Journal of Gerontology: BIOLOGICAL SCIENCES
1999, Vol. 54A, No. 11, B492–B501

Growth Curves and Survival Characteristics of the Animals Used in the Biomarkers of Aging Program

Angelo Turturro,[1] William W. Witt,[2] Sherry Lewis,[3] Bruce S. Hass,[2] Ruth D. Lipman,[5] and Ronald W. Hart[4]

[1]Division of Biometry and Risk Assessment, [2]Division of Veterinary Services, [3]Bionetics Corporation, and [4]Office of the Director, National Center for Toxicological Research, Food and Drug Administration, Jefferson, Arkansas.
[5]USDA Human Nutrition Research Center on Aging at Tufts University, Boston.

The collaborative Interagency Agreement between the National Center for Toxicological Research (NCTR) and the National Institute on Aging (NIA) was aimed at identifying and validating a panel of biomarkers of aging in rodents in order to rapidly test the efficacy and safety of interventions designed to slow aging. Another aim was to provide a basis for developing biomarkers of aging in humans, using the assumption that biomarkers that were useful across different genotypes and species were sensitive to fundamental processes that would extrapolate to humans. Caloric restriction (CR), the only intervention that consistently extends both mean and maximal life span in a variety of species, was used to provide a model with extended life span. C57Bl/6NNia, DBA/2JNia, B6D2F1, and B6C3F1 mice and Brown Norway (BN/RijNia), Fischer (F344/NNia) and Fischer × Brown Norway hybrid (F344 × BN F1) rats were bred and maintained on study. NCTR generated data from over 60,000 individually housed animals of the seven different genotypes and both sexes, approximately half ad libitum (AL) fed, the remainder CR. Approximately half the animals were shipped to offsite NIA investigators internationally, with the majority of the remainder maintained at NCTR until they died. The collaboration supplied a choice of healthy, long-lived rodent models to investigators, while allowing for the development of some of the most definitive information on life span, food consumption, and growth characteristics in these genotypes under diverse feeding paradigms.

A MAJOR objective of the National Institute on Aging (NIA) Biomarkers of Aging Program (BAP), and the collaborative Interagency Agreement between the National Center for Toxicological Research (NCTR) and NIA (1), was to identify and validate a panel of biomarkers of aging in rodents in order to rapidly test the efficacy and safety of interventions designed to slow aging. It was also intended that any panel of rodent-aging biomarkers would also provide some basis for developing biomarkers of aging in humans. This was based on the assumption that biomarkers useful across different genotypes and species would be sensitive to fundamental processes that would extrapolate readily to humans.

Because the BAP biomarkers were to test extension of life span, any validation method that predicted shortened life span as a result of genetic or environmental manipulation was considered inadequate. For that reason, the BAP biomarkers in rodents were validated in animals fed using caloric restriction (CR), the one intervention that is generally accepted to reliably and robustly extend life span and slow the rate of aging as well as delaying many of the processes associated with aging in rodents (1–4). Successful BAP biomarkers should distinguish between young and old ad libitum fed (AL) individuals, as well as between AL and CR animals of the same chronological age (1,5,6).

The rodents used in this program were bred and maintained at the NCTR (7–9). Following a two-year startup and testing phase initiated in 1984, animals either were shipped to NIA investigators with projects funded through BAP or were utilized in studies conducted by staff at the NCTR headquarters, Jefferson, Arkansas. The NIA/NCTR Interagency Agreement was expanded in 1993 to provide AL and CR animals to investigators on a cost basis, and was terminated in 1998 as the last remaining animals were shipped to Harlan-Sprague Dawley, Inc., Indianapolis, IN.

Selections of the work conducted by a number of the BAP-funded and NCTR investigators are presented in this and the December issue of the *Journal of Gerontology: Biological Sciences*.

Materials and Methods

Genotypes

Four genotypes of mice and three genotypes of rats were available to the NIA/NCTR investigators. These included two inbred strains of mice, C57Bl/6NNia (B6) and DBA/2JNia (D2) and two hybrid strains of mice, one of which was derived by crossing B6 and D2 mice (B6D2F1) and the other derived by crossing B6 and another inbred strain, C3H (B6C3F1). The breeding pairs for these animals were obtained from the NIA breeding colonies and were monitored periodically for genetic drift. The three genotypes of rat included two inbred strains, Brown Norway/RijNia (BN) and Fischer 344/NNia (F344), and the F1 hybrid derived by crossing BN and F344 (F344 × BN F1). The breeding pairs for the F344 rat were obtained from the NIA colonies, and the BN rat was derived from frozen embryos shipped from TNO (Rijswik, The Netherlands).

Animal Husbandry Conditions

The animals were divided into three groups. One, the Longevity Group (LG), consisted of AL and CR cohorts of usually 56 male and 56 female mice, or 54 male and 54 female rats. The animals in this group were simply monitored until

they died. The Pathology Group (PG) (usually 108 rats, or 168–210 mice, both sexes, both AL and CR), supplied approximately 15 live mice or 12 live rats at 12 months of age and at 6-month intervals thereafter for comprehensive pathological examinations and clinical tests similar to those previously reported (10–12). In addition, the animals in the LG and those in the PG that died, or were moribund, usually were examined in the same comprehensive manner. The Shipment Group (SG) comprised the cohorts from which live animals were shipped to investigators. The three groups were maintained under identical conditions. The numbers of animals in the SG group for each genotype are given in Table 1. Approximately 58% of the animals in this group were actually shipped to investigators, varying from 42% to 67% in each genotype.

The animals used in these studies were maintained in a specific-pathogen-free (SPF) barrier facility as described previously (9). Caging, bedding, computers, and other equipment entering the barrier were sterilized or sanitized by steam autoclaving, ethylene oxide, or chemical surface sanitizing. Personnel showered before entering and wore sterilized masks, caps, and were otherwise sheathed in autoclaved protective clothing. The air supplied was 100% fresh HEPA-filtered air and conditioned to 72 ± 1° F with relative humidity maintained between 40% and 60%. The animals were checked twice daily for clinical observations and survival. Cage changes were made weekly. Body weights and clinical observations were recorded at least monthly as well as weekly for some periods, such as the first 4 months of age for every animal.

Animals were housed individually in standard caging with modifications. Mice were kept in a standard polycarbonate cage with wooden chip bedding with a stainless steel divider separating the cage longitudinally into two prolate compartments, approximately 11.5 × 3.75 × 5 inches. Each cage had a single water bottle fitted with a two-holed stopper and two sipper tubes. Pelleted food was apportioned independently to each side of the food hopper and partitioned by the steel divider. These cages were placed on racks with 7 shelves, holding 12 cages per row (6 on each side), for a total of 84 cages (168 mice) per rack.

Rats were housed individually in the mouse polycarbonate cages (without divider) to which an additional 2 1/2-inch-high polycarbonate rim was solvent welded, providing approximately 11.5 × 7.5 × 7 inches of space per animal. Standard cage lids and water bottles were utilized. Each rack contained only 6 shelves (due to increased cage height) with 12 cages per rack (6 on each side), for a total of 72 cages (rats) per rack.

