Genetic Differences in Effects of Food Restriction on Aging in Mice[1]

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ABSTRACT Lifelong food restriction to two-thirds of normal ad libitum consumption extended mean and maximum life spans more than 200 d in male B6CBAF1 hybrid mice, already a long-lived genotype. The following biological systems were improved by food restriction, with values for older mice being similar to those previously found for younger individuals: tight wire clinging, a measure of neuromuscular performance; open field movement, a measure of voluntary activity; tail tendon denaturation rate, a measure of collagen solubility; urine concentrating ability, a measure of renal function, and hair regrowth rate, a measure of the frequency of hair follicle cycling. However, wound healing was slower in food-restricted mice than in ad libitum-fed controls. The same food restriction treatment had entirely different effects on longevities of a different genotype, male B6 (C57BL/6J) mice, reducing mean and maximum life spans 265 and 27 d, respectively. This surprising deleterious effect was not predicted by tests of tight wire clinging, open field movement and tail tendon denaturation, but was predicted by hair regrowth rates, as these were lower in restricted B6 mice than in fed controls. In genetically obese (*ob/ob*) B6 mice, food restriction extended mean and maximum longevities 327 and 440 d, yet no biological systems tested performed better than those of food-restricted normal (+/+) mice whose life spans were reduced. Thus the food restriction regimen that increased longevities for individuals of two genotypes decreased them for individuals of a third genotype tested in the same set of experiments. *J. Nutr.* 117: 376–382, 1987.

INDEXING KEY WORDS:

• *food restriction* • *aging* • *genetics* • *longevity* • *physiological age*

McKay et al. (1) first reported that food restriction extends longevities of short-lived rodents if begun early in life, and this has been repeatedly confirmed (2, 3). Recent studies of a variety of biological systems have found beneficial effects of food restriction, even beginning in midadulthood (4, 5). Other studies simultaneously compared a variety of dietary regimens to optimize effects on longevity (6). Since food restriction affects longevity beneficially in many different rodent genotypes and appears to retard aging in many different biological systems, this treatment may affect a central clock or mechanism that times aging processes. However, the data we present here suggest that not all genotypes may be benefited by the same antiaging treatment.

There is no agreement on how the beneficial effects of food restriction are achieved. The classical mechanism by which food restriction is thought to extend longevity is by reducing metabolic rate. This view is supported by studies of metabolic rates in which Sprague-Dawley rats adapted to a restricted diet by reduced oxygen consumption during 28–31 d (7) and the degree of reduction in energy use of Wistar rats underfed in variable degrees was proportional to the reduction in body weight during 18 d (8). Contradictory results were found in studies over longer time periods, in which restricted F344 rats consumed more calories of food lifelong than did fed controls (9) and used the same amount of oxygen per day per gram of lean body mass after 45 mo of food restriction (10). In yet another set of results, food-restricted C3B10RF1 mice consumed fewer calories of food lifelong per mouse than did ad libitum-fed controls, but more calories if data were expressed per gram of body weight and similar numbers of calories if expressed per organ mass (6). Some of these apparent contradictions may have resulted in part from the use of different genotypes of animal. Variation in physiological response due to genotype is common and illustrates the importance of defining genetic aspects of food restriction.

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In previous experiments, we separated effects of food intake from those of adiposity by using genetically obese (ob/ob) B6 female mice. Their longevities were extended by food restriction to match those of restricted +/+ mice, although the obese mice had much higher levels of adiposity, with fat composing about half of their body weights (11). This suggests that beneficial effects of food restriction on longevity do not necessarily depend on low levels of adiposity. In the same study, alterations in physiological changes with age due to food restriction were not consistently correlated with effects on longevities.

Our objectives in the current study were to better define effects of genotype and to compare effects of food restriction in a wide variety of biological systems in the same individuals whose longevities were determined. This required using physiological tests that would not harm a mouse, in order to identify the tests that most consistently predicted effects on lonevity and to thus identify the biological systems that should be used for detailed future studies. We compared male B6CBAF1 mice with their B6-+/+ parent strain, because these are both long-lived genotypes, and the F1 hybrid is heterozygous for genes at all loci that differ between the B6 and CBA inbred strains. We also studied *ob/ob* (obese) mice because they differ from B6 only in the *ob* gene, but this makes them extremely fat and much shorter lived if fed ad libitum. Thus effects of genetic background and of a specific mutant gene on responses to food restriction were determined simultaneously in groups providing a wide range of longevities and physiological values.

