# The Retardation of Aging in Mice by Dietary Restriction: Longevity, Cancer, Immunity and Lifetime Energy Intake[1]

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ABSTRACT We sought to clarify the impact of dietary restriction (undernutrition without malnutrition) on aging. Female mice from a long-lived strain were fed after weaning in one of six ways: *group 1*) a nonpurified diet ad libitum; 2) 85 kcal/wk of a purified diet (~25% restriction); 3) 50 kcal/wk of a restricted purified diet enriched in protein, vitamin and mineral content to provide nearly equal intakes of these essentials as in group 2 (~55% restriction); 4) as per group 3, but also restricted *before* weaning; 5) 50 kcal/wk of a vitamin- and mineral-enriched diet but with protein intake gradually reduced over the life span; 6) 40 kcal/wk of the diet fed to groups 3 and 4 (~65% restriction). Mice from groups 3–6 exhibited mean and maximal life spans 35–65% greater than for group 1 and 20–40% greater than for group 2. Mice from group 6 lived longest of all. The longest lived 10% of mice from group 6 averaged 53.0 mo which, to our knowledge, exceeds reported values for any mice of any strain. Beneficial influences on tumor patterns and on declines with age in T-lymphocyte proliferation were most striking in group 6. Significant positive correlations between adult body weight and longevity occurred in groups 3–5 suggesting that increased metabolic efficiency may be related to longevity in restricted mice. Mice from groups 3–6 ate ~30% more calories per gram of mouse over the life span than did mice from group 2. These findings show the profound anti-aging effects of dietary restriction and provide new information for optimizing restriction regimes. J. Nutr. *116*: 641–654, 1986.

INDEXING KEY WORDS dietary restriction • aging • longevity • cancer • immunity

Dietary restriction started on either weanling or middle-aged mice or rats increases maximum longevity (1–4), reduces the incidence and delays the onset of several cancers and other late-life diseases (3–7), and retards changes in diverse indexes of biologic age (8–9). The extension of maximum life span by weaning-initiated restriction is more reliable and robust than that brought on by any other methods that have been used in an attempt to retard aging in rodents (10–12). The effect appears to depend on a restriction of energy intake (a 25–50% decrease from ad libitum levels in most studies) combined with adequate intakes of essential nutrients (undernutrition without malnutrition).

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The mechanism by which dietary restriction retards aging is unknown. Suggested mechanisms reflect the various theories of aging and include a delay of immunologic aging (10, 13), decreased free radical generation followed by reduced losses of mitochondria with age (9, 14), preservation of protein synthesis capacities in old age (15), neuroendocrine effects (16), as well as others (8).

Optimal nutrient composition and feeding strategies for these low energy, life span–extending diets are not yet established. In our past studies and in the present one, restricted mice ate a purified diet on an intermittent basis, a diet enriched (relative to the diet fed to controls) in protein, vitamins and salts. The enriched diet results in nearly equal per week intakes of these essentials for restricted and control mice. Other workers have fed restricted animals diets enriched only in vitamins (17). Still others have fed restricted and control animals the same nonenriched diet (18, 19).

In the present study, weanling female mice from a long-lived hybrid strain were subjected to one of four different regimens of dietary restriction, or one of two more normal diets. Experimental variables tested included the degree of underfeeding, the protein content of the restricted diet, and preweaning restriction. Longevity, cancer incidence and an age-sensitive immune response were investigated. Also, searches for correlations between body weight and longevity and cancer susceptibility were made. Effects of diet on lifetime energy intake were also determined. We report extreme longevities attained by certain of the restricted mice, which, to our knowledge, exceed those previously reported for laboratory mice.

## METHODS

*Mice.* A long-lived $F_1$ hybrid strain (C3B10RF$_1$) was studied. Females from the C3H.SW/Sn inbred strain were mated with males from the C57BL10.RIII/Sn inbred strain in our animal facility. Female hybrid progeny were weaned at 21–28 d of age, individually caged in plastic cages on wood chip bedding, and assigned to one of six diet regimens. The mice were maintained under conventional (nonbarrier) conditions with temperature (20–24 °C), humidity (50–60%) and lighting (0600–1800) constant throughout this study. To monitor for infectious diseases, sentinel mice were kept in the same room as the experimental mice, and serum samples were screened every 6 mo for titers against a panel of 11 common pathogens. Positive titers were not found during this study.

*Diets.* Six diet groups were studied. *1*) NP: Mice in this group ate a nonpurified diet (NP, Purina Laboratory Chow, Ralston Purina, St. Louis, MO) ad libitum since being weaned at 24 d of age. [Until being weaned and except for group 4 (see below) all mice were allowed free access to their mothers, and the NP diet was provided to the mothers.] The NP diet has a guaranteed minimum analysis of 23% protein and 4.5% fat. These mice were selected from litters of no more than four mice. This population was not considered part of our experimental or control groups but was included by way of reference as most mouse populations are fed this type of diet. *2*) N/N$_{85}$: Mice in this group were fed "normally" (N) both before and after weaning, with the postweaning ration being ~85 kcal/wk (1 cal = 4.18 J). N/N$_{85}$ mice ate a purified, powdered, 20% casein diet (table 1, diet 1) after weaning (d 21) in amounts ~25% less than if given free access to this diet. The ad libitum intake of this diet for 10 female mice at 4–5 mo of age averaged 110 kcal/wk. N/N$_{85}$ mice were fed seven feedings (3.0–3.2 g) per week (one daily feeding on Monday through Thursday mornings; a triple feeding on Friday morning). In contrast to the NP group, mice from litters of any size were used to populate the N/N$_{85}$ cohort. This was our control population, fed at less than ad libitum intake so that total food consumption of the controls would be constant. *3*) N/R$_{50}$: This group was fed normally before weaning and then restricted to ~50 kcal/wk of a purified, powdered, 35% casein diet enriched also in vitamin and mineral content (table 1, diet 2). Each mouse was fed four feedings (3.0–3.2 g) per week (one daily feeding on Monday and Wednesday mornings; a double feeding on Friday morning). The mice in this group ate approximately the same amount of protein, vitamins and minerals per week as did N/N$_{85}$ mice.