Table 1. Numbers of Animals in the Shipment Group

Genotypes	M-AL	M-CR	F-AL	F-CR
B6D2F1	2836	2324	1034	1033
D2	1104	1101	586	582
B6C3F1	1783	1755	1654	1656
B6-NIH	2926	2485	1203	1186
B6-EM	1770	1886	1758	1882
BN	1574	1540	1549	1583
F344 × BN F1	2116	2043	1648	1610
F344-NIH	2792	2542	1858	1814
F344-PUR	438	438	462	462

Notes: M-AL = male ad libitum fed, M-CR = male calorie-restricted, F-AL = female ad libitum fed, F-CR = female calorie-restricted. NIH = animals fed NIH-31, EM = animals fed Emory Morse 911a, PUR = animals fed Purina 5010C. Genotypes are described in text.

In the latter phases of the study (approximately the year 1996), some mice sporadically exhibited a seropositivity in the screening tests for mouse hepatitis virus (MHV). The MHV strain that generated the seropositivity appeared to be nonpathogenic (later verified by Susan Compton, Yale University, personal communication), results of which were consistent with the lack of any observed effects in gross and microscopic pathology, and in both infant and "nude" mice. However, to eliminate the virus from the colony and to allay potential concerns, all breeding in the colony of mice was stopped and the entire mouse colony was subjected to three sequential evaluations for MHV. Any mouse exhibiting seropositivity or that shared a water bottle (e.g., same cage) with a seropositive mouse was killed. The process was successful, but resulted in some losses to the SG group. Approximately 4% of the males and 11% of the females of the B6C3F1 mice, 12% of male and 4% of female B6D2F1, and 5% of male and 1% of female B6-NIH were removed (B6-EM were maintained longer when this occurred).

Food Consumption and Diets

Food consumption was monitored weekly for a cohort of animals, the AL-fed LG rodents (Tables 2–5). These consumptions were used to determine the ration to be fed to the CR groups in the studies, which were given a nominal 60% of the values. Changes in the amount of food actually given to the CR animals were made when a half-gram increase or decrease was indicated. In mice, consumption of an entire cage (usually two animals) was monitored. Near the end of the AL animals' life span, when the number of surviving animals became small (approximately 10% of the cohort), the CR groups were fed 60% of the "static value" (this is the last value in the table for each genotype). This value was determined by estimating an average AL consumption with added amounts of food to prevent malnutrition. Spillage in the food consumption groups was kept below 5% of intake. Although food consumption data are often used to determine caloric intake and "excess" calories in animals, the presence of coprophagy (13) and different energy requirements arising from potential differential activity, body fat, and response to the fixed environmental temperature (14) between the AL- and CR-fed animals, make the use of food disappearance from a feeder (the basis of the food consumption data) a method to accurately characterize caloric needs and consumption problematic.

Three diets (each with two formulations) were used. In seven studies, animals were given NIH-31 (Table 6), the autoclavable form of a commonly used cereal, open formula feed if AL-fed, or NIH-31 formulated with extra vitamin supplementation (1.67 times the vitamin mix) if CR. It can be seen from Table 6 that this diet is adequate for both mice and rats even after autoclaving. The only potential problem is a slight deficiency in Vitamin B_{12} in rats. There were no signs of any problems with Vitamin B_{12}. The rats grew well, with no evidence of anemia in the clinical assays of the sacrificed animals in the PG group. Supplementation to ensure that CR cohorts received adequate amounts of micronutrients to avoid malnutrition was achieved by equalizing vitamin intake on a per/animal (not per/gram body weight [BW]) basis. This approach was based on obser-

Table 2. Male Mouse Food Consumption Data

Age (months)	B6D2F1	D2	B6C3F1	B6-NIH*	B6-EM*
2	4.2	4.4	4.3	4.4	3.6
3	4.2	4.8	5.5	4.9	4.1
4	4.9	4.9	5.8	4.9	4.2
5	5.1	4.8	6	5.1	4.2
6	5.2	4.7	5.8	5	4.3
7	5.1	4.7	5.8	5	4.2
8	5.3	4.7	6.1	5	4.2
9	5.4	4.9	6	5	4.2
10	5.3	5	5.8	5.1	4.1
11	5.3	4.8	5.8	4.8	4
12	5.2	4.8	6	4.7	3.9
13	5.2	4.6	5.9	4.7	3.9
14	5.4	4.6	6	4.9	3.8
15	5.4	4.7	5.8	4.9	4.1
16	5.4	4.5	5.8	4.7	4.4
17	5.3	4.7	5.4	4.8	4
18	5.4	4.8	6	5	4.3
19	5.3	4.6	6.1	4.8	3.9
20	5.2	4.7	6.2	5	4
21	5.1	4.6	6.1	4.8	3.8
22	5.1	4.4	6	4.9	3.6
23	4.9	4.6	5.8	4.5	3.9
24	4.6	4.1	6	4.7	3.9
25	4.5	4	5.6	4.6	3.6
26	4.8	4.2	5.7	4.4	3.1
27	5.1		5.9	4.7	
28	5		5.1	4.4	
29	4.7		4.9	4.1	
30	4.7		4.8	4	
31	5.1		5		
32	5.2		5.1		
Static	5.0	4.2	5	4.2	4.2

Notes: Food consumption, in grams per day for male mice, at the age (in months) listed. Last value in each column is the "static value" (60% of this amount is fed to the restricted animals for the rest of the life span). Genotypes listed are explained in the text.

*NIH = animals fed NIH-31, EM = animals fed Emory-Morse 911a.

Table 3. Female Mouse Food Consumption Data

Age (months)	B6D2F1	D2	B6C3F1	B6-NIH*	B6-EM*
2	3.7	3.9	3.8	4.2	3.5
3	4.1	4.3	4.4	4.6	4
4	4.3	4.3	4.6	4.7	4.1
5	4.4	4.1	5.1	5	4
6	4.5	3.9	5	4.9	4
7	4.5	3.9	5	5	4
8	4.6	4.1	5.2	5	4.3
9	4.7	4.2	5.3	5.1	4.2
10	4.7	4.4	5.4	5	4.1
11	4.8	4.3	5.4	4.9	3.9
12	4.6	4.4	5.4	4.9	3.8
13	4.6	4.2	5.6	4.8	3.9
14	4.9	4.2	5.6	5.1	3.8
15	4.8	4.3	5.4	5.1	3.8
16	4.7	4.2	5.3	5	4.3
17	4.7	4.2	5.2	5.2	4.4
18	4.7	4.4	5.5	5.4	5.1
19	4.6	4.2	5.6	5.3	4.6
20	4.6	4.3	5.7	5.5	4.5
21	4.5	4.4	5.6	5.5	4.5
22	4.5	4	5.5	5.4	4
23	4.4	4.2	5.6	5.3	4.3
24	4.2	3.6	5.8	5.6	4.2
25	4.3	2.5	5.6	5.8	
26	4.6		6	5.5	
27	4.8		6.3		
28	4.7		5.3		
29	4.6		5.2		
30	4.6		5.4		
31	5.1		5.6		
32	5.2		5.7		
Static	5	4.2	5.8	5.0	4.2

Notes: Food consumption, in grams per day for female mice, at the age (in months) listed. Last value in each column is the "static value" (60% of this amount is fed to the restricted animals for the rest of the life span). Genotypes listed are explained in the text.

