOD feeding on body weight, Fig. 3 presents mean body weights in the EOD groups expressed as a percent of mean body weight of their respective AL group.

For groups begun at 2 months, there were no significant differences in their starting body weights. At each survival quartile, however, the analysis revealed both genotype and diet effects. Among AL-fed groups, F_1 mice were always significantly heavier than C57 mice, which in turn were always significantly heavier than A/J mice. Among EOD-fed groups, F_1 mice were significantly heavier than C57 mice at every survival quartile; however, restricted C57 mice were significantly heavier than A/J mice only at time of death. Regarding the effects of diet, the EOD diet significantly reduced body weight in F_1 mice at every survival quartile except the last.

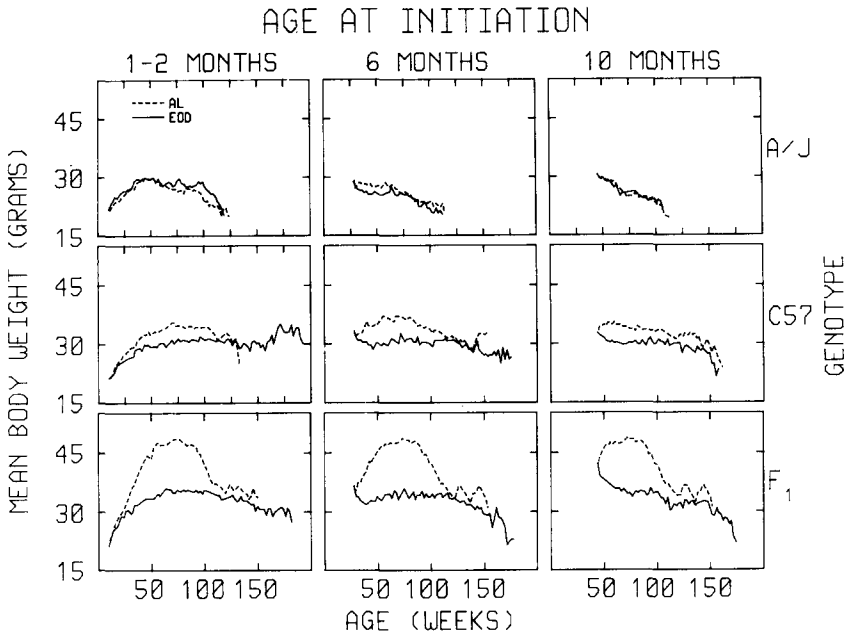

Fig. 2. Mean body weights for male A/J, C57BL/6J (C57) and B6AF$_1$/J (F$_1$) mice fed *ad libitum* (AL) or every other day (EOD) initiated at three ages (ns = 30—40 at the start of each experiment).

Among C57 mice, EOD feeding significantly reduced body weight only at the second and third quartiles. The EOD diet had no significant effects on body weight among A/J mice. Based on percent comparisons of body weight in Fig. 3, it is clear that the greatest effect of EOD feeding occurred among F$_1$ mice, with C57 mice having the intermediate effect and no body weight effect among A/J mice.

For groups begun at 6 months, significant strain differences in body weight existed at the outset of the experiment. Among both diet groups, F$_1$ mice weighed significantly more than C57 mice which in turn weighed significantly more than A/J mice at the outset and at every survival quartile thereafter with one exception. For EOD mice at death, the difference in body weight between F$_1$ and C57 mice was not significant. With respect to diet effects, EOD-fed mice of the C57 and F$_1$ genotypes weighed significantly less than their AL-fed counterparts at every survival quartile beyond baseline. Among A/J mice, EOD-fed mice weighed significantly less compared to AL controls only at the first and last survival quartile.