TABLE 1

*Composition of diets*

| Ingredient[1] | Diet 1[2] | | Diet 2[3] | |
|---|---|---|---|---|
| | *g/kg diet* | *g/(mouse · wk)* | *g/kg diet* | *g/(mouse · wk)* |
| Casein, vitamin-free test | 200.0 | 4.3 | 350.0 | 4.3 |
| Cornstarch | 260.8 | 5.7 | 157.6 | 2.0 |
| Sucrose | 260.8 | 5.7 | 157.6 | 2.0 |
| Corn oil | 135.0 | 2.9 | 135.0 | 1.7 |
| Mineral mixture, USP XIV[4] | 60.0 | 1.3 | 110.0 | 1.4 |
| Fiber | 56.4 | 1.2 | 40.0 | 0.5 |
| Vitamin mixture[5] | 23.0 | 0.50 | 42.2 | 0.52 |
| Brewer's yeast[6] | 4.0 | 0.09 | 7.4 | 0.09 |
| Zinc oxide | 0.05 | $1.1 \times 10^{-3}$ | 0.1 | $1.2 \times 10^{-3}$ |

[1]These ingredients were purchased from ICN Pharmaceuticals (Cleveland, OH): casein (95.5–97.1% crude protein, catalog no. 904520), salt mixture (catalog no. 902850), fiber (cellulose, catalog no. 900453), vitamin mixture (catalog no. 904654) and Brewer's yeast (catalog no. 103312). Cornstarch, sucrose and corn oil [Mazola (Best Foods, Englewood Cliffs, NJ)] were purchased locally. Zinc oxide was purchased from Sigma Chemical Co. (St. Louis, MO). Diets were mixed about once monthly in 20-kg batches by using a Blakeslee CC80 mixer (Chicago, IL) and stored at 4°C until fed. [2]Diet 1: Diet fed to $N/N_{85}$ mice as seven 3.0- to 3.2-g feedings per week providing ~85 kcal/wk. Composition is given as grams of ingredient/kilogram diet. The per week intake of each ingredient for each mouse is also given. Feeding occurred between 0700 and 0900 (one daily feeding on Monday through Thursday, three feedings on Friday). All food was regularly consumed. The caloric density of this diet was 4.1 kcal/g. [3]Diet 2: Diet fed to restricted mice. A purified diet enriched in casein, vitamin and mineral mixtures, brewer's yeast and zinc oxide. Fed as four 3.0- to 3.2-g feedings per week providing ~50 kcal/wk [groups $N/R_{50}$, $R/R_{50}$ and $N/R_{50lopro}$ (until 4 mo old when switched to a 25% casein diet)] or four 2.4- to 2.6-g feedings per week providing ~40 kcal/wk (group $N/R_{40}$). Feeding occurred between 0700 and 0900 (one daily feeding on Monday and Wednesday, two feedings on Friday). All food was regularly consumed. The caloric density of this diet was 3.9 kcal/g. [4]The composition of the USP XIV mineral mixture (in percent) was calcium carbonate, 6.86; calcium citrate, 30.83; calcium biphosphate (monobasic), 11.28; manganese carbonate, 3.52; magnesium sulfate, 3.83; potassium chloride, 12.47; dipotassium phosphate, 21.88; sodium chloride, 7.71; copper sulfate, 0.0078; ferric citrate, 1.53; manganous sulfate, 0.02; potassium aluminum sulfate, 0.01; potassium iodide, 0.004; and sodium fluoride, 0.05. [5]The composition (grams/kilogram diet) of the ICN Vitamin Diet Fortification Mixture was retinyl acetate (500 IU/g), 1.8; ergocalciferol (850,000 IU/g), 0.125; α-tocopheryl acetate (250 IU/g), 22.0; ascorbic acid, 45.0; inositol, 5.0; choline chloride, 75.0; menadione, 2.25; *p*-aminobenzoic acid, 5; niacin, 4.25; riboflavin, 1; pyridoxine · HCl, 1.0; thiamin · HCl, 1.0; calcium pantothenate, 3.0; biotin, 0.02; folic acid, 0.09; and vitamin B-12, 0.001. [6]The composition of ICN's brewer's yeast in percent was protein, 43.0; fat, 1.5; fiber, 1.5; ash, 7.0; and moisture, 5.0. The vitamin levels (mg/kg) were thiamin, 80.0; riboflavin, 35.3; niacin, 499.3; pantothenic acid, 121.7; pyridoxine, 49.8; choline, 4850.2; betaine, 1199.3; biotin, 1.1; folic acid, 15.4; and inositol, 4993.5. The mineral levels were 0.1% calcium, 1.5% phosphorus, 0.9% potassium, 50 ppm iron, 35 ppm copper, 5 ppm manganese, 2 ppm cobalt and 39 ppm zinc.

Mice of this cohort were from litters of any size, weaned at 21 d of age. *4)* $R/R_{50}$: Mice in this group were restricted both before weaning and after weaning (~50 kcal/wk). These mice were selected from litters of at least five (in an attempt to restrict in utero nutrition) and separated from their mother (and their mother's diet) every other day from d 7 until weaned on d 28. They were then fed diet 2 (as per cohort $N/R_{50}$). *5)* $N/R_{50lopro}$: These mice were restricted after weaning to ~50 kcal/wk with dietary protein content decreasing with advancing age. This cohort was fed the same amount of food and via the same schedule as $N/R_{50}$ and $R/R_{50}$. The mice ate diet 2 (35% casein) from weaning until 4 mo, a 25% casein diet from 4 until 12 mo, 20% casein from 12 until 24 mo, and 15% casein from 24 mo until death. Diet 2 was modified to form the 15, 20 and 25% casein diets by replacing casein with carbohydrate (equal amounts of sucrose and cornstarch). Mice were from litters of any size and weaned on d 21. *6)* $N/R_{40}$: This group was more severely restricted (~40 kcal/wk) in postweaning intake of diet 2. Mice were fed 2.4–2.6 g per feeding via the same schedule used for the other restricted

groups. Animals were from litters of any size and weaned on d 21.

Calculations were carried out to determine if these diets met the NRC requirements (20). Adequate per week intakes were found for all nutrients except chromium.

*Body and organ weights.* All mice in the longevity study were weighed once monthly during the initial 4 mo after weaning and about every other month thereafter. Body weight was always recorded on either a Monday or Wednesday afternoon in order to avoid comparison of restricted mice on fasting days with controls on feeding days. Organ wet weights were measured in adult and old mice from groups $N/N_{85}$, $N/R_{50}$, $N/R_{50lopro}$ and $N/R_{40}$ when killed for immunologic study.

*Autopsy and histopathology.* All mice were checked daily for any deaths. Dead mice were immediately stored at −15 to −20°C until examined for the presence of cancer via gross autopsy. All abnormal appearing tissues were further examined microscopically after fixation in 10% buffered formaldehyde, routine processing, and staining with hematoxylin and eosin.