*NIH = animals fed NIH-31, EM = animals fed Emory-Morse 911a.

vations that CR-induced changes in lean body mass (LBM; the body composition component that would require the vitamins) were much less than those seen in BW [(15,16); LBM at some ages was sometimes equal in AL and CR rodents despite large differences in BW]. In addition, B6 mice were used to compare the effects of two different diets, the NIH-31 diet and the autoclavable form of a high-fat, high-protein breeding diet, Emory-Morse 911 [EM; (8,9)]. F344 rats were similarly used to compare the effects of the NIH-31 diet and Purina 5010C (PUR), a diet used by the San Antonio group (e.g., 17). Both diets were fed in the standard formulation to AL animals and with vitamin fortification, similar (i.e., 1.67 times the vitamin mix) to that used for NIH-31, to the CR animals. Because a number of animals were shipped from the studies using EM and PUR, data from these studies were included in this report for completeness. B6-NIH denotes the experiments that fed NIH-31 to the B6 animals, and B6-EM denotes the feeding of EM. Similarly, F344-NIH indicates the F344 animals fed NIH-31, and F344-PUR indicates the animals fed PUR.

Pellets of food were sized accordingly by gram weight [2.0–5.0 gram range; (8)]. Combinations of different-sized pellets were used to provide a predetermined aliquot of food to the CR animals for every species-genotype-sex cohort. Steam autoclaving of the NIH-31, and pasteurization of EM and PUR, were used to manage the microbiological contamination without significantly destroying the vitamins in the diet. To prevent spoilage, the pellets were stored in a temperature-controlled environment at 40°F before and after sizing for no longer than 90 days. Pelleted feed expiration dates were 6 months after manufacture.

Restriction paradigm.—Animals allocated to the CR cohort were introduced to the reduced caloric intake in a stepwise fashion over a period of 3 weeks, beginning at 14 weeks of age in all experiments except F344-PUR, in which the introduction started at 6 weeks of age in order to be consistent with previous experiments using this diet/strain combination (e.g., 17).

Table 4. Male Rat Food Consumption Data

Age (months)	BN	F344 × BN F1	F344-NIH*	F344-PUR*
2	15.3	18	17.2	15.2
3	16.3	19.7	20	16.4
4	15.6	19.4	19.5	15.8
5	15.8	18.6	18.6	15.3
6	14.7	17.8	18.4	14.6
7	15.3	18.1	17.8	15.3
8	15.3	18.4	17.8	15.5
9	15.9	19.1	17.5	15.6
10	15.8	19	18.3	15.4
11	15.8	18.9	18.7	15.7
12	16	19.2	17.9	15.1
13	16.2	19.3	18.3	15.3
14	16.5	19.7	18.9	15.7
15	17	19.9	19.1	15.2
16	17.1	20	18.7	15.7
17	17.4	19.9	19.1	16
18	17.9	19.5	19.5	15.7
19	17.5	20	19.4	15.9
20	17.8	20.4	19.3	15.9
21	17.7	20.1	19.5	16.3
22	17.9	20.2	19.8	16.2
23	17.6	20.2	17.7	15.8
24	16.9	20.3	18.1	15.2
25	17.7	20.4	18.1	
26	17.9	20.9	17.8	
27	18.2	21	17.1	
28	17.9	20.5		
29	18.8	20.8		
30	19.1	21.8		
31	18.5	21.4		
32	18.6	21.4		
33		20.5		
34		20.1		
35		18.4		
36		19		
Static	16.3	19.2	17.5	14.3

Notes: Food consumption, in grams per day for male rats, at the age (in months) listed. Last value in each column is the "static value" (60% of this amount is fed to the restricted animals for the rest of the life span). Genotypes listed are explained in the text.

*NIA = animals fed NIH-31, PUR = animals fed Purina 5010C.

Table 5. Female Rat Food Consumption Data

Age (months)	BN	F344 × BN F1	F344-NIH*	F344-PUR*
2	11.1	12.9	13.5	11.3
3	11.1	13.3	14.3	11.6
4	10.9	13.1	13.4	11
5	11.5	13.2	13.1	10.6
6	11.1	12.8	13.2	10.5
7	11.1	13.2	13	11.3
8	11.1	13.8	12.9	11.5
9	11.6	14.2	12.9	11.6
10	11.4	14.1	13.4	11.6
11	11.4	14.1	13.8	11.9
12	11.4	14.6	13.4	11.3
13	11.5	15	13.9	11.9
14	11.7	15.3	14.3	11.9
15	11.9	15.5	14.5	11.2
16	11.9	15.8	14.1	11.9
17	12.2	15.9	14.3	12.2
18	12.6	15.7	14.8	12.4
19	12.5	16.1	14.7	12.6
20	12.8	16.1	15	12.3
21	12.6	16	15.8	12.6
22	12.8	16.1	15.1	12.6
23	12.6	15.5	14.4	12.5
24	12.4	15.9	15.1	12.6
25	12.8	16.2	15.1	
26	12.9	16.7	15.1	
27	13	16.5	14.6	
28	13.1	16.4	14.4	
29	13.8	16.3	14.3	
30	14.2	17	14.4	
31	14	16.4	15	
32	14	17.3		
33		15.5		
34		15.4		
35		15.3		
36		15.1		
37				
Static	13.5	15.3	14.2	12.5

Notes: Food consumption, in grams per day for male rats, at the age (in months) listed. Last value in each column is the "static value" (60% of this amount is fed to the restricted animals for the rest of the life span). Genotypes listed are explained in the text.

*NIA = animals fed NIH-31, PUR = animals fed Purina 5010C.

Statistical analyses.—AL versus CR cohort comparisons were made using the SAS procedure Proc Lifetest (18). Kaplan-Meier survival distributions were computed, and a two-sided log rank test was used to compare survival times.

Results

The nominal 60% CR reduces BW, as shown in Figures 1–9. These BW growth curves are for the SG cohorts, the groups from which the animals shipped to investigators were drawn. It can be seen that male/female differences were generally much less in the CR groups for each genotype, especially for mice. A large portion of the animals were shipped before 24 months of age; therefore, the numbers of animals that provided data to the BW curves decline with time from shipment and sacrifice, as well as from the mortality resulting from aging.