For groups begun at 10 months, the same general pattern of results prevailed. With respect to strain differences in body weight for both diet groups, F$_1$ mice weighed significantly more than C57 mice at nearly every survival quartile and C57 mice weighed significantly more than A/J mice at nearly survival quartile. Again the exception for F$_1$ mice occurred at the death for EOD-fed mice at which time they did

TABLE III

MEAN BODY WEIGHT (g) AS A FUNCTION OF AD LIBITUM (AL) OR EVERY-OTHER-DAY (EOD) DIET, AGE OF INITIATION AND SURVIVAL QUARTILE IN THREE MOUSE GENOTYPES (NS = 30—40)

Survival quartile	*Diet*	*Genotype*		
		A/J	*C57BL/6J*	*B6AF$_1$/J*
1.5 months				
0	AL	20.8	20.9	21.2
	EOD	21.8	21.3	21.4
1	AL	26.8	29.8†	38.6†
	EOD	28.1	28.4	32.4*†
2	AL	29.3	33.6†	47.1†
	EOD	29.1	29.9*	35.2*†
3	AL	27.2	33.6†	46.3†
	EOD	28.2	30.5*	34.8†
4	AL	21.4	27.8†	32.9†
	EOD	22.1	26.9†	30.5†
6.0 months				
0	AL	29.8	30.0†	35.6†
	EOD	28.8	33.4†	36.5†
1	AL	27.7	34.6†	44.8†
	EOD	25.5*	29.8†*	33.7†*
2	AL	27.2	36.5†	48.2†
	EOD	26.4	31.2†*	34.5†*
3	AL	25.3	33.4†	44.8†
	EOD	24.7	30.6†*	34.1†*
4	AL	21.7	30.5†	32.9†
	EOD	19.0*	26.2†*	27.2*
10.0 months				
0	AL	28.8	32.6†	42.7†
	EOD	30.4	32.7†	42.1†
1	AL	29.1	35.2†	47.1†
	EOD	28.9	29.8*	35.9†*
2	AL	26.1	34.4†	48.4†
	EOD	27.1	30.3†*	34.6†*
3	AL	25.1	33.0†	43.4†
	EOD	25.2	29.8†*	33.7†*
4	AL	20.7	29.9†	32.9†
	EOD	20.7	25.6†*	25.8*

†Significantly higher body weight compared to preceding strain, within same diet group, $P < 0.05$.
*Significantly lower body weight compared to AL-fed counterpart, $P < 0.05$.

not differ significantly in body weight from C57 mice. The exception for C57 mice occurred at the first survival quarter when EOD-fed mice of this strain did not differ significantly in body weight from A/J mice on the same diet. With respect to diet effects, again the EOD regimen was effective in significantly reducing body weight among C57 and F_1 genotypes at every survival quartile beyond baseline, but this regimen did not significantly affect body weight among A/J mice.

