STUDIES OF AGING IN AMES DWARF MICE: EFFECTS OF CALORIC RESTRICTION

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ABSTRACT

Ames dwarf mice, which are small and deficient in growth hormone (GH), prolactin (PRL), and thyroid stimulating hormone (TSH) live much longer (1-1.25 years) than their normal siblings. It was of interest to examine the response of these animals to caloric restriction (CR) because of the possibility that dwarf mice are voluntarily caloric restricted. We are testing the hypothesis that this possible natural caloric restriction will negate any benefits of an imposed CR on lifespan.

Male and female Ames dwarf mice and their normal counterparts have been fed ad libitum (AL) or a 30% CR diet for 25-29 months. Animals were monitored daily and weighed weekly. At 12-15 months of age, CR mice weighed significantly less than their AL fed counterparts (normal females: -42%, normal males: -23%, dwarf females: -18.8%, and dwarf males: -22.2%). Only in dwarf females has this significant difference disappeared with age. At one year of age, a comparison of daily food consumption revealed that female dwarf mice consume significantly more food per gram body weight than normal females and a similar tendency is evident for males. Although they received 30% less food, CR mice ate the same amount as AL mice per gram body weight.

On measures of total locomotor activity, CR mice were significantly more active than their AL-fed counterparts. On an inhibitory avoidance learning task, 18-21 month old dwarf mice exhibited significantly better retention than their age- and diet-matched normal counterparts. Histopathological analysis in aging dwarf versus normal mice suggested that the incidence of tumors does not differ between the two groups but tumors appear to develop later in dwarf than in normal mice.

After 2.25 years on the study 27% of AL normals, 52% of CR normals, 74% of AL dwarfs, and 87% of CR dwarfs are still alive. We conclude that Ames dwarfs are not CR mimetics although they share many characteristics. It remains to be determined whether CR will delay aging and cause a further life extension in Ames dwarf mice.

INTRODUCTION

In the last 100 years the average life span of humans has increased from 60 to 80 years (1). The large increase in average life span is a result of a reduction in infant mortality and environmental hazards, with better nutrition and health care. The principal cause of death is no longer environmental hazards and infectious disease, now most people are dying from the problems related to the processes of aging. The maximal life span, however, has changed little (1). To date, the only intervention which is well documented to reliably extend maximal life span in mammals is a reduction in food intake.

However, intervention is not always necessary. It was observed in our laboratory that Ames dwarf mice stayed in much better general physical condition until late in life compared to their normal siblings. Ames dwarf mice have a primary pituitary deficiency characterized by the absence or near absence of somatotrophs, lactotrophs, and thyrotrophs, the cells producing GH, PRL, and TSH. The defect stems from a deficiency of pit-1 gene expression in the adenohypophysis during fetal development (2). At birth, the Ames dwarf is of normal size but the adult is only 1/3 of the body weight of the normal siblings.

When lifespan of Ames dwarfs and their normal siblings was monitored, it was found that male dwarfs lived an average of 350 days longer than normals and female dwarfs over 450 days (15 months) longer (3). This increase in life span is particularly noteworthy if one considers that dwarf mice exhibit some characteristics of reduced immune function (4). Increased life span has been subsequently observed in other groups of Ames dwarf mice in our lab. Moreover, Snell dwarf mice, which are deficient in the same three hormones as Ames dwarfs, due to a mutation on a different chromosome, also live longer according to data from two different labs (K Flurkey and DE Harrison; RA Miller; personal communication).

Sixty years ago, it was discovered that a very low calorie diet extended rats' maximal life span by 33% (5). It was also reported that CR animals maintained youthful characteristics longer and had fewer late-life diseases than AL-fed counterparts, a situation similar to that in the Ames dwarf. Life extension capabilities of CR have been shown in several species from single-celled protozoans to fish, spiders and rodents (1, 5, 6, 7).

Research on primates is ongoing and indicates several beneficial effects of CR that resemble findings in CR rodents and as such can be viewed as predictive of delayed aging, namely lowered body temp, reduced energy expenditure (8), and improved glucose regulation (9, 10).