Longevity

BW and longevity data for subsets of the LG cohort as well as the PG animals have been previously reported (1,3,9–11, 19–26). Survival curves of the various genotype-diet-sex cohorts are shown in Figures 10–18. These are the right censored mortality data collected on all animals within a cohort of mice or rats in each experiment. The mortality curves for all animals are presented because there were not many animals in the SG after 24 months of age, and presenting mortality curves. Except after 24 months of age, the majority of these animals were usually in the SG. CR extended longevity in every study, for both sexes ($p < .001$). The effect in D2 was least, with almost no effect up to 600 days of age in males. The largest percentage effect in mice was in B6-EM. This was the shortest-lived model, probably as a result of the fast growth by this strain when fed

Table 6. NIH-31 Diet, Nutrient Composition, and Rodent Requirements

Nutrient	Rat Requirement*	Mouse Requirement*	NIH-31† Nutrient Values
Vitamins			
A, retinol, IU	2,300	2,400	17,000
D, cholecalciferol, IU	1,000	1,000	3,881
E, RRR-α-tocopherol, mg	18.00	22.00	53.50
K, phylloquinone, mg	1.00	1.00	ND
Thiamine, mg	4.00	5.00	40.00
Riboflavin, mg	3.00	7.00	8.54
Niacin, mg	15.00	15.00	122.3
Choline, mg	750.00	2,000.00	3,380
Pantothenate, mg	10.00	16.00	33.60
Pyridoxine, mg	6.00	8.00	11.40
Folate, mg	1.00	0.50	2.48
Biotin, mg	0.20	0.20	0.62
B_{12}, μg	50.00	10.00	43.00
Minerals			
Calcium, g	5.00	5.00	14.30
Phosphorus, g	3.00	3.00	9.40
Potassium, g	3.60	2.00	6.90
Sodium, g	0.50	0.50	3.80
Chloride, g	0.50	0.50	6.00
Magnesium, g	0.50	0.50	2.50
Iron, mg	35.00	35.00	260.00
Zinc, mg	12.00	10.00	92.30
Manganese, mg	10.00	10.00	131.30
Copper, mg	5.00	6.00	11.70
Cobalt, mg	ND	ND	0.27
Iodine, μg	150.00	150.00	2,000.00
Molybdenum, μg	ND	150.00	1,600.00
Selenium, μg	ND	150.00	500.00
Protein, %	15.00	18.00	20.10
Fat, %	5.00	5.00	3.60
Kcal/g	ND	ND	4.33

*National Research Council (27) requirements for growing rats or mice; amount, per kg diet. ND = not determined.

†Post-autoclave nutrient values are analyses of 3 production lots. Kcal is gross energy measured by calorimetry.

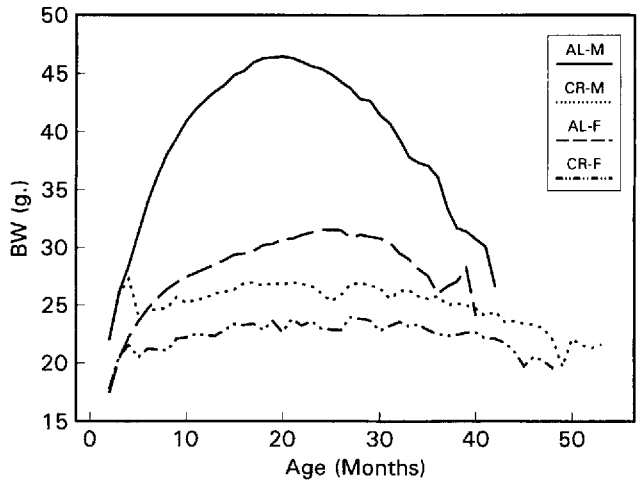

Figure 1. Body weight and age in B6D2F1 mice—Shipping Group. Average body weight (BW) for ad libitum (AL) and calorically restricted (CR) male AL (AL-M), female AL (AL-F), male CR (CR-M), and female CR (CR-F) mice at different ages (in months). Data early in the life span from over 2,000 (male) and 1,000 (female) animals.

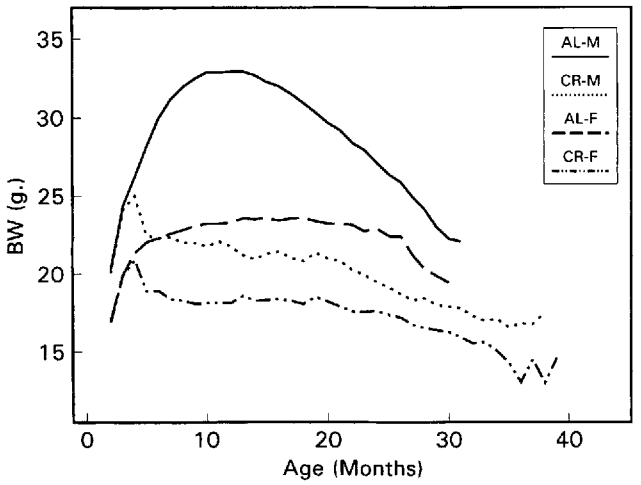

Figure 2. Body weight and age in D2 mice—Shipping Group. Average body weight (BW) for ad libitum (AL) and calorically restricted (CR) male AL (AL-M), female AL (AL-F), male CR (CR-M), and female CR (CR-F) mice at different ages (in months). Data early in the life span from over 1,000 (male) and 500 (female) animals. Note the difference in scale (BW) from Figure 1.

EM (3). The extension of survival of F344 rats by CR was larger in males fed PUR than when fed NIH. This was probably a result of starting CR earlier in the life of the animal, when it was more able to inhibit the onset of the mononuclear cell leukemia that strongly contributes to mortality in the males of this strain (23,24). The oldest male mouse, B6D2F1, lived to 1,628 days and the oldest female mouse, B6D2F1, lived to 1,529 days. The oldest male rat, F344 × BN F1, lived to 1,561 days, and the oldest female rat, BN, lived to 1,686 days (all these animals were from the CR cohorts).

Discussion

The NCTR/NIA collaboration conducted the largest animal study performed to date under the auspices of the Public Health Service. The study collected data on more than 60,000 individually housed rodents of seven different genotypes of two species, in both sexes, and approximately half were CR-fed. Approximately half the animals were shipped offsite to NIA investigators internationally, with the majority of the remainder maintained at NCTR until they spontaneously died. The collaboration supplied a choice of healthy, long-lived rodent models to investigators, while allowing for the development of some of the most definitive information on life span, food consumption, and growth characteristics in these genotypes under diverse feeding paradigms.

The utility of CR in extending life span ubiquitously lends further weight to the use of CR as a "gold standard" for evaluating and stimulating new intervention strategies in aging. In addition, elaboration of the mechanisms by which CR has its effects, because they are so generalized and consistent, will be useful in the application of CR across species.