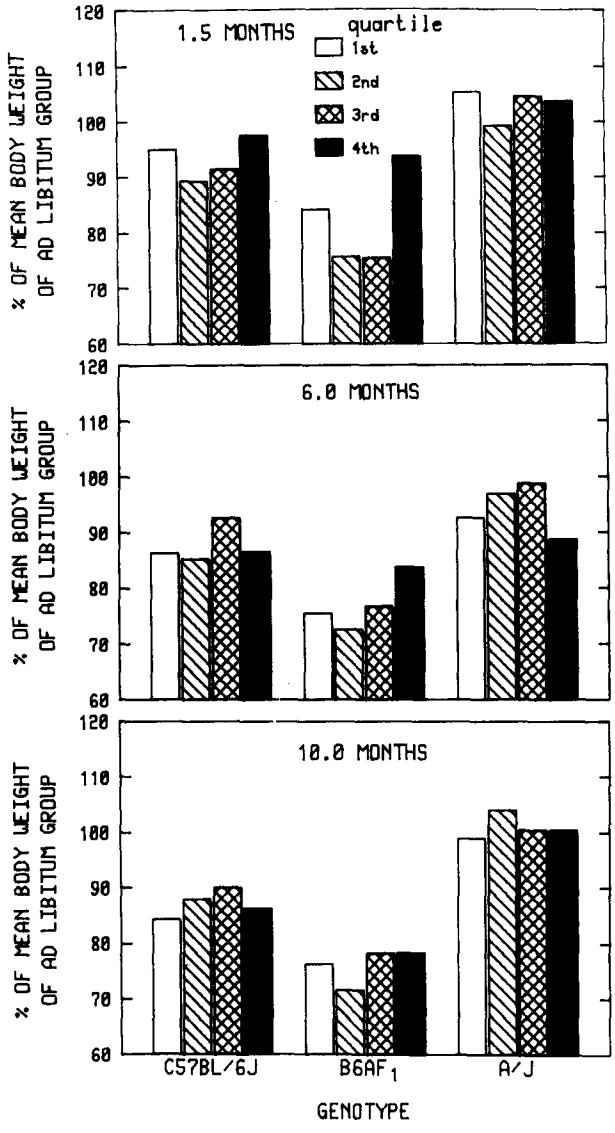

Fig. 3. Body weight of male mice from three genotypes fed every other day initiated at three ages expressed as a percent of mean body weight of comparable *ad libitum* fed groups at different survival quartiles (ns = 30—40).

Maximum body weight

Genotype-diet interactions on body weight could also be analyzed with respect to the maximum body weights attained in different groups. These comparisons are made in Fig. 4. At all three ages, the results of separate 3 (genotype) by 2 (diet) ANOVAs demonstrated significant main effects of genotype and diet, but also sig-

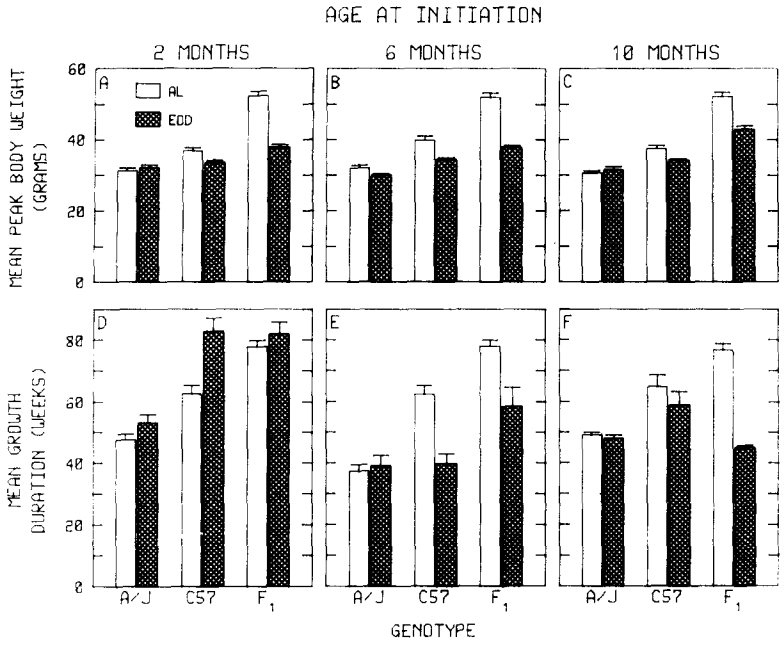

Fig. 4. Mean (S.E.M.) peak body weight and growth duration of male A/J, C57BL/6J (C57) and B6AF$_1$/J (F$_1$) mice fed *ad libitum* (AL) or every other day (EOD) initiated at three ages (ns = 30—40).

nificant genotype by diet interactions, 2 months: $F(2239) = 55.4$, $P < 0.001$; 6 months: $F(2179) = 23.2$, $P < 0.001$; 10 months: $F(2179) = 11.8$, $P < 0.001$. The relative pattern among genotypes was very consistent across ages of initiation. The F$_1$ mice attained the highest maximum body weights with C57 mice of intermediate weight and A/J mice of lowest weight. The effect of diet followed this rank ordering as well. EOD feeding had the greatest effect on maximum body weight for the F$_1$ mice starting at all ages; an intermediate effect on C57 mice; and essentially no effect on maximum body weight among A/J mice.

Growth duration

Genotype-diet interactions were also apparent with respect to the age at which maximum body weight was obtained (growth duration). Although no statistical analysis was conducted on these data, several trends are apparent in Fig. 4. When begun at 1.5 months of age, the EOD diet generally extended growth duration in all three genotypes but to the greatest extent among C57 mice. When the EOD regimen was initiated at 6 and 10 months of age, the growth duration of AL mice was generally longer for both C57 and F$_1$ mice genotypes. For A/J mice growth duration appeared unaffected when EOD feeding was initiated at these older ages.