MATERIALS AND METHODS

Experimental animals. All mice were produced and raised at The Jackson Laboratory, which is fully accredited by the American Association for Accreditation of Laboratory Animal Care. C57BL/6J (B6) females and CBA/CaJ or CBA/CaHT6J (CBA) males were bred in our experimental animal colony to produce B6CBAF1 hybrids. B6 *ob/ob* and +/+ males were produced in Jackson Laboratory Animal Resources colonies and moved to our colony at weaning (4 wk of age). At this age mice of each genotype were divided into two groups: *1*) fed mice allowed unlimited access to food (ad libitum feeding) and *2*) restricted mice given their rations each day in a single feeding between 1200 and 1400, 6 d/wk, with double rations on d 6 and none on d 7. These feeding regimens were continued throughout life. Restricted mice were given two-thirds of the amount of food consumed by ad libitum-fed mice, which was less than half of that consumed by obese mice fed ad libitum. Groups of three or four mice were caged in each side of double-sided boxes. Restricted mice were given one or more food pellets each when fed. There were no unusual losses from fighting among these restricted males, and the variations in body weights of cagemates were similar in fed and restricted groups, suggesting that the dominant mouse did not consume an unusually large share of food in the restricted cages.

All mice were barrier maintained in an isolated, environmentally controlled room with filtered air under positive pressure, temperature at 22°C, and lighting from 0700 a.m. to 1900. Mice were fed a pelleted, pasteurized diet (96WA, Emory Morse, Guilford, CT) containing 22% protein, 7% fat, 50% nitrogen-free extract (mostly carbohydrates), and 357 kcal/100 g. Details of the diet and animal husbandry have been published (12). Mice were not exposed to known pathogens, and the colony was clean when tested for 10 standard mouse viruses by Microbiological Associates. A description of the health monitoring procedures used in this colony has been published (13). Only mice appearing healthy to an experienced observer were used for tests of physiological systems. Health assessment was based on normal activity and responses to handling, normal external appearance including eyes and pelt, absence of palpable lumps, and absence of recent weight loss. Even more important, the tests did not harm the subjects, so subsequent longevities were determined after testing.

Measurements of biological systems. Food is the amount eaten recorded as grams per day per mouse and measured over the 2-wk period when the physiological systems were being tested. Weight is body weight in grams measured when the other tests were performed. Tail length is measured in centimeters from the root of the tail where it joins the rectum to the tip of the tail. Tight wire is the time in seconds that a mouse can hold itself on a wire suspended above foam padding. Timing is begun after the mouse grips the wire with both front paws and one hind paw. There is no exit from the wire, but mice are removed and testing ends if they cling for 240 s. Otherwise the maximum score of five trials is recorded (14). Correlations between tight wire clinging times and body weights among mice of the same age and genotype showed that only a small portion of the variance could be explained by increased weights causing decreased clinging times. For example, in B6 males at 500–614 d of age, correlation coefficients between clinging times and body weights for ad libitum-fed and food-restricted +/+ mice and for food restriction *ob/ob* mice were −0.266, −0.159 and −0.409, explaining 7, 3 and 17%, respectively, of the variances in clinging times.

Open field is a measure (15) of the activity displayed during 5 min in a 80-cm-square, well-lighted, open-topped box, measured as the number of 15-cm-sided squares the mouse crosses. Tail collagen is the number of minutes required for a tendon fiber from the middle of the tail to be denatured in 7 M urea at 45°C so that it cannot support a 2-g weight (16). Urine conc. is the osmolality (mosol/kg) of urine from a mouse after 48 h without water. Urine samples are held in capillary tubes to prevent evaporation, food remains present and

mice receive 1.0 ml water intraperitoneally at time zero (13).

Hair growth is the fraction of a 2-cm-square shaved area centered on the back near the tail into which hair has begun to regrow after 25 d. The subjective element in estimating the area is minimized by defining regrowth as the first appearance of hair and dividing the shaved area to be scored into eight equal portions with a transparent screen. Excellent repeatability was found when 22 indistinguishable mice were randomly recaged and rested an hour after the initial trial; 20 were scored exactly the same way both times, and scores of the other 2 varied by only 1/8.