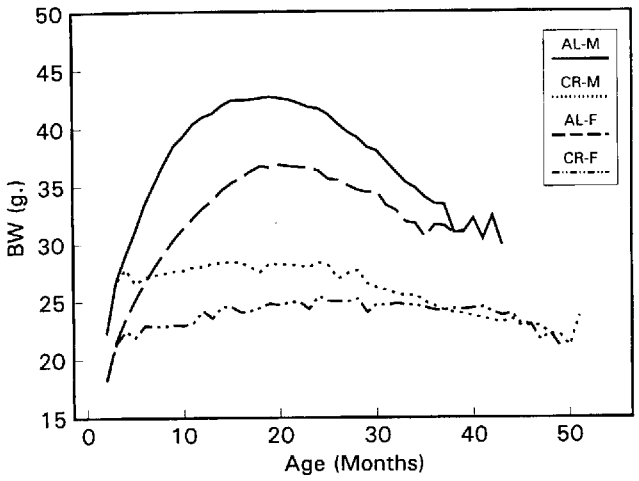

Figure 3. Body weight and age in B6C3F1 mice—Shipping Group. Average body weight (BW) for ad libitum (AL) and calorically restricted (CR) male AL (AL-M), female AL (AL-F), male CR (CR-M), and female CR (CR-F) mice at different ages (in months). Data early in the life span from over 1,500 (male) and 1,500 (female) animals.

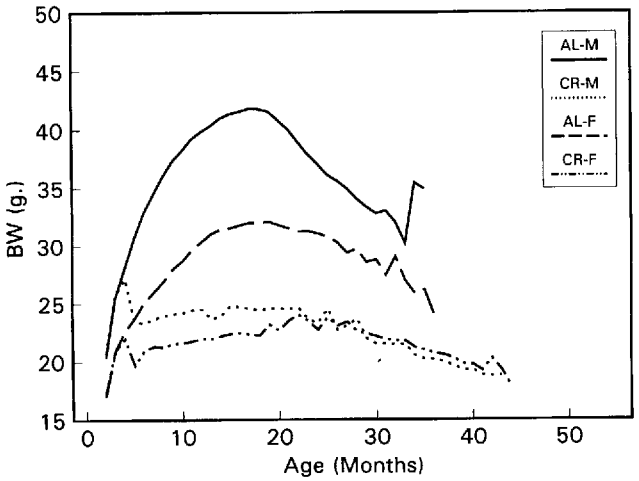

Figure 4. Body weight and age in B6-NIH mice—Shipping Group. Average body weight (BW) for ad libitum (AL) and calorically restricted (CR) male AL (AL-M), female AL (AL-F), male CR (CR-M), and female CR (CR-F) mice at different ages (in months). Data early in the life span from over 2,400 (male) and 1,100 (female) animals.

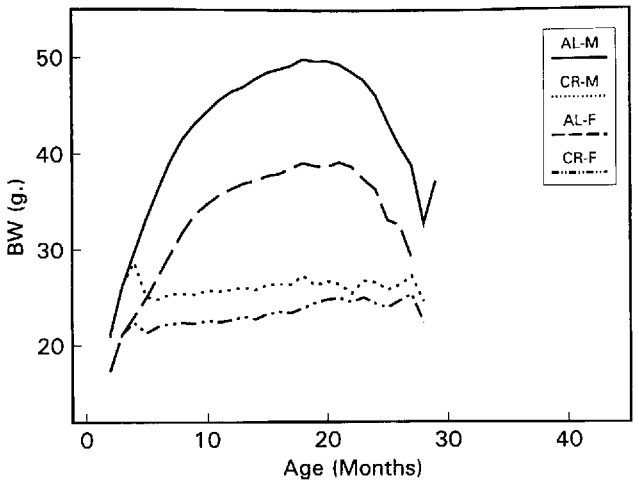

Figure 5. Body weight and age in B6-EM mice—Shipping Group. Average body weight (BW) for ad libitum (AL) and calorically restricted (CR) male AL (AL-M), female AL (AL-F), male CR (CR-M), and female CR (CR-F) mice at different ages (in months). Data early in the life span from over 1,700 (male) and 1,700 (female) animals. Note the difference in scale (BW) from Figure 4.

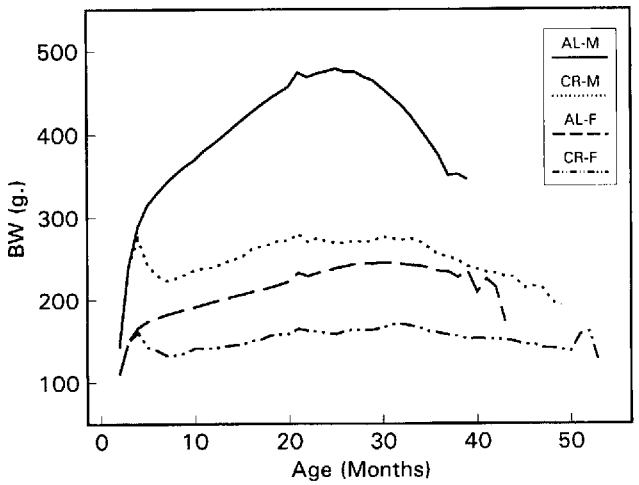

Figure 6. Body weight and age in BN rats—Shipping Group. Average body weight (BW) for ad libitum (AL) and calorically restricted (CR) male AL (AL-M), female AL (AL-F), male CR (CR-M), and female CR (CR-F) rats at different ages (in months). Data early in the life span from over 1,500 (male) and 1,500 (female) animals. Note the difference in scale (BN) from Figure 5.

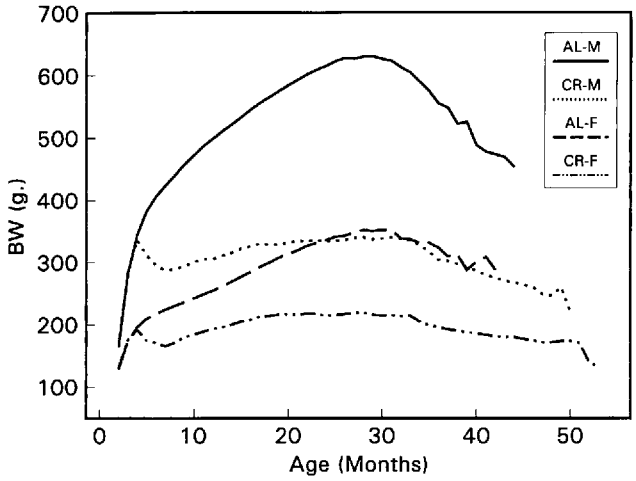

Figure 7. Body weight and age in F344 × BN F1 rats—Shipping Group. Average body weight (BW) for ad libitum (AL) and calorically restricted (CR) male AL (AL-M), female AL (AL-F), male CR (CR-M), and female CR (CR-F) rats at different ages (in months). Data early in the life span from over 2,000 (male) and 1,500 (female) animals. Note the difference in scale from Figure 6.