Considering Ames dwarfs longer life and reduced body size, blood glucose, and body temperature, it seemed plausible that these animals were voluntarily CR, i.e. they were eating less naturally. Thus the extended life span of this mutant may be due to the benefits of CR. We hypothesized that self-imposed natural CR would negate or drastically reduce any of the benefits of an imposed CR on lifespan. To test this hypothesis, we are studying the effects of CR in Ames dwarf mice.

Methods and results

Ames dwarf (df/df) mice are produced in a random bred, closed colony with heterogeneous genetic background which has been maintained for over 20 years. Dwarf mice are derived from matings of phenotypically normal carriers of the recessive mutation (df/+ x df/+). Animals are maintained in a fully AALAC-accredited vivarium and fed Purina Lab Chow (Purina Mills, St. Louis, MO), as specified by diet group, and tap water ad libitum. Tests of serum from sentinel animals housed in the same room revealed negativity for each of fifteen pathogens tested.

Male and female Ames dwarf mice and their normal siblings have been fed an AL or a 30% CR diet (70% of AL intake) beginning at 8 weeks of age for 25-29 months (n=9-17 animals per group at start of study). The animals have been monitored daily and weighed weekly.

Body weight

At 12-16 months of age, CR mice weighed significantly less than their gender- and genotype-matched AL fed counterparts (Table 1). At 2 years of age, body weight of AL-fed normal mice was similar to their weight at one year, while AL-fed dwarf males gained a significant amount of weight ($p<0.05$) in the second year and appeared obese. This finding is consistent with previous unpublished observations that dwarf mice do become obese in old age. Ad lib-fed dwarf females have gained weight but they did not become obese by 2 years which

may reflect a gender difference in age of obesity onset in these mice. Although CR dwarf females weighed less than their AL controls after 2 years, the difference was not significant. Dwarf females may be better suited than males to protect energy stores during the stress of caloric restriction.

Food consumption

Originally it was hypothesized that food restriction slowed the rate of aging by slowing metabolic rate per unit body mass (11). However, it has since been demonstrated that Fischer 344 rats on a 40% restricted diet lived longer but consumed a greater number of calories per gram body weight during their lifetimes than AL fed rats (12). The results were similar if restriction was begun in adulthood (13). McCarter and Palmer (14) found that oxygen consumption per unit lean body mass was the same in 40% restricted rats as AL-fed over most of the life span. Thus it is apparent that a reduction in metabolic rate is not necessary for the life-prolonging benefits of CR.

In the present study, food consumption on a per gram body weight basis was evaluated after mice had adhered to their designated diet for at least 9 months and, again, after 23 months. During the first year, AL-fed dwarf females consumed significantly more food per gram body weight than AL-fed normal females. A similar tendency, although a non-significant difference, existed for the AL-fed males (Table 2). Although the difference in food consumption was not significant by 2 years on the study, a similar trend was evident: AL dwarf mice were eating as much, if not more, than AL normal mice. These data indicated Ames dwarf mice were not voluntarily caloric restricted and their increased life span was not the result of a reduced energy intake.

Similar to previous findings, CR mice in the present study were eating the same amount as AL mice on a per gram body weight basis despite receiving 30% less food. These data indicate that Ames dwarf mice are able to alter metabolic rate and fuel flux to maintain homeostasis despite a reduction in food availability.

Evaluation of locomotor activity

Spontaneous locomotor activity was measured using Opto-Varimex-Mini activity monitors (Columbus Instru-

Table 1: Mean body weight (grams $\pm$ SE) for AL and CR mice at 12-16 months and 25-29 months of age and the percentage difference in weight between AL and CR mice. The number in parentheses represents the number of animals in that group at the time of weighing.