Correlation between body weight and life span

The correlations between life span and body weight are presented in Fig. 5 for those mice monitored since 1.5 months of age. Estimates of body weight were computed for individual survival quartiles to provide relative comparisons of this relationship across different groups with different survival experiences.

Regarding the C57 genotype, the pattern of correlations between life span and body weight was very similar for the AL and EOD conditions. Significant positive correlations were observed within both dietary groups at the first, second and third survival quartiles. Among the A/J genotype, a similar pattern of positive correlations was observed within both dietary groups. Among AL-fed mice of this strain, the magnitude of this correlation diminished across survival quartiles to become negative in direction at death. Regarding the hybrid mice, the pattern of correlations exhibited little similarity to those observed for the parental strains. Positive correlations were observed at the first survival quartile, but the direction changed to clearly negative correlations by the third quartile.

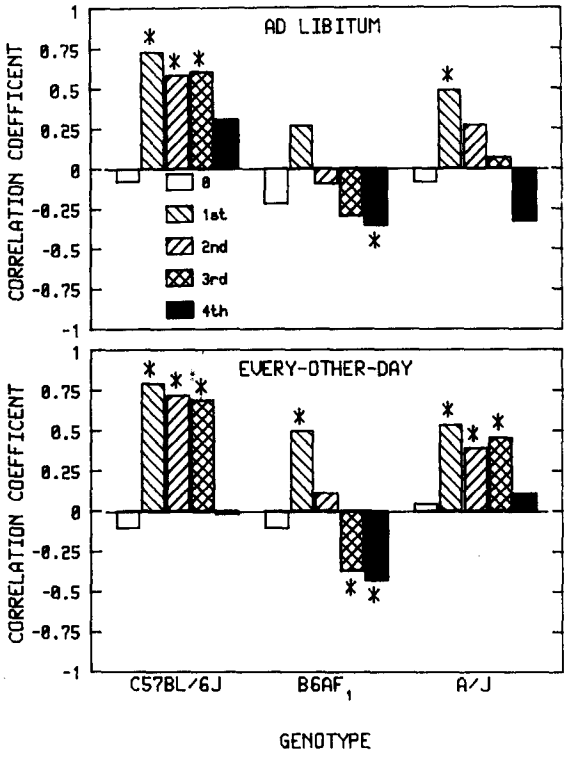

Fig. 5. Correlation (Pearson product-moment) between body weight and life span in male mice of three genotypes on two feeding regimens begun at 1.5 months (ns = 40). Each bar represents a different survival quartile. *$P < 0.05$.

Figure 6 provides the correlations between life span and body weight at individual survival quartiles for the three mouse strains observed from 6 months of age. Among C57 mice in both dietary groups, the pattern of correlations was again positive during the first, second and third quartiles as was observed in Fig. 5. The correlations were significant only among AL-fed mice of this strain. Among A/J mice, the pattern of positive correlations that had been observed in Fig. 5 was still evident in the EOD group, but among AL-fed mice the pattern had converted to a negative direction toward the later quartiles. For F_1 mice on both diets, no positive correlations were observed with significant negative correlations evident at the last two quartiles among AL-fed mice.

Figure 7 presents the pattern of correlations between life span and body weight at individual quartiles for mice followed since 10 months of age. For C57 mice, the pattern of positive correlations observed at earlier ages was maintained for the EOD group, but there were no significant correlations among AL-fed mice of this strain. Among A/J mice on the EOD diet, the pattern of positive correlation was also evident at early quartiles, while the pattern in the AL group remained negative at the