Wound heal is the number of days required until the wound in the tail made in removing a portion of one of the dorsal tail tendon bundles for the tail collagen test feels smooth to the touch when running the index finger along the tail. Tails were checked 7 d after the wounds were made and every 2–3 d thereafter. Since this test is subjective, all animals in an experiment were scored by the same technician, who had no idea of expected results. In a blind test of repeatability, the correlation coefficient between successive runs was 0.88 with 16 mice. In the same experiment, one that was designed to evaluate the technician, wounds were made at different times over 3 wk, but presented as if all had been made simultaneously. Expected healing times were 27.2, 34.5, 41.2 and 34.8 d for four groups of mice; reported times were 24.5, 36.5, 40.0 and 35.6 d, respectively, demonstrating good objectivity.

When changes with age in these biological systems were tested in previous experiments, all changed significantly in B6 and B6CBAF1 males except open field. This test was retained because it and hemoglobin concentrations were the only measures correlating with life expectancy after 22 mo of age in B6 males; weight, wound heal and hematocrit were the only measures that correlated with subsequent longevities in F1 hybrids (17, 18). Hematocrit and hemoglobin levels and sleep time after Avertin anesthesia did not differ significantly in any groups of the same age that were compared in this study, so these data are not shown. Statistical significances were tested by the Student-Newman-Keuls multiple-range test, and correlation coefficients were calculated for linear correlations.

RESULTS

B6CBAF1 males. Food restriction extended longevities of this already long-lived F1 hybrid, increasing the mean by 211 d. The maximum longevity of 1742 d in this group of 34 mice may have set a new record for the genus *Mus* (**Table 1**). **Figure 1** compares the longevities of restricted and ad libitum-fed mice as plots of the percentage alive versus age in days. Death rates increase with age in parallel, but the increase starts later in the food-restricted group. Thus the median, mean and maximum longevities for restricted mice are about 200 d longer than those for fed groups, except for one especially long-lived food-restricted individual (Table 1).

TABLE 1

Summarized longevity data

Genotype	Treatment	Median	Mean	SE	*N*	Maximum 10%
B6CBAF1	Restricted	1191	1185	36	34	1432–1742[1]
B6CBAF1	Fed	985	951	35	35	1188–1296
B6-+/+	Restricted	591	593	40	48	967–1145
B6-+/+	Fed	878	858	19	45	994–1172
B6-*ob/ob*	Restricted	821	817	36	48	1102–1210
B6-*ob/ob*	Fed	515	490	21	53	700–770

[1]The longest-lived mouse was fed ad libitum from 1541 to 1742 d of age.

Effects of food restriction on changes with age in a variety of biological systems are shown in **Table 2**. Most deleterious changes with age ocurred more slowly in the food-restricted group, so values in restricted mice differed from values in ad libitum-fed controls in the direction previously shown by younger mice for the following systems: tight wire clinging time, open field movement, tail tendon denaturation rate, urine concentrating ability and hair regrowth. However, one deleterious change with age occurred more rapidly in restricted mice; wound healing rates were slower, differing from control rates in the direction previously shown by older mice. These differences were apparent after 10 mo of food restriction and continued to be clearly seen 15 mo later, as shown by values for adult and aged mice in Table 2.

B6-+/+ males showed a dramatic genetic difference in effects on longevity from the same food restriction treatment; it reduced their mean longevities from 858 to 593 d, although maximum longevities were little affected (Table 1). Nevertheless, three of the biological systems that showed values more similar to those for young mice in F1 hybrids as a result of food restriction showed the same effects in B6 mice. These were tight wire clinging, open field movement and tail tendon denaturation rate. Hair regrowth and wound healing rates were altered in the opposite direction, with restricted mice giving values more like those previously found in older mice. These effects were apparent after 10 mo of food restriction (**Table 3**) but became greater 10 mo later (**Table 4**).