	12-16 months old	% difference	25-29 months old	% difference
Normal Female				
AL	37.22 $\pm$ 0.65 (17)	43% ^a	36.0 $\pm$ 0.22 (4)	45% ^a
CR	21.28 $\pm$ 0.63 (14)		19.8 $\pm$ 0.90 (7)	
Normal Male				
AL	37.72 $\pm$ 0.69 (9)	24% ^a	38.9 $\pm$ 2.6 (3)	27% ^a
CR	28.56 $\pm$ 0.14 (13)		24.5 $\pm$ 1.1 (7)	
Dwarf Female				
AL	14.88 $\pm$ 0.37 (13)	16.7% ^a	16.2 $\pm$ 0.8 (10)	20%
CR	12.40 $\pm$ 0.30 (13)		13.4 $\pm$ 0.4 (11)	
Dwarf Male				
AL	15.96 $\pm$ 0.61 (10)	23.9% ^a	21.0 $\pm$ 2.1 (8)	27% ^a
CR	12.14 $\pm$ 0.28 (10)		15.3 $\pm$ 1.0 (9)	

^asignificant difference between AL and CR pair; $P<0.001$.

Table 2. Average food consumption (mg food/gram body weight/day $\pm$ SE) of Ames dwarf and normal mice on an AL or 70% CR diet at 11-12 months of age and 25-29 months of age.

Group	11-12 months old		25-29 months old	
	AL	CR	AL	CR
Normal male	121 $\pm$ 8.6	105 $\pm$ 7.4	95.1 $\pm$ 12.9	111.5 $\pm$ 7.6
Normal female	100 $\pm$ 6.4 ^{a,b}	110 $\pm$ 2.7	124.5 $\pm$ 5.1	174.9 $\pm$ 27.1
Dwarf male	138 $\pm$ 8.0 ^a	117 $\pm$ 9.0	140.4 $\pm$ 7.4	127.8 $\pm$ 3.8
Dwarf female	133 $\pm$ 9.7 ^b	120 $\pm$ 10.0	133.4 $\pm$ 21.0	109.7 $\pm$ 9.7

^{a,b}significant difference between same lettered pair; $P < 0.01$.

ments) which record horizontal movements as activity counts. Total output recorded stereotyped (small-sized, repetitive) movements by counting one pulse every time a light beam was broken. Ambulatory output recorded horizontal movement counting one pulse every time a new light beam was broken.

Individual mice, 19-23 months old, were placed in their home cage, a clear acrylic box with a plastic microisolator top, on the activity monitor. Activity was monitored and recorded at 10-min intervals for 30 min. Testing took place during 4 different time periods: 6-8 am, just after lights on, 12-2 pm, just prior to feeding, 6-8 pm, just after lights off, and 12-2 am, midway through the dark period. Because there was no significant difference in activity due to the time of testing, data from the four 30-minute time periods were averaged.

Increased physical activity is considered to be one of the beneficial effects of CR, countering some of the effects of aging. However, increased activity is not likely an important factor in the mechanism for increased life span. McCarter et al. (15) found that neither AL nor 40% restricted Fischer 344 rats had an extended life span due to lifelong running compared to sedentary controls. Previous studies in our lab have shown that Ames dwarf mice, regardless of age, are less active than age-matched normal controls (C Meliska, unpublished observations). In the present study, dwarf females, both CR and AL, were less active on both measures than normal females. There was no difference between male groups. These data would indicate that Ames dwarf mice were not living longer due to the benefits of increased physical activity.

The current data supports previous findings of increased activity in CR animals. Total activity was greater in all groups of CR mice (male dwarf and normal, female dwarf and normal) compared to their gender- and genotype-matched AL controls ($p < 0.0001$) (Figure 1). Ambulatory activity tended to be greater in CR mice as well, however, the difference was significant for normal females only. Yu et al. (13) reported Fischer rats on 40% restriction maintained high levels of activity throughout life, whereas the activity of AL-fed rats progressively declined. Thirty percent restricted Long-Evans rats had higher voluntary running activity than AL-fed rats (16). Similarly, adult rhesus monkeys on long-term CR are more active than those fed AL (17). Thus, although increased physical activity may provide some benefits to the aging animal and a CR diet induces increased

activity, it does not appear to be a primary factor for increased lifespan in restricted animals or Ames dwarfs.