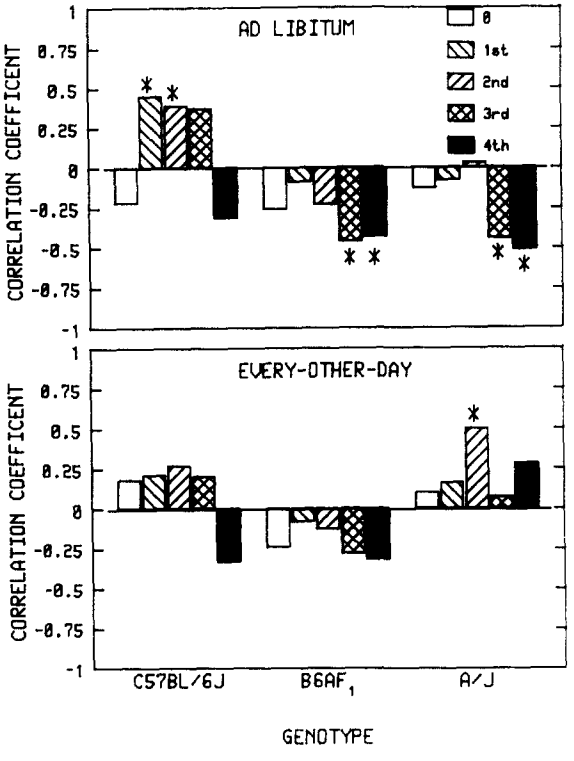

Fig. 6. Correlation (Pearson product-moment) between body weight and life span in male mice of three genotypes on two feeding regimens begun at 6 months (ns = 30). Each bar represents different survival quartile. $^*P < 0.05$.

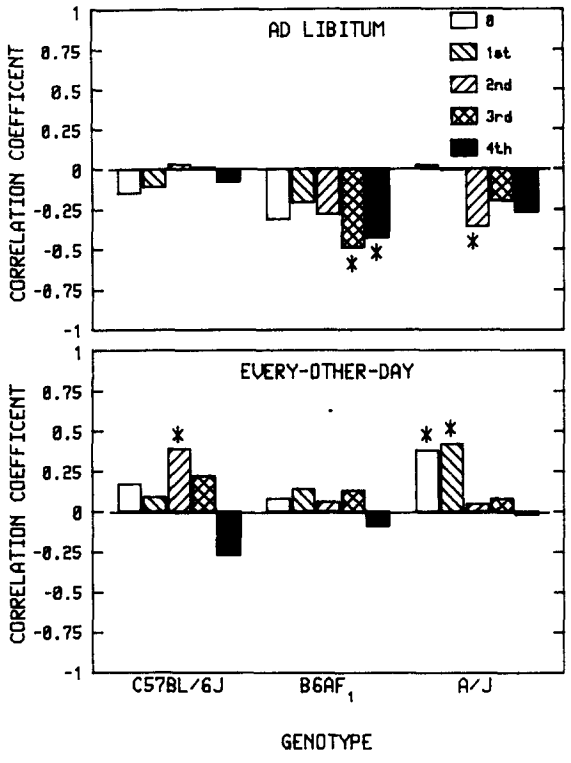

Fig. 7. Correlation (Pearson product-moment) between body weight and life span in male mice of three genotypes on two feeding regimens begun at 10 months (ns = 30). Each bar represents different survival quartile. *$P < 0.05$.

later quartiles. Among AL-fed hybrid mice, the pattern remained negative with the greatest magnitude at later quartiles. Among EOD-fed hybrid mice, little evidence of consistent, significant correlation was seen.

DISCUSSION

The objective of this study was to assess the interaction of feeding regimen, age and genotype upon measures of body weight and survival in inbred mice. The effect of increased survival associated with a regimen of intermittent feeding proved to be a robust phenomenon when initiated shortly after weaning. C57 and A/J genotypes and their F_1 hybrid exhibited increased mean and maximum life spans when fed EOD compared to AL-fed counterparts. These data support numerous other studies using other DR regimens in mice [4,9,11,13,23,36,42]. However, the magnitude of the life span effects observed with the current DR treatment varied across genotypes. C57BL/6J mice exhibited about a 25 percent increase in mean life span; whereas, the

effect in A/J mice was about half this magnitude. The effect on mean life span among the hybrid mice appeared intermediate to the two parental strains. When the mean life span of the C57 genotype was adjusted upward to conform to estimates from previous studies [12], the effect on mean life span of the hybrid strain was closer to the effect observed in this parental strain.

By manipulating the age at which EOD feeding was initiated, it was clear that age was also an important interactive variable affecting survival experience associated with this feeding regimen. These results were similar to those previously reported in parametric studies of rats and mice [2,3,15,28,31,38,41,44].