*Lymphocyte proliferation to mitogens.* Mice were killed by cervical dislocation the day after all groups were fed (i.e., Tuesday or Thursday), to avoid influences of the fasting interval. The spleen was asceptically removed, weighed, and placed in cold media [RPMI-1640 (Microbiological Associates, Los Angeles, CA)] supplemented with antibiotics [100 U/ml penicillin, 100 μg/ml streptomycin (Gibco, Grand Island, NY)]. Spleens were studied individually. Only mice appearing free of disease were investigated for immune parameters. Each spleen was gently pressed through a sterile aluminum mesh screen with a tuberculin syringe plunger. The cell suspensions were washed once, and erythrocytes were lysed with 7% ammonium chloride. Suspensions were then washed three times in medium, counted in a hemocytometer, and adjusted to $6 \times 10^6$ viable cells/milliliter in 95% RPMI + 5% heat-inactivated fetal calf serum (FCS, Gibco). Lymphocyte viability ranged from 80 to 95% as judged by trypan blue exclusion. The in vitro proliferative responses to lymphocyte mitogens were tested via a microculture method (21) to assay mitogen-induced DNA synthesis. Cell suspensions ($6 \times 10^5$ cells in 0.1 ml) were added to microtiter plates (Scientific Products, Santa Ana, CA) along with either 0.1 ml of RPMI + 5% FCS alone or with mitogen. Two T-cell mitogens [phytohemagglutinin (PHA, Difco Labs, Detroit, MI) and concanavalin A (Con A, Miles-Yeda, Kankakee, IL)] and a B-cell mitogen [purified protein derivative (PPD, Statens Seruminstitute, Copenhagen, Denmark)] were tested. Mitogen doses used (PHA, 2.5 μl/ml; Con A, 2.0 μg/ml; PPD, 1 mg/ml) were found in preliminary studies to optimally stimulate spleen cells from 3- to 4-mo-old $C3B10RF_1$ mice eating the nonpurified diet ad libitum. Rigorous mitogen dose–response studies were not carried out in the restricted mice because earlier work did not detect differences with underfeeding (22). Triplicate cultures were carried out for each mitogen stimulated and control test. Total incubation time was 48 h with cultures labelled the last 24 h with 2 μCi of tritiated thymidine ([$^3$H]TdR, 1.9 Ci/mmol activity, Schwarz-Mann, Orangeburg, NJ) in 0.05 ml media/well. Cultures were harvested on glass fiber filters by means of a Multiple Automated Sample Harvester (M.A.S.H. II, Microbiological Associates), washed free of soluble [$^3$H]TdR, and prepared for liquid scintillation counting. Results are reported as (cpm in stimulated cultures) minus (cpm in control cultures) with values rounded to the nearest integer multiple of 25.

*Lifetime energy intake (LEI).* For each mouse, except those in the unrestricted NP group, the known daily caloric intake was divided by the average of adult body weights (measured at ages 5–6, 9–11, 14–17 and 21–23 mo), to obtain kilocalorie/gram per day. This ratio was then multiplied by the life span of the mouse in days, yielding the LEI in kilocalorie/gram per lifetime. LEI per mouse (kilocalories/mouse per lifetime) or kilocalories/(mouse • lifetime) was calculated for each mouse by multiplying its life span (days) by the daily kilocalorie intake.

*Statistical analyses.* For statistical comparisons among the groups we used chi-square tests for dichotomous data (the tumor incidence data) and one-way analysis of variance for other data items. Duncan's multiple-range test (23) was applied when analysis of variance indicated significant

differences. Incidence comparisons used chi-square tests on subtables. SAS programs (24) were used for statistical comparisons.

## RESULTS

*Body weight.* Body weights for mice in the six diet groups are described in figure 1. The NP and $N/N_{85}$ mice gained weight most rapidly during the initial 2 mo after weaning. Then, the NP mice (eating ad libitum) began to weigh more than the $N/N_{85}$ mice (restricted fed). Peak body weight averaged 45–50 g for NP, 35–40 g for $N/N_{85}$ and 20–25 g for the more severely restricted mice. The body weight of $N/R_{40}$ mice was less than that of the other restricted groups during the first two years of life but not thereafter. Influences of preweaning restriction on body weight were apparent for only a brief time (~1 mo) after weaning.

*Organ weights.* Organ weights were determined in adult (10–11 mo) and old (27–31 mo) mice that were used for the immunologic study. Four diet groups ($N/N_{85}$, $N/R_{50}$, $N/R_{50lopro}$, $N/R_{40}$) were studied at each age. As shown in table 2, liver weight was higher in $N/N_{85}$ mice at either age than in mice from any of the three restricted groups. Kidney weight increased with age in all groups except $N/R_{40}$ and was greatest in $N/N_{85}$ mice, lower in $N/R_{50}$ mice (but not significantly so in young mice), and lowest in $N/R_{50lopro}$ and $N/R_{40}$ mice. Spleen weight increased significantly with age only in $N/N_{85}$ and $N/R_{50lopro}$ mice and was largest in $N/N_{85}$ mice at either age. Thymus weight fell with age in all groups but $N/R_{50lopro}$. At 10–11 mo of age, the thymus weight of the $N/N_{85}$ mice was greater than that of the restricted mice. No intergroup differences were observed for thymus weights of old mice.

The organ weight data in table 2 can be used to calculate organ weight–body weight ratios. The liver–body weight ratio and the

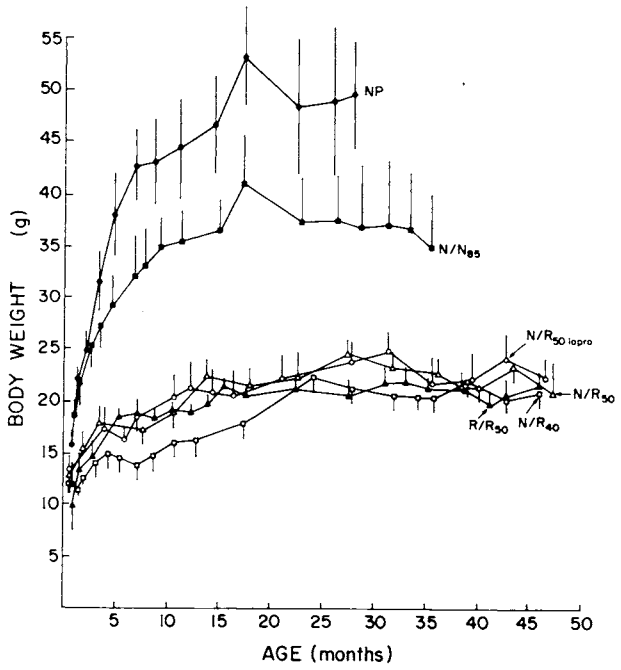


Fig. 1 Influences of diet on body weight. Values represent mean ± SD body weight in grams for all mice alive in each group at the indicated age. Diet groups: NP, fed nonpurified diet (reference group); $N/N_{85}$, fed normally before and after weaning, postweaning fed diet 1 at ~85 kcal/wk (25% less than ad libitum) (controls); $N/R_{50}$, fed normally before weaning, after weaning fed a diet enriched in vitamins and minerals (diet 2) because of their restricted intake (~50 kcal/wk, fed about every other day); $R/R_{50}$, restricted in feeding levels before and after weaning; $N/R_{50lopro}$, restricted after weaning to ~50 kcal/wk with a decrease in protein content with age; $N/R_{40}$, restricted after weaning to ~40 kcal/wk of diet 2, schedule as for $R_{50}$.