Evaluation of inhibitory avoidance learning

Apparatus

Inhibitory avoidance training and testing were carried out in a trough-shaped alley (33.5 cm long, 12.2 cm deep, 9.3 cm wide at the top, and 2.7 cm wide at the floor). The apparatus was divided into a start compartment (9.5 cm long) made of clear acrylic plastic and illuminated from approximately 18 cm above by a 75-watt incandescent lamp, and a shock compartment (24 cm long) made of black acrylic plastic with steel floor plates. These compartments were separated by a guillotine door which opened to a height of 4.2 cm. A Lafayette shock generator delivered a 1-s, 0.20-mA shock to the shock compartment through the floor plates; duration was controlled by a Hunter timer. A Techtronics oscilloscope was used to calibrate shock intensity and duration. The apparatus was wiped clean with a 70% ethanol solution before each trial.

Procedure

The first trial was acquisition training in which the mouse was placed in the start compartment facing away from the guillotine door. When the mouse turned to face the door or 20 sec had elapsed, the door was opened and a stopwatch started. After the mouse entered the shock compartment with all four paws, the door was closed and the footshock was delivered. Shock response was recorded. The shock response was scored as described previously (18): "0" for no response, "1" for flinch, "2" for jump (at least two paws leaving the floor), and "3" for vocalization. The mouse remained in the shock compartment for 30 sec after the shock delivery and then was returned to its home cage.

The 24-h retention test was identical to acquisition training, but without the shock. Entry latency was recorded as the time for the mouse to enter the shock compartment with all four paws. If the mouse did not enter the shock compartment within 300 sec, a maximum score of 300 sec was recorded. A second retention test was administered 7 days after training.

Sixty-seven mice from the on-going caloric restriction study were tested when they were 18-21 months old. Mice were grouped according to gender, genotype and diet; there were 7-10 animals per group. All testing began at 1000 hours. Mean shock response scores were 2.8 ± 0.07 and 2.7 ± 0.08 for normals and dwarfs, respectively.

Impairments of inhibitory avoidance learning are common in aged rats and mice (19, 20) and declines in retention vary with age (21). Caloric restriction has been shown to improve maze performance in aged rats (22) and mice (23).

In the present study, retention scores were similar on the 24-h and 7-day test for any given group (Figure 2). It was likely that memory retention would have been compromised following a longer interval between tests

Figure 1: Comparison of total activity (A) and ambulatory activity (B) in Ames dwarf and normal mice. Data represent mean activity counts of the four time periods tested. *Significant difference from the corresponding AL group, $P < 0.0001$.

as reported in other groups of mice (24). Differences in diet did not affect inhibitory avoidance learning, CR and AL mice performed equally well. When grouped according to diet, dwarfs performed better (longer step through latency) than normals on 3 out of 4 comparisons. Evidence of better retention by the dwarfs is in agreement with previous findings of improved cognitive function in other groups of Ames dwarf mice compared to age-matched normals (B Kinney, unpublished observations). The better performance in dwarf mice indicates that they may experience a delayed onset of age-associated deficits in learning compared to normals.

Blood glucose and insulin

Hyperinsulinemia and/or hyperglycemia are considered to contribute to or cause many age-associated diseases. Similar to diabetes, the high levels of glucose can react nonenzymatically with proteins and nucleic acids forming products that affect function and decrease tissue elasticity (25). Low insulin levels promote fat metabolism and better maintenance of mitochondrial function (26). Caloric restriction has been shown to be effective in reducing plasma glucose and insulin levels and increasing insulin sensitivity in rodents (10, 27, 28). Reductions in glucose and insulin were evident in monkeys as well, but only after 3-4 years of diet restriction

(10, 29). If not merely a result of CR, low glucose and insulin may be contributing to the mechanism for delayed aging.

Similar to CR animals, AL-fed Ames dwarfs are hypoglycemic and insulin sensitive (30). Data from AL-fed mice in the present study agree with previous findings (Table 3). However, glucose levels in CR dwarf and normal mice were not lower than AL controls. Surprisingly, CR dwarf females had higher glucose levels than AL dwarfs. These findings in CR mice may be similar to those seen in monkeys in which there were no differences in glucose and insulin during the first two years of a restricted diet (29). The mice in the present study had been restricted for only 3-6 months.