In B6-*ob/ob* males, the same food restriction regimen increased mean and maximum longevities more than 300 d (Table 1). Despite this beneficial effect, Table 3 and Table 4 show poor tight wire and open field performances in restricted *ob/ob* males, with shorter clinging times and less activity than in restricted +/+ males, although much above the performances of fed *ob/ob* controls. Hair regrowth and wound healing were also

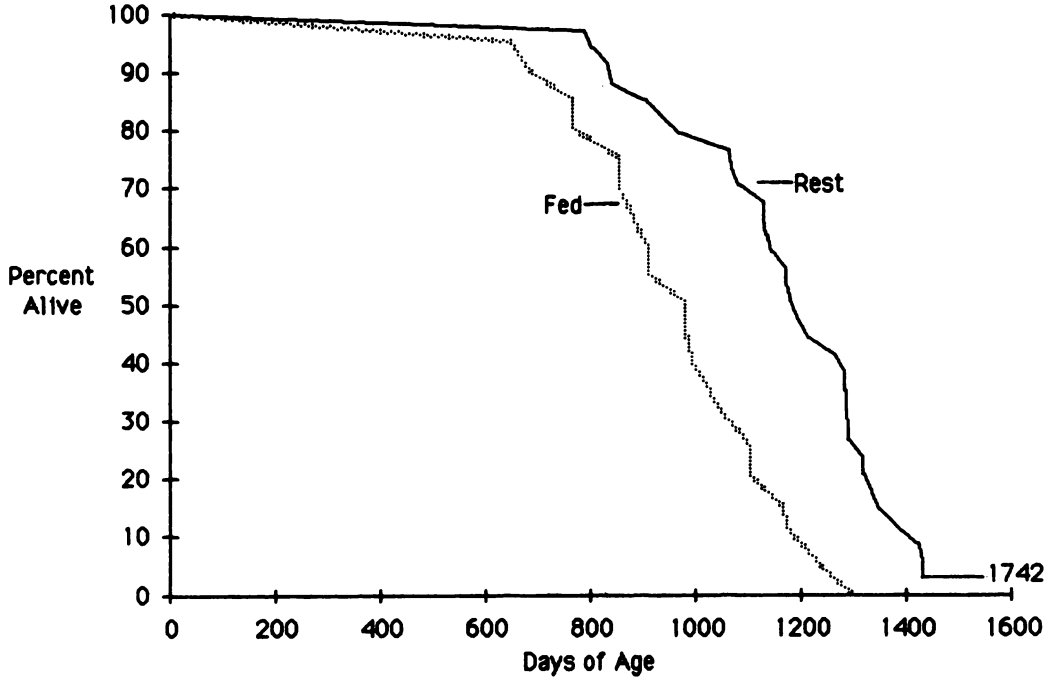

FIGURE 1 Longevity plots as percentage remaining alive versus age in days for B6CBAF1 males. Data for food-restriced mice are plotted as the solid line and data for ad libitum-fed mice as the dotted line.

significantly slower in restricted *ob/ob* than in +/+ mice. Hair regrowth was equally slow in fed *ob/ob* mice, but wound healing rates in these extremely fat animals were higher than when they were food-restricted. Only aging rates of tail tendon collagen fibers appeared to be retarded to the same degree in both the B6- +/+ and -*ob/ob* food-restricted groups (Tables 3 and 4). Urine concentrating abilities did not differ among any of the B6 mouse groups of the same age, so these data are not shown.

Longevity curves of the four B6 mouse groups are shown in **Figure 2**. Death rates increased more slowly with age in the food-restricted groups than in the groups fed ad libitum. This caused maximum longevities of restricted +/+ mice to be almost as long as those of fed +/+ mice, although the former began dying at a much younger age. The decline in mean and median longevities as a result of food restriction in +/+ mice was over 260 d, while the decline in maximum longevity was only 27 d (Table 1). Food-restricted obese mice began dying much later than fed obese mice, giving increases in median and mean longevities of about 300 d; increases in maximum longevities were even greater, over 400 d, as expected if death rates increased