TABLE 2

*Organ weights*[1,2]

| Group | Organ weight | | | |
|---|---|---|---|---|
| | Liver | Kidney | Spleen | Thymus |
| | g | | | |
| *Adult (10–11 mo)* | | | | |
| $N/N_{85}$ | 1.51 ± 0.15[a] | 0.186 ± 0.006[a] | 0.097 ± 0.002[a] | 0.026 ± 0.002[a] |
| $N/R_{50}$ | 1.05 ± 0.02[b] | 0.172 ± 0.011[ab] | 0.040 ± 0.002[b] | 0.016 ± 0.003[bc] |
| $N/R_{50lopro}$ | 0.98 ± 0.02[b] | 0.147 ± 0.002[c] | 0.036 ± 0.002[b] | 0.011 ± 0.002[cd] |
| $N/R_{40}$ | 0.97 ± 0.02[b] | 0.156 ± 0.004[cd] | 0.037 ± 0.003[b] | 0.018 ± 0.004[b] |
| *Old (27–31 mo)* | | | | |
| $N/N_{85}$ | 1.54 ± 0.07[a] | 0.224 ± 0.004[e] | 0.158 ± 0.020[c] | 0.010 ± 0.001[cd] |
| $N/R_{50}$ | 1.06 ± 0.06[b] | 0.202 ± 0.006[f] | 0.054 ± 0.003[bd] | 0.009 ± 0.001[d] |
| $N/R_{50lopro}$ | 1.02 ± 0.02[b] | 0.178 ± 0.011[ab] | 0.065 ± 0.007[d] | 0.010 ± 0.001[cd] |
| $N/R_{40}$ | 0.92 ± 0.02[b] | 0.169 ± 0.008[bd] | 0.046 ± 0.002[bd] | 0.010 ± 0.001[cd] |

[1]Values are means ± SEM for $n = 8$ mice in each diet/age group. For group descriptions, see text. The body weights for the adult mice were 37.2 ± 1.9 g for $N/N_{85}$, 20.9 ± 0.8 g for $N/R_{50}$, 19.7 ± 0.5 g for $N/R_{50lopro}$ and 19.8 ± 0.7 g for $N/R_{40}$. For old mice, the body weights for these groups were (respectively) 41.5 ± 1.1, 24.1 ± 0.5, 24.8 ± 0.5 and 21.5 ± 0.6 g. [2]Statistical significance of differences between means was evaluated by Duncan's multiple-range test, which was applied when one-way analysis of variance indicated significant differences. Means in each column not sharing a common superscript letter were significantly different ($P = 0.05$).

kidney–body weight ratio were higher in the restricted mice than in the $N/N_{85}$ mice at both ages. The liver–body weight ratio fell with age in all groups, whereas the kidney–body weight ratio was stable. The spleen–body weight ratio was lower in restricted mice than in $N/N_{85}$ mice and tended to increase with age in all groups. The thymus:body weight ratio fell with age in all groups and was not overtly influenced by dietary restriction.

*Longevity.* The longevity of mice in the six diet groups is shown in figure 2 and table 3. Each group consisted of 49–71 mice. Mortality during the first 2 mo after weaning is not shown in figure 2 but was very low (<5%) in all groups. Mean life span was shortest for NP mice (~27 mo), longer for $N/N_{85}$ mice (~33 mo), even longer for $N/R_{50lopro}$ mice (~40 mo) and longest for the three other restricted groups (42–43 mo for $N/R_{50}$ and $R/R_{50}$, ~45 mo for $N/R_{40}$). An index of maximum life span is the mean life span of the longest-lived 10% in each group, and this ranked in the same order: ~35 mo for NP, ~40 mo for $N/N_{85}$, 48–49 mo for $N/R_{50lopro}$, ~51 mo for $N/R_{50}$ and $R/R_{50}$, and ~53 mo for $N/R_{40}$. The longest-lived individual mouse was from group $N/R_{40}$ that lived 54.6 mo.

*Cancer incidence.* Influences of diet on overall cancer incidence are shown in table 4. Cancer incidence was highest for $N/N_{85}$ mice (78%) and lowest for $N/R_{40}$ mice (38%). Mice restricted in any of the four ways described above did not differ from NP mice in overall tumor incidence. The mean longevity for tumor-bearing mice in group NP was greater than that of tumor-free NP mice ($P < 0.01$). Early deaths in the NP group occurred largely in tumor-free mice, which were among the most obese. The longevity of tumor-bearing versus tumor-free mice was not significantly different in the other five groups.

Effects of diet on the incidence of the three most common tumors (lymphoma, hepatoma and soft tissue neoplasms) and on the longevity of mice bearing each tumor type are shown in table 5. Lymphoma incidence was highest in the $N/N_{85}$, NP and $N/R_{50lopro}$ groups. Longevity for lymphoma-bearing mice was increased by ~8–12 mo in the four restricted groups. Hepatoma occurred most frequently in $R/R_{50}$ and least commonly in NP mice. Hepatoma-bearing

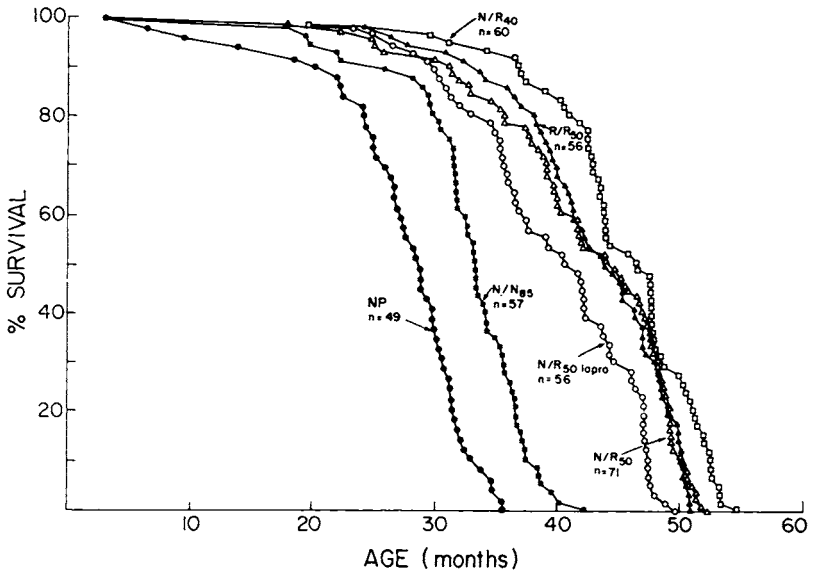


Fig. 2 Influences of diet on survival. Each symbol represents an individual mouse. Diet groups, see fig. 1 or text.

mice from the four most restricted groups showed greater mean life spans (~6–15 mo) than did mice from the other two groups. Soft tissue tumors (mostly sarcomas and breast tumors) and lung tumors (2–7% incidence in the six groups) were far less frequently encountered than were lymphoma and hepatoma.