Results from insulin measurements were inconclusive. Both male and female CR normal mice had lower insulin levels than their AL counterparts, but these

Table 3: Mean blood glucose (ng/ml $\pm$ SE) in AL and 30% CR Ames dwarf mice; $n = 3-5$ mice per group.

	AL	CR
Normal males	175.7 $\pm$ 22.0 ^a	97.3 $\pm$ 20.7
Normal females	112.8 $\pm$ 9.2 ^b	112.9 $\pm$ 23.3
Dwarf males	68.8 $\pm$ 13.8 ^a	87 $\pm$ 16.6
Dwarf females	50.7 $\pm$ 2.5 ^{bc}	78.7 $\pm$ 7.6 ^c

^{abc}significant difference between same lettered pair; $P < 0.05$.

Figure 2: Inhibitory avoidance learning and retention in AL and CR Ames dwarf and normal mice. Data are step through latencies (means + SE) at 24-h and 7-days after acquisition training. *Significantly different from the corresponding normal animals; $P < 0.05$.

apparent differences were not statistically significant. In dwarfs, insulin levels were undetectable. Additional measurements are in progress.

Histopathological data

Histopathological changes in aging Ames dwarf and normal mice have been recorded from an on-going aging study of AL-fed mice. At the time of death (mice were sacrificed if they were near death), mouse carcasses were preserved in Bouin's solution, necropsied, and examined histologically.

The incidence of tumors in animals dying from natural causes did not differ between dwarf and normal mice (Table 4). However, dwarfs live significantly longer than normals; therefore, it was possible that the tumors developed later in dwarfs or that the tumors grew more slowly.

No data is available yet on tumor incidence in CR Ames dwarf mice. Evidence from other studies suggests that CR reduces the occurrence and/or delays the onset of tumors. In four mouse strains, the occurrence of lesions in mice killed at 12, 18, 24, and 30 months of age were lower in 40% restricted mice compared to AL-fed (31). Yu et al. (6) reported that Fischer 344 rats on 40% restriction had delayed onset of renal lesions, testicular Leydig cell tumors, bile duct hyperplasia, and cardiac lesions compared to AL-fed rats. In the same rat strain,

Table 4. Incidence of tumors in Ames dwarf vs. normal mice.

Genotype	Sex	n	% with tumors ^a	Tumor incidence (%)		
				liver	lung	other
Normal	Female	18	72	6	28	39
Dwarf	Female	13	77	15	15	62
Normal	Male	16	44	19	25	19
Dwarf	Male	11	73	36	27	36

^a Some animals had tumors in two or more organs and, therefore, the sum of incidence (%) of different tumors exceeds the percentage of animals with tumors.

Shimokawa et al. (32) found that a reduction in calories was effective in reducing mortality rates due to neoplastic disease.

Survival data

Survival data was calculated after approximately 2.25 years of the study. Data from this study has confirmed previous findings that Ames dwarfs live longer than their normal littermates. Preliminary evidence indicated that normal mice on a CR diet had a greater survival rate than their AL fed counterparts.

Table 5. Survival data of Ames dwarf and normal mice after approximately 2.25 years on an AL or 70% CR diet.

	# of animals starting study	# animals alive 1/01/00	% survival
Normal Males			
AL	9	3	33%
CR	13	7	54%
Normal Females			
AL	17	4	24%
CR	14	7	50%
Dwarf Males			
AL	10	7	70%
CR	10	9	90%
Dwarf Females			
AL	13	10	77%
CR	13	11	85%

DISCUSSION

Many of the characteristics of the Ames dwarf mouse are consistent with those detected in other studies of delayed aging. Therefore these animals may be considered a novel mammalian model of delayed aging. There are several possible contributors to this delay of aging and longer lifespan of Ames dwarf mice. Their small size is consistent with evidence in this and other species that smaller animals live longer than larger individuals from the same species (33).

Ames dwarfs have greatly reduced levels of IGF-I (34). Caloric restricted mice have also been reported to have lower IGF-I levels than AL-fed mice (35, 36). Considering that IGF-I levels decrease with age in AL-fed mice, it is unclear if low IGF-I levels are an important determinant in a longer life span. Evidence that low levels may be beneficial derives from the conclusion that IGFs are stimulants of cell turnover, thus the lower levels may be expected to decrease cancer risk (37). Transgenic mice overexpressing GH, with high levels of both GH

and IGF-I exhibit several traits of premature aging and have reduced lifespan (reviewed by 38).