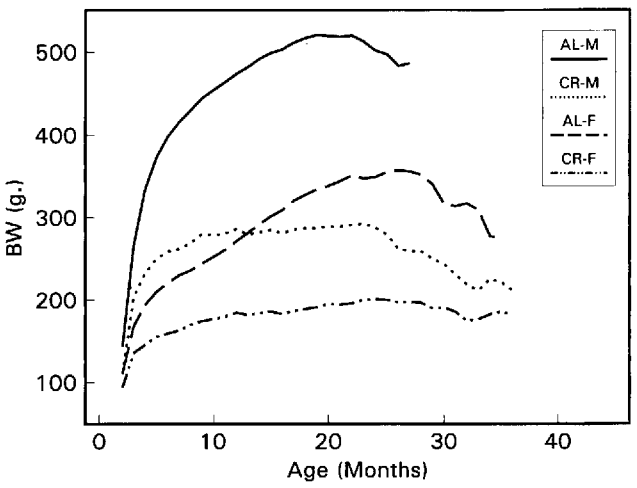

Figure 9. Body weight and age in F344-PUR rats—Shipping Group. Average body weight (BW) for ad libitum (AL) and calorically restricted (CR) male AL (AL-M), female AL (AL-F), male CR (CR-M), and female CR (CR-F) rats at different ages (in months). Data early in the life span from over 425 (male) and 525 (female) animals. Note difference in scale from Figure 7.

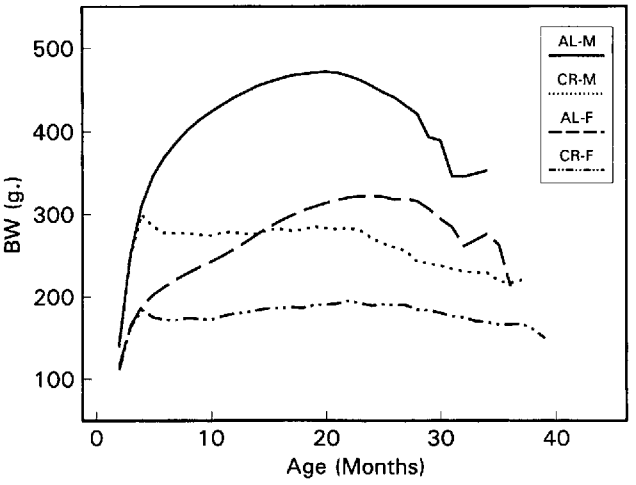

Figure 8. Body weight and age in F344-NIH rats—Shipping Group. Average body weight (BW) for ad libitum (AL) and calorically restricted (CR) male AL (AL-M), female AL (AL-F), male CR (CR-M), and female CR (CR-F) rats at different ages (in months). Data early in the life span from over 2,500 (male) and 1,700 (female) animals. Note the difference in scale from Figure 7.

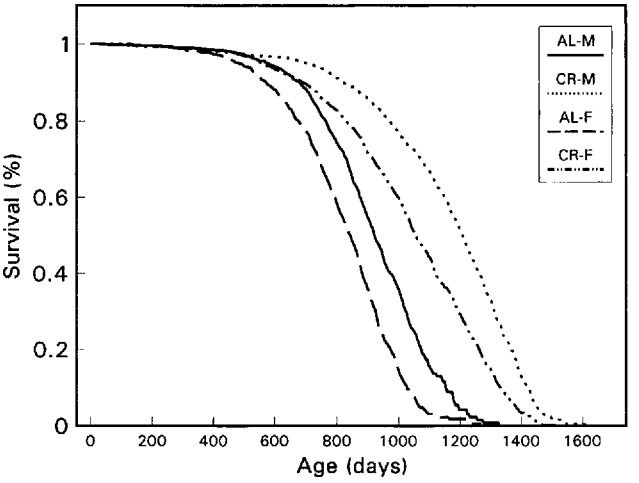

Figure 10. Survival and age in B6D2F1 mice—Total. Survival of ad libitum (AL) and calorically restricted (CR) male AL (AL-M), female AL (AL-F), male CR (CR-M), and female CR (CR-F) mice at different ages (in months). Data from Shipping Group augmented by data from Pathology and Longevity groups.

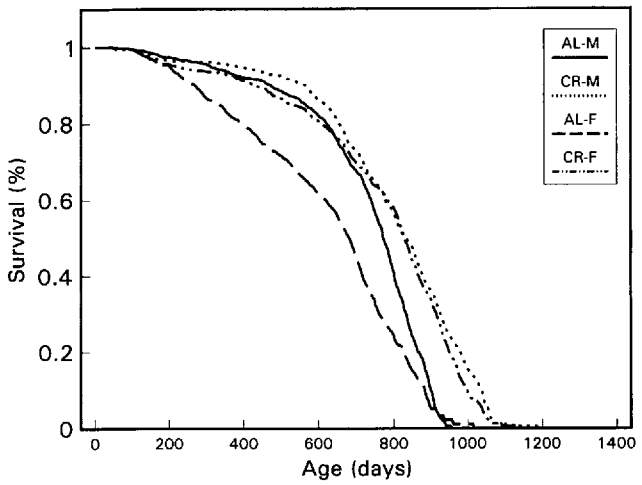

Figure 11. Survival and age in D2 mice—Total. Survival of ad libitum (AL) and calorically restricted (CR) male AL (AL-M), female AL (AL-F), male CR (CR-M), and female CR (CR-F) mice at different ages (in months). Data from Shipping Group augmented by data from Pathology and Longevity groups.

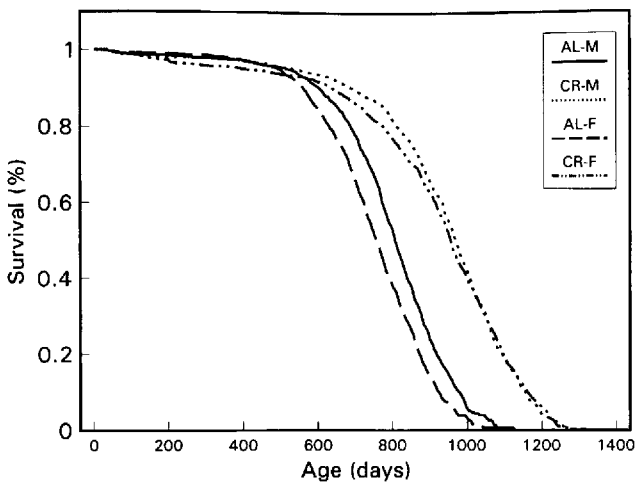

Figure 13. Survival and age in B6-NIH mice—Total. Survival of ad libitum (AL) and calorically restricted (CR) male AL (AL-M), female AL (AL-F), male CR (CR-M), and female CR (CR-F) mice at different ages (in months). Data from Shipping Group augmented by data from Pathology and Longevity groups.