*Correlations between life span, cancer incidence and body weight.* These correlations were determined with data from individual mice. Body weights (BW) from five ages were analyzed: weaning ($BW_W$), 5–6 mo ($BW_5$), 9–11 mo ($BW_{10}$), 14–17 mo ($BW_{15}$) and 21–23 mo ($BW_{22}$). Correlation coefficients were calculated to determine associations between body weight and life span. Data are shown in table 6. No correlation between weaning body weight and longevity reached statistical significance. In contrast, certain of the adult body weights showed a significant positive correlation with life span, but only in the restricted groups. This positive correlation was first seen with $BW_{10}$ for the $N/R_{50lopro}$ mice, and continued to be positive for this group at 15 mo before losing statistical significance at 22 mo. For $N/R_{50}$ mice, $BW_{15}$ showed a significant positive correlation with life span, whereas $BW_{22}$ nearly did ($P = 0.064$). For $R/R_{50}$, only $BW_{22}$ was significantly positively correlated with life span. For $N/R_{40}$, no correlation reached statistical significance, but $BW_{15}$ approached ($P = 0.107$) that level. No statistically significant correlations were observed for NP or $N/N_{85}$ mice.

Correlations between $BW_W$, $BW_5$, $BW_{10}$, $BW_{15}$ or $BW_{22}$ and presence of lymphoma or hepatoma were also determined for mice in each group. Results (not shown) indicated several small positive correlations between body weight and the eventual development of lymphoma, which were either statistically

TABLE 3

*Influence of dietary restriction on longevity*[1,2]

| Group | *n* | Range | Life span | Age of longest-lived 10% |
|---|---|---|---|---|
| | | | *mo* | |
| NP | 49 | 6.4–35.5 | 27.4 ± 0.9[a] | 35.1 ± 0.3[a] |
| $N/N_{85}$ | 57 | 17.9–42.3 | 32.7 ± 0.7[b] | 39.7 ± 0.6[b] |
| $N/R_{50}$ | 71 | 18.6–51.9 | 42.3 ± 0.9[c] | 51.1 ± 0.2[c] |
| $R/R_{50}$ | 56 | 24.2–50.7 | 42.9 ± 0.9[ce] | 50.5 ± 0.1[c] |
| $N/R_{50lopro}$ | 56 | 23.4–49.7 | 39.7 ± 0.9[d] | 48.5 ± 0.5[d] |
| $N/R_{40}$ | 60 | 19.6–54.6 | 45.1 ± 0.9[e] | 53.0 ± 0.3[e] |

[1]Values are in months. Means are followed by SEM. [2]Statistical significance of differences between means was evaluated by Duncan's multiple-range test, which was applied when one-way analysis of variance indicated significant differences. Means in each column not sharing a common superscript letter were significantly different ($P = 0.05$).

TABLE 4

*Influence of diet on tumor incidence and longevity*[1,2]

| Group | n | Mice with a tumor | | Mice with no tumor | |
|---|---|---|---|---|---|
| | | Incidence | Longevity | Incidence | Longevity |
| | | % | *mo* | % | *mo* |
| NP | 45 | $56^{ab}$ | $29.8 \pm 0.6^{a}$ | $44^{ab}$ | $25.5 \pm 1.4^{a}$ |
| $N/N_{85}$ | 54 | $78^{c}$ | $32.3 \pm 0.9^{a}$ | $22^{c}$ | $34.2 \pm 0.8^{b}$ |
| $N/R_{50}$ | 70 | $51^{ab}$ | $42.1 \pm 1.2^{bc}$ | $49^{ab}$ | $42.2 \pm 1.5^{cd}$ |
| $R/R_{50}$ | 54 | $63^{bc}$ | $42.5 \pm 1.2^{bc}$ | $37^{bc}$ | $43.9 \pm 1.3^{cd}$ |
| $N/R_{50lopro}$ | 55 | $53^{ab}$ | $39.3 \pm 1.2^{b}$ | $47^{ab}$ | $40.2 \pm 1.5^{c}$ |
| $N/R_{40}$ | 60 | $38^{a}$ | $43.6 \pm 1.2^{c}$ | $62^{a}$ | $46.1 \pm 1.2^{d}$ |

[1]Values for tumor incidence are the percent of mice in that diet group with or without a tumor. Values for longevity are means ± SEM in months. [2]The statistical significance of differences between values for tumor incidence was evaluated by an overall chi-square test followed by separate chi-square tests between each pair of groups. Values for longevity were statistically compared by using Duncan's multiple-range test after one-way analysis of variance indicated significant differences. Values in each column not sharing a common superscript were significantly different ($P = 0.05$).

significant or very nearly so. For NP mice, $BW_W$ and lymphoma were positively correlated ($r = 0.255$, $P = 0.078$), and for $N/N_{85}$ mice, $BW_{22}$ and lymphoma were positively correlated ($r = 0.258$, $P = 0.065$). Likewise, in group $N/R_{50}$ positive associations with lymphoma occurred for $BW_W$ ($r = 0.188$, $P = 0.117$) and for $BW_5$ ($r = 0.214$, $P = 0.074$). For $R/R_{50}$, the presence of lymphoma was positively correlated with $BW_5$ ($r = 0.282$, $P = 0.035$), $BW_{10}$ ($r = 0.225$, $P = 0.096$), and $BW_{22}$ ($r = 0.209$, $P = 0.121$). $N/R_{40}$ mice showed the same tendency with associations between lymphoma and $BW_{15}$ ($r = 0.233$, $P = 0.073$) and $BW_{22}$ ($r = 0.251$, $P = 0.055$). In contrast, the presence of hepatoma was not so clearly related to body weight at any of the ages in the six diet groups. Only one statistically significant correlation was observed for hepatoma (for $N/R_{40}$ mice, $BW_W$ gave $r = -0.257$, $P = 0.048$) and none of the other 29 correlations even approached significance ($P > 0.135$). Thus, we conclude that there is only chance association between hepatoma presence and body weight.