TABLE 2

***Effects of food restriction on B6CBAF1 males*[1–3]**

	Food-restricted		Ad libitum-fed	
Measure[4]	Adult	Aged	Adult	Aged
Food	2.0	2.1	3.3	3.1
Weight	24.9 ± 0.3[a]	24.8 ± 0.5[a]	39.1 ± 0.7	40.0 ± 0.9
Tail length	10.4 ± 0.04[a]	10.9 ± 0.03	10.8 ± 0.04	11.0 ± 0.04
Tight wire	233 ± 4[a]	199 ± 10[a]	178 ± 11	77 ± 10
Open field	105 ± 7[a]	87 ± 4[a]	49 ± 4	58 ± 4
Tail collagen	33.5 ± 1.4[a]	95.9 ± 7.4[a]	40.6 ± 1.2	205 ± 17
Urine conc.	3929 ± 40[a]	3453 ± 98[a]	3156 ± 68	2831 ± 84
Hair growth	69.4 ± 6.3	67.2 ± 6.8[a]	53.3 ± 5.2	36.3 ± 6.9
Wound heal.	19.4 ± 0.7	32.6 ± 3.6	16.2 ± 0.5[a]	20.0 ± 1.3[a]

[1]Values are means ± SEM, with 30–36 mice per group; measures are defined in the Materials and Methods section.

[2]Adults were 294–389 d old and aged mice 775–804 d old when tested, with ages matched in the two dietary groups.

[3]Superscript a denotes values significantly different from those for others of the same age on the same line, with $P < 0.05$ by the Student-Newman-Keuls multiple range test.

[4]Units are: food, g/d; weight, g; tail length, cm; tight wire, clinging time, s; open field, number of squares crossed; tail collagen, denaturation time, min; urine concentration, mosmol/kg; hair growth, % of shaved area regrowth in 25 d; wound healing, number of days until healed.

TABLE 3

Effects of food restriction on middle-aged adult B6 males[1-3]

Measure[4]	Restricted		Ad libitum-fed	
	+/+	*ob/ob*	+/+	*ob/ob*
Food	1.8	1.8	3.0	4.5
Weight	22.5 ± 0.8[b]	28.3 ± 0.8[a]	33.3 ± 0.4[a]	81.7 ± 1.5
Tail length	9.7 ± 0.06[a]	9.3 ± 0.06[a]	10.0 ± 0.04	10.1 ± 0.06
Tight wire	149 ± 11.8[b]	45 ± 7.0	77 ± 8.4[a]	–[5]
Open field	116 ± 6.2[b]	76 ± 4.0[a]	109 ± 5.2[b]	4 ± 0.2
Tail collagen	25.6 ± 1.3[a]	27.8 ± 1.3[a]	27.8 ± 1.3[a]	46.5 ± 2.9
Hair growth	26.4 ± 3.8[a]	11.9 ± 2.7	68.9 ± 3.7[b]	10.2 ± 2.4
Wound heal.	31.6 ± 2.8[a]	38.8 ± 1.5	20.1 ± 0.7[b]	30.5 ± 2.4[a]

[1-4]Same as Table 2, except 44–53 mice per group; all mice were 260–373 d old when tested, with ages matched in the two dietary and two genetic groups; urine conc. data were the same and so are not included; [a] denotes values significantly different from unmarked values on the same line; [b] denotes a significant difference from those marked with superscript a, with $P < 0.05$ by the Student-Newman-Keuls multiple-range test.

[5]Fed *ob/ob* mice failed to cling to the tight wire long enough to be tested.

with age more slowly in the restricted *ob/ob* group (Table 1).

DISCUSSION

Why did the same lifelong food restriction treatment that significantly extended longevities of B6CBAF1 and B6-*ob/ob* male mice reduce mean longevities of the B6-+/+ strain? An obvious possibility is that an essential nutrient was not present in adequate quantities when B6-+/+ males were restricted to two-thirds of their normal food intake. However, such a deficiency would be a strikingly specific genetic effect because F1 hybrids are not sensitive to this deficiency, the effect is eliminated by the obese mutation and the effect depends on gender since the food restriction regimen used in this study increases longevity in B6-+/+ females (11). In designing food restriction studies, it is impossible to avoid the possibility of malnutrition in food-restricted animals without either overnutrition in ad libitium-fed controls or alterations of nutrient proportions. These proportions were maintained in our study; however, future caloric restriction studies are needed in B6 males in which all essential nutrients are fed at a constant level to determine whether this benefits longevities.