Ames dwarfs have low blood glucose and are insulin sensitive (30). Both of these traits are also characteristic of the CR model (9, 10, 26). Hyperglycemia and hyperinsulinemia have damaging effects on tissue and thus it is possible that the lifetime maintenance of lower insulin and glucose levels in the Ames dwarf may have protective effects.

Ames dwarf mice are hypogonadal with reduced plasma levels of LH and FSH (39), decreased gonadotropin gene expression (40), and decreased production of testosterone by the testes (34). Some males can sire litters, while females are sterile due to PRL deficiency and the resulting luteal failure. Hypogonadism of Ames dwarf mice may contribute to their prolonged survival. Gonadectomy was reported to increase life span in rats (41) and cats (42).

Ames dwarf mice have reduced thyroid hormone levels and a lower body temperature throughout a 24 hour period (43) both of which may contribute to a slower metabolism. Although low body temperature indicates a lower metabolic rate, dwarf mice consume as much or more food per gram body weight than normal animals. This is consistent with other studies of CR which reported reduced body temperature and a longer life span despite consumption of a greater number of calories per gram body weight (15, 27). Because CR rodents can eat as much food per unit of body weight as AL animals, with a higher level of lifetime activity, it indicates that a lower metabolic rate is not essential for the life-prolonging benefits of CR and the same appears to apply to the prolonged survival of Ames dwarf mice.

Deterioration in inhibitory avoidance learning is typical in aging animals and the declines in retention vary directly with age (21). Better performance of 18-21 month old Ames dwarfs than age- and diet-matched normals on inhibitory avoidance tasks suggests that there is a delayed age-related deterioration of cognitive function in the dwarf animals.

Although many traits of Ames dwarf mice resemble a caloric restricted state, the finding that restricted dwarf mice weigh significantly less than AL-fed dwarfs, can survive a 30% food restriction, and that they do become obese as they age indicates that they are not CR mimetics and living longer because they eat less naturally.

Survival data after 2.25 years on the study indicate that normal mice have some life extension benefit from CR. The naturally long life of Ames dwarfs prevents drawing conclusions about the effect of CR in these animals at this time point of our study.

Greatly increased life expectancy, differences from animals subjected to CR, and evidence for improved cognitive function in old Ames dwarf as compared to age-matched normal mice suggest that these animals provide a novel model of genetically controlled delayed aging in a mammal.

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REFERENCES

1. Weindruch, R, and Walford, RL: The retardation of aging and disease by dietary restriction. Springfield, Charles C. Thomas, 1988, p. 5.
2. Andersen, B, Pearce, RV, Jenne, K, Sornson, M, Lin, S-C, Bartke, A, and Rosenfeld, MG: The Ames dwarf gene is required for Pit-1 gene activation. *Dev. Bio.*, 172:495-503, 1995.
3. Brown-Borg, HM, Borg, KE, Meliska, CJ, and Bartke, A: Dwarf mice and the ageing process. *Nature*, 384:33, 1996.
4. Esquifino, AI, Szary, A, Brown-Borg, HM, and Bartke, A: Age-related effects of ectopic pituitary transplants on the activation of Ames dwarf mouse lymphocytes in vitro. *Proc. Soc. Exp. Bio. Med.*, 211:87-93, 1996.
5. McCay, CM, Crowell, MF, and Maynard, LA: The effect of retarded growth upon the length of life span and upon the ultimate body size. *J. Nutr.*, 10:63-79, 1935.
6. Yu, BP, Masoro, EJ, Murata, I, Bertrand, HA and Lynd, FT: Life span study of SPF Fischer 344 male rats fed ad libitum or restricted diets: longevity, growth, lean body mass and disease. *J. Gerontol.*, 37:130-141, 1982.
7. Weindruch, R: The retardation of aging by caloric restriction: studies in rodents and primates. *Toxic. Pathol.*, 24:742-745, 1996.
8. Lane, MA, Baer, DJ, Rumpler, WV, Weindruch, R, Ingram, DK, Tilmont, EM, Cutler, RG, and Roth, GS: Calorie restriction lowers body temperature in rhesus monkeys, consistent with a postulated anti-aging mechanism in rodents. *Proc. Natl. Acad. Sci.* 93:4159-4164, 1996.
9. Kemnitz, JW, Roecker, EB, Weindruch, R, Elson, DF, Baum, ST, and Bergman, RN: Dietary restriction increases insulin sensitivity and lowers blood glucose in rhesus monkeys. *Am. J. Physiol.*, 266:E540-E547, 1994.
10. Lane, MA, Ball, SS, Ingram, DK, Cutler, RG, Engel, J, Read, V, and Roth, GS: Diet restriction in rhesus monkeys lowers fasting and glucose-stimulated glucoregulatory end points. *Am. J. Physiol.* 268:E941-E948, 1995.
11. Sacher GA: Life table modifications and life prolongation, in *Handbook of the Biology of Aging*, edited by Finch, CE, and Hayflick, L. New York, Van Nostrand Reinhold, 1977, pp. 582-638.