TABLE 5

*Influence of diet on the incidence of three types of tumors and longevity for mice in each diet-tumor group*[1,2]

| Group | Type of tumor | | | | | |
|---|---|---|---|---|---|---|
| | Lymphoma | | Hepatoma | | Soft tissue | |
| | Incidence | Longevity | Incidence | Longevity | Incidence | Longevity |
| | % | *mo* | % | *mo* | % | *mo* |
| NP | $31^{ab}$ | $29.5 \pm 1.0^{a}$ | $9^{a}$ | $28.8 \pm 1.0^{a}$ | $9^{ab}$ | $32.2 \pm 1.1^{a}$ |
| $N/N_{85}$ | $46^{a}$ | $31.1 \pm 1.1^{a}$ | $20^{ab}$ | $34.4 \pm 1.7^{a}$ | $15^{a}$ | $34.2 \pm 2.2^{ab}$ |
| $N/R_{50}$ | $23^{b}$ | $39.8 \pm 1.7^{b}$ | $21^{ab}$ | $45.2 \pm 1.9^{b}$ | $0^{c}$ | — |
| $R/R_{50}$ | $19^{bc}$ | $38.7 \pm 2.6^{b}$ | $37^{b}$ | $44.1 \pm 1.3^{b}$ | $6^{ac}$ | $40.3 \pm 4.6^{ab}$ |
| $N/R_{50lopro}$ | $29^{ab}$ | $39.1 \pm 1.5^{b}$ | $18^{a}$ | $40.7 \pm 2.2^{b}$ | $4^{bc}$ | $32.6 \pm 2.7^{a}$ |
| $N/R_{40}$ | $13^{c}$ | $42.2 \pm 1.8^{b}$ | $15^{a}$ | $44.0 \pm 2.0^{b}$ | $3^{bc}$ | $44.4 \pm 2.0^{b}$ |

[1]Tumor incidence values are the percent of mice in that diet group with that type of tumor. Values for longevity are means ± SEM in months. [2]The statistical significance of differences between values for tumor incidence was evaluated by an overall chi-square test followed by separate chi-square tests between each pair of groups. Values for longevity were statistically compared by using Duncan's multiple-range test after one-way analysis of variance indicated significant differences. Values in each column not sharing a common superscript were significantly different ($P = 0.05$).

TABLE 6

*Correlations between body weight (BW) and life span (LS)*[1,2]

| Group | $BW_W$-LS | $BW_5$-LS | $BW_{10}$-LS | $BW_{15}$-LS | $BW_{22}$-LS |
|---|---|---|---|---|---|
| NP | 0.115 | 0.026 | −0.020 | −0.053 | 0.224 |
| $N/N_{85}$ | 0.192 | 0.053 | 0.050 | −0.014 | −0.014 |
| $N/R_{50}$ | −0.016 | −0.063 | 0.045 | 0.257* | 0.223† |
| $R/R_{50}$ | −0.002 | −0.216 | −0.003 | 0.066 | 0.264* |
| $N/R_{50lopro}$ | −0.208 | 0.005 | 0.313* | 0.355‡ | 0.211 |
| $N/R_{40}$ | 0.174 | 0.058 | −0.078 | 0.210 | 0.140 |

[1]Abbreviations used: $BW_W$, body weight at weaning; $BW_5$, body weight at 5 mo of age; $BW_{10}$, body weight at 10 mo of age; $BW_{15}$, body weight at 15 mo of age; $BW_{22}$, body weight at 22 mo of age. All mice studied for longevity were used for this analysis. [2]Pearson product-moment correlations were used. Statistical significance indicated by: †$P < 0.10$; *$P < 0.05$; ‡$P < 0.01$.

*Lymphocyte proliferation.* Responses to the T-cell mitogens fell with age in all diet groups (table 7). PHA and Con A responses were higher for the restricted mice than for $N/N_{85}$ mice at both 10–11 mo and 27–31 mo of age. Old $N/N_{85}$ mice responded very weakly to either of the T-cell mitogens. The PHA and Con A responses of $N/R_{40}$ mice at either age exceeded responses for age-matched $N/R_{50}$ mice (although the difference for the PHA response of old mice in these two groups was not statistically significant). Responses to PPD were not influenced by aging or diet. Background proliferation (i.e., media without mitogen) was also not overtly affected by aging or diet.

The number of nucleated cells that could be harvested from the spleen was greatest for old $N/N_{85}$ mice (average of $135 \times 10^6$ cells/spleen), less for adult $N/N_{85}$ mice ($88 \times 10^6$ cells/spleen), and least for the three restricted groups at either age ($23–51 \times 10^6$ cells/spleen).

*Lifetime energy intake (LEI).* LEI was calculated for mice in five diet groups ($N/N_{85}$, $N/R_{50}$, $R/R_{50}$, $N/R_{50lopro}$ and $N/R_{40}$). Data are shown in table 8. The daily caloric intake per gram of mouse [kilocalories/(gram • day)]

TABLE 7

*Influences of diet and aging on splenic lymphocyte proliferation*[1,2]

| Group | Response to mitogen or medium | | | |
|---|---|---|---|---|
| | PHA | Con A | PPD | Medium |
| | *cpm* | | | |
| *Adult (10–11 mo)* | | | | |
| $N/N_{85}$ | 4,425 ± 500$^{ab}$ | 19,575 ± 2,175$^{abc}$ | 16,750 ± 1,800$^{a}$ | 675 ± 150$^{abc}$ |
| $N/R_{50}$ | 15,700 ± 2,525$^{c}$ | 38,300 ± 4,800$^{de}$ | 13,950 ± 3,575$^{a}$ | 525 ± 100$^{bc}$ |
| $N/R_{50lopro}$ | 22,800 ± 2,525$^{d}$ | 49,200 ± 5,575$^{ef}$ | 16,000 ± 3,750$^{a}$ | 550 ± 100$^{bc}$ |
| $N/R_{40}$ | 26,375 ± 4,550$^{d}$ | 56,575 ± 7,450$^{f}$ | 19,925 ± 2,275$^{a}$ | 1,025 ± 250$^{a}$ |
| *Old (27–31 mo)* | | | | |
| $N/N_{85}$ | 675 ± 200$^{a}$ | 5,225 ± 1,075$^{a}$ | 14,275 ± 2,025$^{a}$ | 500 ± 75$^{bc}$ |
| $N/R_{50}$ | 4,300 ± 1,000$^{ab}$ | 13,475 ± 2,000$^{ab}$ | 15,400 ± 5,000$^{a}$ | 425 ± 100$^{c}$ |
| $N/R_{50lopro}$ | 7,900 ± 2,250$^{b}$ | 25,525 ± 6,575$^{bcd}$ | 19,900 ± 3,325$^{a}$ | 900 ± 200$^{ab}$ |
| $N/R_{40}$ | 10,925 ± 1,450$^{bc}$ | 32,000 ± 5,400$^{cd}$ | 18,200 ± 3,150$^{a}$ | 675 ± 50$^{abc}$ |

[1]Values are means ± SEM for $n = 8$ mice per group (except for PPD, where $n = 5$). Mitogens: PHA, phytohemagglutinin; Con A, concanavalin A; and PPD, purified protein derivative. The values are counts per minute (cpm) of [$^3$H]TdR in mitogen-stimulated cultures minus cpm in unstimulated (media without mitogen) cultures. [2]Statistical significance of differences between means was evaluated by Duncan's multiple-range test, which was applied when one-way analysis of variance indicated significant differences. Means in each column not sharing a common superscript letter were significantly different ($P = 0.05$).