Taken alone, the experiments summarized in Table 1 and Figure 1 would have led to false conclusions. Longevities of the B6CBAF 1 males were extended by food restriction, and values were more like those of young animals in five physiological systems. If this had been the only group studied, it would have seemed reasonable to suggest that these effects were related. However, food restriction reduced longevities in B6-+/+ males while maintaining three of the same five systems at levels characteristic of younger mice (Table 4). Thus effects of food restriction in these three systems are not consistently correlated with effects on longevity.

Studies of food restriction in B6-*ob/ob* males emphasized the absence of consistent correlations between effects on longevities and on changes with age in different physiological systems. These data are given in Table 3 for middle-aged adults and in Table 4 for aged males. No measures correlated with effects on longevities by giving better results in restricted *ob/ob* mice than in restricted +/+ mice, but in comparisons limited to obese mutants, all measures except wound healing correlated with longevity by giving better results in restricted *ob/ob* mice than in fed *ob/ob* mice. Comparing restricted *ob/ob* and fed +/+ mice was complex. Longevities were similar, as were tight wire clinging and open field movement in aged mice. Hair regrowth and wound healing rates were better in fed +/+ mice at both ages, as were tight wire clinging and open field movement in middle-aged adults. Tail tendon denaturation was better in restricted *ob/ob* aged mice.

Taken as a whole, these data suggest that genetic effects may be important in responses to antiaging

TABLE 4

Effects of food restriction on aged B6 males[1-3]

Measure[4]	Restricted		Ad libitum-fed	
	+/+	*ob/ob*	+/+	*ob/ob*
Food	2.1	2.1	2.8	–[5]
Weight	24.0 ± 0.9*	30.2 ± 1.2	35.6 ± 0.6	–
Tail length	10.1 ± 0.04	9.7 ± 0.06*	10.1 ± 0.06	–
Tight wire	60 ± 12.9*	22 ± 4.5	35 ± 6.2	–
Open field	111 ± 10.3*	80 ± 5.8	83 ± 6.1	–
Tail collagen	46.8 ± 4.3*	46.5 ± 2.9*	60.3 ± 2.5	–
Hair growth	18.3 ± 5.8	5.0 ± 2.3	40.0 ± 6.6*	–
Wound heal.	42.7 ± 3.8*	49.4 ± 1.6	20.9 ± 0.9**	

[1-4]Same as Table 3, except 17–24 mice per group; aged mice were 570–679 d old when tested.

[5]Numbers of healthy fed *ob/ob* mice were too small for these analyses.