When EOD feeding was initiated at 6 months of age, the beneficial effect on mean life span declined in C57 mice to 10 percent relative to the effect observed when initiated at weaning. No demonstrable increase in mean life span was observed when EOD feeding was introduced at 10 months of age in these mice. Thus, among this strain a threshold for a prolongevity effect induced by this particular DR regimen appears to occur between 6 and 10 months of age. Weindruch and Walford [41] noted that increased longevity in C57 mice and a hybrid strain could occur as late as 12 months of age when a different DR regimen was used. We have observed beneficial effects of EOD feeding in rats when initiated as late as 12 months [3], and even 18 months of age [15]. It should be noted that maximum life span was incremented in the C57 strain when EOD feeding was initiated at 10 months of age. Thus, it appears that this regimen was too severe for some of the mice at this age, but those that adapted to it managed to survive to older ages compared to controls.

Among A/J mice in the present study, no significant effect on mean life span was observed when EOD feeding was begun at 6 months, and this treatment actually reduced mean life span by about 15 percent when initiated at 10 months. A detrimental effect of DR treatment (50 percent caloric restriction) on survival has been observed previously when begun in 19-month-old rats [1]. A detrimental effect on longevity has also been observed in male C57BL/6J mice begun on restricted feeding (60 percent that of controls) at 4 weeks of age [16]. This finding is in direct contrast to results in the current study when EOD feeding was initiated near weaning and also with that of others previously reported for older mice of this strain [41]. Maximum life span was not affected in the Harrison and Archer (1987) study. The shape of the mortality curve indicated that some early life stress, possibly a deficient nutrient in the diet, accounted for the reduced mean and median life spans of restricted C57 mice that they observed. Analyzing a number of functional measures of aging, these investigators noted that the values of DR mice in this strain were more like that of younger mice than were AL-fed mice of the same age. Thus, it would appear that the rate of aging was affected by the DR regimen that they used, but the mortality analysis did not reflect it.

The survival experience of the hybrid mice begun on EOD feeding at older ages in the present study paralleled that of the C57 parental strain. The effect on mean life span was significant when begun at 6 months (about 20% increase) and was not

significant when begun at 10 months. However, maximum life span in the hybrid mice was incremented with EOD feeding begun at 10 months of age. Comparing the beneficial effects on maximum life span observed in the C57 and F_1 genotypes when EOD feeding was initiated at 10 months to the detrimental effect observed in A/J mice begun at this age, there would appear to be an early negative factor associated with this DR regimen that continues to impact on all A/J mice but which does not affect all C57 and F_1 mice. Further investigation of possible mechanisms, perhaps the neuroendocrine response to this stress, that may vary across these strains would be an important research avenue to pursue.

These results demonstrate that genotype is a potent modulator of the life span effects associated with this form of DR. Moreover, because of the contrasting life span effects observed between the two parental strains and the close association of the life span effects observed between C57 and the F_1 hybrid, additional research might uncover important genetic mechanisms related to this apparent dominant mode of inheritance for these effects.

What is unknown from the current results are the relationships among food intake, body weight and life span. Data on food intake were not available for analysis. Thus, it could be that the variability among genotypes observed in the effects of EOD feeding on body weight could be due to differential food intakes. For example, the body weight data would suggest that A/J mice were very efficient in consuming food during the days that it was available. There was little evidence of a diet-induced reduction of body weight in this strain, yet the EOD regimen nonetheless impacted upon life span when initiated at 1.5 and 10 months of age in this strain. Harrison, Archer and Astle (1983) indicated that the level of food intake rather than the level of adiposity was the more critical factor for affecting survival in mice. On the other hand, when food intake is analyzed on a relative basis (per gram body weight), this physiological variable does not appear to be related strongly to the life span extension effects associated with DR in rats [44]. Even exposure to a DR regimen during certain critical periods is sufficient to increase life span even though food intake may be quite equivalent to control groups during other periods. For example, Yu *et al.* [44] fed rats on a restricted diet until 6 months of age and then fed them *ad libitum*. Thus, while food intake was restricted during early development, it was comparable to control groups during most of the life span, and yet this DR treatment significantly increased mean and maximum life span over control values. Beauchene *et al.* [3] reported similar results when DR was terminated or initiated in rats at 12 months of age. Thus, it might be suggested that the physiological impact of the regimen itself, possibly acting through neuroendocrine mechanisms [25], rather than the actual food intake is the more potent aspect of DR treatments in rodents.