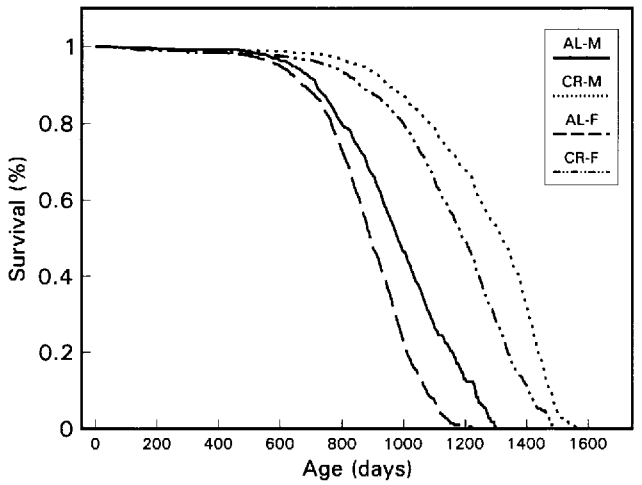

Figure 12. Survival and age in B6C3F1 mice—Total. Survival of ad libitum (AL) and calorically restricted (CR) male AL (AL-M), female AL (AL-F), male CR (CR-M), and female CR (CR-F) mice at different ages (in months). Data from Shipping Group augmented by data from Pathology and Longevity groups.

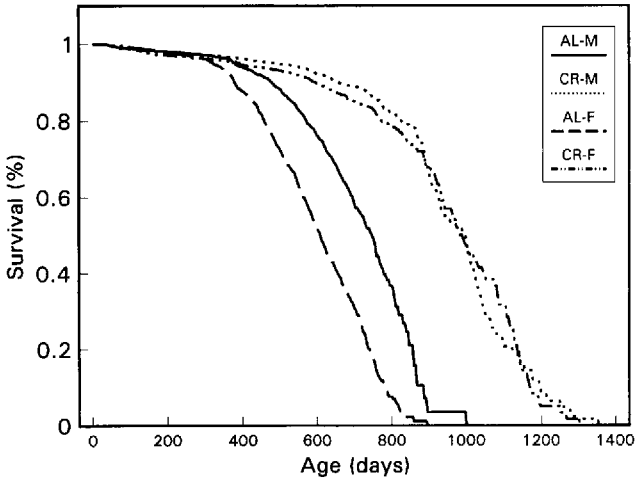

Figure 14. Survival and age in B6-EM mice—Total. Survival of ad libitum (AL) and calorically restricted (CR) male AL (AL-M), female AL (AL-F), male CR (CR-M), and female CR (CR-F) mice at different ages (in months). Data from Shipping Group augmented by data from Pathology and Longevity groups.

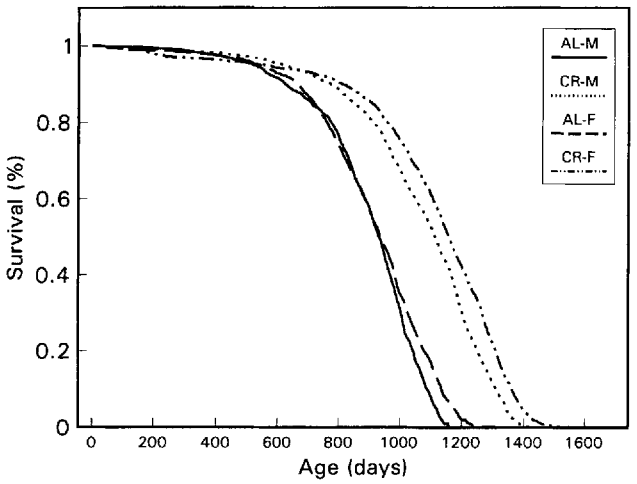

Figure 15. Survival and age in BN rats—Total. Survival of ad libitum (AL) and calorically restricted (CR) male AL (AL-M), female AL (AL-F), male CR (CR-M), and female CR (CR-F) rats at different ages (in months). Data from Shipping Group augmented by data from Pathology and Longevity groups.

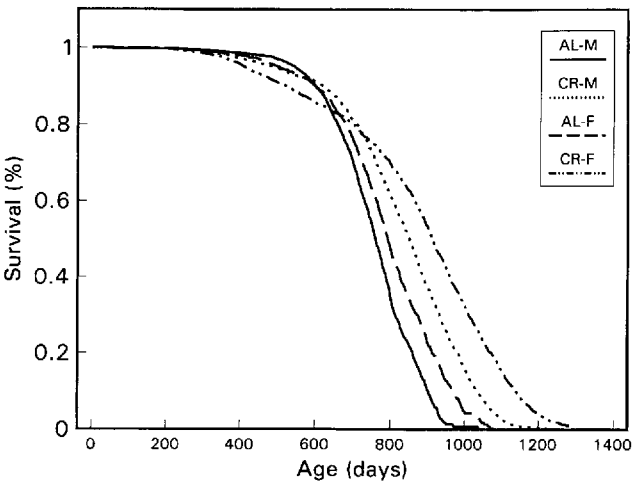

Figure 17. Survival and age in F344-NIH rats—Total. Survival of ad libitum (AL) and calorically restricted (CR) male AL (AL-M), female AL (AL-F), male CR (CR-M), and female CR (CR-F) rats at different ages (in months). Data from Shipping Group augmented by data from Pathology and Longevity groups.

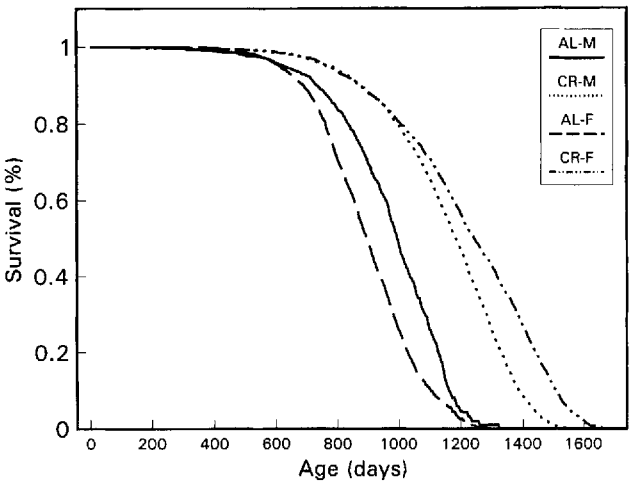

Figure 16. Survival and age in F344 × BN F1 rats—Total. Survival of ad libitum (AL) and calorically restricted (CR) male AL (AL-M), female AL (AL-F), male CR (CR-M), and female CR (CR-F) rats at different ages (in months). Data from Shipping Group augmented by data from Pathology and Longevity groups.

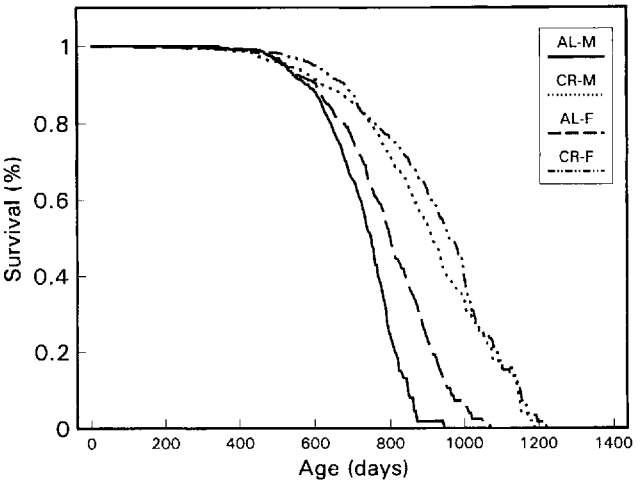

Figure 18. Survival and age in F344-PUR rats—Total. Survival of ad libitum (AL) and calorically restricted (CR) male AL (AL-M), female AL (AL-F), male CR (CR-M), and female CR (CR-F) rats at different ages (in months). Data from Shipping Group augmented by data from Pathology and Longevity groups.