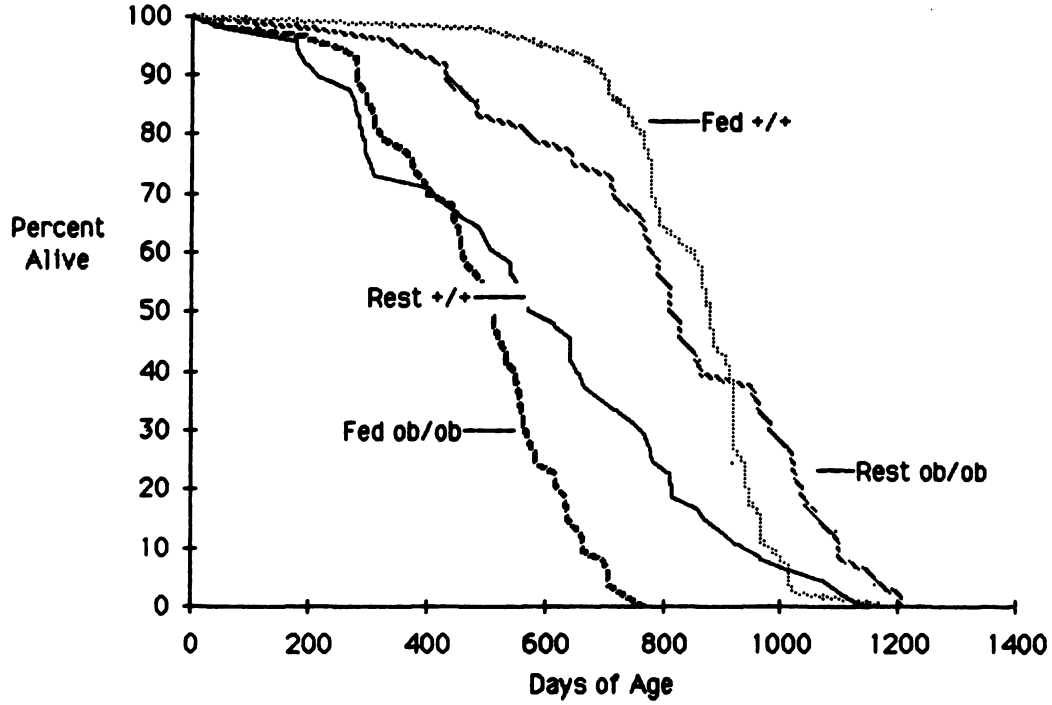

FIGURE 2 Longevity plots as percentage remaining alive versus age in days for C57BL/6J males. Data for food-restricted +/+ mice are plotted as the solid line, data for ad libitum-fed +/+ mice as the dotted line, data for food-restricted *ob/ob* mice as the dashed-dotted line and data for ad libitum-fed *ob/ob* mice as the dashed line.

treatments, even the well-defined treatment of food restriction. A unique aspect of our experimental design was the comparison of three different genotypes in the same set of experiments so that genetic effects could be studied directly. A wider variety of genotypes should be studied in the future, to determine whether different genotypes often show widely disparate responses to antiaging treatments such as food restriction.

It is also possible that each genotype and biological change with age has individual characteristics that must be considered before drawing conclusions about relationships with longevities. For example, *ob/ob* mice have high percentages of fat, even when food-restricted (11), which interfere with performance in the tight wire and open field tests. Collagen aged more slowly in all food-restricted groups, but aging of collagen may be caused by a nonenzymatic reaction with glucose (19). Therefore collagen aging may be proportional to long-term blood glucose levels, which are affected by food restriction independently of other mechanisms of aging. Wound healing was consistently faster in fed mice than in restricted mice of the same age and genotype, perhaps because healing requires protein and that is made less available by food restriction. Healing was slower in *ob/ob* than in +/+ groups, perhaps because the *ob/ob* genotype causes maximum amounts of fat to be deposited at the expense of protein.

Hair regrowth rats were lowered by food restriction in B6-+/+ mice and enhanced by food restriction in B6CBAF1 mice. This was the only physiological test affected in the same way by food restriction as were longevities in mice of these two genotypes. This suggests that neuroendocrine mechanisms may be important in extending longevities by food restriction, since hypophysectomy of adult B6 males improved hair regrowth to young levels (20).

The absence of consistent relationships between the other physiological tests and longevities suggests that changes with age in the biological systems tested were not closely controlled by the same mechanism that controls longevities in the different genotypes. It is possible that other tests would have shown consistent relationships in all genotypes; however, this has never been demonstrated. Our findings suggest that longevity is controlled by different mechanisms in different genotypes so that a unique set of tests would predict longevity for each genotype.

Although there were not enough data for detailed analyses (21), the longevity curves for restricted and fed B6CBAF1 mice (Fig. 1) suggest that food restriction may reduce initial vulnerabilities without affecting aging rates, as the death rates appear to increase with age in parallel. Longevity curves of the four B6 mouse groups (Fig. 2) suggest that food restriction may retard aging rates, because death rates appear to increase more slowly with age in restricted than in fed groups. Food restriction appeared to increase initial vulnerabilities in B6-+/+ mice, since their mean longevities were significantly reduced despite a slower increase in death rate with age, whereas it greatly decreased initial vulnerabilities in B6-*ob/ob* mice.

This study demonstrates that genetic differences such as those between an inbred strain and its F1 hybrid, or those resulting from a single-gene mutation, may de-

termine the outcome of a lifelong food restriction treatment. Clearly the use of different genotypes may cause researchers to obtain conflicting results, and an anti-aging treatment that benefits normal individuals of several different genotypes may not be beneficial to all. Our data suggest, but do not prove, that the beneficial effects on aging processes from food restriction may be caused by a variety of mechanisms in different genotypes. To verify or disprove this suggestion requires more data on these and other genotypes, other food restriction regimens and tests of other biological systems. It is supported by our findings that changes with age in various biological systems may be independently timed and that relationships to longevities are not the same in different genotypes (11, 22).

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