While it is evident that an intermittent schedule of feeding could impact positively upon life span when begun near weaning in several inbred strains of mice, the relationship of life span effects to body weight loss was inconsistent. By examining body weight data for all ages, it was concluded that body weight loss was neither sufficient

nor necessary for effecting increased life span in these mice. The evidence against the sufficiency of body weight loss for increasing life span was observed in the 10-month groups. Body weight loss was evident among C57 and F_1 genotypes; yet, the effect on mean life span was not significant in these strains at this age. Moreover, while significant body weight loss was not evident among A/J mice begun on EOD feeding at this age, the effect on life span was deleterious in this strain. The evidence against the necessity of body weight loss for increasing life span was observed in the A/J mice begun on EOD feeding at weaning. Mean and maximum life span were increased in EOD-fed groups compared to AL controls, yet there were no significant differences in body weight between diet groups. In other comparisons there was little association between the degree of body weight loss and changes in mean life span. For example, the greatest body weight loss was exhibited by hybrid mice on EOD feeding since weaning (about 20%); however, the greatest effect on mean life span was observed among C57 mice that showed about a 10—15% body weight loss compared to AL controls across all ages. The same was true in degree of loss in maximum body weight. The greatest relative loss in maximum body weight was observed among the F_1 mice, but they did not necessarily exhibit the greatest effects on life span associated with this DR regimen.

The complexity of the relationship between body weight and life span was further revealed in the examination of the intragroup correlations between these variables. To support a hypothesis that reduced body weight should effect increased life span, one might expect to observe negative correlations between BW and LS within groups of mice on the same diet. Only among hybrid mice were the data generally consistent with this hypothesis. Among the parental strains on both AL and EOD diets, the correlations between BW and LS were generally positive in direction but modest in magnitude. These findings agree in part with those previously reported for a strain of hybrid mice on a different DR regimen [42].

These seemingly disparate relationships between body weight and life span across genotypes have been addressed more fully in a review of data from our laboratory and others [20]. The theoretical model presented postulated a curvilinear relationship between body weight and life span as an extension of that suggested by Economos and Lints [7] and Sacher and Duffy [33]. For rodent genotypes prone to leanness, generally body weight should be positively correlated to life span. In contrast, for genotypes prone to obesity, body weight should be negatively correlated to life span. In addition, the model accommodates genotypes in which little or no relationship between body weight and life span would be observed within the presumed normal weight range for the species.

Thus, this model would predict the observed negative correlations between body weight and life span among the heavier hybrid mice in this study. The F_1 mice were approximately 20 percent heavier than their parental strains. A survey of several comparative studies of males from various inbred mouse genotypes indicated that relative body weights of A/J and C57BL/6J strains were intermediate to low com-

pared to other strains. In the current study, C57 mice were heavier than A/J mice. Thus, these two parental strains would be considered normal to lean in relative adult body weight [19,33,37]. The observed relationships between body weight and life span are therefore consistent with predictions based on the proposed curvilinear model.

This curvilinear model would appear to be consistent with data from other species as well. For example, Soliman and Lints [35] reported that the magnitude and direction of relationship between adult body weight and life span were greatly dependent upon strain among flour beetles (*Oribolium castaneum*). Among heavier strains, the correlations were significantly negative; among lighter strains, the correlations were significantly positive. Among more normal weight species, no significant correlations were observed. This curvilinear relationship between body weight and life span was again observed in *Drospholia* [6,7]. Many epidemiological studies of human populations also demonstrate a curvilinear relationship between body weight and mortality. Extreme body weights, either obesity or leanness, are associated with higher mortality risk.

In summary, it is clear that DR can alter aging rate as measured by mortality and life span in laboratory rodents. However, the magnitude of the effects observed can be modulated by genotype and age of initiation. Moreover, although body weight loss is usually associated with DR regimens, the relationship between body weight and longevity in both *ad libitum* and intermittently-fed mice is complex such that the fact of having reduced body weight is not sufficient information for predicting effects on longevity.

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