12. Masoro, EJ, Yu, BP, and Bertrand, HA: Action of food restriction in delaying the aging process. *Proc. Natl. Acad. Sci.*, 79:4239-4241, 1982.
13. Yu, BP, Masoro, EJ, and McMahan, CA: Nutritional influences on aging of Fischer 344 rats: I. physical, metabolic, and longevity characteristics. *J. Geron.*, 40:657-670, 1985.
14. McCarter, RJ and Palmer, J: Energy metabolism and aging: a lifelong study of Fischer 344 rats. *Am J Phys.*, 263:E448-E452, 1992.
15. McCarter, RJM, Shimokawa, I, Ikeno, Y, Higami, Y, Hubbard, GB, Yu, BP, and McMahan, CA: Physical activity as a factor in the action of dietary restriction on aging: effects in Fischer 344 rats. *Aging Clin. Exp. Res.*, 9:73-79, 1997.
16. Holloszy, JO and Schechtman, KB: Interaction between exercise and food restriction: effects on longevity of male rats. *J. Appl. Physiol.*, 70:1529-1535, 1991.
17. Weed, JL, Lane, MA, Roth, GS, Speer, DL, and Ingram, DK: Activity measures in rhesus monkeys on long-term calorie restriction. *Phys. Behav.*, 62:97-103, 1997.
18. Yagi, H, Katoh, S, Akiguchi, I, and Takeda, T: Age-related deterioration of ability of acquisition in memory and learning in senescence accelerated mouse: SAM-P/8 as an animal model of disturbances in recent memory. *Brain Res.*, 474:86-93, 1988.
19. Bartus, RT, Dean, RL, Goas, JA, and Lippa, AS: Age-related changes in passive avoidance retention: modulation with dietary choline. *Science*, 209:301-303, 1980.
20. Dean, RL, Scozzafava, J, Goas, JA, Regan, B, Beer, B, and Bartus, RT: Age-related differences in behavior across the life span of the C57BL/6J mouse. *Exper. Aging Res.*, 7:427-451, 1981.
21. Gold, PE, McGaugh, JL, Hankins, LL, Rose, RP, and Vasquez, BJ: Age dependent changes in retention in rats. *Exper. Aging Res.*, 8:53-58, 1981.
22. Goodrick, CL: Effects of lifelong restricted feeding on complex maze performance in rats. *Age*, 7:1-2, 1984.
23. Ingram, DK, Weindruch, R, Spangler, EL, Freeman, JR, and Walford, RL: Dietary restriction benefits learning and motor performance of aged mice. *J. Geron.*, 42:78-81, 1987.
24. Meliska, CJ, Burke, PA, Bartke, A, and Jensen, RA: Inhibitory avoidance and appetitive learning in aged normal mice: comparison with transgenic mice having elevated plasma growth hormone levels. *Neurobio. Learn. Mem.*, 68:1-12, 1997.
25. Preuss, HG: Effects of glucose/insulin perturbations on aging and chronic disorders of aging: the evidence. *J. Am. Coll. Nutr.*, 16:397-403, 1997.
26. Parr, T: Insulin exposure and aging theory. *Gerontology*, 43:182-200, 1997.
27. Masoro, EJ, McCarter, RJM, Katz, MS, and McMahan, CA: Dietary restriction alters characteristics of glucose fuel use. *J. Geron.*, 47:B202-B208, 1992.
28. Harris, SB, Gunion, MW, Rosenthal, MJ, and Walford, RL: Serum glucose, glucose tolerance, corticosterone and free fatty acids during aging in energy restricted mice. *Mech. Age. Devel.*, 73:209-221, 1994.
29. Cutler, RG, Davis, BJ, Ingram, DK, and Roth, GS: Plasma concentrations of glucose, insulin, and percent glycosylated hemoglobin are unaltered by food restriction in rhesus monkeys and squirrel monkeys. *J. Geron.*, 47:B9-B12, 1992.
30. Borg, KE, Brown-Borg, HM, and Bartke, A: Assessment of the primary adrenal cortical and pancreatic hormone basal levels in relation to plasma glucose and age in the unstressed Ames dwarf mouse. *Proc. Soc. Exp. Bio. Med.*, 210:126-133, 1995.
31. Bronson, RT and Lipman, RD: Reduction in rate of occurrence of age related lesions in dietary restricted laboratory mice. *Growth, Dev. Aging*, 55:169-184, 1991.
32. Shimokawa, I, Yu, BP, and Masoro, RJ: Influence of diet on fatal neoplastic disease in male Fischer 344 rats. *J. Geron.*, 46:B228-B232, 1991.
33. Comfort A: *The Biology of Senescence*. New York, Elsevier, 1979, p. 56.
34. Chandrashekar, V and Bartke, A: Induction of endogenous insulin-like growth factor-I secretion alters the hypothalamic-pituitary-testicular function in growth hormone-deficient adult dwarf mice. *Bio. Repro.*, 48:544-551, 1993.
35. Breese, CR, Ingram, RL, and Sonntag, WE: Influence of age and long-term dietary restriction on plasma insulin-like growth factor-I (IGF-I), IGF-I gene expression, and IGF-I binding, proteins. *J. Geron.*, 46:B180-B187, 1991.
36. Sonntag, WE, Lenham, JE, and Ingram, RL: Effects of aging and dietary restriction on tissue protein synthesis: relationship to plasma insulin-like growth factor-I. *J. Geron.*, 47:B159-B163, 1992.
37. McCarty, MF: Up-regulation of IGF binding protein-1 as an anticarcinogenic strategy: Relevance to caloric restriction, exercise, and insulin sensitivity. *Med. Hypoth.*, 48:297-308, 1997.