TABLE 8

*Influences of diet on lifetime energy intake*[1,2]

| Group | Caloric intake | | |
|---|---|---|---|
| | kcal/(g · d) | kcal/(g · lifetime) | kcal/(mouse · lifetime) |
| $N/N_{85}$ | 0.356 ± 0.004[a] | 354 ± 8[a] | 12,080 ± 248[a] |
| $N/R_{50}$ | 0.356 ± 0.003[a] | 455 ± 10[bc] | 9,174 ± 284[bc] |
| $R/R_{50}$ | 0.355 ± 0.002[a] | 464 ± 10[c] | 9,312 ± 195[b] |
| $N/R_{50lopro}$ | 0.360 ± 0.004[a] | 433 ± 10[b] | 8,613 ± 202[c] |
| $N/R_{40}$ | 0.323 ± 0.003[b] | 441 ± 8[bc] | 7,833 ± 147[d] |

[1]Values are means ± SEM. Lifetime energy intake was calculated as either the number of kilocalories ingested per gram of mouse over its lifetime [kcal/(g · lifetime)] or as the number of kilocalories ingested per whole mouse over its lifetime [kcal/(mouse · lifetime)]. The mean body weight for each mouse was estimated by averaging body weights recorded at 5–6, 9–11, 14–17 and 21–23 mo of age. This value for the five groups listed above was (respectively): 34.5, 20.3, 20.1, 20.0 and 17.8 g. The mean adult body weight of each mouse was then divided into the daily caloric intake (12.1 for $N/N_{85}$; 7.1 for $N/R_{50}$, $R/R_{50}$ and $N/R_{50lopro}$; 5.7 for $N/R_{40}$) to give kcal/(g · d). This value was multiplied by the mean life span (in days) to give kcal/(g · lifetime). The kcal/(mouse · lifetime) was calculated for each mouse by multiplying its life span (days) by the daily caloric intake. [2]The statistical significance of differences between means was evaluated by Duncan's multiple-range test, which was applied when one-way analysis of variance indicated significant differences. Means in each column not sharing a common superscript letter were significantly different ($P = 0.05$).

was ~10% lower for $N/R_{40}$ mice than for the other groups (which did not differ significantly). The LEI per gram of mouse [kilocalories/(gram · lifetime)] was ~30% greater in the four most restricted groups as compared to $N/N_{85}$ mice. However, LEI per mouse [kilocalories/(mouse · lifetime)] was ~30–55% greater for $N/N_{85}$ mice than for the four restricted groups. The LEI per mouse for group $N/R_{40}$ mice was significantly less than that of all the other groups.

Energy intake was also calculated on the basis of organ weight by using data from table 2. The liver, kidney and spleen weights for each mouse were added, and this total organ mass (in grams) was used to calculate kilocalories/(organ mass · day) and kilocalories/(organ mass · lifetime) (using the mean life spans from the longevity study). The kilocalories(/organ mass · day) values (mean ± SEM) for the adult mice ranked $N/N_{85}$ (6.93 ± 0.42) > $N/R_{50lopro}$ (6.11 ± 0.11) > $N/R_{50}$ (5.59 ± 0.10) > $N/R_{40}$ (4.88 ± 0.12). The kilocalories/(organ mass · day) values for the old mice ranked $N/N_{85}$ (6.35 ± 0.21) > $N/R_{50lopro}$ (5.64 ± 0.12) = $N/R_{50}$ (5.41 ± 0.11) > $N/R_{40}$ (5.00 ± 0.10). By using an average of the adult and old organ weight values for each group, the kilocalories/(organ mass · lifetime) values were calculated and found to be quite similar for each group: $N/N_{85}$ = 6600, $N/R_{50}$ = 7073, $N/R_{50lopro}$ = 7097, and $N/R_{40}$ = 6772. This small variation (<10%) among the groups in kilocalories/(organ mass · lifetime) contrasts to large differences between restricted and $N/N_{85}$ mice in both kilocalories/(gram body · lifetime) and kilocalories/(mouse · lifetime).

## DISCUSSION

The present findings are in accord with previous ones showing that appropriate dietary restriction of rodents can increase mean and maximum life span (1–4), favorably influence late-life disease patterns (3–7), and delay immunologic aging (13). Our results expand the earlier findings in several ways. These include the following: *1*) As the severity of dietary restriction increased, so did longevity. The maximum life span (mean for the 10% longest lived) of mice fed slightly restricted amounts of a control purified diet (group $N/N_{85}$) was 13% greater than that for mice fed a nonpurified diet (group NP) ad libitum. The maximum life span for the most severely restricted group ($N/R_{40}$) was 51% greater than that of NP mice, 34% greater than that of $N/N_{85}$ mice, and 6% greater than that of $N/R_{50}$ mice. The $N/R_{40}$ regime approximates as severe a restriction as can be well tolerated by weanling female mice of this hybrid

strain. This comparative increase in longevity for the restricted mice occurred even though the $N/N_{85}$ and NP populations themselves were quite long-lived. The amount of chromium, equally deficient in control and test diets, did not prevent either population from obtaining good or great longevity. 2) The mean and maximum life spans observed for $N/R_{40}$ mice exceed, to our knowledge, all previously reported values for laboratory mice. 3) Food intake limited prior to weaning did not further increase longevity for mice subjected to postweaning dietary restriction. 4) Mice restricted in both calorie and protein intake (group $N/R_{50lopro}$) exhibited shorter mean and maximum life spans (~5%) than did mice fed the same number of calories of a high protein diet. 5) Beneficial influences on tumor patterns or on age-related declines in T-lymphocyte proliferation were, like effects on longevity, most striking in the $N/R_{40}$ group.

For the two most commonly occurring tumors, the incidence of lymphoma was reduced by dietary restriction while hepatoma incidence was not. Mice eating near ad libitum amounts of a purified diet showed the highest overall tumor incidence. It is unclear if the NP mice developed fewer tumors than $N/N_{85}$ mice because of protective factors in the nonpurified diet or tumor initiating/promoting substances in the purified diet.

An intermittent feeding schedule was used in this study. It is not known with certainty what effect, if any, intermittent feeding has on longevity. Earlier results in rats (2, 3) suggest that intermittent feeding is not essential for life-span extension with dietary restriction.