Acknowledgments

A study of this scope can never appropriately acknowledge everyone. However, we especially thank Charles Schmieder and Rodney Coats, who were key to shipping animals to investigators, and Kathy Carroll, who was crucial to managing the data that were generated. Dr. Thomas Bucci and his staff, Dr. W. McCallum, and Warren Campbell and his staff were instrumental in evaluating and maintaining animal health. The study would not have been possible without the dedicated long-term commitment of the animal care and diet preparation personnel at NCTR.

Address correspondence to Dr. Angelo Turturro, Division of Biometry and Risk Assessment, National Center for Toxicological Research, 3900 NCTR Drive, Jefferson, AR 72079-9502. E-mail: aturturro@nctr.fda.gov

References

1. Allaben W, Chou M, Pegram R, et al. Modulation of toxicity and carcinogenicity by caloric restriction. *Korean J Toxicol.* 1990;6:167–182.
2. Weindruch R, Walford R. *The Retardation of Aging and Disease by Dietary Restriction.* Springfield, IL: CC Thomas; 1988.
3. Turturro A, Hart R. Dietary alteration in the rate of cancer and aging. *Exp Gerontol.* 1992;27:583–592.
4. Turturro A, Duffy P, Hart R. Antioxidation and evolution: dietary restriction and alterations in molecular processes. In: Basu T, Temple N, Garg N, eds. *Antioxidants in Human Health and Disease.* Oxford, England: CAB International; 1999;83–94.
5. Baker G III, Sprott R. Biomarkers of aging. *Exp Gerontol.* 1988;23:223–239.

6. Reff M, Schneider E. *Biological Markers of Aging*. Publication no. 82-2221. Washington, DC: NIH Press; 1982.
7. Witt W, Brand C, Attwood V, Soave O. A nationally supported study on caloric restriction in rodents. *Lab Animal*. 1989;18:37–48.
8. Hart R, Hass B, Turturro A, Lewis S. The use of nutrition to implement refinement in animal bioassays. *Lab Animal*. 1996;25:29–34.
9. Lewis S, Leard B, Turturro A, Hart R. Long-term housing of rodents under specific pathogen-free barrier conditions. In: Yu BP, ed. *Methods in Aging Research*. Boca Raton, FL: CRC Press; 1999:217–235.
10. Thurman J, Bucci T, Hart R, Turturro A. Survival, body weight, and spontaneous neoplasms in ad libitum fed and dietary restricted Fischer 344 rats. *Toxicol Pathol*. 1994;22:1–9.
11. Sheldon W, Bucci T, Hart R, Turturro A. Age-related neoplasia in a lifetime study of ad libitum-fed and food restricted B6C3F1 mice. *Toxicol Pathol*. 1995;23:458–476.
12. Loeb W, Das S, Harbour L, Turturro A, Bucci T. Clinical biochemistry. In: Mohr U, Dungworth D, Capen C, Carlton W, Sundberg J, Ward J, eds. *Pathobiology of the Aging Mouse, Vol. 1*. Washington, DC: ILSI Press; 1996:3–20.
13. Soave O, Brand D. Coprophagy in animals: a review. *Cornell Veterinarian*. 1991;81:357–364.
14. Duffy P, Feuers R, Pipkin J, et al. The effect of caloric modulation and aging on the physiological response of rodents to drug toxicity. In: Hart R, Neuman D, Robertson R, eds. *Dietary Restriction: Implications for the Design and Interpretation of Toxicity and Carcinogenicity Studies*. Washington, DC: ILSI Press; 1995;127–140.
15. Garthwaite S, Cheng H, Bryan J, Craig B, Holloszy J. Ageing, exercise and food restriction: effects on body composition. *Mech Ageing Dev*. 1986;36:187–196.
16. Duffy P, Feuers R, Leakey J, Nakamura K, Turturro A, Hart R. Effect of chronic caloric restriction on physiological variables that modulate energy metabolism in the male Fischer-344 rat. *Mech Ageing Dev*. 1989;48:117–133.
17. Kristal B, Yu BP. Dietary restriction augments protection against induction of the mitochondrial permeability transition. *Free Rad Biol Med*. 1998;24:1269–1277.
18. SAS (Statistical Analysis System). *SAS/STAT User's Guide, Volume 2*. Cary, NC: SAS Institute; 1990:1027–1069.
19. Sprott RL, Austad SN. Animal models for aging research. In: Schneider E, Rowe J, eds. *Handbook of the Biology of Aging*. 4th ed. San Diego, CA: Academic Press; 1996;3–23.
20. Sheldon W, Bucci T, Blackwell B, Turturro A. Effect of ad libitum feeding and 40% feed restriction on body weight, longevity, and neoplasms in B6C3F1, C57Bl6, and B6D2F1 mice. In: Mohr U, Dungworth D, Capen C, Carlton W, Sundberg J, Ward J, eds. *Pathobiology of the Aging Mouse, Vol. 1*. Washington, DC: ILSI Press; 1996:21–26.
21. Blackwell B-N, Bucci T, Hart R, Turturro A. Longevity, body weight, and neoplasia in ad libitum fed and diet-restricted C57Bl6 mice fed NIH-31 open formula diet. *Toxicol Pathol*. 1995;23:570–582.
22. Turturro A, Hart R. Longevity-assurance mechanisms and caloric restriction. *Ann NY Acad Sci*. 1991;621:363–372.
23. Turturro A, Blank K, Murasko D, Hart R. Mechanisms of caloric restriction affecting aging and disease. *Ann NY Acad Sci*. 1994;719:159–170.
24. Turturro A, Hart R. Modulation of toxicity by diet: implications for response at low-level exposures. In: Calabrese E, ed. *Biological Effects of Low Level Exposures: Dose-Response Relationships*. Chelsea, MI: Lewis Publishers; 1994:143–152.
25. Turturro A, Duffy P, Hart R. The effect of caloric modulation on toxicity studies. In: Hart R, Neuman D, Robertson R, eds. *Dietary Restriction: Implications for the Design and Interpretation of Toxicity and Carcinogenicity Studies*. Washington, DC: ILSI Press; 1995:79–98.
26. Turturro A, Hass B, Hart R, Allaben W. Body weight impact on spontaneous and agent-induced diseases in chronic bioassays. *Int J Toxicol*. 1998;17:79–100.
27. NRC (National Research Council). *Nutrient Requirements of Laboratory Animals*. 4th rev. Washington, DC: National Academy Press; 1995.

Received June 1, 1999
Accepted June 28, 1999