38. Bartke, A, Brown-Borg, HM, Bode, AM, Carlson, J, Hunter, WS, and Bronson, RT: Does growth hormone prevent or accelerate aging? *Exper. Geront.*, 33:675-687, 1998.
39. Bartke, A: Genetic models in the study of anterior pituitary hormones, in *Genetic Variation in Hormone Systems*, Vol. 1, edited by Shire, JGM., Boca Raton, CRC Press, 1979, pp.113-126.
40. Tang, K, Bartke, A, Gardiner, CS, Wagner, TE, and Yun, JS: Gonadotropin secretion, synthesis, and gene expression in human growth hormone transgenic mice and in Ames dwarf mice. *Endo.*, 132:2518-2524, 1993.
41. Talbert, GB and Hamilton, JB: Duration of life in Lewis strain of rats after gonadectomy at birth and at older ages. *J. Geront.*, 20 2518-2524, 1965
42. Hamilton JB: Relationship of castration, spaying and sex to survival and duration of life in domestic cats. *J. Geront.*, 20:96-104, 1965.
43. Hunter, WS, Croson, WB, Bartke, A, Gentry, MV, and Meliska, CJ: Low body temperature in long-lived Ames dwarf mice at rest and during stress. *Phys. and Behav.*, 67:433-437, 1999.