Although dietary restriction is widely viewed as the most potent method available to retard aging in homeotherms (10–12, 25), several individuals have expressed other interpretations of the data. Cherkin suggests (26) that general acceptance of a causal relationship between dietary restriction and increased life span needs to be reexamined because the evidence equally supports the idea that ad libitum–fed laboratory animals are showing accelerated aging, restricted animals being the norm. We agree that it is probably incorrect to view ad libitum feeding as "normal" (although many humans eat ad libitum). For this reason we included one control group ($N/N_{85}$) not eating ad libitum. Cutler (27) also argues that overeating hastens aging suggesting that restriction returns the animal to the aging rate it would normally have in its natural ecological niche. He continues: "Life span extension is most frequently thought to be a process that prolongs life beyond what is the normal genetic potential for the animal. Calorie restriction and/or intermittent fasting does not appear to be such a process." Problems with these views include the following: *1*) Mice in the wild show greatly accelerated mortality as compared to their counterparts in the laboratory (28). Because so many wild mice die so early in life, extensive data on maximum life spans are apparently lacking. Quite likely, the "normal" state in the wild is that state which gives the *species* (not the *individual*) the greatest chance to survive. Since restricted female rats in the laboratory show delayed sexual maturation (but a striking prolongation of reproductive life span) (19), caloric restriction would not appear to be such a state. 2) Even for laboratory mice, the "normal genetic potential" for longevity in a given animal model remains undetermined. Does the longevity of the $N/R_{40}$ mice in the present study fall short of, meet, or exceed the "normal genetic potential" for longevity in this $F_1$ hybrid? One of us has more fully addressed elsewhere (29) these and other contrary views on the capacity of dietary restriction to increase maximum life span.

The present data reveal a tendency for heavier mice in the diet-restricted groups to live longer than lighter diet-restricted mice. Body weight did not serve as a predictor of longevity in the two groups of mice eating at or near ad libitum. The increased longevity of the heavier restricted mice might suggest that metabolically efficient individuals on low calorie regimes are longer lived than individuals less capable of storing ingested calories as body mass. Our results contrast with those of Goodrick and co-workers (18) where ad libitum–fed rats showed significant positive correlations between body weight and life span but restricted rats showed no significant correlation.

We have previously suggested that immunologic sequelae of dietary restriction may contribute to effects on longevity and late-life diseases (13, 30, 31). The immune

systems of mammals show changes with age that might be involved in either the etiology and/or pathogenesis of aging (10, 32). The study of immunologic aging is a rapidly expanding field (reviewed in ref. 33) and two main types of changes occur in humans and mice. A *decreased* capacity to respond to exogenous stimuli (e.g., T-cell mitogens, viruses) occurs with advancing age along with *increases* in autoimmunity. Both aspects of immunosenescence have been reported to stay "younger longer" with dietary restriction in mice (13, 30, 31, 34, 35). In the present study, T-cell proliferation induced by either of two mitogens was observed to decline sharply with age and be increased by dietary restriction in youth and old age. Based on a previous study we carried out on $N/N_{85}$, $N/R_{50}$ and $N/R_{40}$ mice from this $F_1$ hybrid, these increases in splenic proliferation responses may be partly due to a higher proportion of T cells within the spleen (30). In that study no diet effect on Con A–induced proliferation was observed in 5- to 6-mo-old mice, which contrasts to the present findings in 10- to 11-mo and 27- to 31-mo-old mice.

The LEI data may also provide insights into how dietary restriction retards aging. We observed that restricted mice ingested energy at about the same daily rate when expressed per gram of mouse [kilocalories/(gram • day)] as did $N/N_{85}$ mice. Restricted mice consumed ~30% more kilocalories/(gram • lifetime) than did controls but ~30–55% less kilocalories/(mouse • lifetime). These results on kilocalories/(gram • lifetime) agree with those reported in rats by Masoro and colleagues (36), which led these workers to conclude that dietary restriction does not slow the aging rate by slowing metabolic rate. On the other hand, by calculating kilocalories/(gram • lifetime) using data from two of Ross' studies (37, 38), Sacher (25) reached a different conclusion, namely, that kilocalories/(gram • lifetime) was about the same for five groups of rats varying more than fourfold in daily caloric intakes. Sacher deduced that the near constancy of kilocalories/(gram • lifetime) indicated that dietary restriction prolongs life by reducing the metabolic rate per gram of rat and allowing a longer time to reach the limiting number of kilocalories/(gram • lifetime). However, in our view, Sacher's calculations are marred because he combined data from two of Ross' studies and differences existed between those populations. Also, Ross' data appear inappropriate for this type of calculation (36). Finally, to compare our results, the kilocalories/(organ mass • lifetime) showed less than 10% variation among $N/N_{85}$, $N/R_{50}$, $N/R_{50lopro}$ and $N/R_{40}$ mice.

Other measures of metabolic rate such as $O_2$ consumption and heat production have been studied in rodents subjected to short-term dietary restriction but the literature fails to yield a consensus view (discussed in ref. 9). Recent findings (39) show that $O_2$ consumption per gram of lean body mass is the same for food-restricted and ad libitum–fed rats but that $O_2$ consumption per whole rat is reduced in restricted rats. These data, like those on calorie intake, can be difficult to interpret because different ways of expressing the data can drastically change the direction of a dietary effect.

Mitochondria and free radicals generated therein by normal metabolism may play a causative role in the aging process (40, 41). Mounting evidence describes age-related deficits or defects in this organelle including increases in free radical formation and peroxidative membrane damage, lower activities for several inner membrane enzymes, and lowered in vitro respiratory activity. We have previously suggested (9, 14) that dietary restriction may retard aging via mitochondrial effects. We observed (9) that livers from restricted mice gave a lower recovery of mitochondrial protein and cytochrome *c* oxidase activity. Liver mitochondria from restricted mice generally showed increased state 3 rates of oxygen usage (i.e., in the presence of ADP) with no differences from controls in state 4 rates (i.e., after ADP was used) for respiration supported by glutamate or pyruvate + malate. This resulted in an increased respiratory control index for the restricted group. A higher index suggests better coupling of oxidative phosphorylation to electron transport, which could result in reduced free radical generation, less mitochondrial damage, and a postponement of losses with age in mitochondria. These mitochondrial effects could partly explain two of the present findings: that restricted mice ate more kilocalories/(gram • lifetime) and that the heavier, more energy efficient restricted

mice tended to live longer than lighter restricted mice.

The rodent fed a restricted diet appears to provide the best existing model to study the biology of decelerated aging. Understanding the mechanisms behind this deceleration should advance our knowledge about fundamental aging processes. In addition, the profound retardation of aging brought on by these diets may reshape our thinking on what is optimal nutrition for humans.

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